



## NOTE

Wildlife Science

**Behavioral effects of scents from male mature Rathke glands on juvenile green sea turtles (*Chelonia mydas*)**Chiyo KITAYAMA<sup>1)</sup>, Yohei YAMAGUCHI<sup>2)</sup>, Satomi KONDO<sup>1)</sup>, Ryuta OGAWA<sup>1)</sup>, Yusuke K. KAWAI<sup>3)</sup>, Mitsunori KAYANO<sup>4)</sup>, Jumpei TOMIYASU<sup>5)</sup> and Daisuke KONDOH<sup>2)\*</sup><sup>1)</sup>Everlasting Nature of Asia (ELNA), Ogasawara Marine Center, Ogasawara, Tokyo 100-2101, Japan<sup>2)</sup>Laboratory of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan<sup>3)</sup>Laboratory of Toxicology, Obihiro University of Agricultural and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan<sup>4)</sup>Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan<sup>5)</sup>Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn 10-748, Poland*J. Vet. Med. Sci.*

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**ABSTRACT.** Sea turtles can detect airborne and waterborne odors, but whether they recognize scents from the same species and if so, how they affect their behavior remains unknown. The present study evaluated the behavioral effects of odorants on juvenile green sea turtles (*Chelonia mydas*). The odorants were derived from Rathke glands (external scent glands) of mature male green sea turtles, and from two types of food. The activity of the juveniles increased when exposed to food scents, and significantly decreased compared with controls when exposed to scents from Rathke glands. These findings indicated that scents from the same species affect behavior, and that chemical communication via olfaction has important outcomes for sea turtles.

**KEY WORDS:** *Chelonia mydas*, ethology, odor, olfaction, Rathke gland

Green sea turtles (*Chelonia mydas*) are ocean-dwelling Testudines (family: Cheloniidae) that are distributed in tropical and sub-tropical oceans throughout the world [2]. After reaching maturity, they seasonally migrate between foraging and breeding areas [22], and adult males and females return to the sites of their birth (natal homing) to breed [7, 18, 28]. Although the mechanisms of breeding and natal homing behaviors of sea turtles remain moot, one possibility is the detection of chemical cues via olfaction [4, 6, 8, 9, 18], as recently found in the terrestrial painted turtle (*Chrysemys picta*) [12].

Some freshwater turtles, as well as loggerhead sea turtles (*Caretta caretta*) exhibit throat-pumping behavior (moving the throat slightly to transport water from the mouth to nostrils) to detect odors [19, 21, 26, 31]. This behavior was also evident in green sea turtles reared at the Ogasawara Marine Center (OMC) (Supplementary Video), especially when they encountered unfamiliar foods. We previously reported that sea turtles possess the morphologically-complicated nasal cavity [15, 16, 32] and that areas where water easily and hardly enters in the nasal cavity of green sea turtles are lined with different types of sensory epithelia [15], indicating that airborne and waterborne odors play important roles in their behaviors. In fact, sea turtles can detect airborne and waterborne odors [5, 20]. Due to the different properties such as diffusion in the air rapidly to a relatively long distance and diffusion in the water to a fairly close range, it is possible that odorants in the air and water are used properly depending on the situation. However, whether sea turtles can recognize odorants derived from the same species and if so, how their behaviors are affected and whether chemical communication proceeds via olfaction remain unknown.

Rathke glands are external scent glands in Testudines including some sea turtle species [3, 25, 27]. Green sea turtles normally have two pores in the axillary inframarginal region and one in the inguinal region [27]. The roles of powerful scents might include avoiding predators [3] and providing interspecific chemical cues [17], but the exact role remains unknown at 170 years after discovery. Here, we evaluated the behavioral effects of airborne odorants derived from mature male Rathke glands, and scents from two types of food on seven juvenile green sea turtles (age, ~ one year; straight carapace length, 20.1–27.2 cm) of unknown sex that

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were hatched and bred at OMC.

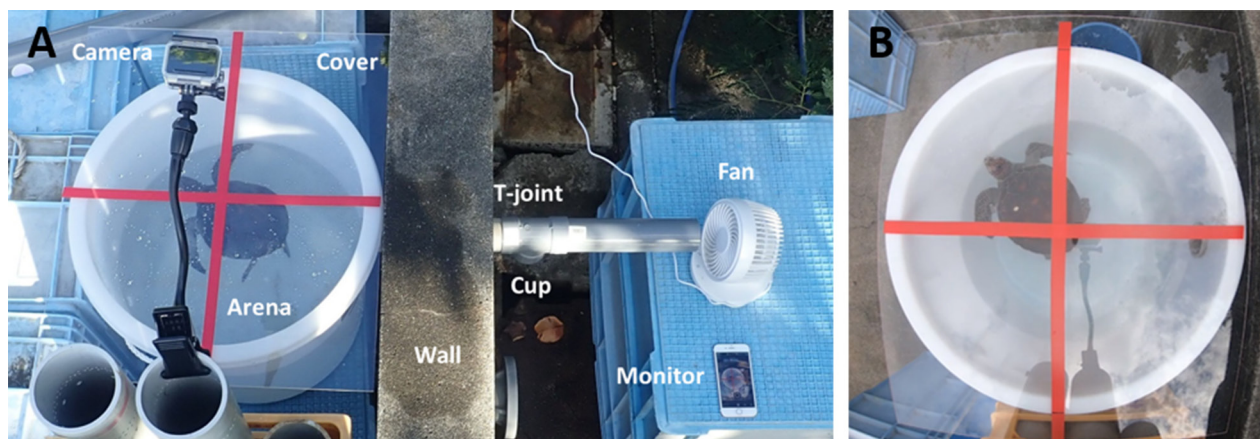
Rathke glands used in this study were sampled from both axillary inframarginal and inguinal regions of a dead mature male green sea turtle that was harvested for meals in Chichi-jima, the Ogasawara Islands in 2018. This harvesting is conducted strictly under the permission of the governor of Tokyo, and it is usually held during the mating season (February to May) of green sea turtles. The fat and muscle around the Rathke glands were carefully removed as much as possible, those sampled from axillary inframarginal and inguinal regions were stored frozen, and the mixture was used for the behavioral experiments described below. The present behavioral experiments proceeded at OMC during November 2018, and the Animal Care and Use Committee at Obihiro University of Agriculture and Veterinary Medicine approved the experimental protocol (Approval No: 19-239).

Turtles were exposed to odors from formula food (Kuroshio float EP-3, Higashimaru Co., Ltd., Kagoshima, Japan), shrimps and Rathke glands or distilled water (control) in a blindly random order. The formula food, shrimps and Rathke glands were dissolved in distilled water, and the intensity of scents was measured using an XP-329m odorant sensor with a minimum–maximum of 0–999 (New Cosmos Electric Co., Ltd., Osaka, Japan) immediately before the experiment. The mean ( $\pm$  SEM) average scent values to which the seven turtles were exposed were  $111.5 \pm 11.3$  and the ranges of intensity of three stimuli were unified within  $\pm 4.5$  for the same individual. The experiments were based on a study by Endres *et al.* [5], and Fig. 1 shows the experimental system. Water was poured to a depth of 40 cm in a round container with a 48 cm diameter, and PVC pipe with a T-joint located at the center was inserted in the container. Air was then propelled via a PVC pipe into the area using a fan. Turtles were placed individually in the container which was covered with the Perspex sheet comprising two crossed strips of red tape, and each turtle was allowed five min to become acclimated to the environment. A camera was placed above the container to record the activities of each turtle, and a cup containing one of the three odors was placed on the T-joint. The experiment started when the first inhalation of the odor (namely, the first respiration after each presentation), and the activity for five min was evaluated, which is a same condition as Endres *et al.* [5]. Among this, the air inside the container was freely fall outside. All of seven turtles could move and breathe freely throughout the experiments. The cup was replaced with an empty cup and the lid was opened to ventilate the container for 10 min. This process was repeated for the next two odors. Two independent observers evaluated indexes related to the turtle activities as follows.

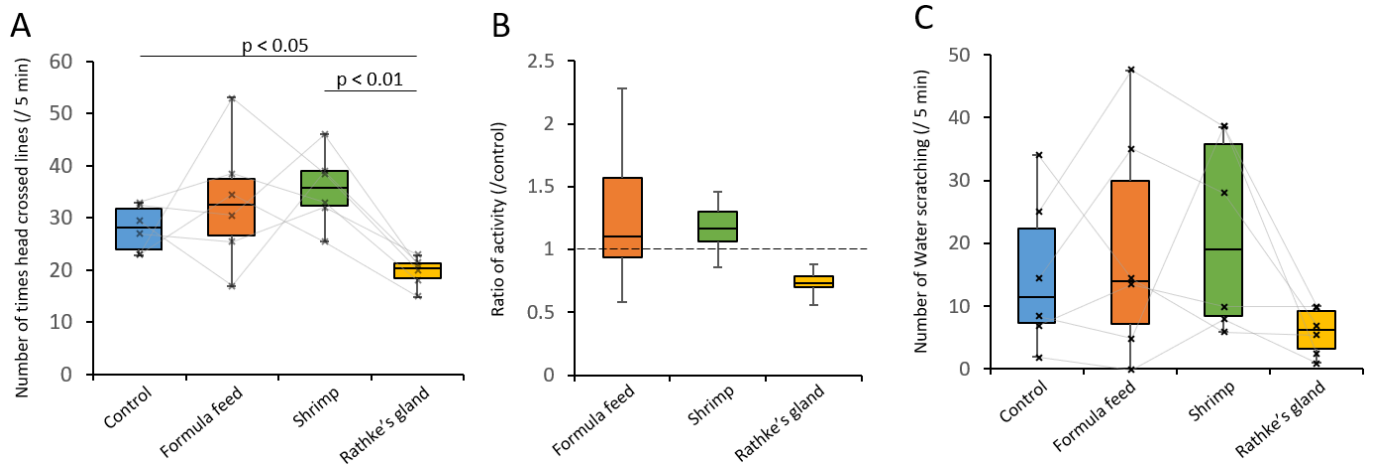
The number of times the head of the turtle crossed the red lines (Fig. 1B) within five mins of inhaling the odorants was established as a primary index (Fig. 2A). There were no significant differences between the order of presentation (Supplementary Fig. 1). One turtle that was hyperactive ( $>40$  times) throughout all odor presentations including distilled water, was determined by Smirnov–Grubb tests to be an outlier among turtles exposed to Rathke gland scents. Thus, data from this individual were excluded. The activities of the juveniles increased when exposed to the formula food and shrimps, and decreased when exposed to that derived from Rathke glands compared with the control (Fig. 2A and 2B). Paired *t*-tests with Holm multiple comparison tests [11] showed that activities significantly differed between turtles exposed to Rathke gland and control scents ( $P < 0.05$ ,  $n = 6$ ), as well as shrimps ( $P < 0.01$ ,  $n = 6$ ; Fig. 2A). All data were statistically analyzed using R version 3.5 [24]. We then counted the number of water scratches with both forelimbs as a secondary index of activity (Fig. 2C). This index also showed decreased activity in turtles exposed to Rathke gland scent (Fig. 2C). These results indicated that scents derived from male mature Rathke glands suppress the activities of juvenile green sea turtles.

The present findings showed that the odor derived from two types of foods activated green sea turtle behavior, as found in loggerhead sea turtles [5]. Sea turtles were fed with formula food and shrimps every day at OMC, and our findings indicated that they can use olfaction to search for food.

In contrast, the activity of the juvenile sea turtles decreased when exposed to the odor of male mature Rathke glands, which was similar to the freezing reaction of mice against predator [23, 29]. One assumption is that the juveniles might be simply confused



**Fig. 1.** Experimental system (A) and crossed lines on cover (B). Animals cannot see the investigators who are located behind a wall. Number of times the turtle head crossed the red lines were counted.



**Fig. 2.** Effects of odorants derived from formula food, shrimps, and mature male Rathke glands on juvenile green sea turtles. (A) Box plots show number of times the head crossed lines within five minutes after initial inhalation of each odorant. Cross plots connected by lines indicate same individual. Significant differences are evident between Rathke gland and control (distilled water) as well as shrimp odors ( $P < 0.05$  and  $P < 0.01$ , respectively; paired  $t$ -tests with Holm multiple comparison tests,  $n = 6$ ). (B) Box plots show prevalence of locomotion in response to each odor compared with control. (C) Box plots show number of water scratches using both forelimbs within five minutes of initially inhaling each odorant. Cross plots connected by lines indicate same individual.

by unknown odor resulted in inactivate reactions, because it was the first time to encounter the odor of mature male Rathke glands contrary to formula food and shrimp. However, it was indicated that some non-marine odors never exposed to sea turtles do not affect their activity [4], and thus the suppressive effects of Rathke gland odor on juvenile turtles shown herein were considered significant in their ecology.

Responses to odors seem to be determined by social relationships between the donors and recipients of chemicals. For example, subadult brown bears (*Ursus arctos*) investigate adult male odors more intensively than other odors [14], probably because subadults process such information to avoid adult males. Another example of odor response regarding to social relationships is reported in fathead minnows (*Pimephales promelas*) [1], and they use chemical disturbance cues as an antipredator signal to create group defenses such as shoaling, freezing and dashing [1]. Sea turtles spend most of their time alone in the wild and the biting behavior is observed under group feeding condition [10], thus the suppressive effects shown herein might be beneficial to maintain proper distance between individuals.

As far as we can ascertain, this is the first study to determine the behavioral effects of odorants derived from adult males on the species of juvenile sea turtles, indicating that green sea turtles use chemical signals in various situations via olfaction. Importantly, the suppressive effects of airborne scents from male mature Rathke glands on the activities of juveniles does not rule out the notion that odorants from the same species are associated with breeding behavior in sea turtles. In fact, responses to chemical cues derived from the same species, even for the same odorants, depend on sex and maturity status from lampreys [30] to mammals [13]. Therefore, further studies using various combinations of scent recipients and donors are required to understand the overall nature of chemical communications via olfaction in green sea turtles.

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