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# Seasonal Feeding Rhythm Associated with Fasting Period of *Pangasianodon gigas*: Long-term Monitoring in an Aquarium

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The Mekong giant catfish *Pangasianodon gigas* is endemic to the Mekong River basin, and is recognized as endangered species, largely due to overfishing and development of the river basin. We monitored food intake of *P. gigas* in a stable environment in an aquarium over a 6-year period and analyzed their feeding rhythm and fasting periods. The daily food intake for each fish was recorded from 18 June 2004 to 17 June 2010. The feeding rhythm or pattern was determined by the fast Fourier transform (FFT) analysis. The FFT analysis revealed that different cycles of feeding rhythm (168.8, 313.1, and 365.3 days) in three catfishes and no observable cycles in two catfishes. However, three catfishes showed subordinate peaks with approximately 365 days (365.3 days for all). These suggest that, at least, four of five catfish had have approximately 365-days feeding cycle. We also showed that all catfish undergo long-term fasting periods (> 20 days). Of note, the feeding/fasting pattern coincides with the wet/dry seasons in Thailand, which also corresponds to the abundance of the catfish food resource (*Cladophora* spp.). We found that *P. gigas* exhibit a seasonal feeding rhythm that is synchronized by food availability. Furthermore, we found that the seasonal feeding rhythm was gradually dampened over time, suggesting that the observed seasonal feeding rhythm with long-term fasting of the catfish is likely controlled by an endogenous clock system. To our knowledge, this is the first case of quantification of the seasonal feeding rhythm with fasting periods in teleost fish.

**Key words:** seasonal feeding rhythm, fasting period, stable environment, FFT analysis, food availability, endogenous clock, *Pangasianodon gigas*

## INTRODUCTION

The Mekong giant catfish *Pangasianodon gigas* (Chevey, 1931), which is endemic to the Mekong River basin, is one of the largest freshwater fishes in the world; its body length can be a maximum of ca. 3 m and its weight can exceed 300 kg (Burgess, 1989; Rainboth, 1996). In the Mekong River basin, *P. gigas* has been a popular food resource for the local people and is thus the most important species for fisheries in the area (Akagi et al., 1996; Hogan, 2004). However, the number of catfish caught from the Mekong River has declined in recent years because of overfishing and development of the river basin (Hogan et al., 2001, 2004; Hogan, 2004). Specifically, the total number of *P. gigas* present in the area is estimated to have decreased by approximately 90% over the past two decades (Hogan et al., 2004), suggesting that this catfish is at risk of extinction

in the wild. In addition, the Mekong giant catfish genetically forms a single population in the Mekong River basin, and mitochondrial and microsatellite DNA analyses have revealed remarkably low genetic diversity in the wild population as well as in blood stocks (Na-Nakorn et al., 2006, 2007; Ngamsiri et al., 2007; Sriphairoj et al., 2007). Due to these findings, this catfish is currently listed as a critically endangered species in both the Conservation on International Trade in Endangered Species (CITES) Appendix I and International Union for the Conservation of Natural Resources (IUCN) Red List.

The Mekong giant catfish have been cultured for preservation in ponds. In Thailand, the first artificial insemination technique for this catfish (F1) was technically established in 1983, and the second filial generation (F2) was successfully produced in 2001 (Hogan, 2004; Meng-Umphon et al., 2006). Both F1 and F2 hatchery-reared juveniles and immature adults have been released into the Mekong River to enhance its fish stock (Hogan, 2004). In 2004, our aquarium also received artificially propagated F1 Mekong giant catfish from Thailand for research purposes. The goal of our study is to understand the ecological features of the catfish.

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Although Mitamura and colleagues have extensively described the movement patterns of this fish by performing telemetry studies (e.g., Mitamura et al., 2007, 2008, 2009), other aspects of its ecology are less well understood (Hogan, 2004). Although many aspects of the ecology of *P. gigas* are known, we have hitherto focused on feeding ecology, because feeding ecology is one of the important information for fish culture management. Here, we document the feeding/fasting cycles of this catfish in an aquarium.

Generally, organisms have various biological rhythms (e.g., circadian, circalunar, circatidal, and circannual rhythms) for reproduction, migration, and feeding (Baggerman, 1985; Boujard and Leatherland, 1992; Foote et al., 1992; Gwinner, 1986, 1996; Heilman and Spieler, 1999; Mizushima et al., 2000; Satoh et al., 2008; Wikelski et al., 2009; Takemura et al., 2010). Seasonal feeding rhythms usually involve a fasting period; such a pattern has been identified in various organisms, especially in avian and mammalian species (Hissa, 1997; Piersma et al., 2008). For example, migratory birds have reduced metabolic rates and body masses because they fast during migration between non-breeding and breeding habitats (Piersma et al., 2008). However, a small number of studies have been reported about the seasonal feeding rhythm associated with long-term fasting periods in teleost fish species, although the daily feeding cycle is well known (Boujard and Leatherland, 1992; Heilman and Spieler, 1999). In this study, we monitored the food intake of the Mekong giant catfish over a 6-year period, and analyzed the feeding rhythm associated with the long-term fasting period.

## MATERIALS AND METHODS

### Specimens and rearing conditions

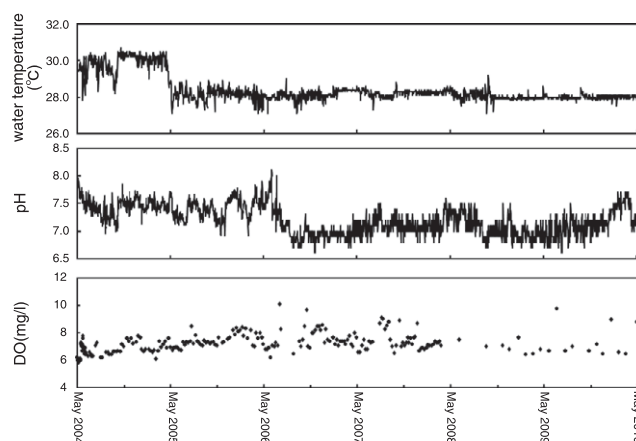
In this study, we examined five artificially propagated F1 Mekong giant catfish that were obtained from Thailand in 2004. The catfish were reared in an exhibition tank (12 × 6 × 1 m, water capacity, 72,000 liters) with a water circulation-filter system at Gifu World Freshwater Aquarium (GWFA) in Gifu Prefecture, Japan, from 17 May 2004. Before transportation to GWFA, the catfish were reared in an outdoor artificial tank (10 × 6 × 1 m, water capacity, 60,000 liters) at Inland Aquaculture Research Institute at Ayutthaya.

The catfish were maintained under stable conditions during this study, which were the same conditions applied before this study, thereby negating the influence of the physical environment and photoperiod on feeding. The physical environment of the exhibition tank was kept stable by maintaining a fixed water temperature (mean ± SD = 28.4 ± 0.7°C), dissolved oxygen level (7.2 ± 0.7 mg/l), and pH (7.2 ± 0.3) (Fig. 1). A 12:12 h light:dark regime (08:00–20:00 in light and 20:00–08:00 in dark) was also maintained throughout the study period. We measured the total length (TL, cm) of all catfish in 2004 only (before this study) using a scale, because we abstained from handling the catfish thereafter to avoid affecting their feeding activity. TL of the catfish ranged from 105 to 124 cm. All catfish were 6+ in age when this study was started. Also, all catfish were immature during study periods. Each fish was identified on the basis of the black spots on its body surface and the shape of its anal fin.

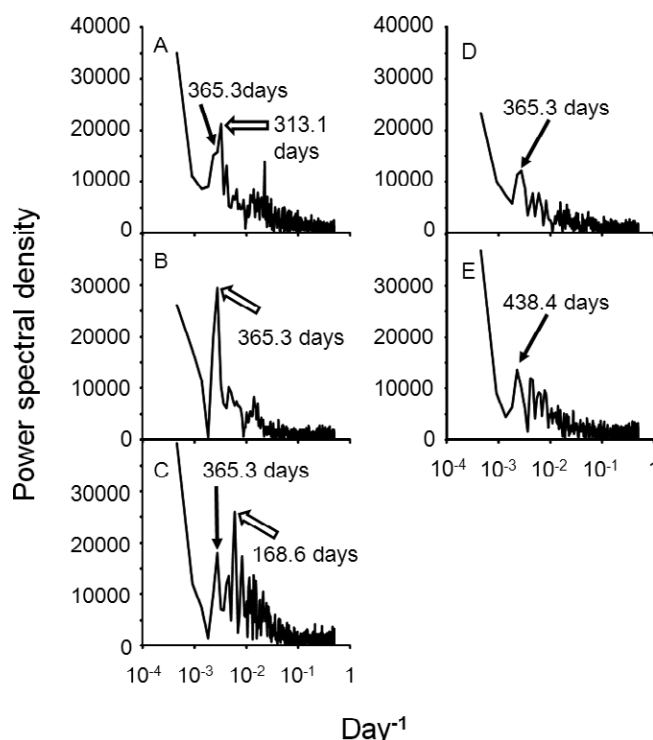
### Feeding protocol

Daily food intake for each individual was recorded over a 6-year period from 18 June 2004 to 17 June 2010. The adults of *Pangasianodon gigas* in the wild are likely to feed mainly on the filamentous attached algae *Cladophora* spp. (Akagi et al., 1996), and the catfish that used in this study were fed formula food (880 g fish pellet with 35% protein, 5 g vitamin complex, 10 g *Spilulina* spp., 5

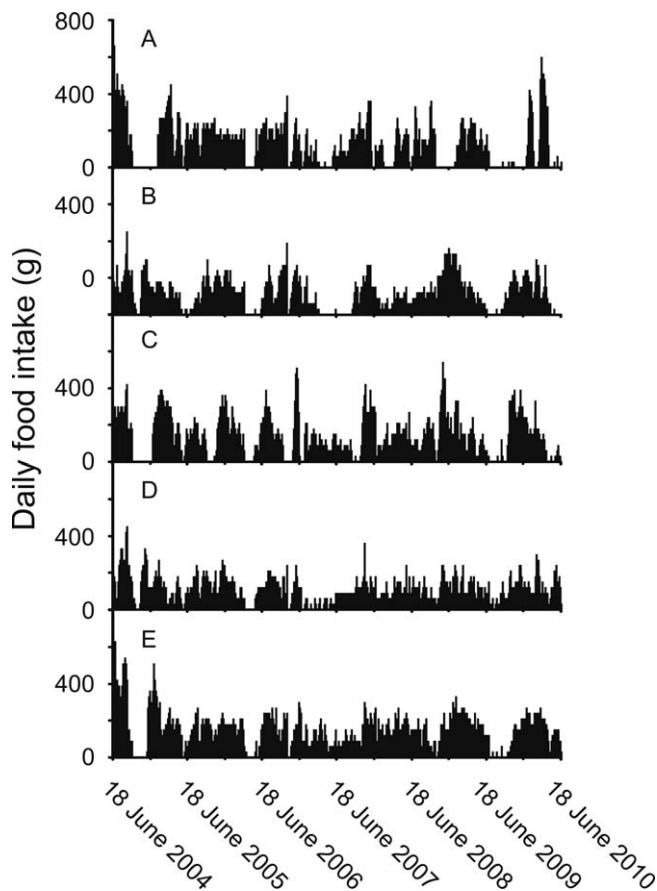
g fish oil, and 100 g wheat flour in total 1,000 g) before transportation. In this study, thus, the catfish were fed the formula food for carp (Nosan Corporation, Kanagawa, Japan) with *Chlorella* liquid (5% in total food weight) once daily (16:00). It is known empirically that fish food with *Chlorella* liquid inhibits fat accumulation and improves immunity. This food was kneaded with water and shaped like a disk prior to feeding. The disks (30 g) were thrown in front of the catfish snouts upon feeding. The number of disks eaten by each catfish was counted until they finished eating; those disks discarded by the catfish were not counted. The daily feeding amounts (g) for each fish were calculated on the basis of the total number of disks consumed. After the catfish finished feeding, uneaten disks were



**Fig. 1.** Annual changes in the physical conditions in the aquarium (May 2004 to June 2010).



**Fig. 2.** Frequency spectra produced by the FFT analysis of the feeding data for the Mekong giant catfish. White and black arrows indicate dominant and subordinate peaks, respectively. (A) No. 1, (B) No. 2, (C) No. 3, (D) No. 4, (E) No. 5.



**Fig. 3.** Annual changes in the daily food intake by the Mekong giant catfish. (A) No. 1, (B) No. 2, (C) No. 3, (D) No. 4, (E) No. 5.

**Table 1.** Food intake of the Mekong giant catfish.

ID	Food intake (kg)							Total
	2004*	2005	2006	2007	2008	2009	2010**	
1	21.24	48.18	31.89	20.55	21.27	17.25	20.55	180.93
2	20.76	29.52	27.49	13.98	27.99	29.58	11.67	160.99
3	16.32	49.05	31.47	25.59	35.43	31.23	8.28	197.37
4	21.87	28.32	21.86	13.5	19.41	22.32	12.78	140.06
5	30.96	41.82	29.18	23.28	27.21	29.16	17.28	198.89

\*data from 18 June to 31 December, \*\*data from 1 January to 17 June.

**Table 2.** Long-term fasting period (mean  $\pm$  SD days) of the Mekong giant catfish (see the Results for definition).

ID	Fasting period			Total
	Wet season	Dry season	Both seasons*	
1	40.0 $\pm$ 17.7 (4)	43.7 $\pm$ 19.6 (3)	73.5 $\pm$ 42.2 (4)	53.2 $\pm$ 31.1 (11)
2	38.8 $\pm$ 22.8 (6)	0	68.5 $\pm$ 23.3 (2)	46.3 $\pm$ 25.3 (8)
3	29.7 $\pm$ 10.6 (6)	24	65.3 $\pm$ 30.0 (3)	39.8 $\pm$ 24.0 (10)
4	23	0	51	37.0 $\pm$ 19.8 (2)
5	29.5 $\pm$ 10.9 (4)	0	45.0 $\pm$ 35.4 (2)	34.7 $\pm$ 19.6 (6)

\*Fasting period overlapped with the wet and dry seasons. Numerous in parenthesis indicates the number of long-term fasting period showing.

removed from tanks to prevent the catfish from eating them.

#### Data analysis

To examine the feeding pattern of the Mekong giant catfish, we used the fast Fourier transform (FFT) analysis to assess the feeding rhythm. This method is widely used in time-series analysis and population studies to identify rhythmic patterns (periodicities) within time-series data. This analysis was performed using IGOR Pro™, version 4 software (WaveMetrics, Oregon, USA). Results are presented as the mean or the mean ( $\pm$  SD).

#### RESULTS

The results of the FFT analysis revealed that the feeding rhythms differed among three individuals (313.1 days for No. 1, 365.3 days for No. 2, and 168.8 days for No. 3), and that the two other catfishes (Nos. 4 and 5) did not exhibit clear feeding cycles (Fig. 2). Also, three catfishes (Nos. 1, 3 and 4) showed subordinate peaks with approximately 365 days (Figs. 2A, C, and D), suggesting that, at least, they had have ca. 365-day feeding rhythms. In addition, oscillation progressively is likely to be dampened throughout the study period, especially from 2007 to 2010 (Fig. 3). Furthermore, food intake of the Mekong giant catfish varied among individuals, as well as across years (Table 1).

Long-term fasting periods (defined as not eating for more than 20 days) were also detected in the catfish during the study period (mean  $\pm$  SD = 44.3  $\pm$  25.51 days; range 20–121 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  $\pm$  15.54 SD (range 14–73 days).

#### DISCUSSION

Seasonal rhythm for reproduction has been well demonstrated in teleost fish (Baggerman, 1985; Takemura et al., 2010), but studies examining the presence of a seasonal feeding rhythm associated with a long-term fasting have not yet been reported. In the present study, we found that the Mekong giant catfish exhibited a seasonal feeding rhythm involving cycles of active feeding and long-term fasting (Table 2; Fig. 3). Furthermore, feeding habits of the Mekong giant catfish are also less understood. Currently, these fish are considered to be herbivorous, as adults in the wild are likely to feed mainly on the filamentous attached algae *Cladophora* spp. (Akagi et al., 1996). In the Mekong River, *Cladophora* spp. grows during the dry season (November to March in the next year). In contrast, during the wet season (April to October), the majority of *Cladophora* spp. in deep waters die and a few of them survive in shallow waters, because light cannot reach the bottom of the river due to turbidity (Prathumratana et al., 2008). It is predicted that this seasonal change in the abundance of *Cladophora* spp. affects the feeding rhythm of the catfish; that is, they prey on the filamentous attached macroalgae during the dry season and fast during the wet season. In fact, the long-term fasting period of the catfish in this study largely coincided with the wet season in Thailand (Table 2). In some cases, however, the catfish showed long-term fasting peri-

ods overlapped between the wet and dry seasons (Table 2). A possible explanation for such a fasting pattern is that the variety of the timing of the opening for fasting period. We think that the catfish may fast during the wet season in the nature, but the timing of the opening for fasting period progressively became misaligned to the wet season throughout the study period in the catfish reared under stable environment. In several teleost fishes, such as the European catfish *Silurus glanis*, the Japanese sea catfish *Plotosus japonicus*, and sea bass *Dicentrarchus labrax*, food availability is an important synchronizer for the feeding rhythm (Sánchez-Vázquez et al., 1995; Bolliet et al., 2001; Kasai et al., 2009). These findings suggest, therefore, that food availability may be one of the important synchronizers of seasonal feeding rhythm in the Mekong giant catfish.

In this study, we did not examine whether other synchronizers (e.g., photoperiod) affect the seasonal feeding rhythm of this catfish. Photoperiod is considered an important synchronizer of biological rhythms in teleost fishes, including rainbow trout *Oncorhynchus mykiss* and threespine stickleback *Gasterosteus aculeatus* (Baggerman, 1985; Bolliet et al., 2001); however, the dominant synchronizer for feeding rhythm differs among fish species, such as those that are nocturnal or diurnal (Bolliet et al., 2001). Thus, further study is needed to clarify the function of environmental factors as synchronizers of the seasonal feeding rhythm in the Mekong giant catfish.

In this study, we found different cycles of feeding rhythm (168.8, 313.1, and 365.3 days) in three catfishes (Nos. 1–3) and no observable cycles in two catfishes (Nos. 4 and 5) (Fig. 2). However, three catfishes (Nos. 1, 3 and 4) showed subordinate peaks with approximately 365 days (365.3 days for all) (Fig. 2). These suggest that, at least, four of five catfish had have approximately 365-days feeding cycle. According to Gwinner (1986), studies that show a nearly 365-day biological cycle such as feeding are not directly in line with those studies that show circannual rhythm, because there is a possibility that an approximately 365 day-cycle may be synchronized by unknown exogenous factor that cannot be regulated during rearing conditions. In many cases, when animals have been maintained in a stable laboratory environment, seasonal rhythms tend to be dampened within a few cycles (e.g., Pisingan and Takemura, 2007). In this study, it is likely that oscillation progressively dampened throughout the study period, especially from 2007 to 2010 (Fig. 3). We hypothesize that two catfishes (Nos. 1 and 3) exhibited a shorter 1-year cycle due to dampened feeding cycles, and two catfishes (Nos. 4 and 5) exhibited no feeding cycle. Thus, the seasonal feeding/fasting cycle in this catfish may be controlled by an endogenous clock system, as seen in other organisms (Gwinner, 1996; Satoh et al., 2008).

Although the feeding protocol we used in this study, which involved giving the catfish food once daily (at 16:00), may not be in phase with the species' natural feeding rhythm, the catfish used in this study were supplied with sufficient quantities of food throughout this study period (Table 1). Thus, our protocol likely had no effect on the feeding activity or feeding rhythm of the catfish. However, food intake is likely to affect their feeding rhythms (Table 1). In addition, a positive correlation between feeding activity/rhythm and growth rate has been demonstrated in sea bass

(Azzaydi et al., 1998), but we did not examine this relationship in this study. However, further studies on these points are needed.

In conclusion, this study shows that the Mekong giant catfish exhibit seasonal feeding rhythms associated with long-term fasting when maintained in a stable environment. Of note, this feeding/fasting pattern coincides with the wet/dry seasons in Thailand, respectively. Food availability for the catfish, which differs between the wet and dry seasons, may be a synchronizer of their seasonal feeding rhythm. To our knowledge, this is the first quantitative study of the periodic feeding rhythm associated with a long-term fasting in teleost fish, although numerous instances have been described in avian and mammalian species (Hissa, 1997; Piersma et al., 2008). According to previous studies, adult catfish use shallow waters as a feeding habitat (Mitamura et al., 2007) and are likely to feed mainly on filamentous attached macroalgae of the *Cladophora* spp. (Akagi et al., 1996). Recently, there have been concerns about damming in the river basin (Hogan et al., 2004). If a dam is built at the upper stream in the Mekong River, *Cladophora* spp. production will be reduced, as damming reduces fluctuations in water level and impacts water quality. These changes in the river will also likely affect the feeding habits of the catfish (i.e., by reducing their feeding habitats and food items). Thus, further studies in the wild are needed to better understand the feeding rhythm of catfish. Such studies should identify the underlying ecological and physiological issues that could affect the conservation and fish culture management of the Mekong giant catfish.

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