Different degrees of lunar synchronization of ovary development between two morphs of a Tanganyikan cichlid fish

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Abstract The degree of lunar synchronization of spawning is thought to be related to a strategy that decreases predation on the brood in Tanganyikan substrate-brooding cichlids. Here, I examined the periodic change of ovary development in two morphs of *Telmatochromis temporalis*: the normal morph uses burrows under stones as spawning nests, whereas the dwarf morph uses holes within empty snail shells. The normal morph showed a significant lunar synchronization of ovary development, but the dwarf morph did not. In the normal morph, spawning prior to the full moon probably decreases the incidence of approaching brood predators and increases the guarding efficiency of parents. In the dwarf morph, however, lunar cyclic spawning may be dispensable, because the spawning nests within shells are probably highly effective for predator avoidance. These two morphs are closely related, and then will be a good model to clarify the widely observed phenomenon of lunar cyclic spawning.

Introduction

Although biological rhythmicity that is synchronized with lunar or semi-lunar cycles is a widespread phenomenon in marine organisms (Leatherland et al., 1992; Hernández-León, 2008), it is rare among organisms inhabiting freshwater bodies, where tidal fluctuation is limited or absent. However, lunar cyclic spawning has been reported in nine substrate-brooding species of cichlid fish belonging to the tribe Lamprlogini in Lake Tanganyika, Africa (Nakai et al., 1990; Rossiter, 1991). In these species, a pair of parents or a female guards the eggs and yolk-sac larvae, which cannot easily escape from predators by themselves. The spawning cycles of these species are thought to improve the survival of the vulnerable brood (eggs and yolk-sac larvae) or dispersing young (Nakai et al., 1990; Rossiter, 1991). Three possible explanations have been proposed: 1) spawning prior to the full moon reduces the predation on the vulnerable brood by nocturnal predators (bagrid catfish) that are not active during the full moon (Rossiter, 1991), 2) spawning prior to the full moon enhances the effectiveness of nocturnal parental guarding of the vulnerable brood under the maximum lunar illumination during the full moon (Nakai et al., 1990; Rossiter, 1991), and 3) dispersal of young during the fourth quarter of the lunar cycle and the new moon improves the survival of young dispersing under the cover of darkness (Nakai et al., 1990). If any of these explanations is true, the degree of lunar synchronization of spawning will vary according to the strategies for decreasing predation on the vulnerable brood and/or dispersing young. Comparison between more closely related populations is better to test this hypothesis because it will minimize the effect of phylogeny.

The algae-feeding Tanganyikan cichlid, *Telmatochromis temporalis* Boulenger, is an iteroparous substrate brooder. Like other substrate-brooding species that are known to exhibit lunar synchronized spawning, this fish also belongs to the tribe Lamprologini (Takahashi, 2003). *T. temporalis* is dimorphic for body size. The two morphs dwell in different habitats, lay eggs and guard the brood in different types of nest, and hide in different types of shelter, respectively (Takahashi, 2004; Takahashi et al., 2009). The normal morph possesses a moderate-sized body for a Tanganyikan rock-dwelling cichlid [88 mm in standard length at maximum size (SL_{max}) in males, 62 mm SL_{max} in females] and is one of the most common fish on rocky shorelines. This morph uses burrows under stones as spawning nests and shelters (Mboko & Kohda, 1999; Katoh et al., 2005). The dwarf morph is about half of the normal morph in body size (45 mm SL_{max} in males, 29 mm SL_{max} in females) and invariably inhabits shell beds, in which the lake bottom is covered by a high density of empty snail shells of the gastropod *Neothauma tanganyicense* Smith (Takahashi et al., 2009: fig. 1d). This morph uses the empty shells as spawning nests and shelters. A population genetic study showed that the normal and dwarf morphs from Wonzye (08°43’31” S; 31°07’55” E; near Mpulungu, Zambia, at the southern end of the lake) were closely related but isolated from each other, although their geographical distributions partly overlap (Takahashi et al., 2009).

In the present study, I observed the periodic changes of ovary development in the normal and dwarf morphs from Wonzye, and examined whether the ovary development was synchronized with the lunar cycle. Based on my findings, I discuss the relationship between the degree of lunar synchronization of ovary development and predator avoidance strategies.

Materials and Methods

Sampling of fish

Using SCUBA diving, 6 to 11 females of the normal morph were collected every 3 to 5 days between 27 September and 2 December of 2005 on rocky shorelines at 1.0 to 4.9 m depth (N = 173), and 5 to 12 females of the dwarf morph were collected every 3 or 4 days between 29 September and 1 December of 2005 on shell beds at 9.5 to 10.3 m depth (N = 174). Fish were transported to the laboratory at Mpulungu and killed in a solution of anesthesia FA 100 (Takeda Pharmaceutical Co. Ltd.) within 6 hours after collection. The ovaries were extracted from all females. The bodies and ovaries were dabbed on tissue paper to remove excess moisture before weighing, and were weighed to the nearest 1 mg. When the ovary was lighter than the minimum readable weight of the electronic balance (1 mg), the weight was taken to be 0 mg. However, zero values cannot be subjected to log-transformation. To avoid this problem, I added 1 mg to all ovary weights. The gonado-somatic index was calculated as $I_G = 100\ W_O\ W_B^{-1}$ (%), where W_B is body weight (mg), and W_O is ovary weight + 1 (mg). Note that I_G is not a useful descriptor of gonad investment in some organisms (Tomkins and Simmons, 2002). However, this index was used in the present study, because the normal and dwarf morphs showed isometric relationships between W_B and W_O (see below in Results).

Test of lunar synchronization

I_G was approximated to a cosine curve:

$$f(T_i) = a \cos[2\pi (T_i - x) / l] + y$$
$$[f(T_i) \geq 0, 0 \leq x < l],$$

where T_i is the number of days from 27 September (the first day of sampling) to the day that individual i was collected, a, x and l are amplitude, phase and length of the cosine curve, respectively, and y is a corrected average of I_G. Appropriate values for parameters were searched by the method of least squares in three models: full model (four parameters: a, x, l and y), lunar-cyclic model with $l = 29.5$ (three parameters: a, x and y) and non-cyclic model with $a = 0$ (one parameter: y). The F-test was used to compare these three models for each morph.

Results

Body weight and ovary weight

The relationship between W_B and W_O is shown in Fig. 1. In the normal morph, all small females lighter than 1000 mg possessed light ovaries. These small females were considered to be immature and were excluded from the following analyses. Some large females heavier than 1000 mg also had light ovaries. However, these females were considered to be mature females having undeveloped ovaries, for example, females just after spawning, and were not excluded from the following analyses. In the dwarf morph, all females were likely mature, although their W_B was much lighter than that of the mature females of the normal morph. The average W_B of the mature females was 2050 mg (N = 160) in the normal morph and 259 mg (N = 174) in the dwarf morph.

Tomkins and Simons (2002) pointed out that I_G was not a useful descriptor of gonad investment in organisms that showed an allometric relationship between the gonad and somatic weights. In the present study, however, the isometric function provided a significantly better fit than the allometric function for explaining the relationship between W_B and W_O in each morph (Fig. 1) (test of difference between allometric and isometric functions: $F = 0.483$, df = 1 and 158, $P = 0.488$ in the normal morph; $F = 0.034$, df = 1 and 172, $P = 0.854$ in the dwarf morph). This result means that I_G is not significantly affected by W_B after maturity; therefore, I_G was used as the descriptor of gonad investment in the present study. In the normal morph, the variance of log(W_O) seemed to increase with log(W_B) (Fig. 1), but this tendency was not significant [the correlation coefficient between log(W_B) and squared deviates was r = 0.071, $P = 0.370$].

Lunar synchronization of ovary development

In mature females of the normal morph, I_G showed a marginally significant difference among sampling days (Kruskal-Wallis: $\chi^2 = 33.3$, df = 19, $P = 0.022$) and a significant fit to the lunar-cyclic model (Fig. 2A) [full model versus lunar-cyclic model: $F = 0.819$, df = 1 and 156, $P = 0.367$; lunar-cyclic model versus non-cyclic model: $F = 6.32$, df = 2 and 157, $P = 0.002$; this result was also supported by analyses using log(I_G)]. I_G peaked prior to the full moon (13 days). In the dwarf morph, some females collected during the second quarter of the lunar cycle (7–15 days) possessed higher I_G than females collected during the fourth quarter (22–0 days), like the normal morph (Fig. 2B). However, the difference of I_G among sampling days was not significant (Kruskal-Wallis: $\chi^2 = 15.6$, df = 18, $P = 0.618$) and I_G did not significantly fit the full and lunar-cyclic models [full model versus lunar-cyclic model: $F = 1.81$, df = 1 and 170, $P = 0.180$; lunar-cyclic model versus non-cyclic model: $F = 2.38$, df = 2 and 171, $P = 0.096$; this result was also supported by analyses using $\log(I_G)$]. This result suggests that the ovary development of the dwarf morph was not synchronized with the lunar cycle, or that the degree of lunar synchronization of ovary development was lower than the detection level of the present test.

Discussion

The present study revealed that 1) the ovary development of the normal morph was significantly synchronized with the lunar cycle, and 2) the ovary development of the dwarf morph was not significantly synchronized with the lunar cycle ($P = 0.096$), or at least the cycle was less pronounced compared to the normal morph. The ovary development of the normal morph peaked during the second quarter of the lunar cycle, suggesting high activity of reproduction during this period, in accord with the findings for nine other substrate-brooding species of Tanganyikan cichlids (Nakai et al., 1990; Rossiter, 1991). Three possible explanations have been proposed for the lunar cyclic spawning of the Tanganyikan substrate brooders, and two of these explanations are applicable to the normal morph.

The first applicable explanation is synchronization of the vulnerable stages of the brood (egg and yolk-sac stages) with a period when nocturnal predators are not active (Rossiter, 1991). The bagrid catfish is one of the main predators in the lake (Fryer and Iles, 1972). Small bagrid catfishes (*Phyllonemus* spp. and young of *Chrysichthys* spp.) are ubiquitous in rocky shorelines, which are the main habitat of the normal morph. Predation by these catfishes will critically affect the survival of the broods of the normal morph. Young of the normal morph hatch out about 3 days after spawning (Katoh et al., 2005), and therefore the vulnerable stages of the brood occur around the full moon, which accords with a period when bagrid catfishes are not active (McKay, 1983; Rossiter, 1991).

The second applicable explanation is synchronization of the vulnerable stages of the brood with a period when the nocturnal parental guarding is effective (Nakai et al., 1990; Rossiter, 1991). The parents of the normal morph attack and repel the brood predators when they approach (Mboko and Kohda, 1999). The ambient light during the full moon may assist the parents in visual detection at night, and in repelling approaching nocturnal brood predators, for example, spiny eels (Ochi et al., 1999).

The other proposed explanation for lunar cyclic spawning, namely, that spawning prior to the full moon improves the survival of young dispersing under the cover of darkness (Nakai et al., 1990), is unlikely in the normal morph. The young of some substrate-brooding species leave the spawning nest immediately after they complete yolk absorption. The period of yolk absorption is about 2 weeks (Kuwamura, 1997), resulting in the dispersal of the young during dark nights during the fourth quarter and new moon (Nakai et al., 1990). However, the young of the normal morph remain in the spawning nest for more than 1 month after yolk absorption (Mboko and

Kohda, 1999). The timing of dispersal of the free-swimming young of the normal morph may be decided by some environmental cue.

As discussed above, the lunar cyclic spawning of the normal morph may improve the survival of the brood by synchronizing the vulnerable stages of the brood with the period when the nocturnal brood predators (bagrid catfish) are not active and the nocturnal parental guarding is effective. On the other hand, the dwarf morph did not show clear lunar synchronization of ovary development. The dwarf morph uses empty snail shells as spawning nests in shell beds. The eggs and yolk-sac larvae were always found with a female close to the end of the hole within a shell (N = 12, observed in November of 2005 and October to November of 2007 by the author), suggesting that females spawn and care for the brood there. The end of the hole is very small and is invisible from the outside. The predators would probably have trouble finding and accessing the brood. Spawning nests within empty shells, therefore, will be very effective for preventing predation on the vulnerable brood of the dwarf morph, and therefore lunar cyclic spawning may be dispensable for predator avoidance. Release from the limitation of the spawning timing may reduce the degree of lunar synchronization of the reproduction of the dwarf morph.

Different degrees of lunar synchronization in spawning have been reported among eight species of Tanganyikan substrate-brooding cichlids, and these variations were suggested to be related to the spawning sites (Nakai et al., 1990). The present statistical test of the lunar synchronization using a cosine-curve function supports this suggestion. However, this explanation for the different degrees of lunar synchronization is based on circumstantial evidence, and other explanations remain possible. In fact, females of *Lamprologus callipterus* and *Altolamprologus compressiceps* use shells as spawning sites like the dwarf morph of *T. temporalis*, but show lunar spawning (Nakai et al., 1990). Other factors, such as spawning position within the shells, may also play a role. The present study was conducted from the end of dry season to the beginning of rainy season covering two lunar cycles. More analyses of other life-history traits and replicates in another season will be needed to reveal the mechanism and the adaptive significance of the lunar synchronization.

Lunar cyclic spawning is a well-documented feature in marine organisms (Leatherland et al., 1992; Hernández-León, 2008). More detailed analyses of the normal and dwarf morphs of *T. temporalis* will help to clarify the mechanism and evolution of this phenomenon.

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References

Fryer, G. & T. D. Iles, 1972. The cichlid fishes of the Great Lakes of Africa: their biology and evolution. Oliver & Boyd, Edinburgh.

Hernández-León, S., 2008. Natural variability of fisheries and lunar illumination: a hypothesis. Fish and Fisheries 9: 138–154.

Katoh, R., H. Munehara & M. Kohda, 2005. Alternative male mating tactics of the substrate brooding cichlid *Telmatochromis temporalis* in Lake Tanganyika. Zoological Science 22: 555–561.

Kuwamura, T., 1997. The evolution of parental care and mating systems among Tanganyikan cichlids. In Kawanabe, H., M. Hori & M. Nagoshi (eds), Fish Communities in Lake Tanganyika. Kyoto University Press, Kyoto: 57–86.

Leatherland, J. F., K. J. Farbridge & T. Boujard, 1992. Lunar and semi-lunar rhythms in fishes. In Ali, M. A. (ed), Rhythms in Fishes. Plenum Press, New York: 83–107.

Mboko, S. K. & M. Kohda, 1999. Piracy mating by large males in a monogamous substrate-breeding cichlid in Lake Tanganyika. Journal of Ethology 17: 51–55.

McKaye, K. R., 1983. Ecology and breeding behavior of a cichlid fish, *Cyrtocara eucinostomus*, on a large lek in Lake Malawi, Africa. Environmental Biology of Fishes 8: 81–96.

Nakai, K., Y. Yanagisawa, T. Sato, Y. Niimura & M. M. Gashagaza, 1990. Lunar synchronization of spawning in cichlid fishes of the tribe Lamprologini in Lake Tanganyika. Journal of Fish Biology 37: 589–598.

Ochi, H., Y. Sato & Y. Yanagisawa, 1999. Obligate feeding of cichlid eggs by *Caecomastacembelus zebratus* in Lake Tanganyika. Journal of Fish Biology 54: 450–459.

Rossiter, A., 1991. Lunar spawning synchroneity in a freshwater fish. Naturwissenschaften 78: 182–184.

Takahashi, T., 2003. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes).

Ichthyological Research 50: 367–382.

Takahashi, T., 2004. Morphological and genetic distinctness of rock and shell-bed dwelling *Telmatochromis* (Teleostei, Cichlidae) in the South of Lake Tanganyika suggest the existence of two species. Journal of Fish Biology 65: 419–435.

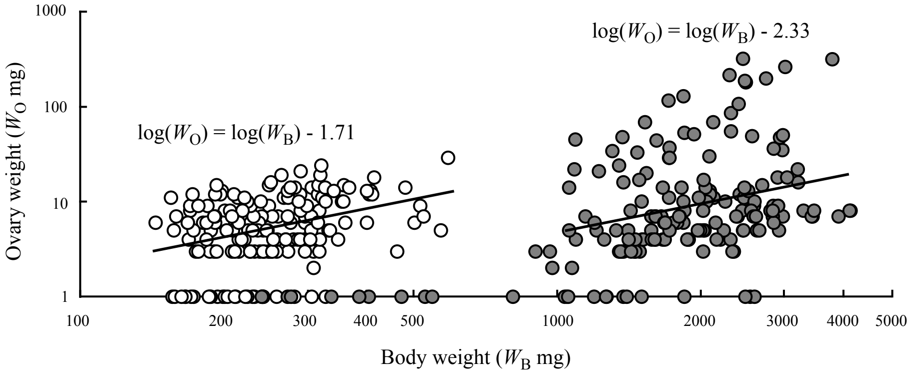
Takahashi, T., K. Watanabe, H. Munehara, L. Rüber & M. Hori, 2009. Evidence for divergent natural selection of a Lake Tanganyika cichlid inferred from repeated radiations in body size. Molecular Ecology 18: 3110–3119.

Tomkins, J. L. & L. W. Simmons, 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. Animal Behaviour 63: 1009–1016.

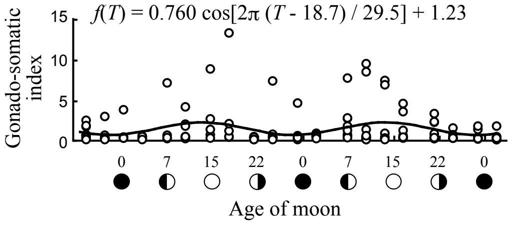
Figure legends

Fig. 1 Relationship between the ovary weight and body weight in the normal (solid circles; *N* = 173) and dwarf (open circles; *N* = 174) morphs. Regression lines and functions in the graph are of the mature individuals of the normal (*N* = 160) and dwarf (*N* = 174) morphs (see text)

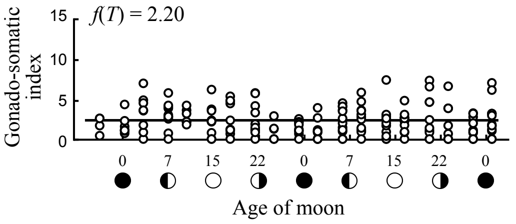
Fig. 2 Periodic change of ovary development during the study period. The normal morph showed a significant lunar cyclic pattern (**A**; *N* = 160), whereas non-significant cyclic pattern was seen in the dwarf morph (**B**; *N* = 174) (see text). A line in the graph and a function above the graph are of the selected model in each morph


log(W_O) = log(W_B) - 2.33
log(W_O) = log(W_B) - 1.71
Ovary weight (W_O mg)
1000
100
10
1
100
200
300
400
500
1000
2000
3000
4000
5000
Body weight (W_B mg)

A


f(T) = 0.760 cos[2π (T - 18.7) / 29.5] + 1.23
Gonado-somatic index
15
10
5
0
0 7 15 22 0 7 15 22 0
● ◐ ○ ◑ ● ◐ ○ ◑ ●
Age of moon

B


f(T) = 2.20
Gonado-somatic index
15
10
5
0
0 7 15 22 0 7 15 22 0
● ◐ ○ ◑ ● ◐ ○ ◑ ●
Age of moon