Received: 22 July 2018 | Revised: 14 September 2018 | Accepted: 24 October 2018
DOI: 10.1111/eth.12828

BEHAVIOURAL NOTE

WILEY ethology

Locomotion is not a privilege after birth: Ultrasound images of viviparous shark embryos swimming from one uterus to the other

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Funding information
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Editor: R. Bshary

Abstract

Underwater ultrasound, a new tool for observing the internal body parts of aquatic animals by scuba divers, allowed us long-term and frequent observations of the embryos of captive aquatic vertebrates. New ultrasound data of captive tawny nurse sharks (*Nebrius ferrugineus*) revealed that their embryos frequently migrate between the right and left uteri during gestation. This report is the first reliable evidence of active embryonic locomotion in live-bearing vertebrates and is contradictory to the concept of "sedentary embryo" which has mainly arisen from studies of mammals. The tawny nurse shark is unique among orectolobiform sharks, in which the embryo develops by feeding on sibling eggs in utero. Thus, we hypothesized that swimming aids in an efficient search and capture of these eggs in the uterine environment.

KEYWORDS
captivity, diagnostic sonography, elasmobranch, nurse shark, oophagy, viviparity

1 | INTRODUCTION

Locomotion, the ability of body displacement, by swimming, walking, or flying, is one of the defining characteristics of animals (Biewener, 2007). However, this ability is generally considered to be highly restricted in embryos developing in the maternal body. This view is well confirmed in mammals: Fetal motion is mostly limited to the local parts of the body, including mouth, limbs and fingers, and movement of the entire body is much less as compared with that after birth (e.g., Nowlan, 2015). Limited locomotion shown by the fetus may be attributed to several reasons, including physical (e.g., limited space in uterus and physical connection between fetus and mother with placenta) and functional (e.g., incomplete development of nerves and locomotor muscles) constraints for the fetus.

Recent advances in ultrasound technology, mainly the increased portability of instruments, have provided a novel opportunity to observe pregnant females of live-bearing elasmobranchs (sharks, skates, and rays), which has revealed the poorly known embryonic behavior of nonmammalian vertebrates (Tomita, Cotton, & Toda, 2016; Tomita, Toda, Uchida, & Nakaya, 2012; Tomita et al., 2018). However, ultrasound devices cannot be used underwater, and specimens should be kept near the surface of the water during observation. Thus, frequent monitoring of females using ultrasound was generally avoided to limit stress to the individuals. To prevent this limitation, we have developed a water- and pressure-resistant system for portable ultrasound, that we named underwater ultrasound (Figure 1).

The present study reports the active locomotion of the embryos of captive tawny nurse shark *Nebrius ferrugineus* (Orectolobiformes; Ginglymostomatidae) observed by underwater ultrasound. Our data shows frequent embryonic migration between the right and left uteri, which is contradictory to the "sedentary" mammalian fetus. The reproductive mode of the tawny nurse shark is characterized by its unique nutrient transfer mechanism from mother to embryo, called oophagy. In this reproductive mode, the embryo develops by consuming unfertilized eggs (nutritive eggs) accumulated in utero (Teshima, Kamei, Toda, & Uchida, 1995). Other aspects of the reproductive biology of tawny nurse shark are still poorly understood, except for some

FIGURE 1 Underwater ultrasound developed in this study (a). Ultrasound observation for the pregnant tawny nurse shark at the depth of 10 m in the exhibition tank of Okinawa Churaumi Aquarium (b)

basic reproductive parameters, such as fecundity of one to four and birth size of 40–60 cm in total length (Compagno, Dando, & Fowler, 2005). The aims of the present study were to first describe locomotion of the embryonic tawny nurse shark and second, to discuss its ecological significance in association with their reproductive strategy.

2 | MATERIALS AND METHODS

Ultrasound experiments were conducted from June 2015 to January 2018 using three tawny nurse shark pregnant females (Females 1–3, c.a. 2.7 m in total length) maintained in the exhibition tank ("Kuroshio tank"; 7,500 cubic meters) at Okinawa Churaumi Aquarium (Okinawa, Japan). These specimens were originally caught from wild in 2001 off Ishigaki Island (Okinawa, Japan). During the observation, the diver remained near the pelvic fin of the specimen (Figure 1b). The time of the observation was mostly between 7:30 a.m. and 8:30 a.m., and the duration of each observation was <5 min to limit the potential stress caused to pregnant females. The observations were conducted throughout the pregnancy for each female, and forty-four ultrasound observations were recorded in total. Ultrasound data were obtained using the portable ultrasound diagnostic imaging system FAZONE M (FUJIFILM Co., Tokyo, Japan)

TABLE 1 Changes in embryonic number in right and left uteri through time for three pregnant females

	Number of embryos		
Date	Rigth uterus	Left uterus	Total
Female 1			
2016.7.9	0	1	1
2016.7.21	0	1	1
2016.7.28[a]	1/0	0/1	1
2016.8.7	0	1	1
2016.9.5	1	0	1
2016.11.7[a]	1/0	0/1	1
2016.11.8	0	1	1
2016.12.1[a]	1/0	0/1	1
2016.12.8	0	1	1
Female 2			
2017.9.21	1	0	1
2017.10.5	1	0	1
2017.10.10	1	0	1
2017.10.17	0	1	1
2017.10.26	1	0	1
2017.11.6	0	1	1
2017.11.27	0	1	1
2017.12.16	0	1	1
2017.12.31	0	1	1
Female 3			
2017.9.12	2	2	4
2017.9.21[b]	3→2	1→2	4
2017.10.10[c]	3→1	1→3	4
2017.10.10	3	1	4
2017.10.17	2	2	4
2017.10.19	2	1	3
2017.10.26	2	1	3
2017.11.6	1	2	3
2017.11.12[d]	3→2	0→1	3
2017.11.27[e]	3→2	0→1	3
2017.12.3	2	1	3
2017.12.4	2	1	3
2017.12.8	0	3	3
2017.12.11	2	1	3
2017.12.12	2	0	2
2017.12.16	1	1	2
2017.12.17[f]	1/0	0/1	1
2017.12.21	0	1	1
2017.12.25	1	0	1
2017.12.31	0	1	1

(*Continues*)

TABLE 1 (Continued)

Date	Number of embryos		
	Rigth uterus	Left uterus	Total
2018.1.6	1	0	1
2018.1.7	0	1	1

[a]Embryonic movement between right and left uterus was observed. [b]Three embryos in right and one embryo in left uterus at 10:20 a.m. Two embryos in each uterus at 10:40 a.m. [c]Three embryos in right and one embryo in left uterus at 10:37 a.m. One embryo in right and three embryos in left uterus at 10:55 a.m. [d]Three embryos in right uterus at 10:27 a.m. Two embryos in right and one embryo in left uterus at 10:29 a.m. [e]Embryonic number was chaged from three to two in right and from zero to one in left uterus during 1 min. [f]Embryo was located across right and left uterus. One pup was born.

and ARIETTA Prologue (Hitachi-Aloka Medical Ltd., Tokyo, Japan). Water- and pressure-resistant housings, with a maximum depth of 10 m for FAZONE M and 20 m for ARIETTA Prologue, were codeveloped by SSP Ltd. (Matsumae, Japan) and Okinawa Churaumi Aquarium (Figure 1a) for their underwater use. The probes used for ultrasound were placed laterally on either side of the trunk to scan the whole length of right and left uteri, and the number of the embryos was recorded for the uterus of each side.

The ultrasound experiment presented in this study was conducted as part of the routine medical checkup for captive animals in the aquarium. Animal handing during the experiment was done in strict accordance with the guidelines for animal experiments of the Okinawa Churashima Foundation, with the same consideration for animal care and welfare as that for "higher" vertebrates (reptiles, birds, and mammals). However, as the guidelines stipulated, the approval from the Institutional Animal Care and Use Committee of Okinawa Churashima Foundation, required for higher vertebrates, is waived for "lower" vertebrates including fishes.

3 | RESULTS

During the experiment, pregnant females carried a maximum of four embryos. In all females, the number of embryos in each uterus frequently changed during gestation (Table 1). According to the change in embryonic number in each uterus, movement of embryos between right and left uteri occurred at least 4, 3, and 24 times in females 1, 2, and 3, respectively.

Ultrasound footage showed that embryos of the tawny nurse shark actively swam in utero (Figure 2a,b; Supporting Information Movie S1). A footage of female 1 showed the process of embryonic movement from the right to the left uterus: At time 0, the head of the embryo is located in the posterior part of the right uterus, but the rest of the body was left inside the left uterus. Thereafter, up to 7.41 s, the embryo can be seen entering the left uterus in the following order: pectoral fin, trunk, pelvic fin, and tail (Figure 3a,b; Supporting Information Movie S2). The speed of the embryonic motion, which was calculated by tracing the tip of the pectoral fin, was c.a. 8 cm/s.

In addition to the footage for embryonic locomotion, we also recorded an ultrasound footage in which the embryo of female 3 exposed its head outside the uterus through the cervix (Figure 4). This behavior was also confirmed externally (Figure 4a; Supporting Information Movie S3). This behavior occurred in the final month of pregnancy as all four embryos of female 3, confirmed by ultrasound, were born within 1 month after the head protruding behavior was observed.

4 | DISCUSSION

Our data strongly suggest that the tawny nurse shark embryo frequently migrates across right and left uteri, which is the first reliable evidence for active embryonic locomotion of viviparous vertebrates. The only previous record of embryonic locomotion in elasmobranchs was seen in the program for Discovery Channel in 1993: The camera crew for the program filmed the swimming embryo of the sand tiger shark (*Carcharias taurus*). However, for this observation, the side of the female body was cut for access to the uteri, and the camera was inserted through the opening (G. Gilmore, Jr., *Pers. Obs.*). Thus, it is not an observation under natural conditions. To the best of our knowledge, the observation of embryonic behavior in natural conditions has been reported for three elasmobranch species, including *Mobula alfredi*, *Squalus japonica*, and *Galeocerdo cuvier* (Tomita et al., 2016, 2012, 2018). In these species, embryonic behavior was restricted to the respiratory mouth movement (i.e., buccal pumping), not embryonic locomotion.

We hypothesize that active embryonic locomotion of the tawny nurse shark may be associated with its unique reproductive strategy. It is known that the tawny nurse shark and the sand tiger shark (*Carcharias taurus*) share a highly specialized mother to embryo nutrient transfer mechanism, called oophagy (Gilmore, 1993; Teshima et al., 1995). Unlike many aplacental viviparous sharks, in which the embryo acquires nutrient solely from its yolk, embryos of oophagous sharks develop mainly by feeding on sibling eggs *in utero* (e.g., Gilmore, 2005). It seems likely that in this mode of reproduction, the active swimming ability of the embryo may allow it to effectively search and capture nutritive eggs in the uterine environment.

Our data also showed that the cervix of the tawny nurse shark sometimes opens, and the embryo exposes its head out of the uterus through the cervix. This phenomenon is in contrast to that seen in mammals where the cervix is tightly closed until birth (e.g., Myers et al., 2015). Interestingly, intermittent opening of the cervix has been hypothesized based on the chemical composition of the uterine fluid in some elasmobranch species: The ionic and protein contents of the uterine fluid during late gestation are similar to seawater in *Squalus acanthias* and *Orectolobus ornatus*, suggesting that some level of fluid exchange occurs between the inside and outside of the uterus through the cervix (Ellis & Otway, 2011; Kormanik, 1992; Kormanik & Evans, 1986).

To summarize, the mechanism of in utero movements seen in the tawny nurse shark is likely to be uncommon among viviparous vertebrates. The embryo of this species has abilities of active locomotion,

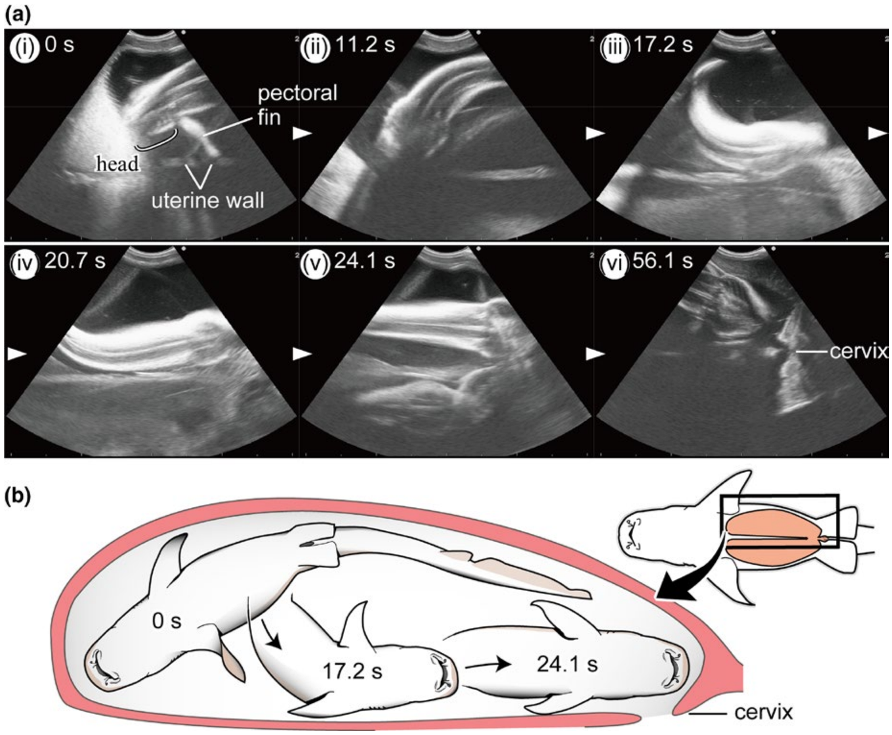

FIGURE 2 Locomotion of the tawny nurse shark embryo. (a) Serial ultrasound images for female 3, showing the embryonic movements from the anterior to the posterior portion of the left uterus. (b) Schematic illustration of embryonic movement found in (a). See Supporting Information Movie S1 for original video clip

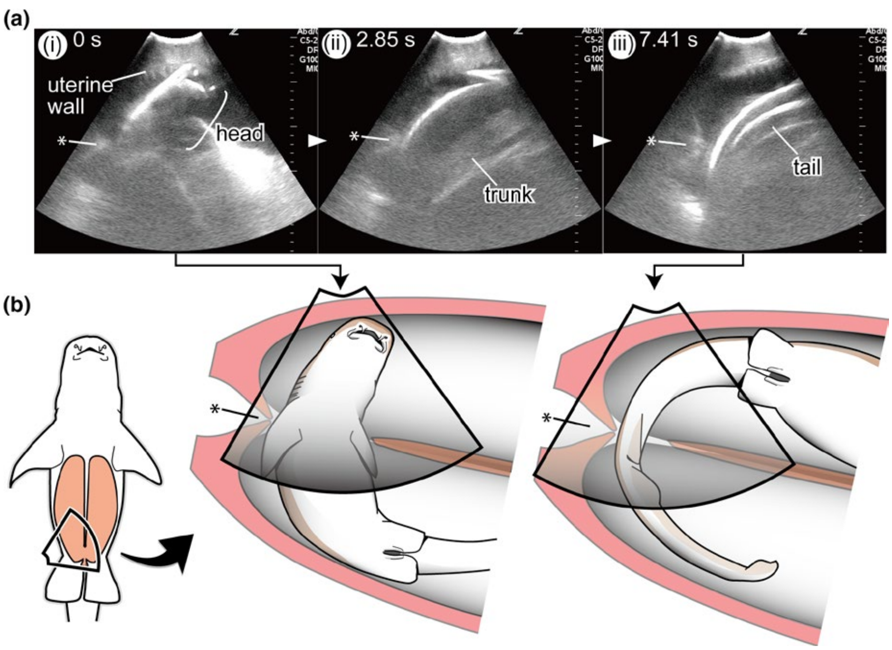

FIGURE 3 Evidence of embryonic movement from one uterus to the other. (a) Serial ultrasound images for female 1, showing embryonic movement from the left to the right uterus. The embryo passed near the cervix (*), which is located at the junction between right and left uterus, in the following order: head (i), trunk (ii), and tail (iii) of the embryo. (b) Schematic illustrations of the embryonic posture in uterus, which correspond to (i) and (iii) in (a). See Supporting Information Movies S2 for original video clips

oral feeding, and exposure to the external environment. These abilities are acquired after birth in mammals and probably in most viviparous elasmobranchs. The early onset of "postnatal behaviors" in the tawny nurse shark might reduce the ecological gap between embryonic and postnatal periods, which may decrease the risk of neonatal mortality.

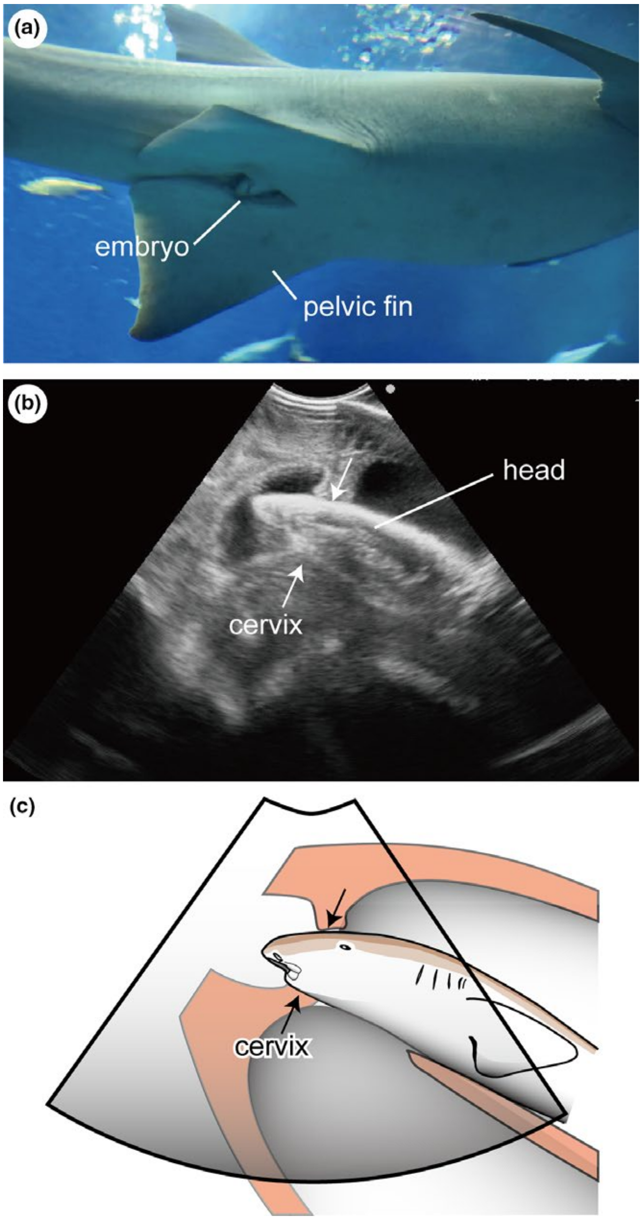

FIGURE 4 Embryo of female 3 exposing its head out of the uterus. External (a) and ultrasound (b) images. (c) Schematic illustration of the embryonic posture in uterus, which correspond to (b). See Supporting Information Movie S3 for original video clip for (a)

ACKNOWLEDGEMENTS

We thank the following for their help and useful comments; Makio Yanagisawa, Rui Matsumoto, Keiichi Sato, and other staff at the Okinawa Churaumi Aquarium and Okinawa Churashima Research Center. We also thank Grant Gilmore, Jr. for sharing data for the embryonic locomotion of the sand tiger shark. This work was supported by the admission fees for the Okinawa Churaumi Aquarium.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Biewener, A. A. (2007). *Animal locomotion*. New York, NY: Oxford University Press.

Compagno, L., Dando, M., & Fowler, S. (2005). *Sharks of the world*. Princeton, NJ: Princeton University Press.

Ellis, M. T., & Otway, N. M. (2011). Uterine fluid composition of the dwarf ornate wobbegong shark (*Orectolobus ornatus*) during gestation. *Marine and Freshwater Research*, *62*, 576–582. https://doi.org/10.1071/MF10138

Gilmore, R. G. (1993). Reproductive biology of lamnoid sharks. *Environmental Biology of Fishes*, *38*, 95–114. https://doi.org/10.1007/BF00842907

Gilmore, R. G. (2005). Oophagy, intrauterine cannibalism and reproductive strategy in lamnoid sharks. In W. C. Hamlett, & B. G. M. Jamieson (Eds.), *Reproductive biology and phylogeny of Chondrichthyes* (pp. 435–462). Enfield, UK: Science Publishers.

Kormanik, G. A. (1992). Ion and osmoregulation in prenatal elasmobranchs: Evolutionary implications. *American Zoologists*, *32*, 294–302. https://doi.org/10.1093/icb/32.2.294

Kormanik, G. A., & Evans, D. H. (1986). The acid-base status of prenatal pups of the dogfish, *Squalus acanthias*, in the uterine environment. *Journal of Experimental Biology*, *125*, 173–179.

Myers, K. M., Feltovich, H., Mazza, E., Vink, J., Baijka, M., Wapner, R. J., … House, M. (2015). The mechanical role of the cervix in pregnancy. *Journal of Biomechanics*, *48*, 1511–1523. https://doi.org/10.1016/j.jbiomech.2015.02.065

Nowlan, N. C. (2015). Biomechanics of foetal movement. *European Cells and Materials*, *29*, 1–21. https://doi.org/10.22203/eCM.v029a01

Teshima, K., Kamei, Y., Toda, M., & Uchida, S. (1995). Reproductive mode of the tawny nurse shark taken from the Yaeyama Islands, Okinawa, Japan with comments on individuals lacking the second dorsal fin. *Bulletin of Seikai National Fisheries Research Institute*, *73*, 1–12.

Tomita, T., Cotton, C., & Toda, M. (2016). Ultrasound and physical models shed light on the respiratory system of embryonic dogfishes. *Zoology*, *119*, 36–41. https://doi.org/10.1016/j.zool.2015.09.002

Tomita, T., Toda, M., Uchida, K., & Nakaya, K. (2012). Live-bearing manta ray: How the embryo acquires oxygen without placenta and umbilical cord. *Biology Letters*, *23*, 721–724. https://doi.org/10.1098/rsbl.2012.0288

Tomita, T., Touma, H., Murakumo, K., Yanagisawa, M., Yano, N., Oka, S., … Sato, K. (2018). Captive birth of tiger shark (*Galeocerdo cuvier*) reveals a shift in respiratory mode during parturition. *Copeia*, *106*, 292–296.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Tomita T, Murakumo K, Ueda K, Ashida H, Furuyama R. Locomotion is not a privilege after birth: Ultrasound images of viviparous shark embryos swimming from one uterus to the other. *Ethology*. 2018;00:1–5. https://doi.org/10.1111/eth.12828