*Journal of Herpetology*, Vol. 42, No. 4, pp. 615–625, 2008

# Male Salamanders *Hynobius leechii* Respond to Water Vibrations via the Mechanosensory Lateral Line System

DAESIK PARK,[1,2] JUNG-HYUN LEE,[3] NAM-YONG RA,[3] AND JUNHO EOM[1]

[1]*Division of Science Education, Kangwon National University, Chuncheon, Kangwon 200-701 South Korea*
[3]*Division of Life Science, Kangwon National University, Chuncheon, Kangwon 200-701 South Korea*

**ABSTRACT.—To test the hypothesis that male Korean salamanders, *Hynobius leechii*, respond to water vibrations via the mechanosensory lateral line system, we conducted a series of experiments. First, we examined behavioral responses of males to 0, 0.5, 1.0, and 1.5 Hz water vibrations generated by a model salamander, and we measured the number of times the males (1) oriented their heads toward the vibrating model; (2) approached within a 15-cm–diameter circle centered on the cloaca of the model; and (3) touched the model, as well as (4) the length of time the male stayed within the circle. To determine whether the mechanosensory lateral line system mediated these responses, we measured the same behavioral responses to 1.0 Hz water vibrations (1) both with and without a transparent vibration blocker placed between the model and test males, and (2) after exposing the test males to 0, 0.1, 0.5, and 1.0 mM concentrations of cobalt chloride for 1 h to disrupt the mechanosensory lateral line system. Test males showed significant responses to water vibrations from the model regardless of the vibration frequencies. Males showed significantly lower responses when a vibration blocker was placed and after cobalt chloride treatments. These results indicate that *H. leechii* males respond to water vibrations via the mechanosensory lateral line system. In addition, we describe aspects of the body undulation of mating males. This is the first clear result in urodeles that the mechanosensory lateral line system plays a role in male-male mating competition.**

Vibration signals play an important role in the daily lives of many animals, being involved in self-, individual, and species recognition, foraging, and reproduction (Vogel and Bleckmann, 1997; Fritzsch and Neary, 1998; Baker and Montgomery, 1999; Hill, 2001). Although most aquatic urodele amphibians have a well-developed mechanosensory lateral line system to detect vibrational signals (Lannoo, 1987; Fritzsch and Neary, 1998), the function of these signals in reproduction is not well understood.

*Hynobius leechii*, like all hynobiids, has external fertilization (Salthe, 1967; Houck and Arnold, 2003). Fertilization is preceded by a number of courtship displays (e.g., snout contact, smelling, tail undulation, chin rubbing, insemination, postinsemination; Park and Park, 2000). *Hynobius leechii* show two different types of tail undulations, one for mating display to the female or male and another for aggressive display to rival males. During mating tail undulation, a male stiffens his body intermittently so that his tail and the hind part of his body move from side to side. Previous studies have termed this display tail vibration, waving behavior, or tail undulation (Usuda, 1993; Park et al., 1996). Hereafter, we will refer to this tail undulation during mating as ''body undulation.'' During the second type of tail undulation, used for aggression, a male waves only the tip of his tail so that the distal third of the tail waves, but the hind part of the body does not move. Males often display this tail undulation before and after attacking other males (Park et al., 1996).

When close to oviposition, *Hynobius* females often approach a male who is defending an appropriate spot for oviposition such as twigs or small rocks, or they approach a site where several males frequently compete for occupancy (Sato, 1992). While a *H. leechii* female attaches her egg sacs to substrates such as twigs after arriving at these sites, several males usually compete to fertilize the eggs, resulting in the formation of a mating ball (Park and Park, 2000). In the field, male *Hynobius nebulosus* who defended oviposition sites fertilize more than 50% of total egg sacs deposited (Usuda, 1997). If a male occupies a ''hot spot'' for female oviposition and effectively succeeds in attracting females to the site, it may increase his reproductive success. Thus, males compete to occupy such sites to gain the exclusive position as a territory-holder. Males without such a territory may try to locate the hot spots of territorial males and intercept an approaching female who is attracted by signals from territorial males.

We expect that, in *Hynobius*, chemical and vibrational signals play a role in this process

---

[2] Corresponding Author. E-mail: parkda@kangwon.ac.kr

since they usually mate in low light (Sato and Iwasawa, 1993). The possible involvement of chemical cues was suggested by several studies: Kim (2000) showed that female *H. leechii* respond to male odors in a Y-maze experiment; Park and Sung (2006) found female odors attract male salamanders; and Hasumi (1996) proposed the possible role of cloacal secretions of *Hynobius nigrescens* females in attracting conspecific males based on histological studies. Meanwhile, several previous studies have suggested that vibrational signals may be involved in these processes. Body undulation generates water vibrations in the form of surface waves during mating and has been reported to be important for several different species, including *Hynobius dunni* (Mashiba, 1969) and *H. nebulosus* (Tanaka, 1987). Male *Hynobius takedai* respond to water vibrations generated by a swinging glass rod on the water surface (Tanaka, 1987). In the field and in the laboratory, male *H. nigrescens* also respond to water vibrations generated by body undulations of other males and by a swinging glass rod on the water surface (Usuda, 1995). Nevertheless, there are several outstanding questions about whether *Hynobius* males directly respond to water vibrations via the mechanosensory lateral line system. First, in the previous studies, the frequency of water vibrations may have been varied since the researchers directly swung a glass rod on the water surface. Second, the shadow or visual cues of the hand or human body that swung the rod may have interfered with the experimental results. Third, chemical cues in the experiments were not controlled. Fourth, those studies did not elucidate whether the mechanosensory lateral line system mediated the responses by chemically disrupting the function of the system. In the present study, we tested the hypothesis that *H. leechii* males respond to water vibrations via the mechanosensory lateral line system by conducting a series of experiments, and additionally, we investigated the nature of the body undulation of mating males.

## MATERIALS AND METHODS

*Animal Collection and Housing.*—Salamanders used in this study were collected using a hand net from the mountain streams located at Cheongwoon-myeon, Yangpyeong-gun, Kyeongki-do (37°31′56.6″N, 127°42′16.52″E) and at Gaduck-myeon, Cheongwon-gun, Chungbuk-do (36°33′09.8″N, 127°35′18.0″E), South Korea. Thirteen females plus 104 males, and 11 females plus 32 males were collected from 10–13 March 2005. We kept the salamanders in refrigerated boxes (5–10°C; 55 × 35 cm and 35 cm high) to transport them to the laboratory. Upon arrival at the laboratory, each individual was sexed based on swelling of the cloacae (Park and Park, 2000). We toe clipped all the males for individual identification after anesthesia with 0.1% tricaine methanesulfonate (MS222, Sigma; Donnelly et al., 1994) and preserved the toes obtained in 10% formalin for a skeletochronology study. We cut the first two digits of one or two toes using sterilized scissors and applied antiseptic solutions (Povidone iodine topical solution, Green Pharmacy Co., Korea) to the digits to reduce the probability of infection. No bleeding occurred during the toe clipping and all individuals successfully recovered from the anesthesia.

Males and females were kept in separate aquaria (for males: 100 cm long, 74 cm wide, 35 cm high; and for females: 33 cm long, 20 cm wide, 25 cm high) containing approximately 50 or 25 liters of aged tap water at a density of no more than 50 males and 10 females per tank. Water in the aquaria containing males was circulated in the aquaria by a pump (PB-38E-D, Hanil Co., Hwasung, Korea). Half of the water for each aquarium was changed weekly. The water temperature of the aquaria was maintained between 8 and 10°C by a cooler (DHI-150, Daeho, Namyangju, Korea), and the photoperiod was modeled after the local photoperiod of approximately 12 : 12 L : D. Hiding places were provided in the form of wet paper towels on the rocks and pieces of broken pottery that were placed on the bottom of the aquaria. Salamanders were fed freshwater amphipod (*Gammarus*) and chopped earth worms ad libitum. All males used in this study were in full breeding condition, as evidenced by swollen cloacae and wide tailfins.

No mortality occurred during this study except for four salamanders sacrificed and used for the scanning electron microscope (SEM) study in experiment III. All salamanders used in the study were released in good health to the same streams where we captured them after the experiments in mid-April 2005. This study was approved by the Korean government. All other experimental procedures followed the guidelines for the use of live amphibians and reptiles in field and laboratory research (ASIH, 2004).

*Experiment I.*—We tested the hypothesis that male salamanders respond to water vibrations generated by a model salamander. The model salamander (total length 13.15 cm, SVL 7.7 cm, body mass 12.5 g) was similar to a real one (total length mean ± SD = 13.21 ± 0.83 cm, SVL 7.25 ± 0.35 cm, body mass 9.83 ± 1.65 g, $N$ = 19). It was made with additional modifications to an existing plastic salamander model (Safari Ltd., USA). The bottom of an experimental aquarium (48 cm long, 27 cm wide, 30 cm high) was covered with sand to a depth of 3 cm and

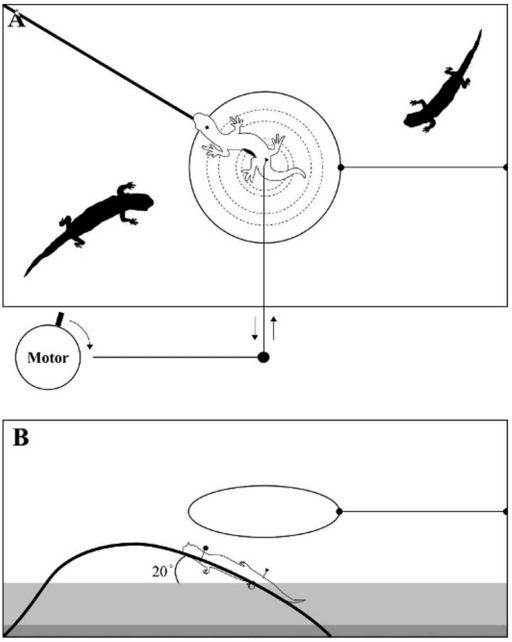

FIG. 1. Diagram of the stimulation setup: aerial view (A) and lateral view (B). Water vibrations were generated from the cloacal region of the model salamander by repeating horizontal intension and extension of a fishing line, connected between the middle of the tail and a motor controller.

filled with aged tap water to a depth of 4.5 cm. An inverse V-shaped twig of wood (33 cm long, 1 cm diameter) was placed from the center to a corner of the aquarium (Fig. 1). Approximately half the twig was above the water surface and the other half was submerged. Males prefer to conduct body undulations at the twig placed along the water surface where a female could easily attach her egg sacs (Usuda, 1993). We placed the model salamander on the twig with a screw placed on the head (Fig. 1). Parts of the main body including the head were outside the water, but the abdomen and tail were submerged. The angle of the body axis of the model salamander to the water surface was about 20° (Fig. 1). Water vibrations were generated from the cloacal region of the model salamander by repeated horizontal intension and extension of a transparent fishing line toward a wall of the aquarium (Fig. 1). The line was extended between a pin nailed at the middle of the model's tail and a hand-made frequency controller (Fig. 1). The controller consisted of various gears and a controllable DC power supply (PWS-30050, Protek, Seoul) to generate different frequencies of water vibrations. In this experiment, we used four different frequencies of water vibrations generated by the model based on a previous study (Usuda, 1995), and these were slightly varied as 0 Hz, 0.5 ± 0.07 Hz SD ($N = 10$), 1 ± 0.03 Hz SD ($N = 10$), 1.5 ± 0.07 Hz SD ($N = 10$). The water vibrations generated by the model were similar to those generated by real salamanders who displayed body undulations on tree twigs or on rocks, which we observed during our laboratory study (Park and Park, 2000). Although the walls of the test aquarium generated minor secondary reflections of water vibrations from the model, we did not attach any antivibration materials to the walls because test salamanders crawled up the attached materials.

For each trial, we used two test males with SVL (snout–vent length) and body masses that differed by 0.5 cm and 1.0 g, respectively. During our previous mating studies, two different sized males showed higher mating activities such as actively chasing females and displaying body undulations as compared to one male or two same-sized males (Park and Park, 2000). Two males were randomly selected from a pool of 136 males. Each male was used only once for a specific frequency experiment. Males who were reused in another frequency experiment were given at least two days of rest. In our previous olfactory and mating studies of this species, two days of rest was enough to obtain robust experimental data (Park et al., 2004; Park and Sung, 2006). The same pairing of males was never used twice in our experiments.

Experiments were executed under 0.1 lux light (YL102, UINS, Seoul) between 1900 and 0100 h from 14–26 March 2005. In the field, *Hynobius* salamanders show the highest levels of mating activity between 2100 and 0000 h (Sato and Iwasawa, 1993). In this experiment, 17 trials (34 test males) for four different frequencies of water vibrations (total of 136 males in 68 trials) were completed. A trial was started with a randomly selected frequency vibration after two test males released at a corner of the aquarium were given a 5-min acclimation period in the experimental aquarium without any vibration stimuli. During this adaptation period, we did not restrict their movements so that several males visited the twig and model salamander. All responses of the test males were monitored by a low-light waterproof B/W camera (Model: 10IR LED, SLCC), displayed on a screen and stored on a video recorder (Daewoo) placed in a remote area, 2 m away from the aquarium. Two investigators directly recorded behavioral responses of the two test males on the screen during a 5-min experimental period when a vibration stimulus was given, and the results were later confirmed using videotape by two experimentally blind observers. If the score differed between direct observation and videotape observations, we used the score obtained by analyzing the videotape. After each test, we washed the sand using tap water and changed the water to prevent possible interference by chemical cues.

We measured the behavioral response of two test individuals using the number of times that individuals (1) oriented their head toward the vibrating model; (2) approached within a 15-cm circle centered at the cloaca of the model; and (3) touched the model; and we also measured (4) the length of time the individual stayed within the circle. Head-orientation was identified when a test male suddenly turned his head toward the vibrating model at more than a 45° angle from the line of his body axis. The behavior was easy to determine because the orientation behavior was distinctive. To measure the approach within the circle, we placed a wire circle 15 cm in diameter, which was horizontally fixed to the wall of the aquarium approximately 5 cm above the model and centered on the cloaca of the model. The 15-cm diameter was arbitrarily selected to be about two times the length of the salamanders' SVLs because we thought that a 7.5-cm distance is sufficient for two salamanders to take notice of each other. An approach within the circle was considered to occur when the snout of a test male entered into the circle. It is known that many lateral line receptors are distributed on the snout of salamanders (Lannoo, 1987). The staying time was evaluated as the time between the entry of the snout of a test male into the circle and the time at which the snout of the test male leaves the circle. If a test male was within the circle at the beginning of a trial, the staying time was immediately measured from the start of the trial. This occurred in six of 170 total trials during the whole study. Touching the model was defined when the snout of a test male touched the model salamander.

*Experiment II.*—To test the hypothesis that the mechanosensory lateral line system mediates the behavioral response of males to water vibrations detected in experiment I, we repeated parts of experiment I and blocked water currents with a rectangular transparent acryl box blocker (13 cm long, 6 cm wide, 5 cm high; thickness 1.5 mm). The box blocker surrounded the model salamander with an inner distance of approximately 1 cm between the blocker and the model, and the bottom of the box blocker was buried in the sand to prevent the generation of water currents from the box. Small secondary reflections in water vibrations were generated by the acryl box during the experiment, but the currents did not extend more than 2–3 cm from the box (pers. obs.).

We observed and recorded behavioral responses of 68 test males in 34 trials to 1.0 Hz water vibrations for a 5-min experimental period, during which each trial was randomly performed either with or without the box blocker. Behavioral responses of individuals were measured as follows: the number of (1) head-orientations toward the model and (2) approaches within the 15-cm diameter circle centered at the cloaca of the model. We selected a 1.0 Hz frequency for water vibration because it is close to the frequency of body undulations evoked by mating males (see experiment IV). In this experiment, we did not measure the staying time within the circle and the number of touches with the model because the box blocker significantly reduced inner distances within the circle and did not allow test males to directly touch the model.

*Experiment III.*—To further test the hypothesis that the mechanosensory lateral line system mediates the behavioral response of males to water vibrations, we repeated experiment I after exposing test males to cobalt chloride in a manner similar to Karlsen and Sand (1987). Test males were exposed to 0, 0.1, 0.5, and 1.0 mM concentrations of cobalt chloride ($Co[II]Cl_26H_2O$, Sigma) dissolved in $Ca^{2+}$-free artificial water (in g/ liter distilled water, NaCl 4, KCl 0.2, $Na_2HPO_4$ 0.015, $KH_2PO_4$ 0.03, $MgSO_4$ 1, pH 7.5 adjusted using NaOH) based on studies of Himé Salmon, *Oncorhynchus nerka*, and *Xenopus laevis* tadpoles (Satou et al., 1994a; Simmons et al., 2004). Cobalt chloride treatment is known to disrupt the mechanosensory lateral line system in fish and amphibians (Baker and Montgomery, 1999; Simmons et al., 2004). During the exposure, we could not observe any obvious abnormal behaviors such as fast swimming or swimming at the water surface (Janssen, 2000). In the tests, we used $Ca^{2+}$-free artificial water (in g/ liter distilled water, NaCl 0.2, KCl 0.025, $Na_2HPO_4$ 0.05, $KNO_3$ 0.05, $MgSO_4$ 0.1), made according to the methods of Karlsen and Sand (1987). Prior to the experiment, we exposed each test individual to cobalt chloride solutions for 1 h in an individual container and then washed the animal for 2 min in fresh artificial water. Because only one big male was exposed to cobalt chloride, we only measured the behavioral responses of the big males to 1.0 Hz water vibrations from a model salamander. However, we placed two different sized test males in an experimental aquarium throughout the experiment. We only exposed one big male to cobalt chloride because we thought the results from one male exposure experiment could be enough to determine whether the mechanosensory lateral line system is responsible for the behavioral response of males to water currents. The behavioral response parameters measured in 17 trials (17 test males) were the same as in experiment I. In this experiment, no mortality occurred following cobalt chloride treatments and all individuals, except four individuals used for the SEM study, fully recovered from the cobalt chloride exposure after two weeks based on their active feeding behaviors and general activities in the stock aquaria, although we did not directly confirm the recovery of damaged lateral line receptors.

In addition, we confirmed whether cobalt chloride exposure disrupts the mechanosensory lateral line system under SEM. Because the nature of the mechanosensory lateral line system of adult *H. leechii* was not known when we conducted this experiment, we arbitrarily selected one individual from each experimental group and compared the conditions of their neuromasts. The four individuals were sacrificed by an overdose of 0.1% MS222 (Sigma), and they were decapitated with a scalpel. The head skin was removed quickly and preserved in 4% glutaraldehyde (Sigma) dissolved in PBS buffer (pH 7.2). The prepared skin tissues were washed two times using 0.1 M cacodylate buffer (pH 7.4) and serially dehydrated in 50, 70, 90, 100, 100% ethanol for 30 min each. Afterward, the media was exchanged with isoamyl acetate two times for 30 min each. The tissues were dried using a critical point dry method, mounted on the specimen stub, coated using Au-Pd, and then observed under the S-3500N Low-vacuum SEM (Hitachi) at the Korea Basic Science Institute, Chuncheon, Kangwon. We only obtained observable results from the individuals exposed to 0 and 1.0 mM cobalt chloride and compared conditions of the neuromasts in the infra-orbital neuromast line (Northcutt, 1992). A recently published abstract showed that *H. leechii* larvae and adults have well-developed neuromasts, pit organs, and ampullary organs (E. S. Choi, J. H. Lee, and D. Park, Morphological Characteristics of the Mechanosensory Receptors of *Hynobius leechii* [Hynobiidae, Urodela], 45th Annual Meeting of the Herpetological Society of Japan, Hisroshima, Japan, 2006).

*Experiment IV.*—To elucidate the nature of male body undulations, we analyzed mating behaviors of seven successful salamander pairs recorded from 16–24 March 2005. To provide proper mating circumstances, we covered the bottom of a mating aquarium (33 cm long, 20 cm wide, 25 cm high) with a layer of sand to about 3-cm depth and placed an inverse V-shaped twig (42 cm long, 1 cm diameter) between the diagonally located two corners of the experimental aquarium to provide egg-attachment sites. Approximately half the twig at the center was 1–2 cm above the water surface and the other half at each corner was submerged. Tanks were filled with aged tap water to a depth of about 4.5 cm. At 2200 h, we placed a female with two males whose SVLs differed by more than 0.5 cm to facilitate mating processes following a previous study (Park and Park, 2000). Mating occurred under $< 0.1$ lux light and were recorded with a low-light waterproof B/W camera (Model: 10IR LED, SLCC) and a video recorder (Daewoo) until 0900 h the following morning.

Seven successful mating events up to the completion of egg fertilization occurred. We arbitrarily selected one body undulation display both before (mean ± SD = 67.66 ± 46.91 min, $N = 7$) and after (about 10 min) the female's oviposition from each mating case during our analysis. We obtained data for a body undulation display only when body stiffening during a body undulation occurred at least 10 times. We recorded the number and period of each body undulation on the screen by analyzing the videotapes. We also recorded the number of (1) head-orientations toward the undulating male, (2) approaches within a 10-cm circle centered at the cloaca of the undulating male, (3) touching of the undulating male. and (4) the staying time within the circle of another male placed in the mating aquarium if other males responded to the undulating male. Because the aquaria were relatively small, we used a smaller 10-cm circle to measure the number of approaches and staying time response of the males. Physical conditions of the mating males, including SVL, body mass, head length, head width, and tail width, were obtained when the mating event was completed.

*Statistics.*—To determine whether different frequencies of water vibrations evoked behavioral responses from a different number of test males in response parameters of head-orientation toward the vibrating model, approach within the 15-cm circle, and touching of the vibrating model, we used the chi-square test. If the difference was significant, the chi-square test was used again to compare the differences between each group. In those tests, differences were considered to be significant at the 0.044 level after Bonferroni's correction. The differences in the times of responses during each experiment were analyzed by the Kruskal-Wallis test or Mann-Whitney *U*-test based on the number of groups compared, because such data did not pass the normality test (Kolmogorov-Smirnov test, $P < 0.05$). If the difference was significant in the Kruskal-Wallis test, we conducted a post hoc test at the 0.05 level, following the method by Siegel and Castellan (1988).

To determine relationships among the number, period, and frequency of body undulations of mating males and their physical conditions, we used a Pearson Correlation analysis. Because the times of body undulations and the staying time within a 10-cm circle before and after female's oviposition met the normality (Kolmogorov Smirnov test, $P > 0.05$) and equal variance (Levene's Test, $P > 0.05$) assumptions, we used a paired sample *t*-test to analyze the data. All statistical analyses were two-tailed and performed using SPSS version 11.0 (SPSS Inc., Chicago, IL). All values are reported with mean ± SE.

## RESULTS

*Experiment I.*—The number of test males that responded to water vibrations of different frequencies was significantly different in each measured parameter of (1) orientation toward a vibrating model ($X^2_3 = 37.91$, $P < 0.001$); (2) approach within a 15-cm circle ($X^2_3 = 9.57$, $P = 0.023$); (3) and touching the model ($X^2_3 = 18.55$, $P < 0.001$, Table 1, experiment I). In particular, the number of test males that showed head orientation and approached and touched the vibrating model during the three different frequency vibrations was significantly larger than the number of males who performed these activities in response to the control stimulus (chi-square test, $P < 0.044$ for the cases). All other comparisons among different frequency groups were not significant (chi-square test, $P > 0.044$ for all cases). The response times in the head orientation toward ($H_3 = 21.35$, $P < 0.001$) and the touching the model ($H_3 = 7.95$, $P = 0.047$) were significantly different at different frequency vibrations, whereas the response times in the approach within a 15-cm circle ($H_3 = 5.40$, $P = 0.145$) and the staying time within the circle ($H_3 = 2.25$, $P = 0.522$) were not different (Table 1, experiment I). In particular, the response times in the head orientation toward the model to the three different frequency vibrations were significantly larger than those that responded to the control stimulus (post hoc test, $P < 0.05$ for the cases). All other comparisons among different frequency groups were not significant (post hoc test, $P > 0.05$ for the cases).

*Experiment II.*—The behaviors of head orientation ($X^2_1 = 24.01$, $P < 0.001$) and approach ($X^2_1 = 16.29$, $P < 0.001$) of test males were significantly lower when the water currents were blocked (Table 1, experiment II). Moreover, the response times in both parameters were significantly different between the presence and absence of a vibration blocker ($U = 325$, $N_1 = N_2 = 34$, $P < 0.001$ for head orientation; $U = 373.5$, $N_1 = N_2 = 34$, $P = 0.004$ for the approach, Table 1, experiment II).

*Experiment III.*—After being exposed to different concentrations of cobalt chloride, which blocked lateral line mechanoreceptors, the number of test males that responded to 1.0 Hz water vibrations varied significantly in each measured parameter, including head orientations toward a vibrating model ($X^2_3 = 159.35$, $P < 0.001$), approach within a 15-cm circle ($X^2_3 = 117.27$, $P < 0.001$), and touching the vibrating model ($X^2_3 = 123.54$, $P < 0.001$, Table 1, experiment III). In particular, the number of test males in all measured parameters after exposure to three different cobalt chloride concentrations was significantly lower than that of test males responding to the control (chi-square test, $P < 0.044$ for the cases). The response times of head orientation toward the vibrating model were significantly different with different concentrations of cobalt chloride ($H_3 = 41.58$, $P < 0.001$, Table 1, experiment III). In particular, the response times in head orientation toward the model after exposure to the three cobalt chloride concentrations were significantly smaller than those that responded to the control stimulus (post hoc test, $P < 0.05$ for the cases). All other comparisons among different cobalt chloride concentration groups were not significant (post hoc test, $P > 0.05$ for the cases). We did not analyze the response times of the approaching the model and touching the model because certain cobalt chloride concentrations completely disrupted individual responses (Table 1, experiment III). The hair cells of neuromasts of a test male exposed to 1.0 mM cobalt chloride were damaged, unlike those of a control male (Fig. 2).

*Experiment IV.*—Male salamanders conducted a mean of 30.14 ± 6.77 (range: 10–67, $N = 14$) body undulations over a mean time of 42.29 ± 7.02 sec (range: 16–89, $N = 14$) per body undulation display. The mean frequency of body undulations was 0.71 ± 0.08 Hz (range: 0.33–1.20, $N = 14$). The frequency of body undulation was positively correlated with the number of body undulations per display ($r = 0.57$, $N = 14$, $P = 0.035$) but not with the period of undulating ($r = 0.06$, $N = 14$, $P = 0.848$). Males that did not undulate responded to the water vibrations generated by undulating males in six of seven mating cases both before and after female oviposition, with head orientation toward an undulating male and approach to the undulating male. Four responding males touched the undulating male before female oviposition, whereas only one male touched the undulating male after female oviposition. The frequency of body undulation was significantly higher before female oviposition (0.86 ±

TABLE 1. Summary of experiments I, II, and III. Behavioral responses of test males to different frequencies of water vibrations generated by a model salamander were measured (experiment I). The same behavioral responses to 1.0 Hz water vibrations were also measured both with and without a transparent vibration blocker placed between the model and test males (experiment II) and after exposing the test males to different concentrations of cobalt chloride to disrupt the mechanosensory lateral line system (experiment III).

| | Response to water vibrations ($N$ = 34 for each group) | | | | |
|---|---|---|---|---|---|
| Experiment I | 0 Hz | 0.5 Hz | 1.0 Hz | 1.5 Hz | Statistics |
| No. of individuals head orienting | 2 | 17 | 16 | 18 | $x_3^2 = 37.91, P < 0.001$ |
| (head orientations/ind) | (0.06 ± 0.04) | (0.77 ± 0.15) | (0.77 ± 0.17) | (0.94 ± 0.18) | $H_3 = 21.35, P < 0.001$ |
| No. of individuals approached | 8 | 16 | 15 | 16 | $x_3^2 = 9.57, P = 0.023$ |
| (approaches/ind) | (0.29 ± 0.10) | (0.65 ± 0.14) | (0.59 ± 0.13) | (0.68 ± 0.16) | $H_3 = 5.40, P = 0.145$ |
| Staying time (sec) | 25.24 ± 9.78 | 39.74 ± 12.08 | 39.47 ± 12.03 | 27.26 ± 11.00 | $H_3 = 2.25, P = 0.522$ |
| No. of individuals touched | 1 | 9 | 8 | 8 | $x_3^2 = 18.55, P < 0.001$ |
| (touches/ind) | (0.03 ± 0.03) | (0.59 ± 0.22) | (0.50 ± 0.22) | (0.38 ± 0.14) | $H_3 = 7.95, P = 0.047$ |

| | Response to water vibrations ($N$ = 34 for each group) | | |
|---|---|---|---|
| Experiment II | with a blocker | without a blocker | |
| No. of individuals head orienting | 5 | 19 | $x_1^2 = 24.01, P < 0.001$ |
| (head orientations/ind) | (0.15 ± 0.06) | (0.85 ± 0.18) | $U = 325, P = < 0.001$ |
| No. of individuals approached | 7 | 19 | $x_1^2 = 16.29, P = 0.003$ |
| (approaches/ind) | (0.27 ± 0.10) | (0.79 ± 0.16) | $U = 373.5, P = 0.004$ |

| | Response to water vibrations after cobalt chloride exposure ($N$ = 17 for each group) | | | | |
|---|---|---|---|---|---|
| Experiment III | 0 mM | 0.1 mM | 0.5 mM | 1.0 mM | |
| No. of individuals head orienting | 16 | 1 | 3 | 2 | $x_3^2 = 159.35, P < 0.001$ |
| (head orientations/ind) | (1.35 ± 0.19) | (0.06 ± 0.06) | (0.18 ± 0.10) | (0.12 ± 0.08) | $H_3 = 41.58, P < 0.001$ |
| No. of individuals approached | 11 | 1 | 3 | 0 | $x_3^2 = 117.27, P < 0.001$ |
| (approaches/ind) | (0.77 ± 0.16) | (0.06 ± 0.06) | (0.18 ± 0.10) | – | – |
| Staying time (sec) | 47.71 ± 14.42 | 0.71 ± 0.71 | 7.41 ± 6.98 | – | – |
| No. of individuals touched | 7 | 0 | 0 | 0 | $x_3^2 = 123.54, P < 0.001$ |
| (touches/ind) | (0.53 ± 0.17) | | | | |

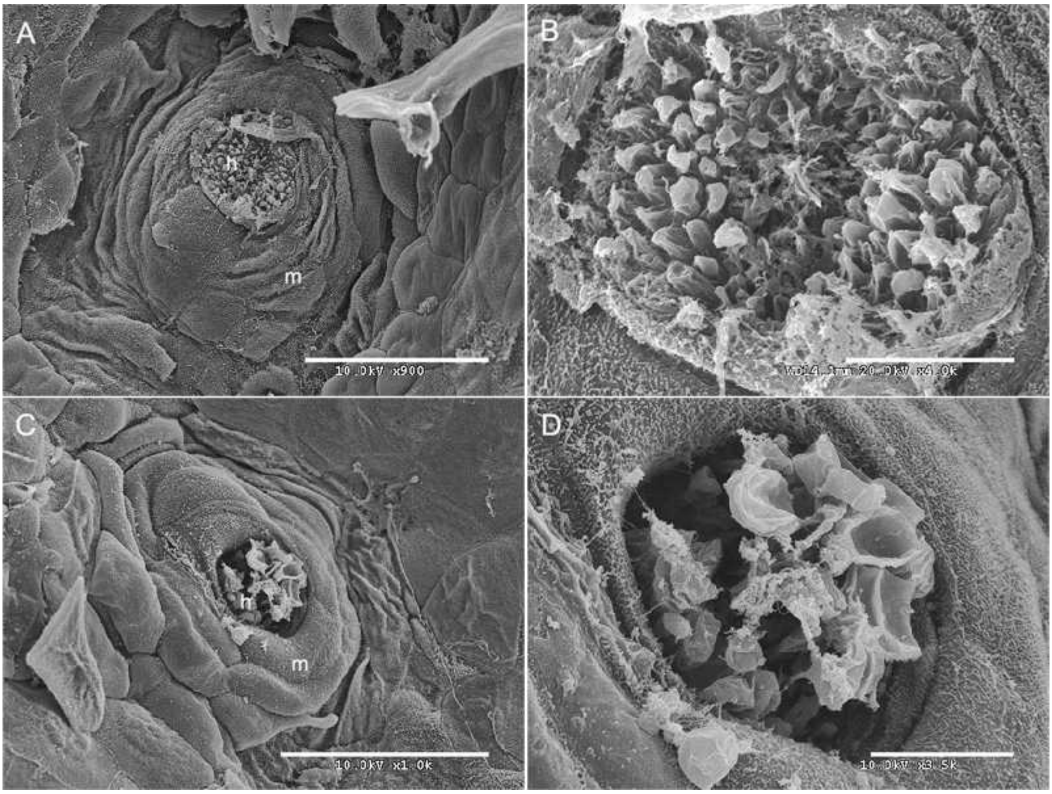

FIG. 2. Scanning electron micrographs of neuromasts under low (A, C) and high (B, D) magnification. (A, B) Intact hair cells are visible in the control group. (C, D) Exposure to 1.0 mM cobalt chloride for 1 h damaged hair cells. h; hair cell, m; mantle cell. Scale bars; 50 µm in A and C, 10 µm in B and D.

0.05) than after female oviposition (0.56 ± 0.11; $t_{12} = 2.50$, $P = 0.028$). However, the staying time of responsive males within a 10-cm diameter circle centered at the cloaca of the undulating males was not different before (22.17 ± 5.08) or after female oviposition (19.00 ± 2.96; $t_{10} = 0.54$, $P = 0.602$). The frequency of body undulation did not show significant correlation with the total length, SVL, head length, head width, tail width, and mass of males (Pearson Correlation analysis, $P > 0.05$ for all cases).

## DISCUSSION

Our results show that *H. leechii* males respond to water vibrations via the mechanosensory lateral line system and suggest that vibrational signals may play a role in male-male mating competition. This is the first clear result that urodeles use the mechanosensory lateral line system for male-male mating competition.

Male *H. leechii* responded to water vibrations via the mechanosensory lateral line system. Detection of vibrational signals via the mechanosensory lateral line system has been reported for fish (Montgomery et al., 1997; Coombs et al., 2001), arthropods (Joanidopoulos and Marwan, 1999; Maklakov et al., 2003; Virant-Doberlet and Cokl, 2004), anurans (Elepfandt et al., 1985), urodeles (Usuda, 1995), and reptiles (Soares, 2002). In our study, male salamanders showed positive responses to water vibrations generated by a model salamander and by body undulations of mating males. In most experiments, about 50% of test males oriented toward and approached the vibrating model or undulating salamanders. The rate of the response was similar to the value of 59% observed in a previous field result (Usuda, 1995). On the contrary, when we blocked water currents and disrupted the mechanosensory lateral line systems using cobalt chloride, males' responses to water vibrations were significantly decreased, indicating that the mechanosensory lateral line system is involved in the responses.

The mechanosensory lateral line system of *H. leechii* might be sensitive to the exposure of cobalt chloride. Several studies have used high concentrations of cobalt chloride to disrupt mechanosensory lateral line systems of fish and tadpoles (Montgomery et al., 1997; Simmons et al., 2004) and pointed out possible toxic effects of cobalt chloride (Janssen, 2000). For example, the Mexican Cave Fish *Astyanx fasciatus*, when exposed to 2 mM cobalt chloride for 3 h, swam faster than control fish and tended to swim at the water surface (Janssen, 2000). The lowest effective concentration previously known was 0.1 mM cobalt chloride with a 14-h exposure in the study of Himé Salmon (Satou et al., 1994a). In our study, after exposure to 1.0 mM cobalt chloride for 1 h, several male salamanders showed some awkward movements at the very beginning of the experimental trials, but none of individuals exposed to 0.1 and 0.5 mM cobalt chloride showed any behavioral abnormalities. We detected significantly reduced behavioral responses to water vibrations in all exposed groups. With exposure to 1.0 mM cobalt chloride, some neuromast cells were damaged, and this might be linked to decreased vibration responses of male salamanders. Considering previous studies regarding the disruptive effects of cobalt chloride on the mechanosensory lateral line system (Baker and Montgomery, 1999; Simmons et al., 2004), 0.1 and 0.5 mM cobalt chloride exposure may also disrupt mechanosensory lateral line receptors, depending on the dose administered. Meanwhile in this study, we only examined four individuals under SEM to determine morphological damage of lateral line receptors by cobalt chloride exposure. To further explore the relationship between reduced behavioral responses to water vibrations and damaged lateral line receptors in *H. leechii*, we need to increase our sample size for morphological observations following cobalt chloride exposure and to study the effects of various concentrations of cobalt chloride on general activities and side effects. At the moment, the results of the cobalt chloride exposure study tentatively suggest that the *H. leechii* mechanosensory lateral line system is highly sensitive to cobalt chloride exposure without side effects, and it might be directly responsible for impaired behavioral responses to water vibrations.

Why do male salamanders respond to water vibrations generated by another male? Detecting vibration signals could generally be advantageous for successful mating because they live in murky water and mate in low light in most cases. Other specific advantages can also be inferred. First, approaching a vibration source may allow the male to locate eggs if the vibrations are caused by an ovipositing female. Second, if water vibrations are generated by an undulating male who defends a territory, approaching this site may allow the male to court a female who is attracted to the site or promote competition with other males. Third, males may respond to water vibrations to disrupt the undulating male's display so that he can reduce the probability that a female directly approaches the undulating male.

In several salamanders, tail vibrations of male urodeles have been suggested to deliver chemical cues to a female mate, although clear evidence does not exist (Toyoda et al., 1993; Halliday, 1977). During courtship, the male *Triturus* often lashes his tail against his side to send a wave of water toward the female's snout (Halliday, 1977). In the Fire-Bellied Newt, *Cynops pyrrhogaster*, a male often blocks the path of a female and vigorously vibrates his tail possibly to deliver pheromones to the female (Toyoda et al., 1993). However, tail vibrations of these species are different from the body undulations described in *H. dunni* (Mashiba, 1969), *Salamandrella keyserlingii* (Takayama, 1978), and *Ambystoma mexicanum* (Park et al., 2004). The tail vibration in *Triturus* is more similar to the tail undulation of *Hynobius*, where a male waves only the tip of the tail to indicate aggression (Park et al., 1996). Also, tail vibration occurs only when a female is close to a male (Toyoda et al., 1993), but body undulations occur even when a female is more than 10 or 30 cm away from the undulating male. Although *H. leechii* females could respond to male odors (Kim, 2000), whether male body undulations deliver such chemical cues to females remains to be studied. In our study, water vibration from a model salamander itself functioned to induce behavioral response from other males, and it is highly possible that water vibrations generated by a male might deliver information about his territoriality to other males or his location to females regardless of the chemical delivery functions of body undulation.

Vibrational signals play a critical role in swimming, foraging, and predatory responses in amphibians (Fritzsch and Neary, 1998), but the involvement of these signals in reproduction is not well studied. The reproductive function of vibrational signals has been studied in rotifers, spiders, and fish. Successful reproduction of a rotifer was achieved only when both chemical and vibration cues were available (Joanidopoulos and Marwan, 1999). In spiders, vibration signals generated by males initiate mating behaviors in females (Maklakov et al., 2003). In salmon, when either the male or female vibration system is disrupted, successful mating does not occur (Satou et al., 1994b). Even though most amphibians have a well-developed mechanosensory lateral line system, the reproductive functions of such a system is not well understood. Thus, further study of behavioral connections between mating behavior and vibration displays (i.e., female behavioral responses to water vibrations, oviposition site selection based on vibration signals, and functions of the mechanosensory lateral line system on the mating success of males and females) could increase our understanding about the function of the mechanosensory lateral line system in amphibian mating.

*Acknowledgments*.—We would like to thank J.-K. Jeon for his help in the study, M.-S. Park and N.-I. Kang (KBSI-Chuncheon) for technical assistances in LV-SEM analysis, and M. Sparreboom and S. Coombs for valuable comments on earlier versions of this manuscript. This work was supported by the Korea Research Foundation Grant by the Korean Government (MOEHRD, KRF-0807015-1-1).

## LITERATURE CITED

ASIH. 2004. Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research. 2nd ed. Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, Lawrence, KS.

BAKER, C. F., AND J. C. MONTGOMERY. 1999. The sensory basis of rheotaxis in the blind Mexican Cave Fish, *Astyanax fasciatus*. Journal of Comparative Physiology A 184:519–527.

COOMBS, S., C. B. BRAUN, AND B. DONOVAN. 2001. The orienting response of Lake Michigan Mottled Sculpin is mediated by canal neuromasts. Journal of Experimental Biology 204:337–348.

DONNELLY, M. A., C. GUYER, J. E. JUTERBOCK, AND R. A. ALFORD. 1994. Techniques for marking amphibians. *In* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. A. C. Hayek, and M. S. Foster (eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, pp. 277–284. Smithsonian Institution Press, Washington, DC.

ELEPFANDT, A., B. SEILER, AND B. AICHER. 1985. Water wave frequency discrimination in Clawed Frog, *Xenopus laevis*. Journal of Comparative Physiology A 157:225–261.

FRITZSCH, B., AND T. NEARY. 1998. The octavolateralis system of mechanosensory and electrosensory organs. *In* H. Heatwole and E. M. Dawley (eds.), Amphibian Biology. Vol. 3, pp. 878–922. Surrey Beatty and Sons, Chipping Norton, New South Wales Australia.

HALLIDAY, T. R. 1977. The courtship of European newts: an evolutionary perspective. *In* O. H. Taylor, S. I. Buttman, and K. Yamamoto (eds.), The Reproductive Biology of Amphibians, pp. 185–232. Plenum, New York.

HASUMI, M. 1996. Times required for ovulation, egg sac formation, and ventral gland secretion in the salamander *Hynobius nigrescens*. Herpetologica 52:605–611.

HILL, P. S. M. 2001. Vibration and animal communication: a review. American Zoologist 41:1135–1142.

HOUCK, L. D., AND S. J. ARNOLD. 2003. Courtship and mating behavior. *In* D. M. Sever (ed.), Reproductive Biology and Phylogeny of Urodela, pp. 383–424. Science Publishers Inc., Enfield, NH.

JANSSEN, J. 2000. Toxicity of Co2+: implications for lateral line studies. Journal of Comparative Physiology A 186:957–960.

JOANIDOPOULOS, K. D., AND W. MARWAN. 1999. A combination of chemosensory and mechanosensory stimuli triggers the male mating response in the Giant Rotifer *Asplanchna sieboldi*. Ethology 105: 465–475.

KARLSEN, H. E., AND O. SAND. 1987. Selective and reversible blocking of the lateral line in freshwater fish. Journal of Experimental Biology 133:249–262.

KIM, N. G. 2000. Studies in the Sexual Selection and Reproductive Dynamics of Korean Salamander (*Hynobius leechii*). Unpubl. master's thesis, Korea National University of Education, Chungbuk Korea. [In Korean with English Abstract.]

LANNOO, M. J. 1987. Neuromast topography in urodele amphibians. Journal of Morphology 191:247–263.

MAKLAKOV, A. A., T. BILDE, AND Y. LUBIN. 2003. Vibratory courtship in a web-building spider: signaling quality or stimulating the female? Animal Behaviour 66:623–630.

MASHIBA, S. 1969. Ecology of *Hynobius dunni* Tago. Saishu to Shiiku 31:122–135. (in Japanese).

MONTGOMERY, J. C., C. F. BAKER, AND A. G. CARTON. 1997. The lateral line can mediate rheotaxis in fish. Nature 389:960–963.

NORTHCUTT, R. G. 1992. Distribution and innervation of lateral line organs in the Axolotl. Journal of Comparative Neurology 325:95–123.

PARK, D., AND S. R. PARK. 2000. Multiple insemination and reproductive biology of *Hynobius leechii*. Journal of Herpetology 34:596–600.

PARK, D., AND H. C. SUNG. 2006. Male *Hynobius leechii* (Amphibia: Hynobiidae) discriminate female reproductive states based on chemical cues. Integrative Biosciences 10:137–143.

PARK, D., J. M. MCGUIRE, A. L. MAJCHRZAK, J. M. ZIOBRO, AND H. L. EISTHEN. 2004. Discrimination of conspecific sex and reproductive condition using chemical cues in Axolotls (*Ambystoma mexicanum*). Journal of Comparative Physiology A 190:415–427.

PARK, S. R., D. PARK, AND S. Y. YANG. 1996. Courtship, fighting behaviors and sexual dimorphism of the salamander, *Hynobius leechii*. Korean Journal of Zoology 39:437–446.

SALTHE, S. N. 1967. Courtship patterns and the phylogeny of the urodeles. Copeia 1967:100–117.

SATO, T. 1992. Reproductive behavior in the Japanese Salamander *Hynobius retardatus*. Japanese Journal of Herpetology 14:184–190.

SATO, T., AND H. IWASAWA. 1993. Oviposition activity and time of the Japanese Salamander *Hynobius retardatus* during the breeding season. Science Report of Niigata University: Series D Biology 30: 25–30.

SATOU, M., H. A. TAKEUCHI, J. NISHII, M. TANABE, S. KITAMURA, N. OKUMOTO, AND M. IWATA. 1994a. Behavioral and electrophysiological evidences that the lateral line is involved in the inter-sexual vibrational communication of the Himé Salmon

(Landlocked Red Salmon, *Oncorhynchus nerka*). Journal of Comparative Physiology A 174:539–549.

SATOU, M., H. A. TAKEUCHI, K. TAKEI, T. HASEGAWA, T. MATSUHIMA, AND N. OKUMOTO. 1994b. Characterization of vibrational and visual signals which elicit spawning behavior in the male Himé Salmon (Landlocked Red Salmon, *Oncorhynchus nerka*). Journal of Comparative Physiology A 174:527–537.

SIEGEL, S., AND N. J. CASTELLAN JR. 1988. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, New York.

SIMMONS, A. M., L. M. COSTA, AND H. B. GERSTEIN. 2004. Lateral line-mediated rheotactic behavior in tadpoles of the African Clawed Frog (*Xenopus laevis*). Journal of Comparative Physiology A 190:747–758.

SOARES, D. 2002. An ancient sensory organ in crocodilians. Nature 417:241–242.

TAKAYAMA, S. 1978. *Salamandrella keyserlingii* from the Kushiro Marshland. Dobutsu to Shizen 8:5–10. [In Japanese.]

TANAKA, K. 1987. The tail vibration directed by males towards females in *Hynobius nebulosus* in the breeding season. Japanese Journal of Herpetology 12:84–85. [In Japanese.]

TOYODA, F., M. ITO, S. TANAKA, AND S. KIKUYAMA. 1993. Hormonal induction of male courtship behavior in the Japanese newt, *Cynops pyrrhogaster*. Hormones and Behavior 27:511–522.

USUDA, H. 1993. Reproductive behavior of *Hynobius nigrescens*, with special reference to male midwife behavior. Japanese Journal of Herpetology 15:64–70. [In Japanese with English Abstract.]

———. 1995. Waving behavior and its effect on the reproductive behavior of *Hynobius nigrescens*. Japanese Journal of Herpetology 16:19–24. [In Japanese with English Abstract.]

———. 1997. Individual relationship of male aggressive behavior during the reproductive season of *Hynobius nigrescens*. Japanese Journal of Herpetology 17:53–61. [In Japanese with English Abstract.]

VIRANT-DOBERLET, M., AND A. COKL. 2004. Vibrational communication in insects. Neotropical Entomology 33:121–134.

VOGEL, D., AND H. BLECKMANN. 1997. Water wave discrimination in the surface-feeding fish *Aplocheilus lineatus*. Journal of Comparative Physiology A 180:671–681.

Accepted: 10 March 2008.