Reproductive biology of the introduced sailfin catfish *Pterygoplichthys pardalis* (Pisces: Loricariidae) in peninsular Malaysia

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ABSTRACT

Reproductive biology of the introduced sailfin cat fish *Pterygoplichthys pardalis* in the Langat River of peninsular Malaysia was studied. Fish samples were collected monthly from Langat River during March 2003 - June 2004. The sex ratio observed for *P. pardalis* was female-biased with 1: 2. Gonadal maturity stage is achieved at 12.5 cm standard length (SL) for males and 13.0 cm SL for females. This species appeared to have continuous spawning that peaked when water levels were high between September and January. Egg sizes were found to reach as large as 3.3 mm in dia in the ovary; suggesting that they spawn in batches. Fecundity varied from 1297 to 18791 numbers per brood, proportional to individual size. Data obtained from this study indicate that the introduced sailfin cat fish has successfully colonised in Langat River in Malaysia.

Keywords: Introduced cat fish, Peninsular Malaysia, *Pterygoplichthys pardalis,* Reproduction

Introduction

Catfishes of the family Loricariidae are ecologically diverse and widely distributed within South America (Suzuki *et al*., 2000). Further from its native tropical region of South America, the sailfin catfish *Pterygoplichthys pardalis* has been reported in south-east Asia including peninsular Malaysia (Chavez *et al*., 2006; Page and Robin, 2006; Samat *et al.*, 2008). One specimen was recently recorded in Siberia (Simonovic *et al.*, 2010). According to Agostinho *et al.* (1995) fishes of this family are able to tolerate a wide range of habitat conditions because of diversity in reproductive strategies. Their successful establishment in new habitats beyond native regions are also attributed to absence of natural predators and no human exploitation (Rueda-Jasso *et al*., 2013).

Reproduction in fishes can be easily affected by environmental variables such as temperature and water flow regimes (Devine *et al*., 2000; Chellappa *et al*., 2003; Schaan *et al*., 2009). In temperate waters, daily development generally increases with increasing temperature (Pepin, 1991; Dorts *et al*., 2012), but for tropical waters, several other environmental variables such as availability of food and crowding might also affect life history parameters (Lowe-McConnell, 1987; Tedesco and Hugueny, 2006).

Studies on reproduction evaluates size at maturity, sex ratio, gonadosomatic index (GSI), egg size and fecundity, and permit quantification of the reproductive capacity while providing some insight into a population's reproductive potential (Paugy, 2002; Venturelli *et al*., 2010). Although peninsular Malaysia has considerable numbers of oviparous non-native fishes (n=22), there is no information available about their reproductive biology (Chong *et al*., 2010). Studies that consider the reproduction of oviparous fish outside their natural range provide the parameters for understanding their establishment in invaded ecosystems. Thus our aim in this study was to examine the reproductive biology of the non-native sailfin catfish *P. pardalis* in Langat River, Malaysia.

Material and methods

The study was conducted at Langat River that is located in the west coast of peninsular Malaysia (Fig. 1). Climate in the region is humid with mean annual temperatures
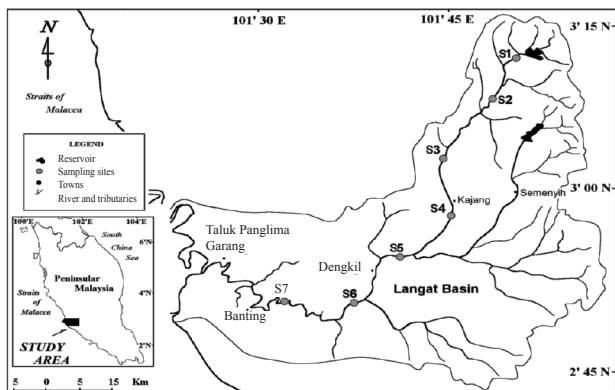
Fig. 1.   Map showing Langat River and the sampling sites S1 to S7

varying between 25 and 32°C. Two seasons prevail namely, the south-west monsoon with winds blowing from May to September, and the north-east monsoon that generally occurs from November to February. March and October are the transition months between the monsoons. Monthly rainfall in the study area during these two monsoon seasons ranges from 180 to 330 mm.

Wet and dry seasons were indicated by mean monthly water level measured to the nearest 0.1 m using a data logger (HOBO-Model-U20-001-01) placed in the river at Site-6. Fish samples were collected monthly from the main Langat River, for 16 months from March 2003 to June 2004, using cast nets thrown within the area between the shoreline and the middle part of the river. Standard length (SL) and weight (W) of each individual fish caught were measured to the nearest 0.1 cm and 0.1 g, respectively.

The abdomen of each sample was dissected to expose the genital organs and male and female samples were identified based on macroscopic observation of gonad. Temporal variations in sex ratio were also determined to observe the occurrence of productive and non-productive periods. To determine the sex ratio variations for different individual size (SL) and categories (or size structure), a frequency distribution of the SL was constructed with 3 cm class intervals.

The gonadosomatic index (GSI) used for determining the reproductive status or phenology of a fish species (Mazzoni and Caramaschi, 1995; Chellappa *et al.*, 2003) was calculated as a ratio of weight of gonad to the weight of the individual fish. GSI was calculated using the formula: GSI = 100 x (W$_g$/W) (Solomon and Ramnarine, 2007; Simon *et al.*, 2009) where W$_g$ is weight of wet gonad (g), W is total wet body weight without gonad (g).

The reproductive season of the fish was determined by examining the monthly dynamics of the GSI value based on gonad maturity stages as suggested by Kesteven (1960). Monthly values of the gonadosomatic index (GSI)

were also plotted and analysed, together with temporal variation in sex ratios. The GSI values for both male and female individuals were represented in logarithmic scale in order to clearly show their temporal patterns.

The size at first maturation (Lp$_{50}$), was estimated according to the distribution of relative frequencies of the fishes by length classes (Martins and Haimovici, 2000; Liang *et al.*, 2005). A frequency distribution of the SL was constructed at 3 cm intervals for fish with GSI values equal to or higher than 0.1. The coefficient of determination (r^2) of the linear curves for both sexes was above 95%.

Ovaries were excised from fresh samples of gravid females, weighed to the nearest 0.1 g and kept in Gilson's fluid (Bagenal, 1978). Fecundity was estimated from the ripe ova by a combination of gravimetric and volumetric methods (Lagler, 1978). After weighing the ovaries, small sub-samples from the anterior, middle and posterior portions of the ovary were removed, weighed and the eggs in the sub-samples were counted, averaged and extrapolated to the egg count of the entire ovaries using the formula: Fecundity = Average number of eggs in subsample x Weight of ovary/Weight of subsample. The number and size of eggs were determined under a binocular dissecting microscope.

Eggs from the ovary subsamples were then sorted and counted in two size classes; Sc-1 clutch (larger than 2.0 mm dia) and the Sc-2 clutch (smaller and equal to 2.0 mm in dia). The Sc-1 clutch of eggs, with full yolk and yellow in colour, were considered as eggs ready to be spawned. The remaining eggs (the Sc-2 clutch), which were whitish to pale yellow in colour were considered to be in the recruitment statge. Quantity of eggs for each clutch is presented as relative frequency (%). Any temporal change between these two clutches of eggs was evaluated according to water levels. The pattern produced from this analysis was taken to suggest the spawning season for *P. pardalis*.

Relationship between fecundity and the selected reproductive parameters was described as: $F = aX^b$ where *F* is fecundity, *X* is standard length (cm)/weight (g)/gonad weight (g)/water level (m), *a* is intercept and *b* is the slope. The software, Origin (version 6.0) was employed to determine the relationship between fecundity and standard length, weight and gonad weight (Simon *et al.*, 2012).

To study sex ratios, χ^2- test (goodness of fit) was used to determine whether the sex ratio differed from the expected ratio of 1:1. Size structure of the fish population assessed by length frequency distribution in size classes with numerical fish abundance and was also compared

by χ^2 test for each class. Relationships among the fishes, fecundity and egg size and water level were examined by means of correlation coefficient using Pearson correlation.

Results

The overall male to female ratio over the period of sixteen months for *P. pardalis* population in Langat River was 1:1.7 (χ^2 = 28.01; p<0.05), indicating that the sex ratio for this species was female biased. Size of male *P. pardalis* individuals during the study were between 16.0 and 38.0 cm SL and of females between 10.8 and 36.0 cm SL. The SLs of both males and females were normally distributed according to size classes (Fig. 2). Statistically, the sex ratio departed from 1:1 ratio for fishes measuring between 21 and 33 cm in SL, a size range where females outnumbered males (Table 1).
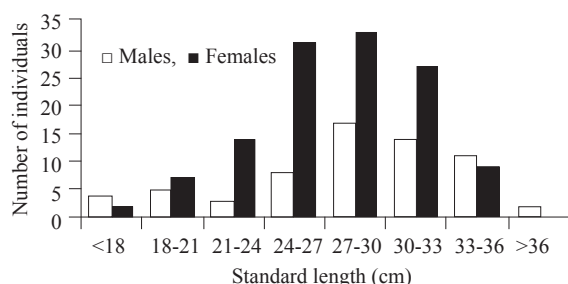
Fig. 2. Distribution in size classes of *Pterygoplichthys pardalis* of different sexes in Langat River

Table 1. Chi-square (χ2) test for *Pterygoplichthys pardalis* sex ratio comparisons by size class in Langat River, (F = expected frequency)

Size class (cm)	Males	Females	Total	F	χ^2
<18	4	2	6	3.0	0.67
18-21	5	7	12	6.0	0.33
21-24	3	14	17	8.5	7.12*
24-27	8	31	39	19.5	13.56*
27-30	17	33	50	25.0	5.12*
30-33	14	27	41	20.5	4.12*
33-36	11	9	20	10.0	0.20
>36	2	0	2	1.0	2.00

* p<0.05

Relatively higher GSI values for both male and female individuals of this species were detected from August to October 2003, which coincided with period of high water level in Langat River (Fig. 3). The study indicated that the number of *P. pardalis* in the gravid condition was more frequently found during wet season than dry season. In general, during wet season between August 2003 and January 2004 gravid females recorded for each month was more than 85%. During dry season, percentage of gravid females for each month was less than 70%.
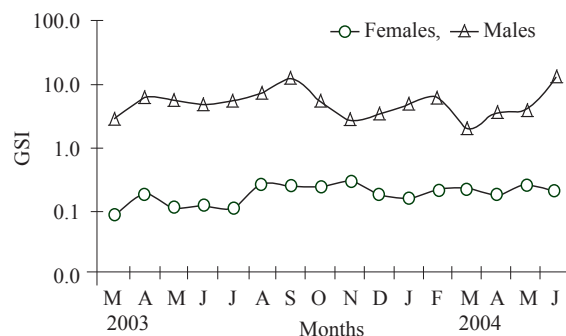
Fig. 3. Temporal variation of the gonadosomatic index (GSI for male and female *Pterygoplichthys pardalis*

In this study, the size at first maturity (Lp$_{50}$) was estimated to be at 12.5 cm SL in males and 13 cm SL in females (Fig. 4). Analysis revealed no difference in LP$_{50}$ between males and females of *P. pardalis* (χ^2 = 0.010; p>0.05). Furthermore, the size of the fish measured did not correlate significantly with the gonad weight (r^2 = 0.0372; p>0.05), suggesting year-round maturation.

The mean length, weight and fecundity of 225 gravid females of *P. pardalis* are presented in Table 2. The mean body length of the fish ranged between 24.6±4.4 and 30.8±3.3 cm (SL) and weight between 260.0±123.0 and 552.9±132.7 g. Ovary weights were recorded between 0.73 and 114.12 g and fecundity was

Table 2. Number of individuals, mean length, mean weight, and mean fecundity of *Pterygoplichthys pardalis* during the study period

Month	N	SL (cm)	Weight (g)	Fecundity
March 2003	6	29.0±2.6	450.9±76.7	4132±1162
April	15	27.1±2.3	369.3±87.2	5154±1663
May	11	25.4±3.8	327.1±115.3	4972±1786
June	11	27.6±3.4	404.6±130.0	5637±2821
July	13	28.6±2.0	428.2±81.7	6718±1881
August	15	29.9±4.3	492.8±187.4	8526±3296
September	22	26.6±4.2	361.0±136.9	7081±2961
October	10	30.8±3.3	552.9±132.7	11480±2458
November	24	30.5±4.0	472.7±142.6	9395±3767
December	23	30.3±3.1	448.5±110.9	6301±3492
January 2004	19	24.6±4.4	260.0±123.0	7129±2566
February	12	27.9±4.6	406.5±177.6	8273±3839
March	8	29.5±2.0	422.8±73.0	10428±4061
April	13	29.7±3.8	386.1±29.6	9698±4247
May	10	26.9±4.2	324.5±143.3	7659±3530
June	13	29.0±6.3	476.6±213.2	8918±3256

between 1,297 and 18,791 with monthly averages higher than 4,000 nos. of ripe egg. The egg size in Sc-1 group was found to be as large as 3.3 mm in dia, with full yolk and distinguished by its bright yellowish colour. Eggs in Sc-2 group were smaller than 2 mm in dia and appeared cream to whitish in colour.
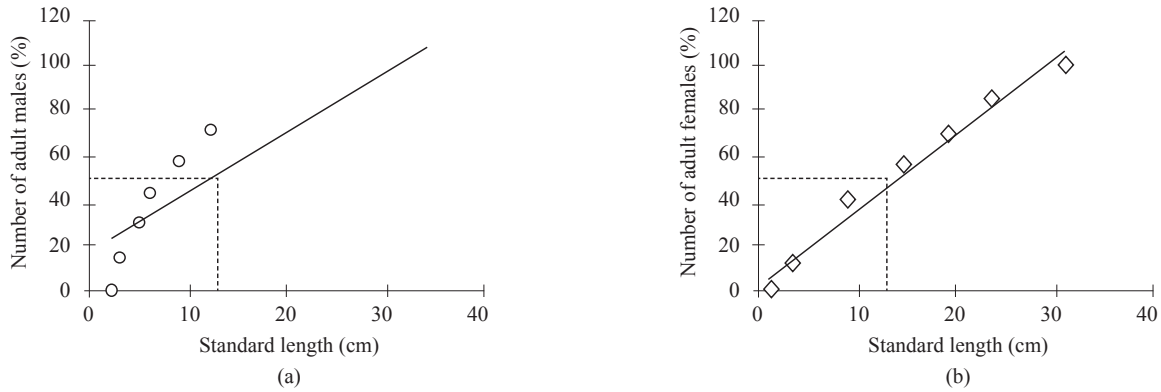
Fig. 4. Size at first gonadal maturation based on length frequency distribution of adult *Pterygoplichthys pardalis* in Langat River (a) male; (b) female

A larger number of ripe eggs (Sc-1) were observed in the ovary during the period of low water level than during the period of high water levels (Fig. 5). This suggests the likelihood that the species keep their ripe eggs at low water levels and later release them when water levels are high. This finding that the GSI value of this fish is influenced by high and low water levels is consistent with the findings of other researchers (Goulding, 1981; Lowe-McConnell, 1987).
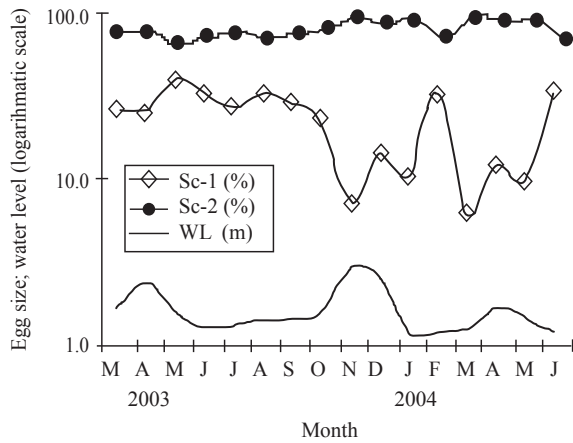

Fig. 5. Temporal pattern of frequency distribution of eggs (Sc-1 and Sc-2) of *Pterygoplichthys pardalis* and water level (reflecting rainfall) over the period of 16 months

Fecundity of *P. pardalis* from Langat River was positively correlated with body size and weight, as well as with gonad weight. However, a negative relationship was found between fecundity and water level. This could be due to the release of ripe eggs (Sc-1), while the developing eggs (Sc-2) were left behind in the ovary during high water level period.

Discussion

Female *P. pardalis* in the Langat River were found to outnumber males throughout the year. Similar trend

was also reported for other Loricariids such as *Liposarcus multiradiatus*; an introduced armored catfish in Taiwan (Liang *et al*., 2005). However, Mazzoni and Caramaschi (1995) estimated the overall sex ratio for two species of armored catfish *Hypostomus affinis* and *H. luetkeni* (Family: Loricariidae) in their native habitat as 1:1.

The smaller number of male fish recorded at high water levels might be due to lesser chances of the fish being caught since at high water levels the males may have still been guarding their nest while the females were living free in the open water. This is in line with the findings of Mazzoni *et al*. (2002), Power (2003) and Liang *et al*. (2005) that the males are responsible for the protection of eggs and juveniles.

Relatively, more male than female individuals were recorded for fish smaller than 18 cm SL. Sex of fish may change at their first maturity (Lp_{50}), as it is common for fishes (Allsop and West, 2003; Gardner *et al*., 2005). If this is the case for *P. pardalis* of Langat River, the sex ratio would be balanced, as has been estimated for its congeners (Mazzoni and Caramaschi, 1995). Males outnumbering females was also found for *P. pardalis* individuals larger than 330 mm. At this size it has been suggested that female mortality may be higher than male mortality due to the females' greater reproductive investment (Liang *et al*., 2005).

Fish can become sexually mature at various ages, depending on the species. For most fishes, reproduction starts when they have reached about half of their likely maximum size. Our estimated Lp_{50} values for *P. pardalis* are comparable with those estimated for *Hypostomus affinis* and *Hypostomus luetkeni* at 14.4 cm SL and 16.0 cm SL, respectively (Mazzoni and Caramaschi, 1995). However, the estimated Lp_{50} values for *P. pardalis* were much lower than that of its congeneric *Pterygoplichthys*

multiradiatus, which has Lp_{50} value at 25.0 cm SL (Liang *et al*., 2005).

Liang *et al*. (2005) suggested that *Liposarcus multiradiatus* in Taiwan invested more energy for environmental adaptation rather than for reproduction unlike *H. affinis* and *H. luetkeni* in Brazil (Mazzoni and Caramaschi, 1995, 1997). Our findings agree to an extent with Mazzoni and Caramaschi (1995, 1997) as Malaysia and Brazil are in the tropical climate realm, while Taiwan is subtropical. Differences in climatic conditions may potentially interfere with or delay the species' reproductive processes including the onset of sexual maturity.

Generally, change in water temperature affects the reproduction of a fish (Yoneda and Wright, 2005; Pankhurst and Munday, 2011). Therefore, a habitat with a stable water temperature (*e.g*., tropical rivers) is expected to be a better place for fish to reproduce than habitats with fluctuating water temperature (*e.g.,* subtropical rivers). When water temperature fluctuates, fishes spawn only when the temperature remains within the specific range conducive for spawning of the species and hence fluctuating temperatures would limit the length of the spawning period and thus the reproductive rate of the fish. Temperature may not be an important limiting factor for spawning of fish that live in a habitat with stable or less fluctuating water temperature.

It may be presumed that decreasing water temperature during high water levels trigger the spawning mechanism of this species. Such a phenomenon is a common reproductive strategy in most tropical and neotropical fishes (Merigoux *et al*., 1999; Ferreira, 2000). In addition, food is always much more abundant, and space more available during wet season which enhance fish population growth (Goulding, 1981; De-Merona and De-Merona, 2004).

Spawning in batches has also been reported for other *P. pardalis* congeners such as *Loricariichthys platymetopon, Loricariichthys* sp. and *Loricaria* sp. (Suzuki *et al*., 2000), and *Hypostomus affinis* (Duarte and Araujo, 2002). In the mature ovaries of a batch spawner like *P. pardalis*, mature eggs with size >2.0 mm (Sc-1) are found together with immature ones with size less than 0.5 mm in dia known as pre-vitellogenic eggs (Sc-2). This Sc-2 group of eggs is expected to remain in the ovaries after the first spawning, and, together with those in the primary growth, form the egg stock for the next spawning season. Only a single cohort of Sc-1 is recruited and spawned during a specific spawning period. However, not all Loricariid fishes spawn in batches. Suzuki *et al*. (2000)

have reported that several others such as *Hypostomus ternetzi, Megalancistrus aculeatus* and *Rhinelepis aspera* are total spawners.

The way the armored catfishes fertilise their eggs differs across species For *Hoplosternum littorale* (Callichthyids), there are indications that female drink the sperms and the fertilisation takes place after the sperms have passed through the digestive tract (Hostache and Mol, 1998). On the other hand, loricariids have external fertilisation and development (Suzuki *et al*., 2000), and thus egg characters are important especially in terms of their resistance to the environmental conditions where fertilisation takes place. According to Dorucu *et al*. (2001), egg size may affect fecundity, embryo survival rate and fry size. Naturally, egg size of each species of fish is strongly related to internal (*i.e.,* genetic) factors and may be influenced by environmental conditions or both (Kaeriyama *et al*., 1995).

The number of eggs per brood of *P. pardalis* in the Langat River was higher than other loricariids of its native habitat of South America such as *Hypostomus affinis* with 2,350 (Mazzoni and Carasmachi, 1995), *H. ternetzi* with 2,800 and *Plecostomus ancistroides* with 970 eggs. However, it is far lower than the eggs per brood of *Rhinelepis aspera* with 47,370 eggs (Suzuki *et al*., 2000). Positive relationships between fecundity in an individual species and its size, body weight and gonad weight found in this study are common for fish in general (Bagenal, 1978), including loricariids (Mazzoni and Caramaschi, 1995; Duarte and Aroujo, 2002).

Among freshwater species, it is usually observed that hatching success is low in unprotected eggs than in species which exhibit parental care (Dahlberg, 1979). Moodie and Power (1982), Suzuki *et al*. (2000), and Hoover *et al*. (2004) described at least three reproductive strategies of loricariids during early stages of their life cycle *viz*., no parental care (*e.g., Rhinelepis aspera*), external bearers (*e.g., Loricaria* and *Loricariichthys*), and guarders (*e.g., Hypostomus*, *Liposarcus* and *Pterygoplichthys*).

This study has described several key aspects of the reproductive biology of sailfin catfish *P. pardalis* in Malaysian waters. It is clear that *P. pardalis* has made its way in Malaysian waters to propagate, survive and finally become an important component in the local fish community. The overall environmental condition of Langat River facilitates the reproductive activities of this introduced sailfin catfish, thus making it a successful invader.

Acknowledgements

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