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Individual Identification of Medaka, a Small Freshwater Fish, from the Dorsal Side Using Artificial Intelligence

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Abstract: Individual identification is an important ability for humans and perhaps also for non-human animals to lead social lives. It is also desirable for laboratory experiments to keep records of each animal while rearing them in mass. However, the specific body parts or the acceptable visual angles that enable individual identification are mostly unknown for non-human animals. In this study, we investigated whether artificial intelligence (AI) could distinguish individual medaka, a model animal for biological, agrarian, ecological, and ethological studies, based on the dorsal view. Using Teachable Machine, we took photographs of adult fish (n = 4) and used the images for machine learning. To our surprise, the AI could perfectly identify the four individuals in a total of 11 independent experiments, and the identification was valid for up to 10 days. The AI could also distinguish eight individuals, although machine learning required more time and effort. These results clearly demonstrate that the dorsal appearances of this small spot-/stripe-less fish are polymorphic enough for individual identification. Whether these clues can be applied to laboratory experiments where individual identification would be beneficial is an intriguing theme for future research.

Keywords: medaka; individual identification; artificial intelligence; Teachable Machine

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1. Introduction

Individual identification requires the ability to recognize subtle differences between conspecific animals (morphology, coloration, behavior, etc.). Humans primarily identify others by looking at their faces, so their facial recognition abilities are highly developed [1]. However, humans also have faces that are difficult to distinguish from one another. For example, it takes longer to identify an unfamiliar face than a familiar one [2], and it is more difficult to distinguish between faces of a different race than those of the same race [3,4]. This trend is seen not only in adults but also in children [5].

It is not only the face but also other parts of the human body which are polymorphic, and the technology that uses characteristic body parts for individual identification is called "biometric authentication". Various parts, such as fingerprints [6], the iris [7], the ear [8], and the tongue [9], can be used in biometric authentication. Biometrics is used not only in security but also for medical purposes, where it verifies patient identity [10] or manages patient data [11].

Individual identification of non-human animals is also important for humans in the fields of research and industry to keep records of each individual. Because many non-human animals look the same to non-experts, various methods have been developed for individual identification. In mice and rats, for example, there is a method called ear punching, which involves punching holes in the ears at different locations [12]. However, since it is often carried out without anesthesia, the punching causes pain to the experimental animals, and the pain could affect experimental results. The same could be said for other invasive methods, such as tagging, tattooing, or microchip implanting. For this reason, less invasive methods are more preferable, such as painting/shaving the fur or looking at the arrangement pattern of blood vessels in their ears [13].

Another alternative that has recently attracted attention is biometric authentication using artificial intelligence (AI), which has been practically used in domestic/wild animals such as sheep [14], cows [15], whales [16], and giant pandas [17]. However, it is still challenging in animals with little intraspecific polymorphism in coat/skin color.

Individual identification of aquatic animals, such as fish, is even more challenging. Neither oil- nor water-based inks can be used to mark their wet body surfaces, and they do not have fur that can be shaved (scales may be an alternative, but its removal leaves a wound). It can be even more difficult for small fish, such as zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), where invasive methods (e.g., implanting elastomers) could cause serious (often lethal) damage, although some protocols have been proposed [18–20].

Non-invasive methods have been applied for larger aquatic animals. For example, the Atlantic salmon (*Salmo salar*) [21] and smalleye stingray (*Megatrygon microps*) [22] can be identified according to the dot/spot patterns on their skin. Basking sharks (*Cetorhinus maximus*) can be identified by their fin shapes or by scars on their fins [23]. Such attempts for small fish are scarce [24], but they would be technically beneficial and ethically important.

The wild-type medaka is brown, and its skin has no dots, spots, or stripes. It is very difficult even for medaka experts to identify individuals by their appearances, except that their sex can be determined by their fin shape. Nevertheless, recent studies have demonstrated that medaka can distinguish individual medaka. Females prefer to mate with visually familiar males [25,26], and this identification depends on the anterior part of the male's body [27]. Therefore, there must be clear differences between medaka individuals that aid their social interactions (e.g., fighting and mating), although these differences are very difficult for humans to recognize.

Medaka has been widely used as a research model [28,29], and researchers often encounter situations where individual identification is desirable, e.g., via genotyping, growth measurement, or behavioral experiments. We also believe that individual identification enables a rigorous comparison of results. If individual identification is possible, experimental (e.g., treated) and control (e.g., untreated) groups can be kept under the same conditions (i.e., in the same aquarium).

In this study, we investigated whether a non-invasive and experimentally practical method for identifying medaka individuals could be developed by using a freely available and easily operable web tool, Teachable Machine ver. 2, provided by Google (https://teachablemachine.withgoogle.com, accessed on 8 June 2024). Teachable Machine enables "transfer learning", where the accuracy of AI can be increased more efficiently by utilizing existing learning models rather than learning from scratch. This means that less time and fewer sample data are required to train the AI, although there are potential risks if the existing models have little relevance to the new AI being created.

2. Materials and Methods

2.1. Photographing Medaka

We used an orange–red variant of medaka, the *b* mutant, which has a spontaneous mutation in the solute carrier family 45 member 2 (slc45a2) gene [30], purchased from a fish farm. The mutants lack visible black pigment cells (melanophores) in the skin and have no dots, spots, or stripes on the body surface. The adults were kept in a 50 $\times$ 34 cm container with a water level of about 30 cm until being used for experiments.

The fish to be individually identified were chosen from the stock, excluding individuals that were clearly different from the others (e.g., big/small, fat/thin, curved spine, or unusually dark/bright coloration). The fish were individually placed in a small acrylic case (either 5 $\times$ 5 cm or 3 $\times$ 3 cm) with a water level of about 1.0–1.5 cm. The bottom of the case was either matte black or matte gray in color, and all the sides were transparent. Light was provided by a fluorescent light on the ceiling.

A webcam (C615n [Logicool, Lausanne, Switzerland] or ELP-USB4KHDR01-MFV [Shenzhen Ailipu Technology, Shenzhen, China]) was connected via cable to a PC (OMEN by HP 880-000jp [Hewlett Packard, Palo Alto, CA, USA]) with Windows 10 Pro and fixed directly above the case at a height so that the photographing range only covered the bottom of the case (Figure 1). Photographing parameters (brightness, contrast, hue, sharpness, etc.) of the ELP-USB4KHDR01-MFV were adjusted and fixed using the AMCap application (ver. 9.23-build-300.6).

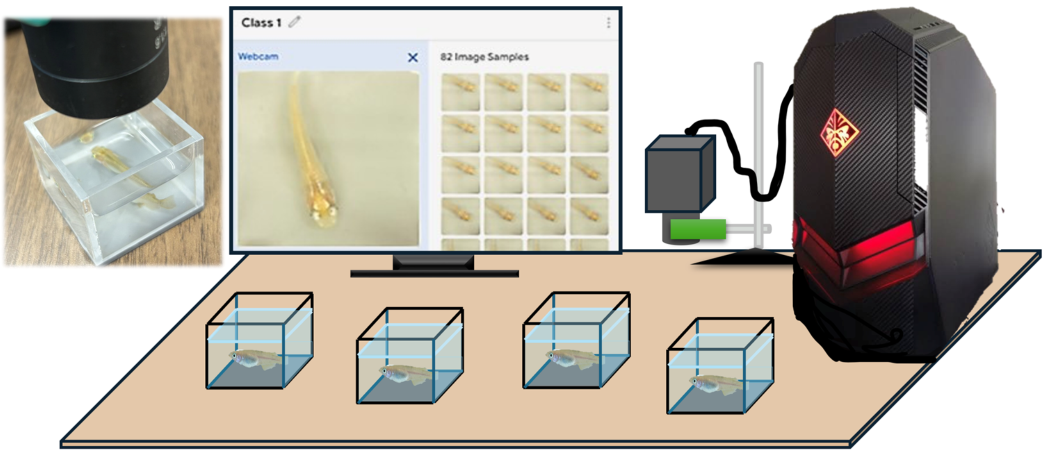


Figure 1. Diagram of the experimental setup. Medaka was placed in a small case with water, and a webcam connected to a PC was fixed directly above it at a certain height. We launched the Image Project of Teachable Machine and photographed the fish from the dorsal side. The images were used for training a model, and the model was tested in an identical setup by showing the fish individually to the AI through the webcam. The results of the tests were quantified according to the identification score defined in Table 1.

Table 1. Identification score for evaluating the accuracy of individual identification.

Point	Definition
5	The AI correctly identified the individual with a 100% accuracy rate.
4	The accuracy rate fluctuated but eventually reached100% with the correct individual.
3	The accuracy rate did not reach 100%, but it was highest with the correct individual.
2	The accuracy rate was highest with an incorrect individual but did not reach 100%.
1	The AI incorrectly identified an individual with a 100% accuracy rate.

2.2. Machine Learning

To train a model that could identify medaka individuals, we used the Image Project of Teachable Machine ver. 2 (https://teachablemachine.withgoogle.com (accessed on 8 June 2024)) operated on Google Chrome (ver. 110.0.5481.105). Teachable Machine processes color images of 224 $\times$ 224 pixels (best for most users) or grayscale images of 96 $\times$ 96 pixels (best for microcontrollers). We took color photographs of the four or eight fish to be individually identified at a speed of 24 frames per second (fps), which were used for training a model with the default settings (i.e., epochs: 50, batch size: 16, learning rate: 0.001).

After each training session, we tested if the AI could identify each fish correctly by presenting the fish individually. The accuracy of the individual identification was evaluated per fish using the identification score defined in Table 1, ranging from five points (perfect identification) to one point (complete misidentification). The training and testing (i.e., machine learning) were progressively carried out. A typical procedure using four fish was as follows: (1) select two fish and train/test a model, (2) repeat the training/testing until both individuals can be identified with identification scores of four points or higher, (3) take photographs of the third fish and train/test the model, (4) repeat the training/testing until all three individuals achieved four points or higher, (5) take photographs of the fourth fish and train/test the model, and (6) repeat the training/testing until all four individuals achieved five points. The procedure using eight fish was basically the same as that using four fish, i.e., starting with two fish, we added additional fish one by one after verifying that the model could distinguish all the fish.

2.3. Periodic Analysis of the Identification Score

In any model, the average identification score immediately after machine learning (on Day 0) was 5.0 $\pm$ 0.0 (mean $\pm$ standard error of the mean [SEM]; n = 4 or 8). After learning, each fish was transferred from the acrylic cases to small plastic cups (for individual identification by humans) and used for further testing on the following days (Days 1, 2, 3, 4, 10, and 20). The tests were carried out by transferring the fish from the cups to the cases (the cases used during machine learning were not necessarily used for the same fish) and presenting them individually to the AI via the webcam (i.e., the same process as that of the tests on Day 0). The results were evaluated using the identification score (Table 1). The average identification scores were expected to decrease in the following days, and significance (in comparison with the score on Day 0) was statistically tested using the Wilcoxon signed-rank test.

2.4. Orientation Analysis

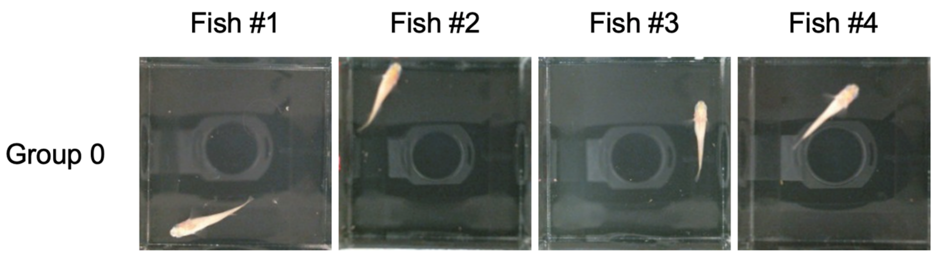
For ordinary machine learning (Experiments 1–3), we took photographs so that the body orientation of medaka was not biased. In Experiment 4, we intentionally photographed each medaka in a different body orientation (e.g., Fish #1 always faces 12 o'clock, whereas Fish #2 always faces 6 o'clock). To quantify the bias, we manually drew a straight line along the medaka body in each image (if the body was curved, we drew a line perpendicular to the line connecting the eyes) and measured its angle (direction of the head) with respect to the 3, 12, and 6 o'clock directions as 0, 90, and -90 degrees, respectively. We regarded the 9 o'clock direction as 180 (instead of -180) degrees. The angles were classified into 12 categories of 30 degrees each, and the percentages for each category were calculated and illustrated.

3. Results

3.1. Individual Identification of Four Fish—Experiment 1 (Group 0)

We selected four fish (two females and two males, with body sizes of 2.7 $\pm$ 0.04 cm [mean $\pm$ SEM]) of the orange-red variant [30], which are very difficult, if not impossible, for humans to identify individually. Each fish was placed into a small acrylic case with a black bottom measuring 5 $\times$ 5 cm (with a water level of about 1.5 cm) and photographed from the dorsal side using Teachable Machine with a generic webcam (C615n [Logicool]). The images captured were used to train a model for individual identification. Examples of these images are shown in Figure 2a.

Machine learning progressed step by step as follows, which is summarized in Figure 2b. First (column 1 in Figure 2b), we, respectively, captured 100 images for Fish #1 and #2, and these 200 images were used to train a model. It is worth noting that the images were captured at a speed of 24 fps, meaning that 100 images could be captured within 4.2 s within the shortest possible time. Subsequently, we tested the model by alternately presenting each fish. However, the AI consistently identified both fish as Fish #2 with a confidence level of 100% (i.e., identification scores of five points for Fish #2 but one point for Fish #1; see Table 1).



(**a**)

Group 0

Fish	the number of images used for training, and the identification score in testing													total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
#1	100	51		38	75		35	101		50			49	449
#2	100			53	51		97				60		28	389
#3			100	69	30		100					60		359
#4						160			100			31		291

5 points 4 points 3 points 2 points 1 point no test

(**b**)

Figure 2. Individual identification of four medaka in Experiment 1. (**a**) Examples of the images of four medaka (Fish #1–4 in Group 0), which were actually used for training a model. The black bottom of the case reflected the image of the webcam, which were not necessarily identical among the images and might disturb the machine learning process. The brightness, contrast, and focus of these images seemed not to be uniform. (**b**) The procedure and results of the machine learning. The training and testing progressed in 13 steps (see Results for details). Numbers indicate the number of images used for each training, and colors indicate the result (the identification score) in each testing.

Second (column 2), we additionally captured 51 images of Fish #1 and trained the model. The AI became capable of distinguishing between Fish #1 and #2, with identification scores of four and five points, respectively. Third (column 3), we additionally captured 100 images for Fish #3 and trained the model. However, this additional training decreased the overall identification scores, i.e., three, four, and three points for Fish #1, #2, and #3, respectively. Fourth (column 4), we trained the model with 38, 53, and 69 images for Fish #1, #2, and #3, respectively, but the AI still could not identify Fish #2. Fifth (column 5), we used 75, 51, and 30 images for Fish #1, #2, and #3, respectively, to train the model, and the AI became capable of distinguishing all three fish with identification scores of four points or higher.

Sixth (column 6), we trained the model with 160 images of Fish #4, which decreased the identification scores of Fish #2 and #3 to three points. After the seventh, eighth, and ninth trainings and testings (columns 7–9), the AI became capable of distinguishing all four fish with identification scores of four points or higher. We continued the machine learning process until the AI could distinguish all four fish with identification scores of five points, which was achieved after the thirteenth training/testing (columns 10–13). The average number of images per fish used to complete machine learning was 384.5 ± 37.5 per fish.

However, this individual identification did not last long; the identification scores decreased to one, two, five, and one points for Fish #1, #2, #3, and #4, respectively, on the fourth day after machine learning (Day 4). Although the average identification score of 2.3 ± 0.8 points (n = 4) was not significantly lower than that on Day 0 (5.0 ± 0.0 points; p = 0.102, Wilcoxon signed-rank test), it was clear that the AI was no longer able to identify the four fish individually.

3.2. Individual Identification of Four Fish—Experiment 2 (Groups 1–10)

We hypothesized that the AI's rapid inability to distinguish the medaka individuals within four days was unlikely due to morphological changes in the objects. Four days would have been too short for changes in size, shape, or color of the body to occur, although this did not rule out the possibility of minute changes recognizable by AI but not by humans, which could confuse identification. Instead, we suspected that unstable photographing conditions might bias machine learning and individual identification. For instance, the autofocus or auto-brightness functions of the webcam could unnecessarily vary photographing conditions, the reflection of the webcam image on the black bottom of the case (see Figure 2a) could vary in position/orientation, or the size of the case (i.e., 5 × 5 cm) could allow the fish (about 3 cm in length) to localize various positions, which might be biased among the fish or between the days. Hence, we reduced the size of the case to 3 × 3 cm and changed the color of its bottom to gray. Furthermore, we used a new webcam that allowed manual control and fixing of all photographing conditions (e.g., focus, brightness, contrast, and color/white balances) in the following experiments.

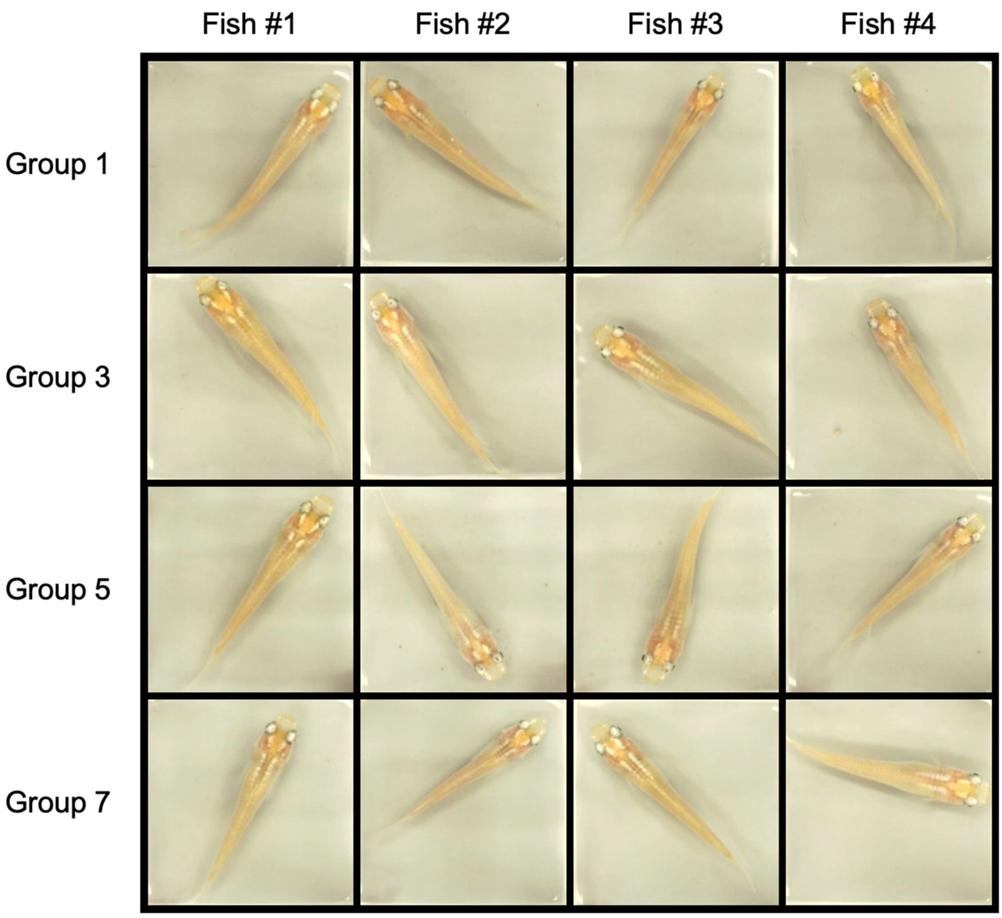
We conducted 10 independent experiments using a total of 40 fish (Groups 1–10), whose sex and body length are summarized in Table 2. Examples of the images taken under the new photographing conditions and used to train a model are shown in Figure 3a. Results of the training/testing are summarized in Figure 3b. The number of images per fish required to complete machine learning ranged from minimally 50 (Fish #4 in Group 6) to maximally 316 (Fish #3 in Group 3). The average was 137.9 ± 14.9, which was only 35.9% of that in Group 0 (384.5 ± 37.5; Figure 2b), suggesting that the new photographing conditions increased the efficiency of machine learning.

Table 2. Fish and number of images used for machine learning in Experiment 2.

Group	Sex (Female + Male)	Body Length (cm) *	The Number of Images per Fish *
1	1 + 3	3.2 ± 0.18	152.0 ± 14.0
2	0 + 4	3.2 ± 0.15	137.8 ± 26.9
3	0 + 4	3.1 ± 0.15	234.8 ± 37.0
4	1 + 3	3.1 ± 0.04	165.0 ± 13.2
5	0 + 4	3.1 ± 0.02	104.3 ± 12.3
6	0 + 4	3.2 ± 0.08	69.8 ± 7.8
7	0 + 4	3.1 ± 0.07	101.0 ± 5.7
8	2 + 2	3.1 ± 0.02	168.8 ± 13.5
9	1 + 3	3.1 ± 0.04	163.8 ± 21.6
10	3 + 1	3.1 ± 0.05	81.8 ± 7.2

* The mean ± SEM are shown.

Then, we investigated the duration of individual identification (Table 3 and Figure 4). On Day 4, seven out of nine groups (Group 10 was not tested on Days 3 and 4) maintained an average identification score of four points or higher, indicating accurate identification. The average scores of the remaining two groups (Groups 2 and 4) were more than three points, which can be considered as just barely accurate (refer to the definitions in Table 1). We also conducted tests on Days 10 and 20 for four groups (Groups 5, 6, 8, and 10), and all these groups retained identification scores of more than four points on Day 10. However, by Day 20, the scores had decreased to 1.8–3.8 points, which no longer ensured correct individual identification.



(**a**)

Group 1

Fish	training and testing									total
	1	2	3	4	5	6	7	8	9	
#1	21	60				47	40		32	200
#2	26		30			50			35	141
#3				65		48			24	137
#4					51			50	29	130

Group 2

Fish	training and testing							total
	1	2	3	4	5	6	7	
#1	32		30		20			82
#2	32		40		5	13		90
#3		64	18		55		73	210
#4				50	87		32	169

Group 3

Fish	training and testing													total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
#1	35			60				41			24			160
#2	40	25						68			29			162
#3			61					41	65	33	48	28	40	316
#4					75	71	57	14			43	41		301

Group 4

Fish	training and testing								total
	1	2	3	4	5	6	7	8	
#1	37	20			36		33	33	159
#2	45		15		21		40	53	174
#3				31	45		16	35	127
#4						96	49	55	200

Group 5

Fish	training and testing								total
	1	2	3	4	5	6	7	8	
#1	50	12		38	13		7	10	130
#2	55	35			10			27	127
#3			45		30				75
#4						56	29		85

Group 6

Fish	training and testing						total
	1	2	3	4	5	6	
#1	42	17					59
#2	45	25	17				87
#3				64	19		83
#4						50	50

Figure 3. *Cont.*

Group 7

Fish	training and testing									total
	1	2	3	4	5	6	7	8	9	
#1	42	22			7		20		9	100
#2	44		16		25		10		7	102
#3				51	24	21			21	117
#4								53	32	85

Group 8

Fish	training and testing											total
	1	2	3	4	5	6	7	8	9	10	11	
#1	30	28			42	23	49				12	184
#2	30	24			32		31		30		9	156
#3			41		21	37	24	27	10	43		203
#4				30	12	50	16				24	132

Group 9

Fish	training and testing											total
	1	2	3	4	5	6	7	8	9	10	11	
#1	45		17				34		14			110
#2	44		19				34	33	21		14	165
#3		48	14			34		35	39		60	230
#4				50	33			39	10	18		150

Group 10

Fish	training and testing								total
	1	2	3	4	5	6	7	8	
#1	40			19				16	75
#2	40	11		10	4	25		10	100
#3			40	22				28	90
#4							42	20	62

5 points 4 points 3 points 2 points 1 point no test

(**b**)

Figure 3. Individual identification of four medaka in Experiment 1. (**a**) Examples of images (Fish #1–4 in Groups 1, 3, 5, and 7), which were used for training models. It should be noted that medaka do not stay still while being photographed, and the set of images consists of fish in various orientations. Unlike those in Figure 2a, the bottom of the case did not reflect the image of the webcam, and all the photographs have uniform brightness, contrast, and focus. (**b**) The procedure and results of the machine learning. Numbers indicate the number of images used for each training, and colors indicate the result (the identification score) in each testing.

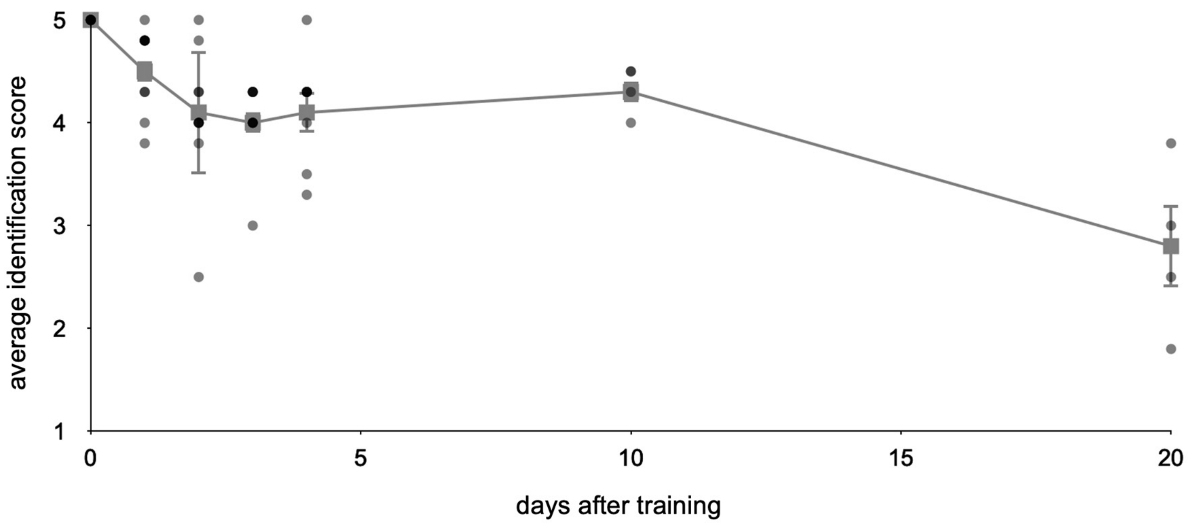


Figure 4. Daily changes in the averaged identification scores in Experiment 2. Each light-gray dot represents each identification score shown in Table 3 (dark gray indicates multiple overlapping dots). Gray squares with error bars indicate the average identification score for each day (n = 10 on Days 0–2, 9 on Days 3 and 4, or 4 on Days 10 and 20) with SEM. The decrease on Day 20 was apparent but not statistically significant according to the Wilcoxon signed-rank test (p = 0.068).

Table 3. Daily changes in the identification scores in Experiment 2.

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 10	Day 20
1	5.0 ± 0.0	4.3 ± 0.6	4.0 ± 0.4	4.0 ± 0.6	4.3 ± 0.4	n.t.	n.t.
2	5.0 ± 0.0	4.0 ± 0.4	4.0 ± 0.9	3.0 ± 0.8	3.3 ± 0.7	n.t.	n.t.
3	5.0 ± 0.0	3.8 ± 0.8	2.5 ± 0.8	4.0 ± 0.4	5.0 ± 0.0	n.t.	n.t.
4	5.0 ± 0.0	4.8 ± 0.2	5.0 ± 0.0	4.3 ± 0.6	3.5 ± 0.4	n.t.	n.t.
5	5.0 ± 0.0	5.0 ± 0.0	4.3 ± 0.6	4.3 ± 0.4	4.0 ± 0.5	4.3 ± 0.6	3.8 ± 0.8
6	5.0 ± 0.0	4.8 ± 0.2	4.3 ± 0.2	4.0 ± 0.4	4.3 ± 0.2	4.5 ± 0.3	1.8 ± 0.4
7	5.0 ± 0.0	4.8 ± 0.2	4.0 ± 0.6	4.0 ± 0.4	4.3 ± 0.4	n.t.	n.t.
8	5.0 ± 0.0	4.3 ± 0.4	4.0 ± 0.4	4.3 ± 0.4	4.3 ± 0.6	4.5 ± 0.3	2.5 ± 0.8
9	5.0 ± 0.0	4.8 ± 0.2	3.8 ± 0.6	4.3 ± 0.4	4.3 ± 0.6	n.t.	n.t.
10	5.0 ± 0.0	4.8 ± 0.2	4.8 ± 0.2	n.t.	n.t.	4.0 ± 0.5	3.0 ± 0.6

n.t.: not tested.

3.3. Individual Identification of Eight Fish—Experiment 3 (Groups 11 and 12)

The results of Experiments 1 and 2 demonstrated that the AI could effectively discriminate four medaka from their dorsal views. To investigate how the AI would perform with an increased number of fish, we conducted additional experiments using eight fish. The sex and body length of the eight fish in each group (n = 2) are summarized in Table 4.

Table 4. Fish and number of images used for machine learning in Experiment 3.

Group	Sex (Female + Male)	Body Length (cm) *	The Number of Images per Fish *
11	3 + 5	3.0 ± 0.05	376.4 ± 16.6
12	3 + 5	3.0 ± 0.09	150.5 ± 7.6

* The mean ± SEM are shown.

Although more steps (24 or 15 steps) and images (276.4 ± 16.6 or 150.5 ± 7.6 images per fish) were required to complete machine learning (Table 4 and Figure 5) compared to experiments using four fish (9.0 ± 0.6 steps and 137.9 ± 14.9 images per fish [n = 10]; Table 2 and Figure 3b), the AI successfully discriminated the eight medaka in both groups (11 and 12).

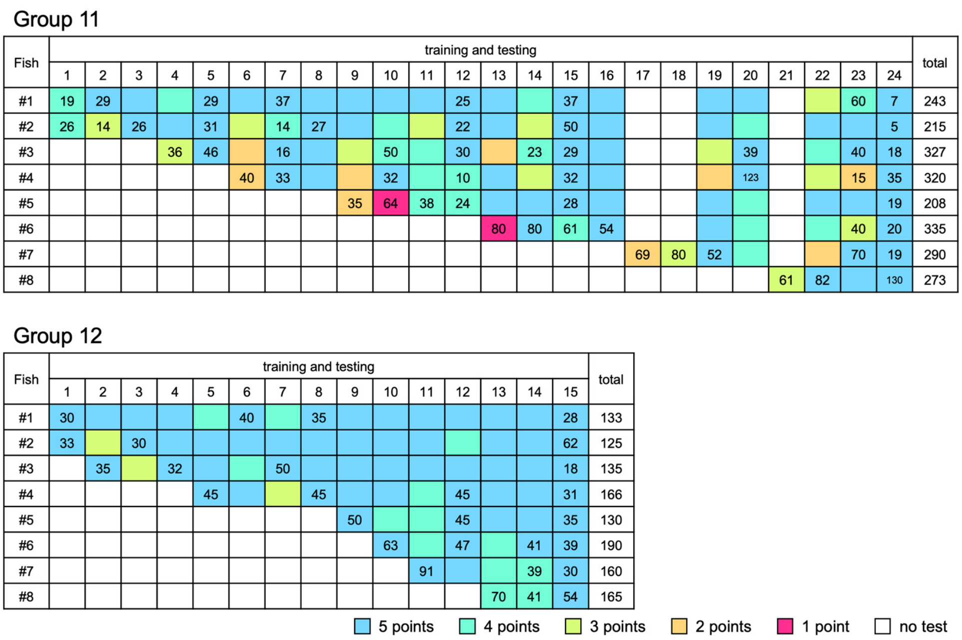


Figure 5. The procedure and results of machine learning in Experiment 3. Numbers indicate the number of images used for each training, and colors indicate the result (the identification score) in each testing.

However, the machine learning process took more than two hours, whereas it took less than 30 min for four fish. Moreover, the average identification scores of Group 12 (Group 11 was not tested) rapidly decreased to fewer than four points by Day 3 (Table 5), indicating that individual identification might not be reliably achievable.

Table 5. Daily changes in the identification scores in Experiment 3.

Group	Day 0	Day 1	Day 2	Day 3	Day 4
12	5.0 ± 0.0	4.8 ± 0.2	4.4 ± 0.4	3.4 ± 0.5	3.5 ± 0.4

3.4. Effects of Fish Orientation on Machine Learning—Experiment 4 (Groups 13 and 14)

During machine learning, we paid particular attention to photographing medaka so that their body orientations were not biased. This was because orientation biases could have hindered machine learning and individual identification; the AI might have learned and identified individuals based not on size, shape, color, or pattern, but on the orientation of the body. Indeed, when we intentionally introduced orientation biases in the photographs, the AI could not distinguish between four fish even after 13 or 12 steps of training/testing, using 236.0 ± 9.6 or 251.5 ± 8.8 images per fish in Groups 13 and 14, respectively (Figure 6a; Table 6). Unlike those shown in Figure 2b, Figure 3b, and Figure 5, the identification scores did not increase after each step.

Table 6. Fish and number of images used for machine learning in Experiment 4.

Group	Sex (Female + Male)	Body Length (cm) *	The Number of Images per Fish *
13	ND **	ND **	236.0 ± 9.6
14	2 + 2	2.4 ± 0.05	251.5 ± 8.8

* The mean ± SEM are shown. ** no data.

Figure 6b shows the learning curves obtained from machine learning in Groups 6, 10, and 14. Teachable Machine divides the photographed images into training samples (85%) and test samples (15%), using the former for machine learning and the latter for model testing. The horizontal axis of the graph indicates the number of learning epochs, and the vertical axis indicates the accuracy in testing the samples. As shown, the AI in Groups 6, 10, or 14 accurately identified the test samples. Therefore, the reason the identification score could not reach five points in Group 14 (Figure 6a) should not be attributed to failed machine learning. Because the AI had only learned to recognize medaka facing a certain direction, it could not identify the same fish facing different orientations as the same individual.

The actual orientation bias is visualized in Figure 6c,d. In Group 14, the bias was apparently greater (note that Fish #1 always faced 7–11 o'clock, whereas Fish #4 mostly faced 4–6 o'clock) than that in Groups 6 or 10. The orientations were not strictly even among fish in the successful groups, and it could even be said to be biased for Group 6, where Fish #4 never faced 6–9 o'clock, indicating that this level of orientation bias is acceptable for machine learning.

Group 13

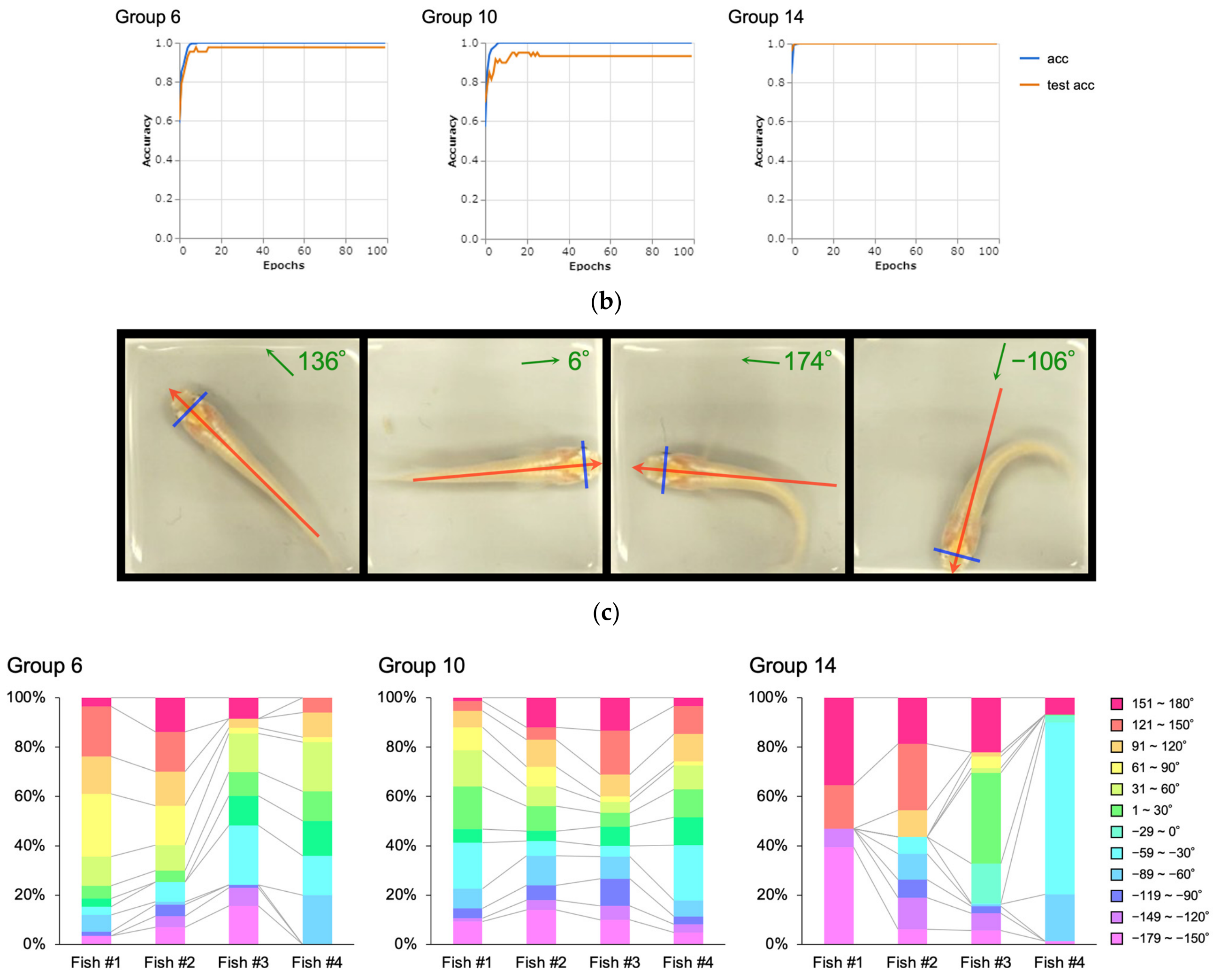
Fish	training and testing													total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
#1	31	28	60				29			52	20	28		248
#2	30	44	23		25	28	35			32	30	14		261
#3				41	26	23	33	27		26	24	20		220
#4									44	78	28	40	25	215

Group 14

Fish	training and testing												total
	1	2	3	4	5	6	7	8	9	10	11	12	
#1	31	27	33			41			53	40	22	24	271
#2	38	29	33			39			30	41	18	31	259
#3				41	42	30	39		23	28	27	23	253
#4								53	27	42	31	70	223

5 points 4 points 3 points 2 points 1 point no test

(**a**)



(**d**)

Figure 6. Individual identification of four medaka in Experiment 4. (**a**) The procedure and results of machine learning. Numbers indicate the number of images used for each training, and colors indicate the result (the identification score) in each testing. Note that the identification scores rarely reached five points, indicating that the AI trained with biased orientations could not identify fish in different

orientations. (**b**) Learning curves in Groups 6, 10, and 14 produced by Teachable Machine. (**c**) Measuring the body orientation in the images used for the machine learning. Regardless of whether the body was straight (left) or curved (right), we drew a line (red) perpendicular to the line connecting the eyes (blue) and measured the angle of the red line (green). (**d**) Quantification of the orientation bias. The body angles in all the learned images (as exemplified in (**c**)) are categorized and shown as percentages. Machine learning was successful in Groups 6 and 10 (Figure 3b), but not in 14 (Figure 6a). If there was no bias, the percentage proportions should have been identical among Fish #1 to 4 in each group and all the connecting lines (gray) should have appeared horizontally.

4. Discussion

4.1. Individual Identification from the Back of Medaka

The most important finding in this study is that the AI could perfectly discriminate up to eight medaka based solely on their dorsal appearances (Figure 5). Strictly speaking, however, we cannot know exactly which parts of the image (including the background) the AI trained with Teachable Machine relies on for accurate discrimination. Therefore, it would be more appropriate to state that the AI is "classifying images" rather than "discriminating fish", although both descriptions are essentially the same for the purpose of this study.

Given that the AI consistently succeeded in individual identification (Groups 0–12), except when trained with biased information (Groups 13 and 14), it suggests that there are distinct differences (e.g., sizes, shapes, colors, and/or patterns) on the dorsal side of each individual. However, the precise features the AI uses for identification are not clear from the current results, and this remains an interesting subject for future research.

It is also unknown whether medaka themselves are aware of and utilize these dorsal differences for individual identification. Unlike terrestrial animals (including humans) that move primarily in two dimensions on the ground, fish (and birds) that move in three dimensions in water (and air) should have more opportunities to view other animals from above and below (i.e., the dorsal and ventral sides). For such animals, it would be unreasonable to assume that individual identification is only possible when they see others horizontally. Indeed, it is likely that top and bottom views could contain comparable (or even more) visual information than horizontal (front/side/rear) views, particularly in flat-shaped animals, such as rays [22,31]. This might also be true for fish with more rounded bodies, such as pufferfish, frogfish, and monkfish.

The body of medaka is thin (Figures 2a, 3a and 6c), and previous studies on individual identification did not seem to focus on dorsal information [23,24,29]. In those studies, visual contact was typically achieved by partitioning an aquarium with transparent walls or by placing fish in a transparent container with an open top, which was floated in an aquarium. Therefore, it was surprising that fewer than 100 dorsal photographs per fish (which could be taken in five seconds) were sufficient for completing machine learning (Figure 3b). We had initially expected to rely on side views, which are broader and might contain more information for individual identification than dorsal views. Exploring whether side views enable more efficient machine learning and longer-lasting individual identification would be another interesting direction for future research.

Additionally, it would be worthwhile to investigate whether medaka actually use dorsal views for individual identification. This could be tested by examining if familiarization [25,26] occurs when medaka are shown only the dorsal side.

4.2. Practical Aspects of the Present Study

The present study demonstrated that there are apparent intra-specific polymorphisms in the dorsal view of medaka, enabling the AI to identify fish individually (Figures 2b, 3b, and 5). However, this individual identification by the AI cannot immediately aid our research endeavors, such as genotyping or behavioral experiments. The main issues are (1) the time and effort required for machine learning and (2) the limited validity period for individual identification. The two-hour training/testing period needed to distinguish eight fish for only a few days (Figure 5 and Table 5) is not cost-effective.

For instance, we often carry out genotyping of mutants at the scale of 32 or 48 fish. Given that the time required for machine learning increases rapidly with the number of fish (it took only one minute for two fish; see Figure 2b, Figure 3b, and Figure 5), it could take a very long time (days?) to train the AI to identify 32 or 48 fish. This study demonstrated that it is not only the photographing conditions (see Experiments 0 and 1), but also the photographing skills of the trainer (see Experiments 1 and 4), that critically affects the efficiency of machine learning. Although more optimized conditions and a more skilled trainer could reduce the time and effort required for machine learning, some technological innovations would be needed to replace the traditional method of isolating individuals into small, numbered cups.

Regarding the validity period for individual identification, the maximum of 10 days for four fish (Table 3 and Figure 4) might be useful for short-term and small-scale experiments. The inability to maintain identification for 20 days could be attributed to body changes due to normal growth or mental stress (which could affect food intake or body-color regulation) in new (isolated) environments, which differ from the stock container. New environments could also induce physiological or morphological adaptations in body color, as pigment cells (chromatophores) in the skin are known to respond rapidly to background colors [32]. For example, black melanophores disperse and increase, while white leucophores aggregate and decrease against dark backgrounds. Such body-color adaptations could explain the decreased identification scores on Day 20 (although the fish used in this study, the *b* mutants, largely lack melanophores). Therefore, if the fish could be returned to the stock container after machine learning, these body-color changes might be minimized, potentially extending the validity period.

A recent study has shown that adult medaka can individually be distinguished based on the melanophores on their heads [24]. The micro-distribution of melanophores can remain stable for up to 34 weeks, which would be sufficiently long for many experiments requiring individual identification. A promising direction for individual identification using Teachable Machine could involve machine learning of the melanophore distribution pattern on the head, using a high-magnification camera. This approach would require developing specialized image-capture conditions and techniques for accurate individual identification, similar to what was performed in this study. Using a strain with color patterns, such as the variegated orange–red strain (*B′*) [33], instead of a patternless strain, might also be effective if the patterns are polymorphic and stable. These individual identification methods using Teachable Machine could save researchers from manually matching images to objects, especially when a large number of individuals need to be identified quickly.

5. Conclusions

This study has demonstrated that AI, specifically using Teachable Machine, can accurately identify individual medaka from dorsal view images. The AI was able to perfectly distinguish up to eight individuals, provided the training images were not biased in terms of body orientation. This finding suggests the presence of clear, distinguishable features on the dorsal side of medaka that the AI can learn to recognize.

Optimizing the photographing conditions and developing specialized image-capture techniques, such as focusing on the melanophore distribution pattern on the head, could enhance the efficiency and longevity of individual identification. Additionally, using medaka strains with stable and polymorphic color patterns might improve identification reliability. While there are challenges to overcome, AI-based individual identification holds promise for improving the efficiency of research involving large numbers of animals, reducing the need for manual identification and enabling more accurate tracking of individuals over time.

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