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Preliminary result of ontogenetic change of wave orientation of green turtles in the initial growth stages

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ABSTRACT
Head-starting is the practice of growing hatchlings in captivity to protect them from the high rates of natural predation that would occur in their early stage. It is concern whether the head-started turtles disperse to the open sea after the release like wild turtles. During offshore migration, hatchlings use refracted wave as an orientation cue to go straight to the open sea. This conditioned response to the wave results in movement away from land towards the open sea, because waves and swells entering shallow coastal areas are refracted until they approach a beach directly. Therefore, it is considered that the ability of orientation to the wave is an important key for their efficient migration. In order to investigate the growth stages, in which hatchlings keep their willingness to orientate toward the direction of surface wave approach, we conducted a preliminarily wave tank experiment using green turtle (Chelonia mydas) in their initial growth stages. The results of the preliminary experiment indicated that the green turtle keep the response to the wave until 2 weeks of age. We need to continue the experiment hereafter.

KEYWORDS: Chelonia mydas, headstarting, wave orientation

INTRODUCTION
A lot of the activities for conservation and population enhancement of sea turtles have been made around the world. Head-starting is the one of these activities with the practice of growing hatchlings in captivity and then releasing to the sea, in order to protect them from the high rates of natural predation that would have otherwise occurred in their initial growth stage (Mortimer 1995). After the emergence, sea turtle hatchlings crawl dawn to the ocean, and swim rapidly away from shore (Lohmann et al. 1997). After reaching the offshore, most turtles subsequently undertake a mostly passive, denatant migration drifting pelagically in oceanic gyre systems (Musick & Limpus 1997). In order to introduce the head-started turtles into the natural populations, therefore, the turtles released from shore should experience passive drifting on the same route as wild hatchlings.

During offshore migration, hatchlings use refracted waves as an orientation cue to go straight to the open sea (Salmon and Lohmann 1989, Lohmann and Lohmann 1992, Lohmann et al. 1995). This conditioned response to the wave results in movement away from land towards the open sea, because waves and swells entering shallow coastal areas are refracted until they approach a beach directly (Lohmann & Lohmann 1996). Therefore, it is considered that the ability of orientation to the wave is an important key for their efficient migration. However, do head-started turtles, which spend time in a tank until the release, keep the ability of wave orientation? The lack of this ability would bring negative results for their survival after release such as the increase of predation rate or the wrong migration to different destinations from that of wild hatchlings.

Therefore, the preliminary study was conducted to investigate the ontogenetic change of wave orientation of green turtle (Chelonia mydas) in the initial growth stages by wave tank experiment.

MATERIALS AND METHODS
Experimental turtles
Eggs of green turtles laid at Ibaruma beach located in the northeastern part of Ishigaki Island, Japan were collected. These eggs were carried to Yaeyama Station, Seikai National Fisheries Research Institute, Fisheries Agency, and then implanted into an artificial beach at this station. About 90 % of them hatched regularly. After the emergence, the turtles were reared for each experimental period (0 to 56 days) in containers. Experimental periods were 0 day, 1-3 days, about 1 week, about 2 weeks, about 1 month and about 2 months. From the third day, we started to feed them once a day. Diets were mixtures of anchovies, mysids, and clams which were supplemented with vitamins and calcium. Active and healthy looking turtles were selected from the reared ones and were employed as experimental turtles. The sample number, mean straight carapace length (SCL) and mean weight of turtles are summarized in Table 1. After the experiments, all turtles were collected and
Table 1 Summary of the sample number and physical information of turtles in each experiment.

<table>
<thead>
<tr>
<th>Reared period</th>
<th>Experiment</th>
<th>n</th>
<th>SCL (cm)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>Wave</td>
<td>6</td>
<td>5.0 ± 0.2</td>
<td>25.9 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6</td>
<td>5.0 ± 0.2</td>
<td>27.4 ± 4.0</td>
</tr>
<tr>
<td>1-3 days</td>
<td>Wave</td>
<td>9</td>
<td>4.9 ± 0.2</td>
<td>25.9 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9</td>
<td>4.9 ± 0.3</td>
<td>24.8 ± 3.7</td>
</tr>
<tr>
<td>ca.1 week</td>
<td>Wave</td>
<td>6</td>
<td>5.0 ± 0.2</td>
<td>26.9 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5</td>
<td>5.1 ± 0.2</td>
<td>27.1 ± 2.8</td>
</tr>
<tr>
<td>ca.2 week</td>
<td>Wave</td>
<td>5</td>
<td>5.7 ± 0.2</td>
<td>36.2 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4</td>
<td>5.7 ± 0.1</td>
<td>36.7 ± 2.8</td>
</tr>
<tr>
<td>ca.1 month</td>
<td>Wave</td>
<td>12</td>
<td>6.6 ± 0.2</td>
<td>52.6 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13</td>
<td>6.6 ± 0.3</td>
<td>50.2 ± 3.6</td>
</tr>
<tr>
<td>ca.2 months</td>
<td>Wave</td>
<td>9</td>
<td>7.6 ± 0.7</td>
<td>78.1 ± 15.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>7.8 ± 0.6</td>
<td>79.5 ± 11.7</td>
</tr>
</tbody>
</table>

then re-released at the Ibaruma beach.

**Experimental apparatus**

In order to investigate the wave orientation ability of turtles, we made a wave tank (Fig.1). Waves were generated by the rhythmic movement of a paddle at the end of the tank. This paddle was driven by a DC motor. A sloping platform at the opposite end of the tank dissipated wave energy and minimized wave reflection. Thus, waves moved through the tank only in this direction. To measure orientation, we fitted each turtle with a lever arm using a small fishing hook and nylon line, but it did not impede swimming movements. The lever arm was free to rotate in any direction and could easily be pulled by a swimming turtle. A data logger recorded the swimming direction of turtles every 1 s. via rotation arm and rotary encoder. To make a complete darkness condition, we used blackout curtain which covered the whole tank. Thus, they could not detect the light, using only waves as a orientation cue.

**Experiment design**

The wave was designed to move from the south (exactly, 172 degree). We conducted 2 types of experiments as a wave experiment and a control one. Each experiment consisted of 3 periods. In the first period, we exposed the turtles to still water for 30 min. in order to measure the orientation before the experiment. However, the turtles in the 0 day group did not have an initial still water period, because they had just hatched. Next, the wave tank was immediately turned on so that waves approached the turtle, and the turtle was allowed to swim into the waves for 60 minutes. After this, the wave tank motor was turned off. The surface of the water became still within a few minutes. Then, the orientation of each hatching was monitored for an additional 30 minutes as it swam in still water and complete darkness. In the control experiment, we exposed turtles to the noise from the wave motor, but not to the waves (the wave paddle was disconnected from the motor). By this experiment, we can conclude that the turtles orientated to the direction of wave approach, not to motor noise.

**RESULTS**

In the wave experiment, the turtles in the 0 day, 1-3 days and 1 week groups exposed to waves in complete darkness significantly oriented to the direction of wave approach (Rayleigh test, all case $P < 0.05$, Fig. 2 a, b, c), and subsequently when tested in still water (third period), they kept swimming towards the same direction significantly (Rayleigh test, all case $P < 0.05$, Fig. 2 a, b, c). As for the turtles reared for 2 weeks and 1 month, significantly orientation was not observed, but the mean orientation angles of these groups were similar to the direction of wave approach (Fig. 2 d, e). Also, the turtles in the subsequent still water period seemed to keep the same heading direction to that in the wave period as well as the turtles for 0 day, 1-3 days and 1 week. The turtles reared for two months kept the heading direction in the previous period rather than orientating to the wave direction (Fig. 2 f). In the first period (Fig.2 b, c, d, e, f), the turtles tended to orient towards north to east, except for those in 1-3 days group.

The turtles that experienced motor noise, but no wave motion, were not significantly oriented to the wave in all of groups (Fig.2 b, c, d, e, f). Interestingly, in the still water period including the motor noise period, the turtles in all periods of all of the groups tend to orient to around the northeast (Fig.2 a, b, c, d, e, f) and sometimes significantly. (Rayleigh test, Fig. 2b, c, f, all case $P < 0.05$), which indicated that the turtles did not change their heading in the experimental condition of no wave and
Fig. 2 The orientation angle of turtles reared for 0 day to about 2 months in three periods of the wave and motor noise experiments. The dots indicate the mean orientation angle of each turtle. The arrows indicate the mean orientation angle in each group. The length of the arrow represents the intensity (r) of orientation in each group. Black arrows represent the turtles significantly oriented, and grays not significantly. White triangles indicate the direction of wave approach or motor noise. NS means “not significant”, and NES means “not enough sample”.
complete darkness. Moreover, this direction corresponded to that in the first period of the wave experiment.

**DISCUSSION**

A significant orientation of the turtles to the wave occurred in the growth stages from emergence to 1 week. In the subsequent growth stages from 2 weeks to 1 month, the turtles tended to, but not significantly orientate to around the direction of wave approach. In the stage of 2 months, the turtles did not orientate to the wave, and kept the heading direction of the previous period. These results demonstrated that the conditioned response of the wave orientation would occur until the 1 week growth stage, and then would attenuate gradually by the 2 months growth stage.

In the experimental condition of no wave and complete darkness, turtles reared for over 1 day tended to orient to the around northeast. The result of this study was unable to clarify this reason. But the possible reasons are the environments of experimental tank and rearing tank. The experimental tank might have some cue to draw the turtle to the northeast, although turtles orientate to the approach direction when the wave comes because the wave motion seems to be stronger to attract the turtles than some other cue. Otherwise, the environment of rearing tank might have some cue. In this study, the rearing condition of the experimental turtles (e.g. light, water flow in the tank) was not uniform or completely controlled. Sea turtle hatchlings were known to use the light (Mrosovsky and Shettleworth 1968, 1975, Witherington and Bjorndal 1991) and wave motion (Salmon and Lohmann 1989, Lohmann and Lohmann 1992, Lohmann et al. 1995) as an orientation cue. Therefore, sun light and water flow in the tank might have influenced the orientation angle in the experiment.

For the turtles reared for 0 day to 1 week which oriented to the wave, in the subsequent period, in still water, they oriented to the same direction. Additionally, the turtles which did not experience wave motion also tended to maintain their heading to the same direction during all three periods of the experiment. These establishments of their swimming course were reported in the previous field studies (Frick 1976, Salmon & Wyneken 1987). Also, the laboratory experiment using orientation arena demonstrated that sea turtle hatchlings memorized their heading direction and kept it using their geomagnetic compass, even in the still water and complete darkness, once they orientated to a direction by light or wave (Lohmann 1991, Light et al. 1993, Goff et al. 1998). In this study, therefore, the turtles would maintain their heading using their geomagnetic compass. Moreover, these results of this study indicated that the course establishment of turtles was sustained at least until 2 months growth stage. This might relate to the passive migration in the open sea.

In this study, the number of samples was varied in each experimental group and the wave was exposed to the turtles only from south. For theoretical demonstration of wave orientation, we have to expose the turtles to the wave from at least two directions. In order to demonstrate the ontogenetic change of wave orientation, therefore, we should conduct more experiments which employ waves from another direction. In the near future, we will do, and hope to demonstrate the ontogenetic change of wave orientation of sea turtles.

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