Individual Identification of Japanese Giant Salamanders (*Andrias japonicus*) and Detection of Their Hybrids by Image Recognition Using Deep Learning

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2024

TABLE OF CONTENTS

CHAPTER 1 General introduction

1.1 Background

1.1.1 Estimating abundance of wildlife populations

Estimating the abundance of wildlife population size is a fundamental issue in animal ecology (Aubry & Francesiaz, 2022). The measuring abundance enables population trends to be assessed and provides information for evaluating anthropogenic and natural threats, such as disease, over-harvesting, or changing land use patterns (Fuentes et al. 2015). The most classical way to estimate abundance is the direct count. For example, all individuals in a given area can be counted from populations of plants and sessile invertebrates such as barnacles. However, direct counts are unsuitable for surveys over large areas and most animals because they move inconspicuously and avoid humans (Elphick 2008). Therefore, the sampling methods from the population are widely used. One example is the quadrats method (Krebs, 2001). The general procedure in this technique is to count all individuals in certain quadrats and then extrapolate the average count to the whole area. Another example is the capture-recapture method (Begon et al. 2009). This technique is also called the mark-recapture method, and the following steps are used to estimate the number of individuals, especially for mobile animals. First, individuals are randomly captured from the population and marked. Second, the marked individuals are released to the population. Finally, the target species is randomly captured again, and the population size is estimated from the proportion of marked individuals in this capture. Many ecological studies depend on sampling methods with individual identification to estimate abundance because a direct count of all individuals is impractical.

1.1.2 Individual identification of animals

Individual identification of animals provides the opportunity to answer ecological and evolutionary questions (Dębicki et al., 2021). For example, does a sea turtle return to its natal beach to lay eggs? Is the beak length of finches in the Galápagos inherited from parents to offspring? These questions related to life history and natural selection can be answered by individual variation data obtained from recognizing individuals (Grant & Grant, 2002; Richardson et al., 1999). Moreover, individual-level events such as birth, reproduction, and death are related to fitness (Reid et al., 2003; Ross et al., 2021) and thus ultimately determine the genes passed to the next generation (Ross et al., 2022). In addition, individual identification is essential for estimating reliable abundance of animals for their management and conservation (Crouse et al., 2017; Koivuniemi et al., 2019). For example, incorrect individual identification is an issue for endangered species because it leads to biased estimation (Johansson et al., 2020). From this background, methods to identify specific individuals within a population are required.

1.1.3 Individual identification by invasive methods

In general, there are two approaches to animal identification: invasive and non-invasive methods. An invasive method involves capturing animals and applying artificial markings, such as physical tags, tattoos, or removed tissue (Anderson et al., 2010; Davis & Ovaska, 2001; Gannon et al., 2007). Physical tags are usually made of metal or plastic, and researchers can identify individuals from the numeric and alphabetic codes on the tags. This method uniquely identifies each individual, and tags can be attached to many individuals because small tags are low cost. Therefore, many techniques have been developed for tagging individuals in wildlife studies. After capturing animals, tags can be attached in various ways depending on the target species, such as ear tags on mammals (Gannon et al., 2007), leg bands on birds (Hobson, 2008), and dart tags on fish (Runde et al., 2022). Technological advances have led to the application of telemetry and GPS satellite tags; these tags enable an individual to be tracked for a long period with high accuracy. However, physical attachment of tags sometimes pose problems from the perspective of objective data acquisition and ethical aspects (Zemanova, 2020). For example, a previous research about such device effects found that a tag attachment can lead to the negative impacts on animals relating to behavior, reproduction, and survival rate (Gannon et al., 2007; Hobson, 2008; Soulsbury et al., 2020). In addition, the statistical model assumes that tagged individuals represent the population (Iijima, 2020; McCarthy & Parris, 2004); therefore, tagging effects on animals will lead to biased estimates of population parameters, such as under- or overestimates of population sizes. Furthermore, it is difficult to attach physical tags when the body size is small (Gamble et al., 2008). For small animals, such as rodents, reptiles, and amphibians, toe clipping is a classic method of marking individuals (Davis & Ovaska, 2001). In this way, researchers can identify individuals by the unique combination of toes removed (Caorsi et al., 2012). Toe clipping is a quick, easy, and inexpensive method; therefore, it has been used for a long time, and some studies indicate that it is harmless to animals (Funk et al., 2005). However, several studies have reported a reduced probability of recapture (McCarthy & Parris, 2004), survival rate (McCarthy et al., 2009), and growth rate (Davis & Ovaska, 2001) for toe-clipped animals. Moreover, there is increasing awareness and consideration of animal welfare in research, and scientists are required to minimize stress on organisms for ethical reasons (Soulsbury et al., 2020; Zemanova, 2020). This issue has also been raised by ecologists; for example, eminent theoretical ecologist Dr. Robert May stated, “We need to think more carefully about some present practices” (May, 2004). Furthermore, these methods require capture of the target species. The capture event itself is stressful for wild animals (Wilson & McMahon, 2006), and capture-related mortality and injury have been reported (Soulsbury et al., 2020). Considering these effects, an invasive identification method is undesirable or even prohibited, especially for animals with small populations, such as endangered species (Gannon et al., 2007).

1.1.4 Individual identification by non-invasive methods; DNA analysis

Non-invasive individual identification methods, namely the collection of biological samples without having to catch or disturb the animal (Taberlet et al., 1999), are valuable and attractive to ecologists because this approach minimizes impact on the target species (Lindsjö et al., 2016; Zemanova, 2020). In recent years, molecular genetic methods to identify individuals from DNA samples have become popular in wildlife biology (Hohenlohe et al., 2021). This is because genetic samples, such as hair, feces, and feathers, provide DNA that can be utilized to identify individuals without capturing or observing the target species (Carroll et al., 2018). Therefore, many researchers have applied this approach to individual identification for various species, including vertebrates (Chambers et al., 2014; Taberlet et al., 1997; Walker et al., 2020) and invertebrates (Emery et al., 2001). In addition, molecular analysis can reveal the genetic structure, gene flow, relatedness, and sex ratio of the population (Dutta et al., 2012). However, there are challenges to using genetic analysis for individual identification. First, this approach can only be applied when sufficient genetic markers (e.g., microsatellite markers) exist in the population, because if the target species does not have many markers, it results in low statistical power to differentiate between individuals (Morin et al., 2004). Second, fresh tissue is needed for accurate DNA sequencing (Peralta et al., 2020). For example, DNA degradation is affected by factors such as exposure time, DNA type (e.g., mitochondrial or nuclear DNA), and locus length (DeMay et al., 2013). In studies focusing on carnivores, nuclear DNA was successfully amplified by ≥70% through day 21 in winter but declined to <50% by day 7 in summer (Lonsinger et al., 2015). Third, the researcher must wait until the target species produces samples such as feces and feathers, making this sampling strategy opportunistic (Caudron et al., 2007; Wedrowicz et al., 2013). Finally, although genetic analysis has become cheaper, it is still expensive for large sample sizes and some techniques; therefore, applying this approach over extensive areas is challenging (Sittenthaler et al., 2020).

1.1.5 Individual identification by non-invasive methods; Photo-identification

Historically, researchers have used natural markings to identify individuals because some animals have unique coat patterns, such as tigers (*Panthera tigris*) and cheetahs (*Acinonyx jubatus*), that can act as a “fingerprint” (Karanth & Nichols, 1998; Kelly, 2001). Although sketches of individual features are available (Donnelly et al., 1994), photo-identification is a popular technique, and researchers can identify individuals by matching photos with those on a database (Speed et al. 2007). This approach has long been used in population parameter assessments for cetaceans (Hammond et al. 1990), and it is now applied to many species, such as other marine mammals (Vincent et al., 2001), terrestrial mammals (Hiby et al., 2009), reptiles (Knox et al., 2013), amphibians (Gould et al., 2021; Hoque et al., 2011), fish (Holmberg et al., 2009), and even insects (Romiti et al., 2017). Recent technical advances in camera traps allow wildlife photographs to be obtained in the field, and the use of camera traps in ecological research has grown exponentially (Steenweg et al., 2017). Because this method is inexpensive, cameras are easy to install, and continuous monitoring can be performed over long time scales, camera traps are among the most used devices in recent animal ecology research (Tuia et al., 2022). However, one of the drawbacks of camera traps is the huge amount of data generated, which remains a bottleneck in camera trap studies because data processing is time-consuming and error-prone (Glover-Kapfer et al., 2019; Harris et al., 2010; Weinstein, 2018). To address this issue, scientists have sought the help of non-specialists who perform data handling (Thel et al., 2021). This involvement of volunteers in research, called citizen science, has made substantial contributions to science (Kobori et al., 2016). In addition, ubiquitous access to the internet and information technologies has created new opportunities for citizen science projects to invite large numbers of the public globally (Hochachka et al., 2012). For example, the Zooniverse (www.zooniverse.org), the world’s most well-known citizen science platform, provides researchers access to millions of volunteers (Willi et al., 2019). Snapshot Serengeti is one of the projects on this platform; over 28,000 registered users have contributed 10.8 million classifications from camera trap images taken in Serengeti National Park, Tanzania (Swanson et al., 2015). Although citizen science is valuable, some scientists are often skeptical of the data quality because volunteer ability is variable, which can be a source of bias (Dickinson et al., 2010; Kelling et al., 2015). Furthermore, many volunteers are needed when there are numerous images; however, most citizen science projects are not able to recruit a large number of volunteers (Norouzzadeh et al., 2018).

1.1.6 Machine learning

To overcome the challenges of manual photo-identification, computer vision techniques began to be applied to identify individual animals in the 1990s (Schneider et al., 2019). Computer vision is a field of computer science that deals with gaining insights from digital images (Wäldchen & Mäder, 2018). This technology aims to automate human visual systems, such as detecting objects in images, and perform feature extraction and classification based on specific algorithms (Jordan & Mitchell, 2015). For example, Whitehead (1990) developed computer-assisted methods for identifying individual sperm whales (*Physeter macrocephalus*) using fluke images. In this method, the user observes images of sperm whale tail fins and records the location of a unique characteristic, such as a nick or fluke. The software then calculates the similarity between images using a proprietary algorithm and returns the most similar individual. This approach identified individuals with 32% accuracy from images collected from the Galápagos Islands in Ecuador. Similar methods have been applied to other animals, including cheetahs (Kelly, 2001), whale sharks (Arzoumanian et al., 2005), elephants (Ardovini et al., 2008), tigers (Hiby et al., 2009), polar bears (Anderson et al., 2010), zebras (Lahiri et al., 2011), and chimpanzees (Loos & Ernst, 2013). However, conventional machine-learning-based computer vision has some challenges. First, these systems require substantial preprocessing, such as image cropping and brightness and contrast enhancement, which can be time-consuming and cause errors (Kelly, 2001; Whitehead, 1990). Second, the researcher requires high programming skills and knowledge of the target species. Image data are composed of millions of pixels with associated color information (Wäldchen & Mäder, 2018). The machine learning approach requires a suitable algorithm that extracts the features (e.g., whale tails and tiger stripes) for classification from images. For example, Arzoumanian et al. (2005) used an astronomy algorithm for recognizing star patterns to analyze the characteristic flank spot patterns of whale sharks (*Rhincodon typus*). Bendik et al. (2013) used the scale-invariant feature transform (SIFT) algorithm to identify individuals of Jollyville Plateau salamanders (*Eurycea tonkawae*). Since the features used for image classification depend on the animal, the algorithm should be selected according to the target species. The design of algorithms requires biological knowledge as well as programming skills. Therefore, feature extraction has been performed by specific algorithms created by experts, and sometimes those processes are subjective (Wäldchen & Mäder, 2018). Finally, the low generalizability is a limitation of this approach (Hiby et al., 2009). Although experts have attempted some feature engineering methods to classify animals, once a feature extraction algorithm has been designed for a species, it is not transferable to other species (Schneider et al., 2019).

1.1.7 Deep learning

Recently, deep learning, a branch of machine learning, has revolutionized almost all areas of society and science (Jordan & Mitchell, 2015). This approach is based on findings from a study on artificial neurons that examined how neurons in the biological brain process information (McCulloch & Pitts, 1943). Neural networks, one of the machine learning algorithms, were created to mimic biological neurons, and this network structure was inspired by the human brain (LeCun et al., 2015). A neural network consists of three types of layers: an input layer, one or more hidden layers, and an output layer. Deep learning addresses the complexity of information by increasing the number of hidden layers (Borowiec et al., 2022) and has attracted attention in the field of computer vision (Schmidhuber, 2015). It has some differences from conventional approaches to feature extraction (Guo et al., 2016). Deep learning automatically extracts features from input images, whereas conventional machine learning requires an operator to define features (Najafabadi et al., 2015), which leads to the problem of human biases. Deep learning overcomes this problem by interpreting large amounts of data, allowing the automatic extraction of features (Suzuki, 2017). Individual identification using deep learning has also been applied to many ecological studies. For example, Schofield et al. (2019) used a deep convolutional neural network approach to wild chimpanzees (*Pan troglodytes verus*) and identified 23 individuals with an accuracy of 92.5% from a 14-year dataset. Hou et al. (2020) applied the VGGNet model to recognize giant pandas (*Ailuropoda melanoleuca*), accurately identifying more than 90% of individuals from face images. Clapham et al. (2020) applied the same approach to brown bear (*Ursus arctos*) images obtained from national parks and identified 132 individuals with an accuracy of 83.9%. Similar studies have been conducted on green turtles (*Chelonia mydas*; Carter et al., 2014), great white sharks (*Carcharodon carcharias*; Hughes & Burghardt, 2017), lemurs (*Lemuroidea spp*.; Crouse et al., 2017), great tits (*Parus major*; Ferreira et al., 2020), killer whales (*Orcinus orca*; Bergler et al., 2021), mugger crocodiles (*Crocodylus palustris*; Desai et al., 2022), harbor seals (*Phoca vitulina*; Birenbaum et al., 2022), snow leopards (*Panthera uncia*; Bohnett et al., 2023), and slow lorises (*Nycticebus bengalensis*; Guan et al., 2023). Although deep learning has been used to identify individuals for various animals, no study has applied this technique to amphibians, which are the most at risk of extinction among the vertebrates (Cordier et al., 2021).

1.1.8 Japanese giant salamander

The Japanese giant salamander (*Andrias japonicus*) is one of the largest salamander species in the world, with a maximum body length of 1.5 m, and is endemic to Japan (Okada et al., 2015). This species spends its entire life in the water and rarely uses the terrestrial environment. *A. japonicus* are nocturnal predators, preying on shrimps, crabs, other amphibians, and fish at night. The matured males, called den masters, occupy burrows in the riverside from late August to mid-September. Mating begins when a female enters the burrow, where the den master guards against other males. They are residents in their home range during the non-breeding season but migrate upstream in the breeding season. The population of this species is declining because of habitat fragmentation due to artificial structures and the loss of suitable breeding habitats. Therefore, they are protected as a National Natural Monument under the Law for the Protection of Cultural Properties and also classified as a vulnerable species by the Ministry of the Environment's Red List. Currently, passive integrated transponder (PIT) tags are inserted into the body to identify individuals, but this method requires their direct capture. This species has spots all over its body, and these patterns vary among individuals. Thus, combining images and deep learning to identify *A. japonicus* individuals based on spot patterns is a feasible identification method that would minimize stress on animals. In addition, a similar approach may identify hybrids; hybridization between the non-native Chinese giant salamander (*Andrias* cf. *davidianus*) and *A. japonicus* has recently become a factor contributing to the decline of *A. japonicus*. The existence of hybrid individuals has already been confirmed in eight prefectures (Kyoto, Mie, Nara, Shiga, Okayama, Hiroshima, Aichi, and Gifu), making the early detection and capture of hybrid individuals in the field necessary. Individuals of potential hybrids are currently being captured on the basis of visual information using spot patterns. Although the identification of hybrids based on visual information is limited to specialists, deep-learning-based computer vision could enable the detection of hybrids by the general public.

1.2 Aim of this thesis and research questions

The aim of this thesis is to clarify whether computer vision technology based on deep learning can be applied to identify individuals of *A. japonicus* from images. Although some studies have attempted to identify individuals by conventional machine learning methods, such as SIFT (Bendik et al., 2013; Gamble et al., 2008; Matthé et al., 2017), no studies have applied deep learning to amphibians. This thesis is the first study to apply deep-learning-based automatic photo-identification to the individual identification of amphibians. In addition, I test whether the same approach can be utilized to identify hybrid individuals between native *A. japonicus* and non-native *A.* cf. *davidianus*. Finally, I tested whether deep learning techniques are practical for individual identification in actual ecological investigations. To achieve these objectives, I examine the following three questions (Figure 1.1).

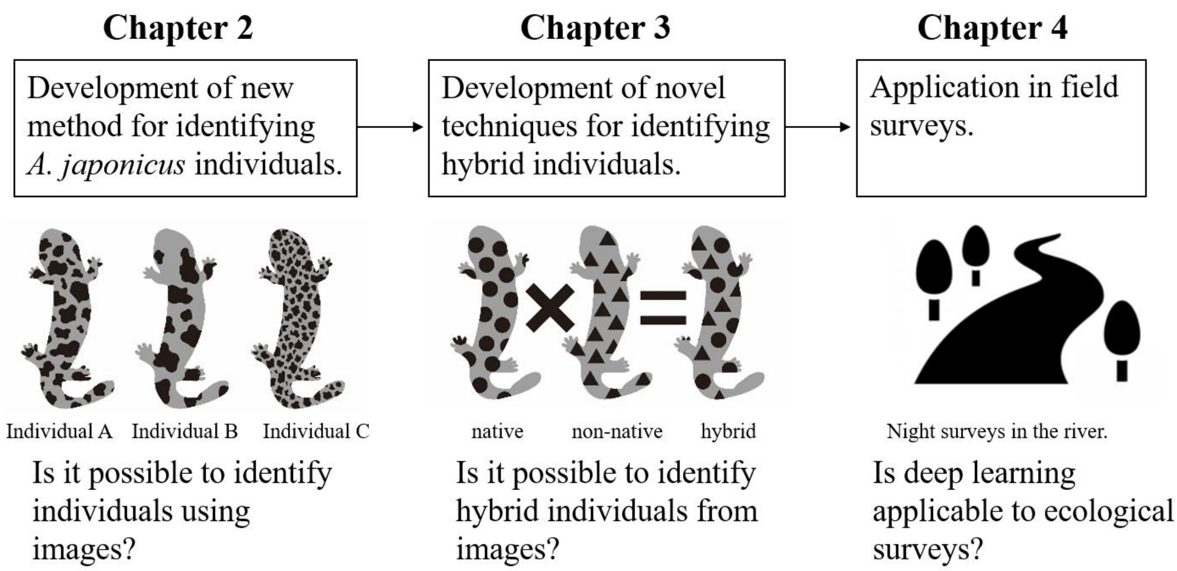

Figure 1.1 Structure of this thesis.

(1) Does automatic photo-identification based on deep learning effectively work for *A. japonicus*?

For small amphibians, invasive identification methods such as toe clipping have been applied, and there is concern about the negative impact on the target species. Although PIT tags are employed for relatively large species, this method requires the capture of individuals. In Chapter 2, I apply deep learning to images obtained from a smartphone to verify the possibility of individual identification of *A. japonicus* based on their spot pattern.

(2) To what extent can computer vision techniques using deep learning identify hybrid individuals between *A. japonicus* and *A.* cf. *davidianus*?

Currently, morphological and DNA information is mainly utilized to identify hybrid individuals. By combining images and deep learning, it may be possible to develop a method to detect hybrid individuals without specialized knowledge. Therefore, I have developed a novel technique to identify hybrid individuals between *A. japonicus* and *A.* cf. *davidianus* from images.

(3) How can image-based identification methods be applied to ecological surveys?

Previous work on deep-learning-based computer vision has focused primarily on technological development, with few studies applying this technology to actual ecological studies. The application of these techniques to field surveys would provide a new non-invasive method of automatic photo-identification for biodiversity assessment. In Chapter 4, I tested the practicality of my method in a field survey of *A. japonicus*.

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CHAPTER 2 Individual identification of endangered amphibians using deep learning and smartphone images : case study of the Japanese giant salamander (*Andrias japonicus*)

2.1 Introduction

Individual identification of wildlife provides fundamental information for ecological studies and conservation efforts (Alberts, 2019; Festa-Bianchet et al., 2019). For example, individual-based studies combined with mark-recapture methods offer estimates of population size, survival and reproduction rates, and immigration and emigration rates. In addition, health status indicators, such as weight and presence of parasites, and behavior variations are important in behavioral evolution and urban adaptation and can be revealed through individual identification. These findings can contribute to answering ecological and evolutionary questions and facilitating the conservation of endangered species (Clutton-Brock et al., 2010).

Individual identification is essential for collecting information at the individual level. There are two main types of approaches: invasive and non-invasive. Invasive methods include blood and tissue sampling and the attachment of physical tags, GPS, and radio transmitters. However, it can be difficult to capture target species in the field, and concerns have been raised about the negative effects of tag attachment (Burley et al., 1982; Funk et al., 2005; Gauthier-Clerc et al., 2004; Moorhouse & MacDonald, 2005). Additionally, research permits are required for certain animals, and obtaining such permits is particularly difficult for endangered species. Furthermore, tags often have a short lifetime and are sometimes lost (Carter et al., 2014), which represents a challenge when applied to long-lived organisms. Although these issues can be addressed by non-invasive methods, such as genetic analysis using DNA in feces and hairs, collecting samples from aquatic organisms, such as amphibians, is not easy because feces diffuse in the water. In addition, DNA analysis is expensive and requires fresh samples.

Biometric identification techniques, such as manual photoidentification, are inexpensive and can identify individuals without harming animals. These methods have several advantages, such as no animal capture, no loss of tags and no effect on animal behavior, because individual-specific patterns, such as stripes and spots, can be utilized for individual identification, which is beneficial for the study of endangered species including individual identification of cetaceans (Bichell et al., 2018), sea lions (Osterrieder et al., 2015), lions (Pennycuick & Rudnai, 1970), polar bears (Anderson et al., 2007), African elephants (Chui & Karczmarski, 2022), and sea turtles (Carter et al., 2014; Schofield et al., 2008). Such methods are alternatives to invasive methods, although they cannot be applied when natural markings are absent (Dorning & Harris, 2019). However, as the number of individuals increases, image classification requires more time and effort; because it is difficult to process large datasets.

In recent years, computer vision has attracted attention as a method of overcoming the challenges of manual photoidentification. Deep learning, such as convolutional neural networks (CNNs), is a new approach for automatically extracting features from large amounts of data. Its implementation in various fields is rapidly advancing with improvements in computing power, such as graphics processing units (GPUs). This technique, which has been used for human facial recognition, was first applied to animal identification in 2014 (Schneider et al., 2019). The target species for individual identification have mainly been mammals (Birenbaum et al., 2022; Chen et al., 2020; Clapham et al., 2020; Schofield et al., 2019), although the method has also been applied to birds and reptiles, with large research bias observed according to the taxonomic group (Bichell et al., 2018; Ferreira et al., 2020). Pattern recognition has been used to identify amphibian individuals (Gamble et al., 2008), but image recognition based on deep learning has not yet been applied. Amphibian populations are declining globally, with 41% of amphibians listed as threatened by extinction on the IUCN Red List (IUCN 2022). Therefore, the application of deep learning is needed for this taxon which is a high conservation priority (Cordier et al., 2021; Nori et al., 2015).

The Japanese giant salamander (*Andrias japonicus*) is one of the world's largest amphibians and endemic species and reaches 150 cm in total length, and it is distributed in the up-streams and middle rivers of western Japan (Matsui & Hayashi, 1992). This species has primitive morphological features similar to those of fossil species and a life span of over 60 years. Their diet is carnivorous, including fish and crabs, and they are top predators in the stream ecosystem. Although this species is protected by law, its population has declined because of habitat modification and fragmentation (Taguchi & Natuhara, 2009). Therefore, it has been listed as a vulnerable species by the IUCN and Ministry of the Environment's Red List. Furthermore, hybridization of this species with the Chinese giant salamander (*Andrias* cf. *davidianus*) has become a problem that requires immediate conservation efforts. Currently, PIT tags are mainly used to identify individuals of this species; however, capture is necessary to insert tags. Before PIT tags became popular, spot patterns on their bodies were mainly used for identification by experts capable of identifying individuals from their unique patterns.

In this study, I aimed to identify individuals of *A. japonicus* via deep learning using spot patterns captured by smartphone. Individual identification from images is a non-contact, non-destructive, and low-cost method that can be applied to other species. Because conservation practices are expensive, inexpensive identification methods will enable conservation measures to be applied for more species. In particular, the conservation of charismatic species, mainly mammals, has generally captured public interest and been the focus of efforts. However, less charismatic animals, such as amphibians, have not received sufficient financial support for their conservation (Bennett et al., 2015; Clucas et al., 2008). Therefore, inexpensive identification can significantly contribute to the conservation of amphibians.

2.2 Materials and Methods

2.2.1 Natural markings

The markers used for individual identification should be permanent, distinctive of the animals, and universally displayed throughout the population, and they should also be measurable using a recording device (Kühl & Burghardt, 2013). In addition, a suitable measurement region for the target species must be determined. *A. japonicus* has spot patterns all over its body, and the head and tail spots have been used to identify individuals (Kobara, 1985). A previous study showed that about eight years of continuous identification could be conducted by their body pattern (Tochimoto, 1996). Therefore, I selected the head as the measurement region because spots were clearly observed and easily photographed by the camera.

2.2.2 Image acquisition

In this study, 11 individuals kept at the Conservation breeding facility of *A. japonicus* in Hiroshima City Asa Zoological Park were used (Figure 2.1). This facility has been used for researching and breeding *A. japonicus* successfully in captivity since 1971. A smartphone (iPhone11 equipped with a 12-megapixel camera) was used for image acquisition. Obtaining optimal images on land was difficult because of the body surface reflection and active movement of the individuals. Therefore, I photographed *A. japonicus* in water using a camera above the water. The head spot of *A. japonicus* was recorded at approximately 60 cm from the camera, and the water depth was about 20 cm. In our experiment, 60 cm was the appropriate distance because they were about 60-90 cm in total length, and the head spot could be clearly photographed. Although I photographed individuals in the zoological facility, the optimal distance could differ depending on the size of the individual and the water clarity, especially in the field. Because reflections on the water surface were a problem with this method, the photographer held a black umbrella to suppress reflections on the water surface and conducted shooting under the umbrella (Figure 2.2). This method was effective because I photographed in an environment without water flow. The water's surface will be more complex in the natural environment, but blocking direct light on the surface with an umbrella would work adequately. In severe water surface reflection, deflection (polarization) filters could also be effective. For example, the effectiveness of the polarization filter has already been confirmed for salmon surveys in rivers (Kudo et al., 2012). Spots were photographed on video and converted to images at ten frames per second using the Free Video to JPG Converter software program, and these images were used as training and test images. In this study, I photographed images three times a day to investigate the effect of light conditions on model accuracy. Photographing was performed on August 20-21, 2022. Each video recording lasted approximately 30 s, and shooting was conducted three times a day at approximately 11:00, 15:00, and 18:00, which are defined as morning, afternoon, and evening, respectively. The solar radiation measured at the Hiroshima Local Meteorological Observatory during each hour on August 20 was 2.00, 0.78, and 0.23 (MJ/m^2). Similarly, August 21 was 0.98, 2.72, and 0.70 (MJ/m^2). Although testing at night light conditions is important because *A. japonicus* are nocturnal, our study aimed to verify the feasibility of individual identification based on the spot patterns. Therefore, I used images during the daytime because photographing is relatively easy.

The framework employed in this study is illustrated in Figure 2.3. The head in the image was automatically detected using YOLOv5 (Redmon et al., 2016). Annotation data were created using the LabelImg annotation tool. First, an image of the *A. japonicus* was loaded using this software. In this case, ten images per individual were selected from 11 individuals and used for the annotation data. Next, a rectangle was created to include only the head. Finally, the rectangles were labeled “head” and the output format was the YOLOv5 format to train the model. The detection model was tested against all of 11 individuals to quantify the performance. After detecting the head in this model, a rectangle in a similar shape to the detected region was created in the center of the region. The size of a similar rectangle was set to 60% area of the detected region and cropped automatically (Figure 2.4; Table 2.1). Although the head image detected by YOLOv5 may contain a background, only an image of the spot patterns were created by cropping at 60%. The training and test images were resized to 224 × 224 pixels because the size varied from image to image. This study used the images obtained on August 20 for training and August 21 for testing. I augmented training datasets and randomly divided those images into training (70%) and validation (30%). Details about augmentation are described in the next section.

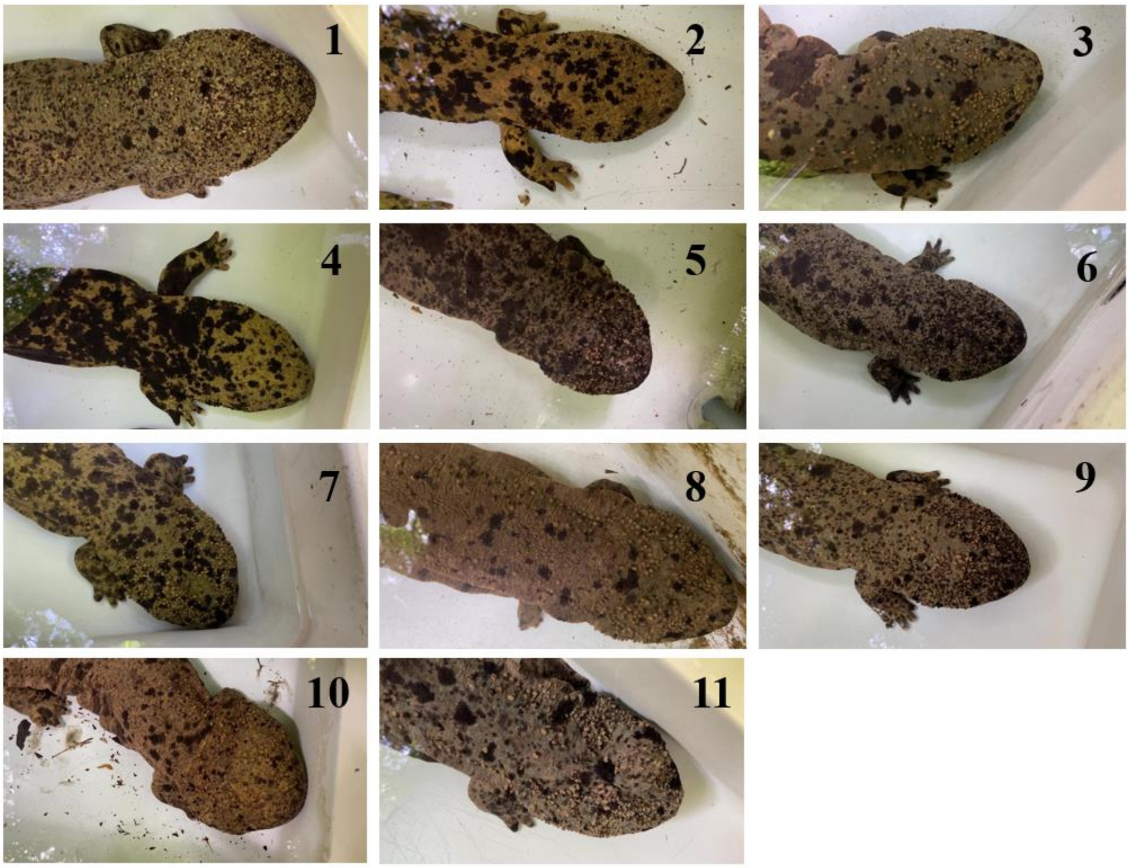


Figure 2.1 Eleven individuals were used in this study.

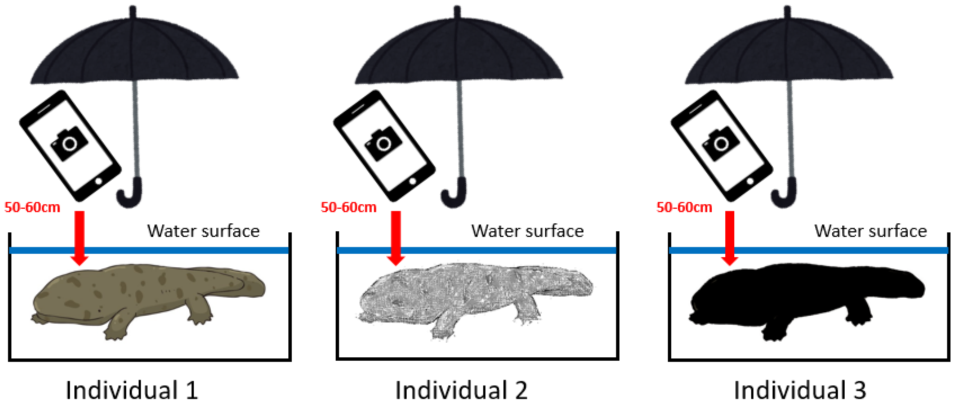


Figure 2.2 Methods of photographing salamanders. Photographs of underwater individuals were taken from under the umbrella to reduce reflections on the water's surface. An iPhone11 equipped with a 12-megapixel camera was used for the photo shoot.

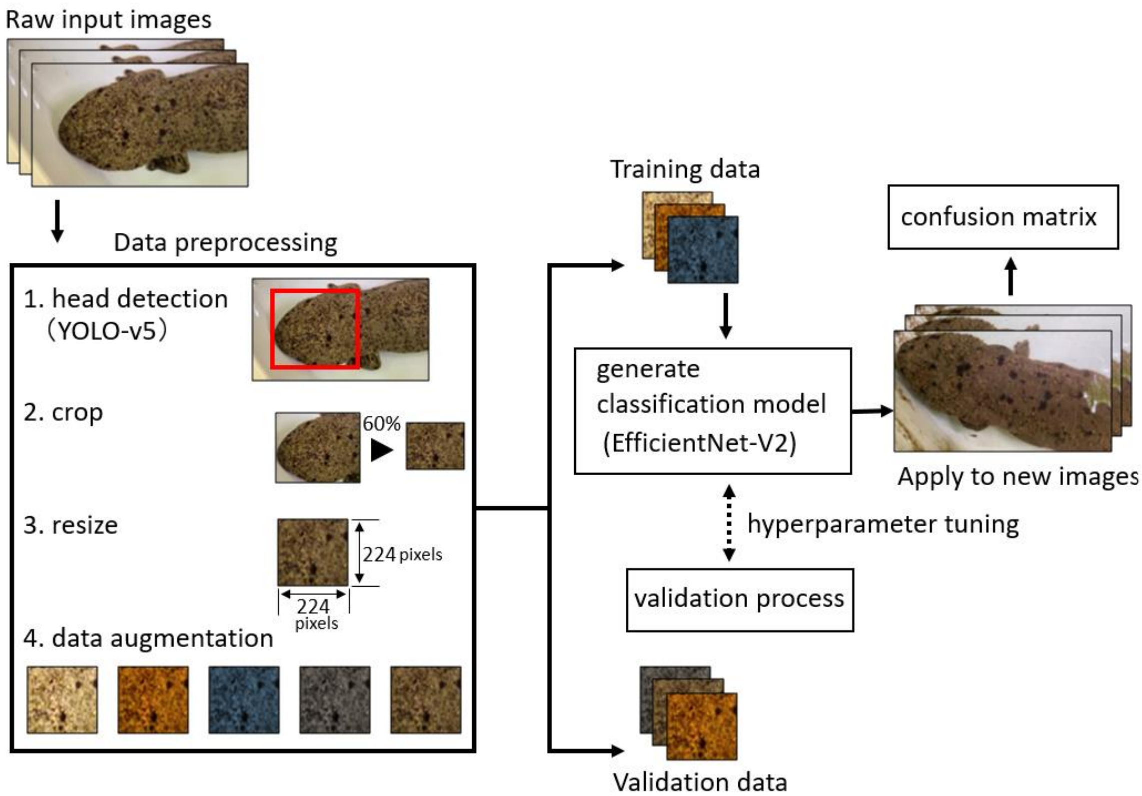


Figure 2.3 Framework of the classification model for identifying *A. japonicus* using smartphone photographs.

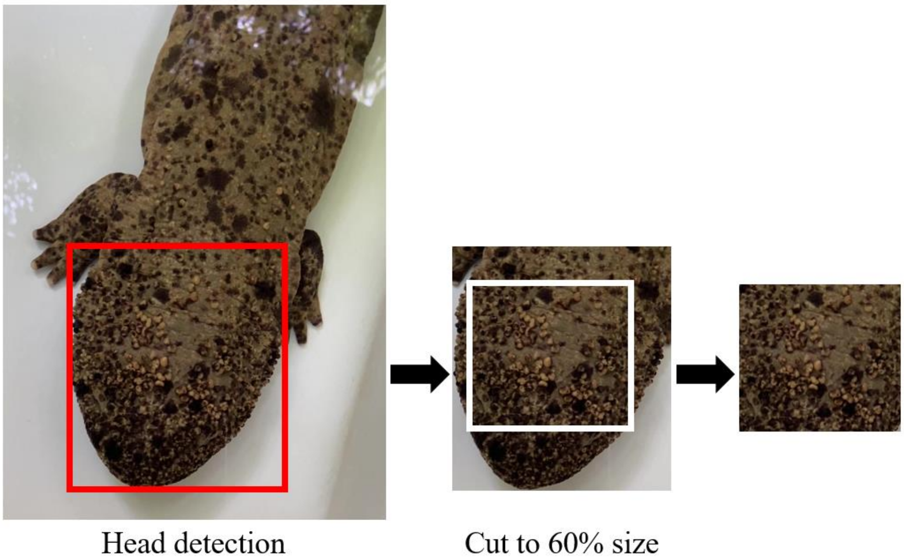


Figure 2.4 The head was detected by Yolov5 (red box); the image also includes the background. Cropping the head image to 60% of its original size (white box) produced an image that only included spots.

Table 2.1 Dataset summary.

Individual	Training（August 20）			Test（August 21）		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening
1	112	110	98	107	87	106
2	109	105	121	106	100	103
3	87	100	101	102	86	108
4	94	110	115	105	82	91
5	97	124	104	103	105	103
6	112	104	104	108	105	100
7	107	108	98	107	106	101
8	77	158	127	103	88	187
9	102	108	98	105	135	101
10	103	160	106	103	104	101
11	109	122	103	104	83	147

2.2.3 Image augmentation

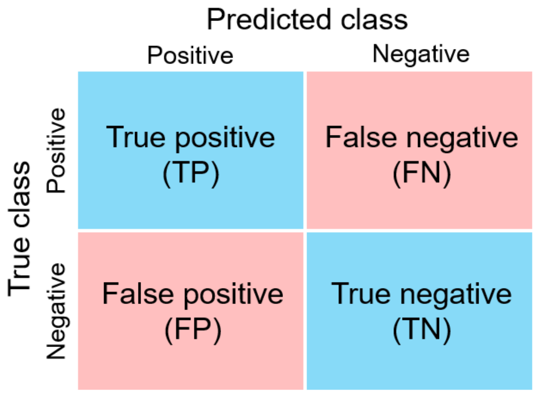
Augmentation is performed to prevent overfitting. The orientation of the individual in the image differs because it moves during the shooting; therefore, rotation and crop processing were added to identify, regardless of the direction of the measurement region. In addition, brightness, Gaussian noise, color jitter, and saturation processing were performed because the light conditions in the image were not uniform. The augmentation process was applied to each parameter with a probability of 50%. For example, applying rotation and cropping will result in the following three patterns of images: (1) both processes are applied, (2) either process is applied or not, and (3) neither process is applied.

2.2.4 EfficientNetV2

In this study, EfficientNetV2, an improved version of EfficientNet, was used for classification. EfficientNet is a convolutional neural network model that achieves more efficient performance by uniformly scaling up the depth, width, and resolution while scaling down the model instead of arbitrarily scaling these factors, as observed in conventional practice (Tan & Le, 2019). For example, the ResNet architecture is scaled up by adding more layers to improve accuracy. However, this approach results in increased computational complexity and vanishing gradient problems. EfficientNet addresses this issue by exploring the relationship between increases in each dimension using a compound coefficient. In addition, unlike other CNN models, it uses a new activation function called Swish instead of a Rectifier Linear Unit (ReLU). EfficientNetV2 is an improved version of EfficientNet with better training speed and parameter efficiency (Tan & Le, 2021). The EfficientNetV2 model employs a neural architecture search (NAS) to optimize model accuracy, size, and training speed. In this case, the EfficientNetV2-B0 model was used as the network, and fine-tuning was performed using a pretrained model with the Imagenet21k dataset. Fine-tuning uses the weights of the trained model and can thus achieve high accuracy with a small number of training images. The number of epochs was set to 50, and the batch size was set to 32 for training. Adam was used as the optimizer, and the dropout was set to 0.3. In this study, early stopping was also employed to prevent overfitting and automatic termination was performed when the validation loss did not improve by more than 0.001 for five consecutive epochs. These analyses were performed using the NVIDIA DGX Station A100. The environment was as follows: OS: Ubuntu 18.04.4, GPU: Tesla V100 32 GB, CUDA Version: 11.2, CPU: Intel Xeon CPU E5-2698 v4. In addition, I used the version 1.2.2 of Keras-efficientnet-v238 on the TensorFlow backend.

2.2.5 Evaluation metrics

The accuracy, Cohen's Kappa coefficient, and macro average F1 score were used for evaluation. I calculated these evaluation metrics using equations (1)-(7). The results were presented as confusion matrices, which are summaries of predictions on a classification (1). For example, if individual A is correctly classified as individual A, it is defined as a true positive (TP). Similarly, if an individual other than A is correctly classified as not A, it is defined as a true negative (TN). On the other hand, if an individual other than A is classified as A, it is defined as a false positive (FP). In addition, if individual A is not classified as A, it is defined as a false negative (FN). Although the accuracy is widely used in assessments, proper evaluations become difficult in cases of imbalanced data. Therefore, I also used Cohen's Kappa coefficient and the macro average F1 score, which can be used to assess unbalanced data. For evaluation, the following equations were used. Regarding the formula of Cohen's Kappa, *Po* is the observed agreement between ground truth and prediction. *Pe* is the hypothetical chance of the ground truth and predictions arriving at the same number. The models were evaluated at different periods: morning, afternoon, and evening. For example, test images taken in the morning were used to assess the models trained based on the morning images; in addition, mixed models that contained images from all periods were trained and tested.



(1)

$$\text{Accuracy} = \frac{(\text{TP} + \text{TN})}{(\text{TP} + \text{FP} + \text{TN} + \text{FN})} \tag{2}$$

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}} \tag{3}$$

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}} \tag{4}$$

$$\text{Kappa (k)} = \frac{\text{P}_o - \text{P}_e}{1 - \text{P}_e} \tag{5}$$

$$\text{F1 score} = 2 \cdot \frac{\text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}} \tag{6}$$

$$\text{Macro F1 score} = \frac{\sum_{i=1}^{n} \text{F1 score}_i}{\text{Precision} + \text{Recall}} \tag{7}$$

2.3 Results

2.3.1 Morning model

The results of the morning model are shown in Figure 2.5 and Table 2.2. All individuals were correctly identified with an accuracy of 97.04%, a Kappa coefficient of 0.97, and an F1 score of 0.98. The identification results for each individual are presented in a confusion matrix. The vertical axis represents the ground truth, the horizontal axis represents the class predicted by the model, and each number represents an individual number. The number in each cell represents the number of identified images, and the color of each cell indicates the percentage of images per ground truth. For example, light blue indicates a ratio of 0.0, indicating that no image was classified as that cell. In contrast, dark blue indicates a ratio of 1.0, meaning that all ground truth images were classified to that cell. The morning model misclassified individual No. 1 as No. 10 in 20/107 (18.69%) images and individual No. 3 as No. 10 in 8/102 (7.84%) images. In addition, individual No. 9 images were misclassified as No. 11 by 2/105 (1.90%) images.

2.3.2 Afternoon model

The results of the afternoon model are shown in Figure 2.6 and Table 2.2. All individuals were correctly identified with an accuracy of 94.36%, a Kappa coefficient of 0.94, and an F1 score of 0.92. The model misclassified 58/105 images (55.24%) of the individual No. 6, 36/105 images as No. 1, and 22/105 images as No. 9 (Figure 2.6). In addition, individual No. 3 were misclassified as No. 8 by 3/86 images (3.49%).

2.3.3 Evening model

The results of the evening model are shown in Figure 2.7 and Table 2.2. Compared to the morning and afternoon models, the accuracy was lower, with an accuracy of 86.86%, Kappa coefficient of 0.85, and F1 score of 0.98. The evening model misclassified 124/147 images (84.35%) of individual No. 11 (Figure 2.8), 122/147 images (82.99%) as No. 9, and 2/147 images (1.36%) as No. 8. In addition, 36/187 images (19.25%) of No. 8 individuals were misclassified, 35/187 images as No. 3, and 1/187 images as No. 4. Futhermore, 3/103 images (2.91%) of individual No. 5 were misidentified as No. 8 and 1/103 images (0.97%) of individual No. 2 were misclassified as No. 4.

2.3.4 Mixed model

The mixed-model results are shown in Figure 2.9 and Table 2.2. The accuracy was 99.86%, Kappa coefficient was 0.99, and F1 score was 0.99. Although there were some misidentifications in the images of individuals No. 2, No. 6, and No. 8, the model correctly identified almost all individuals. In addition, the accuracy was reduced when images were used without cropping, especially in the evening model (Table 2.3).

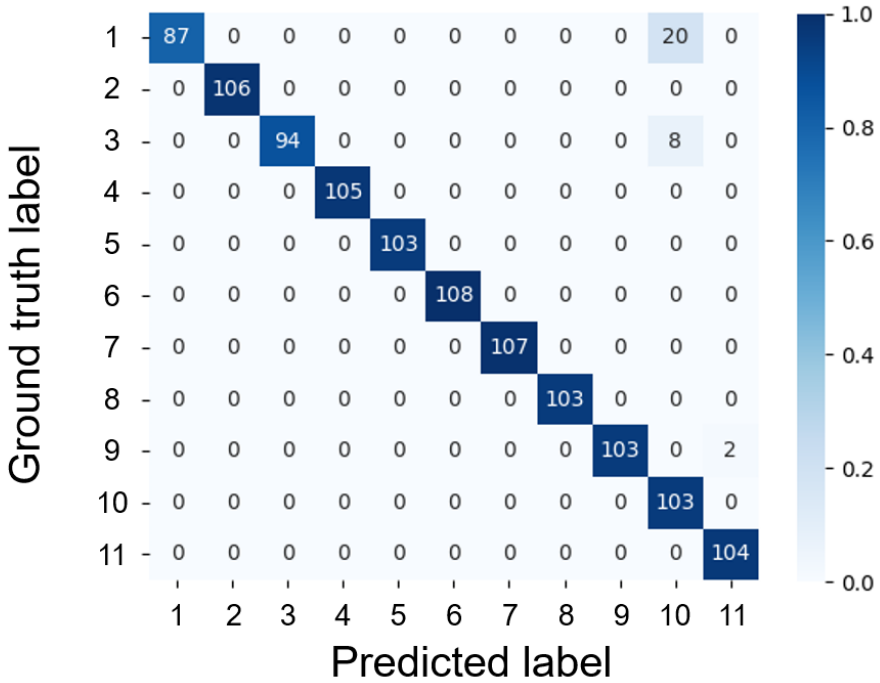


Figure 2.5 Confusion matrix of morning model. The vertical axis shows the ground truth, and the horizontal axis shows the model's prediction results. The numbers on the axes indicate the individual numbers of *A. japonicus*; the number in each cell indicates the number of classified images; and the color of each cell indicates the percentage of images in each class.

Table 2.2 Identification results and comparison of each model.

	Overall accuracy	Kappa	F1 score
Morning model	97.04	0.97	0.98
Afternoon model	94.36	0.94	0.92
Evening model	86.86	0.85	0.98
Mixed model	99.86	0.99	0.99

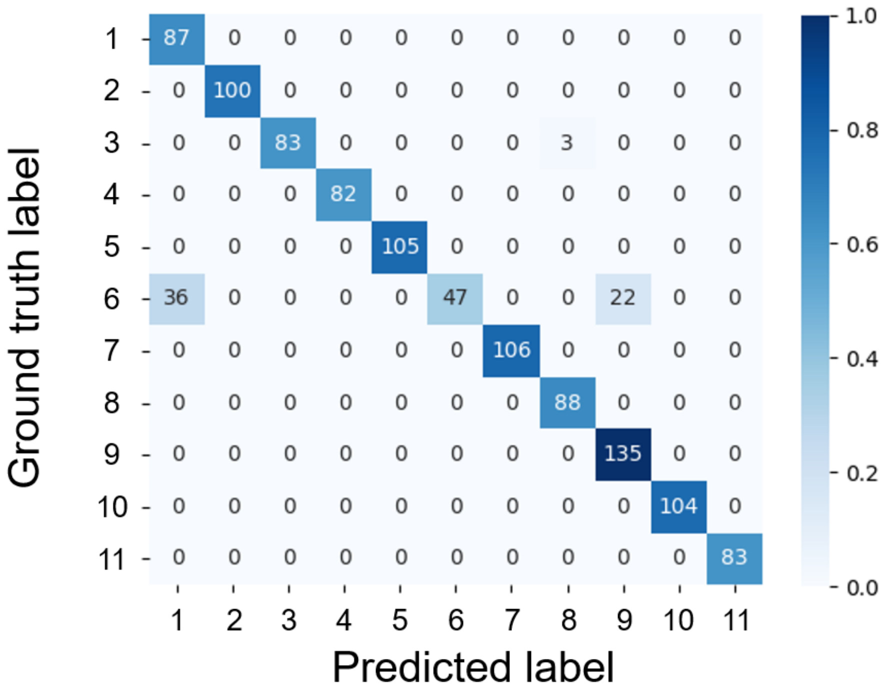


Figure 2.6 Confusion matrix of the afternoon model.

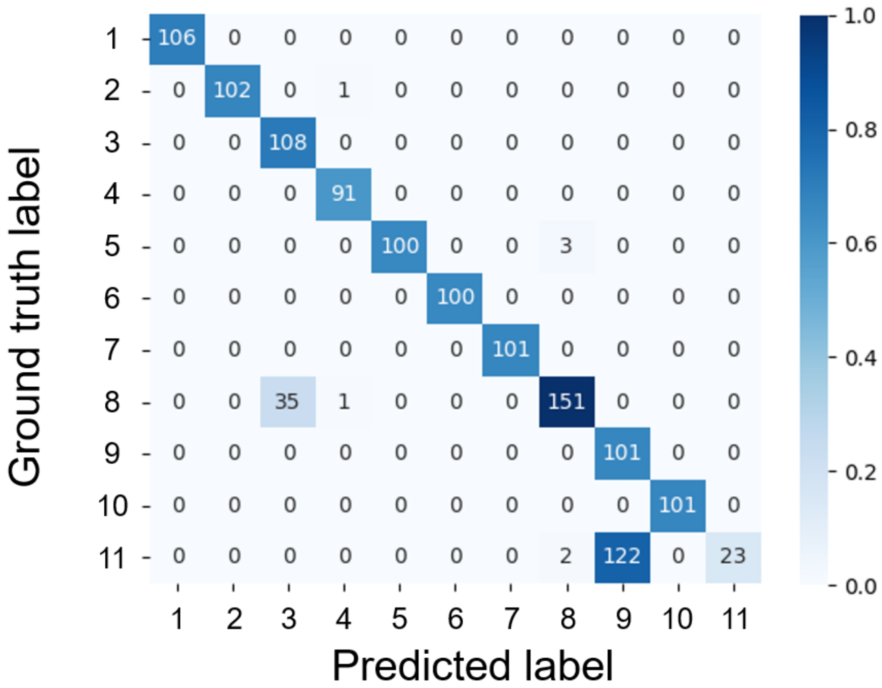


Figure 2.7 Confusion matrix of the evening model.

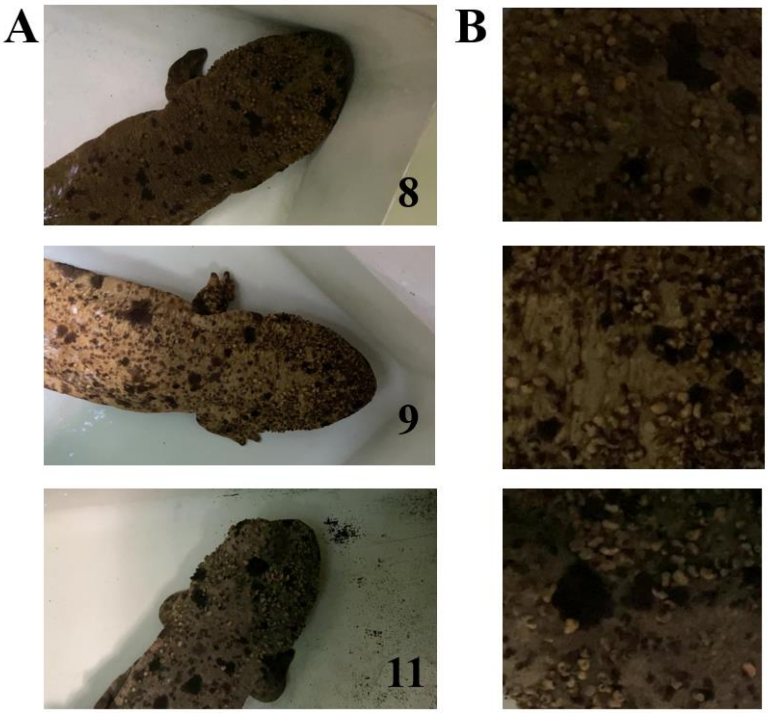


Figure 2.8 Misclassifications of the evening model, including 124/147 images (84.35%) of individual No. 11, 122/147 images (82.99%) as individual No. 9, and 2/147 images (1.36%) as individual No. 8. A shows the spots for each individual, and B shows the test image of each individual.

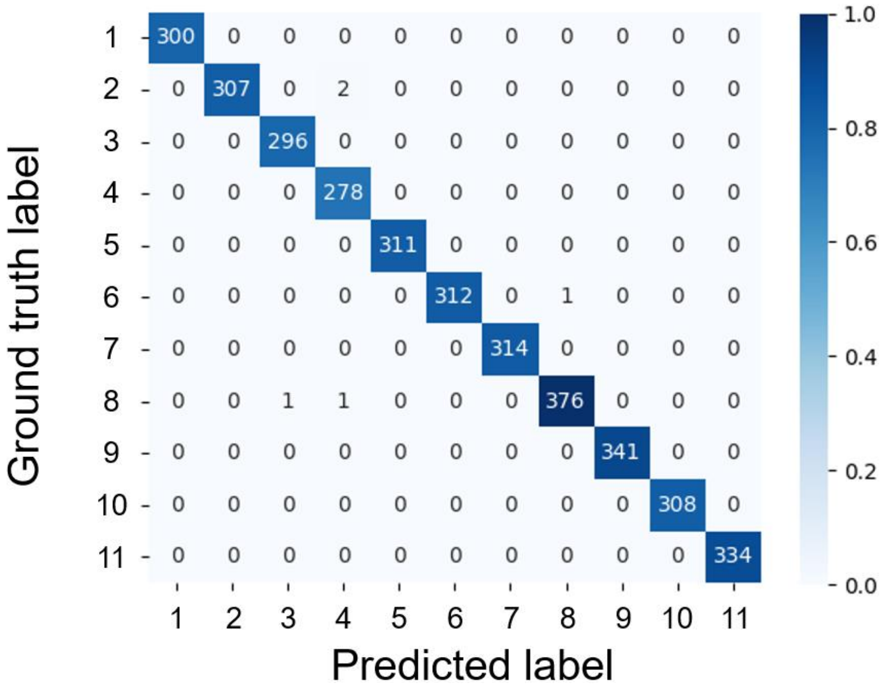


Figure 2.9 Confusion matrix of the mixed model.

Table 2.3 Performance comparison of models created with 60% cropped and head image models. Except for the afternoon model, the performance of the model created with the 60% cropped image was better.

	Accuracy	
	60% images	Head images
Morning model	97.04	95.84
Afternoon model	94.36	95.10
Evening model	86.86	70.67
Mixed model	99.86	98.79

2.4 Discussion

This study applied deep learning approaches for the individual of endangered amphibians using deep learning. Most wildlife studies employ artificial tag attachments to identify individuals by capturing animals (Schofield et al., 2008). However, physical tags have several issues, such as the stress associated with capture and tag attachment and the impact of the tags itself. In contrast, non-invasive methods, such as photoidentification, have a lower impact on animals, although they also present certain disadvantages. For example, photographic matching requires identification skills and cannot be applied to large datasets because of its labor-intensive nature and human errors. Although deep learning can overcome these challenges, it has only been applied to a few taxa, which mainly include mammals. Thus, research on amphibians using deep learning is lacking despite its importance for conservation. Our study demonstrated the effectiveness of a new identification method using deep learning for one of the world's largest amphibians that is currently threatened with extinction. I found that the head spot pattern was suitable for individual identification, and an accuracy of 99.86% was achieved by applying EfficientNetV2 to smartphone images without conventional feature extraction. The high performance obtained with smartphone images suggests that combining this method with citizen science could contribute to amphibian conservation. Moreover, our approach could be applied to other amphibian species at a low cost.

The accuracy of the mixed model was 99.86%, the Kappa coefficient was 0.99, and the F1 score was 0.99. Previous studies that used deep learning for individual identification achieved accuracies of 92.5% for chimpanzees (Schofield et al., 2019), 96.3% for pandas (Chen et al., 2020), and 83.9% for brown bears (Clapham et al., 2020). One reason for the high accuracy in this study was the high similarity between the training and test images. Whereas there was significant variation in the date and location of training images in the previous studies, the training and test images used in this study were obtained over only two days. Furthermore, the high accuracy was probably due to the low variation in the images because the *A.japonicus* did not move much during shooting and the clear images obtained for the spots in water in a captive environment. When photographing *A.japonicus* on land, individuals move relatively fast and their body surfaces reflect light. These problems can be mitigated in water. Because markings for individual identification can be recorded under natural conditions when reflection from the water surface is suppressed, photographing underwater individuals is recommended for aquatic amphibians. Another factor contributing to the high accuracy was that the images contained only the spots used for analysis. In fact, the accuracy was reduced when images were used without cropping, especially in the evening model (Table 2.3).

The performance of the evening model was lower than that of the morning and afternoon models, which may be due to the quality of the images resulting from the light conditions. In particular, individual No. 11 in the evening model was misclassified in 124 out of 147 (84.35%) images. These images could not be classified correctly because the spots were not clearly photographed (Figure 2.8). In addition, confusion may occur because the features of different individuals extracted by AI are similar. Furthermore, the fact that only the head was used for analysis may have contributed to the limited information in the images. In the case of the expert, observers identify individuals based on the characteristic pattern of spots on the whole body, not just the head, to isolate slight differences between individuals. Therefore, it is necessary to verify how the performance varies depending on the areas for individual identification. The selection of the measurement region for identification is important and has a significant effect on the accuracy of the model. For example, in the case of seals, the accuracy was 59% for fur-based identification (Cunningham, 2009) but improved to 88% for face-based identification (Birenbaum et al., 2022). Arzoumanian et al. (2005) achieved more than 90% pair image matching using flank spot patterns for the identification of whale sharks but reported that image matching failed when photographs were obtained at oblique angles of more than 30°.

The mixed model exhibited the best results. One possible reason is the quality of the training images.

Since training images of the morning and afternoon models clearly photographed spots in relatively luminous environments, the image brightness could have contributed to the performance. Although the light intensity in our study was not measured, the solar radiation (MJ/m^2) recorded at the Hiroshima Local Meteorological Observatory was high in the morning and afternoon when the images were captured. Previous studies also indicated that the accuracy of AI models is affected by light intensity and shadow in training images (Ferreira et al., 2020). Therefore, it is important to obtain images in the appropriate environment, depending on the research purpose. As the second reason, the size of the training data in the mixed model could also have increased the accuracy. Tabak et al. (2019) also indicated that the ability of the model to recognize species increased with the size of the training dataset for the species. Although it is challenging to collect training images of animals with small populations, such as endangered species, cooperation with zoos and aquariums will enable the efficient creation of training datasets. Thus, collaboration among ecologists, information scientists, and zoo curators will be required in the future.

This study demonstrated the benefits of using deep learning to identify individuals; however, certain limitations should be noted. First, I used individuals in captivity, which facilitated uniform shooting conditions. In situations where images are captured in the wild, the background, light conditions, water flow, and direction of the target species differ, which leads to high image variation, thereby affecting the identification accuracy. Prior studies have shown that the accuracy is lower when individual identification is conducted in the field than in captivity, with 92% accuracy reported for chimpanzees in captivity and 77% accuracy in the wild (Freytag et al., 2016). In the future, individual identification using the proposed method should be verified for practical application in the field. Second, the data were obtained over two days; therefore, the verification of this method for long-term individual identification is needed. In particular, long-term monitoring is important for surveying long-lived target species and for their conservation. Thirdly, relatively large adult *A.japonicus* with a total length of over 50 cm generally show little change in their spots over time. However, few studies have examined changes in the spot patterns of *A.japonicus* over their lifetime, although the study of Tochimoto (1996) demonstrated continuous identification from the body pattern over eight years. For other species, regions that do not change over long periods should be used for individual identification. For example, Bauwens et al. (2018) analyzed images of over 900 European adders (*Vipera berus*) over 12 years and found that head scale patterns did not change and were useful markers. Therefore, future studies are needed to determine the effects of aging and weight changes on the spot visibility of *A.japonicus*. In addition, our method uses pre-captured images to identify individuals. Therefore, identifying new individuals not included in the training images is challenging. This means that applying our predictive model to other *A.japonicus* will be difficult because our training datasets contained only 11 individuals. In the future, more individuals must be annotated to utilize our model for field surveys. Finally, the ability to identify unknown individuals was not tested in this study. However, it can be tested by the following procedure. If there are images from five individuals (e.g., A to E), an AI model can be created without including the images of one individual (e.g., E). When E's image is tested using this model, if it is classified as a particular individual (e.g., A) with high probability, the model may not be able to sufficiently identify unknown individuals. On the other hand, if the probabilities of E's classification as known individuals (A to D) are uniformly low, this indicates that the model correctly identified the unknown individual. Applying the same process to other individuals will enable evaluation of the ability of the model to identify unknown individuals.

The approach implemented in this study, which combines images and deep learning, can identify target species inexpensively without the need for capture. Moreover, although conventional machine learning requires the design of species-specific algorithms for feature extraction, which are challenging to implement (Bolger et al., 2012; Schneider et al., 2019), highly accurate individual identification was achieved in our study using images alone. This simplicity is a major advantage, and

I believe that similar methods can be applied to other species of amphibians. The findings of this study can contribute to the conservation of amphibians, which are threatened with extinction worldwide. Furthermore, our technique enables applications such as estimating population size. For example, the proportion of marked individuals is needed when applying the mark-recapture method. Previously, target species were marked by attaching tags (e.g., PIT tags) to distinguish between new and re-captured individuals. Our method suggested the possibility of using photographs as an alternative to tags. In addition, our research demonstrated the feasibility of individual identification using smartphone images. Smartphones are widely available at a low cost; therefore, applying this methodology will significantly advance conservation via combination with citizen science. For example, whale shark research has accumulated over 43,000 images with the help of 3,400 researchers and citizen scientists, and over 3,800 individuals have been identified (Holmberg et al., 2009). Such a large database can prevent the illegal release of individuals and trade in wildlife (Hiby et al., 2009). For example, *A. japonicus* can go away on land from the streams after heavy rain, and sightings of such animals have attracted attention on social networking sites, such as Twitter. In this case, individual identification from the images may assist in determining and releasing the original habitat. In addition, our technique could be applied to detect invasive species. Recently, hybridization between *A. japonicus* and non-native *A.* cf. *davidianus* has become a serious issue. Although capturing hybrids in the wild is necessary, they have a spot pattern that inherits the characteristics of both native and non-native species if they are F1 offspring. Therefore, suspected hybrids are identified by visual screening and DNA analysis. However, the visual screening of hybrids is challenging for the public because it requires identification skills. In the future, if hybrid species could be identified from the images, our approach would be a useful tool for the rapid assessment of hybrid species by citizens. Additionally, the identification of individuals may be possible after their illegal capture. For example, a case of human transportation of an individuals from Hyogo Prefecture to Shiga Prefecture (a distance of over 100 km) in 2022 was revealed by its PIT tag. The purpose of its transportation is unknown; however, the unauthorized capture and movement of this species are prohibited by law. A citizen science survey can prevent the illegal trade and release of *A. japonicus* by constructing a database that allows the matching of individuals. Moreover, ecological information such as age at maturity and migration patterns can be obtained by collecting images over a long period. Understanding the life history is essential for conservation, particularly for long-lived species. Combining images with deep learning enables inexpensive long-term monitoring and offers new opportunities to contribute to ecology.

2.5 Conclusion

In this study, I applied deep learning to identify the endangered amphibian, *A. japonicus*. Our study revealed that deep learning-based individual identification, which has previously focused on mammals, is feasible for amphibians and that high performance can be achieved using smartphone images. Individuals were identified with high accuracy by clearly photographing the head spots while suppressing reflection from the water surface. This method enables the stress-free capture of images of the target species in natural conditions without interfering with their movements. Image-based individual identification is non-invasive and inexpensive, and large datasets can be automatically processed when combined with deep learning. In addition, owing to the widespread popularity of smartphones, this method can contribute to the conservation of species with natural markings.

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CHAPTER 3 Identification of hybrids between the Japanese giant salamander (*Andrias japonicus*) and Chinese giant salamander (*Andrias* cf. *davidianus*) using deep learning and smartphone images

3.1 Introduction

Although significant effort has been devoted toward conservation, biodiversity loss remains a global challenge (Johnson et al., 2017). Anthropogenic activities such as urbanization, agricultural intensification, and species exploitation reduce biodiversity, and species extinction rates are progressing much faster than in the past (Ceballos et al., 2015). In addition, globalization has led to the introduction of organisms into new environments, establishing non-native populations in new areas (Pyšek et al., 2020). These non-native species negatively affect the ecosystem through direct and indirect effects such as predation, niche displacement, and introduction of diseases (Doherty et al., 2016; Haubrock et al., 2021; Kortz & Magurran, 2019; Scheele et al., 2019). Moreover, non-native species are recognized as a further driver of the extinction of local species (Bellard et al., 2016). Therefore, the mitigation of biological invasions is essential to conserve biodiversity because the impact of non-native species on biodiversity and ecosystems is expected to increase in the future (Pyšek et al., 2020).

When non-native species are introduced into a new habitat, they sometimes encounter close relatives. In such cases, hybridization occurs owing to incomplete reproductive isolation from closely related species (Todesco et al., 2016). Hybridization in non-native species is frequently observed and considered an evolutionary mechanism that determines invasion success (Bock et al., 2021). For example, native California tiger salamanders (*Ambystoma californiense*) and introduced barred tiger salamanders (*Ambystoma tigrinum mavortium*) have hybridized and formed a hybrid swarm in California. Fitzpatrick and Shaffer (2007) reported that hybrid tiger salamanders exhibited higher fitness than individuals containing mostly native or mostly introduced alleles (hybrid vigor). Hybrid vigor is defined as the superior growth or reproduction of hybrids compared with parental lineages (Vilà & D'Antonio, 1998); this genetic admixture can increase the fitness of colonizers in biological invasion (Qiao et al., 2019). In addition, hybrids sometimes have intermediate traits or different traits from the parent species (Hayden et al., 2011), and some traits may determine the establishment success of non-native species (Coulter et al., 2020). For instance, a meta-analysis of plants, animals, and fungi demonstrated that non-native hybrids have a larger body size and are more fecund than their parent species (Hovick & Whitney, 2014). Although early non-native populations are affected by density-dependent processes such as the Allee effect (Camacho-Cervantes et al., 2023), hybridization provides mating partners for non-native species, which could reduce the Allee effect and promote invasions (Yamaguchi et al., 2019).

Hybrids of similar species pose a threat to genetic diversity because introduced alleles may eventually replace the native alleles (Fitzpatrick & Shaffer, 2007). Although it is necessary to control hybrids to conserve biodiversity, the difficulty in distinguishing between native and hybrid species is one of the critical issues in managing and controlling hybrids. Hybrids can often be detected using morphological characteristics (Allendorf et al., 2001). However, morphological characteristics cannot be used to determine whether an individual is a first-generation or backcross-generation hybrid. In addition, the misidentification of species can also cause conservation problems. For example, incorrect identification of target species could negatively impact native species; native frogs have been killed in Australia because of misjudgments while removing the non-native cane toad (*Rhinella marina*) (Somaweera et al., 2010).

The development of molecular genetic techniques, such as PCR and eDNA, has overcome these challenges (Allendorf et al., 2001; Rees et al., 2017). DNA analysis allows accurate species identification and can reveal the degree of hybridization, previously difficult to determine using morphological traits. However, the cost of molecular analysis remains high for some methods, and laboratory work and expertise are required to analyze samples (Martinez et al., 2020; Stein et al., 2014). On the contrary, visual identification of target species using photographs is less expensive, and data can be easily collected with minimal disturbance for the individuals (Hou et al., 2020). In addition, citizen science surveys using photographs are a valuable approach for the early detection of non-native species because they can be used to collect data over large areas (Werenkraut et al., 2020). For example, new tools and datasets such as iNaturalist and eBird are emerging that allow people to report observations at any time and from any location (Larson et al., 2020). Despite these advantages, photographic identification is time-consuming when the observer must check large databases (Bogucki et al., 2019).

In recent years, deep learning image recognition technology, a novel group of artificial intelligence approaches, has begun to be utilized to identify both species and individuals in ecology. Identifying and counting animal species in images provides basic but essential information (Tuia et al., 2022). Many previous studies have combined camera traps and deep learning to identify species. For instance, Norouzzadeh et al. (2018) identified wild mammals and birds using 3.2 million images obtained from camera traps in the Serengeti National Park. In addition, these techniques have been applied to individual identification, such as green turtles (Carter et al., 2014), chimpanzees (Schofield et al., 2019), and brown bears (Clapham et al., 2020). Furthermore, deep learning algorithms have already been used to detect non-native species. For example, Ashqar and Abu-Naser (2019) classified *Hydrangea* with a dataset containing approximately 3,800 images taken in a Brazilian national forest, demonstrating the feasibility of this approach. Guo et al. (2022) also developed a novel deep learning model to identify common reed (*Phragmites australis*) from unmanned aerial vehicle (UAV) images. In another study, tall goldenrod (*Solidago altissima*) was detected from action camera images using the chopped picture method, and the suitability of this method in citizen science was considered (Takaya et al., 2022). Although a similar approach may provide a new method for identifying hybrids, studies have yet to apply deep learning models to their identification.

Deep learning has achieved remarkable success in various fields, although its lack of transparency is a major disadvantage (Kakogeorgiou & Karantzalos, 2021; Petch et al., 2022). This technique is sometimes considered a "black box" method in that it is unclear how and why a particular classification decision is arrived at (Brunese et al., 2020; Montavon et al., 2017). Recently, several approaches have been developed to overcome this challenge. For example, gradient-weighted class activation mapping (Grad-CAM) provides a heatmap visualization of the regions that influenced the model's predictions, giving valuable information for the interpretation of results (Selvaraju et al., 2017). In ecological research, Grad-CAM is applied in individual re-identification (De Silva et al., 2022) and species identification (Banan et al., 2020). Although this technique provides visual information for classifying hybrids, research applying this technique to detect hybrids in biological invasions is lacking.

The Japanese giant salamander (*Andrias japonicus*) is an amphibian endemic to Japan and is threatened with extinction as a result of decreasing population due to habitat degradation and fragmentation (Taguchi & Natuhara, 2009; Tochimoto et al., 2007; Yamasaki et al., 2013). In the 2022 IUCN Red List, the conservation status rank of this species was changed from Near Threatened to Vulnerable (IUCN, 2022). One reason for the status change in *A. japonicus* is the hybridization with the congeneric but non-native Chinese giant salamander (*Andrias* cf. *davidianus*). This species is also threatened with extinction in its original habitat, but individuals introduced to Japan in the early 1970s have become wild and hybridized with *A. japonicus*. For example, a Kyoto City government survey revealed that only four (2%) out of 244 individuals captured in the Kamo River Basin in Kyoto were native *A. japonicus*, and the remaining 240 (98%) were *A.* cf. *davidianus* or hybrids between *A. japonicus* and *A.* cf. *davidianus* (HYB), a problem requiring rapid action (The Kyoto City Government, 2015). Moreover, the number of areas where HYB have been caught is increasing, with hybrids already confirmed in eight prefectures in western Japan (Kyoto, Mie, Nara, Shiga, Okayama, Hiroshima, Aichi, and Gifu). Currently, HYB is identified by visual screening and DNA analysis (Fukumoto et al., 2015). Although detecting HYB by spot patterning would allow their rapid identification in the field, this approach requires specialized knowledge (Figure 3.1). Generally, *A. davidianus* has a darker body color with paler spots than *A. japonicus*, although the body color and spot patterning differ among individuals of both species. The accurate identification of HYB from images would require less time and expense than DNA analysis. It would also facilitate the early detection and effective capture of suspected HYB individuals via citizen science, particularly in areas where hybrids have not yet been found, thereby contributing to the effective conservation of *A. japonicus*.

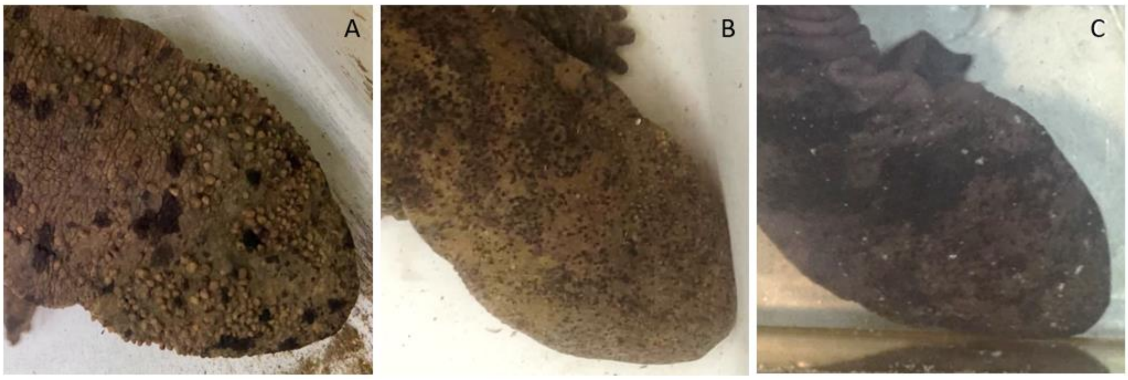

Figure 3.1 Dorsal head spot patterning of *A. japonicus* (A), HYB (B), and *A. davidianus* (C). The image of *A. davidianus* was provided by Dr. Benjamin Tapley, Zoological Society of London.

Our aim was to identify HYB using a computer-based algorithm employing deep learning. The wide availability of the Internet and smartphones provides the opportunity for identifying species and reporting their locations (Larson et al., 2020). Our approach allows the public to photograph and detect HYB individuals without specialized knowledge because *A. japonicus* and HYB often appear in rivers flowing through urban areas and less populated rural areas. In recent years, citizen science has been adopted to manage non-native species (Larson et al., 2020), and a similar method could be applied to HYB. Secondly, I developed an efficient method to recognize *A. japonicus* and HYB. Spot patterns are more difficult to quantify than morphological traits such as body size; thus, few people can utilize this information. However, techniques such as Grad-CAM allow visualization of the important region for predicting whether the species is *A. japonicus* or HYB by the AI model. If specific essential areas for identifying HYB can be clarified, that information will be valuable for helping the general public to identify HYB.

3.2 Materials and Methods

3.2.1 Image acquisition

In this study, 11 individuals of native *A. japonicus* and 20 individuals of HYB were used (Figures 3.2 and 3.3). The Chinese giant salamander has been categorized into several species in recent years (Chai et al., 2022; Turvey et al., 2019; Yan et al., 2018). Because it is unknown which Chinese *Andrias* species was introduced to Japan, it will be referred to as *Andrias* cf. *davidianus* in this study. The native individuals were kept at the Conservation Breeding Facility in Hiroshima City Asa Zoological Park, which is a leading facility in Japan for the research, conservation, and breeding of this species. The HYB were captured in the wild and then transferred to this facility.

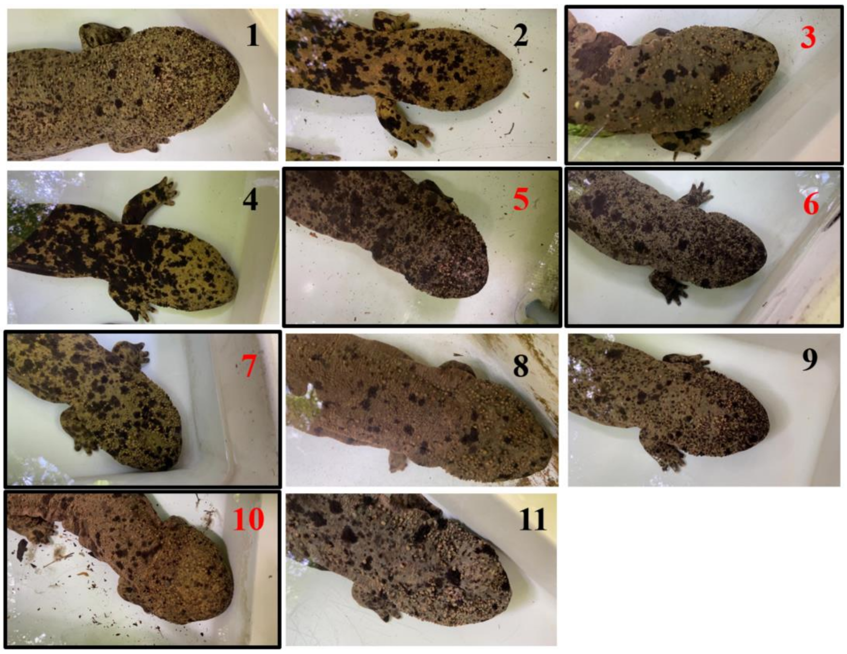

Figure 3.2 Eleven individuals of *A. japonicus*. Individuals marked with red and black numbers were used for training and testing, respectively.

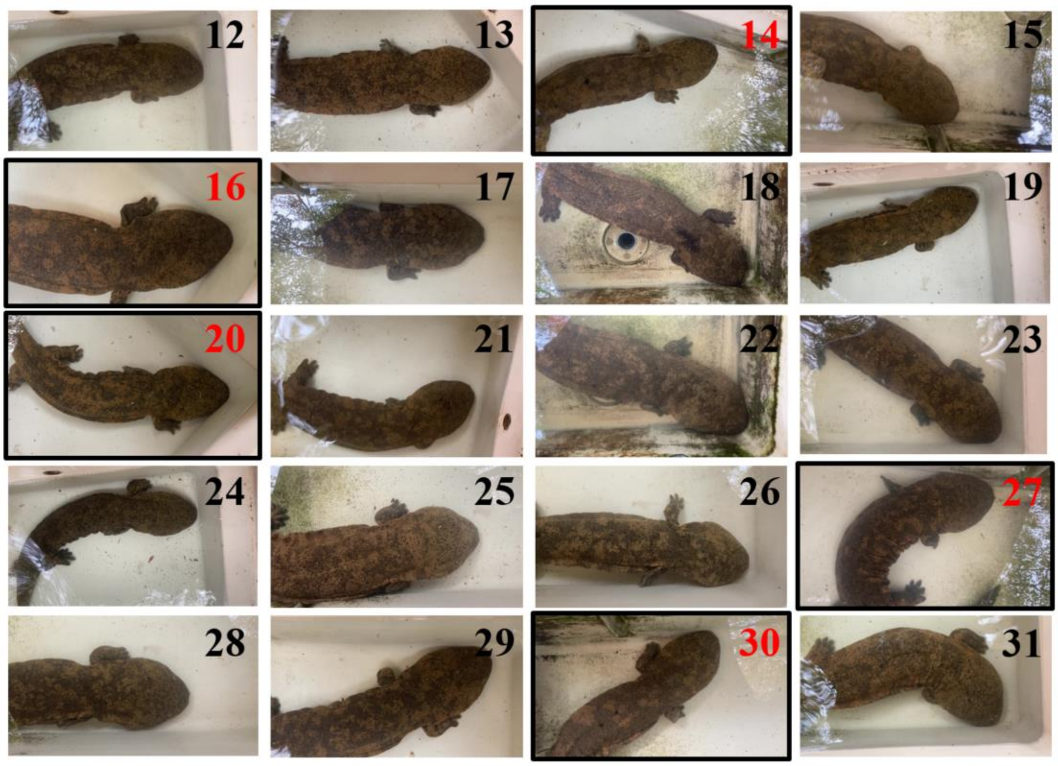

Figure 3.3 Twenty individuals of HYB. Individuals marked with red and black numbers were used for training and testing, respectively.

The 11 *A. japonicus* individuals were photographed on August 20, 2022, at 11:00 a.m. Each individual in the water was recorded on Full HD video from above for approximately 30 seconds using an iPhone 11, from which still images were obtained for analysis. The dorsal head spot patterning of *A. japonicus* was recorded at approximately 60 cm from the camera, and the water depth was about 20 cm. To reduce glare due to reflection from the water surface, the recording was performed under a black umbrella. The videos were converted to 10 still JPEG images (1920 × 1080) per second using Free Video to JPG Converter version 5.0.101 (DVDVideoSoft Ltd.). The 20 HYB were recorded on Full HD video on November 19, 2022, at 2:00 p.m. using an iPhone SE 2020. The method of image collection was the same as that for *A. japonicus*.

3.2.2 Framework

The heads in the images were automatically detected using YOLOv5 (Redmon et al., 2016) and used as either training or test images (Figure 3.4). The training data comprised five individuals of *A. japonicus* and five HYB individuals selected randomly from the two groups (Table 3.1; Figures 3.2 and 3.3). The six remaining *A. japonicus* individuals and 15 remaining HYB individuals not used in training were selected as test images. These images were resized to 224 × 224 pixels to ensure consistency in size. Additionally, augmentation (rotation, crop, brightness, Gaussian noise, color jitter, and saturation) was applied to the training data to prevent overfitting. Each type of augmentation was applied with a probability of 50%. For example, applying rotation and cropping resulted in three patterns of images with (1) both processes applied, (2) one of the two processes applied, and (3) neither process applied. After augmentation, 70% of the images used for training and 30% of the images used for validation were randomly separated for analysis. In this study, I trained EfficientNetV2 to classify images. This convolutional neural network scales down the number of layers while scaling down the model (Tan & Le, 2019). EfficientNetV2 is an improved version of EfficientNet with increased training speed and parameter efficiency (Tan & Le, 2021). The number of epochs was set to 50, and the batch size was set to 32 for training. Adam was used as the optimization algorithm (optimizer), and dropout was set to 0.3. I employed early stopping to prevent overfitting. Automatic termination was performed when the validation loss did not improve by more than 0.001 for five consecutive epochs. These analyses were performed using an NVIDIA DGX Station A100. Finally, overall accuracy was used for evaluation.

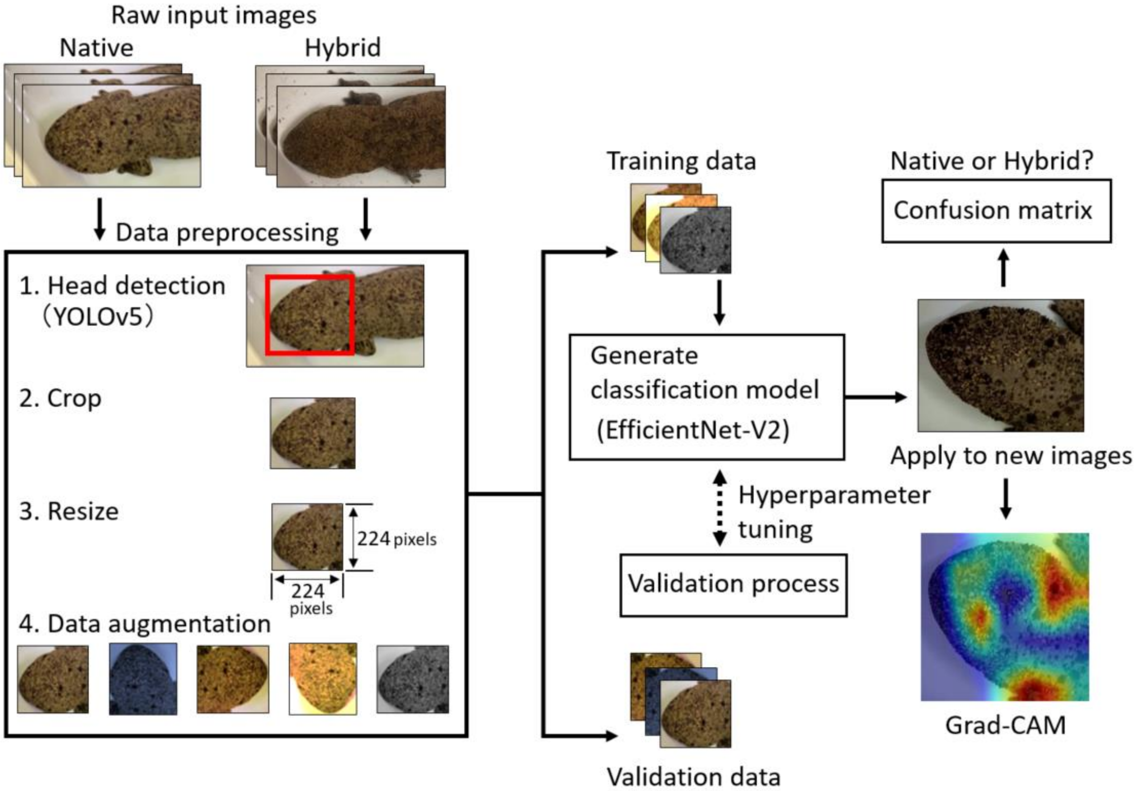

Figure 3.4 Framework of the classification model for *A. japonicus* and HYB using smartphone photographs.

Table 3.1 Dataset summary showing the date and purpose of the images taken.

Species	Year	Date	Training	Test
A. japonicus	2022	August 20	468	525
Hybrids	2022	November 19	439	1,356

3.2.3 Visualization using Grad-CAM

Gradient-weighted class activation mapping generates a heatmap that indicates the importance of pixels in the feature maps of an input image (Selvaraju et al., 2017). These highlighted regions in an image provide an explainable view of deep learning models. Using this method, I extracted the feature maps of the final convolutional layer in the model and calculated the gradients. These gradients were subjected to global average pooling to obtain the weights. I used a weighted combination of feature maps to form output images using the ReLU (rectified linear unit) function, which allows features with a positive effect on the category of interest to be identified.

3.3 Results

3.3.1 Classification of native species and HYB

The six individuals of *A. japonicus* and 15 HYB used in the test (1,881 images) were all correctly classified, namely with an accuracy of 100%, in our experimental environment. The classification results are presented in a confusion matrix (Figure 3.5), where the vertical axis is the ground truth and the horizontal axis is the model's prediction. The number in each cell indicates the number of images classified as native species or HYB, and the color of each cell indicates the percentage of images per ground truth. For example, pale blue indicated a ratio of 0.0, meaning that no images were classified for that cell. In contrast, dark blue indicated a ratio of 1.0, meaning that all images were classified for that cell.

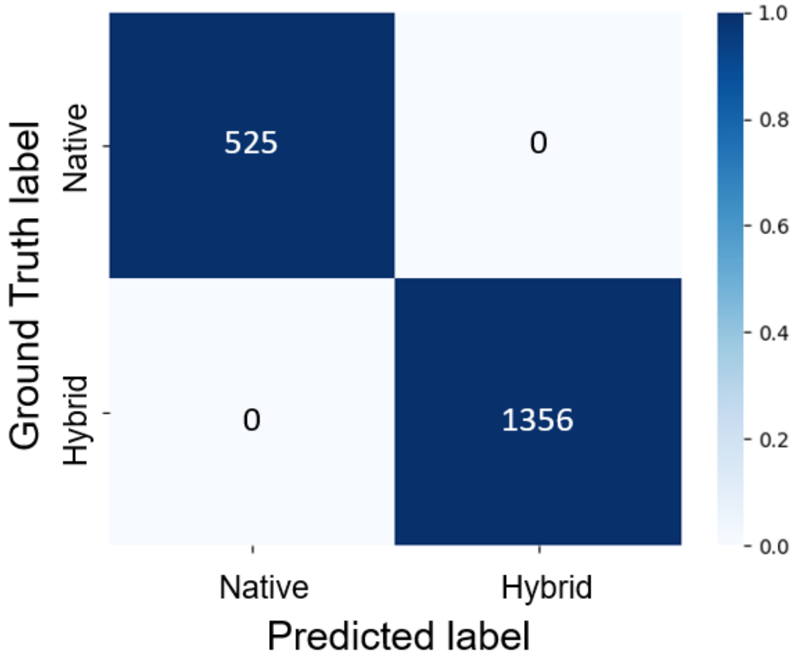

Figure 3.5 Binary classification of the native *A. japonicus* and HYB.

3.3.2 Visualization using Grad-CAM

The red regions are those considered by the model when outputting the prediction results (Figure 3.6 and Figures 3.7 and 3.8). The dorsal head spot patterning was a key area for classifying *A. japonicus* and HYB. The visualizations for *A. japonicus* indicate that the network learned to recognize the relatively distinctive large black spots. On the contrary, the visualizations for the HYB indicate that the network learned to focus on the pale and ambiguous wide region rather than the black spots. However, because the dorsal head spot patterning differed individually, the results of Grad-CAM visualization also varied among them (Figures 3.7 and 3.8).

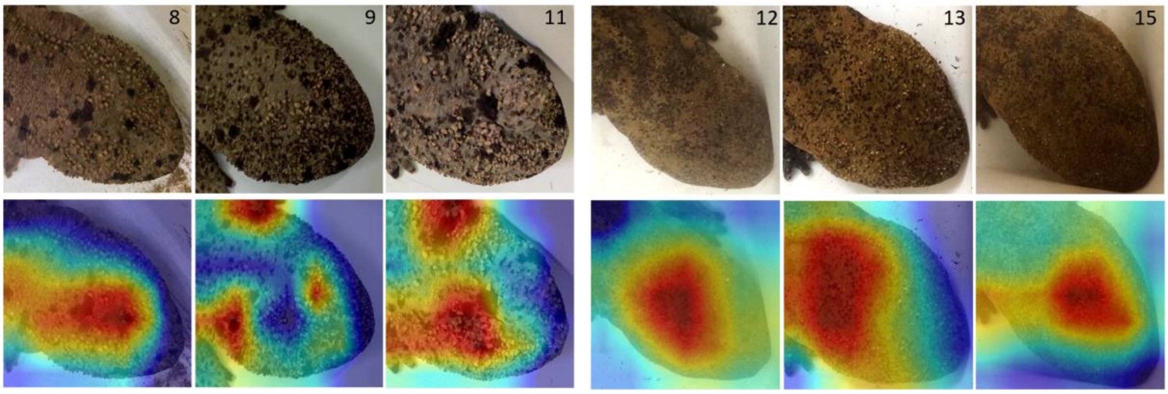

Figure 3.6 Visualization images generated by gradient-weighted class activation mapping (Grad-CAM). The three individuals on the left (8, 9, 11) were *A. japonicus*. Heatmaps indicate that the network learned the relatively distinctive large black spots of *A. japonicus*. The three individuals on the right (12, 13, 15) were HYB. In the case of HYB, the network learned the pale and ambiguous wide region.

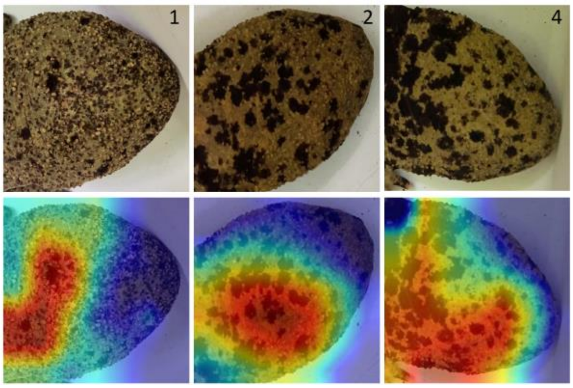

Figure 3.7 Visualization of an *A. japonicus* obtained using Grad-CAM on the trained model. The individuals depicted are different from those in Figure 3.6. The top row shows original images, and the bottom row shows heatmaps generated using Grad-CAM.

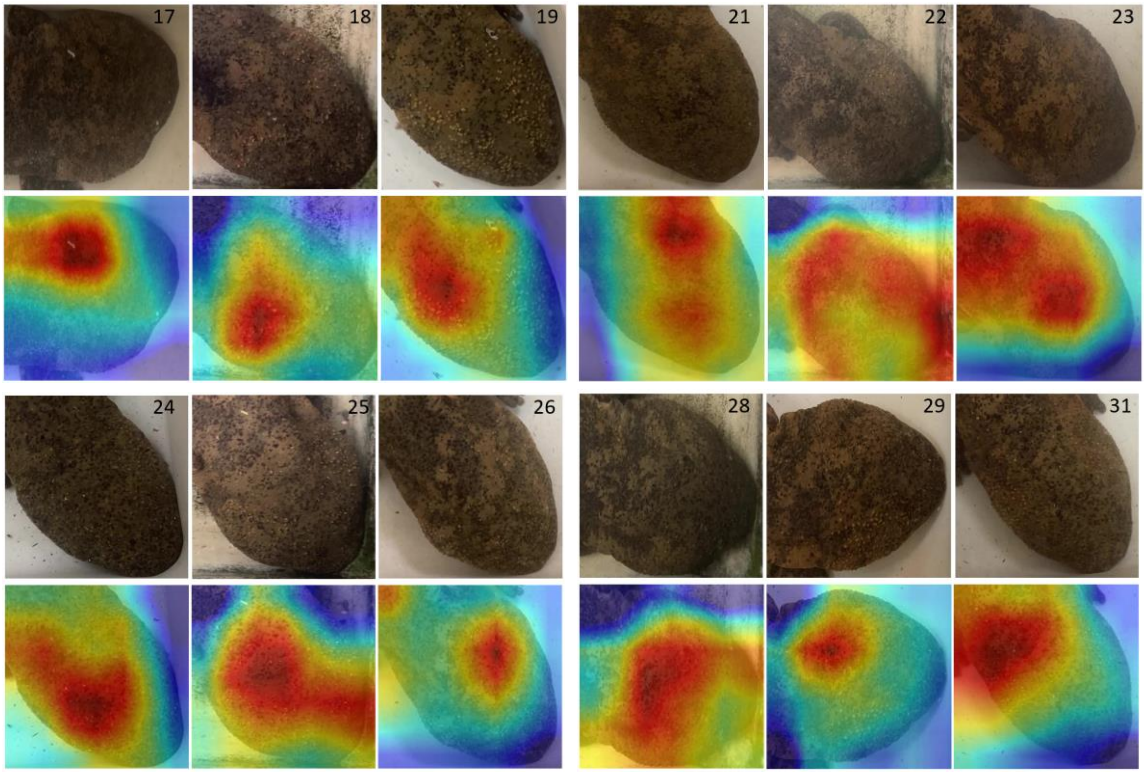

Figure 3.8 Visualization of HYB individuals obtained using Grad-CAM on the trained model. The individuals depicted are different from those in Figure 3.6. The top row shows original images, and the bottom row shows heatmaps generated using Grad-CAM.

3.4 Discussion

I identified HYB from images using deep learning. Historically, visual screening by experts and DNA analysis have been applied to identify HYB. However, the scarcity of experts and the time and cost of DNA analysis have been barriers to effective screening. Therefore, I proposed a novel approach to identifying HYB that uses an image recognition technique. A total of six native *A. japonicus* and 15 HYB individuals were used, and all were correctly classified by the AI model, namely the accuracy was 100%, in our experimental setting. Furthermore, highlighted regions that affect the AI model's prediction suggested that the model distinguished between native *A. japonicus* and HYB on the basis of spot patterns. Although deep learning has already been applied to identify species and individuals, to our knowledge, this is the first study in which it is used to identify hybrid individuals.

EfficientNetV2 demonstrated that dorsal head spot patterning can be used to identify *A. japonicus* and HYB. One reason why all individuals were successfully classified was the quality of the training and test images. In this study, photographs were taken from a short distance; thus, the high accuracy can be attributed to the clear spot patterns in the images. Another reason is that the heads were photographed from a similar angle. For example, previous studies have demonstrated that taking photographs from different angles reduces identification accuracy (Arzoumanian et al., 2005). Instead, I photographed all individuals from directly above the dorsal head and used them for training and testing images. Training and test images were also obtained on the same day, which could also have contributed to the high performance. In the future, the performance of our approach should be carefully evaluated in a varied environment, using images from different dates and locations, before it is implemented in the field.

The visualized distribution of the heatmaps was different for *A. japonicus* and HYB. For the former, the model focused on their distinctive large black spots, whereas for latter, it focused on the pale and ambiguous wide region. These results suggest that the differing spot patterns between *A. japonicus* and HYB can be utilized for classification. In general, *A. japonicus* have such black spots (Figure 3.2), whereas the spots of HYB are more indistinct (Figure 3.3). Experts use these spot pattern differences as one of the criteria to identify HYB individuals. Our study revealed that deep learning distinguishes between *A. japonicus* and HYB using the same pattern recognition as experts. The heatmap could be used as an instruction guide for the general public on HYB identification because the highlighted graphical figures are visually comprehensible. However, I could not analyze *A.* cf. *davidianus* because they are rarely found in Japan. Therefore, the dorsal head spot patterning of *A.* cf. *davidianus* should also be analyzed in cooperation with Chinese research institutions.

Although our approach achieved high accuracy in identifying *A. japonicus* and HYB in this study, several challenges still exist. Firstly, I did not consider the hybridization degree, which affects the spot pattern in HYB. The HYB used in this study were first generation (Shimizu et al., 2022), suggesting that these individuals have intermediate traits between *A. japonicus* and *A.* cf. *davidianus.* Since the HYB is fertile, the dorsal head spot pattern will depend on several factors, such as the generation. Future work should examine the relationship between the degree of genetic introgression and the identification accuracy. Secondly, it is essential to combine this method with DNA analysis because deep learning-based identification has limitations. For example, due to hybridization, some HYB have spots indistinguishable from those of *A. japonicus*. DNA analysis is the only suitable method to determine the species in such cases. However, our technology can be applied for the early detection of suspected HYB through citizen science and rapid identification by computer vision. In addition, further advances in deep learning might enable the identification of backcrossed hybrids that are difficult to distinguish even for experts because their spots are extremely close to those of *A. japonicus*. Thirdly, the sample size was small because only five individuals were used for training. Therefore, greater accuracy can be expected by using additional training datasets. However, all HYB could be identified even when the sample size was small, suggesting that image recognition is an effective approach to detecting HYB. Finally, this study was conducted in the daytime under uniform photographic conditions, whereas *A. japonicus* and HYB must be photographed under artificial light in field surveys because they are nocturnal. In the future, it is necessary to determine whether images obtained under various light conditions can be used to identify HYB.

Hybridization between native and non-native species is one of the major causes of biodiversity loss (Bourret et al., 2022). Moreover, hybrid individual detection is challenging when they are similar to the native species. Deep learning image recognition techniques can be a valuable tool to support the visual identification of hybrids. I proposed a new approach for classifying *A. japonicus* and HYB using smartphone images that could be utilized in citizen science. The artificial intelligence employed in this approach identifies HYB on the basis of spot patterns, a technique previously limited to experts, thus allowing the public to detect HYB easily. In particular, the distribution of HYB is expanding, meaning that managing them is a priority task for the conservation of *A. japonicus*. Our findings can potentially prevent their future spread by providing a method for the efficient discovery of such individuals. For example, citizens can find *A. japonicus* or HYB in the field and upload photos to social media such as X (Twitter), and our technology can facilitate the utilization of such online images.

3.5 Conclusion

I applied deep learning to identify *A. japonicus* and HYB. It was successfully demonstrated that the dorsal head patterning is an effective region for classification by Grad-CAM. Although the visual identification of HYB has historically been restricted to specialists, our approach enables the public to identify them, making it particularly useful within the context of citizen science.

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CHAPTER 4 Assessment of the impact of agricultural dams on the seasonal migration of Japanese giant salamanders: application of image recognition techniques to field survey

4.1 Introduction

Among the vertebrates, amphibians are recognized as one of the most threatened groups because of the alarming extinction rates, with more than 41% of species currently being threatened (Pimm et al., 2014). Recent rapid declines in amphibian populations have been caused by factors such as habitat fragmentation (Scroggie et al., 2019), pathogens (Cohen et al., 2019), chemical pollution (Egea-Serrano et al., 2012), and climate change (Alves-Ferreira et al., 2022). The physiological and ecological characteristics of amphibians are also associated with the population decline (Joly, 2019). Amphibians depend on a humid environment because they rely on cutaneous respiration (Gargaglioni & Milsom, 2007), and their skin is sensitive to chemical pollution due to its high permeability (Joly, 2019). In addition, species with complex life cycles that require multiple habitat types are exposed to various extinction risks (Harper & Semlitsch, 2007). Amphibians are a prime example of such species: they utilize different habitat types with aquatic and terrestrial environments through their life cycle (Goldspiel et al., 2019; Liedtke et al., 2022; Stuart et al., 2004). Furthermore, amphibians generally have limited dispersal abilities and, therefore, they are strongly affected by habitat fragmentation (Smith & Green, 2005; Wake & Vredenburg, 2008). The degradation of ecosystems is likely to accelerate further in future; therefore, immediate conservation of amphibians is required (Johnson et al., 2017).

Recognizing individuals allows researchers to understand demographic parameters required for effective conservation strategies, such as emigration and immigration, reproduction, and fitness (Bolger et al., 2012). Historically, ecologists have relied on capture-mark-recapture (CMR)-based approaches to gain such ecological information (Jung et al., 2020). Although this method is widely employed (Lindberg, 2012), it is applicable only when target species can be marked or have distinctive natural markings for distinguishing between individuals (Chauvenet et al., 2017). If there are no significant differences between individuals, artificial identification through the attachment of physical tags and GPS can be beneficial and provide various insights (Kays et al., 2015; López-López 2016). Furthermore, toe clipping is sometimes used for small amphibians. This method involves removing one or more toes for individual identification (Chauvenet et al., 2017). However, alternative approaches are sometimes required because of the cost of capture and tagging as well as animal welfare perspectives (Zemanova, 2020).

In particular, amphibians pose unique challenges in artificial marking due to their small body size, sensitive skin, and ability to regenerate clipped toes (Bendik et al., 2013). Passive integrated transponder (PIT) tags have been used extensively for identifying individual amphibians (Roberts et al., 2021). However, this method is not applicable to small amphibians and juveniles. In addition, for some species, the expulsion or movement of PIT tags within the body of animals has been confirmed (Gibbons & Andrews, 2004). This loss of PIT tags has been documented for many animal groups, including mammals (Mayer et al., 2022), birds (Jamison et al., 2000; Ratnayake et al., 2014), fish (Gheorghiu et al., 2010), reptiles (Omeyer et al., 2019), and amphibians (Ribas et al., 2022). For amphibians, Weber et al., (2019) reported that 15.7% of great crested newts (*Triturus cristatus*) expelled the tag from their bodies. Furthermore, Ribas et al. (2022) reported that all fire salamanders (*Salamandra salamandra*) and Iberian ribbed newts (*Pleurodeles waltl*) retained their PIT tags, whereas 66.6% of Pyrenean brook newts (*Calotriton asper*) lost their tags. The proportion of PIT tags lost differs among species. This is a problem when applying CMR-based approaches, because it is assumed that the artificial tags are not lost during the survey period.

The use of photography in a CMR-based approach is one potential solution (Jackson et al., 2006). Photo-identification is a non-invasive method that can recognize specific individuals from their distinctive features (Towner et al., 2013). After their first capture by a camera, individuals are considered “marked” based on natural characteristics such as stripes, spots, and scars. Secondly, if the individual is photographed another time, the photograph can be matched to an image in the database to identify the individual. This approach has also been applied to a variety of species with unique natural characteristics, including the humpback whale (*Megaptera novaeangliae*; Gabriele et al., 2022), leopards (*Panthera pardus*; Morris et al., 2022), West Indian manatees (*Trichechus manatus*; Landeo-Yauri et al., 2020), and other species (Armstrong et al., 2019; Bond et al., 2021; Morrison & Bolger, 2012). Furthermore, computer-assisted approaches such as deep learning have begun to be applied to photo-identification (Schofield et al., 2019; Clapham et al., 2020; Guan et al., 2023). However, these studies remain just technological developments, and there have been few applications to actual ecological research.

Here, I applied deep learning to photo-identification in a survey of the Japanese giant salamander (*Andrias japonicus*). These salamanders are a fully aquatic species characterized by a black spot pattern on the body that appears specific to an individual and is stable over time (Tochimoto, 1996). Although I have already developed an automatic photo-identification approach for this species, as reported in Chapter 2, the study was based on images of captive individuals obtained during the daytime. However, it is necessary to verify the applicability of this technique for night river surveys because this species is nocturnal. My objectives in Chapter 4 are (1) to verify that the developed identification method based on deep learning can be applied to images from night surveys; (2) to demonstrate the practicability of this approach for actual ecological investigations. Toward achieving the second objective, I calculated the movement distance of *A. japonicus* within the river, because the movement patterns of wild animals are crucial for understanding their ecology and effective conservation (Rubenstein & Hobson, 2004). Although *A. japonicus* migrate within the river for reproduction, structures such as small agricultural dams prevent their migration (Taguchi, 2009). Therefore, information on migration is particularly important for this species.

4.2 Materials and Methods

4.2.1 Study species

A. japonicus are resident during the non-breeding season and generally inhabit a 40-70 m home range (Kawamichi, 1997). They migrate about 200 m upstream in July and August, and the males find suitable sites for breeding (Taguchi, 2009). The mature female moves into the burrow and lays eggs while the male defends the burrow from predators. Only one male, called the den-master, is able to occupy an appropriate site because the competition between males for the burrow is severe (Okada et al., 2015). Although females move out of the burrow after laying eggs, the males exhibit paternal care behaviors such as tail fanning to provide oxygenated water to the eggs. After the breeding season, they migrate about 200 m downstream to return to their home range, where they live from September to December. The seasonal migration for breeding is sometimes prevented by river structures such as small agricultural dams, because this species does not have claws or suckers on its limbs (Taguchi, 2009).

4.2.2 Study sites

The study site was Shijihara River in Kitahiroshima-cho, Hiroshima Prefecture (Figure 4.1). The river is surrounded by rice paddies and is set in a typical rural landscape. I set up an 830 m study section. This section was separated by small agricultural dams about 2 m in height with a slope of about 50° located at points 1 and 2. Hiroshima City Asa Zoological Park has investigated this study section, which revealed a relatively high abundance of *A. japonicus*. I performed the survey with the zoo's permission because this species is protected by law. The river's width is about 4-8 m and the depth is about 50-100 cm, although both vary with the location and season.

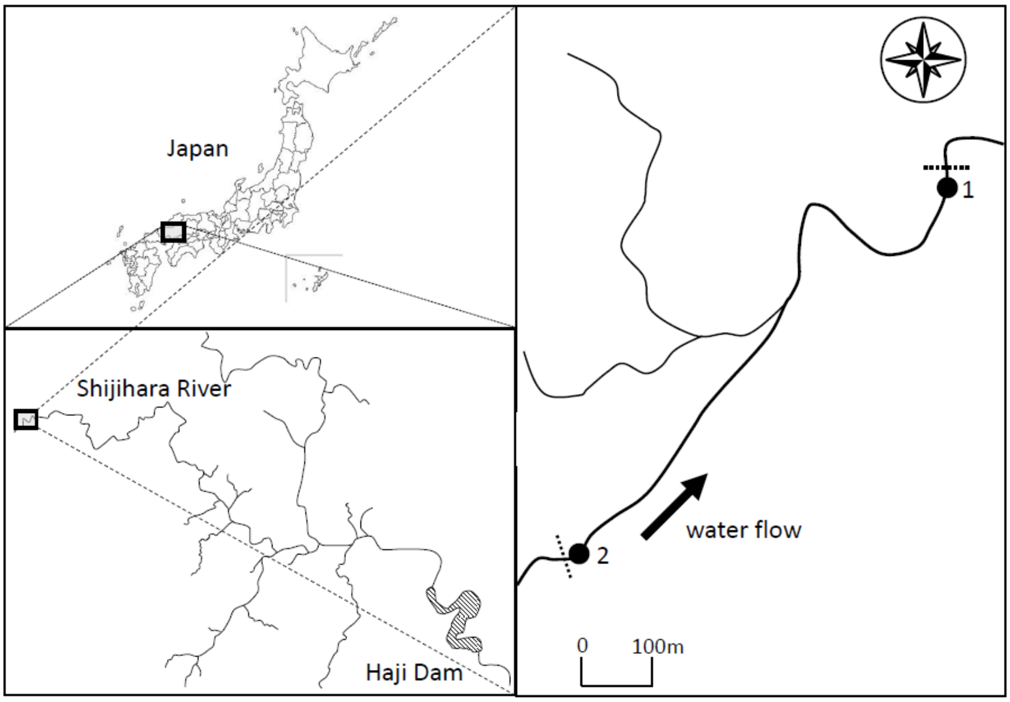

Figure 4.1 Location of the study site (Shijihara River) in northwest Hiroshima, Japan. I established an 830 m section in this study site. Dashed lines in the right figure represent small agricultural dams approximately 2 m in height with a slope of about 50°. The survey started at point 1 and finished at point 2.

4.2.3 Image acquisition

I conducted surveys on 28 May, 4-5 June, 17 June, and 21-22 October in 2023. Each survey was scheduled after sunset because this species is nocturnal. I walked upstream in the river using a flashlight to search for *A. japonicus*. In this study, individuals were photographed in two conditions: underwater and on land. The following steps were performed for individuals in the river (Figure 4.2). First, a smartphone was placed in a box with a glass bottom for underwater viewing to photograph *A. japonicus*. Next, light from a flashlight (1,280 lumens) illuminated the head, and the smartphone recorded the individual in video format for approximately one minute. Although a 1,280 lumen flashlight was used in May and June, it was changed to a 1,900 lumen flashlight in October. Finally, I captured the individuals and measured their length, weight, and PIT tag presence. At the same time, the capture location was recorded by a GARMIN eTrex 30xJ GPS. Individuals on the land were photographed by the following procedure (Figure 4.3). First, an *A. japonicus* individual was placed in a chute to measure its body length. Next, I photographed the individual under flashlight illumination. All images in this study were recorded in Full HD video using an iPhone SE 2020.

Figure 4.2 (a) Photographing *A. japonicus* in the river. Individuals in the water were recorded in video format with a smartphone set up in a box with a glass bottom. A flashlight with an intensity of 1,280 lumens was used in May and June and one with an intensity of 1,900 lumens was used in October. (b) Actual situation of photographing *A. japonicus* in the water.

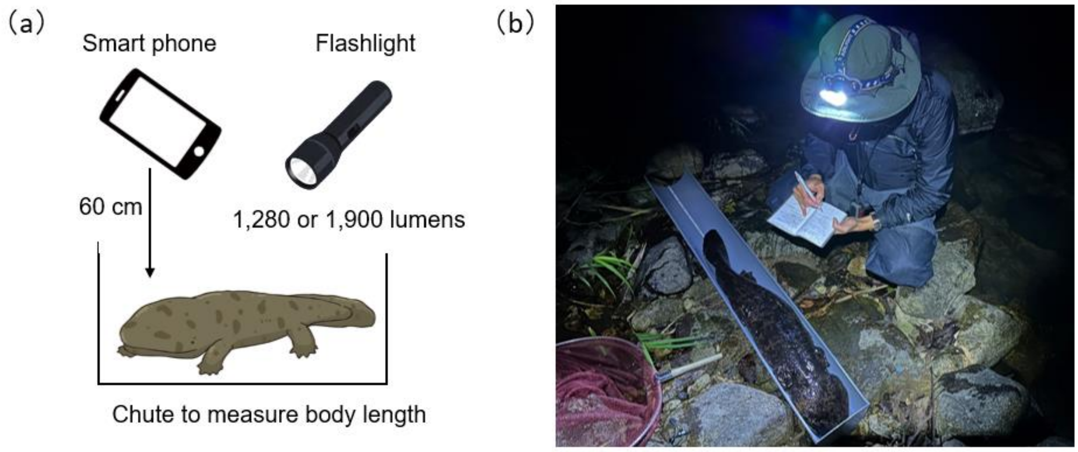

Figure 4.3. (a) Photographing *A. japonicus* on land. Individuals on land were recorded in video format with a smartphone at a distance of 60 cm from the head. (b) Actual situation of photographing *A. japonicus* on land.

4.2.4 Development of AI models for individual identification and biological application

The objectives of this chapter are to compare the performance of AI models created from images obtained in two conditions (underwater and land) and to calculate the movement distance of *A. japonicus* within the river. In this study, the movement distance was defined as the distance between the most upstream and downstream locations where the same individual was captured several times. I created the AI model using the following steps (Figure 4.4). First, only individuals captured at least twice were used. I utilized the images taken on the days when the dorsal head spot patterning was most clearly photographed for training and the other images as test images. Thirty images were used in the test for each individual. In addition, the raw data was cleaned to remove blurred images (Figure 4.5). Next, I created AI models with underwater images (underwater model) and land images (land model) by the same procedure as in Chapter 2 and compared their performance. The parameter settings are summarized in Table 4.1. In this study, successful individual identification was defined as at least 70% (21/30 images) of an individual's test images being correctly classified. The movement distance within the river was calculated using QGIS version 3.18 (QGIS Development Team, 2022). Since a small agricultural dam marked each end of the study section (Figure 4.1), I hypothesized that individuals would move only within the section and not move from downstream to upstream. In addition, I performed linear regression with maximum movement distance as the objective variable and body weight as the explanatory variable.

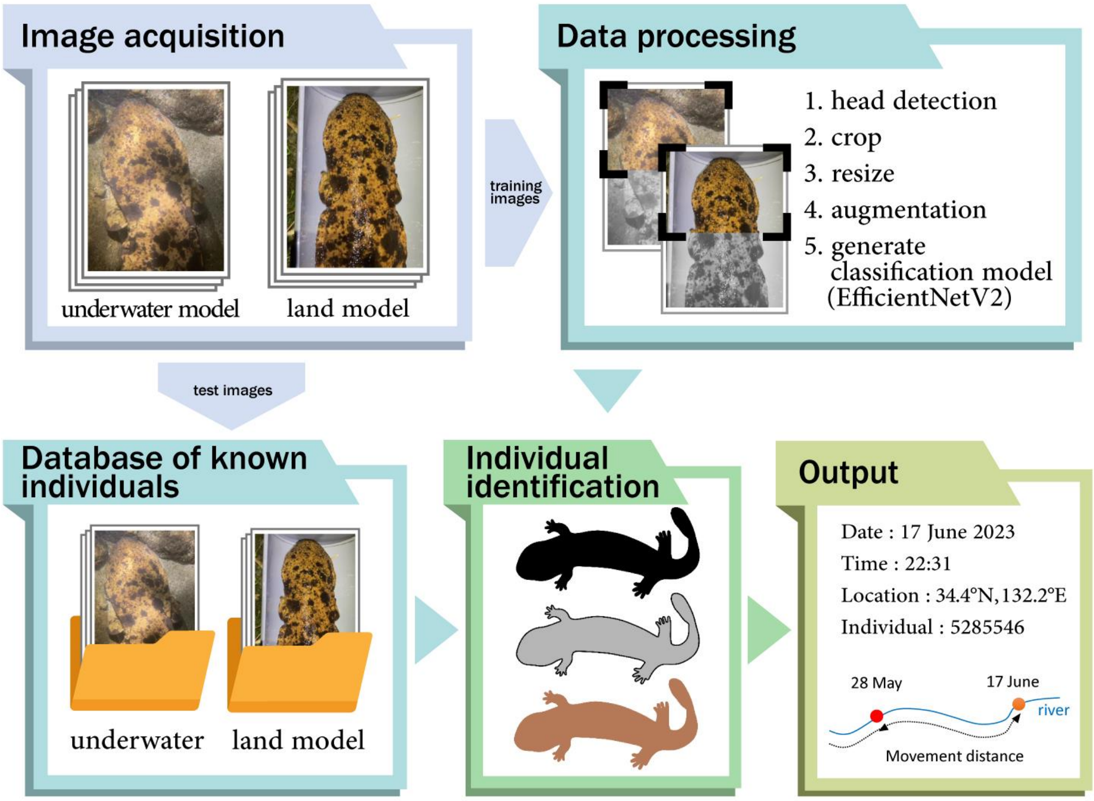

Figure 4.4 Workflow of individual identification in this study.

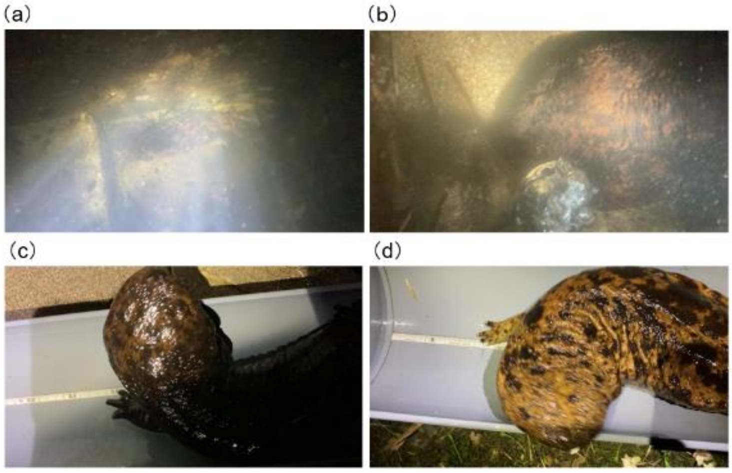

Figure 4.5 Examples of images removed from training and test images. In (a) the dorsal head spot patterning could not be seen due to the depth of the river. In (b) the water is less clear, and air bubbles exhaled by the individual are photographed. (c) and (d) are blurred images due to the active motion of the individual.

Table 4.1 Parameter settings.

Parameter	Value
Epoch	80
Batch size	32
Drop out	0.3
Optimaizer	Adam

4.3 Results

4.3.1 Underwater model

A total of 24 individuals were captured, and ten individuals were captured at least twice (Table 4.2). The average number of recaptures was 2.7 (median = 2, n = 10, SD = ± 1.19, range = 2-6), and individual No. 502 was captured in all surveys. In addition, the average total length of the individuals was 758 mm (median = 619 mm, n = 10, SD = ± 102.27 mm, range = 557-889 mm) and the average weight was 1.63 kg (median = 1.67 kg, n = 10, SD = ± 0.35 kg, range = 1.13-4.63 kg). For the underwater model, nine individuals were used for analysis because no images of individual No. 124 could be acquired. A data summary of training and validation images in this model is shown in Table 4.3. Figure 4.6 shows the confusion matrix of the underwater model. Although eight of the nine individuals were correctly classified (88.9%), individual No. 272 failed to be identified. Example images of individuals No. 920, which was identified correctly, and No. 272 are shown in Figure 4.7.

4.3.2 Land model

Eight individuals were used for analysis in the land model because photographing on land was not conducted in the June 17 survey. As a result, the land model did not include individuals No. 348 and No. 363. Table 4.4 is a data summary of the training and validation images in this model. The classification results are shown in Figure 4.8. This model correctly identified seven out of eight individuals (87.5%). However, individual No. 502 was not classified correctly. Images of this individual are shown in Figure 4.9.

4.3.3 Application of deep learning approach to calculate movement distance

The movement distances of the ten recaptured individuals are shown in Table 4.5. The average movement distance was 51.6 m (median = 10.8 m, n = 10, SD = ± 73.3 m, range = 3.1-238.8 m). The maximum movement distance of seven of the ten individuals (70%) was less than 20 m. On the other hand, the maximum movement distance of three individuals was over 198 m. All capture locations for individual No. 999 were downstream of the small agricultural dam at point 1 (Figure 4.10). Although all individuals except No. 108 were captured within the study section between points 1 and 2, individual No. 108 was captured upstream of a small agricultural dam at point 2, contrary to the hypothesis (Figure 4.11). Linear regression results showed no significant relationship between body weight and maximum movement distance (Figure 4.12).

Table 4.2 Recaptured individuals. The survey was conducted six times, twice each in May, June, and October.

Individual ID	First capture	First recapture	Second recapture	Third recapture	Fourth recapture	Fifth recapture	Total length (mm)	Body weight (kg)
108	28 May	17 June	22 October				778	3.58
124	28 May	4 June	22 October				758	2.13
187	28 May	4 June					No data	1.75
272	28 May	4 June					625	1.9
348	28 May	17 June					614	1.66
363	5 June	17 June					619	1.13
502	28 May	4 June	5 June	17 June	21 October	22 October	889	4.63
741	28 May	4 June					612	1.68
920	5 June	21 October					557	1.25
999	28 May	4 June	5 June				615	1.21

Table 4.3 Data summary of training and validation images in underwater model.

Individual ID	Number of recaptures	Training	Validation
108	2	2,100	900
187	1	2,100	900
272	1	2,100	900
348	2	2,100	900
365	2	2,100	900
502	5	2,100	900
741	1	2,100	900
999	2	2,100	900
920	1	2,100	900

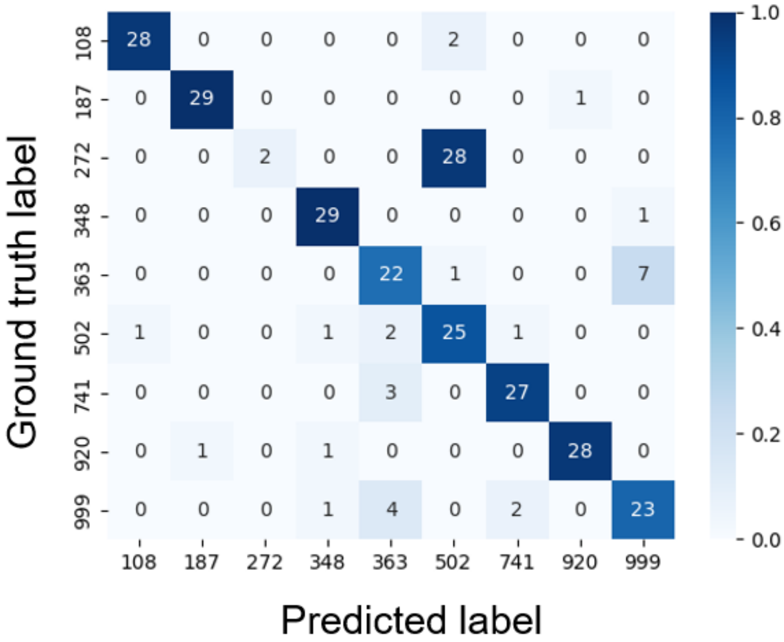

Figure 4.6 Confusion matrix of the underwater model. The vertical axis shows the ground truth, and the horizontal axis shows the model's prediction. The three-digit numbers on the axes indicate individual numbers, numbers in cells indicate numbers of classified test images, and the color of each cell indicates the percentage of images in each class.

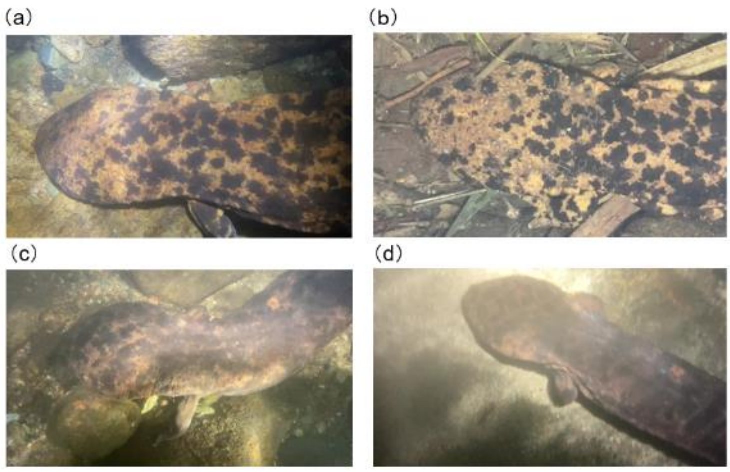

Figure 4.7 Images of individuals No. 920 and No. 272, which the underwater model correctly identified and failed to identify, respectively: (a) training image (5 June) and (b) test image (21 October) of individual No. 920; (c) training image (28 May) and (d) test image (4 June) of individual No. 272.

Table 4.4 Data summary of training and validation images in land model.

Individual ID	Number of recaptures	Training	Validation
108	2	3,220	1,380
124	2	3,640	1,560
187	1	3,710	1,590
272	1	2,870	1,230
502	5	3,500	1,500
741	1	3,150	1,350
999	2	3,290	1,410
920	1	3,430	1,470

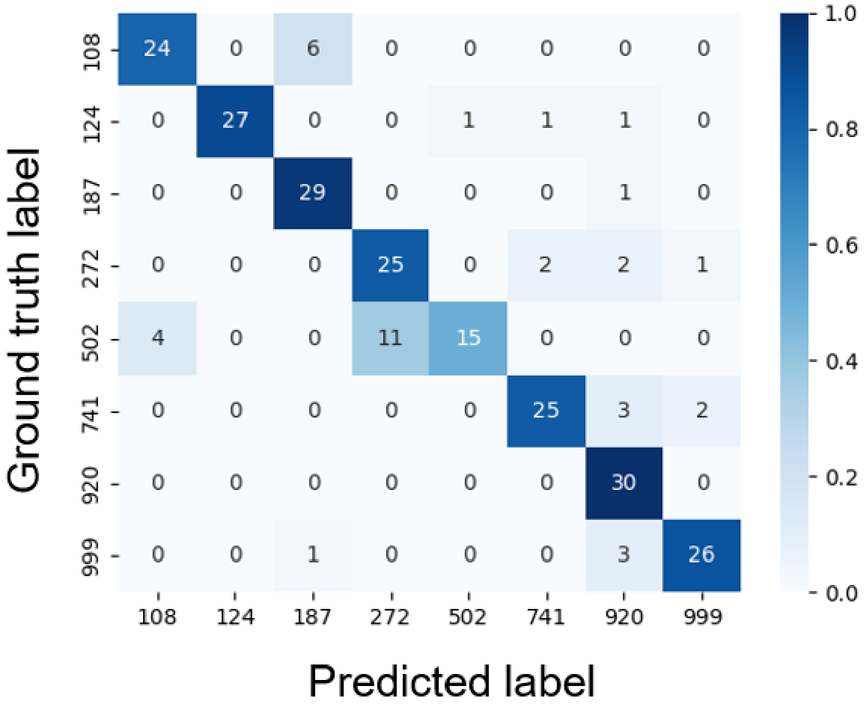

Figure 4.8 Confusion matrix of the land model.

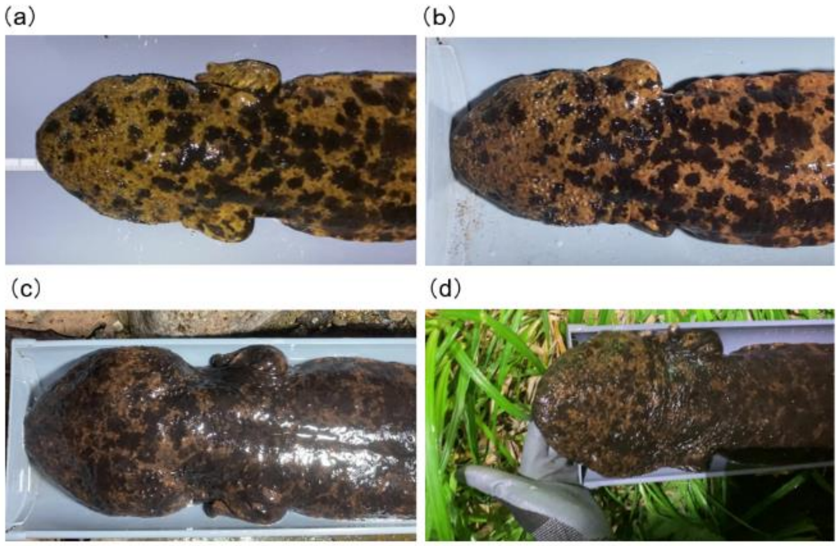

Figure 4.9 (a) Training image (5 June) and (b) test image (21 October) of individual No. 920, which was correctly identified in the land model. (c) Training image (21 October) and (d) test image (21 October) of individual No. 502, which was not correctly identified. The head angle in the test image differed from that in the training image due to the individual's active motion.

Table 4.5 Average and maximum movement distances of individuals captured multiple times. The maximum movement distance was defined as the distance between the most upstream and downstream points when the same individual was captured multiple times.

Individual ID	Date	Average movement distance ± SD (m)	Maximum movement distance (m)
272	28 May 4 June	3.1	3.1
187	28 May 4 June	4.8	4.8
999	28 May 4 June 5 June	9.1 ± 2.79	11.4
502	28 May 4 June 5 June 17 June 21 October 22 October	9.3 ± 22.55	198.4
741	28 May 4 June	9.5	9.5
920	5 June 21 October	12.4	12.4
124	28 May 4 June 22 October	12.0 ± 3.32	16.7
348	28 May 17 June	16.8	16.8
108	28 May 17 June 22 October	159.3 ± 78.14	238.8
363	5 June 17 June	231.3	231.3

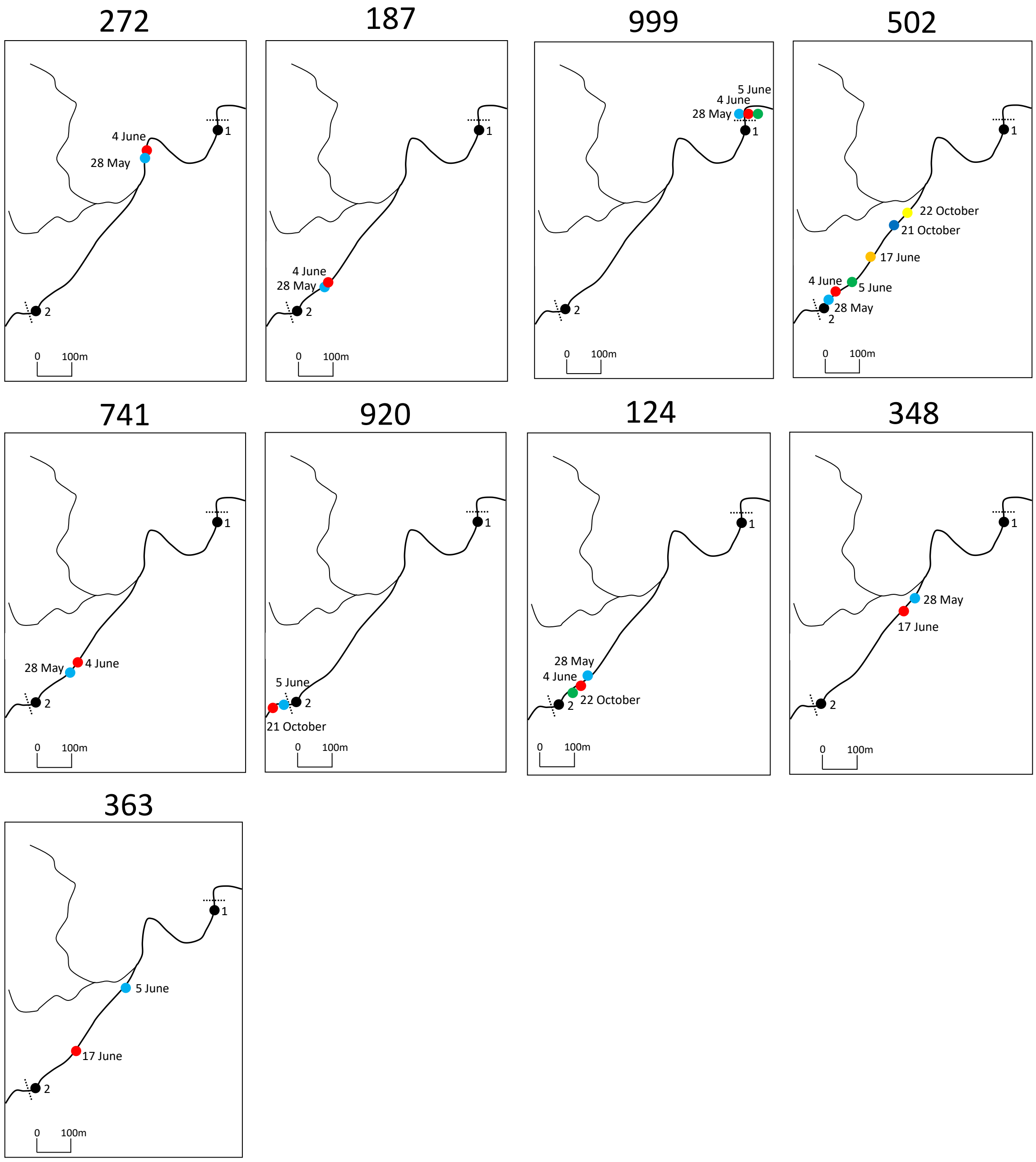

Figure 4.10 Capture locations of individuals. The numbers above the figures indicate individual IDs.

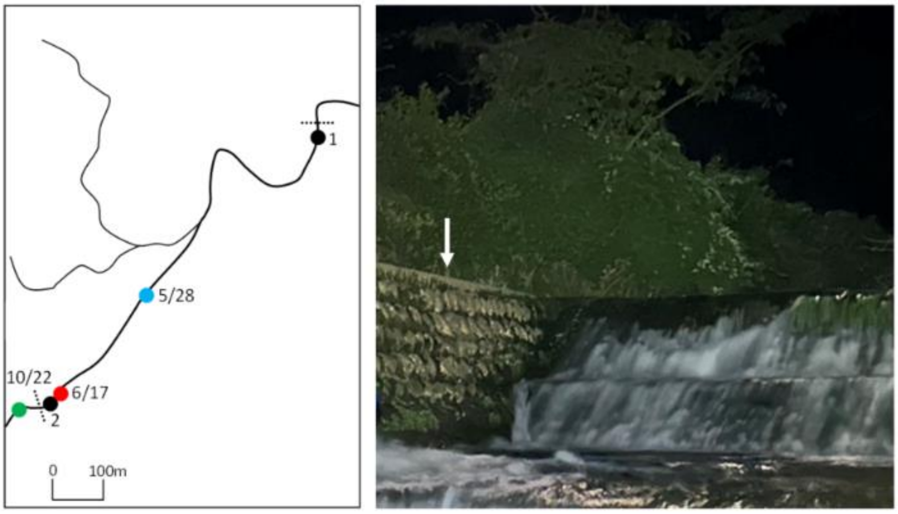

Figure 4.11 Capture location of individual No. 108. Although this individual was captured downstream of the small agricultural dam at point 2 during May and June, it was captured upstream in October. The picture shows the small dam at point 2, and the white arrow indicates the concrete structure that this individual might have climbed to move upstream.

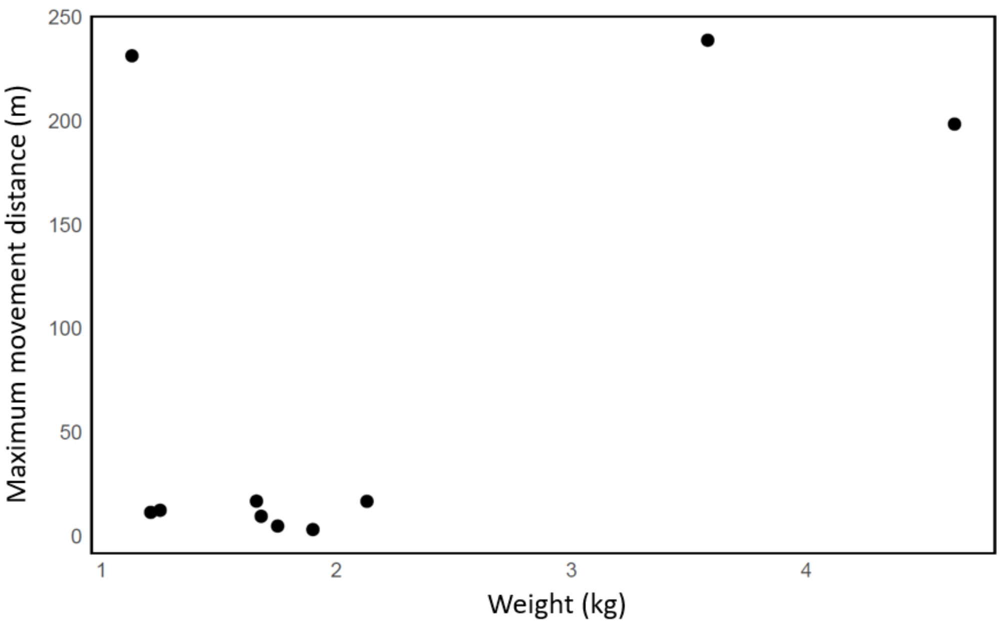

Figure 4.12 Relationship between the body weight and maximum movement distance obtained by linear regression (y = -34.64 + 52.08x, n = 10, F = 3.91, p = 0.08).

4.4 Discussion

With advances in AI technology, deep-learning-based image recognition techniques are beginning to be applied in the academic field, including ecology. Previous research employing deep learning in the identification of individual animals has been limited to the development of technology, which has been rarely used in actual ecological research. In this study, I applied this approach to a survey of *A. japonicus* conducted at night and revealed that more than 80% of the individuals could be identified. Additionally, I found the movement of one *A. japonicus* over a small agricultural dam, which was considered a potential barrier. Seasonal migration for breeding is essential for the conservation of this species, and the methods I have developed have great potential as new tools for applying AI techniques to ecological research.

The underwater and land models were able to identify individuals from images (Figure 4.6 and 4.8). Although the underwater model successfully identified eight of the nine individuals (88.9%), it was difficult for it to classify individual No. 272. This could have been due to the quality of the image. When photographing this individual, the river's water was muddy, and I could not provide sufficient illumination because this individual moved to the bottom of the river quickly. In the land model, seven of the eight individuals were successfully classified (87.5%), but individual No. 502 could not be identified. This could be due to the different head angles of the training and test images. Since previous studies have reported that different photographic angles reduced accuracy (Arzoumanian et al., 2005), it would be possible to identify this individual if images with a uniform photographing angle could be acquired. However, this AI model successfully identified most individuals without anesthesia to stop their movements, which is an advantage of the proposed approach.

The maximum movement distance for seven of the ten individuals was less than 20 m (Table 4.5). This could be attributed to the survey schedule. I surveyed in May and June, in the non-breeding season of *A. japonicus*, during which the species inhabit a 40-70 m home range (Kawamichi, 1997).

Five of the seven individuals with a maximum movement distance of under 20 m were only captured in May and June, suggesting that this short movement distance reflects the highly resident characteristics of the species. The average movement distance was 51.6 m (SD = ±73.3 m). On the other hand, three *A. japonicus* exhibited a maximum movement distance of approximately 200 m. Previous studies have also indicated a similar migration pattern in *A. japonicus* and other salamanders, with most individuals moving only short distances but some moving long distances (Bar-David et al. 2007; Taguchi, 2009). Long-distance migration by a small number of individuals could contribute to population persistence through source-sink dynamics (Lowe & McPeek, 2012; Van Houtan et al., 2007). This point is important for management and conservation because the removal of the source population should result in the extinction of the sink population (Tittler et al., 2006). In other words, conservation in local areas is a high priority for this species because there is little immigration of new individuals from other sources when local populations become extinct. There was no relationship between body size and maximum movement distance (Figure 4.12). However, this may be due to the small sample size, and additional research is required. Furthermore, individual No. 108 moved upstream over the small agricultural dam located at point 2, contrary to the hypothesis (Figure 4.11). Although this dam is approximately 2 m in height and has a slope of about 50°, previous studies have reported that river structures with a height of approximately 80 cm and slope of ≥ 40° prevent seasonal migration of *A. japonicus* (Taguchi & Natuhara, 2009). The river structures that *A. japonicus* can negotiate depend on the surface structure as well as the height and slope (Taguchi & Natuhara, 2009). Therefore, the concrete structure beside the river may have assisted the movement of this individual. However, the likelihood of this small dam functioning as a barrier to migration is unclear; therefore, additional research should be conducted in the future.

Although this study successfully identified individuals using photographs obtained from field surveys, some limitations still need to be addressed. First, the images used for automatic identification cannot always be obtained in field surveys due to varying photographing conditions, such as water depth, brightness, and distance from individuals. For example, it is sometimes impossible to photograph a dorsal head spot patterning clearly in a muddy river after rainfall. Furthermore, wild individuals escape from humans; thus, images cannot be acquired if they hide under rocks or escape to the bottom of deep rivers. The availability of images was also an issue in previous studies and is a challenge when applying deep learning automatic identification to wildlife (Schofield et al., 2019). The accuracy of deep-learning-based identification also depends on the quality of the images, and in such cases, manual photo-identification is sometimes more accurate (Morrison et al., 2016). Second, the feasibility of this method for long-term monitoring is unknown because this study only included images obtained during May, June, and October 2023. Individual characteristics could change due to aging, injuries, or weight fluctuations during surveys across years. *A. japonicus* have a lifespan of over 60 years (Tochimoto et al., 2007), and the robustness of AI models needs to be assessed in the monitoring of long-lived species. Schofield et al. (2019) successfully identified 23 chimpanzees over four years using images collected in the field. Therefore, long-term automatic identification would be feasible if images suitable for training and testing were available. Finally, the applicability of my method to several hundred individuals is unknown because of the small sample size in this analysis. However, my research is expected to be useful for ecological studies and conservation efforts in small rivers such as this study site, even if there are only about ten individuals, because most *A. japonicus* rarely migrate long distances.

I examined the feasibility of individual identification through deep-learning-based image recognition techniques obtained from a nocturnal survey. Most previous studies have been limited to just developing the technology, making it a challenge to use this technology for biodiversity conservation. I demonstrated that this method can be applied to wild populations and identify more than 80% of the individuals in the analysis. In addition, this study revealed the movement of individuals using photo-identification, which is essential for understanding population structure and conservation. Since photo-identification does not require the capture of target species, this method could be applied to large-scale ecological surveys employing citizen science. For example, a database obtained by citizen science revealed that reef manta rays (*Mobula alfredi*) migrate over 1,000 km, which is the maximum migration distance of manta rays (Armstrong et al., 2019). Migration is especially important for *A. japonicus* because this species migrates seasonally for breeding. The techniques in this study provide a novel opportunity to identify individuals, allowing the acquisition of population parameters for endangered species.

4.5 Conclusion

I have demonstrated that the image-based individual identification method can be applied to field surveys at night. The results of this study indicated that individual identification is feasible if the unique spot patterns can be recorded as images. Furthermore, I also revealed that one *A. japonicus* moved beyond the small agricultural dam. However, all other individual capture locations were within the section separated by two dams. River fragmentation is one factor contributing to the declining population of this species, and my research shows that deep-learning-based approaches can be effective in gaining the information needed for conservation.

4.6 References

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CHAPTER 5 General discussion

I proposed a method to identify individuals of the target species without capture, using computer vision technology that utilizes deep learning. In this study, I attempted to identify *A. japonicus*, an endemic and endangered amphibian species in Japan, based on each individual's dorsal head spot patterns, as described in Chapter 2. In addition, I applied a similar approach to identify hybrid individuals between native and non-native species in Chapter 3. Furthermore, I applied my image recognition technology to actual ecological surveys, as reported in Chapter 4, which had been limited to just technological developments in previous studies. The following sections provide a summary of each chapter.

5.1 Summary of each chapter

Chapter 2 describes the development of an image-based identification method based on deep learning that uses the dorsal head spot pattern of *A. japonicus*. I trained and evaluated a dataset collected over two days from 11 individuals in captivity, which included 7,075 images taken by a smartphone camera. Individuals were photographed three times a day at approximately 11:00 (morning), 15:00 (afternoon), and 18:00 (evening). As a result, individual identification using the EfficientNetV2 neural network achieved 99.86% accuracy, a kappa coefficient of 0.99, and an F1 score of 0.99. The performance was lower for the evening model than for the morning and afternoon models, which were trained and evaluated using photographs taken at the corresponding times of the day. The proposed method does not require direct contact with the target species, and the effect on the animals is minimal; moreover, individual-level ecological information can be obtained under natural conditions.

In Chapter 3, I aimed to identify hybrids between native *A. japonicus* and non-native *A.* cf. *davidianus* using EfficientNetV2 and smartphone images of 11 native *A. japonicus* (five training and six test images) and 20 individuals of hybrids (five training and 15 test images). In my experimental environment, an AI model constructed with EfficientNetV2 exhibited 100% accuracy in identifying hybrids. In addition, gradient-weighted class activation mapping revealed that the AI model was able to classify *A. japonicus* and their hybrids on the basis of the dorsal head spot patterning. My approach thus enables the identification of hybrids against *A. japonicus*, which was previously considered difficult by non-experts. Furthermore, since this study achieved reliable identification using smartphone images, it is expected to be applied to a wide range of citizen science projects.

In Chapter 4, I applied deep learning to photo-identification in a survey of *A. japonicus*. Previous research employing deep learning for individual identification has been limited to the development of technology, which has been rarely used in ecological research. Furthermore, it was unclear whether the methods developed in this doctoral dissertation would also be practical for field surveys because *A. japonicus* is nocturnal. Therefore, I verified that the developed method based on deep learning can be applied to images from night surveys of Shijihara River in Hiroshima Prefecture. In addition, I calculated the movement distance of *A. japonicus* within the river to demonstrate the practicability of this approach for actual ecological investigations. I set up an 830 m study section separated by small agricultural dams. The survey was conducted on 28 May, 4-5 June, 17 June, and 21-22 October. A total of 24 individuals were captured, and ten were captured at least twice. More than 80% of individuals could be identified in images obtained underwater and on land. In addition, I found that most individuals moved only distances within the study section, but one was observed outside the study section, having climbed over a small agricultural dam, which was considered a potential barrier. The results demonstrated that the method of identifying individuals from images can be applied to field surveys at night and that deep-learning-based approaches can effectively obtain the ecological metrics needed for conservation, such as migration distance.

5.2 Comprehensive discussion

In this doctoral dissertation, I applied image recognition techniques to ecological research to identify individuals of *A. japonicus* and their hybrids. Deep learning substantially affects many areas of society and science (Jordan & Mitchell 2015). Although this approach has been used to identify species, classify animal behavior, and estimate biodiversity in large datasets from camera-trap images in ecology (Christin et al. 2019), its application to individual identification has been limited. Individual identification is the basis of ecological research, and fundamental information gained from long-term monitoring studies provides valuable insights (Dell et al. 2014). However, there are challenges in implementing such individual-based long-term studies. The first issue is the impacts of individual identification methods on animals as described in Chapter 1. Whereas invasive and non-invasive methods have advantages and disadvantages (Peralta et al. 2020; Zemanova 2020), my proposed technique offers a new approach that overcomes these disadvantages. The next issue concerns the challenges of establishing a long-term research program due to data objectivity and funding issues. There are limitations to the effectiveness of a single researcher remaining involved in a study requiring long-term monitoring over decades (Clutton-Brock & Sheldon 2010), and changing the research member could lead to biased data (Burghardt et al. 2012; Johansson et al. 2020). Unlike humans, automated matching techniques are consistent, free from subjective bias, and unaffected by fatigue (Town et al. 2013). Therefore, my proposed method contributes to long-term monitoring through automatic identification with a photographic database. In addition, the most significant problem in long-term studies is the difficulty of maintaining funding without interruptions (Clutton-Brock & Sheldon 2010). Funding for research institutions is declining in Japan, intensifying concerns about its dwindling international standing in science (Ikarashi 2023). Some scientists indicate that support for basic research in Japan has been compromised because the government desires to make academia more responsive to the needs of society and industry (Phillips 2017). Therefore, gaining continued funding is difficult for long-term monitoring in basic research such as ecology. However, my method allows low-cost identification and can be applied to actual ecological studies, as demonstrated in Chapter 4. Since animal identification and monitoring are costly (Witmer 2005), this method could help projects that are difficult to continue financially.

I also attempted to identify hybrid individuals from images in my doctoral dissertation. Non-native species are one of the threats to biodiversity conservation, but no studies have used deep learning to classify hybrid individuals between native and non-native species. I demonstrated that the dorsal head patterning is an effective region for classifying native *A. japonicus* and their hybrids. Early detection and a rapid response are essential countermeasures against non-native species and hybrids, because it is difficult to control target species that have become established in the wild (Foxcroft et al. 2009). However, effective detection is challenging in the early stages of establishment because the founding population size and density are often low (Martinez et al. 2020). Moreover, if a hybrid individual is morphologically similar to a native species, such as a hybrid between *A. japonicus* and *A.* cf. *davidianus*, it becomes difficult to detect visually. My approach provides new opportunities for large-scale, low-cost, image-based identification of hybrid individuals through citizen science. For example, mobile applications using deep learning have already been applied to determine the distribution of non-native species (Kress et al. 2018). The findings of this dissertation will contribute to biodiversity conservation by facilitating the detection of hybrid individuals, which was previously difficult.

5.3 Future perspectives

The application of image recognition techniques to ecological studies is beneficial and has the potential to become a major tool for gaining a variety of ecological metrics related to breeding, predation, and mortality (Schneider et al. 2019). As new devices such as UAVs and camera traps become more advanced and less expensive, the affinity between the large datasets generated by these devices and deep learning approaches will increase (Tuia et al. 2022). Further applications of deep learning in ecology are required from the following perspectives.

(1) Photo-identification is non-contact and non-invasive; therefore, it is valuable for animals with small populations, such as endangered species. However, image recognition techniques using deep learning require datasets with many images, which makes obtaining the training images an issue when dealing with endangered species. I also faced this challenge; only 11 *A. japonicus* could be used in the analysis in Chapter 2. In the future, it is necessary to verify the feasibility of this method by increasing the number of individuals used for analysis in the future. Collaboration between research institutions, zoos, and aquariums would also be beneficial for the application of deep-learning-based approaches to ecological studies because the acquisition of images is easier for animals in captivity.

(2) An automatic method to identify individuals and species through images is beneficial for biodiversity conservation, but the areas where images are collected could be biased geographically. For example, most of the images included in iNaturalist, a mobile application based on the concept of mapping and sharing records of species on the planet, were taken in the Northern Hemisphere (Tuia et al. 2022). Therefore, it is necessary to resolve regional biases and collect images from biodiversity hotspots, such as tropical regions. Recently, language has been identified as a barrier to the global understanding of biodiversity conservation (Amano & Sutherland 2013). For example, 35.6% of

scientific documents on biodiversity conservation are not in English (Amano & Sutherland 2013). Therefore, ignoring non-English documents can cause biases in our understanding of biodiversity. In contrast to language, images can be shared easily, regardless of the region in which they are collected. It may be necessary to build a platform for sharing images from mobile applications in different countries to take advantage of such image data.

(3) I compared the accuracy of AI models based on images taken at different times of the day and at different locations in the river and found that the quality of the images had a significant influence on the identification performance. In particular, variations in the quality of images from field surveys are generally high due to weather conditions, camera flash, distance from the object, and changes in the animal posture (Anderson et al. 2010; Cheeseman et al. 2022; Chen et al. 2020; Ferreira et al. 2020; Gallo et al. 2022; Schneider et al. 2019). Therefore, it may be necessary to prepare manuals on photography procedures to obtain images suitable for automatic identification. *A. japonicus* are suitable for large-scale surveys in citizen science because their motion is relatively slow and their heads can be photographed easily from the same angle. In addition, utilizing various photographs as training images could contribute to the robustness of the AI model. The application of the deep-learning-based approach to the field study addressed in Chapter 4 demonstrated that the accuracy of automatic identification decreased for images with different head angles. Therefore, it is necessary to improve the practicality of the AI model in the field by including a variety of images in the training images.

Although direct observation of animals in nature remains important in ecology, new technologies provide new observation opportunities (Kays et al. 2015). Combining deep learning and images will be a major approach to generating ecological insights in the future. The approach in this doctoral dissertation accurately identifies individuals and hybrids and can be applied in field surveys. The utilization of new technologies, such as those in this study, can contribute to biodiversity conservation in the Anthropocene.

5.4 References

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ACKNOWLEDGMENTS

Firstly, I express my sincere gratitude to my main advisor, Dr. Takeshi Ise, for the continuous support of my doctoral studies. A few years ago, I had hoped to get a Ph.D. someday while working. He provided me the opportunity to research again and taught me the spirit of science. With the help of his excellent supervision and guidance throughout my studies, I was able to finish my doctoral dissertation without giving up. Definitely, his invaluable advice was crucial to my growth as a researcher. I learned the attitude toward science from him. He always reminds me of the importance of questioning myself about the novelty of my study. I never forget his words, "There is no perfect research that everyone will agree on. But that is why we should not give up but rather make an effort to improve our research as much as possible!" I will make the most of this lesson in my future career. I could not have imagined having a better advisor and mentor for me. I also appreciate Professor Naoko Tokuchi for supporting my research. She warmly welcomed me at the Laboratory of Silviculture, provided an excellent environment to devote myself to research, and offered many helpful comments. The environment allowed me to focus on my research, which resulted in the publication of two papers. I would like to extend my sincere thanks to Professor Reiji Masuda for his insightful comments and encouragement. His attitude toward his research inspired me and taught me the importance of direct observation of organisms in the field. I also thank Professor Yusuke Onoda for his time and effort in reading and for his constructive comments on improving my thesis. He constantly inspires me with his approach to improving his research with new technologies, such as UAVs and LiDAR. The time I talked with him about research at the Tomakomai experimental forest was very beneficial to me. I would also appreciate Associate Professors Hisashi Hasegawa and Masae Ishihara for their feedback on my thesis. Their helpful advice in the lab helped me improve my research and contributed to the publication of valuable articles.

I would also like to thank Dr. Yuki Taguchi. During my fieldwork through my doctoral course, he greatly supported me in collecting field data. When a tough situation happened in the field, it was a great experience and gave me confidence because we were able to solve the problem together. He was kind enough to assist me in researching nocturnal Japanese giant salamanders after his work finished. I really appreciate his support until midnight. His warm support and continuous encouragement enabled me to enjoy my doctoral course. He provided me with many broad perspectives, not only on science but also on life planning. I also thank the Japanese giant salamander team members in Hiroshima City Asa Zoological Park for providing valuable information and supporting my study.

I thank all members of the Laboratory of Silviculture and Forest Informatics, Kyoto University, for their advice on my study, and goofing helped me forget all the efforts. Without the help of Dr. Soyoka Makino and Dr. Tomika Hagiwara, I could not carry out this study. They gave me really valuable suggestions and kind words as a researcher who received their Ph.D. a few years ago. Ms. Akane Kawasaki and Mr. Kazuya Tsubota, I am grateful for your friendships and times of laughter.

I would like to thank all those Japanese giant salamanders for their patience in cooperating with my studies. They always taught me a lot of important things about nature and gave me a chance to feel myself as one of nature. I could not have written this thesis without Japanese giant salamanders.

My research was supported by many people who contributed to my thesis in many ways; I cannot name all of them here. None of the work I have presented would have been done without them. I have been lucky to be surrounded by many kinds of people. Thank you all for supporting my studies.

Finally, I would appreciate my family greatly for their spiritual and financial support throughout my student life. Thanks to their support, I have become the person I am today. I am deeply grateful to my family for providing me with this "life."