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Reproductive Biology of Vermiculated Sail Fin Cat Fish *Pterygoplichthys disjunctivus* in Victoria & Kalaweva Reservoirs in Sri Lanka

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Abstract: *Pterigoplichthys disjunctivus* had been accidentally or intentionally introduced to Sri Lankan freshwaters possibly through ornamental fish trade. *P. disjunctivus* native to South America has established populations in many water bodies in Sri Lanka including Victoria & Kalaweva reservoirs. Live specimens of the species were obtained from commercial catch from 2015 to 2017 & 2016 to 2017, from Victoria & Kalaweva reservoirs respectively. Total & standard lengths were measured to nearest 0.1cm and weighed to nearest 0.1g. and dissected to obtain the gonads which were preserved in 5% buffered formalin. A maturity scale was identified on the macroscopic/microscopic characteristics of gonads for both male and female by examining transparency, overall color, and vascularization, visibility of oocytes and size of gonads. Gonadosomatic (GSI) indices, total fecundity (TF) and relative fecundity (RF) were also studied. Mean gonad weight of females and males are 7.604±9.107 & 12.784±16.699 in Victoria and 0.5162±0.5373 & 0.9012±1.0778 in Kalawewa, respectively. Mean GSI for females and males are 0.04109±0.05220 & 0.04361±0.05042 in Victoria and 0.002347±0.002105 & 0.002362±0.001914 in Kalawewa, respectively. Mean fecundity was 956±261 in Victoria & 1856±817 in Kalawewa, respectively. Mean relative fecundity was 4.3783±1.3242 in Victoria & 6.564±2.841 in Kalawewa. Mean egg diameter was 1712±955.8 in Victoria & 1747±884.8, in Kalawewa, respectively. *P. disjunctivus* shows extended peak reproductive period from April to October. Harvesting this species during its spawning season may aid in controlling population size.

Keywords: invasive, *Pterigoplichthys disjunctivus*, Reproductive biology, controlling

Introduction

Invasive alien species (IAS) has been found out to be main reasons of biodiversity loss in freshwater eco systems (Clavero and Garcia-Berthou 2005). Reproductive biology studies of invasive fish species have found out that they expand well in exotic ecosystems. (Garcia-Berthou 2007). Gonad features make available identifications on reproductive strategies and observing gonadal changes help in determining reproductive array and spawning periodicity (Brown-Peterson et al. 2011). The information on spawning season duration, peak spawning, sex ratio and size or age at maturity is essential for management measures such as mechanical control to prevent their further spread in exotic environments (Morgan, 2008). Visual observations of gonads either macroscopically and microscopically, provide identification of gonad developmental stages (Yoneda et al., 1998; Smith and Walker, 2004; Gundersen et al., 2010; Ellender et al., 2012). Macroscopic methods of gonad analysis means inspecting characteristics of whole gonads with the naked eye including quantitative measurement of relative gonad size i.e. gonadosomatic index (IG; McPherson et al., 2011). Heavy exploitation during spawning season or during reproductive cessation period using a suitable fishing gear, this invasive species can be controlled in inland water bodies of Sri Lanka. The aim of study is to provide scientific information of the reproductive success of *P. disjunctivus* for sustainable management. .

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Materials and Methods

Total length and standard length of live specimens of *P. disjunctivus* obtained from commercial catch, were measured to nearest 0.1cm and weighed to nearest 0.1g.Then the fish was dissected to obtain the gonads which were preserved in 5% buffered formalin. Following preservation, each pair of ovary and pair of testes were examined, weighed to the nearest 0.1 g and the gonadosomatic index: (GSI = [(gonad weight)/(body weight)] x 100) was calculated (Isaac-Nahum and Vazzoler, 1987; Wootton, 1998) for males and females separately to acertain spawning period (Snyder, 1983; Ferrara, et al 2001). The maturity scale based on macroscopic characteristics (White, et al., 1998) was used to classify the gonads. Features examined to identify the maturity stages were the degree of opacity of the gonads, consistency and vascularization, oocytes or sperm visibility and overall coloration of the gonads. The microscopic characteristics of gonads were identified according to Brown-Peterson et al. (2011). Gonad developmental stage was determined according to visual observations. Fecundity was determined by preserving the ovaries of stages IV to V in Gilson's fluid and left for at least 24 hours to be liberated from ovarian tissues after which the eggs were washed with 70% alcohol (Bagneal, 1978). The fully liberated eggs were then counted by gravimetric sub-sampling (wet method) as described by Bagneal, (1978). Eggs from each jar containing the clean eggs of an ovary were weighed after removing excess water on filter paper. One half a portion of the eggs of ovary was weighed and counted. From this, the number of eggs in a whole ovary was calculated. The egg diameter was measured in μm using a trinocular microsope. Relative fecundity (number of mature eggs per gram body weight) for females for each locality was compared. The spawning season was identified by plotting monthly mean GSI values. The correlation among GSI, fecundity and egg diameter with standard length, body weight and gonad weight was established.

Results and Discussion

Table 1 shows six arbitrary stages of oocyte maturity of female *Pterygoplichthys disjunctivus* based on external morphology of the ovary, colour and size of oocytes, % coverage of ovary in the coelomic cavity. Table 2 shows five scale maturity stages in males of the species species based on external morphology, colour and size of testicles and % coverage of testicles in the coelomic cavity. Table 3 and table 4 show microscopic ovary and testicular maturity stages in *P. disjunctivus.*

Table 1. Six arbitrary stages of oocyte maturity of female P. disjunctivus based on external morphology of the ovary, colour and size of oocytes, % coverage of ovary in the coelomic cavity are shown below.

Immature Stage (I): Ovaries are small, occupy < 10% of body cavity. Ovary surface is thin, pink to transparent without visible opaque oocytes.	
Developing Stage (II): Ovaries occupy 15% of the body cavity in females and, are pale yellow to opaque oocytes.	
Developed Stage (IV): Ovaries are large, occupying up to 40% of the body cavity. Thin ovarian walls, highly vascularised, asymmetry between the left and right ovaries. Bright yellow oocytes.	
Spawning Stage V: Ovaries are slightly flaccid, occupying up to 75% of body cavity in mature females. Oocytes large, bright yellow.	
Spent Stage VI: Ovaries occupy < 20-40% of body cavity. Ovaries large but flaccid, appear dark purple, like deflated sacs with few or no oocytes. Ovarian wall is thick.	

Table 2. Five scale maturity stages of male P. disjunctivus are shown below.

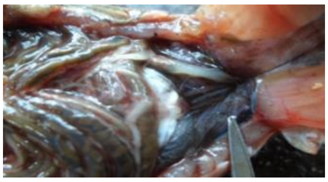
Immature stage (I): Testicles are small in males, occupying < 5% of body cavity and dark in color.	
Developing Stage (II): Testicles are small and turning milkfish in colour.	
Developed Stage (III): Growing testicles are milkish in colour and occupies about 8% of body cavity.	
Spawning Stage (IV): Testicles are milkish, occupies about 10% of the body cavity	

Table 3 Ovarian maturity stages in P. disjunctivus.

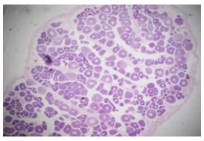
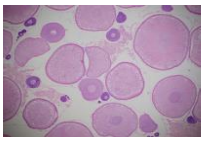
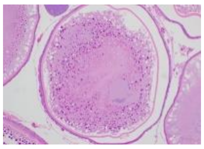
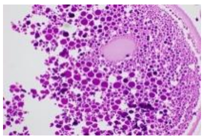
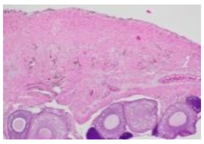
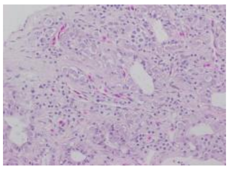
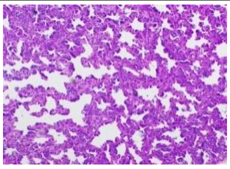
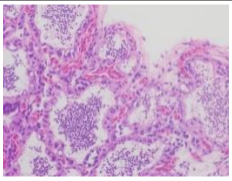
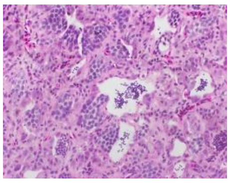
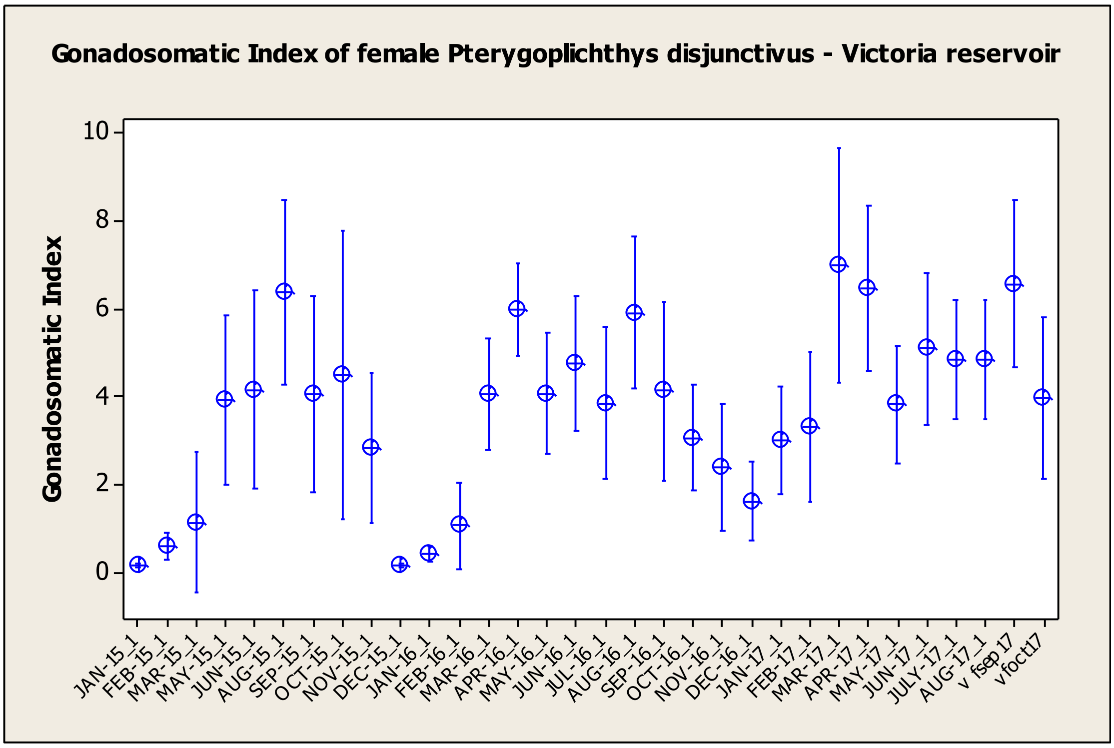
Stage	Histologic features
Immature (Stage 1) Ovaries contain mainly oocytes of primary growth stage. No evidence of prior spawning activity. Cells densely packed, in close association with the ovarian wall	
Developing (Stage 2) Majority of oocytes are in primary vitellogenic stage (secondary growth phase) . Oil droplets visible.	
Developing (Stage 3) Oocytes in secondary vitellogenic stage.	
Mature / spawning (Stage 4) Oocytes in third vitellogenic stage with migrating nucleus Yolk globules coagulated.	
Spent & regenerating (Stage 5) No fully yolked oocytes only primary and secondary growth phase oocytes can be seen. Thick ovarian wall present.	

Table 4. Testicular maturity stages in P. disjunctivus.

Stage	Histologic features
Immature (Stage 1) Testicular lining thin with cysts composed mostly of primary/secondary spermatogonia held in cysts.	
Developing (Stage 2) Increased diameter of individual tubules due to increased number of cysts. Lobules contain cysts of all germ cell types (spermatocytes and spermatids), though not consistently present together in all lobules	
Spawning capable (Stage 3) Lobules are full of Spermatozoa	
Spent & regenerating (Stage 5) Repopulation of the germinal epithelium with spermatogonia; spermatocytes and spermatids continue to repopulate lobules in the mid recovering spent stage. Thickening of lobule walls & occasional spermatozoa can be seen.	

A.


Gonadosomatic Index of female Pterygoplichthys disjunctivus - Victoria reservoir
Gonadosomatic Index
10
8
6
4
2
0
JAN-15_1
FEB-15_1
MAR-15_1
MAY-15_1
JUN-15_1
AUG-15_1
SEP-15_1
OCT-15_1
NOV-15_1
DEC-15_1
JAN-16_1
FEB-16_1
MAR-16_1
APR-16_1
MAY-16_1
JUN-16_1
JUL-16_1
AUG-16_1
SEP-16_1
OCT-16_1
NOV-16_1
DEC-16_1
JAN-17_1
FEB-17_1
MAR-17_1
APR-17_1
MAY-17_1
JUN-17_1
JULY-17_1
AUG-17_1
v fsep17
vfoct17

B.

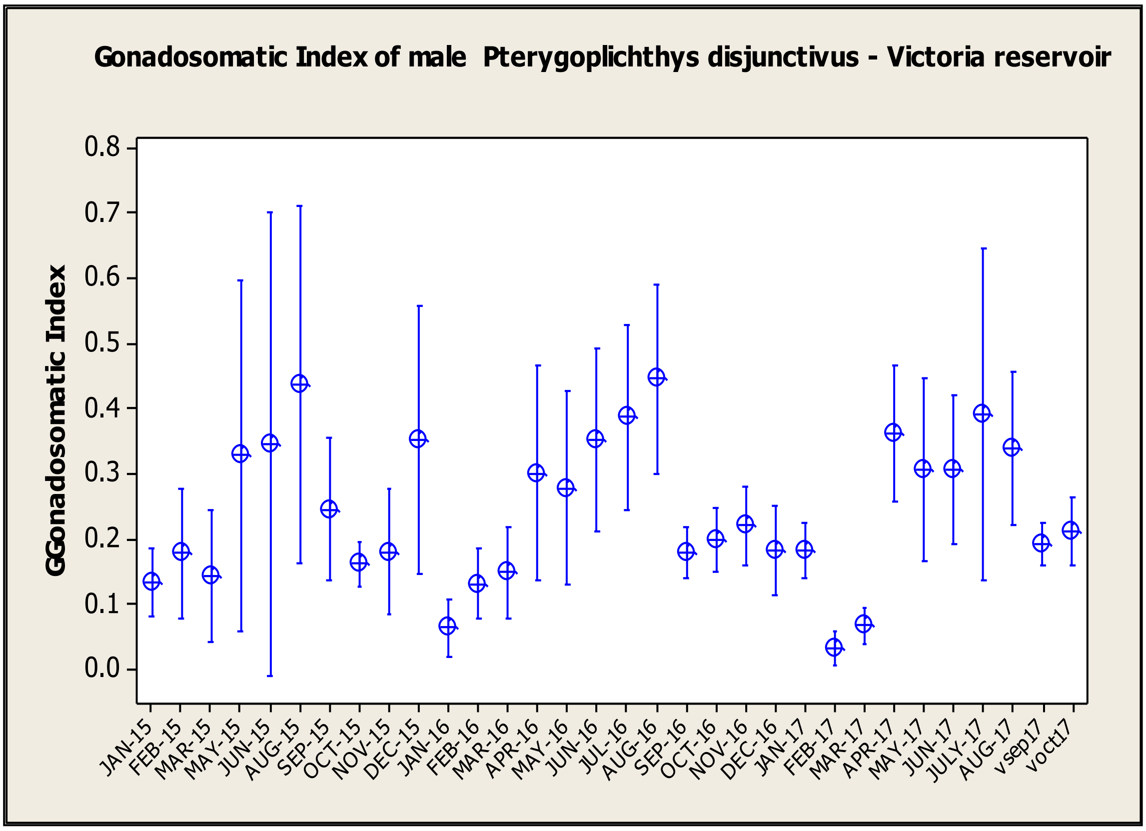
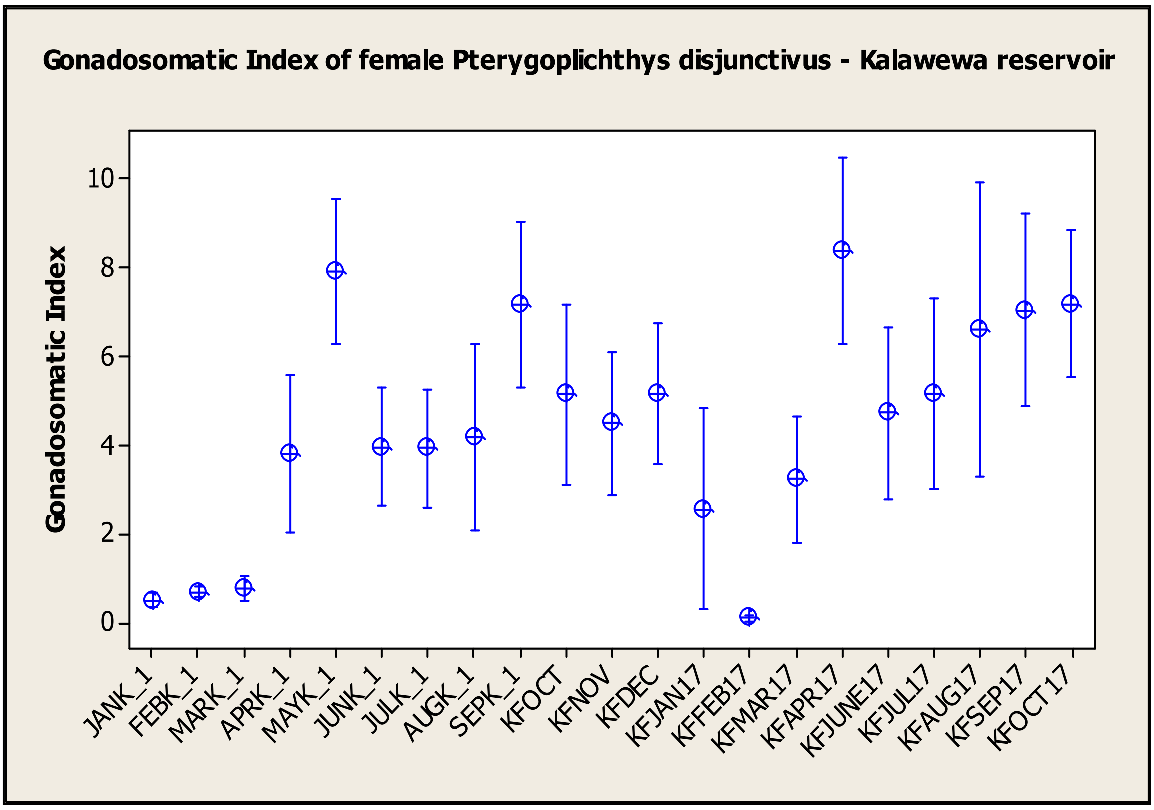

Gonadosomatic Index of male Pterygoplichthys disjunctivus - Victoria reservoir
GGonadosomatic Index
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
JAN-15
FEB-15
MAR-15
MAY-15
JUN-15
AUG-15
SEP-15
OCT-15
NOV-15
DEC-15
JAN-16
FEB-16
MAR-16
APR-16
MAY-16
JUN-16
JUL-16
AUG-16
SEP-16
OCT-16
NOV-16
DEC-16
JAN-17
FEB-17
MAR-17
APR-17
MAY-17
JUN-17
JULY-17
AUG-17
vsep17
voct17

Figure 1. Mean gonadosomatic index values of females (a) and males (b) of P. disjunctivus in Victoria reservoir

A.



B.

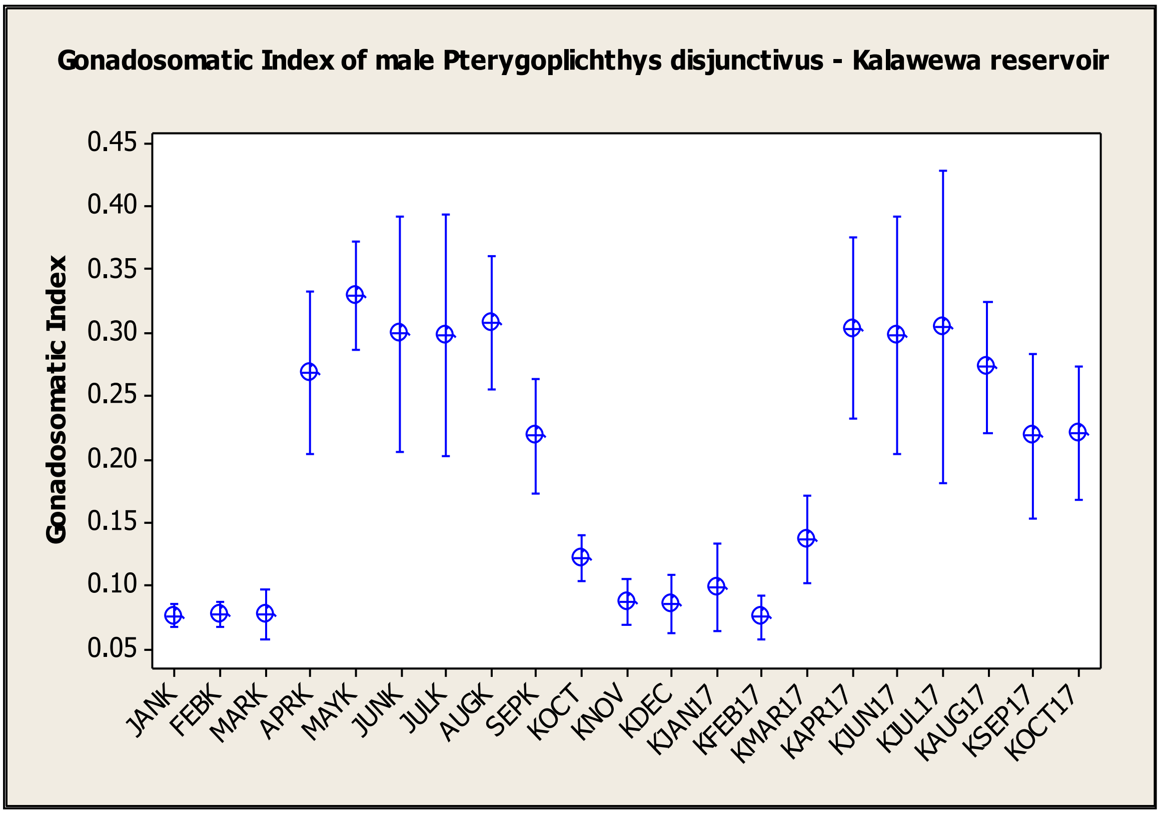
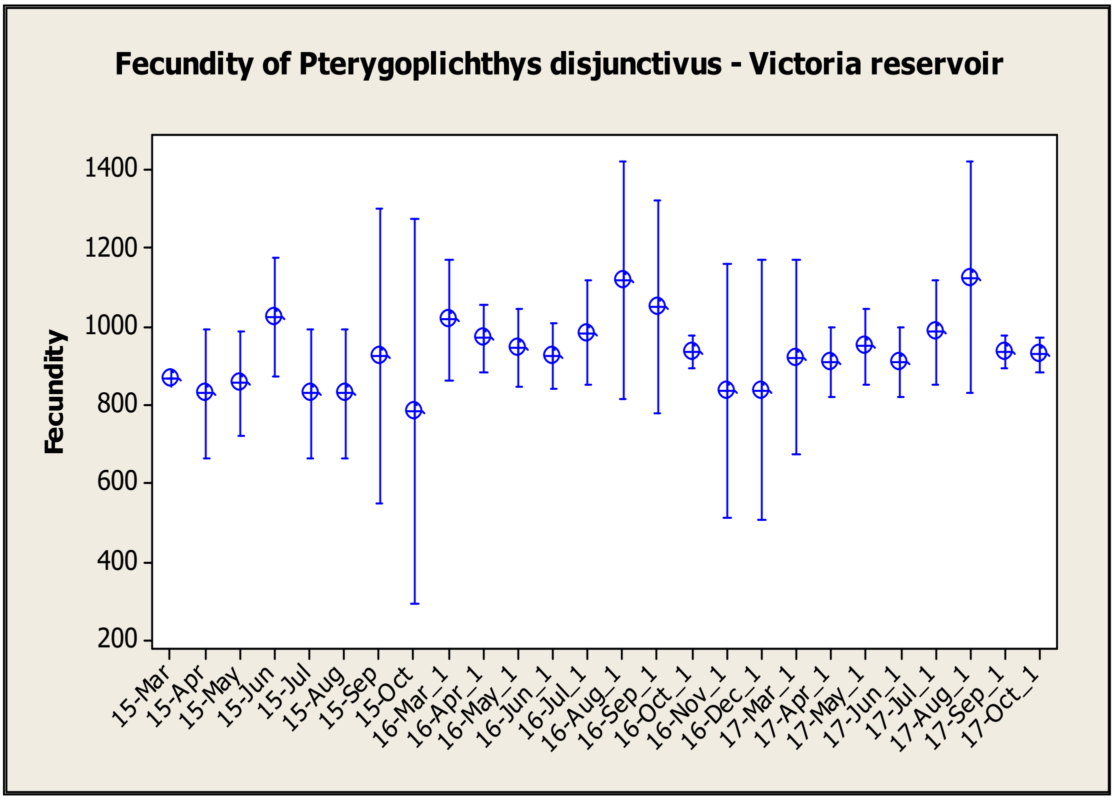


Figure 2. Mean gonadosomatic index values of females (a) and males (b) of P. disjunctivus in Kalawewa reservoir

A.



B.

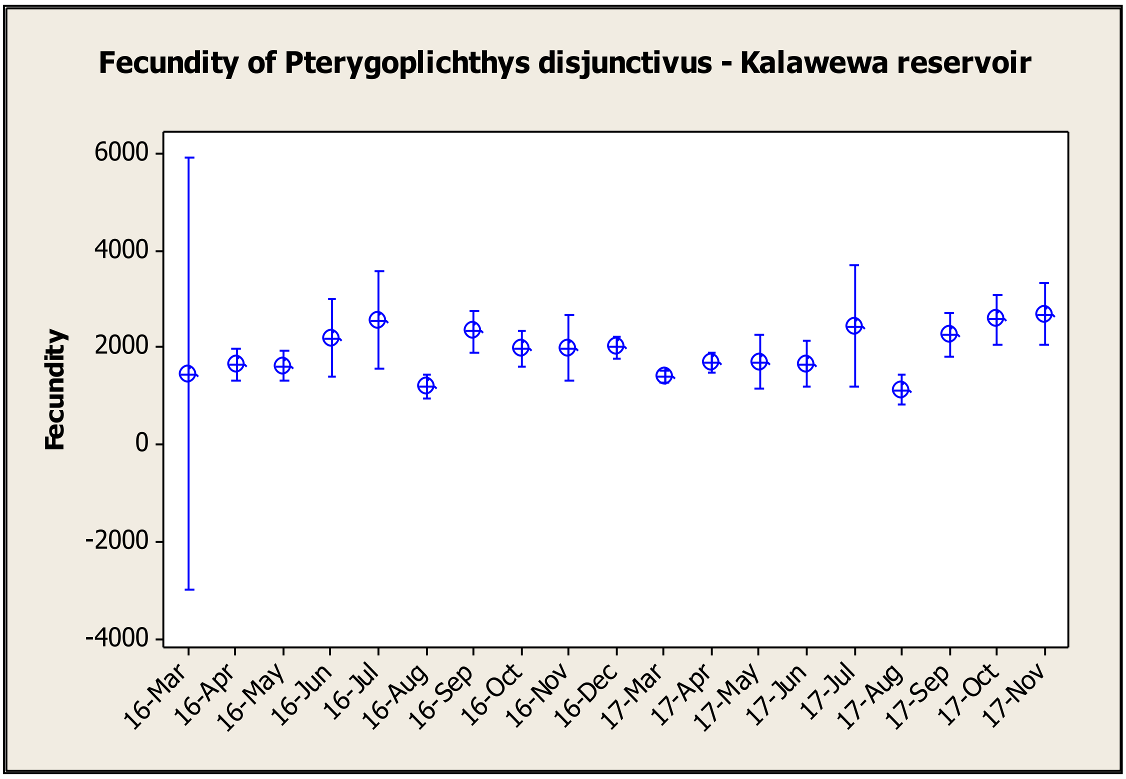
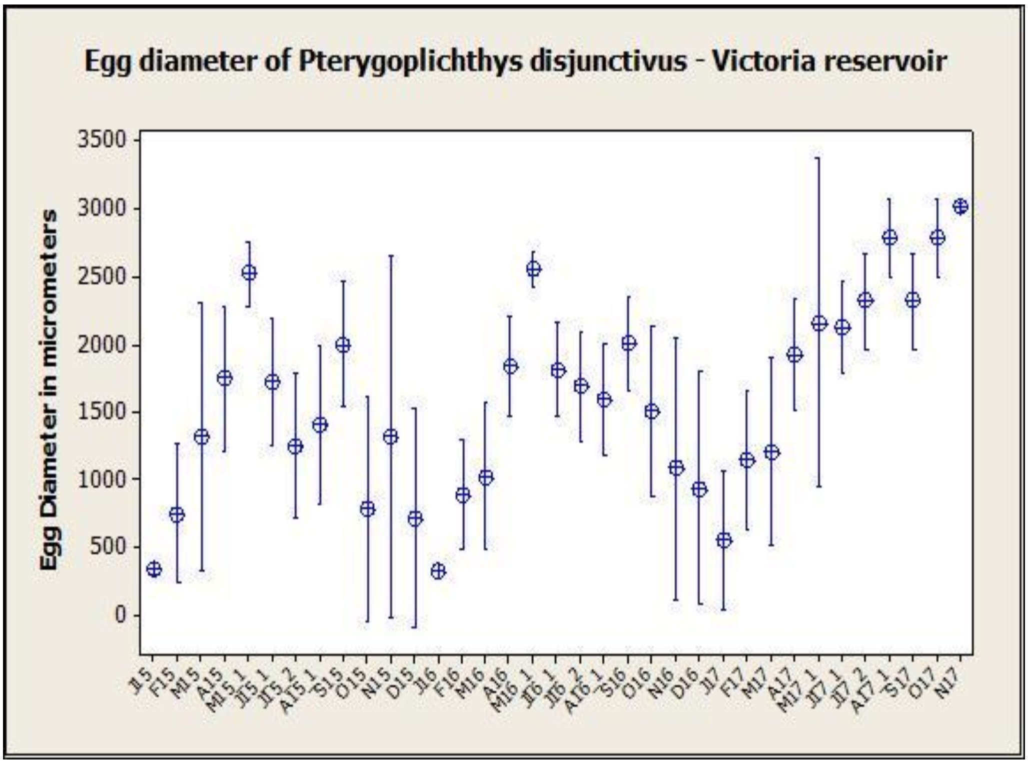


Figure 3. Fecundity of Pterygoplichthys disjunctivus in Victoria (a) and Kalawewa (b) reservoirs

A.



B.

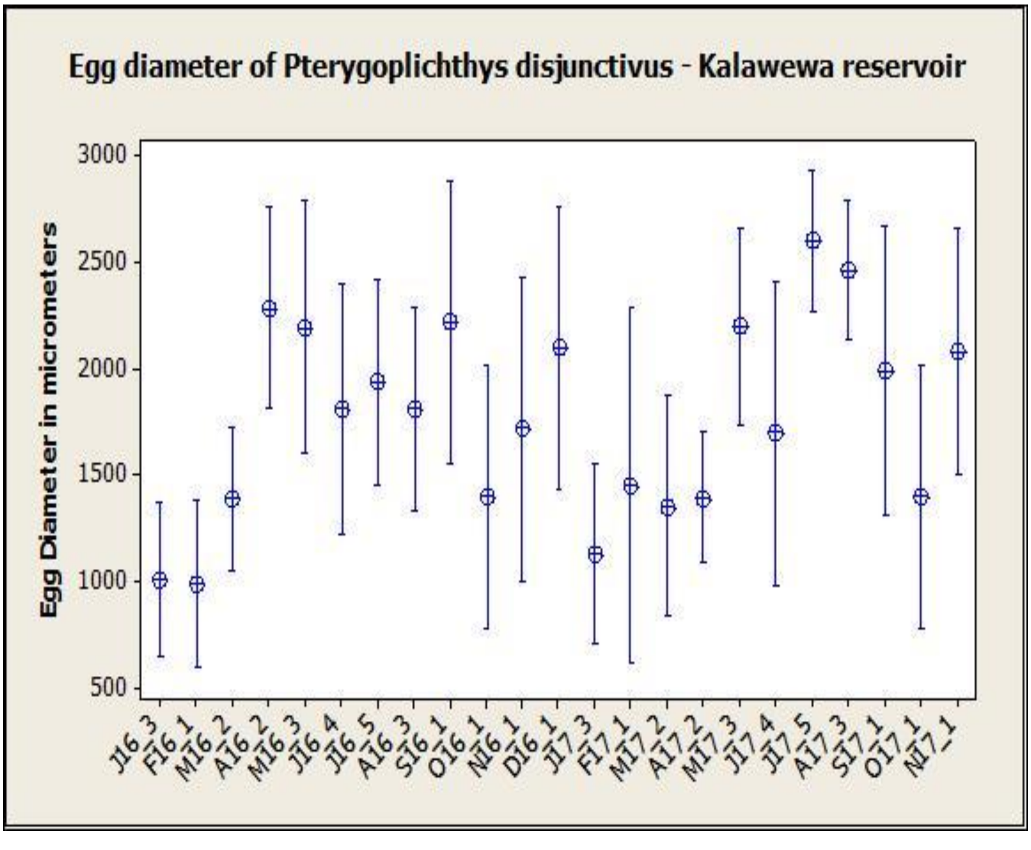


Figure 4. Egg diameter of P. disjunctivus in Victoria (a) and Kalawewa (b) reservoirs.

Table 1. Length weight relationships, condition factor values of both females and males of P. disjunctivus Kalawea & Victoria reservoirs.

		Victoria	Kalaweva
Length-weight relationship	females	log W = - 0.2211 + 1.918 log SL	log W = 0.1800 + 1.692 log SL
	males	log W = - 0.6322 + 2.218 log SL	log W = - 0.6540 + 2.298 log SL
Condition factor	females	0.024274±0.005552	0.03320 ± 0.03277
	males	0.022393 ± 0.004622	0.024439 ± 0.006749

Table 2. Relationships of Gonadosomatic index, Fecundity and Egg diameter with Gonad weight, Standard Length and Body Weight of P. disjunctivus in Victoria and Kalawewa reservoirs.

	Victoria		Kalawewa	
	Gonadosomatic Index (GSI)			
		R2		R2
Gonad weight of Females	Y=0.0046x+ 0.0061	0.6238	Y= 0.0025x+ 0.0121	0.6670
Gonad weight of Males	Y = 0.0036x + 0.0005	0.8323	Y = 0.0012x + 0.0013	0.4193
	Fecundity			
Gonad weight	Y = 16.989x + 574.08	0.5058	Y= 31.698x + 862.32	0.4704
Body Weight	Y= 3.2154x + 821.38	0.4148	Y= 1.8311x+ 526.88	0.4781
Standard Length	Y = 64.007x – 383.41	0.3684	Y= 122.54x – 90.17	0.3280
	Egg Diameter			
Gonad weight	Y = 0.0137x – 6.255	0.6889	Y = 0.0333x + 11.133	0.0254
Body Weight	Y = 0.1341x + 9.0503	0.5297	Y = 0.1285x + 294.14	0.0045
Standard Length	Y= 0.0031x + 15.645	0.5461	Y = 0.0059x + 21.932	0.0105

Impact of invasive species all over world is high (IUCN 2011) and previous studies have reported that Loricariids have a great capacity of geographic dispersion due to their physiological and adaptive characteristics that have led them to become a plague (Capps et al., 2011; Krishnakumar et al., 2009). The Loricariid catfishes are a group of the alien invasive species (AIS) in Sri Lanka, which have been introduced accidentally through ornamental fish industry (Sumanasinghe & Amarasinghe 2013). Armored sail fin catfishes are also known as 'tank cleaner' or 'janitor fish.' (Krishnakumar et al. 2009). In Victoria and Kalawewa reservoirs, impact of *Pterygoplichthys disjunctivus* on the fishery is significant because it entangle in gill nets and effect the fish harvest while damaging the gill nets (pers.obs.). Sumanasinghe & Amarasinghe 2013 have mentioned that it is difficult to eradicate

Loricarid catfishes from the inland water bodies where they have already established but it might be possible to control increment of the population. They have also stated that investigation of population dynamics of Loricarid catfish in different localities is important to find out the likelihood to support commercial scale exploitation. There is no studies on population dynamics of this genus except Sumanasinghe & Amarasinghe 2013. Findings of present study are therefore useful to support whether *Pterygoplichthys disjunctivus* population in Victoria and Kalawewa reservoirs can be effectively exploited. In the present analysis, although length frequency data were collected from gillnet catches, the estimates of growth and mortality parameters were reliable as there is a fair consistency of the estimates from Powell-Wetherall and ELEFAN methods. As Loricariid catfish were entangled in gillnets from their spines, and as fishers used gillnet of considerably wide series of mesh sizes (especially Kalawewa; pers.obs.), catch samples had been revealed not pretentious by gillnet selection (Sumanasinghe & Amarasinghe 2013). Present analysis has shown that the *Pterygoplichthys disjunctivus* population in Victoria and Kalawewa reservoirs is presently underexploited and there is a possibility for further increase of exploitation level. It can be proposed that suitable fishing gear for instance fish trap near its burrows may be an effective way for eradication of this species.

Length Weight Relationship

P. disjunctivus shows exponential growing pattern. In the commercial catch juveniles were rare may be they are indolent during the daylight hours may be to avoid predators or may be due to the selectivity of the mesh size of the gill nets. The b values for the relationship between the standard length and the weight were below 3 indicating negative allometric growth. This finding is consistent with the outcomes of Liang et al. (2005), Samat et al. (2008), and Wakida-Kusunoki and Amador-del Angel (2011). It has been suggested that the b values show ongoing variation in response to several intrinsic and extrinsic factors, even for the same species (Samat et al., 2008).

Reproductive biology

The overall sex ratio is female biased (female to male; Victoria: 1.5:1.0, Kalawewa: 1.1; 1.0). During the uttermost of the reproductive season, mature females can be only eminent from males by swollen abdomen. There are no other distinctive external features between males and females.

The size at first maturity of the armored catfish in Florida was reported to be 260 mm, whereas the size at first maturity of females and males were 180 cm for Victoria; 195mm and 200mm for Kalwawewa, respectively in the present study. This difference could be due to the higher temperature reported at the two reservoirs, whereas lower temperatures could have delayed the commencement of sexual maturity and reproduction in the Florida population.

Gonadosomatic Index

The GSI values of females are higher than males due to heavy weight of ovaries with eggs. Also gonad weight was high in spawning period because of maturing of gonads in both sexes. In the present study, it was found that overall mean GSI values for both females and males in the two reservoirs were high in several months of the year indicating an extended spawning period, declining towards the year beginning and end. (in Victoria high female GSI were recorded from March to

November 2015 and 2016; During 2017 GSI were relatively low in January and February but higher than 2015 and 2016; male GSI high from May to December in 2015; April to August in 2016; April to October in 2017; In Kalawewa females showed high GSI in April 2016 to January 2017 and again March 2017 to October 2017 while males showed high GSI in April to October in 2016 and in 2017).

These results were reliable with the extension of the reproductive period for the same species (Gibbs et al., 2008; Rueda-Jesso 2013). Overall, when food resources are abundant, many fishes, accumulating fat energy in the coelom, liver and muscles (Junk, 1985). When food resource becomes limited, fat stores are eventually consumed for the development and growth of gonads (Winemiller, 1987). Comparably, the reproductive period of an intrusive population of

L. multiradiatus in Taiwan extended from March to September (Liang et al., 2005), and *P. pardalis* extended reproductive heights from June to September (Wakida-Kusunoki and Amador-del Angel, 2011). GSI was higher in the rainy season than in the dry season, additionally endorsing that breeding and spawning in these species takes place for the period of the rainy seasons as reported by Mazzoni and Caramaschi, 1997, and that the reproductive series is yearly as informed by Laleye et al. (2006) and Araoye (1999). GSI of females of two reservoirs showed a good relationship with gonad weight than body weight and length. Ikomi (1996) and Saliu and Fagade (2003) conveyed greater GSI values in bigger samples of *Brienomyrus longianalis* in upper Warri River and *Brycinnus longipinnis* in Asa reservoir, respectively. Mean GSI of both females and males of Victoria and Kalawewa are 0.04109±0.0522:0.04361±0.0504 & 0.002347±0.0021:0.002362±0.001, respectively and are significantly higher in Kalawewa than those of Victoria ($p = 0.00$). Mean gonadal weight of both females and males of Victoria and Kalawewa are 7.604±9.107:12.784±16.699 & 0.5162±0.537:0.9012±1.077, respectively and are significantly higher in Kalawewa than those of Victoria ($p = 0.00$).

Fecundity and Egg Diameter

In Kalawewa, female specimen (SL: 27.1 cm, W: 670.5g) having highest fecundity was 4100 while specimen (SL: 32.1 cm, W: 765.5g) having highest ovarian weight was 70.2952g; Male specimen (SL: 35.2 cm, W: 710.2g) having highest testicle weight was 3.9112g. In Victoria, female specimen (SL: 21.2 cm, W: 264.5g) having highest fecundity and highest gonadal weight was 1900 and 34.21g respectively while male specimen (SL: 25.5 cm, W: 215.0g) having highest testicle weight was 2.5558g. The largest armored catfish collected at CHU was 520 mm in TL and 1 280 g in W; Rueda-Jesso 2013. Absolute Fecundity in Victoria ranged from 500 – 1900 and Kalawewa 605 – 4100.

Mean egg diameter was 1712±955.8 (n=255) and 1747±884.8 (n=252) for Victoria and Kalawewa, respectively ($p= 0.146$). Mean fecundity 956±261 (n=311) and 1856±817 (n=167) ($p= 0.000$); Mean relative fecundity 4.37±1.32 (n=311) and 6.56±2.84 (n=167) ($p= 0.000$) for Victoria and Kalawewa, respectively which were lower than what was reported by Gibbs et al. (2008). Fecundity showed weak correlation with Standard length body weight than gonad weight, with increasing correlation with these three variables respectively suggestive of that gonad weight was a well forecaster of fecundity in this study than total weight & total length. Egg diameter also showed weak correlation with standard length body weight and gonad weight for the two reservoirs with growing correlation with these three variables respectively. Mean fecundity of Kalawewa is significantly higher than that of Victoria but Egg diameter. There are wide dissimilarities in the number of eggs, with large female fish samples with more eggs than the small samples, but the highest number of eggs was not found in the largest fish, nor was the lowest number of eggs found in the smallest fish. Fish species exhibit variable fecundities, sometimes among individuals of the same species (Bagenal, 1957), due to different feeding achievements before spawning (Bagenal, 1978) and owing to release of the eggs in batches where different stages of eggs are present in mature (spawning capable) and regenerating stages. Also variable fecundities may be due to the existence of mixture of fish cohorts (Saliu et al., 2007).

Large size of eggs may be associated to the parental care of eggs stated for the fish species (Ochi, 2001). Egg size distribution and nonconformity in individual months in this study may be a further authentication of batch spawning (fig.4.) and extended spawning period. A similar finding was described for C. gariepinus by Abayomi and Arawomo (1996)

There is no noticeable correlation with egg size to fecundity, suggesting fecundity was independent of the egg size in this species. Fecundity displayed high relationship with gonad weight than with total length or total weight. This was inconsistent with the findings of Imevbore (1970), from a study on the fishes of River Niger and Laleye et al., (2006) have found that fecundity was reliant on on the size of fish. Similar was informed for Liza parsia by Rheman et al., (2002). As said by Hulata et al., (1974), large egg size increases fry and larval sustainability owing to its greater yolk content, and large egg size perhaps a suggestion of better larval sustainability and low fecundity in the species.

The larger eggs are the stocks probably to be shed first, while the ripening stocks will mature subsequently. The results of the regression analysis between egg size and other parameters suggest that gonad weight is a better predictor of egg size than the other parameters. The r values indicated that the higher the gonad weight, the larger the eggs.

This study make available basis on information on specific features of reproduction of *P.disjunctivus* in study areas. These are significant parameters for stock assessment and indulgent of the population dynamics, which could be castoff to stimulate the executive management of the species in the wild.

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