Title: Seasonality in Mekong giant catfishs’ fasting cycles: A comment to Ikeya and Kume 2011

1. Introduction

Reserved (Write last)

1. Methods

*2.1 Study site and subjects*

Reserved for Sandra.

A number of six Mekong Giant Catfish (*Pangasianodon gigas*) that were obtained from Thailand in 2010 were examined from 2019 to 2022 at River Wonders, Mandai Wildlife Reserve, Singapore. The MGC's have been housed in an outdoor exhibition tank with 600, 000 litres water capacity with a water circulation-filter system. The catfish were reared in a mixed-species exhibition tank that includes fourteen other compatible species (e.g.: *Urogymnus polylepis, Catlocarpio siamensis, Pangasius sanitwongsei, Balantiocheilos melanopterus, Probarbus jullieni, Scleropages formosus, Tor tambroides* and more with approximately 112 individuals in total. Physical environment of the water tank was maintained at a constant water temperature (mean ± SD = 28.4 ± 0.7°C), dissolved oxygen level (7.2 ± 0.7 mg/l), and pH (7.2 ± 0.3) throughout the year by performing 5% water exchanged daily. Water parameters were measured weekly to ensure ideal water condition for the fishes. The MGC's were reached maturation stage as they were >10 years in age with total length ranged between 150cm to 170cm when this study conducted. Each individual's catfish were identified by the black spots on its body surface and the shape of its anal fin since 2022.

**Feeding**

Monthly food intake was recorded over a 4-year period from 2019 to 2022. The catfish that used in this study were fed with formula food (540g flax seed, 700g herbivore pellet and 200g oat mixed with 2L warm water moulded in tray and keep in the fridge for overnight before cut into a pellet cube). In captivity, Mekong giant catfish adapt well to a variety of different foods, including fish meat, shrimp meat, and commercial fish food (Pholprasit and Tavarutmaneegul 1998). In this study, the catfish also observed to eat fish meat and prawns that were provided for other fishes in the exhibit. Both pellet cube and the number of fish/ prawns eaten by the catfish was counted until end of the feeding session. The pellet cube was target feed by thrown in front of the catfish snouts during feeding. Behavioural and physiological data of swimming pattern/speed and body coloration respectively were collected as it is reflecting the decreasing of the feeding behaviour of the catfish.

* Long-term fasting periods (defined as not eating for more than 20 days)

**Swimming pattern (U-turn) /speed**

Swimming pattern - measured by U-turn made by each catfish in 60s

Swimming speed, Swimming erratically/nervously as it rubs on the acrylic was measured using a video camera (60s) and measurement tape length of the exhibit

**Body coloration evaluation**

* Image-J?
* Size of tank
* Mixed tank so will need to report each captive species and estimated number during study period..................................................................................................................
* Estimated age and size of each fish; all adult fish and growth rate has peaked during the course of the study
* Water condition of the aquarium, note stable unchanging conditions/parameters despite being outdoors.
  + DJ: to report mean across years with SE; temperature, pH etc
* Food consists of what?
  + Report an estimated food consumed per fish based on anecdotal observation/reporting?
* Ask Sandra again about tank water conditions from 2019—2022.
* Rainfall data for 2022.

*2.2 Data collection protocol*

Reserved for Sandra.

* Seasonality in the fasting cycles of the studied subjects was examined across four years of data, 2019—2022
* That said, behavioural and physiological changes associated with the studied fasting cycles was only examined across 2021—2022.
* How feeding behaviour is quantified so it relates to Section 2.3

*2.3 Statistical analysis*

The analysis of the seasonality of fasting cycles in the MGC was conducted by first calculating the monthly average proportion of feeding behaviour observed from 2019 to 2022. As the studied catfish could not be differentiated individually, the proportion of feeding behaviour observed across the studied group (*n* = 6) was simply derived as the number of fasting fish divided by the total number of fish. Subsequently, the *monthglm*() function from the package “season” ((Barnett et al. 2022) was employed to model the seasonality of the catfish’s fasting cycles from 2019—2022. Here, *monthglm()* command fits a generalized linear model using a categorical month variable as independent variable (i.e., *refmonth* function; see Barnett et al. 2022), and is suitable when dealing with non-sinusoidal time series. Beta coefficients from the fitted *monthglm*() model were then exponentiated to obtain the odds ratio (as well as the 95% C.I.) of a catfish being in a state of appetence in each month. Lastly, an odds ratio could not be calculated for the designated *refmonth* (i.e., November) where fasting behaviour is observed across all studied individuals.

The monthly average proportion of change in swim behaviour (i.e., from normal to erratic; see Supplementary Video 1) and body colouration (i.e., from light to dark colouration; see Supplementary Figure 1) observed from the six studied individuals from 2021—2022 were calculated and depicted graphically through circular plots. Mann-Kendall tests was first used to explore for a monotonic trend in the behavioural and colouration change across months and the non-parametric Spearman correlation was used to assess the relationship between swim behaviour and body colouration to feeding behaviour. All statistical analysis and graphic visualisations were conducted within the R language and environment (R Core Team 2021).

1. Results

Chart, line chart

Description automatically generated

Figure 1. The seasonal rhythmicity of adult Mekong giant catfish (*n* = 6) entering a state of inappetence in an outdoor aquarium at River Wonders. The red, blue and green dotted-dashed lines represent data collected from 2019, 2020 and 2021.

<Some overview results of the fishes pertaining to food intake and size; Sandra?>

Results from the seasonality analysis found that the studied catfish exhibited a consistent fasting cycle from 2019—2022 (Figure 1). Here, the catfish will engage in long-term fasting periods from July to November although the duration of the fasting cycle can vary between individuals. That said, most, if not all, individuals were observed to resume the feeding behaviours by December and the consistency of the pattern across 2019—2022 suggests that Mekong giant catfish are likely to have seasonal feeding rhythms that are constrained within feeding periods ranging between 8—9 months (i.e., December—July; Figure 1) that precedes a corresponding fasting period of approximately 3—4 months (i.e., August—November; Figure 1).

~~During the periods of inappetence (i.e., August—November; Figure 1), a gradual yet distinct change in the swimming behaviour and body colouration of the studied catfish was also noticeable in the studied catfish from 2021—2022 (Table 2, Figure S1 and Video S1).~~

~~Long-term fasting periods (defined as not eating for more than 20 days) were also detected in the catfish during the study period (mean ± SD = 44.3 ± 25.51 days; range 20121 days; n = 37 in total) (Table 2; Fig. 3). Of the 37 cases of long-term fasting we recorded, 21 instances occurred during the wet season in Thailand (April to October), four instances were during the dry season (November to March in the next year), and 12 instances overlapped with both the wet and dry season. The periods overlapped with the wet season averaged 37.5 days with ± 15.54 SD (range 14–73 days).~~

Table 1. Overview data of the studied fishes? Up for consideration.

\* *Behavioural and physiological changes during inappetence cycles\**

Reserved text; DJ.

Figure 2. Picture collage of colour change with the MGC; same fish pictured twice, one for fasting and another for non-fasting. Repeat across 6 fishes if possible (Maybe in Supplementary Images 1—3).

Chart, radar chart

Description automatically generatedFigure 3. The proportion of change observed in A) fish colour, B) swim behaviour and C) feeding behaviour in adult Mekong giant catfish (*n* = 6) housed in an outdoor aquarium at River Wonders in 2021.

Discussion

1. Effects of such fasting cycles
   1. See injuries
   2. Coloration and behavioural changes
   3. Immunity response: see (Liu et al. 2013)
      1. Although not explored specifically with the *P. gigas*, short-term food deprivation in the channel catfish (*Ictalurus punctatus*) resulted in an increase in susceptibility towards several bacterial pathogen (Liu et al. 2013). Here, the authors noted that 7 d fasting cycles imposed on the channel catfish significantly altered the expression of critical innate immune factors in a manner that is consistent with reduced immune fitness and dysregulating key genes involved in energy metabolism and cell cycling/proliferation.
2. Justifying change in swim behaviour to some form of biological significance
3. They reported that with many species, feed intake could be reduced or inhabited completely prior to and/ or during the spawning period (Kadri et al. 1996)

References

Barnett, A.G., Baker, P.J., and Dobson, A.J. 2022. season: Analysing Seasonal Data R Functions. Berlin, Heidelberg.

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Liu, L., Li, C., Su, B., Beck, B.H., and Peatman, E. 2013. Short-Term Feed Deprivation Alters Immune Status of Surface Mucosa in Channel Catfish (*Ictalurus punctatus*). PLOS ONE **8**(9): e74581. Public Library of Science. doi:10.1371/journal.pone.0074581.

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