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Title: Can red sea bream *Pagrus major* learn about feeding and avoidance through the observation of conspecific behavior in video playback?

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Abstract

The present study investigated whether red sea bream *Pagrus major* could learn about feeding and avoidance area through video model observation. In Experiment 1, 45-mm standard length (SL) fish were trained to learn about a feeding area in a tank. In Experiment 2, 114-mm SL juveniles were conditioned to avoid a hand net by moving into a shelter. Three treatments were established in each experiment: (i) live model observer: fish observed the behavior of a real fish in an adjacent tank; (ii) video model observer, fish observed video playback of a conspecific on a monitor; and (iii) non-observing control. Ten observational trials were performed in both experiments for the live and video model observer. Afterwards, fish from all treatments were conditioned by feed or avoidance conditioning. In Experiment 1, there was no difference in the feed learning process among treatments. In contrast, in Experiment 2, live and video model observers acquired avoidance learning more quickly than control. The result indicates that the video model can be as efficient as the live model for observational learning in fish. This study suggests that video playback may be useful for anti-predator training of seedlings for stock enhancement.

Keywords: conditioning; observational learning; social transmission; Sparidae; stock enhancement; training

Introduction

In stock enhancement, released seedlings often suffer high mortality due to maladaptive behavior towards natural preys and predators [1-3]. Such behavioral deficiencies in reared fish can possibly be improved before release. Training has been considered as one of the options to improve the quality of seedlings [4-6]. Through feed training before release, released fish can forage more effectively for natural foods in their living environment. Moreover, fish trained to respond adequately to a threat stimulus would be able to avoid novel predators.

Observational learning is the acquisition of behavior through the observation of other individual(s). For instance, nine-spined stickleback *Pungitius pungitius* learns food patch quality by observing the success of others [7]. Fish can acquire information more effectively by the observational learning than no-observed learning [8]. The observational learning has drawn attention as a training method for released seedlings in stock enhancement, especially for the conditioning of predator information [9, 10]. In practice, however, it is difficult to train fish by observational learning using a live model because of the limitations of time and space.

In this study, we propose training method by observational learning using video playback model. Video playback can be an effective tool of observational learning because it is easily repeatable in a limited space. Past studies have shown that fish can recognize conspecific and heterospecific fish in video playback as much as live fish in an adjacent tank [11-15].

Whereas past studies have found that fish show certain responses to model fish in video playback, to the best of our knowledge, no study has revealed whether fish can acquire the information by observational learning of video model. The present study investigated the observational learning of video model in *Pagrus major* for feed conditioning (Experiment 1) and avoidance conditioning (Experiment 2). In each experiment, the observational trials were established the following treatments: (i) live model observation treatment, where the observer fish was allowed to directly observe behavior of a live fish in the adjacent model fish tank; and (ii) video model observation treatment, where the observer fish observed fish behavior on video playback. Their learning processes for these observational treatments were compared with (iii) non-observing control fish in both experiments.

Materials and methods

Experiment 1: Feed conditioning

Fish

Fertilized *P. major* eggs were purchased from Pacific Trading Co. (Fukuoka, Japan), and the eggs were stocked in four 500 l transparent polycarbonate tanks supplied with filtered seawater at the Maizuru Fisheries Research Station (MFRS) of Kyoto University. After hatching on October 13, 2010, larvae were provided with rotifers *Brachionus plicatilis*, *Artemia* sp. nauplii, and dry pellets (N400 and N700, Kyowa Hakko Bio Co., Ltd., Tokyo, and Otohime S1, Marubeni Nisshin Feed Co., Ltd., Tokyo, Japan), in accordance with growth. The water temperature was maintained at 24 °C using a heater and thermostat. Fish attained about 40 mm standard length (SL) on January 6, 2011. The size is suitable for the experiment; that is, when they could feed on enough pellets at one time. At the experiment, fish SL was 44.6 ± 5.8 mm (average ± standard deviation), and there was no difference of size among live model observer, video model observer and control treatments (ANOVA: $F_{2, 14} = 1.15$, $P > 0.05$).

Experimental tank

Four transparent polypropylene experimental tanks (length × width × height: 30 × 20 × 20 cm) were set in a room with a 12:12 h light/dark regime. These tanks were covered with black vinyl sheets, and seawater was filled to a depth of 15 cm with circulating filtered seawater. Each tank was used as model fish tank, live model observer tank, video model observer tank and control fish tank. The live model observer tank was located next to the model fish tank, and that of the video model observer faced a 26-inch waterproof monitor (Disign, Inc., Kanagawa, Japan). One of the long sides of the each observer tank faced a model fish tank or on a video monitor, in respectively. The black vinyl sheets between each observer tank and model tank or video monitor were removable, and the sheets were used as blind sheets except for the observational trial.

A grey polyvinyl chloride (PVC) pipe (diameter × height: 3 × 2 cm) covered by a white PVC board was set at the center of each tank as a feeding base (Fig. 1). A grey PVC pipe (diameter × height: 2.5 × 15 cm) was placed on the feeding base. In experiments, three to five pellets (Otohime S1) were dropped on the feeding base through the pipe which prevented feeding of fish before the conditioning. On a conditioning, the pellets on the feeding base were exposed to the fish by removing the PVC pipe.

Model fish conditioning

A single fish randomly selected from a rearing tank was introduced into the model fish tank. The fish was conditioned to forage the pellets on the feeding base. We defined the conditioning trial as a sequence that fish starts to forage the pellets on the feeding base after the removal of the PVC pipe. Afterwards, the PVC pipe was placed back on the feeding base. Conditioning trials were repeated at 30-min intervals, and then the model fish had been conditioned to feed on pellets on the feeding base within 30 s after PVC pipe removal in four consecutive trials.

For the video model, the feeding behavior of the model fish was recorded from the lateral side by a video camera (HDR-CX550, SONY Co., Tokyo, Japan); ten unique events of the model fish performing the task were recorded.

Observational trials

The observational trials were established the live model observer and video model observer treatments (Fig. 2). A single fish randomly selected from a rearing tank was introduced into each model observer and control fish tanks on the day before the experiment. These fish were allowed to acclimatize overnight, and then a few pellets were provided before initiating the experiment. If the fish ate these pellets, the observational trials were started except for control fish. The blind sheet between the each observer tank and model fish tank or the monitor was removed 30 min before the beginning of observational trials; thereby, each observer fish was visible to live model fish or video monitor through transparent wall of tanks, in respectively.

The observational trial for the live model observer was a sequence where model fish foraged pellets on the feeding base after removing the PVC pipe, in the adjacent model tank. In the video model observer treatment, the observer fish observed the above sequence on the video monitor. An observational trial lasted 1 min, and ten trials were conducted for both observational treatments, with 5-min intervals.

Test and conditioning trials

After the tenth observational trial, blind sheet was set between each observer tank and model fish tank or video monitor, and 30 min later, we tested whether the each observer fish and control fish could respond to the feeding base without pellets as follows. The test trial lasted 60 s following removal of the PVC pipe. If fish pecked on the feeding base within the 60 s, the fish was considered to have learned about the feeding base. If fish not, the fish was considered as unlearned fish, and then the fish was conditioned to forage the pellets on the feeding base after the test trial. Conditioning trials were provided same manner as the model conditioning. Conditioning trials were repeated four times at 30-min intervals followed by a next test trial. Four conditioning trials the following one test trial was defined as one session. If the fish did not forage the pellets within 30 min, the fish was considered to be under stress and was replaced by a new one. Two sessions were conducted in a day, and the experiment was repeated for a maximum of four consecutive days until the fish met the definition of learning, equivalent to a maximum of nine test trials. At the end of the experiment, fish body length was measured. Five replications were conducted for each observer and control treatments.

Analyses

The proportion of fish to have learned the feeding base was compared among live model observers, video model observers, and non-observing controls from the first to the ninth test trial, using survival analysis. In the survival analysis, the Cox proportional hazard model likelihood ratio test with the Breslow method was performed using the “Survival” package for R statistical software, version 3.0.0 (R Development Core Team 2013).

Experiment 2: Avoidance conditioning

Fish

Hatchery-reared *P. major* juveniles, hatched on June 10, 2010, were transported from Miyazu National Center for Stock Enhancement to the MFRS. Fish were kept in 500 l transparent polyethylene tanks. The fish were fed as in Experiment 1, until December 26, 2010. Fish SL was 114.2 ± 6.7 mm (average ± standard deviation), and there was no difference of size among treatments (ANOVA: $F_{2, 17} = 0.05$, $P > 0.05$).

Experimental tank

Eight glass experimental tanks (length × width × height: 60 × 30 × 36 cm) were set in a room with 12:12 h light/dark regime. Each two tanks were used as model fish tanks, live model observer tanks, video model observer tanks and control fish tanks. These tanks were covered with black vinyl sheets, and seawater was filled to a depth of 25 cm with circulating filtered seawater. The live model observer tank was located next to the model fish tank, and that of the video model observer faced a 26-inch waterproof monitor (Disign, Inc., Kanagawa, Japan). One of the long sides of the each observer tank faced a model fish tank or on a video monitor, in respectively. The black vinyl sheets between each observer tank and model tank or video monitor were removable, and the sheets were used as blind sheets except for the observational trial.

A half-cut transparent polyethylene case (length × width × height: 15 × 20 × 20 cm) attached to a black polyethylene board (length × height: 30 × 20 cm) with a hole (length × height: 5 × 10 cm) was set in the experimental tank as a shelter (Fig. 3). A black PVC board (length × height: 7 × 30 cm) was set as a door in front of the hole to prevent fish from entering the shelter, before experiment.

Model fish conditioning

A single fish randomly selected from a rearing tank introduced into the model fish tank. The fish was conditioned to escape into the shelter when chased by a hand net (length × height: 30 × 30 cm), after removing the door. A conditioning trial was composed of the following sequence: the door was removed, and after 30 s, a hand net was introduced at the opposite side of the shelter and the net was left for 15 s; the hand net was then moved slowly to 22.5 cm from the shelter during the following 15 s. If the fish did not enter the shelter after moving the net, the hand net was moved to 3 cm from the shelter until the fish escaped into the shelter. The escaped fish was allowed to stay inside the shelter for 5 min. If the fish did not go out from the shelter within 5 min, fish was gently forced out using a black polyethylene board. The door was placed back in front of entrance, and the entrance closed. Conditioning trials were repeated at 30-min intervals, and then the model fish had been conditioned to escape into the shelter from hand net within 30 s after removing the door at least four consecutive trials.

For the video model, the escaping behavior of the model fish was recorded by a video camera (HDR-CX550, SONY Co., Tokyo, Japan); ten unique trials of the model fish performing the task were recorded. Video playback from the first to the fifth trial was recorded from the lateral side, and a recording from the oblique backward side was conducted from the sixth to tenth trial. This was because the observer fish might have difficulty understanding the entrance to the shelter in a two-dimensional video monitor.

Observational trials

The same three treatments as in Experiment 1 were conducted. One fish was introduced into the each model observer and control fish tank from the stock tank on the day before the experiment. These fish were allowed to acclimatize overnight, and the fish were provided with observational trials 30 min after removing the blind sheet except for control fish. An observational trial lasted 1 min, and ten trials were conducted for both observational treatments with 5-min intervals. The observational trial for the live model observer was a sequence where model fish escaped into the shelter from the hand net within 30 s, in the adjacent model tank. In the video model observer condition, the observer fish observed the above sequence on the video monitor. An observational trial lasted 1 min, and ten trials were conducted for both observational treatments, with 5-min intervals.

Conditioning trials

After the tenth observational trial, blind sheet was put back, and conditioning trials was started 30 min afterwards. Conditioning trials were provided a same manner as the model conditioning. For each conditioning trial, the period from removing the door to escaping into the shelter was recorded as the escape latency. Ten conditioning trials were conducted per day, with 30-min intervals, for two consecutive days. This means that avoidance conditioning consisted of twenty trials for each fish. A single fish was used for one replication, and six replications were conducted for all treatments. Fish body length was measured after the experiment.

Analyses

The escape latency was used to evaluate avoidance learning; latency is expected to decrease as the fish learns how to avoid the hand net by entering the shelter. The escape latency from the first to the twentieth trial was analyzed using generalized linear mixed models (GLMM) with the "lme4" package for R statistical software. The error distribution of response variables was fitted to the Poisson distribution, with restricted maximum likelihood parameter estimation. The two fixed factors were "trial" (1 to 20) and "treatment" (live model observer, video model observer, and control). We treated "individual" as random factor since individual fish were repeatedly measured. Tukey's test was performed for "treatment" by general linear hypotheses (GLHT) using the "multcomp" package.

Results

Experiment 1: Feeding conditioning

For the feed learning, the proportion of trained fish during nine test trials was not significantly different among observational treatments (Cox proportional hazard model likelihood ratio test = 0.03 on 2 df, $P > 0.05$; Fig. 4). Neither live model nor video model observer were improved the learning efficiency, compared to control fish.

Experiment 2: Avoidance conditioning

The escape latency of the control fish was significantly longer than that of the live model observer (Tukey's test by GLHT for GLMM: $Z = -13.73$, $P < 0.001$; Fig. 5 & Table 1) and video model observer (Z = -14.87, $P < 0.001$). There was no significant difference in escape latency between the fish observed live model and video model (Z = -1.16, $P > 0.05$).

Discussion

In Experiment 1, 45-mm SL *P. major* juveniles did not improve their feed learning ability through the observation of conspecific individuals feeding, either in an adjacent tank or displayed on a video monitor. Therefore, it was not possible to evaluate the efficiency of video model for observational learning. However, in Experiment 2, the escape latency of 114-mm SL juveniles decreased through the observation of live model and video model, compared to non-observing control fish. This result shows that *P. major* juveniles can acquire avoidance and sheltering information by observing conspecific fish in video playback. The video model has been reported to work as effectively as a live model for other fish species and innate behavioral aspects [11-17]. For example, a male swordtail *Xiphophorus helleri* shows a courting behavior to a female displayed in video playback [16], and conspecific model in video playback would induce aggression behavior in *Betta splendens* [17]. In addition to these studies, the present study indicates that watching video model can work for observational learning of avoidance information.

Past studies revealed that by observing a predation event on a live conspecific in an adjacent tank a fish can acquire information about predator threat without risking themselves [18-20]. Watching predation event on a video model, observer fish may be able to learn anti-predator behavior. Indeed, Johnson & Basolo [21] found that *X. helleri* recognized a predation event on a conspecific in video playback, and their mating responses were altered after watching the video. Observational learning for predation event in a video playback should be studied to develop a practical training technique. Furthermore, the duration of such memory also has a high priority for further study to improve the efficiency of training in hatchery-reared fish.

The size of fish may have induced the different results of observational learning between Experiment 1 and 2. Our previous studies showed that learning capability in fish changes ontogenetically and between conditioned stimuli [22, 23]. We also found that the ontogenetic change of observational learning in *T. japonicus* coincides with that of social interaction [24]. Further studies using juveniles in several developmental stages are required to evaluate observational learning through video model on fish feeding behavior.

For establishing observational learning in fish, the appearance of model would be important [25, 26]. Using animation techniques, it is possible to manipulate the model appearance, e.g., size, color, and motion in video model. Fishes are reported to react to animated fish in video model just as to live models [27, 28]. Such image manipulation may play an important role in furthering investigations on the mechanisms of observational learning and thus for the application of this technique in the practice of stock enhancement.

Acknowledgements

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Figure Captions

Fig. 1 Schematic drawings of treatments in Experiment 1: (a) live model observer, (b) video model observer, and (c) control. A PVC pipe was placed on a feeding base at the center of the tank. The pipe was removed, and the pellets were presented to the fish

Fig. 2 Flowchart of the procedure in Experiment 1. The Experiment 2 had same procedure except for having 20 conditioning trials

Fig. 3 A schematic drawing of experimental tank in Experiment 2. A sheltering area was placed at one end of the tank. On a conditioning trial, the fish was chased by a hand net from the opposite end of the tank towards the shelter

Fig. 4 The proportion (%) of fish to have learned the feeding base in the course of nine test trials in Experiment 1: control (■), live model observer (◆), and video model observer (◯)

Fig. 5 Average avoidance latency (s) in the course of 20 conditioning trials in Experiment 2: control (■), live model observer (◆), and video model observer (○). Bars indicate standard deviation (n = 6)

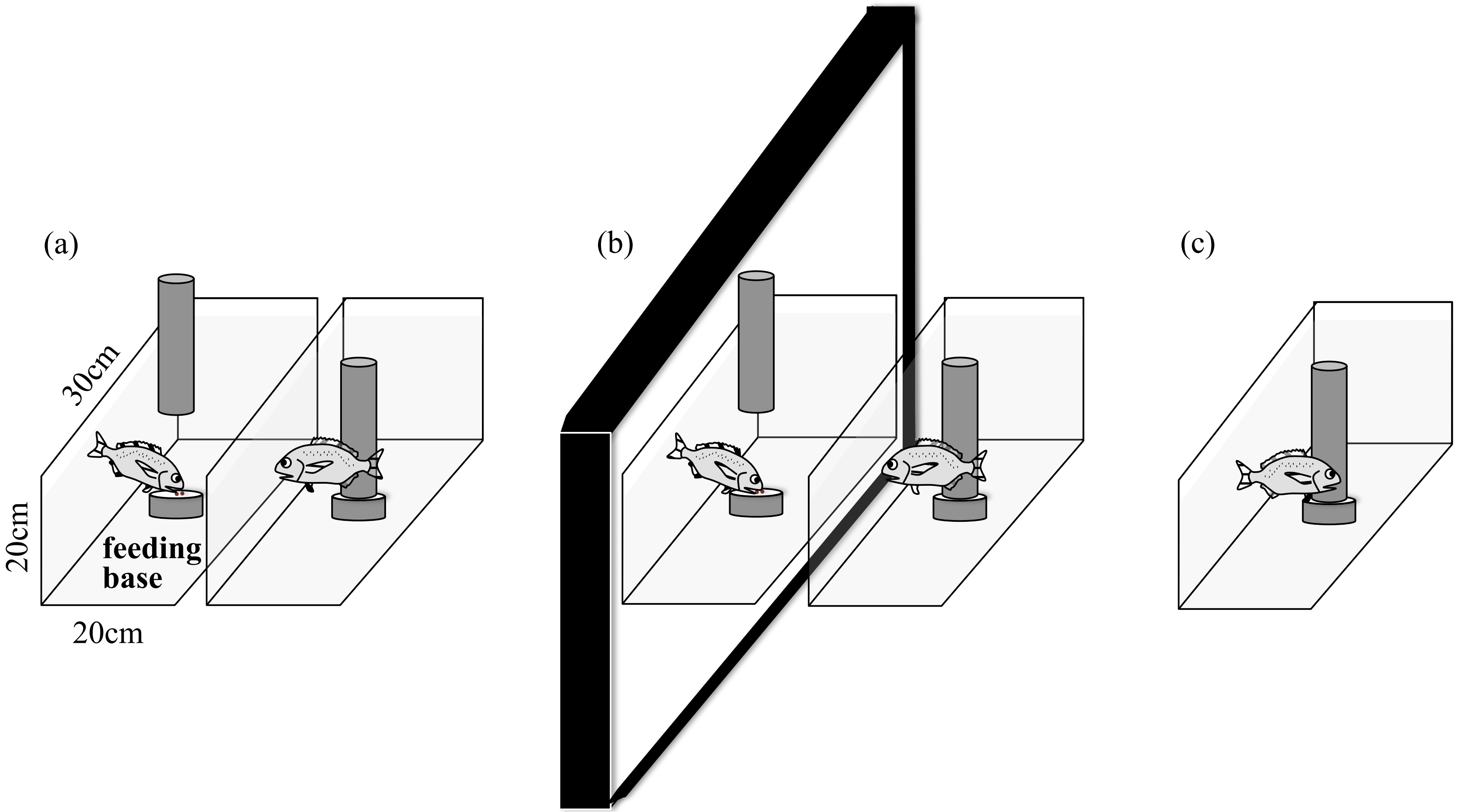
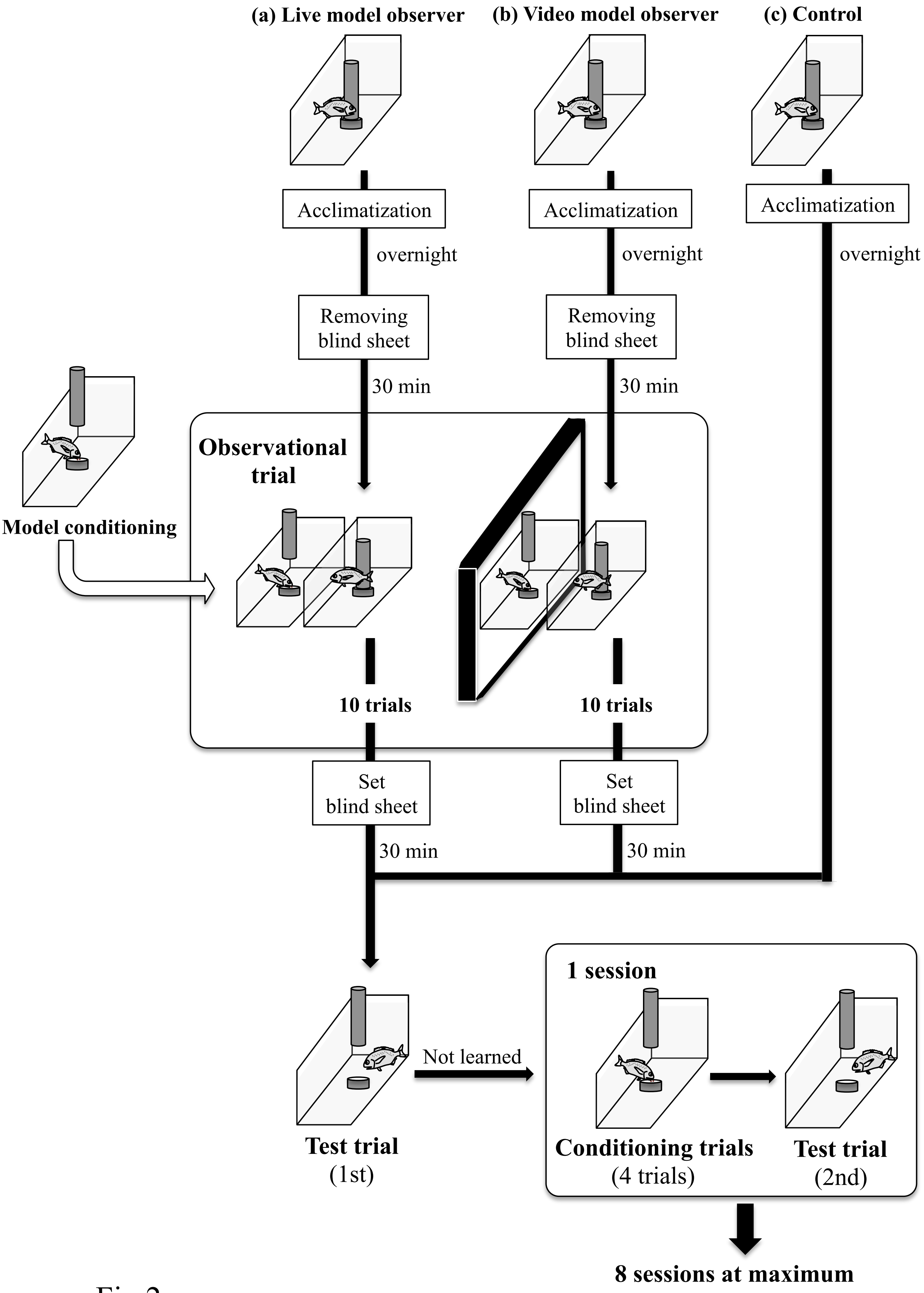


Fig.1



(a) Live model observer
(b) Video model observer
(c) Control
Acclimatization
Acclimatization
Acclimatization
overnight
overnight
overnight
Removing blind sheet
Removing blind sheet
30 min
30 min
Observational trial
Model conditioning
10 trials
10 trials
Set blind sheet
Set blind sheet
30 min
30 min
1 session
Not learned
Test trial (1st)
Conditioning trials (4 trials)
Test trial (2nd)
8 sessions at maximum

Fig.2

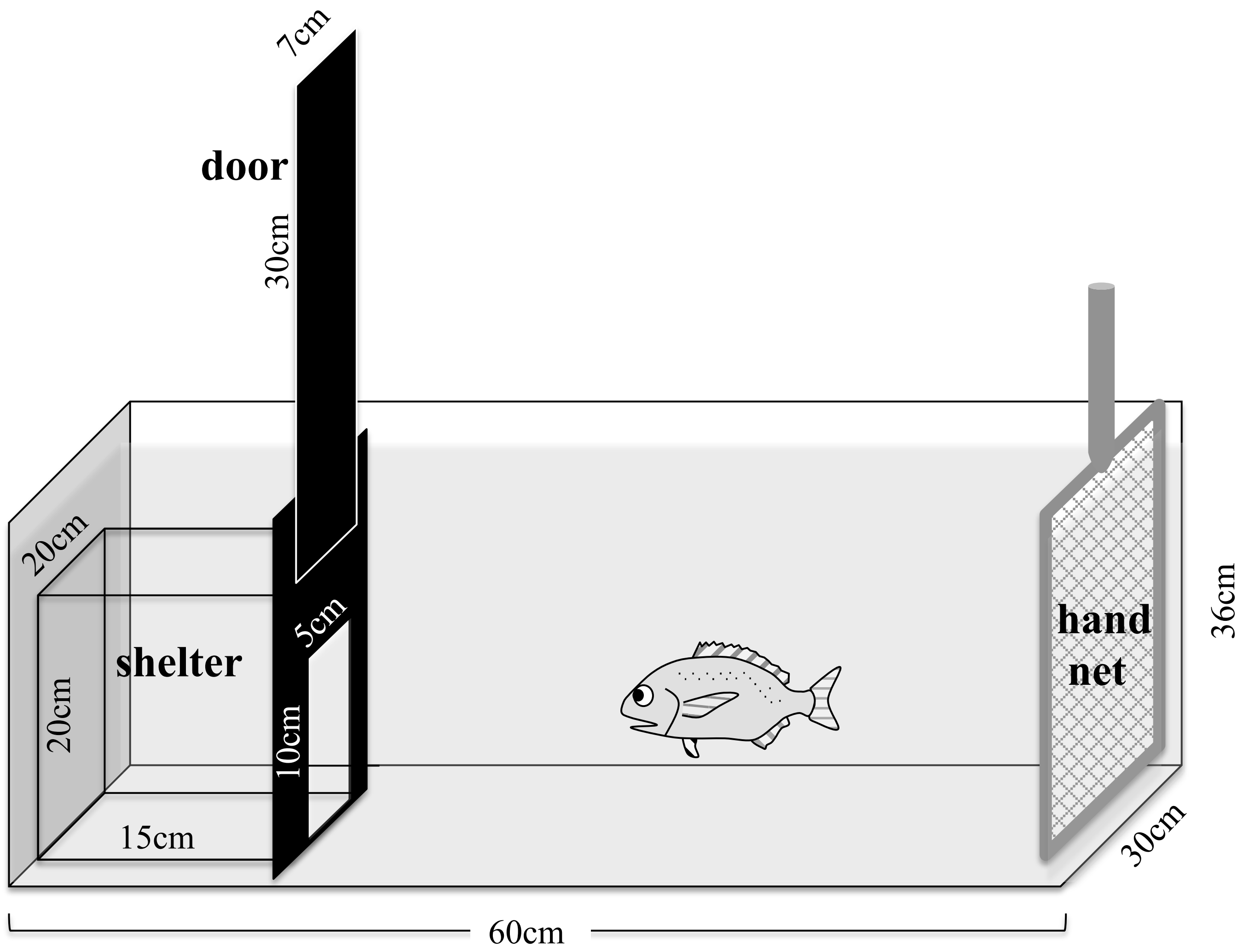

7cm
door
30cm
20cm
shelter
5cm
20cm
10cm
15cm
hand
net
36cm
30cm
60cm

Fig.3

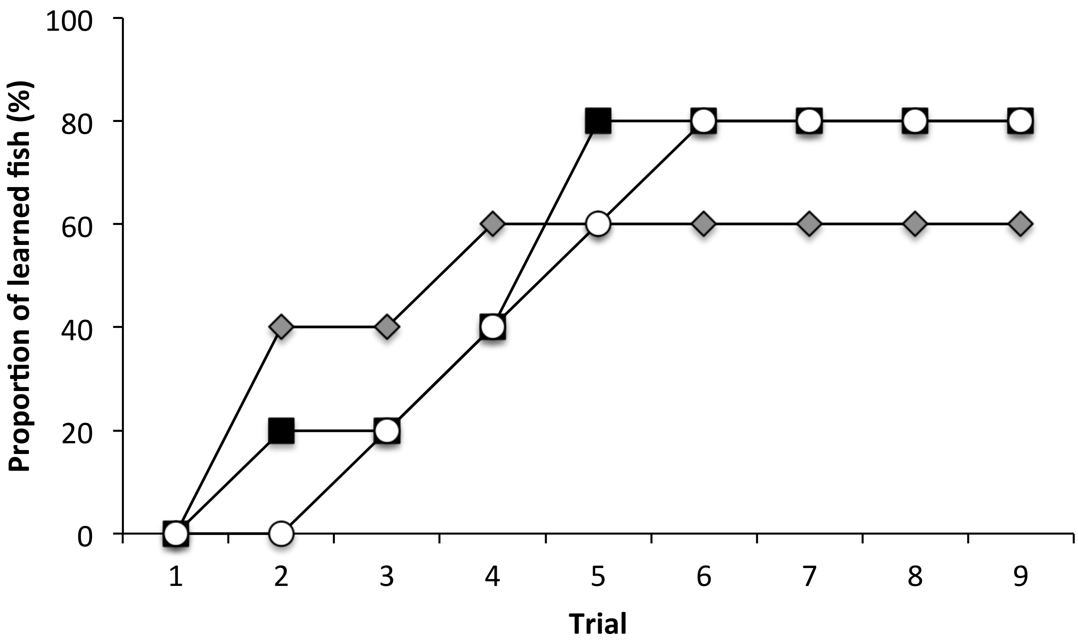
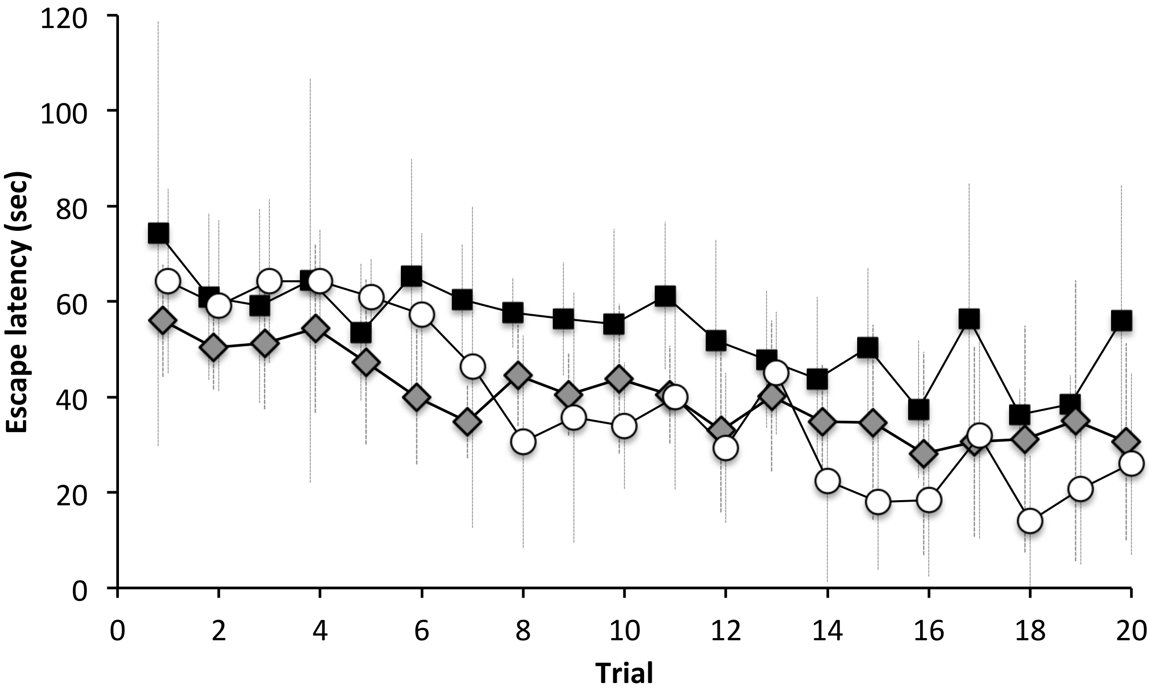


Fig.4



120
100
80
60
40
20
0
Escape latency (sec)
0
2
4
6
8
10
12
14
16
18
20
Trial

Fig.5