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Identification of *Notophthalmus viridescens* Individuals from Photographs Using the Interactive Individual Identification System (I^3S) Software

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Abstract

More efficient methods for estimating the population size and density of salamander species are becoming increasingly important. Amphibian species across the world are experiencing massive population declines and extinctions due to factors such as habitat loss, climate change, and diseases such as *Batrachochytrium dendrobatidis* (*Bd*), or the chytrid fungus. *Batrachochytrium salamandrivorans* (*Bsal*) is an emerging pathogenic chytrid fungus that has been documented in both wild and captive salamanders across Europe and is likely to be spread across the world through the pet trade, posing a significant threat to salamander populations. Estimates of population size (such as Mark-Recapture) are important to track changes in populations over time. However, conventional methods of population size estimation may suffer from drawbacks such as being labor intensive, being invasive, or causing mortality. I tested the effectiveness of the Interactive Individual Identification System (I^3S) software at identifying *Notophthalmus viridescens* (Eastern Newt) individuals from images. I caught and photographed the ventral surfaces of 33 Eastern Newts across six survey days between the months of March and July 2023. I captured all newts at Sandy Bottom Preserve, located south of Asheville, North Carolina. I took at least three photographs of each individual. I used the software I^3S Straighten to ensure all individuals were straight and facing the same direction in images. I then tested the effectiveness of the software I^3S

Pattern+ in correctly matching images of the same individual. I found that the software correctly matched images of the same individual in the first match result 91% of the time. I also found match accuracy to be directly related to image quality. Additionally, I provide future recommendations for conducting Mark-Recapture studies on this species at Sandy Bottom Preserve and effective software use.

Introduction

Estimates of population size and density are key to monitoring trends in animal populations over time. The Mark-Recapture method is one of the most well-known and effective methods of estimating population size and standardizing count statistics (Nichols 1992). The procedures used in Mark-Recapture studies vary greatly depending on what animal is being studied. Data deficiency is a major obstacle to the formation and implementation of effective conservation strategies worldwide, and more efficient methods of population estimation could decrease knowledge gaps in the status of threatened species and could lead to swifter responses in the conservation of species.

The modern biodiversity crisis, often called the Sixth Mass Extinction (Wagler 2012), highlights the importance of more efficient and accurate methods of population estimation. Amphibians have experienced some of the most severe global population declines and extinction rates out of any other major group (Wake & Vredenburg 2008). Many of the threats faced by amphibian species are anthropogenic in nature and their effects are highly complex, making it difficult to predict impacts on amphibian populations. Such threats include habitat loss, climate change, introduced species, and emerging pathogens (Blaustein et al. 2010).

New and emerging diseases pose a major risk to global amphibian biodiversity. Diseases of amphibians are vast and may have viral, bacterial, mycotic, protozoan, or metazoan etiologies (Densmore and Green 2007). Environmental changes such as those caused by climate change can increase disease virulence and transmissibility, leading to sudden die-offs. An amphibian disease of recent concern is chytridiomycosis, a skin infection caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). Approximately 700 vertebrate species belonging to three orders can be infected by *Bd*, and it has contributed to population declines in almost 400 species of amphibians worldwide (Lips 2016). The discovery of *Bd* and its epizootic nature is relatively recent. Some of the first amphibian declines associated with *Bd* occurred in the tropical rainforests of Australia throughout the 1980s, and *Bd* itself was first described in 1997 (Lips 2016). It is currently found on every continent except Antarctica and its virulence varies significantly. Modern *Bd* epidemics are limited to Australia, South America, Central America, Southern Europe, and Western North America. Because the virulence and transmissibility of the pathogen varies greatly across regions, outbreaks are highly unpredictable and future epidemics are a major concern.

Closely related to *Bd*, *Batrachochytrium salamandrivorans* (*Bsal*) is another species of chytrid fungus that is of particular concern among salamander species. *Bsal* appears to infect Caudata species with greater frequency than Anura species, while *Bd* more frequently infects Anurans (Moubarak et al. 2022). First described in 2013, *Bsal* has been shown to infect 1,300 species of amphibians (Moubarak et al. 2022). Thought to have spread from Asia to Europe through the pet trade, it has since led to severe population declines of fire salamanders (*Salamandra salamandra*) and northern crested newts (*Triturus cristatus*) across Northern Europe. Fire salamander declines in Northern Europe are as high as 99.9% in regions such as the Netherlands (Spitzen-van der Sluijs et al. 2016). *Bsal* has not yet been found in the North America, but the likelihood of *Bsal* introduction to the region is high. More species of salamander are found in the Appalachian Mountains of the United States than anywhere else in the world, with about a third of all described salamander species being found in this region, and a *Bsal* outbreak in this region could lead to catastrophic loss of biodiversity (Bishop 2020).

Other anthropogenic impacts such as climate change and habitat loss are also major contributors to amphibian declines. Climate change is a global phenomenon that has numerous direct and indirect effects on amphibians worldwide. The consequences of climate change occur over a vast temporal and spatial scale, making it difficult to pinpoint impacts on specific amphibian populations. Direct effects include changes in precipitation, temperature, and weather patterns (Blaustein et al. 2010). In response to the direct effects of climate change, amphibians will likely experience worsening altitudinal and latitudinal range shifts and alterations in behavior, development, and reproductive and life cycles (Blaustein et al. 2010). The indirect effects of climate change on amphibians include alterations to habitat and community composition, changes in food availability, increased disease transmission, and increased exposure to UV-B radiation (Blaustein et al. 2010). Climate change and its impacts are vast and complex, making its effects on amphibian populations unpredictable. Like climate change, habitat loss is a global phenomenon impacting amphibian populations. Amphibian biodiversity is highest in the tropics, where many biodiversity hotspots are located. Biodiversity hotspots have lost more than 70% of their original geographic extent and now only cover 1.4% of Earth's land surface (Mittermeier 2002). Furthermore, 57% of terrestrial vertebrates are endemic to biodiversity hotspots (Mittermeier 2002). As the major threats to amphibian biodiversity are expected to worsen, so are amphibian declines and extinctions.

A major hurdle to conservation initiatives is a lack of data. Data deficiencies on populations impede conservation efforts and can lead to "silent extinctions". Amphibians have the highest proportion of species listed as Data Deficient (DD) on the IUCN Red List out of all terrestrial vertebrate groups; about 25% of all amphibian species are listed as DD on the IUCN Red List, and some studies estimate that as many as half of all amphibian species listed as DD are threatened with extinction (González-del-Pliego et al. 2019). DD species are often considered low priority for protection. Because threats such as *Bd*, *Bsal*, and climate change are highly unpredictable and can cause catastrophic losses over a short period of time, DD species could experience irreversible declines or even extinction before enough data has been collected to properly inform protection efforts.

Traditional procedures used in methods of population estimation such as Mark-Recapture, while effective, may suffer from drawbacks such as having a high margin of error, being labor intensive, being invasive to the organism and its habitat, and causing high mortality. Mark-Recapture studies obtain data for population estimation by capturing, marking, and releasing individuals within a period of time, recapturing individuals within a second period of time, and counting the number of marked individuals that were recaptured (Nichols 1992). A common method used to mark amphibians such as salamanders is toe clipping, in which one or more toes are cut in such a way that is unique to each individual. While it is generally considered that toe clipping does not significantly affect salamanders, it can impair reproduction and locomotion in some species, and toe regeneration as well as naturally caused toe loss can lead to inaccurate population estimates (Ott & Scott 1999). Using photography and image identification to identify individuals in Mark-Recapture studies has the potential improve both efficiency and accuracy. Photography and imaging are commonly used in population estimation and monitoring; camera traps have been used to monitor wildlife for decades (Kucera & Barrett 2011). Camera traps make handling wildlife unnecessary, limiting mortality, disturbance, and disease transmission. Often used to observe large terrestrial mammals, camera trapping is used in Mark-Resight surveys of population size and density, utilizing pelage patterns unique to each individual, like a fingerprint (Alonso et al. 2015).

Similar to pelage patterns, *Salamandridae* species exhibit coloration and spot patterns that are unique to each individual. A study on large populations of two *Salamandridae* species, *Ichthyosaura alpestris* and *Lissotriton vulgaris*, found that spot patterns did not significantly change over time and were varied enough to allow for accurate identification of individuals (Mettouris 2016). The invasiveness of Mark-Recapture studies can be greatly reduced by utilizing individual spot patterns instead of applying markings such as toe clippings. While individuals would still need to be caught and photographed, body clippings would not be necessary. The accuracy of Mark-Recapture studies could be increased by utilizing photo-identification software instead of manual identification by researchers in the field. Standardized images run through an identification software could minimize human error. Mapping of several key points in markings provides much more detail in individual identification when compared to methods such as toe clipping. Photography and photo identification software can also increase the efficiency of Mark-Recapture studies by decreasing labor requirements, as less time would be spent in the field marking individuals and identifying and tallying recaptures.

There are a multitude of computer programs used to process and identify photographs of wildlife. One such software is the Interactive Individual Identification System (I^3S). I^3S uses patterns that occur naturally on the bodies of animals to identify and distinguish between individuals. A study on the use of I^3S for the identification of sea turtles found that 97% of captured and photographed turtles were correctly identified with I^3S Pattern (Calmanovici et al. 2018). The goal of this study is to test the accuracy of I^3S Pattern+ at identifying photographs of Eastern Newt individuals, explore methods of newt photography and capture, and determine how image quality affects accuracy of identification.

Methods

Capture Site

I captured and photographed all newts at Sandy Bottom Preserve. The preserve consists of floodplain communities such as montane alluvial forest and a swamp-forest bog complex. Salamander and turtle diversity are particularly high here; 15 species of salamander and 5 species of turtle have been found here (Petranka *unpubl.*). Ownership of the property has changed multiple times in the past century. Sandy Bottom was acquired by George Vanderbilt as part of the Biltmore Estate between 1900-1907, but was acquired by the US Forest Service in March 1917, becoming part of Pisgah National Forest (Petranka *unpubl.*). In 1967, the US Forest Service transferred the property to the Daniel Boone Council of Boy Scouts. In 1980, the property was sold to private landowners who donated parcels of land to various organizations, including UNCA, to protect floodplain communities. The entirety of Sandy Bottom became UNCA property by 2005, when The Nature Conservancy transferred the rest of the property to UNCA (Petranka *unpubl.*).

Newt Capture and Photography

I captured and photographed newts with a team of six researchers. We captured and photographed a total of 33 Eastern Newt individuals over the course of six non-consecutive days between the months of March and July 2023. The six capture dates were as follows: March 6 (Day 1), May 4 (Day 2), May 20 (Day 3), June 8 (Day 4), June 12 (Day 5), and July 6, 2023 (Day 6). We captured all newts in the morning to early afternoon. We photographed the ventral surfaces of all captured newts. Adult Eastern Newts have lighter coloration on the ventral side compared to the dorsal side, meaning spot patterns on the ventral side are easier to distinguish from the background coloration.

Our goal for the first capture date (Day 1) was to determine the most efficient method of capturing and photographing newts. No newts captured on Day 1 were included in the image database. We captured all newts on Day 1 using minnow traps set in the vernal pools the previous day. We wore nitrile gloves while handling all newts, and newts were kept in a bucket filled with site water when not being photographed. We first tested photographing newts without placing them on a petri dish. We then tested the use of a small tube or straw cut in half longways to stabilize and straighten newts placed on a petri dish. We decided to photograph all future newts by placing each individual in a petri dish and either gently holding the newt down from the top with a moist paper towel or holding the newt straight with the index and ring finger while gently pressing down on the newt with the middle finger (Figures 1 and 2).

Figures 1a (left) and 1b (right). Tory Ash photographing an Eastern Newt while I hold the individual straight, with the ventral side flat against the petri dish.

We captured and photographed 19 individual newts on Day 2, more than all other capture dates. We captured newts on Day 2 using both minnow traps and large dip nets in the vernal pools. Most of these newts were captured using dip nets. We tested the use of three separate cameras: a Canon EOS 70D DSLR, a Google Pixel 6 stock camera, and a Samsung Galaxy stock camera with a built-in macro lens for close-up pictures. We took at least three pictures of each newt included in the database, taking more pictures if the first pictures taken were of poor quality. The newts photographed on Day 2 were the first to be included in the database.

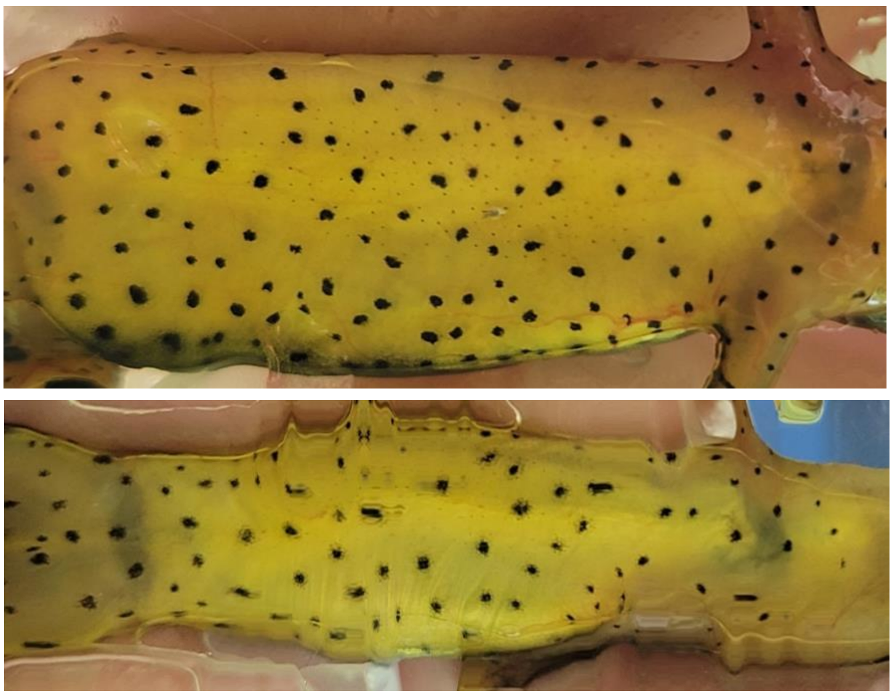
Day 3 was the last date that newts captured in the vernal pools were included in the database. The vernal pools in the wetland area dried significantly over the 16 days since Day 2. Only a fraction of the water was left in the vernal pools, and only two individual newts were captured in dip nets over the course of 6 person-hours. Day 3 was the last date of newt capture in the wetland area. The vernal pools were almost completely dry, and no newts were captured on this day. On day 4, we shifted away from the wetland area and began netting in the pond. We tested a new method of capture using large minnow seine nets about 10 feet in length. No newts were captured on Day 4. Switching back to using large dip nets, we captured and photographed a total of 11 newts in the pond in Day 5. Individuals caught on Day 5 were the last to be included in the database. No newts were caught in dip nets on Day 6, and newt capture and photography were concluded.

Image Processing Using I^{3}S

I compiled the images from each device and organized them by date in a Google Drive folder. I entered the file names of all photographs into a Microsoft Excel spreadsheet organized chronologically by date and time captured. I assigned a quality score between zero and four to each photograph and entered it in the spreadsheet. A quality score of four indicates an ideal photo that clearly displays the full ventral pattern of the individual, and a quality score of zero indicates a photo in which most or all of the ventral pattern of the individual is obscured (Figure 3). I also entered the capture date, photographer name, capture ID, location captured (Wetland or Pond), and any applicable notes into the spreadsheet. The capture ID denotes the individual to which an image represents and consists of the number captured chronologically and the sex separated by an underscore (for example, 22_male is the 22nd newt to be photographed and is a male). I determined sex by rear leg width and the presence of nuptial pads on the rear legs. I reviewed all images and picked the three highest quality images of each individual to be included in the database, for a total of 99 images.

Figures 2a (left) and 2b (right). Variation in ventral spot patterns between two male Eastern Newts caught in the vernal pools of Sandy Bottom Preserve. Nuptial pads are visible on the inner thighs of both individuals.

I processed the three images with the highest quality scores for each individual using I^{3}S Straighten to ensure that the individual was straight, with the snout directly lining up with the cloaca. Because not every individual was photographed at the exact same angle, I^{3}S Straighten also rotated photographs so that newts were positioned with the snout pointed to the right and the tail pointed to the left. After editing images in I3S Straighten, I created a new database in I3S Pattern+. Three reference points are required to correct for variations in scale and rotation among images; I set these to the central posterior abdomen, left upper armpit, and right upper armpit. I set three metadata settings manually: warp size, relative key point distance, and number of key points. I initially set these to 700, 0.03, and 100, respectively. However, due to software crashes, I lowered the number of key points to 95 and changed the relative key point distance to 0.05. All images labeled as “top three” were then annotated using I^{3}S. Annotating in I^{3}S Pattern+ maps the unique pattern of each individual and generates a ‘fingerprint’ for each image. Annotation is done by circling the region of interest (the abdomen) and highlighting 6 references for background (lighter regions of the abdomen) and foreground (the dark spots). I annotated the top three images of each individual and added them to the database, for a total of 99 annotated images and 33 individuals. I named each annotated image included in the database after the corresponding capture ID.



Figures 3a (top) and 3b (bottom). An example of a straightened image with a quality score of zero (top) and a quality score of 4 (bottom). In the top image, I^{3}S Straighten significantly distorted the spot pattern during the straightening process.

Image Search and Individual Matching

After annotating and entering all images into the database in I^{3}S Pattern+, I randomly selected one image out of the top three images of each individual included in the database (33 randomly selected images total, one for each individual). Using the ‘search in database’ function in I^{3}S Pattern+, I searched the database for images of individuals that match the newt in the randomly selected image. After searching for an individual in the database that matches the newt in the randomly selected image, the top matches are listed in order. I^{3}S provides two indices of similarity between photographs: the number of matching **key points** and the score (referred to here as **match score**). Images with a high number of matching key points are likely the same individual. The number of key points mapped by I^{3}S in each image is configurable; for this study, I set the number of key points to 95 to prevent software crashes. Thus, the number of matching key points between two images is a value out of a total of 95 mapped key points. The match score indicates the differences in key point sizes, so images with match scores close to zero have a high likelihood of being the same individual (Figure 4). I visually compared all match results to verify that no individuals were recaptured and falsely labeled as a separate individual. I entered the file names of all randomly selected images into a separate spreadsheet labelled ‘test’ and organized them by capture ID (1_male to 33_male). To organize and compile the results, I listed the file names of the top three match results for each randomly selected image in order along with the match score and number of matching key points. Since three images per individual were included in the database and one randomly selected image for each individual was used in the search, only two correct matches are possible. I compared match results to the spreadsheet listing the capture ID of images to determine whether each match result was correctly matched to the same individual. I used Microsoft Excel for all calculations.

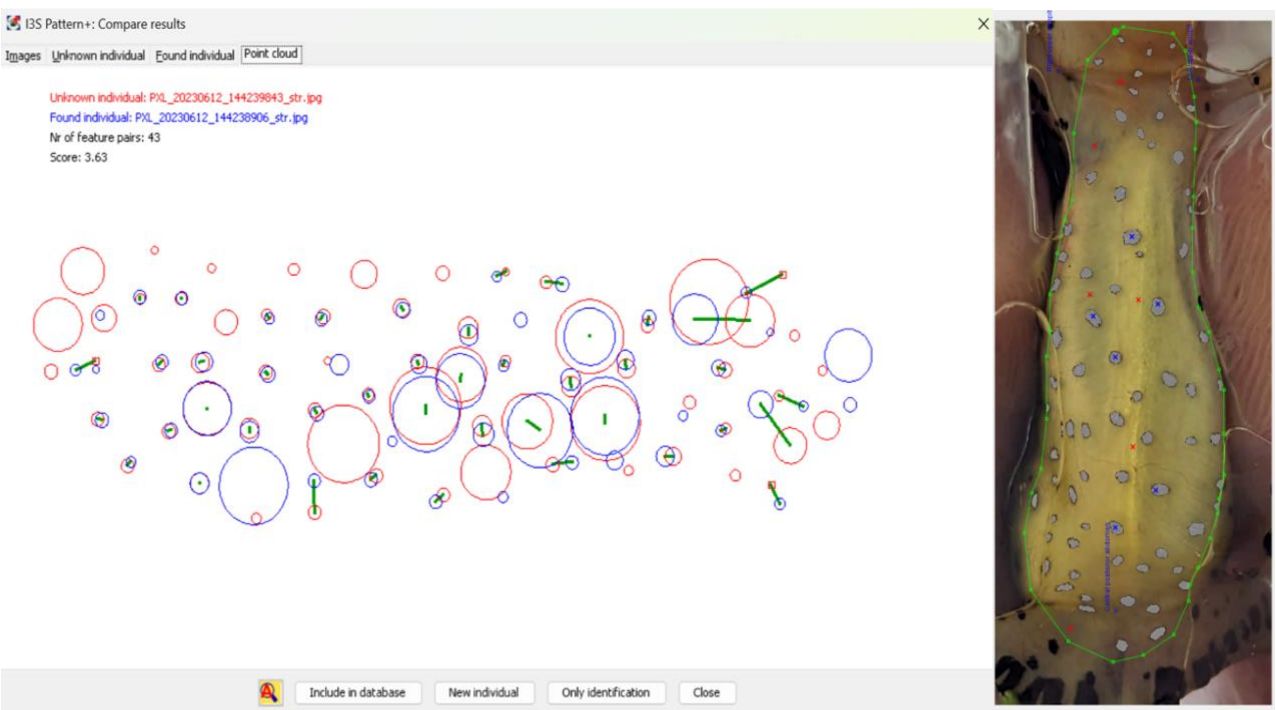


Figure 4. I^{3}S match result window (left) displaying the key points (spots) of the randomly selected image (red circles) and the top match result (blue circles). The green lines indicate matching pairs, of which there are 43. This is a correct match, with a match score of 3.63. The randomly selected picture is shown on the left with annotations.

Results

Newt Capture and Photography Methods

Out of all tested Eastern Newt capture methods, I found the use of dip nets to be the most effective for capturing newts in both the pond and the wetland areas of Sandy Bottom Preserve. Minnow traps were initially effective in the ephemeral wetland area, but no newts were captured in minnow traps after Day 2. Minnow traps with a mesh size large enough for newts to become stuck also posed a mortality risk. Seine nets were not effective at capturing newts, likely due to the difficulty of dragging the net fast enough to trap newts in the net.

Holding newts still by hand or with a paper towel on a petri dish was most effective. Using a cut tube or straw did not prevent newts from moving and compressed the abdomen, obscuring the ventral spot patterns. Out of the three cameras tested, the Samsung Galaxy was the most effective at newt photography. The Google Pixel 6 had difficulties with variation in brightness between images, and the Canon EOS 70D DSLR was not equipped with a macro lens and thus could not bring the ventral surfaces of the newts into focus at close distance.

Newt Individual Recognition Performance in I^{3}S Pattern+

I^{3}S Pattern+ correctly matched selected images with a different image of the same individual in the first match result 90.91% of the time (30 correct matches out of 33 total searches). The second match result identified the same individual 63.64% of the time (21 correct matches out of 33 searches). The third match identified the same individual 6.1% of the time (2 correct matches out of 33 searches; Figure 5). I expected percent correctness of the first and second matches to be high and the third matches to be low because there are only two other photographs of a given individual in the database, and with perfect recognition these would be the two highest matches. In both cases of a correct third match, the first match was correct, and the second match was incorrect. At least one image of the same individual was included in the top three matches 90.91% of the time. For the three remaining cases in which no correct individual was among the top three matches, the quality scores for the original photograph were all 2, and the match scores were above 11, indicating a low potential for agreement between the original and matched images.

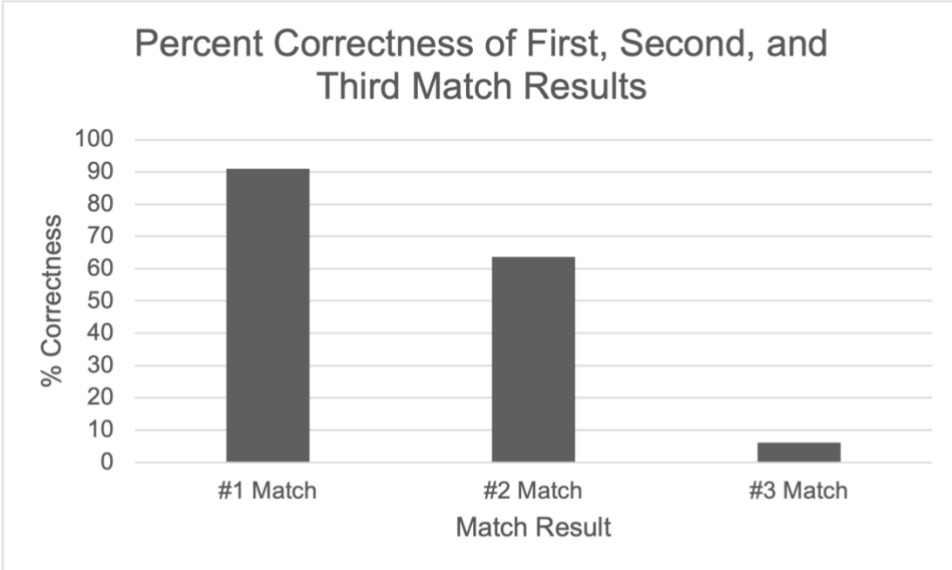


Figure 5. The percent correctness of the first, second, and third match results. The top match was correct 90.91% of the time, the second match was correct 63.64% of the time, and the third match was correct 6.1% of the time. Percent correctness is high in the first result, but sharply declines from the second result down. While none of the third results were expected to be the same individual as the one in the randomly selected image, two search results displayed another image of the same individual as the third result. In both of these cases, the first match result was correct, and the second match result was incorrect.

Effects of Photograph Quality on Individual Recognition Performance

The quality of the photographs directly affected the accuracy of individual recognition in I^{3}S Pattern+. Randomly selected images with low quality scores that were used in searches consistently had less accurate match results than selected images with higher quality scores. There were three cases in which all the top three matches were incorrect; in all three cases, the randomly selected image used in the search had a quality score of 2 or below. Two of the randomly selected images used in searches had a quality score of 4, and both images were correctly matched to the other two images of the same individual in the first two match results. There was a significant correlation between quality scores and match scores.

Discussion

I^{3}S Pattern+ is an effective and efficient tool for identifying individual Eastern Newts; however, field photography must be meticulously conducted, photography methods must be standardized as much as possible, and cameras capable of focusing on objects closer than a foot to the lens must be used to ensure all photographs are taken uniformly and display the entire pattern of the individual. The quality of images used in this study varied significantly, yet individual recognition was still mostly

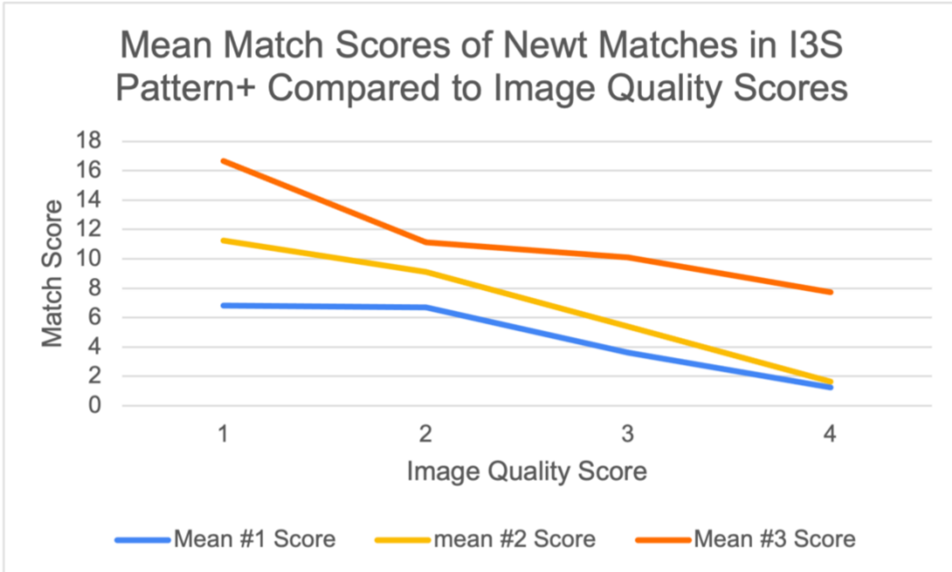


Figure 6. Relationship between the mean match scores for the first, second, and third match results and the quality score of the image used in the search. Match scores closer to zero indicate a good match between the match result image and the image used in the search. Higher match scores indicate larger variations in spot patterns, meaning the individual in the match result is not the same individual as the one in the image being used in the search. Match score decreases as quality score increases. Linear regression revealed that the R^2 values of mean #1, #2, and #3 are 0.91, 0.99, and 0.90, respectively.

accurate. 90.91% of top matches were the same individual that was pictured in the selected reference image used in the search (an increase of 5.2% compared with a similar salamander study referenced in the I^3S Manual). Although the first match result was the same individual 90.91% of the time, accuracy dropped by 27.27% in the second match, with only 63.64% of second results being the same individual. While all third match results were expected to be images of a different individual, two searches displayed an image of the same individual as the third match result, and in both cases a correct first match result and an incorrect second match result were displayed. The frequency of searches containing at least one correct match in the top three results was 90.91%. There was a strong correlation between match score and image quality score. Thus, matching errors in I^3S can be directly attributed to poor photography of newts. Accuracy of individual recognition when using I^3S depends on the standardization and control of all variables that have the potential to decrease the quality of images in the field.

A major obstacle to the completion of this study was newt capture and abundance over the summer months. We noticed a steady decrease in the number of newts caught over from Day 1 to Day 6 of newt capture and photography. Eastern Newts have a triphasic life cycle that includes an aquatic larval stage, a terrestrial juvenile stage (during which they are called red efts), and an aquatic adult stage (Beane et al. 2010). Eastern newts typically breed in spring and fall, and larvae usually transform into terrestrial efts by the summer or fall (Beane et al. 2010). Eastern newts are usually done breeding by early summer and some vernal pools begin to dry up around the same time. Thus, for future studies involving the capture of Eastern Newts from the vernal pools at Sandy Bottom Preserve, I recommend conducting all captures between the months of February and April. Regarding capture methods, I would first recommend the use of minnow traps since this method is the least labor intensive. The use of dip nets also proved to be effective in newt capture, while seine nets did not.

Quality scores assigned to images were determined based on factors impacting image quality. These factors include present glare, blurriness, angle of capture, posture of newts being photographed, brightness, and visibility of the ventral spot pattern. Glare, blurriness, angle of capture, and brightness can all be effectively controlled for and standardized. I recommend standardizing photographs by (a) only using artificial lighting that can be controlled and adjusted, (b) photographing all newts from the exact same distance while using the same focus, (c) using a tripod to stabilize the camera, and (d) properly restraining newts or using a light anesthetic to prevent movement. Other studies that have tested photography in individual identification often use an anesthetic to prevent movement during photography. For example, a study testing individual identification of *Calotriton asper* (Pyrenean brook newt) using photographs of ventral patterns achieved low error rates and high recognition rates by anesthetizing all newts, using a tripod, and using flash in all images (Dalibard et al. 2021).

In addition to field photography methods, image quality can be increased by using editing software such as I^{3}S Straighten. Ensuring newts are oriented the same in all photos is important to ensure accuracy. It can be difficult to control all factors in the field, and using photo editing software like I^{3}S Straighten can ensure image quality. However, if not used correctly, I^{3}S Straighten can distort images, decreasing individual recognition performance. I found that photographs of newts that were severely curved could not be properly straightened in I^{3}S Straighten without significantly distorting the image and further obscuring the ventral spot pattern. While software such as I^{3}S Straighten is useful in increasing image quality, it cannot be fully relied upon in place of careful photography.

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