

Directing the turning behavior of carp using virtual stimulation

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Abstract. Fishes detect various sensory stimuli, which may be used to direct their behavior. Especially, the visual and water flow detection information are critical for locating prey, predators, and school formation. In this study, we examined the specific role of these two different type of stimulation (vision and vibration) during the obstacle avoidance behavior of carp, *Cyprinus carpio*. When a visual obstacle was presented, the carp efficiently turned and swam away in the opposite direction. In contrast, vibration stimulation of the left or right side with a vibrator did not induce strong turning behavior. The vibrator only regulated the direction of turning when presented in combination with the visual obstacle. Our results provide first evidence on the innate capacity that dynamically coordinates visual and vibration signals in fish and give insights on the novel modulation method of fish behavior without training.

Keywords: fish behavior; behavior control; carp (*Cyprinus carpio*); obstacle avoidance; virtual stimulation

1. Introduction

Recently, outstanding progress in electronics, mechanics, and biological sciences has facilitated research on the use of animals as mobile platforms, similar to that of drones and micro robots. Animal platforms have the merit of locomotion and energy efficiency, because their bodies have evolved through natural selection over millions of years. Thus, there have been several trials to control the movement of insects and other lower animals (Daly *et al.* 2010, Sanchez *et al.* 2015, Sun *et al.* 2013, Krause *et al.* 2011, Talwar *et al.* 2002, Britt *et al.* 2011, Tsang *et al.* 2010).

In our previous study, we showed that it is possible to control the behavior of animal by evoking instinctive behaviors through virtual stimulation (Lee *et al.* 2013, Kim *et al.* 2017). Using a special device that provides visual stimulation, obstacle avoidance behavior in freshwater turtles (*Trachemys scripta elegans*) was evoked, allowing us to control their movement trajectories. This result led us to question whether similar virtual stimulation could be employed to control other animals, such as fish.

For the purpose of fishery and aquaculture, research on fish behavior has focused on certain

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aspects, such as the structure of the sense organs, locomotion, and social behavior. Various methods of eliciting fish movement have been explored, including the use of sound, light, electrical/chemical stimuli, and water flow (Popper *et al.* 1998, Popper *et al.* 2004, Parkyn *et al.* 2003, Hodgson *et al.* 1971, Gardiner *et al.* 2007, Gardiner *et al.* 2010, Liao *et al.* 2006, Engelmann *et al.* 2000). Mechanosensory hair cells on the lateral line sensory organs allow fish to detect hydrodynamic pressure differences created by flow velocity gradients (Coombs *et al.* 1989, Dijkgraaf *et al.* 1963). The ability of the lateral line to encode hydrodynamic information is critical for many fundamental behaviors, such as rheotaxis (Dijkgraaf *et al.* 1963, Kanter *et al.* 2002, Montgomery *et al.* 1997), predator avoidance (Blaxter *et al.* 1989), prey detection and capture (Conley *et al.* 1998, Coombs *et al.* 2001, Montgomery *et al.* 1998), and schooling behavior (Pitcher *et al.* 1976). Vision and the lateral line sensory organs are highly important for fish behavior, including searching for prey, evading predators, and school formation (Bisazza *et al.* 1997, Partridge *et al.* 1980, Shaw *et al.* 1978).

Like most animals, vision is the dominant sense for many fishes. In particular, fish rely on vision to initiate and modulate locomotion (Douglas *et al.* 1989, Fernald *et al.* 1985, Hobson *et al.* 1981, Roeser *et al.* 2003). However, fish behavior tends to be regulated by multiple sensory modalities, with the simultaneous contribution of vision and the lateral line being important for locomotion (Partridge *et al.* 1980, Janssen *et al.* 1993, Montgomery *et al.* 2003, Sutterlin *et al.* 1975).

Therefore, our study has come at the way of control in two different stimuli: visual and vibration stimulations. Through these stimuli, we expect that we could remotely guide a fish's moving path by controlling its instinctive behavior (e.g., obstacle avoidance behavior) using a non-invasive stimulation device. In particular, the obstacle avoidance behavior is connected to those instincts which protect the body and which must be evoked and directed in a consistent manner by a stimulus (Alcock *et al.* 1993, Arbib *et al.* 1990, Tinbergen *et al.* 1951). Also in our previous research, this instinctive behavior was utilized to control the turtle's path. As a result, coherent patterns in the turtle's trajectory were observed (Lee *et al.* 2013).

In this study, the common carp (*Cyprinus carpio*) was chosen to examine the obstacle avoidance behavior by the visual and vibration stimulations. To control the carp's moving path using its obstacle avoidance behavior, we should comprehend its behavior mechanism and reactions to each stimulation. So in this paper, we implemented two experimental tests: (1) the obstacle avoidance test and (2) the turning behavior test. The obstacle avoidance test was performed in a flow chamber to determine how carp reacted to the obstacle objects. We then attached a stimulation device to carp to evoke the obstacle avoidance instinct of carp through the virtual stimulation. The turning behavior test was performed in a water tank to analyze the movement trajectories of carp. Based on our results, we discuss the potential to control fish behavior using innate sensory stimuli, without any form of training. Finally, we discuss about our experimental result to develop a remote control system for fish, then propose further research topics on this subject.

2. Material and methods

2.1 Fishes

Carp (*Cyprinus carpio*) were chosen because: (1) they move actively, (2) are easy to obtain,

and (3) are easy to maintain in the laboratory environment. Fifteen carp (length=30~40 cm, weight=550~730 g) were housed indoors in laboratories at Korea Advanced Institute of Science and Technology (KAIST). The carp were housed together in water-filled glass tubs (1.5×3×1 m). The tank was fitted with a water filter and maintained at 18°C. The carp were fed commercial pellets three times a week. After at least 12 hours without any feed in the tank, the fish were moved to the flow chamber and experimental water tank for the experiments (Figs. 1 and 2).

2.2 Experimental protocol and apparatus

When running the experiments, each carp was only used within bounds that they did not experience fatigue during the experimental session. Fifteen carp were selected at random for each set of tests. In each test, their behavior was video recorded for 10~30 s (obstacle avoidance test) and 300 s (turning behavior test) using an experimental camera (Firefly FMVU-13S2C, Pointgrey, Canada).

As the first test, the obstacle avoidance test was implemented in a flow chamber with semi-laminar flow at 1.0-1.5 cm/s. The flow chamber was 1.5 m long and 0.75 m wide, with a water depth of 0.6 m. This chamber had opaque white sides, and three different wall type obstacles. The wall type obstacles were made of transparent, black/transparent, and transparent/black acrylic, and were of 60 cm height (Fig. 1). This test was performed to identify the type of objects that carp recognize as obstacles, and how they avoided the recognized obstacles along their moving route. We examined carp movement trajectories when three kinds of wall type obstacles were initially placed 100 cm in front of each individual.

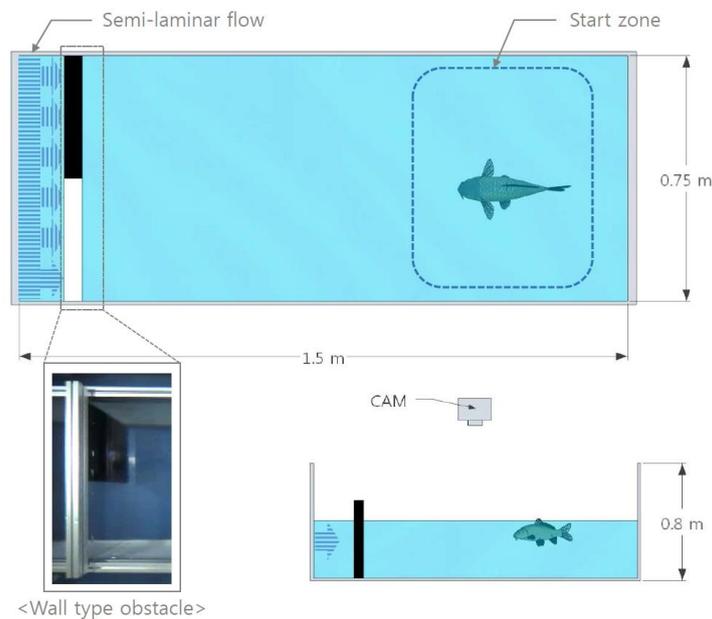


Fig. 1 Experimental setup for the obstacle avoidance test. In the flow chamber, there was a semi-laminar flow of 1.0-1.5 cm/s from the opposite end to the point where carp would start swimming. Then, the carp (*C. carpio*) swam against the current (showing positive rheotaxis)

Secondly, for the turning behavior test, a simple stimulator (Fig. 2(a)) was designed to provide the carp with a stimulus causing obstacle avoidance. This device looks like a full face helmet and consists of a visual stimulator (blinder), a vibration stimulator (vibration motor [$\Phi 10$, 9000 ± 2000 rpm]), and a 1.5 V button cell (LR44, Toshiba, Japan). The stimulator weighed 24 g (with the pair of vibration motors weighing 2 g and the batteries, 4 g), representing 3.3–4.5% of the carp's weight. Using this stimulator, we examined the movement trajectories of carp when visual and vibration stimuli were given. For the test, we allowed the carp to acclimate this device first, and then the carp were allowed to swim in a square water tank (1.5 m \times 1.5 m, water depth 0.6 m) without flow (Fig. 2(b)). The stimulation device is shown in Fig. 2(c).

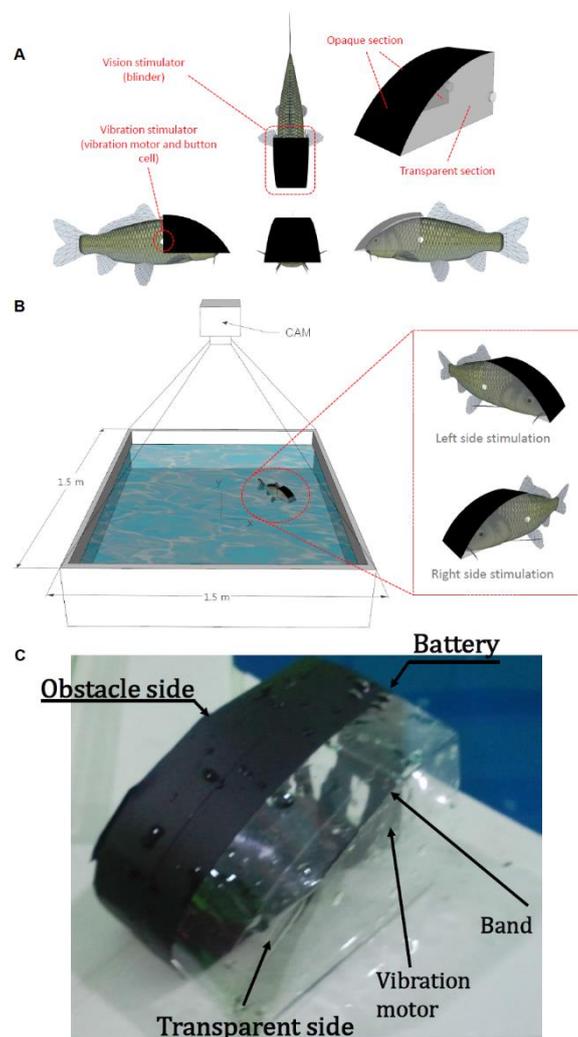


Fig. 2 Schematic of the experimental device and settings for the turning behavior test. (a) The stimulation device used to induce carp (*C. carpio*) obstacle avoidance behavior. The device consists of a blinder, vibration motor, and button cell. (b) Experimental setup for the turning behavior test. The water depth was 0.6 m. (c) Stimulation device

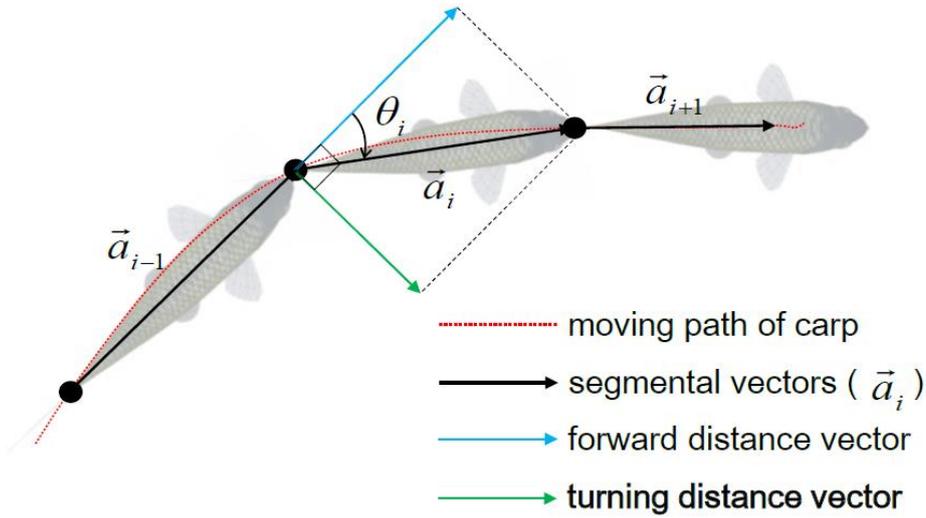


Fig. 3 Schematic of the turning distance (TD) vector. To measure the carp's (*C. carpio*) turning behavior, a TD vector was defined (see the methods section for details)

2.3 Data analysis

Throughout all of the tests, the path used by the carp was tracked by a camera using a color-based tracker based on a MATLAB (The Mathworks Inc., USA) image processing program developed by Matpic. During the tests, a Kalman filter with linear models was used to describe the trajectory of the carp.

In the obstacle avoidance test, fish behavior was recorded for 30 s, and each session was repeated 20 times. We also calculated the “avoidance rate” (defined as the percentage of number of times that the fish avoided visual obstacles throughout the trials) from the trajectory of the carp. Then, in the turning behavior test, the behavior of each individual was recorded for 300 s, and each session was repeated 50 times. This information was used to calculate “the amount of turning” in each experiment; specifically, “Turning Distance (TD)” and “Average Turning Velocity (ATV).” These parameters are defined by the following equation

$$ATV = \frac{TD}{(\text{total travel time})} \quad \text{with} \quad TD = \sum_{i=1}^n |\vec{a}_i| \sin \theta_i .$$

The TD is derived by dividing the entire path into n-segmental vectors by sampling each second, after which, the ATV under the condition $\theta \leq 90^\circ$ is defined by this equation (see Fig. 3 for example). These measures quantify the amount of displacement from the carp's previous heading per unit of time (Lee *et al.* 2013). Using these measures, we could calculate the amount that each carp turned during its movement trajectory (see Fig. 5)

We used various statistical tests to analyze our data (Fig. 6), depending on the assumptions of the tests met by the data. We used a Mann-Whitney U test (M-W U test), with $P < 0.05$. It is a non-parametric test that is used to compare two population means that come from the same population.

It is also used to test whether two population means are equal or not. Using this test, we verify the difference between two types of behavior. In all cases where data were compared multiple times, we used a Bonferroni correction for multiple comparisons of each trial after stimuli presentation. Lower case letters represent statistically homogeneous groups (Robie *et al.* 2010). For example, in Fig. 6, groups “a” and “b” are significantly different, whereas “a” is not significantly different to “ab,” which shares membership with group “a.” All statistical analyses were performed using Minitab (Minitab Inc., USA).

2.4 Ethical note

The animal experiments were approved by KAIST (Korea Advanced Institute of Science and Technology) Institutional Animal Care & Use Committee Board (Permit Number: KA2014-27). The personal certification numbers were as follows: Cheol-Hu Kim (2010-OE01), Dae-Gun Kim (2011-OE01), Daesoo Kim (2008-BS7), and Phill-Seung Lee (2014-OS01). Our target animals (common carp: *C. carpio*) were manipulated in strict accordance with KAIST Animal Experiment Ethical Law RR0303 (revised 24/07/2013), and all efforts were made to minimize suffering.

3. Results

3.1 Obstacle avoidance test

We first analyzed the carp movement trajectories when wall type obstacles (width=75 cm, height=60 cm) were initially placed 100 cm in front of each individual. We found that carp recognized the black wall as an obstacle, but not the transparent wall. The carp faced toward the transparent side, but avoided the black side (Fig. 4). The avoidance rate calculated from each experiment generated a score of more than 90% (N=20), regardless of the position of black wall obstacle.

3.2 Turning behavior test

In this test, we examined the movement trajectories of carp when visual and vibration stimuli were given consistently (see Figs. 5 and 6). The preceding test showed that carp avoid black wall obstacles, moving toward the transparent side. Based on these results, we designed a simple stimulator (Fig. 2(a)), and performed four kinds of experiment. Each experiment was conducted 50 times, and each session was recorded for 300 s, with 15 carp being used at random. We analyzed the results of each experiment using ATV and the M-W U test.

3.2.1 Free swimming

First, we examined the free swimming condition of carp without any stimulation. This test was implemented 50 times (N1=50) by replacing 15 carp each time. Under these conditions, the ATV value was almost zero (+0.0194 cm/s) for free swimming (“+” and “-” signs indicate left and right turning directions, respectively; left turning was dominant in this moving trajectory). Thus, carp movement was inconsistent under free conditions. These data were used as a baseline data for comparison with the subsequent experiments (Fig. 6(a)).

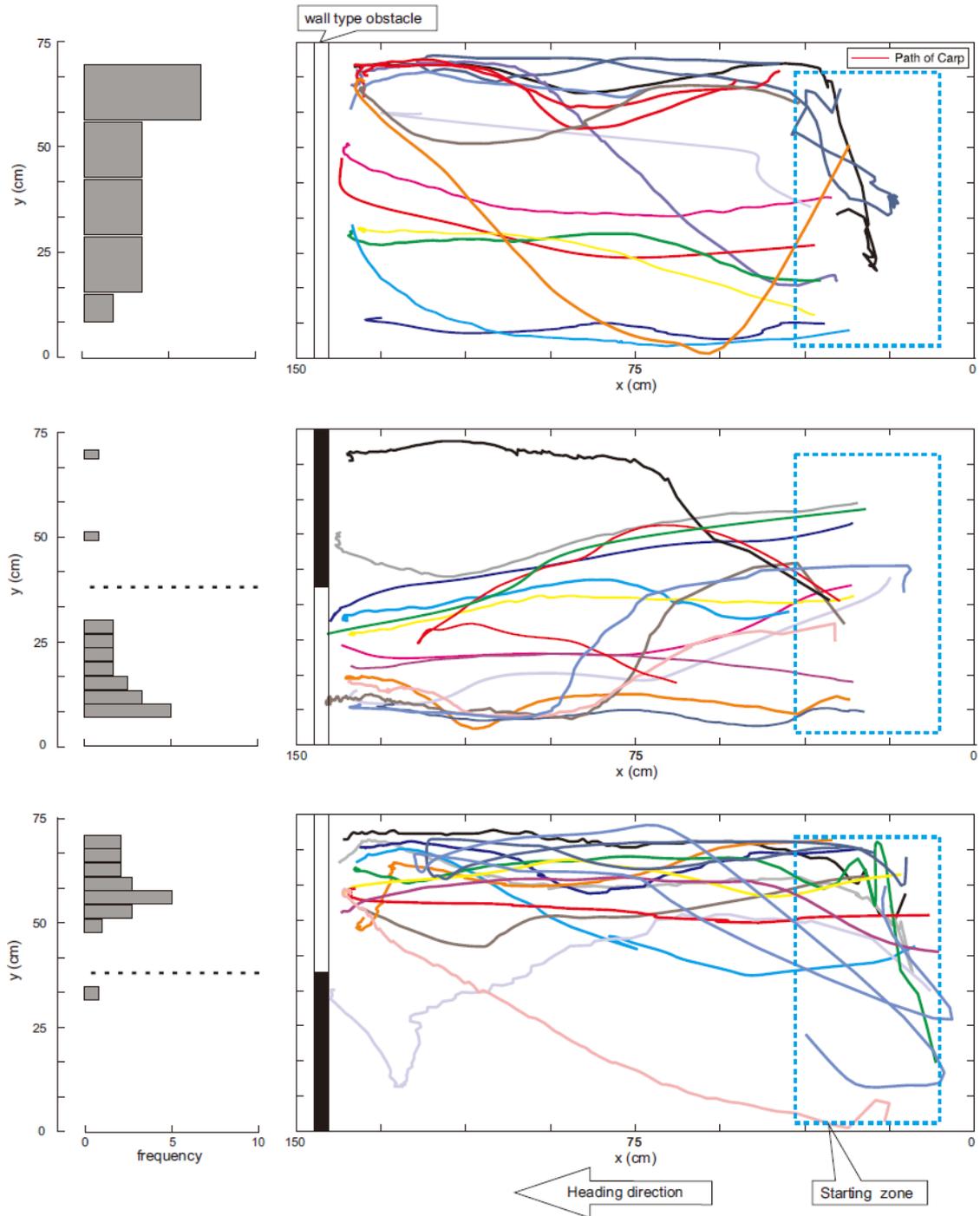


Fig. 4 Movement trajectories of carp (*C. carpio*) in the obstacle avoidance test. Three types of obstacles (transparent, black/transparent, and transparent/black) were used in this test

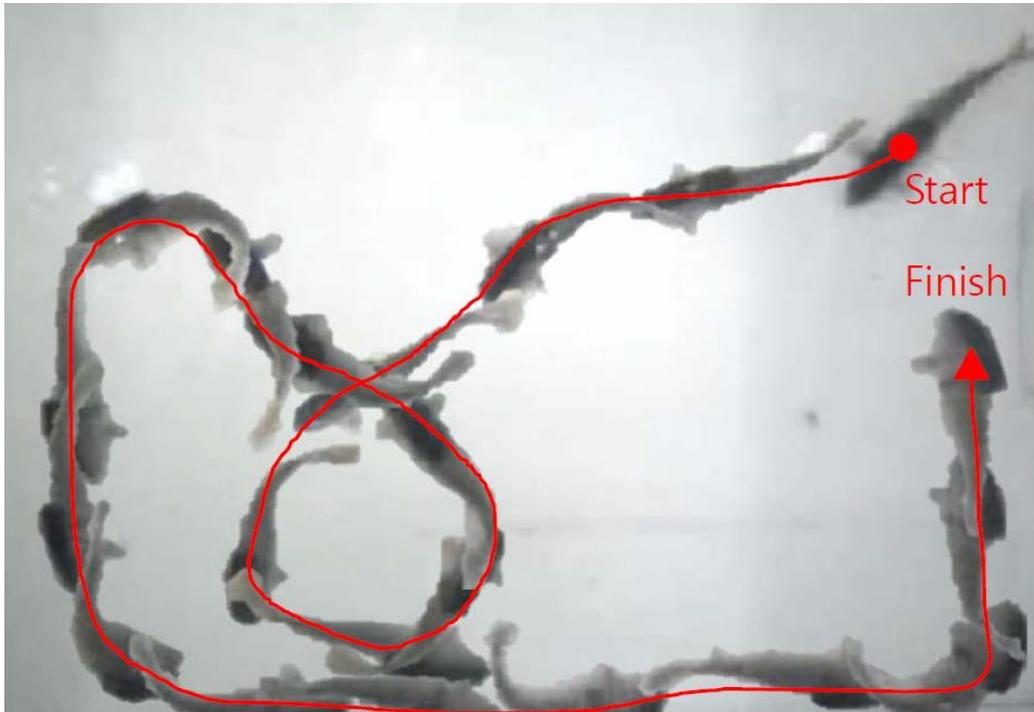


Fig. 5 Movement trajectory during the simultaneous stimulation of the right side (right vision was blocked and the right side vibration motor was operated). Carp (*C. carpio*) consistently turned to the left, as if avoiding a virtual obstacle

3.2.2 Vibration stimulation

Carp movement trajectories were examined when vibration stimulation on each side of its body. The pair of vibration motors vibrated at 150 Hz, changing the water flow near each side of carp. When we stimulated the right side, the ATV value was +0.2573 cm/s. When we stimulated the left side, the ATV value was -0.1103 cm/s. The M-W U test results were similar for the right ($W=2534$, $U=1259$, $N1=N2=50$, $P=0.9533$) and left ($W=2499$, $U=1224$, $N1=N3=50$, $P=0.8605$) sides. Therefore, these stimulations were not significant under our experiment condition.

3.2.3 Visual stimulation

In this test, we blocked the vision (right and left) of carp using our visual stimulation device, and analyzed their moving paths. Throughout the obstacle avoidance test, we found that carp tend to move to the transparent side. As a result, using our device, the overall moving path of carp tended to rotate in the direction that was not blocked. When we blocked the right vision, the ATV value was +0.9788 cm/s, and when we blocked the left vision, the ATV value was -0.6244 cm/s. However, the absolute ATV value was higher than when vibration stimulation case. The M-W U test showed that the control group (free swimming; $N1=50$) was not significantly different to the right ($W=2408$, $U=1133$, $N1=N4=50$, $P=0.4219$) and left ($W=2639$, $U=1364$, $N1=N5=50$, $P=0.4340$) blocked vision stimulation groups.

3.2.4 Simultaneous stimulation: vision and vibration

In this test, we gave the visual and vibration stimulations simultaneously to the carp. When an obstacle was presented virtually through simultaneous stimulation, the carp efficiently turned to move in the opposite direction. Following simultaneous stimulation, the ATV value on the right side was +1.3208 cm/s, while it was -1.1832 cm/s on the left side. These values were considerably higher than those obtained in the previous experiment. The M-W U test showed that the control group (free swimming; N1=50) was significantly different to the right side (W=2092, U=817, N1=N6=50, P=0.0029) and left side (W=2837, U=1562, N1=N7=50, P=0.0318) stimulation groups.

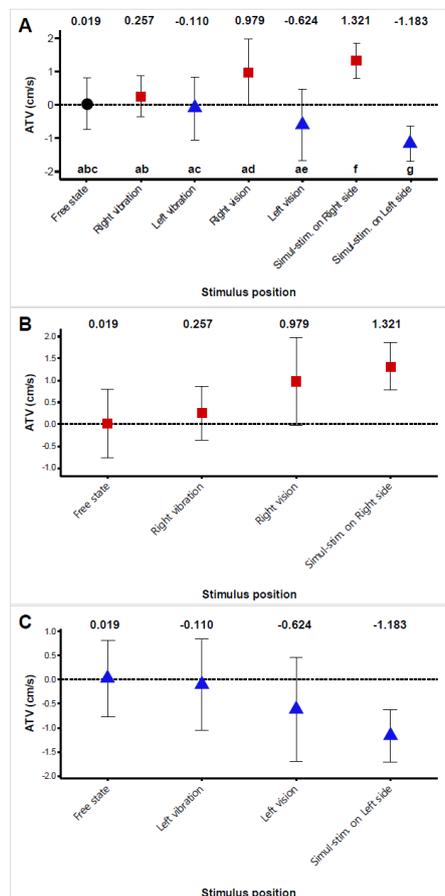


Fig. 6 Relationship between carp (*C. carpio*) movement and the different types of stimulation. (A) The average turning velocities (ATV) of the tracked trajectories in the turning behavior tests (e.g. Figure 5) were plotted from the mean and standard deviation, even though they were not always normally distributed. The positive ATV value meant that left turning was dominant in the moving path, while the negative ATV meant that right turning was dominant. The Bonferroni correction for multiple comparisons (lower-case letters) showed that the “right vibration”, “left vibration”, “right vision” and “left vision” groups were not significantly different from the “free state” group (comparison group). In contrast, the “simul-stim. on the right side,” and “simul-stim. on the left side” were significantly different from the “free state” group and the other groups. (B) and (C) Comparison of the ATV for stimulations on the same side. The absolute ATV value increased with different stimulation types (vibration < vision < simultaneous stimulation)

4. Conclusions

This study was designed using the simple concept that fish movement patterns may be controlled by virtually stimulating their avoidance instinct. Through two basic experiments, we demonstrated that the visual and vibration stimulations could be used to guide fish along specified moving paths or towards goal positions. These results confirm the potential utility of fish as mobile living platforms, similar to mobile artificial agents.

In this study, our stimulator gave continuous stimulation to carp and made a specific swimming pattern of the carp. When the carp was stimulated, such as obstacles was located in each side of carp, it turned constantly as if avoid virtual obstacles. Especially, the results indicate that carps avoid visual obstacle stimulation more effectively than vibration stimulation. Theoretically, most moving animals show obstacles avoidance behavior in their surroundings, and, in general, fast moving animals use vision as the sensory basis for this behavior (Robertson *et al.* 1993, Errigo *et al.* 2001, Griffin *et al.* 2001, Schindler *et al.* 2004). Slow moving animals often employ other senses, such as touch (Migita *et al.* 2005). This generalized concept fits well with our observations of obstacle avoidance in carp being visually guided, since these animals are nimble swimmers (Spierts *et al.* 1999).

Generally, it is well known that fish's ear and lateral line do have some functional overlap. The ear detects sound frequencies from well below 50 Hz to over 2,000 Hz in some species. It also responds to position and acceleration of the body. The lateral line responds to differences between motion of the fish and motion of the surrounding water, including stimuli produced by other swimming fish and other organisms; these frequencies range from less than 1 Hz to several hundred Hz (Popper *et al.* 2004). Also, there are previous study that using both visual and water flow stimuli together, fishes are able to decide their moving path or position (Evelyn Shaw *et al.* 1978). In our experiments, we used the 150 Hz vibrator which exceeds the scope of frequency detection limit for lateral line sense. Nevertheless, the results of experiments showed that the carp determine its turning direction clearly through the simultaneous stimulation; visual and vibration stimulation. Therefore, we surmise that our stimulator caused the ear sense stimulation which is associated with carp's turning movement immediately. Also, there is possibility that our vibration stimulus gave an influence to carp's muscles or lateral line sense indirectly. We think that further studies are necessary to investigate interrelation of visual and vibration stimulations clearly in the future.

The current study examined obstacle avoidance, which is one of the essential responses for an organism's survival. By providing a visual and vibration stimulation that induces obstacle avoidance behavior, we evoked carp's turning movement. Our experiments demonstrate that fish behavior could be effectively guided by evoking instinctive behavior, with our results providing the first evidence about the modulation of fish behavior through the simple combination of innate sensory stimuli, without any form of training.

Previous studies have trailed controlling fish movement by using electrical brain stimulation (Kashin *et al.* 1974, Kobayashi *et al.* 2009). However, these techniques were invasive, requiring surgical intervention. To overcome these limitations, we developed devices and platform technologies to modulate fish innate behavior by stimulating external senses. Our devices were designed to be non-invasive, which would be suited for submersible control systems. Our findings are expected to contribute information to control fishes with a stimulation/control device attached to the fish body in the future. To develop effective devices and achieve the desired level of control, further research is required on the interrelations of fish sense organs. A number of challenges

remain, including telecommunication, miniaturization, attachment method and waterproofing. However, ultimately, this technology could be used in underwater exploration, and could replace our dependence on robotic probes. This technology could also be used to observe fish movement from an ethological perspective and to understand their complicated interactive behavior with conspecifics.

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