

Captive Birth of Tiger Shark (*Galeocerdo cuvier*) Reveals a Shift in Respiratory Mode during Parturition

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Ram ventilation, which is characterized by the production of a respiratory water current over the gill by locomotory effort, widely occurs among pelagic sharks. However, the process by which the juvenile starts this respiratory mechanism remains largely unknown. The captive birth of a Tiger Shark (*Galeocerdo cuvier*) at the Okinawa Churaumi Aquarium on 23 March 2017 permitted the examination of the parturition process and behavior of newborn juveniles of this species. Sonographic examination of the pregnant female showed that, unlike adult Tiger Sharks, embryos used buccal pumping, revealing that Tiger Shark shifts its respiratory mechanism from buccal pumping to ram ventilation after birth. Our observation also showed that ram ventilation of the neonate was not continuous, and buccal pumping was also used when the neonate intermittently rested on the bottom of the tank. We speculate that this behavior is the transitional phase in the shift from buccal pumping to ram ventilation in neonates, and continuous ram ventilation may be established after the development of continuous and fast swimming abilities.

DURING parturition, viviparous vertebrates experience an abrupt change in their surrounding environment. Along with this environmental change, the embryo shifts its physiology to adapt to a new environment. However, it is difficult to examine this process, especially for large, aquatic vertebrates, in the wild (Parsons, 1991).

Public aquaria have offered a rare opportunity to observe the parturition process of viviparous elasmobranchs (Uchida et al., 1990). On 23 March 2017, a Tiger Shark (*Galeocerdo cuvier*), the largest (maximum size, >5.5 m in total length, TL) of the carcharhiniform sharks (Compagno, 1984), gave birth at the Okinawa Churaumi Aquarium (Okinawa, Japan). To the best of our knowledge, this is the first natural birth of this species in captivity. The only previous record of captive birth is found in the book *The Lady and the Sharks* (Clark, 1969), which reports that one of 37 neonate Tiger Sharks survived for three months in captivity at the Cape Haze Marine Laboratory (now the Mote Marine Laboratory). However, these neonates were extracted from the dying mother and was not a natural birth. Captive births of viviparous sharks were previously described for four species, including *Carcharias taurus*, *Carcharhinus plumbeus*, *Triaenodon obesus*, and *Sphyrna tiburo* (Wass, 1973; Gilmore et al., 1983; Uchida et al., 1990; Parsons, 1991). However, knowledge of the parturition process of the viviparous elasmobranchs is still fragmented.

The captive birth of the Tiger Shark provides the first evidence of the shift in respiratory mode after parturition among sharks. Shark respiration has been categorized into two modes: buccal pumping and ram ventilation (e.g., Carlson et al., 2004). The former mode is characterized by the generation of a respiratory water current by rhythmical buccal motion. In contrast, the latter mode is characterized by a lack of buccal motion, with water current forced over the gills by swimming forward with the mouth open. Many non-benthic sharks, including Tiger Sharks, are known to use ram ventilation as the major mode of respiration, though they

potentially have the ability to use buccal pumping when necessary (Carlson et al., 2004). The necessity of continuous locomotion for the achievement of ram ventilation raises the hypothesis that embryos of aplacental sharks, which exhibit highly restricted locomotion in utero, should use a respiratory mode different from that of postnatal individuals. This hypothesis was previously confirmed in the manta ray (*Mobula alfredi*, Batoidea): Tomita et al. (2012) used an ultrasonogram to show that this species actively pumps the uterine fluid using buccal movement in utero, and shifts its respiratory mode from buccal pumping to ram ventilation after birth. However, such ontogenetic shift in respiratory mode from buccal pumping to ram ventilation has not been confirmed in sharks. Therefore, the present study aimed to describe the parturition process of the captive Tiger Shark, and to trace the shift in its respiratory mode after parturition.

MATERIALS AND METHODS

Pregnant Tiger Shark and newborn juveniles.—A female Tiger Shark (3.2 m in total length, TL; Fig. 1A) was incidentally captured in a net set off Okinawa Island, Japan, on 3 March 2017. On the same day, the female was transported to an 800,000 l exhibition tank at the Okinawa Churaumi Aquarium.

Parturition occurred on 23 March 2017. Water temperature of the aquarium was 21.8°C. The newborn juveniles were transported to another tank (10,000 l) located beside the original tank immediately after birth. Total length and body weight were measured for all newborn juveniles on the day following parturition. At the same time, sex was determined by the presence of claspers for each specimen. Determination of buccal pumping was based on the rhythmic opening and closing of mouth and gill slits, and that of ram ventilation was based on continuous swimming without rhythmic opening and closing of the mouth. These criteria have been widely used for the determination of respiratory mode in the previous studies (e.g., Steffensen, 1985).

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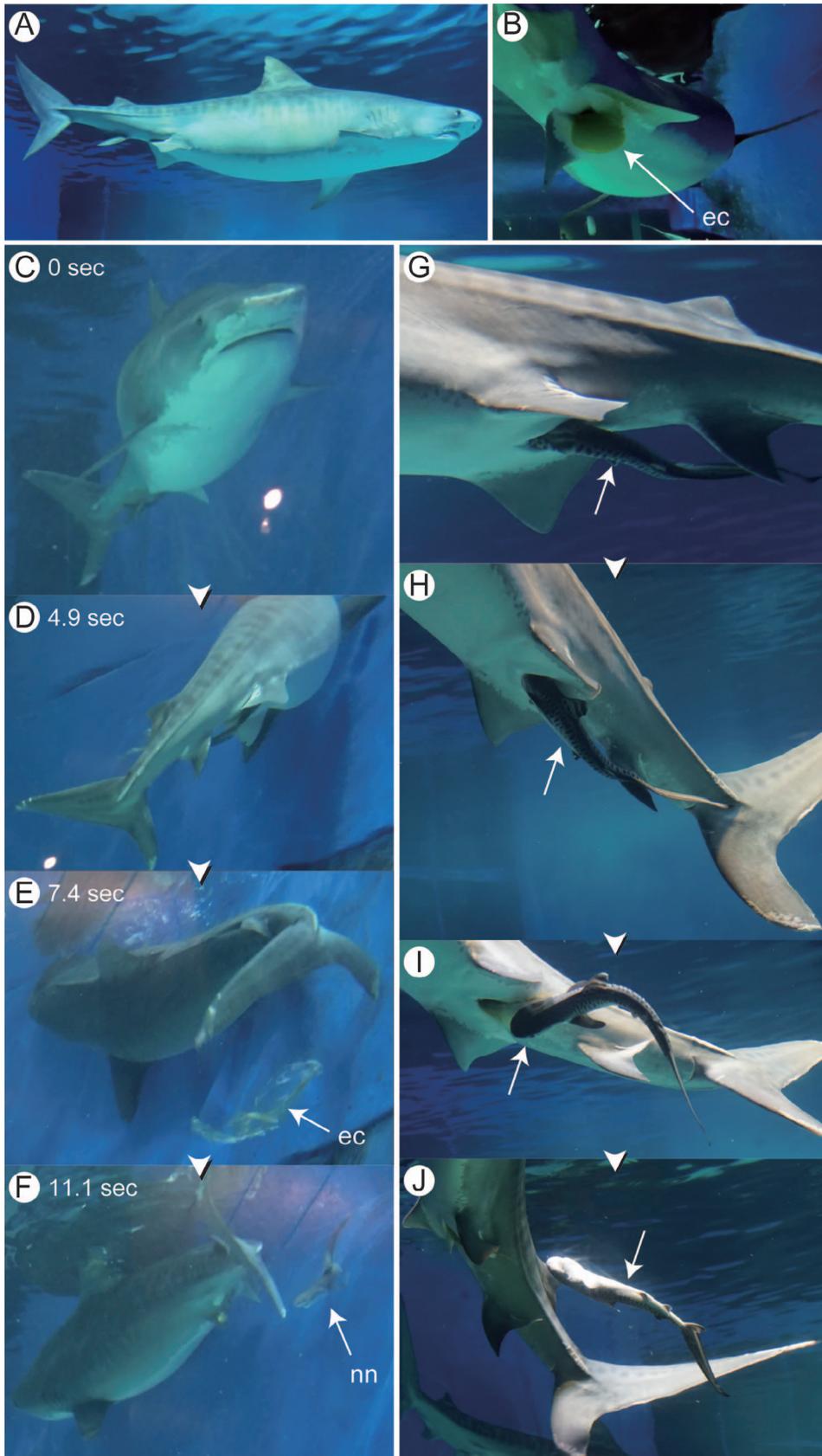


Fig. 1. Captive birth of a Tiger Shark at the Okinawa Churaumi Aquarium. (A) Photograph of captive pregnant female taken on 12 March 2017. (B) A part of the egg capsule (ec) is protruded from maternal cloaca. (C–F) Serial images of parturition behavior. A female quickly turned (0 to 4.9 s, C, D), egg capsule (ec) was excreted (7.4 s, E), and neonate (nn) was separated from maternal body (11.1 s, F). (G–J) Serial images of parturition process of a neonate. Posterior part of the neonate (arrow) was visible from outside of cloaca (G, H), and neonate was separated from maternal body (I, J).

Ultrasonography.—Sonographic observations were performed in the pregnant female using a SonoSite S-serve ultrasound machine (SonoSite, Inc., Bothell, WA) during transportation to the exhibition tank, and in two neonates using a Fazone M sonographic diagnostic imaging system (Fujifilm,

Tokyo, Japan) on 24 March 2017. The transducer of the sonography machine was placed on the body surface just lateral to the left uterus for the pregnant female, and on the upper surface of the head for the two neonates. For kinematic analysis, we obtained the coordinates (x, y) from upper and

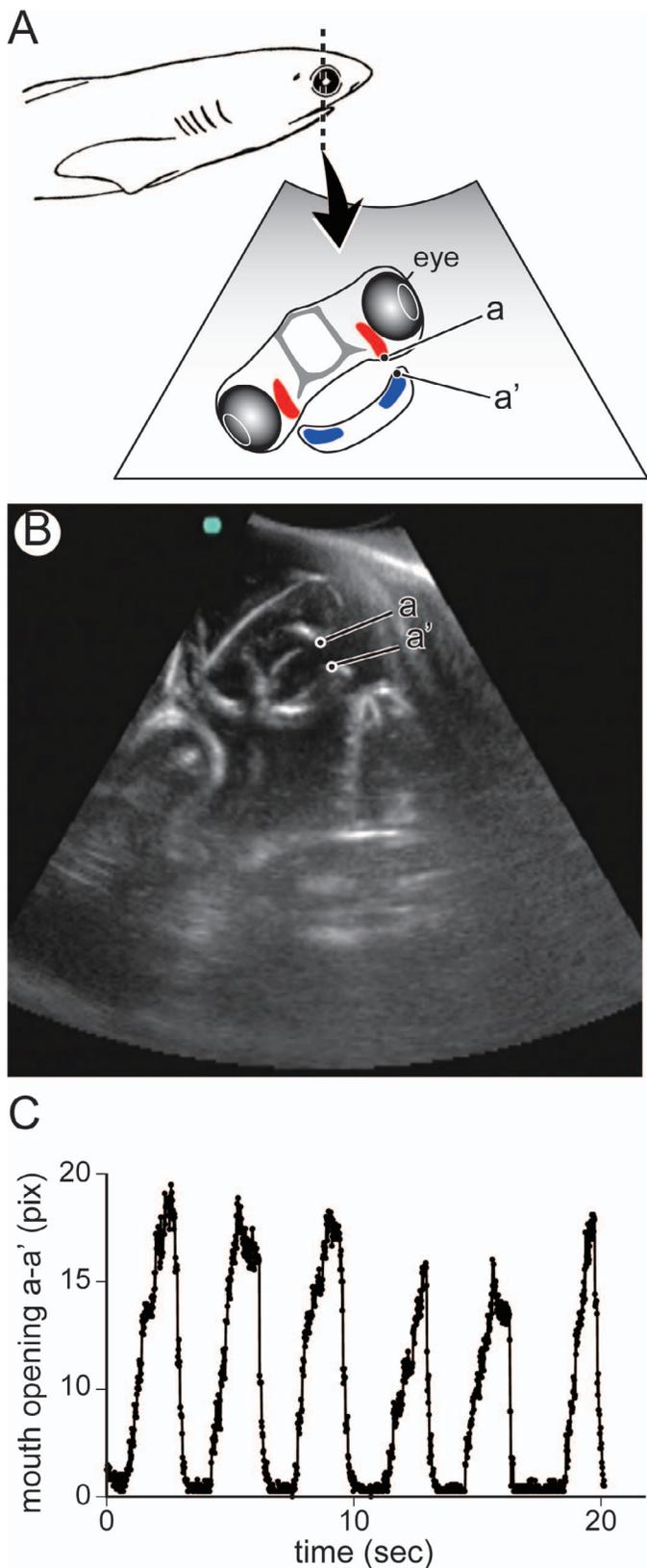


Fig. 2. Sonographic examination to determine the buccal pumping of the embryonic Tiger Shark. (A) Line drawings of the embryo showing the arrangement of embryo in sonogram. (B) Still image taken from sonographic video footage showing the locations of upper (a) and lower jaw (a') used for kinematic analysis. Upper jaw is shown in red and lower jaw in blue. (C) Opening and closing movement of an embryonic mouth (the y-axis indicates the distance between a and a' in panel B).

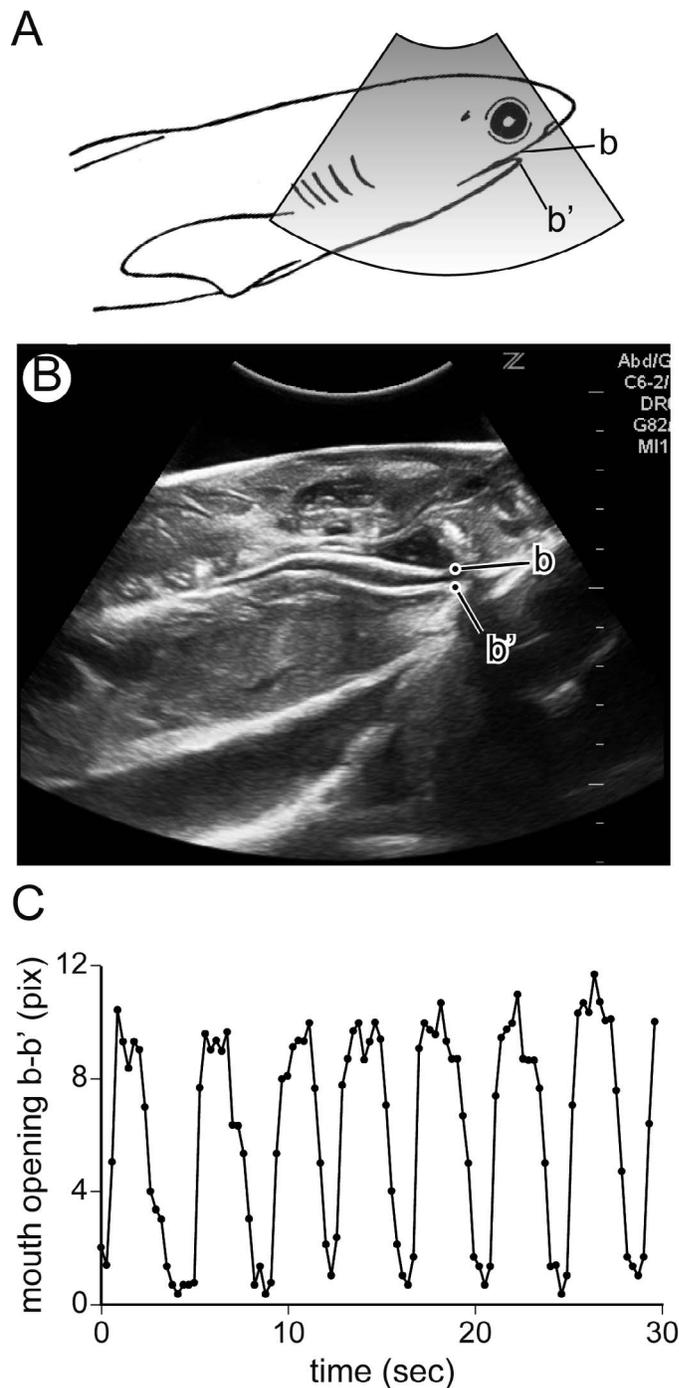


Fig. 3. Sonographic examination to determine the buccal pumping of the newborn Tiger Shark. (A) Line drawings of the newborn juvenile showing the arrangement of newborn juvenile in sonogram. (B) Still image taken from sonographic video footage showing the locations of upper (b) and lower jaw (b') used for kinematic analysis. (C) Opening and closing mouth-movement of newborn Tiger Shark (the y-axis indicates the distance between b and b' in panel B).

lower jaws (points a and a' in Fig. 2 and b and b' in Fig. 3) for each frame of ultrasound footages using Image J (US National Institutes of Health, Bethesda, MD).

RESULTS

Parturition events.—The female gave birth to 30 offspring, which included three still births, one individual preyed upon by another species (*Negaprion acutidens*), and two abnormal



Fig. 4. Neonates “resting” on the bottom of the tank.

individuals presenting lordosis and kyphosis. Six unfertilized eggs were also shed during parturition. The total duration from the first to the last parturition event was over 3.5 h. Birth time of each individual is shown in Table 1. The time interval between successive parturitions ranged from 1 to 22 min (average \pm SD = 6.8 ± 5.6 min).

The size of the neonates, excluding two abnormal individuals, ranged from 71 to 82 cm TL (average \pm SD = 77.2 ± 3.2 cm) and from 1.1 to 2.0 kg BW (average \pm SD = 1.45 ± 0.26 kg). Sex ratio (males/females) of the neonates was 1.25, which was not significantly different from 1.0 ($P = 0.56$, Chi-square test). All neonates lacked an external yolk sac.

Twenty-six parturition events were observed in 59 video footages (total recording time was 109 min). All neonates were born tail-first. All parturition events followed the same sequences: 1) A part of the egg capsule appears outside of the cloaca (Fig. 1B). 2) Embryonic body becomes visible in the order of caudal, pelvic, and pectoral fins (Fig. 1C, D). 3) The neonate is separated from maternal body (Fig. 1F). Egg capsule is excreted from cloaca simultaneously with juvenile separation (Fig. 1E). Neonate separation and egg capsule excretion sometimes occurred after the mother quickly turned (Fig. 1C). Out of 23 parturition events, in which the birth posture was confirmed in video footage, seven individuals were born abdomen-up, nine abdomen-down, and seven in abdomen-sideways positions.

For at least the first 5.5 h after parturition, neonates alternated between swimming in the water column and “resting” on the bottom of the tank (Fig. 4). According to the data from 3.5 to 5.5 h after the last parturition event for three neonates, they spent 54.5%, 60.0%, and 63.3% of the total

Table 1. Parturition time of Tiger Shark neonates born at Okinawa Churaumi Aquarium.

Specimen no.	Parturition time	Time interval (min)
1	12:21	
2	12:23	2
3	12:27	4
4	12:44	17
5 ^a	12:53	9
6 ^a	12:54	1
7	12:57	3
8 ^c	13:10	4
9	13:12	2
10	13:26	14
11	13:29	3
12 ^c	13:30	1
13	13:40	10
14	13:50	10
15	13:54	4
16	13:55	1
17	14:04	9
18	14:07	3
19 ^b	14:25	18
20 ^b	14:27	2
21	14:40	13
22	14:49	9
23	14:56	7
24	15:18	22
25	15:21	3
26	15:26	5
27	15:29	3
28	15:38	9
29	15:42	4
30	15:48	6

^{a,b} Tails of two neonates were visible from outside at the same time.

^c Stillbirths (Parturition time of one of three stillbirths is unknown.)

observational time (ca. 30 min for each individual) lying still on the bottom of the tank. During swimming, the neonates used ram ventilation for respiration. In contrast, the neonates in resting phase continuously opened and closed their mouth. From the top view of the head, rhythmic expansion and compression of pharyngeal area and opening and closing of gill slits were also observed. Repeated resting behavior ceased on the day after parturition. The first feeding occurred for five neonates on 26 March 2017.

Sonographic experiments.—Sonographic experiment on the pregnant female showed that embryonic Tiger Shark rhythmically expanded and compressed its buccal cavity (Fig. 2). Duration of a single buccal-pumping cycle, which was defined by the interval between successive maximum gapes, was 3.40 s (± 0.66 s SD). Similar buccal pumping was also observed for neonates (Fig. 3). Duration of a single buccal-pumping cycle was 4.25 s (± 1.03 s SD).

DISCUSSION

The present study is the first report, to our knowledge, on the captive parturition of Tiger Shark. This event is unlikely to be a result of premature birth caused by an artificial environment because: 1) The size of the offspring agrees with the birth size previously published for Tiger Shark embryos and the smallest free-swimming juveniles (76–89 cm TL; Whitney and Crow, 2007; Castro, 2011). 2) A previous study showed

that the absorption of the external yolk-sac is completed at the very end of gestation (Castro et al., 2016: figs. 1, 2; Sato and Tomita, unpubl. data). The lack of external yolk sac observed in our newborn specimens suggests that they were born fully developed.

The captive birth of Tiger Shark presented in this study has permitted the preliminary comparison of the parturition process between Tiger Shark and other viviparous elasmobranchs. Previous study showed that the parturition process of the viviparous Carcharhiniformes was characterized by tail-first and abdomen-up birth posture (Wass, 1973; Uchida et al., 1990; Parsons, 1991). Our observation showed that all juveniles of Tiger Shark were born in the tail-first position, which is thus consistent with other carcharhiniform species. In contrast, the abdomen-up birth posture was observed in approximately one-third of all birth events for Tiger Shark, revealing that the abdomen-up birth posture is not the typical case in this species.

Sonographic examination of embryonic Tiger Shark showed that, unlike postnatal individuals, the embryo uses buccal pumping for respiration. To the best of our knowledge, this is the first observation on the ontogenetic shift of respiratory mode from buccal pumping to ram ventilation in viviparous sharks. In many viviparous elasmobranchs, including Tiger Shark, embryos lack a placental connection with the mother (Castro et al., 2016). For such aplacental species, production of continuous water flow over the gills via buccal movement is probably essential for efficient oxygen uptake from the uterine fluid (Tomita et al., 2012, 2016).

The present study also revealed that neonates experience a transitional period shifting from buccal pumping to ram ventilation just after parturition. Unlike in adult Tiger Sharks, ram ventilation of the neonates was not continuous, and buccal pumping was also used when they “rested” on the bottom of the tank. Previous studies show that ram ventilation is energetically advantageous compared to buccal pumping, but requires relatively fast and continuous swimming for the acquisition of enough oxygen from ambient water (Roberts, 1975; Steffensen, 1985). This was originally based on experiments with several species of bony fishes, though it is now widely applied to non-benthic fishes in general (e.g., Carlson et al., 2004). Thus, we speculate that the neonates start continuous ram ventilation only after the establishment of continuous and fast swimming abilities. Based on our observation, Tiger Shark neonates continuously swam on the day following parturition; thus, the shift of respiratory mode from buccal pumping to ram ventilation may be completed within one day after parturition.

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