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

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3
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10 Abstract

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

25

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

28 Introduction

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

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

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

46 Whereas past studies have found that fish show certain responses to model fish in
47 video playback, to the best of our knowledge, no study has revealed whether fish can acquire
48 the information by observational learning of video model. The present study investigated the
49 observational learning of video model in *Pagrus major* for feed conditioning (Experiment 1)
50 and avoidance conditioning (Experiment 2). In each experiment, the observational trials were
51 established the following treatments: (i) live model observation treatment, where the observer
52 fish was allowed to directly observe behavior of a live fish in the adjacent model fish tank; and
53 (ii) video model observation treatment, where the observer fish observed fish behavior on video

54 playback. Their learning processes for these observational treatments were compared with (iii)
55 non-observing control fish in both experiments.

59 Materials and methods

61 **Experiment 1: Feed conditioning**

62 Fish

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

75 Experimental tank

76 Four transparent polypropylene experimental tanks (length $\times$ width $\times$ height: 30 $\times$ 20 $\times$ 20 cm)
77 were set in a room with a 12:12 h light/dark regime. These tanks were covered with black vinyl
78 sheets, and seawater was filled to a depth of 15 cm with circulating filtered seawater. Each tank
79 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 $\times$ 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 $\times$ 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 $\pm$ 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 $\times$ width $\times$ height: 60 $\times$ 30 $\times$ 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 $\times$ width $\times$ height: 15 $\times$ 20 $\times$ 20 cm) attached to a black polyethylene board (length $\times$ height: 30 $\times$ 20 cm) with a hole (length $\times$ height: 5 $\times$ 10 cm) was set in the experimental tank as a shelter (Fig. 3). A black PVC board (length $\times$ height: 7 $\times$ 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 $\times$ height: 30 $\times$ 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.

209 Analyses

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

219

220

221 Results

222

223 **Experiment 1: Feeding conditioning**

224

225 For the feed learning, the proportion of trained fish during nine test trials was not significantly
226 different among observational treatments (Cox proportional hazard model likelihood ratio test =
227 0.03 on 2 df, $P > 0.05$; Fig. 4). Neither live model nor video model observer were improved the
228 learning efficiency, compared to control fish.

229

230 **Experiment 2: Avoidance conditioning**

231

232 The escape latency of the control fish was significantly longer than that of the live model
233 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.

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279 References

- 280 1. Masuda R, Tsukamoto K (1998) Stock enhancement in Japan: review and prospective. Bull
- 281 Mar Sci 62: 337–358
- 282 2. Steingrund P, Ferno A (1997) Feeding behaviour of reared and wild cod and the effect of
- 283 learning: two strategies of feeding on the two-spotted goby. J Fish Biol 51: 334–348
- 284 3. Stunz GW, Minello TJ (2001) Habitat-related predation on juvenile wild-caught and
- 285 hatchery-reared red drum *Sciaenops ocellatus* (Linnaeus). J Exp Mar Biol Ecol 260: 13–25
- 286 4. Olla B, Davis MW (1989) The role of learning and stress in predator avoidance of
- 287 hatchery-reared coho salmon (*Oncorhynchus kisutch*) juveniles. Aquaculture 76: 209–214
- 288 5. Brown C, Day RL (2002) The future of stock enhancements: lessons for hatchery practice
- 289 from conservation biology. Fish Fish 3: 79–94
- 290 6. Wisenden BD, Klitzke J, Nelson R, Friedl D, Jacobson PC (2004) Predator-recognition of
- 291 hatchery-reared walleye (*Stizostedion vitreum*) and a field test of a training method using yellow
- 292 perch (*Perca flavescens*). Can J Zool Aqua Sci 61: 2144–2150
- 293 7. Coolen I, Bergen Yv, Day RL, Laland KN (2003) Species difference in adaptive use of public
- 294 information in sticklebacks. P Roy Soc Lond B Biol 270: 2413–2419
- 295 8. Takahashi K, Masuda R, Yamashita Y (2012) School for learning: sharing and transmission of
- 296 feeding information in jack mackerel *Trachurus japonicus* juveniles. Fish Sci 78: 269–276
- 297 9. Suboski MD, Templeton JJ (1989). Life skills training for hatchery fish: social learning and
- 298 survival. Fish Res 7: 343–352.
- 299 10. Brown C, Laland K (2001) Social learning and life skills training for hatchery reared fish. J
- 300 Fish Biol 59: 471–493
- 301 11. Kodric-Brown A, Nicoletto PF (1997) Repeatability of female choice in the guppy: response
- 302 to live and videotaped males. Anim Behav 54: 369–376
- 303 12. Clark DL, Stephenson KR (1999) Response to video and computer-animated images by the
- 304 tiger barb, *Puntius tetrazona*. Environ Biol Fish 56: 317–324

- 305 13. Robinson-Wolrath SI (2006) Video playback versus live stimuli for assessing mate choice in
306 a pipefish. *Environ Biol Fish* 75: 409–414
- 307 14. Trainor BC, Basolo AL (2006) Location, location, location: stripe position effects on female
308 sword preference. *Anim Behav* 71: 135–140
- 309 15. Gonçalves DM, Oliveira RF, Korner K, Poschadel JR, Schlupp I (2000) Using video
310 playbacks to study visual communication in a marine fish, *Salaria pavo*. *Anim Behav* 60: 351–
311 357
- 312 16. Trainor BC, Basolo AL (2000) An evaluation of video playback using *Xiphophorus helleri*.
313 *Anim Behav* 59: 83–89
- 314 17. Verbeek P, Iwamoto T, Murakami N (2007) Differences in aggression between wild-type
315 and domesticated fighting fish are context dependent. *Anim Behav* 73: 75–83
- 316 18. Arai T, Tominaga O, Seikai T, Masuda R (2007) Observational learning improves predator
317 avoidance in hatchery-reared Japanese flounder *Paralichthys olivaceus* juveniles. *J Sea Res* 58:
318 59–64
- 319 19. Webster MM, Laland KN (2008) Social learning strategies and predation risk: minnows
320 copy only when using private information would be costly. *P Roy Soc Lond B Biol* 275: 2869–
321 2876
- 322 20. Brown C, Laland KN (2011) Social learning in fishes. *Fish cognition and behavior* (Edited
323 by Brown C, Laland K and Krause J). Blackwell Publishing Ltd, UK 2011, 240–257
- 324 21. Johnson JB, Basolo AL (2003) Predator exposure alters female mate choice in the green
325 swordtail. *Behav Ecol* 14: 619–625
- 326 22. Takahashi K, Masuda R, Yamashita Y (2010) Ontogenetic changes in the spatial learning
327 capability of jack mackerel *Trachurus japonicus*. *J Fish Biol* 77: 2315–2325
- 328 23. Takahashi K, Masuda R, Matsuyama M, Yamashita Y (2012) Stimulus-specific development
329 of learning ability during habitat shift in pre to post-recruitment stage jack mackerel. *J Ethol* 30:
330 309–316

- 331 24. Takahashi K, Masuda R, Yamashita Y (2014) Development of observational learning during
332 school formation in jack mackerel *Trachurus japonicus* juveniles. Behav Process 103: 52–57
- 333 25. Duffy GA, Pike TW, Laland KN (2009) Size-dependent directed social learning in
334 nine-spined sticklebacks. Anim Behav 78: 371–375
- 335 26. Takahashi K, Masuda R, Yamashita Y (2014) What to copy: the key factor of observational
336 learning in striped jack (*Pseudocaranx dentex*) juveniles. Anim Cog 17: 495–501
- 337 27. Shashar N, Rosenthal GG, Caras T, Manor S, Katzir G (2005) Species recognition in the
338 blackbordered damselfish *Dascyllus marginatus* (Rüppell): an evaluation of computer-animated
339 playback techniques. J Exp Biol Ecol 318: 111–118
- 340 28. Baldauf SA, Kullmann H, Thunken T, Winter S (2009) Computer animation as a tool to
341 study preferences in the cichlid *Pelvicachromis taeninatus*. J Fish Biol 75: 738–746
- 342

343 Figure Captions

344

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

348

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

351

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

355

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

358

359 **Fig. 5** Average avoidance latency (s) in the course of 20 conditioning trials in Experiment 2:

360 control (■), live model observer (◆), and video model observer (○). Bars indicate standard

361 deviation (n = 6)

362

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5