



# Ultrasound and physical models shed light on the respiratory system of embryonic dogfishes



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## ARTICLE INFO

### Article history:

Received 8 June 2015

Received in revised form 24 July 2015

Accepted 10 September 2015

Available online 15 September 2015

### Keywords:

Buccal pumping  
Oxygen diffusion  
Respiration  
Uterine fluid  
Uterine villi

## ABSTRACT

Embryos of live-bearing elasmobranchs (sharks, skates, and rays) must acquire oxygen in the uterus for several months to more than a year, but the mechanisms of delivery and uptake are still largely unknown. Diagnostic sonography performed on a captive Japanese dogfish (*Squalus japonicus*) showed that a late-stage embryo used buccal movement to pump uterine fluid, suggesting that the embryo acquires oxygen from uterine fluid via gill ventilation. It has been assumed that embryonic respiration in aplacental sharks depends on oxygen supplied by the uterine wall. To test this hypothesis, the rate of oxygen diffusion was estimated by applying a physical model to the uterine wall of two dogfish species (*Squalus cf. mitsukurii* and *Squalus cubensis*). The model calculations indicate that the supply of oxygen via diffusion through the uterine villi contributes less than 15–30% of the total oxygen demand of late-stage embryos. Some previous authors have suggested that pregnant dogfish intermittently exchange uterine fluid with external seawater during late gestation. Thus, late-stage embryos may acquire oxygen primarily from uterine seawater introduced from the external environment.

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## 1. Introduction

Viviparous (live-bearing) elasmobranchs (sharks, skates, and rays) sustain their embryos inside the female's body for several months to more than a year (e.g., Castro, 2011). Placental viviparity is restricted to five carcharhiform families, providing a mechanism of oxygen transfer analogous to that of the mammalian placenta (Hamlett and Koob, 1999). However, the majority of viviparous elasmobranchs lack such a maternal–embryonic connection (Musick and Ellis, 2005) and the process of embryonic oxygen uptake is poorly understood for these aplacental species. Recently, Tomita et al. (2012) used diagnostic sonography to describe buccal pumping behavior of a captive manta ray (*Manta alfredi*) embryo. This observation provided the first direct evidence of the respiratory behavior of a viviparous elasmobranch embryo by revealing that the embryo acquires oxygen from the uterine fluid using gill ventilation.

Documentation of buccal pumping in the manta ray embryo may also have evolutionary implications in the transition from

oviparous (egg laying) to viviparous reproductive modes. Buccal pumping has also been reported in late-stage embryos of several oviparous elasmobranchs, such as *Chiloscyllium plagiosum*, *Heterodontus portusjacksoni*, *Scyliorhinus canicula*, and *Scyliorhinus torazame* (Diez and Davenport, 1987; Tullis and Peterson, 2000; Rodda and Seymour, 2008; Tomita et al., 2014). This implies that viviparous and oviparous elasmobranchs develop embryonic respiratory mechanisms through similar processes. However, the scarcity of data on embryonic respiratory behavior, particularly for viviparous species, precludes confirmation of this hypothesis.

Public aquaria offer rare opportunities to examine specimens on demand. The present study reports the first sonographic examination of a captive pregnant female Japanese dogfish (*Squalus japonicus*) performed at the Okinawa Churaumi Aquarium (Okinawa, Japan). To the best of our knowledge, this is the second viviparous elasmobranch to date in which the embryonic respiratory behavior was observed in utero.

The reproductive mode of dogfishes (*Squalus* spp.) is categorized as yolk-sac viviparity. In this reproductive mode, the embryo grows to a large size inside the uterus by consuming yolk without additional nutrient input from the mother (Musick and Ellis, 2005). Studies of embryonic development in yolk-sac viviparous sharks are limited (Frazer et al., 2012) and those investigating embryonic oxygen demand are non-existent in the literature.

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It has been hypothesized that dogfish embryos depend on the uterine wall for gas exchange (Burger, 1967; Jollie and Jollie, 1967; Hamlett and Hysell, 1998). Comparison of the uterine microstructure among pre-fertilization, pregnant, and postpartum females of *S. acanthias* revealed that villi develop on the inner surface of the uterine wall during gestation (Jollie and Jollie, 1967). These structures are thought to increase surface area for gas exchange (Jollie and Jollie, 1967; Hamlett and Hysell, 1998); however, this hypothesis has never been tested quantitatively. Therefore we developed a physical model to estimate the respiratory capacity of dogfish uterine villi.

## 2. Materials and methods

### 2.1. Diagnostic sonography

Sonography (Fazone M sonographic diagnostic imaging system; Fujifilm, Tokyo, Japan) was conducted on a captive pregnant *S. japonicus* (ca. 70 cm in total length [TL]) on April 1st and 14th, 2010, at the Okinawa Churaumi Aquarium (Okinawa, Japan). The sonograph transducer was placed on the lateral surface of the body, just lateral to the uterus. The specimen, which was maintained in a tank at the Okinawa Churaumi Aquarium, was transferred to a small aerated tank prior to the experiment.

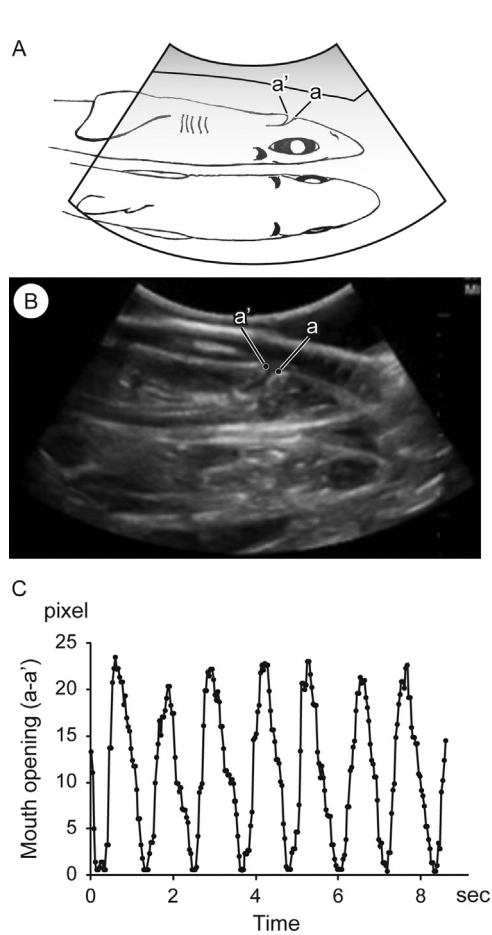
After the experiment, still images were captured from the sonographic videos using the movie-editing software KMPlayer v. 2.9.4.1.1435 (Jelsoft Enterprises Ltd, Pangbourne, UK). The capture

rate was 29 frames per second. Mouth opening (distance between the symphyses of the upper and lower jaws = distance between points a and a' in Fig. 1) and head width (distance between the lateral-most points of the right and left hyoid arches = distance between points b and b' in Fig. 2) were measured from each still image using ImageJ (US National Institutes of Health, Bethesda, MD, USA).

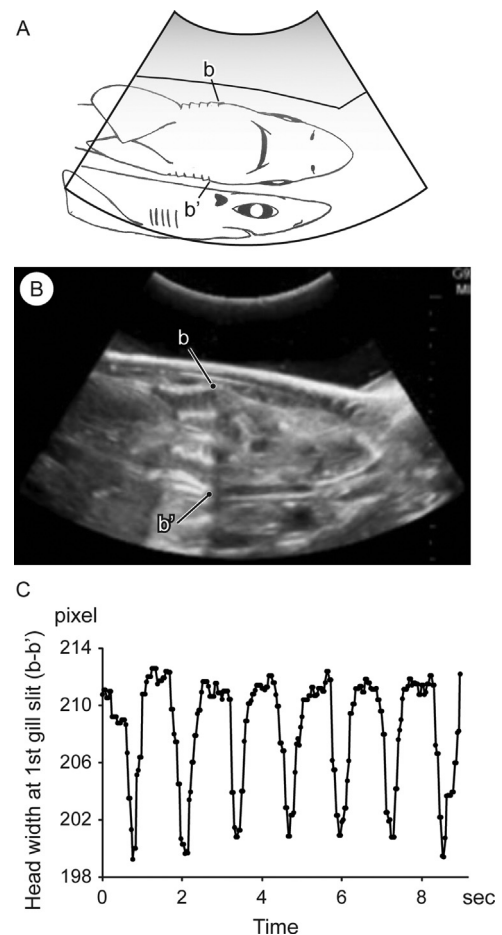
### 2.2. Measurements of the uterine villi

Morphological measurements were required to estimate respiratory capacity of the dogfish uterus. We examined five pregnant female *Squalus cf. mitsukurii* specimens and three pregnant female *Squalus cubensis* specimens collected in April 2012 along the West Florida Slope north of 27°N (~250–500 m) in the DeSoto Canyon region. Uteri of *S. cf. mitsukurii* (with late-stage embryos, 10–17 mm TL) and of *S. cubensis* (with late-stage embryos, 17–19 mm TL) were fixed in 10% formalin shortly after capture.

Tissue samples of the uterine wall (approximately 1 cm × 1 cm in size) were obtained from three sites from each uterus (sites 1–3 in Fig. 3). For these tissue samples, length and width at the midpoint (Fig. 4) of each villus and density of the villi were measured using a digital microscope (SteREO Discovery V12; Carl Zeiss AG, Oberkochen, Germany) (Fig. 4) to the nearest 0.01 mm. Measurements were obtained from 20 villi at each site (60 villi per uterus). In addition, the thickness of the diffusion barrier (Fig. 4), defined as



**Fig. 1.** Sonographic video recording showing buccal movements of the embryonic Japanese dogfish (*Squalus japonicus*). (A) Line drawing showing the arrangement of the embryos in the sonograms and (B) a still image from the sonograms. Points a and a' indicate the symphysis of the upper and lower jaws in the midsagittal plane. (C) Rhythmic mouth opening/closing movements were observed.



**Fig. 2.** Sonographic video recording showing branchial movement of the embryonic Japanese dogfish (*Squalus japonicus*). (A) Line drawing showing the arrangement of the embryos in the sonograms and (B) a still image from the sonograms. (C) Head width at the first gill slits (the distance between points b and b') rhythmically increased and decreased.

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