



Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
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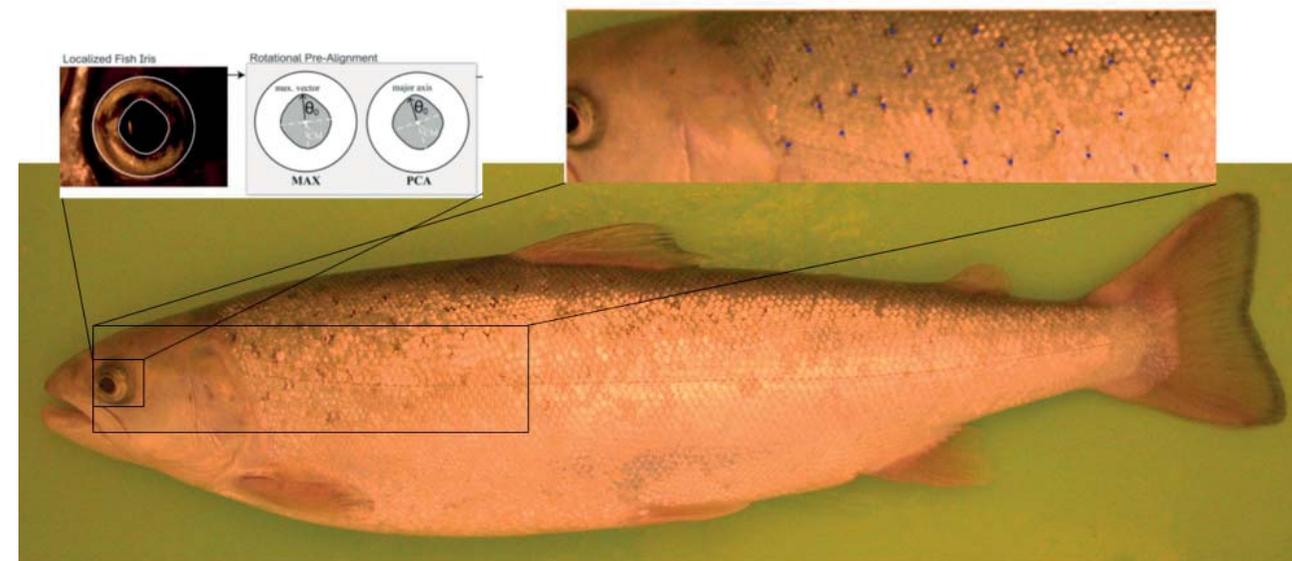


Doctoral thesis

Fish biometrics using machine vision

Rybí biometrie s využitím metod počítačového vidění

Fish biometrics using machine vision



Doctoral thesis by
Dinara Bekkozhayeva

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FOREWORD

Dear reader,

I still remember the day when I was sitting in the Laboratory of Zoology at the Institute of Biology and Biotechnology in Almaty undertaking fish morphological analysis and wondering whether nowadays in this technological century somewhere in the world somebody could do this time-consuming manual work faster by using computers. So, I thought that I had to go there to learn from them. I was so obsessed with this idea and luckily, I was successful. So, as you may guess, that place was the of Signal and Image processing Laboratory in Nové Hradky, Czech Republic, and the people who dealt with machine vision fish biometrics were Petr Císarš and his team.

Could it even have occurred to me at that moment that Petr and I together would come up with a new approach to fish identification which will have a tremendous impact in different areas of aquaculture? I guess NO. However, I am convinced that we have been successful in our method of making aquaculture production easier, economically profitable, and more environmentally friendly. Our study is just a first step towards developing a real system, but we have done a great job in proving that it is possible and could be used. Our journey is not over yet. Our task now is to apply our approach with confidence in real conditions.

In this thesis, you will not only find scientific research but see the enormous work undertaken during this study. It is not only about becoming a scientist, but also about the long way to get to this point. It concerns leaving the parental home, independent life in a foreign country, making new friends, colleagues and even love. You can see that this thesis is not only the story of the research, but my study is indeed a multi-faceted work. From enthusiastic (or naive) student to independent scientist.

I hope you will enjoy reading the thesis because for me it was a great pleasure to have undertaken this study to the point where I could write the "conclusion" of this fascinating stage called "Ph.D. thesis".

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CHAPTER 1

GENERAL INTRODUCTION

Importance of individual fish identification

Nowadays, the role of aquaculture in the world is significant. An increasing population leads to rise in food production. According to FAO (2001), the production of all aquatic organisms in the world grows fast, and this trend will not decline. Different types of aquaculture form an essential component within agricultural and farming systems development (e.g. FAO, 2000; Prein and Ahmed, 2000). Profitability diminishes the risks of disease and stress considerably for all types of aquaculture. That is why the role of rapid, inexpensive and non-invasive methods in measuring the quality of fish production is crucial (Saberioon et al., 2016). Fish behaviour monitoring can give important information about fish nutrition, welfare, health condition and environmental interaction with the aquaculture system (Zion, 2012). Different fish behaviour studies can be used for fish production optimization (Pautsina et al., 2015).

Aquaculture production of finfish has seen rapid growth in production volume and economic yield over the last decades and is today a key provider of seafood. As the scale of production increases, so does the likelihood that the industry will face emerging biological, economic and social challenges that may influence the ability to maintain ethically sound, productive and environmentally friendly production of fish. Therefore, the industry must aspire to monitor and control the effects of these challenges to avoid upscaling potential problems when upscaling production. The identification is critical in the precise aquaculture. Precision Fish Farming (PFF) (Fore et al., 2017) concept aims to apply control-engineering principles to fish production, thereby improving the farmer's ability to monitor, control and document biological processes in fish farms.

Fish farmers need information about individual fish traits like length, weight, sex and maturity and fish skin colour during different growth stages to monitor growth status for better stock management (Saberioon et al., 2016). These measurements can be done using Machine Vision Systems (MVS). Today, MVSs are becoming cheaper, more comfortable for farmers, less stressful fish, and even more accurate alternative than traditional methods (Delcourt et al., 2012).

One of the significant parameters of physiological, behavioural and ecological status is the skin colour of aquatic organisms (Pavlidis et al., 2006). Moreover, the skin colouration pattern in fish exhibits fishes welfare. When fish are under stress, they transfer different metabolic changes expressed by various parameters, including skin colour change (Saberioon et al., 2016). There are some examples of using MVS to estimate fish colour changes. Colihueque et al. (2011) created a method to evaluate skin colour, spottiness, and darkness using computer-based image analysis to classify cultured rainbow trout (*Oncorhynchus mykiss*). Zatkova et al. (2011) showed the implementation ability of MVS for monitoring skin colour changes due to diet alteration. They utilized an MVS to evaluate skin colour changes of wels catfish (*Silurus glans*). Urban et al. (2012) developed an algorithm for measuring fish skin colour in a stand-alone application. Their algorithm can give the dominant wavelength of fish skin.

In addition, fish identification and traceability are essential in managing food production. There are a lot of different ways to the identification of fish. All these methods have advantages and disadvantages. The widespread and popular methods of fish identification are tagging and marking (PIT, RFID, VIE). This identification based on attaching or implanting chips can cause technical, health, and animal behavioural problems.

The recognition of individual specimens has been essential to scientific discoveries in several fields of ecology, evolution and behaviour (Monteiro et al., 2014). However, it remains a challenge for research in animal ecology. Several techniques have been developed and successfully used to mark individuals or groups of individuals for later identification (Silvy et al., 2012). Such markings have been used, for example, to estimate population size (Haines and

Modde, 1996; Moore et al., 2010), growth (Linnane et al., 2012), survival (Monk et al., 2011) and recruitment rates (Pearson and Munro, 1991), to monitor populations for conservation management (Dutton et al., 2005; Biggins et al., 2006) and to identify individuals for natural history studies (Franz and Fontana, 2013). However, artificial marks have limitations that may affect the results.

Individual recognition of fishes by markings and tags has been made for many years (Pine et al., 2003). Most commonly used methods are invasive and can have adverse effects on fishes, increasing the risk of sequelae or mortality (Ombredane et al., 1998; Murray and Fuller, 2000), mainly for small and sensitive fishes (juveniles). An added mark or tag can also affect fish behaviour (Mesa and Schreck, 1989), an undesirable side effect. Moreover, tagged individuals can often lose their tags compromising data uptake (Arnason and Mills, 1981).

Non-invasive fish identification is an identification from the images. Non-invasive fish identification is cheap, less stressful for the fish and accurate. A further advantage is that digital images may be easily stored in association with other data (sex, size, and weight) and used for reassessments, new research questions and long-term investigations. The importance of non-invasive identification is crucial and developing in aquaculture production because of advantages such stressless for fish and less time consuming for farmers. The perspective of non-invasive identification of individual fish is increasing and for now the studies which deal with this topic was low therefore we started to work in this direction.

1.1. Common (Invasive) fish identification

For the identification of fish are usually used external tags and marks. External tags for identification are the oldest method and are widely used today. They are easy to use and detect, and most of them do not need special equipment. Simple ones are cheap. Those tags are visible and applied externally to the fish. Examples of these tag types include ribbons, threads, wires, plates, and disks (McFarlane Schreck, 1990). External tags have been used for both scientific and assessment purposes. The best-known examples of external tags are probably T-Bar Anchor Tags (Jones, 1979; Morgan and Walsh, 1993) and Carlin tags (Carlin, 1955) and various modifications. An external mark is a mark visible on the outside of the fish to identify individual fish or groups of fish, but without any information to report. Examples of external marks are visual modifications of the fish body (or fins), pigments, dyes, stains, brands, and meristic or morphometric characteristics. Usually, marks used to identify a small number of individuals and are easy to use in field studies. The use of external marking of individual fish has been limited in scope. Marks are often simple, cheap and quick to apply, but they carry limited information (Thorsteinsson, 2002).

Today, most research on individual fish identification is based on invasive methods like tagging (Cousin et al., 2012). Many different tagging methods exist: marks (by dye injection, branding, tattooing, spray painting, fin clipping, injection), external or internal mechanical tags (dart and anchor tags, streamers, clips and discs), implanted internal tags (Passive Integrated Transponder Tags, X-ray microtags, coded wire tags). These types of tags can be used to monitor migratory habits of the tagged fish which is observed by measuring the distance and direction travelled between tagging and recapture. Growth patterns are also monitored using fish tagging.

The more advanced tags are a PIT tags. It can be read without removing the tag. The need to identify fish individually and to identify groups of fish with minimal influence on behaviour, health or survival has led to the development of internal tags. These tags are extensively used for tagging large numbers of fish, but special detection equipment is needed. Magnetic Body Cavity

Tags (MCTs) are steel plates inserted into the fish's body cavity. The tags are detected during fish processing with magnets placed at specific positions in the industrial units. Today, state of the art in animal identification is using RFID tags or applying other identifying tags. However, attaching or implanting chips can cause technical, health, and animal behaviour problems.

Disadvantages of invasive fish identification

Many commonly used marking techniques are invasive (e.g., subcutaneous chemical markings, tattoos, amputations, insertion of transponders and subcutaneous tags). They may pose a risk to animal health or survival (Silvy et al., 2012). Even non-invasive artificial marks such as tags, collars or external colourants may cause behavioural alteration, increasing the risk of predation and reducing fitness (Gauthier-Clerc et al., 2004; Carlson and Langkilde, 2013). Additionally, the possible loss of artificial marks may represent the loss of desired data (Reisser et al., 2008). All invasive methods of individual identification of fishes have main disadvantages like those methods stressful for fishes; fish must be a catch for identification, it is time-consuming; the tags can remain in the fish in the case of escapes, and there is also size limit for the different tags. The use of invasive fish tagging is complicated for the intensive aquaculture in sea cages because of the high number of fish.

Image-based non-invasive identification (alternative to invasive methods)

Alternative to invasive methods of fish identification is photo-identification (video). Camera-based solutions are non-invasive, not stressful and can provide real-time information but are challenging to implement. Non-invasive fish identification has become an essential tool for monitoring fish behaviour and getting more individualised information about fish (Speed et al., 2007); it also reduces animal stress if live specimen manipulation is necessary. An additional advantage of photo-identification is that a digital image database can be compiled and then used for a precise identification process based on examining several images of one specimen, allowing eventual comparisons with other species or with previous images of the same species. It means making the long-term identification of individual fish. Work on animal biometrics (based on different visual identifiers) is emerging and has started to be applied in many fields (Kuhl and Burghardt, 2013). For instance, face recognition also has been investigated as a biometric trait for sheep (Corkery et al., 2007a), beef cattle (Noviyanto and Arymurthy, 2013) and chicken (Corkery et al., 2007b).

Biometric identification of fish individuals has only been used for fish species classification (Spampinato, 2015; Salman et al., 2016) and fish stock identification (Cadrin and Friedland, 1999). Few papers have been published on identifying individual fish using (dorsal) fin shape (Durban et al., 2010; Marshall and Pierce, 2012) and skin pattern (Marshall and Pierce, 2012) for marine biology. However, the individualized (biometric) automatic approach based on identifying the fish in aquaculture has not been studied to the best of our knowledge.

The reliable use of non-invasive fish identification requires at least two assumptions to be met (modified from Bolger et al., 2012). First, individuals must bear patterns on some region of the external surface of their body that is sufficiently variable to discriminate among individuals. Second, an individual's pattern should be stable over the study period (preferably throughout the individual adult life span) and unambiguously photographed under differing conditions. Non-invasive fish identification can be a useful alternative to traditional marking techniques for many species. Non-invasive fish identification has already been used for individual identification of marine teleost fish (e.g. Martin-Smith, 2011; Correia et al., 2014; Giglio et al., 2014), and non-invasive fish identification as a technique for recognition of individual specimens of a Neotropical freshwater fish species, *Rineloricaria aequalicuspis* Reis and Cardoso (Loricariidae) (Dala-Corte et al., 2016). Also, the identification of Atlantic salmon

was made based on an image (Stien et al., 2017; Schraml et al., 2020; Cisar et al., 2021), common carp (Huntingford et al., 2013), zebrafish (Al-Jubouri et al., 2018), European perch (Hirsch and Eckmann, 2015) and catshark (Navarro et al., 2018).

1.2. Fish skin and colour change

Fish has different patterns and enormous diversity of colour due to unique structures in the fish skin. The variation of colour between species is vast. Still, it can also differ within species, change during maturation, habitat changing for long or short-term reasons, and changing the environmental conditions. The skin colour can also change during ontogeny (Baldwin, 2013). Colour patterns on fish skin can be highly complex, and prints may be very labile, changing with the stage of maturity or sex, social dominance and with the background.

Fish skin colouration is produced either by pigments in specialized cells called chromatophores. Skin colour in fish is multilayer, multicomponent signals (Grether et al., 2004). The basic unit of colour in these taxa is the dermal chromatophore, which is generally composed of three cell layers: the xanthophore (contains carotenoid and pteridine pigments), the iridophore (reflects colour structurally), and the melanophore (contains melanin). Pigments are compounds that absorb particular wavelengths of light and can contribute to the colour of biological patches. Two pigments commonly studied in fish are carotenoids (usually yellows, orange, and red) and melanin (browns, blacks, and greys); they are deposited in the integument. Short wavelength (blue and violet) and silvery colouration in vertebrates are almost always structurally based. The result of selective light scatter is owing to variable refraction within the integument, and only one blue pigment has been described in fish (Bagnara et al., 2007).

As many fish undergo a colour change, the colour itself is not necessarily an excellent taxonomic characteristic, although it can contribute to identification. Fish can change their colour during their life cycle by varying numbers of pigment amounts or types of chromatophores. They can briefly adjust the colour by intracellular changes in response to background or stress. Individual fish can also show changes in their skin during their lifetimes which may be ontological, cyclical, sexual or related to nutrition (Burton and Burton, 2018).

Pigment-based colour patterns can change through direct regulation of pigment-containing cells or indirectly through adjustment of the light interacting with the pigment through regulation of iridophores. The behaviour of pigment-containing cells is controlled by both the nervous and endocrine systems, with more rapid changes typically reflecting neural control (Fujii, 2000). This flexibility in expressing colour patterns means that colouration can go beyond signalling static properties such as species identity, sex, or developmental stage and indicate an individual's current quality and motivational state (Price et al., 2008).

Formation of colours, patterns and colour change

When a specific colour dominates one skin area, this can be attributed to an accumulation of pigment cells of the type producing that colour. White stripes are often due to an abundance of iridophores. The red spots of particular carp are local aggregations of erythrophore cells, and black lines in the angelfish are due to melanophores. Despite few available pigments, a glance through an encyclopedia of fishes (Dakin, 1992) reveals many different colours. The macroscopically perceived colour can be attributed to the microscopic organization of pigment cells in the skin. In amphibians and reptiles, chromatophores are arranged in ordered layers in the dermis to form the dermal chromatophore unit (Bagnara and Hadley, 1973). These structures can create colours like green which cannot be formed by the available pigments alone. Similar arrangements exist in fishes. Increases and decreases in the number

of dermal chromatophores lead to morphological colour changes. The changes result from the proliferation of chromatophores and the degradation of terminally differentiated pigment cells (Parker, 1948).

Different models of visible patterns

Several visible patterns exist on the fish, which could be used for identification. The visual pattern which could be used for individual fish identification exists on the fish skin (Kondo et al., 2009) for several species and the iris of the eye, colour pattern at the body surface, e.g. Syngnathidae (Martin-Smith, 2011; Correia et al., 2014), and pattern of spots and scarring marks, e.g. whale sharks (Arzoumanian et al., 2005; Holmberg et al., 2008). The formation of the visible patterns is related to the fish genotype, and it is not explored to the level to determine the uniqueness of the individuals. The digital camera and machine vision development level enable the fingerprint, facial geometry, or iris pattern remote detection. Therefore, the individual fish identification using the visible patterns is possible in the case of the pattern uniqueness for the specific group. It would enable a non-invasive approach to fish identification applicable directly in the tanks or sea cages without fish sampling. Identification of the individuals underwater in real-time is then a step to precision fish farming (Fore et al., 2017) which has a lot of advantages such as early disease detection and early water quality detection based on individual behavioural and appearance changes. The modelling techniques exist to model the fish patterns, which means that the patterns can be mathematically described, which is useful for computer processing. Figure 1 describes different visible patterns which could be used for identification.

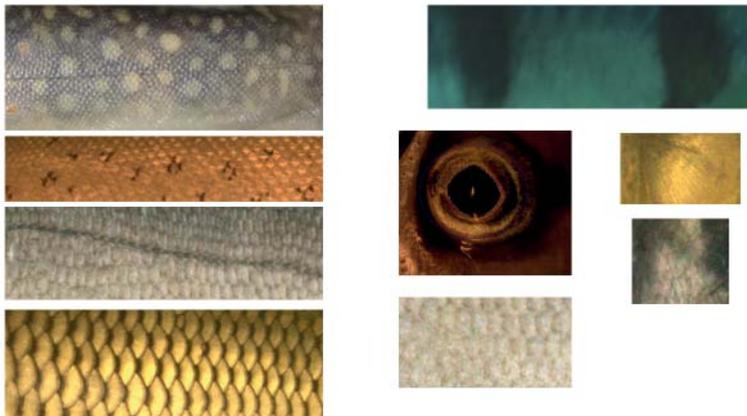


Figure 1. Different visible models of patterns were used in this study for individual identification procedure. Left images are the body patterns on a fish body (side view) top – Atlantic salmon, middle – European seabass and bottom image is a common carp. Top middle region is eye of Atlantic salmon. Bottom middle image is a scale pattern on a European seabass body. Right top region is an operculum of common carp and the bottom image is seabass operculum part.

1.3. Machine vision/image processing methods (pattern analysis)

As was written before, the substitute for the invasive methods of fish identification is non-invasive identification from the images. It is possible by using image processing and computer vision methods. This chapter describes existing techniques and methods which were used in our research.

The current development of the hardware (cameras) and computer vision and machine learning methods is appropriate for taking the pictures of the patterns and analysing them for identification. One of the possible schemes of the fish identification process is based on the visible patterns shown in Figure 2. First a high-quality picture of the fish pattern has to be taken. The object of interest (fish/fish body part) is then detected on the image using the segmentation methods. One of the existing shape/texture parameterizations describes the pattern, which converts the image to the vector of numbers usable for the classification. To solve the problem of close group identification (which is typical for aquaculture research), the database of the fish representation can be constructed. The identification of the particular fish is then performed to classify the fish into one of the existing classes in the database.

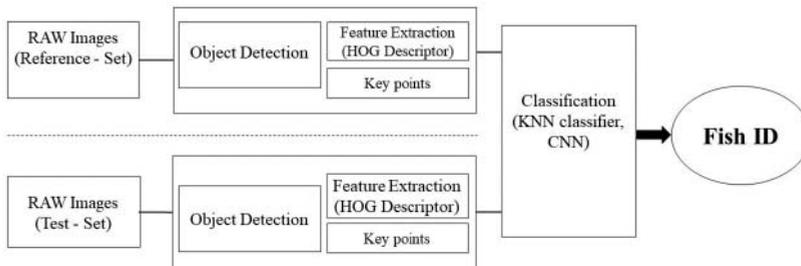


Figure 2. Scheme of the process of analysis.

Data collection (Photographing)

Different devices like video cameras, digital cameras, and the digital microscope can be used for data collection. It is critical to obtain high-quality data for identification. To simplify the next steps of the data processing, the conditions of image collection can be fixed as much as possible (laboratory conditions). This simplification can be done to develop the method, but finally, the methods and system have to be adapted to the real conditions of aquaculture fish farming. The lighting, the background and the fish position can be fixed to minimize the influence of these factors on the final identification result. Fixed background and lighting simplify the segmentation task and make the parametrization more robust. The data are usually represented as the images in the tiff or raw format to minimize details distortion and keep the original bit depth of the data.

Object detection (Image segmentation)

Image segmentation aims to extract the outlines of different regions in the image, that is, to divide the image into an area made up of pixels that have something in common. For instance, photos may have similar brightness or colour, indicating that they belong to the same object or facet of an object (Petrou and Petrou, 2014).

Object detection, such as face detection and pedestrian detection, are well-researched domains. Object detection algorithms typically use extracted features and learning algorithms to recognize instances of an object category. Object detection has applications in computer vision, such as image retrieval and video surveillance (Singh and Shubham, 2015). Object detection is a technique for detecting the specific object in an image. It involves background subtraction and extraction of an object from an image (Vijayalakshmi and Durairaj, 2013). One of the simplest methods to divide an image into uniform regions is based on histogram and thresholding. We create the image's histogram if we plot the number of pixels with a specific grey value versus that value. Correctly normalized, the histogram is essentially the probability density function of the grey values of the pictures.

“Detect” an object in an image means identifying the pixels corresponding to the thing. Create an array of the same size as the original image and give each pixel a label to express this information. All pixels that correspond to the object are given the same label, and all pixels that correspond to the background are given a different label.

- Histogram-based image segmentation is the most often used segmentation technique. It uses the histogram to select the grey levels for grouping pixels into regions. In a simple image, there are two entities: the background and the object. The background is generally one grey level and occupies most of the image. Therefore, its grey level is a large peak in the histogram. Another grey level represents the object or subject of the image, and its grey level is another smaller peak in the histogram.

- Segmentation based on the model of the background

A static camera observing a scene is a common case of a surveillance system. Detecting intruding objects is an essential step in analyzing the scene. A usually applicable assumption is that the images of the scene without the intruding objects exhibit some regular behaviour that a statistical model can well describe. If we have a statistical model of the scene, an intruding object can be detected by spotting the parts of the image that don't fit the model. This process is usually known as “background subtraction” (Zivkovic, 2004). The most commonly used algorithm for background subtraction is the “Gaussian Mixture Model” (GMM) (Sharon Femi and Thaiyalnayaki, 2013).

- Segmentation based on the image homogeneity

Initially, the whole image is considered as one region. Suppose the variance of the pixel intensities within this region is greater than a predetermined value. In that case, the area is split into four quadrants. Each quadrant is tested in the same way until every square region created contains pixels with intensity variance within the given value. In the end, all adjacent regions with similar intensity may be merged.

After the segmentation, the image of the object can be processed to remove the noise or other undesirable objects. Morphological operations are used for the post-processing of binary images. The operations like erode and dilate are applied to the image's detected objects (binary mask). Erode operation is used to remove noise in an image and dilate to fill the holes of the object's boundaries in the image (Song et al., 1989).

Parametrization (Feature extraction)

Parametrization is a process when object (fish) has to be described by its specific feature of it. The pixel corresponding to the object is usually transformed into the vector of numbers (feature vector) that parametrize the most significant features of the object (shape, edges, corners, colour, etc.). Several methods how to parametrize the object exists. The two main groups of parametrization methods are based on the geometrical features (shape parametrization) and texture parametrization (colour distribution parametrization). The main difference between the two groups of methods is that the object border (essential parts of the object – eye, mouth, gills) has to be detected for shape-based parametrisation. The texture-based parametrization parametrizes the whole image (object appearance). Shape-based parametrization (border-based descriptors) is referred to as an external 2D outline, appearance or configuration.

The shape is independent of colour and translation, rotation, and scale. There are different methods for shape representation (border-based descriptors): chain code, Fourier descriptor, chord distribution, B – splines or profiling. Below we will describe some of these methods briefly for better understanding. Profiling is a 2D method of shape representation. For measuring the vertical profiles along with the object, it has to be divided into the equidistant sections along the length. The profile is determined for each section as the distance between

the first and last point of the line perpendicular to the central line (length); after obtaining the feature vector for further analysis constructed, the length of the profiles. All methods for shape representation are sensitive and do not give complete accuracy for tasks.

The texture-based parametrization describes the colour (grayscale) patterns on the object. There are different algorithms like Scale Invariant Feature Transform (SIFT), Histogram of Oriented Gradients (HOG), Local Binary Patterns (LBP), Discrete cosine transform (DCT) and others for texture parametrization. In general, parametrization converts important patterns into the feature vector used for object classification. Below is the description of some of the methods we tested in our study. Scale Invariant Feature Transform (SIFT) is a method for extracting distinctive invariant features from images that can be used to perform reliable matching between different views of an object or scene. The features are invariant to image scale and rotation. They can provide robust matching across a substantial range of affine distortion, change in 3D viewpoint, addition of noise, and change in illumination. The features are highly distinctive because a single feature can be correctly matched with high probability against a large database of features from many images (Lowe, 2004). This method identifies "strong" corners in the image.

Histogram of Oriented Gradients (HOG) is a texture descriptor often used for object detection. The idea is to describe the local object's appearance and shape by the orientation distribution of intensity gradients (Dalal and Triggs, 2005). To calculate the HOG descriptor, first, calculate the horizontal and vertical gradients; after all, calculate the histogram of gradients orientation. At every pixel, the gradient has a magnitude and a direction. The image is divided into 8×8 cells, and a histogram of gradients orientation is calculated for each 8×8 cell. The representation is compact and calculating a histogram over a patch makes this representation more robust to noise. The next step is to create a histogram of gradients orientations in these 8×8 cells. A bin is selected based on the direction, and the vote corresponds to the magnitude. The contributions of all the pixels in the 8×8 cells are added up to create the 9-bin histogram. Gradients of an image are sensitive to overall lighting. We need to "normalize" the histogram so they are not affected by lighting variations.

Local Binary Patterns (LBP) compute a local representation of texture (Ojala et al., 2002). This local representation is constructed by comparing each pixel with its surrounding neighbourhood pixels. Building the LBP texture descriptor is to convert the image to grayscale. For each pixel in the grayscale image, select a neighbourhood of size r surrounding the centre pixel. LBP value is then calculated for this centre pixel and stored in the output 2D array with the same width and height as the input image. The binary code is created from the values of neighbour pixels, and the decimal number is made. This process of thresholding, accumulating binary code, and storing the output to decimal value in the LBP array is repeated for each pixel in the input image. The last step is to compute a histogram over the output LBP array. Then, add a histogram that reflects the number of times each LBP pattern occurs. Finally, this histogram can be treated as our feature vector.

From the described approaches only, HOG worked well on our data.

1.4. ID classification

Once the feature vectors describe all the images, it is possible to perform the task of object identification. The problem of individual identity can be characterized as finding the best match between the unknown feature vector and the existing feature vectors (existing database). Therefore, the classification methods can be used to solve the identification problem. The feature vectors corresponding to one object represent the class in the classification approach.

Several methods exist for object classification based on the machine learning approach.

For identification usage, the concept of supervised learning. Supervised learning is the machine learning task of inferring a function from labelled training data (Mohri et al., 2012). The training data consist of a set of training examples. In supervised learning, each example is a pair consisting of an input object (typically a vector) and the desired output value (also called the supervisory signal). A supervised learning algorithm analyses the training data and produces an inferred function, which can be used for mapping new examples. An optimal scenario will allow the algorithm to determine the class labels for unseen instances correctly. This requires the learning algorithm to generalize from the training data to unseen situations in a “reasonable” way. A wide range of supervised learning algorithms is available, each with its strengths and weaknesses.

Support vector machines (SVMs) have supervised learning models with associated algorithms that analyse data used for classification and regression analysis in machine learning. Given a set of training examples, each marked as belonging to one or the other of N categories (2 for the illustration of the principle), an SVM training algorithm builds a model that assigns a new sample to one category or the other, making it a non probabilistic binary linear classifier. An SVM model represents the sample as points in space, mapped so that the examples of the separate categories are divided by a clear gap. New samples are then mapped into the same space and predicted to belong to a category based on which side of the gap they fall. In addition to performing linear classification, SVMs can efficiently perform a non-linear classification using the kernel trick, implicitly mapping their inputs into high-dimensional feature spaces.

All machine learning methods determine the similarity of the model (known data points) and the unknown data point. The forms can work with the feature vectors or the whole images. One of the examples of similarity determination is the cross-correlation method. Cross-correlation is a standard method of estimating how two series are correlated. For our research (image of the fish), we selected the region with patterns (reference pattern) and selected the minor part (test pattern), then moved the test pattern overall locations or reference pattern. After that, calculate cross-the correlation coefficient between reference and test patterns for all positions. This data plotted the confusion matrix to visualize the similarity for each pair of the reference image and test image.

1.5. Convolutional neural networks (CNN)

The CNN based approaches are nowadays very popular and powerful methods used in many areas of data processing and analysis. CNN is the network of connected artificial neurons. Each neuron has several inputs and one output. The neuron itself is an activation function which transforms the input signals to the output value. The CNN (learning with supervisor case) is trained using the labelled training set using the backpropagation algorithm. The algorithm tune the weight of the inputs of the neurons to minimize the prediction error of the network. The differences between the neural network and its target usage are based mainly on the architecture of the network. Many different architectures exist for the specific tasks of object detection, classification, segmentation, or identification. The general network architecture for image processing is the concatenation of input convolutional layer (feature extraction) followed by pooling layer (decrease the size of the features from input layer), fully connected layer (connection between different network stages, usually placed before output layer), dropout layer (reduces network overfitting). The traditional approach is to use some already existing network and adapt it to the specific task. The advantage is that the network is already trained (by thousands of images) to extract the features in the image.

The classification neural networks (Shaf et al., 2018) are trained to classify the input image into a predefined number of classes. The input is the image, and the output is the probability that the image belongs to the class. The networks used for object identification are usually based on the triplet loss function (Cheng et al., 2016). The triplets of the input images are used for network training. The reference image is compared with a positive example, and negative example and the network tries to minimize the distance between the reference and positive and maximize the distance between reference and negative. The advantage of this approach is that we need not have a lot of training images for each individual.

Aims and objectives of the Ph.D. thesis

The aim of the thesis is non-invasive individual fish identification from images.

The objectives are listed below:

- Identify individual fish in the close group of fish (aquarium, tank).
- Test different patterns (body patterns – dots, stripes; iris of the eye, lateral line, scale patterns, scale formation) on a fish body of a different fish species.
- Develop the method for automatic fish individual identification.
- Prove automatic concept of non-invasive individual fish identification from images.
- Test the stability of the chosen patterns during the cultivation period.

The structure of the dissertation thesis is based on an idea of our concept. Firstly, we tried to test the photo-identification approach of the fish individuals for the small group of ornamental fish with a strong obvious pattern on a body (Sumatra barb *Puntigrus tetrazona*). The study is presented in Chapter 2. After obtaining the promising results, the next step was to apply the concept to the commercially important fish species such as Atlantic salmon *Salmo salar*. Also, we had increased the number of fish to 330 fish individuals. Those researches are in Chapters 3 and 5. The next step of our complex study was to test this approach for the fish without an obvious pattern on a body; we used the scale and lateral line on a fish body for this study. European seabass *Dicentrarchus labrax* and common carp *Cyprinus carpio* were used in this study. The results are presented in Chapter 4.

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CHAPTER 2

AUTOMATIC INDIVIDUAL NON-INVASIVE PHOTO-IDENTIFICATION OF FISH (SUMATRA BARB *PUNTIGRUS TETRAZONA*) USING VISIBLE PATTERNS ON A BODY

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My contribution to this work was about 85%.



Automatic individual non-invasive photo-identification of fish (Sumatra barb *Puntigrus tetrazona*) using visible patterns on a body

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Abstract

Non-invasive fish identification of individuals can provide new possibilities for the monitoring of fish cultivation, improve and make fish production technologies less demanding for farmers, and increase fish welfare. The aim of this research is to confirm the idea of automatic non-invasive image-based fish identification of individuals using visible features on a fish body and prove the pattern stability during the fish cultivation period. Visible patterns, such as black stripes along the body of a Sumatra barb (*Puntigrus tetrazona*), were used for machine identification of individual fish. Two experiments were completed: a short-term experiment (43 fish) to show the uniqueness of the stripe patterns for identification, and a long-term experiment (25 fish) to test the stability of patterns during the cultivation period. The overall accuracy of classification was 100% for data collection in one day and 88% between two data collection times. This study shows that visible patterns and image processing methods can be used to automatically identify individual fish of the same species. This is not just limited to Sumatra barb—the concept should work for any fish with unique visible skin patterns, for example, for commercial fish species like Atlantic salmon (*Salmo salar*) and European perch (*Perca fluviatilis*).

Keywords Precision fish farming · Machine vision · Individual identification · Non-invasive identification · Skin pattern

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Introduction

Nowadays, increasing consumption of fish and aquatic organisms pushes aquaculture to produce fish more intensively. Based on an FAO report (Food and Agriculture Organization 2018), almost half of global fish production (47%) comes from aquaculture. The trend of increasing aquaculture production leads to the automation of processes to increase profits and facilitate the cultivation process. Automation of fish cultivation processes helps to control general issues of aquaculture such as feeding, fish sampling, fish size-dependent sorting, and controlling fish welfare and diseases. The idea of automation of fish production is the general aim of the precision fish farming concept, where controlled engineering principles are applied to fish production processes, increasing the ability of fish farmers to monitor, control, and document all biological processes of the fish cultivation process (Føre et al. 2018). One of the main parts of automation of the process of fish cultivation is fish identification and tracking the fish trajectory. Automation of fish behavior monitoring can reduce unnecessary expenses (Pautsina et al. 2015). Fish identification is used to monitor and control cultivation processes and management of fish food production and controlling disease (Lai et al. 2012).

Today, image-based animal identification systems are widely used all over the world in different fields. For example, in wildlife research, image-based animal identification has been implemented for studies of green sea turtles (*Chelonia mydas*) to enable long-term identification of individuals and control the population dynamics (Carpentier et al. 2016), photo-matching of the Balearic lizard (*Podarcis lilfordi*), and the northern spectacled salamander (*Salamandrina perspicillata*) in ecological studies (Óscar et al. 2015), and the identification of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) based on pictures of the pronotum (Díaz-Calafat et al. 2018). Animal identification is already used in agriculture as well, for instance, in automatic individual identification of Holstein dairy cows using tailhead images (Li et al. 2017).

The identification of fish is also well studied. The first fish recognition study, from 1990, was focused on fish species recognition by shape analysis in digital images (Strachan et al. 1990). They computed fish body shape from images of seven different fish species and computed the geometrical shape descriptors to prove that species classification can be done using image analysis automatically. The accuracy of the classification was 90%. Later, many types of research were focused on fish species identification; for example, image-based fish recognition was performed by Saitoh et al. (2015). They collected images of 129 species of fish under natural conditions and used features for fish recognition, such as geometrical features, bags of visual word model, and five kinds of texture features. Shafait et al. (2016) described fish species identification from videos captured in uncontrolled underwater environments. The real-time underwater video system for species recognition was developed by Hsiao et al. (2014). This is an advanced fish species recognition system for fish population studies and can be used for long-term study. The fish were detected using multiple bounding-surrounding boxes and the partial ranking method, based on the sparse representation-based classification that was used for species identification. The results are robust and accurate for use in fish species recognition and identification. The modern methods based on the deep learning approach are nowadays used for many classification tasks. Villon et al. (2018) applied the approach to the classification of coral reef fish species. The convolutional neural network (CNN) was trained by 900,000 images for nine species. The identification accuracy of 94.9% was better than the identification made by humans (89.3%). The main advantage of the deep

learning approach is the robust classification and generalization. The disadvantage is the need of many training examples to train the network.

The identification of fish individuals of the same species is a more challenging problem because of the similarity among fish. The typical approach of individual identification of fish from the same species is marking and tagging (Pine et al. 2003). These methods are invasive and can negatively affect fish by increasing mortality, causing injury, and causing stress (Ombredane et al. 1998). The non-invasive identification of fish individuals of the same species is an approach which avoids all the negative consequences of tagging. To the best of our knowledge, just a few papers have dealt with non-invasive identification of individual fish with a limited number of individuals (Huntingford et al. 2013; Hirsch and Eckmann 2015; Stien et al. 2017; Al-Jubouri et al. 2018; Navarro et al. 2018).

Most previous studies were based on manual fish identification with no automated process of fish detection, parametrization, and identification. Hirsch used stripe patterns for the identification of individuals of Eurasian perch (*Perca fluviatilis*) (Hirsch and Eckmann 2015). They worked with six groups of eight fish in each group over a 6-week experiment, although they were larvae; nevertheless, the accuracy of identification was high (no exact number). Humans made the identification for just the eight fish within the groups.

Navarro et al. (2018) used the tail (caudal area) of the catshark (*Scyliorhinus canicular*) with spot color patterns for individual identification. They collected three images of 92 fish individuals. The human experts performed the identification for 25 randomly selected individuals manually. Identification was successful for 99.6% of images. Experts used the location of the scales on 15 common carp (*Cyprinus carpio*) bodies for identification by Huntingford et al. (2013). The matching accuracy was 95.76%. The most extensive study (Stien et al. 2017) of non-invasive identification of individuals used consistent melanophore spot patterns on Atlantic salmon (*Salmo salar*). They tested the long-term stability of these patterns during the time. They worked with 246 individuals and two data collection periods recorded over 10 months of cultivation. The study manually proved the stability of the feature with 93% accuracy, but the number of individuals used in one identification task was 30. All studies tested the stability of the patterns during fish growth. Based on the above-mentioned studies, which have already been successful, we can say that non-invasive identification of individuals is possible and can be used in the future as a desired replacement of tagging and marking methods. Non-invasive methods of fish identification, such as image-based identification, are increasingly important because they can reduce the various adverse effects on fish welfare.

The only study of computer-based individual identification was based on the identification of five individuals of zebrafish (*Danio rerio*) using HSV (hue, saturation, value) color model (Al-Jubouri et al. 2018). The accuracy of identification was 99% for the five fish for the images collected on the same day. No pattern deformation caused by fish group was studied. The study of Stien et al. (2017) also included semi-automated computer-based identification. The dot pattern of the Atlantic salmon was manually connected by hand and the angles between the lines were used for identification. The accuracy of semi-automated identification of a maximum of 30 individuals was 85%.

This paper aims to prove the concept of automatic non-invasive image-based fish identification of individuals using the visible skin patterns on a fish body, and to test the stability of these patterns during the cultivation period. Sumatra barb (*Puntigrus tetrazona*) is used in this study due to its black stripes along the body representing a unique skin pattern.

Materials and methods

Experimental animal

Sumatra barb (Fig. 1b), a small ornamental fish species, was used in this study. The skin pattern consists of black vertical stripes on the body. These stripes are similar for all individuals but vary enough to be used to test the identification approach. The fish were bought in the local pet shop and kept for one month in 60 L aquaria. Two groups of adult fish were used in this study. The first group contained 43 fish and the second group contained 25 fish. Both groups were a mixture of males and females with an average size of 4.1 mm. The fish have four black vertical stripes on their body. These patterns can be used as visible features for individual identification of commercially important fish with this kind of pattern, such as pike-perch (*Sander lucioperca*) and European perch (*Perca fluviatilis*).

Experimental setup and dataset

For data collection, a digital camera (Camera Nikon D90) was used in a setting with controlled lighting and background, and where the fish was able to swim (Fig. 1a) in the aquarium. A fixed background (green uniform foam) and lighting was used to simplify the automatic fish localization task. Each fish was moved from the cultivation aquarium to the small data collection aquarium (10 × 15 × 30 cm) to take the pictures to simulate the real underwater conditions of fish identification. The swimming space was limited by the movable green background. Five pictures of the lateral view of the fish were collected for each individual. An image of the left side of the fish was captured for all fish.

The camera was set to manual mode to control the focus, shutter, and ISO. These parameters influence the final image quality and must be controlled to simplify automatic fish localization. An indirect incandescence light was used for scene illumination to avoid direct reflections from the fish skin. Data were collected in two scenarios: a short-term and long-term scenario. The short-term scenario was designed to test the classification power of the fish stripe pattern. Five pictures of each of 43 fish individuals were taken during the session. The fish position and orientation were different for each picture of the individual. A total number of 215 images were used for later analysis. The long-term scenario was designed to test the stability of

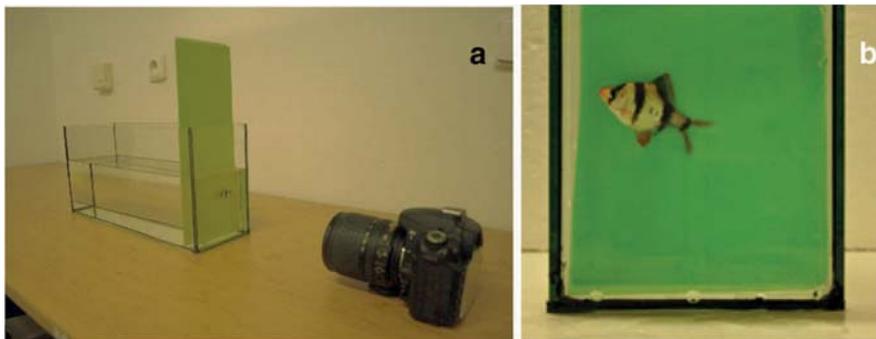


Fig. 1 **a** Data collection design. A digital camera takes picture of the lateral view of the fish swimming in the small aquarium with mobile background. **b** The fish inside the small aquarium with a uniform background. This image is used for automatic fish localization

the patterns for identification during the cultivation period of 2 months. The stability of patterns shows the ability of identification of individuals in a durable time. It means that the group of the fish can be individually identified in some period of time. The pattern can be changed by the fish growth, body deformation, skin color changes, or scales loss. Twenty-five fish individuals were used for data collection for 2 months. The data were collected in the first data collection (1) period and then again in the second period (2) two months later. Three images of each individual were taken from the lateral left side view at different angles and positions.

The data from long-term and short-term scenarios were captured in RAW format to avoid pattern distortion by image compression. The images of the same fish were labeled by a unique identifier together with the information about the date of image collection.

Data processing

Automatic data processing consists of two steps: fish detection and region of interest (ROI) selection in the image and feature extraction to describe the skin pattern of the selected region (Fig. 2). The image processing methods implemented in Matlab were used for data processing. The images were first converted from the RAW format to the tiff format using the demosaicing method (Kimmel 1999).

Fish detection

The automatic detection of the fish body in the image was based on the detection of the green background and then the detection of the fish body inside the background area (Fig. 3). The area of the background was detected as the object with known color in the HSV representation of the image (Šonka et al. 2008). The method segmented the largest object of this color in the image. This approach localized the area of possible fish appearance. The same segmentation (color based) was done again just for the area of the localized background. The noise filtration based on the minimal size of the segmented areas (16 pixels) was used to remove the pixels, which did not correspond to the fish. The filtered image was processed by morphological close (Matlab—function `imclose` with the structural element 3*3) operation to concatenate the fish body parts which could be separated in the segmentation step. The output image is represented as the binary mask where pixels with value 1 represent the fish body and pixels with value 0 represent the background. The orientation of the fish body was determined (Matlab—function `regionprops` (“Orientation”)). The fish was rotated to be horizontal position on the image. The

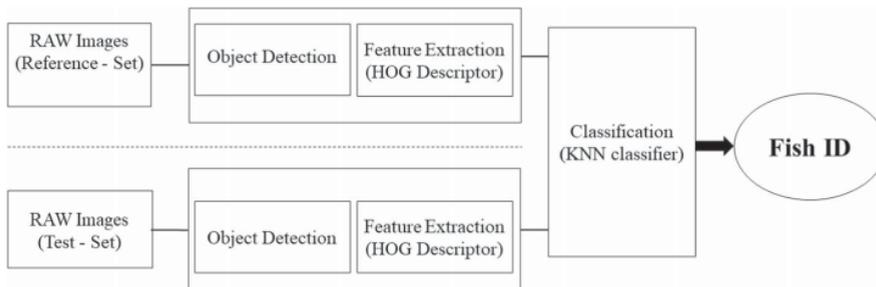


Fig. 2 Identification scheme

output of the detection is the binary mask of the localized fish body horizontally aligned. The fish body deforms (mainly the tail) during swimming. The fish were freely moving in the aquarium during the data collection. To precisely detect the region of the fish stripe pattern on the body, the fin tail was removed from the mask. The last $\frac{1}{4}$ of the fish body was used for the localization of the point with the minimal height of the fish body before the tail (Fig. 3). The point is detected as the position of the vertical line connecting the upper and bottom fish shape border with the minimal length. The final fish mask contains the full fish body without the tail. The mask was used for localization of the fish in the original color image.

Feature extraction

Four different descriptors were used for detected fish parametrization, one shape-based and three texture-based descriptors. The shape-based descriptor parametrizes the shape of the fish. The full fish body was divided into ten equidistant sections along the horizontal axis (it is used to eliminate the scaling). The body height was measured in these sections and used as the description of the body shape (BS).

Four different ROIs were tested for the parametrization based on the texture descriptors. The ROI was localized as the rectangle of the normalized fish body. The rectangle covered the same part of the fish body for all fish. The ROIs covering the whole fish body (ROIW), from head to tail (ROIH), two middle stripes (ROI2), and one stripe only (ROI1) were used. The ROI is localized automatically as part of the mask (Matlab function “Bounding.box”).

The three texture descriptors were selected for parametrization of the selected ROI of the fish body: horizontal intensity profiles (HP), histogram of oriented gradients (HOG) (Dalal and Triggs 2005), and local binary patterns (LBP) (Ojala et al. 2002). The descriptors parametrize the color patterns of the fish body in the specified ROI. This codes two-dimensional textures of ROI into one-dimensional feature vector. The ROI was (Fig. 4a) transformed into a rectangle of size 64×64 pxl for the efficient calculation of the descriptors (Fig. 4b) and for image scale compensation. Several different settings of the LBP (different sizes of the neighborhood: 8 and

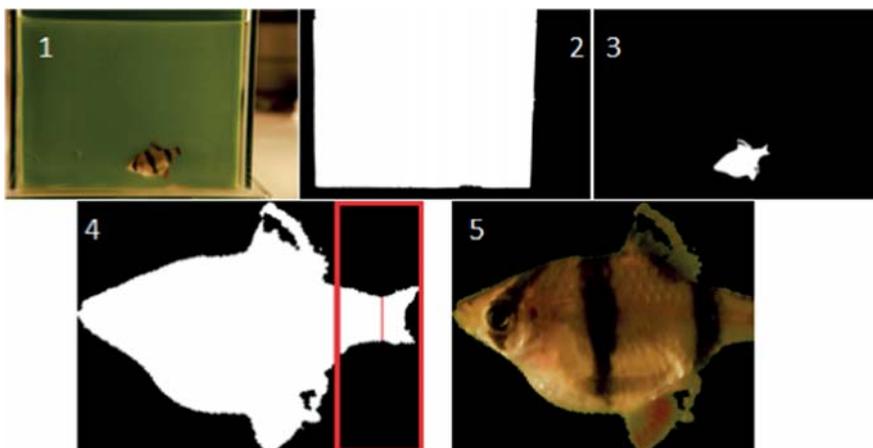


Fig. 3 Fish localization. 1—original image, 2—segmented background, 3—segmented fish inside the background, 4—rotated mask of fish, rectangle—the area for beginning of fin search, line—detected narrowest part inside the rectangle, 5—segmented fish used for ROI selection

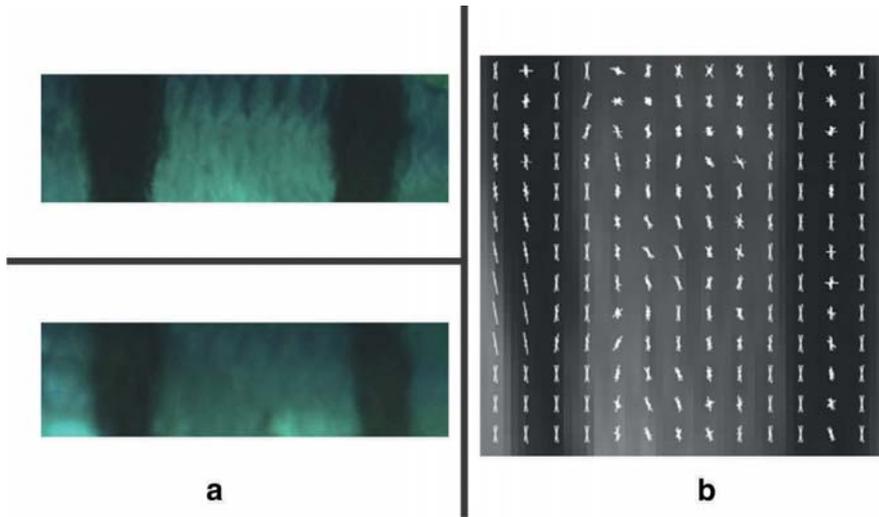


Fig. 4 **a** Region of interest (ROI2) of two different fish from one data set covering the two middle stripes of the fish body; **b** Visualization of the histogram of oriented gradients (HOG) of the 64×64 rectangle of ROI2. The lines correspond to the direction of the edges in the subregion

16) and HOG (different sizes of the cell size: 4 and 8) were tested. The example of the HOG descriptor is shown in Fig. 4b. The output of all parametrization is the feature vector describing the fish appearance based on the selected ROI.

Classification

The fish identification task was done as a classification in the closed group (known number of individuals) where the number of classes corresponds to the number of fish individuals (43 fish in short-term scenario and 25 fish in long-term scenario). The classification was done separately for both the short-term and long-term datasets. The nearest-neighbor classifier was used for the classification of the fish.

Five images for each 43 fish (classes) were used for the short-term dataset (totally 215 images into 43 classes). Three out of five images for each fish (class) were randomly selected as the reference set, and two images as the test set. The task aimed to test how unique the pattern is for individuals in a group of fish. All descriptors described in the “[Feature extraction](#)” section were tested. The distance (similarity) of the feature vectors used for classification was calculated as the norm (Matlab norm function) of the feature vectors. The sliding window of 10×10 pixels was used to find the best match of the two compared images to eliminate errors in the ROI detection for texture-based descriptors.

The aim of the long-term dataset analysis was to test how the pattern changed during the study period and how it can influence identification. Therefore, the images of one fish from the first data collection were compared with the images of the same fish from the second data collection. Three images of the fish from the second data collection were used as the reference set, and three images of the fish from the first data collection were used as a test image. The same similarity measure as for the short-term dataset was used. The test of the uniqueness of the pattern was done for the first and second data collection separately, using the same

approach as described for the short-term classification of 25 fish. The results of the classification are confusion matrixes describing the similarity between all combinations of individual fish.

Results

The automatic fish body localization worked for all images in all datasets. The displacement of the ROI is in units of pixel. In the first task of the identification of 43 individual fish (short-term experiment), the different descriptors and different regions of interest were tested to explore the classification power of the fish skin pattern. The best result, 100% classification accuracy, was obtained using a combination of the HOG descriptor (4 pixels cell size) and ROI2 (Table 1). All 43 fish were identified correctly without any error. The other descriptors and other regions of interest obtained lower classification accuracies. The best result for BS features with different ROIs was 65% accuracy. The combination of BS and HP improved the accuracy to 89%. The use of LBP descriptor reached 93% accuracy using ROI2.

Based on the results of the short-term scenario, only the HOG descriptor was used for the classification of the long-term dataset for 25 fish. First, the identification within the first and second long-term data collection was performed. Table 2 shows the results of identification using different ROIs and HOG features. The best accuracy of 100% was obtained using the combination of ROI2 and HOG with a cell size of 4 pixels, like for the short-term scenario. The fish in the first and second data collection were identified without any error. To test pattern stability, the final classification was done for the first and second data collection periods. The combination of HOG descriptor and ROI2 achieved a top accuracy of 88% (false acceptance rate = 0.0048 and false rejection rate = 0.16)

Discussion

This study successfully shows that fish individuals of the same species can be automatically identified based on the visible skin pattern on their body using computer vision during the period of fish cultivation among a limited number of fish. We proved that the selected pattern could be used for individual identification because the classification accuracy within one data collection was 100% (for all data collections). This means that the pattern is unique and can automatically be distinguished between individual fish. Different regions of interest (parts of the fish) were selected to test the uniqueness of the patterns. The best results were achieved using the ROI containing central stripes of the fish. The lower accuracy of the ROI covering the whole fish is probably caused by the deformation of the pattern at the tail end, which is

Table 1 Results of identification for the short-term dataset using different descriptors and ROI2. Whole fish body was used for BS and HP descriptors

Descriptors	Accuracy (%)
HOG	100
BS	65
BS and HP	89
LBP	93

Table 2 Results of identification for the long-term dataset with different HOG descriptors and ROIs

Number of data sets	Region of interest	Accuracy	Number of data sets	Region of interest	Accuracy
1 to 1	ROIF	96%	1 to 1	ROIW	96%
2 to 2	ROIF	96%	2 to 2	ROIW	96%
1 to 1	ROI2	100%	1 to 1	ROI1	96%
2 to 2	ROI2	100%	2 to 2	ROI1	96%
1 to 2	ROI2	88%	1 to 2	ROIW	80%
1 to 2	ROIF	80%	1 to 2	ROI1	80%

moving as the fish is swimming. The ROI covering only one stripe, on the other hand, does not contain enough information to distinguish between very similar-shaped stripes.

The selected parameterizations represent both shape-based and appearance-based descriptors. The best results were achieved using the HOG descriptor because the patterns mainly consisted of the edges of the stripes and their positions. The use of the shape-based descriptor did not achieve acceptable results, mainly because of the dependence on the fish body height, which is affected by the fish orientation to the camera.

The test of pattern stability for long-term identification was represented by the identification between two data collection periods. This achieved an accuracy of 88% (three fish out of 25 were identified incorrectly) for ROI2. The identification was performed as the classification within the closed group of fish. This means that the unknown fish had to be classified as one of the fishes in the database. The classification accuracy of 88% was calculated for this case where no rejection threshold was applied. A rejection threshold can be applied to avoid bad quality images. Poor-quality images or images containing deformed fish patterns (caused by swimming) should have low similarities to the fish images in the database. This approach can reduce the rate of incorrect fish identification and the risk of impossible-to-identify images. This approach can also be used under real conditions because of the nature of the identification application. The images of the fish can be taken repeatedly until the fish is identified. The false acceptance rate (FAR) – false rejection rate (FRR) diagram (Fig. 5) shows the change of correctly and incorrectly identified individuals based on the change of the similarity threshold. The application of the threshold increases the accuracy to 96%.

The lower classification accuracy of 88% is mainly caused by the less than optimal quality of the collected images; the reflections in some images covered part of the selected region. The selected parameterization methods were designed to be invariant to the overall object intensity differences. The differences between the first and the second data collection period in the long-term experiment can be seen in Fig. 6. Furthermore, our matching before machine classification was done manually and could cause some errors. This matching was used as ground truth for the identification. No tags were used due to the fish size. Figure 6 shows the different ROI of the fish and some stripes look unique even by eye (e.g., fish 1), but usually these vertical stripes on a body look almost the same for all fish, as for fish 2 and 3 (Fig. 6). Nevertheless, Fig. 6 shows how the data can differ, and the experimental condition is crucial for identification and classification accuracy. The classification accuracy clearly shows that the fish pattern is unique, as it could distinguish between 25 individuals of the same species on the same data collection.

The approach based on deep learning was not tested in this study. The reason is that the number of images for each fish individual is very low in the comparison to the number of images the method typically needs for proper training of the network.

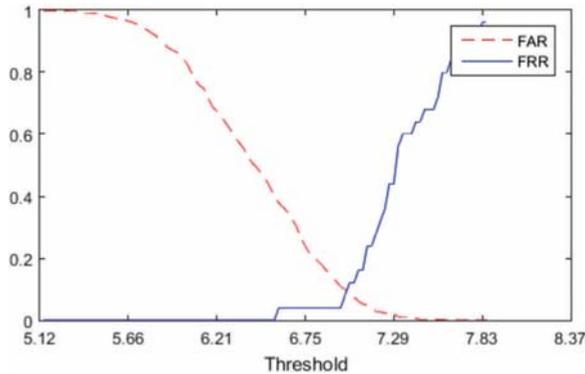


Fig. 5 FAR-FRR diagram. The change of correctly and incorrectly identified individuals

There are two ways to compare the approach with state-of-the-art methods. The conventional method used for fish identification is invasive fish tagging. The accuracy of identification is 100% (except in cases of tag damage). This approach has disadvantages caused by the invasive application of tags. The non-invasive fish identification approach is, therefore, a better alternative to fish tagging. The reason for this is that no tags need to be used for fish marking, and no physical manipulation is needed for fish identification. Studies dealing with fish identification within the same species based on visible patterns are very limited. They were based on human identification (Huntingford et al., 2013; Hirsch and Eckmann 2015) or used a limited number of fish of the same species. The highest number of fish used for identification was reported by Stien (2017), who used a semi-automated method of pattern parametrization (manually detected spots) for identification. The maximum number of fish used for

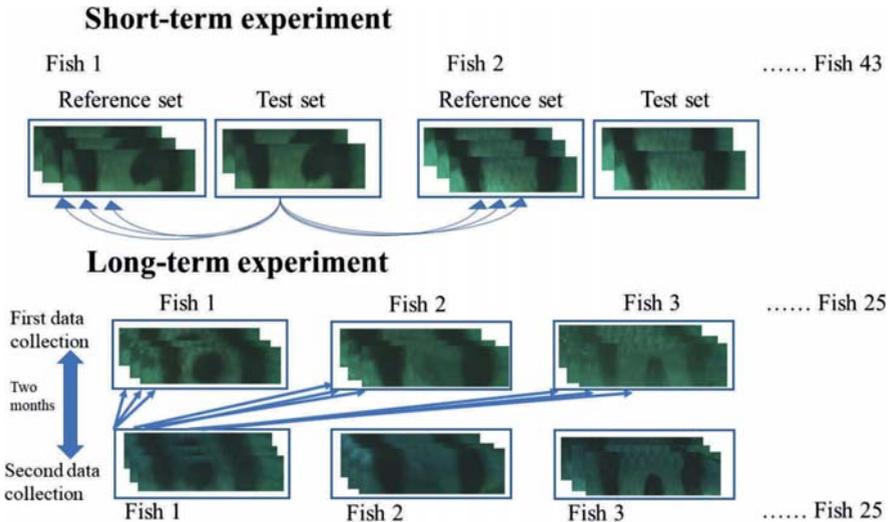


Fig. 6 Classification scheme. The top of the figure illustrates the classification of the individuals within one data collection. The test images of the fish (2 images) are compared with all reference images (3 images) of all fish. The bottom of the figure illustrates fish identification for two datasets. The images of one fish (3 images) from the second data collection are compared with images (3 images) of all fish in the first data collection

identification was 30 individuals. The reported accuracy was 85%. This result can be compared with our test of the pattern stability performed for 25 fish. The best accuracy achieved by our fully automated method was 96% using a similarity threshold.

The only fully automated approach was described by Al-Jubouri (2018), who made the identification of five fish only with a 99% accuracy. This result can be compared with the results of short-term experiments with 43 fish individuals. The achieved accuracy was 100%. Therefore, the presented approach outperformed all of the state-of-the-art approaches and demonstrated the usability of computer-based fish individual identification.

Small-scale research studies usually use a comparable number of fish; therefore, the method is promising to substitute conventional procedures of fish identification (e.g., tagging or marking). The restrictions during data collection (illumination, background) do not allow us to generalize the results to real conditions. To fully automatize the identification based on the visible pattern under production conditions, more fish should be involved in the study and the data should be collected under real conditions of the experimental studies or fish cultivation. Data collection is a vital part of the experiment, and better data collection will facilitate improved results. The visible patterns—in our case vertical stripes—can be used for the identification of Sumatra barb and these patterns are stable during the growth of the fish. However, this approach is not limited to Sumatra barb and could be applied to any fish species with a visible pattern on the body (stripes or dots). The approach could be beneficial for commercial fish species and even for pike-perch and European perch.

Fish have pigment cells on their body which are responsible for coloration and their ability to change it. There are two mechanisms for color changes: physiological color change (rapid motile responses of chromatophores) and morphological color change (changes in the chromatophores morphology and density) (Cal et al. 2017). The long-term adaptation of fish for different conditions of abiotic factors is mostly caused by morphological color changes (Sugimoto 2002). Usually, all abiotic factors which mostly affect pigmentation such as light and background are controlled and not much changing during the cultivation period of fish, and for approach which we had used for identification of small groups of individuals during short period of the experiment, these changes were not critical. Research about the stability of used patterns during a longer period of time is the next step of our work.

Conclusion

This study shows that fish individuals of the same species with a limited number of fish can be automatically identified by visible skin patterns and image processing methods. The visible patterns, in our case, were vertical stripes of Sumatra barb that can be used for the identification of individual fishes in the long-term context of fish cultivation. The uniqueness of the patterns was proved, together with their stability over time. Automatic processing and parametrization of the patterns enable the implementation of an automatic method for non-invasive fish identification based on fish appearance only. This concept provides an alternative to the standard invasive fish identification based on fish tagging. However, this is not just limited to Sumatra Barb; the concept is applicable for any fish with visible and unique patterns, especially for commercial fish species like salmon and pike-perch. Moreover, if minor modifications were made, most other species of fish could work in the program too. With current success and the constant amelioration of camera and video technology, the program will get better results due to better clarity and color from camera images in the future.

Code availability Available on request to the corresponding author

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Data Availability Available on request to the corresponding author

Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Conflict of interest The authors declare no competing interests.

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CHAPTER 3

COMPUTER VISION BASED INDIVIDUAL FISH IDENTIFICATION USING SKIN DOT PATTERN

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My contribution to this work was about 35%.

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Computer vision based individual fish identification using skin dot pattern

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Precision fish farming is an emerging concept in aquaculture research and industry, which combines new technologies and data processing methods to enable data-based decision making in fish farming. The concept is based on the automated monitoring of fish, infrastructure, and the environment ideally by contactless methods. The identification of individual fish of the same species within the cultivated group is critical for individualized treatment, biomass estimation and fish state determination. A few studies have shown that fish body patterns can be used for individual identification, but no system for the automation of this exists. We introduced a methodology for fully automatic Atlantic salmon (*Salmo salar*) individual identification according to the dot patterns on the skin. The method was tested for 328 individuals, with identification accuracy of 100%. We also studied the long-term stability of the patterns (aging) for individual identification over a period of 6 months. The identification accuracy was 100% for 30 fish (out of water images). The methodology can be adapted to any fish species with dot skin patterns. We proved that the methodology can be used as a non-invasive substitute for invasive fish tagging. The non-invasive fish identification opens new possibilities to maintain the fish individually and not as a fish school which is impossible with current invasive fish tagging.

The trend toward increased automation in agriculture is significant¹ and the aquaculture sector is not exception. The development of new methods for machine vision and digital cameras enables the automation of several tasks under the non-trivial conditions of fish cultivation². The main idea of the automatized aquaculture concept is implementation of precision fish farming³ (The automation of aquaculture production improves the control, monitoring and documentation of the biological process of fish growth. Based on automatically extracted knowledge, the farmers can increase the profits by controlling diseases and monitor fish welfare and fish growth. Early disease detection and the prediction of outbreaks can safeguard the livestock of fish⁴. One of the critical components of automation is individual fish identification⁵ (Yusup et al., 2020).

The standard approach to the identification of individual fish of the same species is tagging⁶. There are several disadvantages and limitations of this invasive approach: the high mortality after injection in specific cases^{7,8} (Bolland et al., 2009; McMahon et al., 1996), stress caused the fish by the application of the invasive approach, need for the fish to be caught for identification, time-consuming nature of the method⁹ (Whitfield et al., 2004), and limited fish size. The possibility of the non-invasive identification of individuals becomes an alternative for fish tagging¹⁰ and address all the previously listed disadvantages.

Today, photo- or video-based identification systems are used in agriculture¹¹ and wildlife for ensuring population welfare and estimating the size of the population^{12,13}. All systems use the visible pattern of the animal for the identification of the species or individuals of the same species. The identification principle is based on human biometric identification as document for cow iris-based identification¹⁴. Similar issues of aging¹⁵ are therefore recognized in animal identification.

In the aquaculture sector, machine vision is usually used to identify fish species¹⁶. Several studies^{17–23}, exist on the non-invasive individual identification of fish using images but only few of them use machine vision. The majority of the studies use human experts for identification based on the fish images^{20,22}. These studies proved that fish appearance based individual identification is feasible for the carp (*Cyprinus carpio*) (15 fish, 95.76% accuracy)²⁰ and catshark (*Scyliorhinus canicular*) (25 fish, 99.6% accuracy)²² in the short-term. A long-term study of wild populations of cutthroat trout²¹, in which datasets from 1997 and 1999 were compared, showed that two

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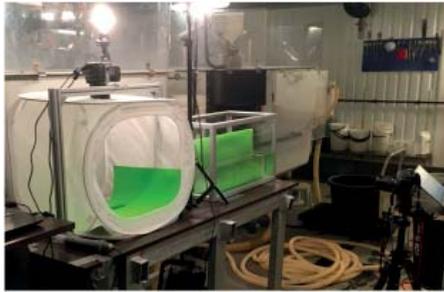


Figure 1. Experimental setup for data collection. Photo tent with camera installed on top, aquarium with the camera installed in front of it, and LED lights for scene illumination. The green background was installed in the tent and aquarium.

adult fish can be identified using the skin spots after two years. The stability of the pattern was also proved by Stien¹⁸. They manually labeled the dots on salmon and performed the identification of 25 fish for ten months. The identification accuracy was 85%.

Only a few papers have presented results for semi-automatized identification, with a minimal number of fish^{17,19} and it has been tested only for short-term periods.

A computer-assisted approach for the identification of 30 individuals was used for armored catfish¹⁷. Computer-assisted means that the 20 most similar images were ranked based on the SIFT (scale-invariant feature transform). The humans used this ranked list for identification. The identification accuracy was 99% for the images taken on two days.

The only fully automated approach has been described by Al-Jubouri¹⁹, who performed the identification of five zebrafish (*Danio rerio*) with 99% accuracy. The histogram of the hue-saturation-value color space of the part of zebrafish stripes was coupled with the KNN (*K*-nearest neighbour) classifier. All images were collected for one day.

To the best of our knowledge, there is no study using fully automated computer vision for individual fish identification working with a high number of fish or tested for a long-term period. The abovementioned studies showed the feasibility of appearance-based identification but for a limited number of fish or species with low value for the aquaculture industry.

Therefore, this study's aim goes beyond the mentioned studies and introduces a fully automatic method for the long-term individual identification of the commercially important species, Atlantic salmon (*Salmo Salar*).

Methods

Experimental animal. The Atlantic salmon (*Salmo salar*), one of the most economically important fish species, was used in the study. The experiment was conducted at the NOFIMA experimental infrastructure in Sunndalsora, Norway. A total of 328 farmed Atlantic Salmon were used in the experiment. The average fish weight was 251 ± 21 g and the length 29.5 ± 2.5 cm. The age of the fish was 5 months. The 328 fish were used for short-term identification to test the identification power of the pattern. Thirty of the fish were tagged with PIT (passive integrated transponder) tags and used for long term identification to test pattern stability. The tagged fish were cultivated in a 2m³ recirculation freshwater tank for six months. The fish were only manipulated for data acquisition. The experimental procedure followed the standard operational procedures and regulations of NOFIMA. The experimental protocol was approved by the Norwegian Animal Research Authority and by the ethical advisor of the AQUAEXCEL²⁰²⁰ project. The study is in accordance with ARRIVE guidelines.

Experimental design. A total of 4 data collections (session—s) were performed over 6 months at 2 months intervals. Two types of data were taken in each session: lateral view images of the fish out of the water (in a photographic tent; see Fig. 1) and underwater (in a small aquarium; see Fig. 1). During each session, the fish were caught in the tank, anesthetized (FINQUEL MS-222) and moved to a single-layer white fabric photographic tent with controlled illumination and a foamy green background. The photographic tent was used to ensure a uniform light intensity over the fish skin. The foamy green background allowed for easier background segmentation in the image pre-processing phase. The scene was illuminated by LED bulb light to control the light conditions in the tent. Only the left sides of the fish were photographed. The NIKON D90 digital camera was used to take approximately eight images of each fish in RAW format. The fish were moved and rotated (± 45 degrees) in the tent to take pictures of the fish in different positions and rotations (Fig. 2). The resolution was 4288×2848 pixels, with 12 bits/pixel, and three color channels (i.e., red, green and blue). After data collection in the tent, the fish were moved into an aquarium with dimensions of $60 \times 35 \times 30$ cm. The illumination was changed during data collection to simulate real production conditions. The Canon EOS 5D Mark II digital camera was used to take approximately eight images of each fish in aquarium. The fish were moved and rotated (± 45 degrees) in the aquarium to take their pictures in different positions and rotations (Fig. 2). The resolution was 5616×3744



Figure 2. Example of images from data collection. Left—tent data, middle—aquarium data SL1, right—aquarium data SL3. Middle and right images show the different illumination conditions for data collection.

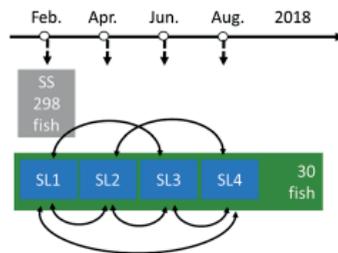


Figure 3. Visualization of data collection sessions. SS—298 fish data collection, SL1, SL2, SL3, SL4—30 tagged fish data collection over the time. The arrows between the SL datasets indicate the identification tasks performed for all combinations of the datasets.

pixels, with 12 bits/pixel and three color channels. Both cameras were set to the automatic mode. After data collection, the fish were moved back to the cultivati tank for recovery. All the fish fully recovered.

The first data collection session was different from the subsequent three sessions. Images (tent and aquarium) were collected for 328 fish in the first data collection for five days. Of these, 298 fish were not tagged, and their images were collected only in the first session. This image dataset is marked SS (see Fig. 3). Images of the 30 tagged fish were also collected as the long-term dataset during the next six months with two months intervals. The first dataset is marked SL1 and the rest are numbered according to their session (see Fig. 3).

Identification design

Two specific identification tasks were performed with the recorded datasets. The first focused on testing the uniqueness of the skin dot pattern for individual identification and is called short-term. The identification was performed for the fish images collected for five days. The SS dataset (298 fish), together with SL1 dataset (30 fish), was used for this task. The images for each fish in both datasets were divided randomly into templates and test images. The templates were used as the representative images of fish, and the test images were used for the identification. All fish were compared with all the others within the SS and SL1 datasets. In total, 328 fish were used for short-term identification.

The second identification task was focused on the testing of the long-term stability of the patterns for identification. The datasets SL1, SL2, SL3 and SL4 were used for this task. Two identifications were performed for these datasets. The within dataset identification was performed for each dataset separately. The same procedure as used for the SS dataset was used. This task tested the identification within the S1, S2, S3 and S4 datasets independently. The results of the within-dataset identification show the identification accuracy according to fish age. The more important task was to test the long-term stability of the patterns. Therefore, the identification was performed with the combinations of all four datasets. This task was to test if the pattern was stable over six months of fish cultivation. All possible combinations of the datasets were used for identification (see Fig. 3).

ROI selection

Before the images in the dataset were processed, they were manually checked to remove images corrupted during data collection. The number of images per fish varied from 5 to 9.

The regions of interest (ROIs) were automatically extracted for all images in all datasets using image processing methods. The green background was used for the segmentation of the fish and background. First, the area with the specific colour (green) was detected based on the known hue and saturation values of the background in terms of hue, saturation and lightness colour space. Thresholding was used for background detection. The second step was to localize the fish within the background area. The fish was detected as the largest object inside the background area. The detected fish object is shown in Fig. 4. The object was rotated using an estimated ellipse

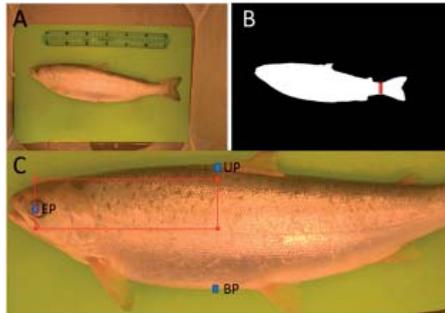


Figure 4. Process of ROI localization. **A** Original image of the fish in the tent. **B** Localized objective image of the fish with the narrowest area before the tail fin. **C** Fish with localized points EP (eye position), UP (upper fin beginning) and BP (belly point at the vertical position of UP); red rectangle represents the ROI.



Figure 5. Localization of the upper fin beginning. Left—Segmented fish with selected region of upper fin beginning. Right—Magnification of selected region; blue line—horizontal center of the image, red lines—lines fitted to the left and right halves of the border pixels. Produced by Matlab R2020b.

around the object (Matlab R2020b – function `regionprops (image,'Orientation')`) to compensate for rotation of the fish in the image. Because the fish tail fin is semi-transparent and the segmentation of this part is not stable, the narrowest area of the fish before the tail fin was detected (see Fig. 4B). The length of the fish was then defined from the head tip to the narrowest area. The approximate area of the upper fin was estimated to be in the region of $3/8$ to $5/8$ of the length of the detected object (without the tail fin). This area was used to search for the beginning of the upper fin. The segmented fish border of the area was divided into the left and right half.

The line was fitted to the left and right pixels and the intersection was taken as the beginning of the upper fin (see the example in Fig. 5). The localized upper fin position is marked UP.

The approximate area of fish eye was estimated between 0 and $1/3$ of the UP position (in horizontal axes). The exact area of the fish eye was detected by the thresholding of the grayscale image of the selected area. All pixels with intensities lower than 20 (the threshold was determined experimentally and works for all datasets) were marked as eye pixels. The eye position EP was calculated as the centroid of the eye pixels.

The belly point (BP), which represents the height of the fish at the horizontal position of UP, was detected as the point at the border of the fish belly. This point was used to determine the height of the ROI.

Based on the determined points UP, BP and EP, the ROI was selected in the image. The ROI was selected as the area between EP and UP in the horizontal direction and between $UP + (BP - UP)/20$ and $BP/2$ in the vertical direction. See an example of a ROI in Fig. 4.

DOT based approach

Identification based on the dots' exact position on the fish skin consists of several steps (see Fig. 6). First, the dots in the normalized ROI are localized using convolutional neural network (CNN) for all images of the particular fish. The localized dots are used for the identification of individuals for the SS dataset. Then the representative dot pattern is created as a subset of the detected dots for the particular fish from the images of that fish. The representative dot pattern is used for the identification of individuals for the SL datasets.

DOTs localization. The first step in dot detection was the normalization of the ROI to the length of 1000 pixels. The height of the image was calculated to maintain the height/width ratio. Normalization eliminates the change in ROI size during fish growth. The CNN was used for dot localization. The network performs the classification into two classes, dots or no-dots. The network architecture contains five trained layers: three convolutional and two fully connected layers. The rectified linear unit (ReLU) activation function was used for convolutional layers, and the Softmax activation function, for the classification layer. Max pooling layers were used between the convolutional layers. The training was performed with the Matlab R2020b Deep learning toolbox function "trainNetwork". The architecture of the network with training examples is shown in Fig. 7. A total of

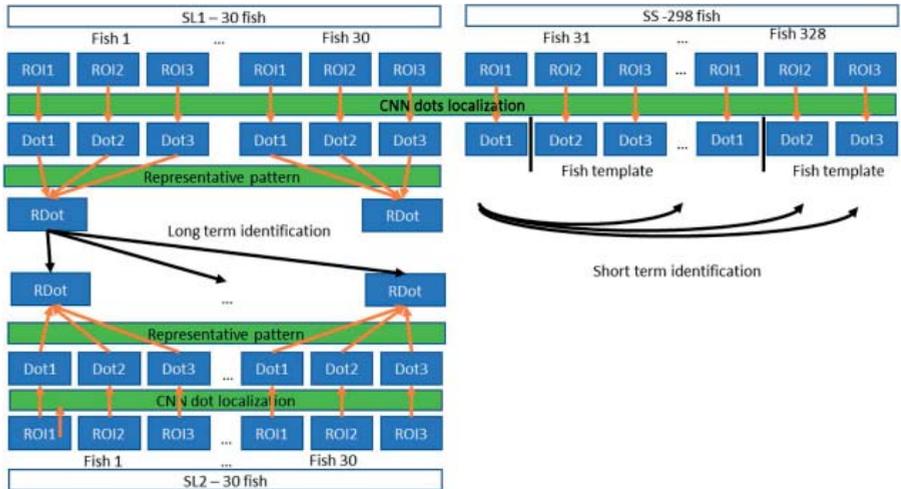


Figure 6. Scheme of dot localization-based approach to identification. The dots (Dot) for each ROI of each fish are detected using CNN. The dots are used for short-term identification, where two images are used as the pattern and one image as an unknown image. The representative dot pattern (RDot) is calculated for each fish from all fish dot patterns. The representative dot pattern is used for long-term identification for the SL1, SL2, SL3 and SL4 datasets.

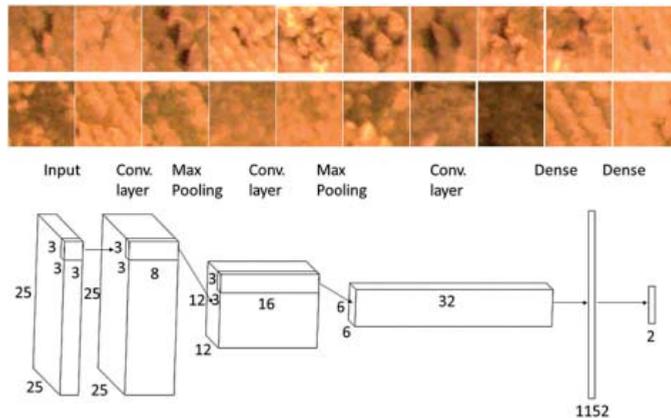


Figure 7. Examples of data for dots localization CNN training. Upper row – dots, bottom row – areas without dots.

535 examples of the area with dots and 535 examples without dots were used for CNN training. The resolution of the images was 25×25 pixels. These images were manually randomly selected from the images of ROIs from all SL datasets. The network was trained using 2/3 of the images. The accuracy of the CNN, tested on the testing dataset (1/3 of the images), was 99%.

The localization of the particular dot in the ROI was performed as the classification of the sliding window (with 5 pixel step of the window in the x and y directions) over the ROI into the dot or no dot class. The sliding window was classified as dot if the dot class' probability was higher than 30%. The dots were detected between 1/3 and 3/3 of the length of the ROI to exclude the head region (there is no dot pattern on the head). The method identifies a high number of dots, and some dots are detected more than once. Therefore, the dots' clustering

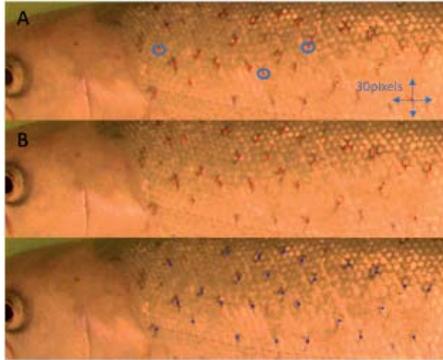


Figure 8. Top and middle images—example of dot detection for two (A and B) images of the same fish. The arrows indicate the pattern shift for alignment. The dots (blue circle) of the A pattern do not have close points in the B pattern, and therefore, they are not used in the representative pattern. Bottom—representative dots selected as a subset of the dots detected for all images of the same fish.

was used to cluster the dots closer than 15 pixels using the Matlab function *clusterXYpoints*. The final dots are represented as centroids of the detected clusters (see Fig. 8). The coordinates of the dots were saved as the dot pattern describing the fish.

Short term identification. The dot pattern was detected for all images for all fish in the SS and SL1 datasets. SS+SL1 dataset was collected for the 328 fish, where the rotation and translation of the fish were applied during the data collection. One dot pattern was selected as a comparative image for each of the 328 fish. Two other dot patterns for the fish were used as the fish template. Identification was performed to compare the relative pattern with two templates for each fish (nearest neighbour classification). The distance between the dot patterns was measured as the average distance between $\frac{1}{3}$ of the points of the two compared patterns. For each dot in the first pattern, the closest dot in the second pattern (according to Euclidean distance) was determined. The distances of all dots were sorted by size, and the average distance was calculated for the first $\frac{1}{3}$ of the points of the first pattern. The last $\frac{1}{3}$ of the distances were not used, to eliminate the effects of outliers (incorrectly detected dots). Because of the possibility of the dots shifting in the x and y axes (the ROI not being correctly determined), the calculation of the distance between the two patterns was repeated for the second dot pattern shifted ± 30 pixels with a step of 5 pixels in the x and y directions. The determination of the closest point and calculation of the average distance was performed for all shifts. The minimal distance was then used as the final distance between the two patterns. The fish was then identified as the fish with the minimal final distance between the comparative pattern and one of the two template patterns. The number of dots in the pattern varied from 4 to more than 30. A heuristic to minimize the calculation time was applied for the fish comparison. The dot pattern of fish A was compared with that of fish B only if the number of dots for B was up two times lower or higher than that of A. This approach compared only similar patterns.

Two-point cloud registration methods (iterative closest point (ICP)²⁴ and coherent point drift (CPD)²⁵ implemented in Matlab (function *pregisterict* and *pregistercpd*) were tested for the matching of the dot patterns, but they achieved lower accuracy than the sliding window method.

Representative pattern. The representative dot pattern for each fish was determined to improve the identification and reduce the time of the calculations. All dot patterns of one fish were compared, and the points appearing on $\frac{1}{3}$ of the patterns were selected as the best representatives. The same approach used for the comparison of patterns for short-term identification was used. The pattern with the minimal average distance from all other patterns was used as the reference pattern. The best match to the other patterns of the same fish was detected based on the shift in x and y and calculating the average minimal distance. This step aligned all the dot patterns. For each point of the reference pattern, the closest points in all other patterns were detected. If the distance to the closest point was smaller than 20 pixels (it meant that the point appeared in more images of the fish) then it was selected for the representative dot pattern. The point was selected if it appeared on more than $\frac{1}{3}$ or at least three images (for low numbers of images). See an example of a representative dot pattern in Fig. 8. The representative patterns represent detected dots for the particular fish.

Long term identification. The long-term identification task involved the dot pattern stability for long-term fish cultivation. Based on the manual analysis described in the Sect. 2.7., it was determined that the automatic fish rotation compensates the different poses of the fish in the original image. Therefore, the main difference between the dot patterns caused by fish growth is in the horizontal/vertical direction and scale. No other distor-

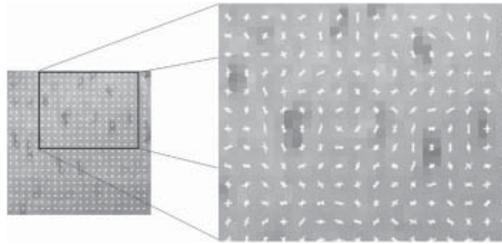


Figure 9. Visualization of HOG descriptor for the dots pattern. Left – normalized image of ROI (zoom of 64×64 pixels image). Right – orientation of the edges in the image coded by HOGs (zoom of left image). Produced by Matlab R2020b.

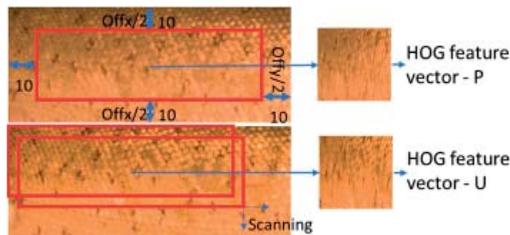


Figure 10. Similarity measure of two fish skin patterns using HOG feature descriptor. Upper row (P)—image of the identified fish. The subpart of the pattern is used for parametrization. The subpart is normalized to a 64×64 pixel image, and the HOG feature vector is calculated. Bottom row (U)—images of the unknown fish. The subpart of the pattern is selected from the image repeatedly by scanning over the image. The subpart is normalized to a 64×64 pixel image, and the HOG feature vector is calculated. The similarity between the P and U images is the best similarity between the P subset and one of the U subsets from the scanning.

tions corrupted the dot pattern over the six months period (missing scales are discussed in the Discussion). The long-term identification used the same approach as described for short-term. The only difference was that the representative patterns were used for identification instead of the detected dot patterns and the maximal shift (used for dot alignment) was 50 pixels. The identification was made separately for all combinations of the SL datasets to test the identification for different fish ages and different periods (see Fig. 3).

Histogram of oriented gradients (HOG) approach

Histogram of oriented gradients³⁶ is a feature descriptor of image pattern parametrization mainly used for object detection in computer vision. The HOG feature descriptor codes the gradients of the image, which mainly represent the edges or points in the image, and therefore, it is also a good texture descriptor invariant with the illumination. The identification using HOG consisted of the following steps: (1) feature vector calculation for the pattern from the ROI of the known fish (see Fig. 9), (2) scanning of ROI for unknown fish, (3) the calculation of the best similarity between the known and unknown fish, (4) the classification of unknown fish into one of the known fish. The classification using HOG was based on the calculation of the similarity of the HOG vectors of the ROIs for both known and unknown fish. The HOG vector was first calculated for known fish (i.e., the fish with known ID). The only subset of the ROI was used for parametrization (see Fig. 10). The subset was selected as the ROI without the border areas of the size of $Offx$ and $Offy$. The subset was resized to a $sizeN \times sizeN$ pixel image, and the HOG feature vector was calculated. Then, the HOG feature vector was calculated from a subpart of the ROI of the unknown fish (fish we identified). The same size of subpart as for the known fish was used. The subpart was repeatedly selected using a sliding window over the ROI of the unknown fish. The similarity (distance) of the two HOG vectors (known and unknown fish) was calculated as the norm between the two vectors. The similarity was determined as the minimal distance between the known HOG vector and all HOG vectors generated by scanning over the unknown fish ROI. The function `extractHOGFeatures`—Matlab R2020b was used for HOG calculation.

Identification. The same tasks of identification were performed as for the dot pattern approach described in Section 2.5. The short-term identification was performed using the SS and SL1 datasets together. The first image of all images for the particular fish was used as the unknown image. The second and third images of all for the

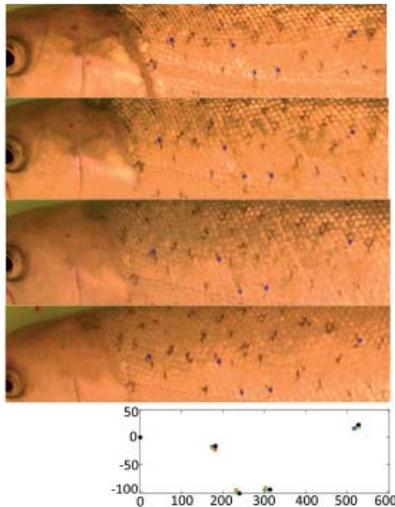


Figure 11. Manual localization of dots for one fish for four sessions, SL1–SL4. Blue dots were manually localized. Bottom plot shows aligned dots from SL1–SL4. Manually localized dots were used for analysis of pattern stability.

particular fish were used as the known images. For each fish the similarity to all other fish was calculated based on the scanning described above. The fish was identified as the most similar fish. For long-term identification, all images of the fish from one SL dataset were used as the known images and all images of the fish from another dataset were used as the unknown images. The similarity between all combinations of the images for the two fish was calculated using the scanning approach, and the best similarity was taken as the similarity between these two fish. The similarity was calculated for all fish and the two SL datasets. Each fish from first SL dataset was identified as the most similar fish from the second SL dataset.

Different settings of the parameters of HOG feature calculation were tested: the cell size, resolution of normalization image and the offset Off_x and Off_y . Different subregions of the ROI were also tested for HOG feature calculation. The right half, right 2/3 and right 4/3 of the ROI in the horizontal direction were tested as subregions. Only the best results and settings are described in the Results section.

Pattern validation

ROI localization and dot detection were performed automatically, which could influence the identification accuracy. Therefore, we performed a manual analysis of the dot pattern changes during the six months of fish growth. Five fish out of the 30 from the SL datasets were randomly selected for manual analysis. The same five dots at different places of the ROI were selected for each fish. The dots were manually localized in one image for all four SL datasets for all five fish (see Fig. 11). The x and y shift, rotation and scale were applied to the localized dots to obtain the best alignment for each fish separately. The average displacement of the five points was calculated after the alignment to analyze the pattern changes over time.

Results

Manual evaluation. The average displacement of the four points for all five fish was 9 pixels for the x axes and 8 pixels for the y axes. The displacement was calculated for each fish and for each point separately as the distance of the point in the first image and other images. The image resolution was 1000 pixels in the x axis and 269 (average) pixels in y axes. See the displacements of the points in Fig. 11. The displacement is partially caused by the change in the pattern (fish growth) and partially by the manual localization. The dot pattern does not have any exact shape, and the shape of the dots is mainly changed during fish cultivation. The labeler selected one exact point in the dot, which was used for the labeling. We estimate that half of the displacements were due to the labeling itself. The displacement of 5 pixels proved that the dot pattern was stable and usable for image-based identification for at least six months.

ROI localization. The localization of the ROI is the most critical part of the identification. Manual analysis proved that the dot pattern was stable. For successful identification, the same part of the pattern has to be selected. The ROI was detected with the maximal displacement of 50 pixels in both directions. It was 5% for the

HOG-based identification										Dot-based identification				
Tent data					Aquarium data					Aquarium data				
Session	SL1	SL2	SL3	SL4	Session	SL1	SL2	SL3	SL4	Session	SL1	SL2	SL3	SL4
SL1	100	100	83.3	36.6	SL1	100	83.3	73.3	70	SL1	100	100	96.7	70
SL2		100	100	53.3	SL2		100	76.6	66.6	SL2		100	100	100
SL3			100	93.3	SL3			100	60	SL3			100	100
SL4				100	SL4				100	SL4				100

Table 1. The accuracy of automatic identification using hog descriptor of the pattern and dot approach. The accuracy in % is shown for the combinations of the SL datasets.

x axes and approximately 12% for the y axes. The localization depended mainly on fish bending in the z axes (distance from the camera) and the change on fish proportions caused by fish growth.

Identification. Two methods were used for the automatic identification of the individuals. Both methods used the same ROI for identification. Two tasks of identification were performed. First, the pattern uniqueness was tested using the SS + SL1 dataset. Both methods obtained 100% accuracy for identification from the 328 fish tent and aquarium data. It is proof that the pattern is unique for a high number of fish and can be used for identification. The second task was to test the stability of the pattern during fish growth. Four SL datasets were used for this task with the data of the fish in the tent and aquarium. The best results were achieved using the identification based on dot detection. The accuracy was 100% for all the combinations of all datasets for the tent data. The accuracy for aquarium data was also 100% for all combinations except for the identification for the SL1/SL3 (accuracy 96.7%) and SL1/SL4 (accuracy 70%) datasets, representing identification after 4 and 6 months of growth, respectively. The results of the method based on HOG are summarized in Table 1. The best results were obtained using the following settings: normalized image size, 64*64; offset, 10*10; cell size, 2; ¼ROI for the SL1/SL4 dataset and 2/3ROI for all other combinations of datasets.

It can be seen that both methods obtained an accuracy of 100% for the datasets taken over two months. The accuracy for the other combinations (more distant in time) decreased and was correlated with the time interval between the data collections for HOG approach.

The identification of one individual into 30 classes using the dot approach took 0.8 s (CPU – Intel i5-6300CPU). The whole code was implemented in Matlab R2020b without any optimization or precalculated representations of the patterns.

Discussion

This study is the most extensive study of automatic individual fish identification based on fish skin patterns. The study was large because 328 fish were used to test the feasibility of using the lateral skin dot pattern of Atlantic salmon for individual identification and a 6 month period of fish growth was used to study the pattern stability during fish cultivation. Previous studies had only identified a maximum of 30 fish with semi-automated methods¹⁸ and five fish with fully automated methods¹⁹, and there were none using fully automated methods for long-term identification. This study is the first in which images of fish out of the water (i.e., in the tent) and underwater (i.e., in the aquarium) were used for long-term automatic identification. All other studies used images of fish taken out of water only.

Individual fish identification is widely used in aquaculture research where it is necessary to study individual behaviour, growth or fish states according to environmental changes or feeding strategies. The benefit of remote fish identification based on appearance only is evident for research purposes because of its non-invasiveness and the possibility of identifying fish too small to be tagged¹⁰. The need for individual fish identification is also becoming more critical for aquaculture production with the automation of production monitoring and control. It is not yet widely used, mainly because of the limitations of the existing invasive tagging methods. The fish must be caught to be tagged, which is time consuming and often impossible in high-density fish cultivation (200 000 fish in the cage) in the sea cages²⁷. The price of such a large number of tags is enormous for fish farmers. Another limitation is tag reading underwater and the need for tag removal before the fish are delivered to the market. The remote individual identification method, which could be used in the tanks or sea cages without the need to manipulate the fish could enable new methods of monitoring fish growth and state. Individual identification can be used to improve biomass estimation, fish sorting based on the signs of disease or individual growth. The approach of individual identification based on appearance must be fully automatic, accurate for long-term fish cultivation and able to work under production conditions to be useful for the aquaculture industry. This study examined the possibility of the automatic individual identification of Atlantic salmon over 6 months of cultivation.

Two datasets were recorded for the study: a tent dataset representing high quality images of the fish out of the water; an aquarium dataset representing lower quality data due to the light scattering by the water and varying illumination in each recording session.

Two methods were used for fully automatized individual fish identification: a dot localization method and HOG feature-based method. The accuracy of both methods for the short-term identification of 328 individuals

for tent and aquarium images was 100%. The accuracy of long-term identification with the tent data was 100% for six months and that with the aquarium data was 70% (100% for four months), for the dot localization method.

Originally, more texture descriptors (local binary patterns, scale invariant feature transform), and an approach completely based on CNN (end-to-end) were tested for fish identification. The best accuracy was obtained using the HOG descriptor. Because the HOG-based long-term identification did not reach 100% accuracy, the specific dot localization method was developed, which shows better accuracy for most identification tasks.

Different approaches were used for dot alignment for dot based identification. The methods for point cloud registration^{24,25} obtained lower accuracy than the simple but effective method of using point shift and scale. The lower accuracy was caused by the incorrect alignment of the dot patterns with low numbers of dots. The methods expect that the point clouds used for registration contain the inliers and outliers, which is not the case with the low numbers of dots patterns.

The short-term identification of the 328 fish proved that the fish skin's melanophore dot pattern is unique for distinguishing all the fish without any error. The fish rotation, translation and illumination changes did not cause any misidentification. The identification was performed for all the SS and SL datasets, and the fish growth did not influence the accuracy. The dot pattern had better contrast for older fish than for the fish in the first session. The result cannot be directly generalized for more fish but the differences in the numbers of dots and the positions are high, which is a good assumption for the identification of a higher number of fish.

The long-term stability of the pattern is critical for accurate identification in aquaculture production. The fish are usually cultivated for several months/years and should be identifiable for the whole cultivation period. The four SL datasets were recorded to cover the fish growth, which could influence the skin dot patterns. Six months of data collection started at the fish age of 5 months and finished at the age of 11 months. Visual inspection of the data recorded at the different sessions showed that the dot pattern is more visible on older fish. Especially, the contrast between the dots and the surrounding skin is higher. This is consistent with the observations of Stien¹⁸. During the 6-month period the fish length changed 1.6 times and the fish height changed 1.9 times, on average. Manual verification of the pattern stability was therefore performed to analyze pattern changes. The dot displacement was estimated to be 9 pixels for the image of 1000 pixels in width—0.9% of the image's width. The translation, rotation and scale were applied to align the patterns for all sessions. The result of the analysis was that the dot pattern was stable based on the dot's position for 6 months and could be used for long-term identification.

Both methods (HOG and dots) were used to test the fish's identification for 6 months. All possible combinations of SL datasets were used for testing. The combination SL1/SL2 represents the identification after 2 months of cultivation. The combination SL1/SL4 represents the identification after 6 months of cultivation. The dot-based identification method achieved 100% accuracy for all combinations using the tent data. Using the aquarium data, the accuracy dropped for the combination SL1/SL3 and the SL1/SL4 dataset, representing 4- and 6-month differences, respectively. The main reason for the lower accuracy with the aquarium data could be the lower data quality and high difference in the illumination in each session. The main difference was between the sessions SL3 and SL4. The accuracy of identification using the HOG approach was the same or lower than that with the dot approach for all combinations. For the tent data, the HOG approach could correctly (except SL3/SL4 - accuracy 93.3%) identify all fish in the 2-month data collections. The accuracy for the 4 months period was 83.3% and 53.3%. The accuracy for the 6 months period was only 36.6% only. The accuracy with the aquarium datasets was lower than that with the tent data. The average accuracy for 2 months was 73.3%; for 4 months, it was 70%; and for 6 months, it was 70%. The HOG approach's lower accuracy is explained by coding not only the dots but also the reflections and missing scales on the fish's bodies. The dot approach successfully eliminates these problems. The long-term identification showed that the dot approach could identify all fish without any errors over a period of 6 months using high quality (tent) data. The 100% accuracy could be achieved for lower quality data (aquarium) for a 4 month period. The HOG approach could correctly identify all fish in 2 months with high quality data. Using the lower quality data, the identification for all periods was on average 71%. Automatic long-term fish identification (for at least 6 months) based on appearance is possible using the dot approach with high quality data. The HOG approach can also be used but mainly for 2 months periods. Both methods' results can be improved by updating the fish images that represent the individual fish. Because of the 100% accuracy for the 2-month period (SL1/SL2, SL2/SL3 and SL3/SL4), the old images of each fish can be substituted by the new images after the identification. This approach will update the representative images for the growing fish and will increase the identification accuracy during fish growth. The accuracy for the tent data for the 6-month period would be 93.3% with the HOG approach using the image update method. This approach expects that the system detects all fish during the period of 2 months.

The HOG based approach uses image with resolution of 64×64 pixels for identification. Therefore, there is high probability that the method will work under real conditions where the quality of the fish images will be decreased by fish movement, changing light conditions and fish overlaps. The advantage of the HOG approach is the possibility of using the method for the parametrization of the different skin patterns of the fish. The method was successfully used to identify the ornamental fish Sumatra barb (*Puntigrus tetrazona*) with the stripe pattern²⁸. The dot approach can only be used for the fish with the dot pattern and the CNN for dot detection must be trained for particular species.

The HOG approach is also sensitive to the localization of the ROI. A shift in the ROI highly influences the identification accuracy. This was observed during the experiments with the selection of the best ROI for identification. Finally, the upper left part of the fish was selected because it contains the dot pattern (there are just few dots in the left bottom part) and does not deform with fish movement. The pattern on the right (tail part) part of the fish deforms due to the tail bending, which is natural during fish swimming.

The study proved that automatic individual fish identification based on dot patterns for Atlantic salmon is possible. The limitation of the current approach is that the images of the immobilized fish were used. The approach is directly useful in all studies where the fish are caught and sampled. It can be used as a substitute for the tagging

method. The main potential of the method is for remote identification in aquaculture production. To apply the method inside the tanks and sea cages, high-quality data capture for the swimming fish must be achieved. This can be performed using high resolution cameras and deep learning methods, as shown by Schellewald²⁹. The usability of the automatic identification method under the real conditions is also influenced by the method's speed. The speed is not too critical. The real-world scenario of identification is that the system captures images of the fish while they are swimming around and performs the identification. The speed of identification into 30 classes was 0.8 s without any optimization. The time needed for identification of one individual in the out-of-water scenario depend mainly on the time of fish imaging. The time is from 3–10 min including fish immobilization. The HOG-based approach is general for any species with the visible pattern. Therefore, the method can be used also for laboratory and wild species. In general, the more structured (strong edges or isolated dot) pattern on the fish body the better results of the identification. The method can be also used for field experiments because the only condition of successful identification is to take good quality pictures of the fish.

The comparison of the results of the study with the state-of-the-art is complicated because of low number of studies in this field. The standard method used for fish identification is tagging. The accuracy of the developed approach and tagging is the same for 30 fish for the period of 6 months and for 328 fish for short-term identification. The main advantage of the dot approach is the non-invasiveness. The main disadvantage is that pictures of the fish have to be taken. Once the image-based identification is implemented in the tank/sea cage without the need for catching the fish, the advantage of the approach will be much higher. All studies of individual identification based on the skin pattern use low numbers of fish, are performed manually and use images of the fish out of water. The only study where a fully automatic approach was developed is that of Al-Jubouri¹⁹. They used a total of 50 images (10 per fish) of five zebrafish in an aquarium under controlled illumination to perform short-term identification. The reported accuracy was 99%. The dot based short-term identification of 328 individuals using aquarium data introduced in this study has a higher number of fish, greater variability in the data collection conditions and better identification accuracy. For the long-term identification, there is no study reporting automatic fish individual identification.

Future research will be focused on the implementation of image-based individual identification under real aquaculture production conditions to harness the potential of the approach. The possibility of identifying the fish without visible skin patterns will be tested to cover more species important in aquaculture.

Conclusion

In this paper, we tested the possibility of short- and long-term automatic fish individual identification based on skin pattern for Atlantic salmon. We developed a new fully automated approach for dot-based individual identification, with an accuracy of 100% the short-term identification of for 328 individuals and 100% for long-term (6 months) identification of 30 fish. The approach was tested for fish out of the water and fish in the aquarium to approximate real conditions. The approach can be used as a substitute for identification with invasive tagging. It can be used for any species with the dot pattern. The more general HOG-based identification was also tested and shown to have lower accuracy. The HOG-based identification can be used for fish species with patterns different from dots. Future work will be focused on the adaptation of the approach to tank/cage real conditions. Image-based fish identification under real aquaculture conditions could open new possibilities for fish maintenance and treatment.

Data availability

Data will be made available upon request through our data management system bioWES.

Code availability

The source code for Matlab environment for dot-based fish identification is available upon request.

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Author contributions

Corresponding Author: P.C.: conceptualization, methodology, supervision, manuscript writing. D. B.: data processing, manuscript editing. R. S.: data collection, M. S.: data collection, experimental design, manuscript editing. O. M.: CNN data processing, visualization.

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Competing interests

The authors declare no competing interests.

Additional information

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CHAPTER 4

IMAGE-BASED AUTOMATIC INDIVIDUAL IDENTIFICATION OF FISH WITHOUT OBVIOUS PATTERNS ON THE BODY (SCALE PATTERN)

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My contribution to this work was about 85%.

Article

Image-Based Automatic Individual Identification of Fish without Obvious Patterns on the Body (Scale Pattern)

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Abstract: The precision fish farming concept has been widely investigated in research and is highly desirable in aquaculture as it creates opportunities for precisely controlling and monitoring fish cultivation processes and increasing fish welfare. The automatic identification of individual fish could be one of the keys to enabling individual fish treatment. In a previous study, we already demonstrated that the visible patterns on a fish's body can be used for the non-invasive individual identification of fishes from the same species (with obvious skin patterns, such as salmonids) over long-term periods. The aim of this study was to verify the possibility of using fully-automatic non-invasive photo-identification of individual fish based on natural marks on the fish's body without any obvious skin patterns. This approach is an alternative to stressful invasive tagging and marking techniques. Scale patterns on the body and operculum, as well as lateral line shapes, were used as discriminative features for the identification of individuals in a closed group of fish. We used two fish species: the European seabass *Dicentrarchus labrax* and the common carp *Cyprinus carpio*. The identification method was tested on four experimental data sets for each fish species: two separate short-term data sets (pattern variability test) and two long-term data sets (pattern stability test) for European seabass (300 individual fish) and common carp (32 individual fish). The accuracy of classification was 100% for both fish species in both the short-term and long-term experiments. According to these results, the methods used for automatic non-invasive image-based individual-fish identification can also be used for fish species without obvious skin patterns.

Keywords: precision fish farming; machine vision; individual-fish identification; non-invasive identification; scale pattern; intensive aquaculture



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1. Introduction

The use of automatization systems in aquaculture is not novel but is becoming increasingly necessary to reduce human maintenance. The precision fish farming concept [1] is based on automation processes, in which controlled-engineering principles are applied to fish-production processes, which increases fish farmers' abilities to control and monitor all stages of fish cultivation. This process enables farmers to make data-based decisions. The applications of automation in fish cultivation are very broad and include feeding control, fish welfare and disease monitoring, fish sampling, and fish sorting.

Automatization has also been used in many biological research studies. For example, new technologies (camera based system and automatized image processing) were successfully applied to fish behavior and welfare monitoring [2–4]. One main advantage of using such novel technologies is fully automated data processing. For example, Zhou et al. [5] developed an automatic monitoring system for feed consumption to maintain high-quality water parameters for fish welfare. In such studies, it would be beneficial to obtain information on individual fish, to monitor and maintain individual fish instead of fish groups. Individual identification is a broad research area that is related to humans and other animals (e.g., cows and whales) [6,7]. Individual identification has also been applied to fish

with significant differences to other species due to challenging aquaculture conditions. Identification is a standard research approach as it allows one to individualize the fish. Currently, there are very limited options for the real-time identification of individuals under real conditions (in tanks and cages). Solving the issue of real-time individual identification under real-world conditions would open a new area of research for individual fish treatment. Our study was focused on testing if the non-invasive (photo) identification of individual fish without obvious pattern could work, and moreover, on testing the stability of chosen features on a fish body during the cultivation period.

The most widely used method for individual-fish identification is invasive tagging [8]. There are many negative impacts of tagging, such as being traumatic for the fish (as it is an invasive method), increasing mortality and injury, being a time consuming procedure, applicability to limited fish sizes, and the need to catch fish for tagging and identification [9,10]. Modern methods of individual-fish identification based on non-invasive principles, such as photo identification, can minimize all the negative consequences of tagging. Photo-recognition is often used not only as a tool to track morphological changes and behavioral monitoring but also for individual identification [11]. The principle of photo recognition has already been successfully used for fish species identification. Fish species identification methods are very broad, but the main criteria for classification are morphological and meristic features [12,13].

Several species identification systems were developed for use under real conditions and in real time [14–16]. At present, the most popular method for species identification is the deep learning approach [17,18]. This approach has been very successful, but the limitations of this approach include the need for a large number (hundreds/thousands) of training examples.

The identification of individuals of the same species is a more challenging task than the identification of species. Typically, few images of an individual are available, and the similarities within one species are much higher than the similarities between species. Several studies demonstrated that non-invasive photo-based identification of individual fish is possible for different fish species, such as catshark (*Scyliorhinus canicular*) [19], zebrafish (*Danio rerio*) [20], Eurasian perch (*Perca fluviatilis*) [21], common carp (*Cyprinus carpio*) [22], Atlantic salmon (*Salmo salar*) [23], brown trout (*Salmo trutta*) [24], whale shark, white shark, and spotted-edge ray [25]. The stability of the chromatophore and melanophore patterns of individuals has also been studied [23,26]. Identification approaches are based on visible patterns on the fish's body (stripes, dots, iris of the eye). Previous studies were performed with relatively low numbers of individual (maximum 30) but demonstrated that skin and eye patterns are unique and can be used for identification. It was also confirmed that these patterns are stable for reasonable time periods (several months) of fish cultivation. The common factor of these studies is the use of manual or semi-automatic image processing. This means that identification must be performed by human experts, which is time consuming and not practical for automation in commercial aquaculture.

The objective of this research was to test the method of non-invasive individual identification of the fish without a pattern on their body from the images and see if this approach is stable within a certain time.

Therefore, in this study, we developed a fully automatic approach for the machine-vision photo-identification of individual fish and demonstrated this method on the ornamental fish Sumatra Barb [27]. This automatic approach uses the unique position of stripes on a fish's body for identification. This work studied 43 ornamental fish only; therefore, we updated the approach for the commercial species of Atlantic salmon. We [28] developed an automated system for individual-fish identification using a fisheye pattern. The identification accuracy was over 95% for 330 individuals over a short-term period. The long-term stability of the iris pattern was also studied for two, four, and six months of fish cultivation. The identification accuracy for 30 fish varied from 31% to 80%, decreasing with cultivation time. Ultimately, we focused on automatic identification based on the skin dots of Atlantic Salmon within the same fish collection. We [29] developed an approach

combining convolutional neural networks for the detection of skin dots and dot distances, which were used for the identification. The identification accuracy was 100% for 328 fish under short-term cultivation and 100% for 30 fish under long-term cultivation (six months). We demonstrated that the skin patterns should be stable and unique for automatic long-term (six months) individual-fish identification and that this technique can be used as substitute for fish tagging. There are also other commercially important species such as the common carp *Cyprinus carpio* and European seabass *Dicentrarchus labrax* that do not have any obvious skin patterns and do not need individual identification. Some studies showed that the pattern of the scale position can be used for individual-fish identification. In a previous study [30], a rhombic squamation pattern was used to identify two genera of fish. The best results of the MANCOVA test ($p = 0.079$) were effective in detecting differences in the rhombic lamination patterns of scale between species, and cross-validated quadratic discriminant analyses (DAs) provided values of 75.8% ($p = 0.001$) based on the shape and 75.8% based on the form. Huntingford et al. [22] demonstrated the possibility of identifying individual common carp using the scale position. Both studies were conducted manually by humans. The aim of this paper is to show that automatic photo-based identification is possible based on the patterns of the scale position without any other distinctive features.

Organization of the paper follows the chapters: Introduction; materials and methods (which consists of the information about experimental animals, design of experiments, used methods and setups); results; discussion; and conclusions, limitations, and future research.

2. Materials and Methods

2.1. Experimental Animal

Two important and economically beneficial commercial fish species were used in this study: European seabass, as representative of marine aquaculture; and common carp, representing freshwater aquaculture.

2.1.1. European Seabass

Data were collected at the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR) in Heraklion, Greece. In total, 300 sea bass were used during the experiment. The initial fish size was 70–90 g for approximately one-year old fish. Three-hundred fish were used for testing short-term identification (short-term experiment (ST)). Thirty-two individual fish were selected from among the 300 fish and tagged with PIT tags (Trovan, 2×11.5 mm, ISO 11784/85 FDX-B) for the long-term experiment (LT) to test the stability of the chosen patterns for individual identification during the cultivation period. The 32 fish were moved to a separate tank (500 l) for two months and kept under standard cultivation conditions.

2.1.2. Common Carp

Fish for the experiment were cultivated in our laboratory at the Institute of Complex systems in Nove Hradky, Czech Republic, in a recirculation aquaculture system (RAS). Thirty-two individual fish of common carp were used during our study. The initial fish size was 347 ± 126 g. The age of the carp was approximately two years. All 32 fish were tagged by the same PIT tags used for sea bass. The fish were cultivated under standard cultivation conditions for four months in the RAS system.

2.2. Experimental Setup and Data Sets

Two rounds of data collection were performed during the two months of the seabass identification experiment. Images were taken of the whole fish out of water (lateral view of the left fish side, Figure 1). Each fish was caught in the cultivation tank, anesthetized in a bucket using Phenoxyethanol, and moved to a green background (Figure 1) for imaging. Natural light conditions were used during data collection. Ten images of each individual were taken using a Nikon D90 digital camera for each round of data collection. Each fish was moved and rotated on the green background to simulate different object-camera

positions and angles. The fish head was oriented to the left for all images. There was no obvious pattern on the fish (both sea bass and carp). The main pattern was a lateral line for seabass. For some fish, this line was interrupted (Figure 1, top). Some fish were also scratched on the body. These scratches were visible as lines on the body (Figure 1, middle). Parts of the tail and fins were sometimes missing due to fish cannibalism (Figure 1, bottom). Pictures were saved in the RAW format. The manual camera mode was used to control the focus, shutter, and ISO. The resolution of each picture was 4288×2848 pixels, with 12 bits/pixel and 3 color channels.



Figure 1. Examples of seabass images. (Top) example of the interrupted lateral line. (Middle) example of a fish with scratches visible on its body. (Bottom) example of a missing upper-tail part.

In the first round of data collection, all 300 fish were photographed, and this set was labelled as the short-term seabass (STS) database. The 32 randomly-selected fish were PIT-tagged and separated into RAS. The rest of the fish were returned to the tanks. All fish recovered after the experiment.

After two months, the second round of data collection was performed with 32 fish. The procedure was the same as that for the short-term experiment (STS).

Common Carp

Four rounds of data collection were performed over the four-month experiment for carp identification. Images were taken of the whole fish out of water (lateral view of the left fish side, Figure 1). Each fish was caught in the cultivation tank, anesthetized in a bucket using clove oil, and moved to the green background (Figure 2) for imaging. A single-layer white-fabric photographic tent with controlled illumination was used for photography. The fish heads were oriented to the left for all images.



Figure 2. (Left image) seabass data collection design. Digital camera photographing the lateral view of the fish. Image of the seabass (middle, bottom) and carp (middle, top) on the uniform green background. This image was used for automatic fish localization. (Right image) experimental design for carp imaging using a tent with controlled lighting conditions.

2.3. Data Processing: Identification Procedure

Automatic data processing consisted of three steps: fish detection, region of interest (ROI) selection, and feature extraction to describe the skin patterns of the ROI (Figure 3). The image-processing methods implemented in MATLAB R2020b were used for data processing. First, the images were converted from the RAW format to the PNG format.

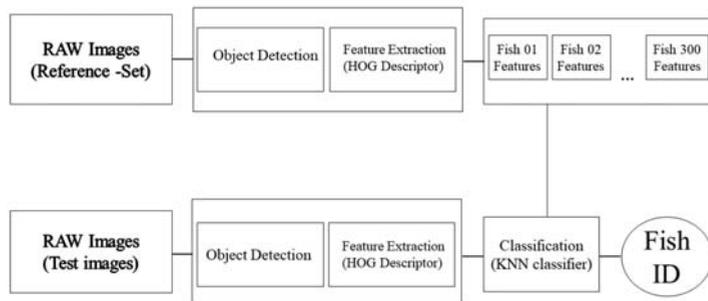


Figure 3. Identification scheme.

2.3.1. Fish Detection and Feature Extraction

The detection of the fish body was based on subtracting the background (green) followed by detecting the object (the largest) inside the area of the background. The area of the background was detected as an object with a known color based on the hue, saturation, value (HSV) representation of the image [31]. The hue and saturation channels were used for segmentation. The rest of the pixels belonged to the fish. Then, size-based noise filtration was used to remove the pixels that did not represent the fish. The morphological close operation (the MATLAB R2020b function `imclose`) was applied to the filtered image to connect the fish body parts that could be divided during the segmentation procedure. The output image was represented as a binary mask of the fish object. Fish body rotation was estimated via the MATLAB R2020b function `regionprops` ('Orientation') to align the fish horizontally. The final fish mask contained the full fish body (Figure 4, left images). The mask was used for localization of the fish in the original color image.

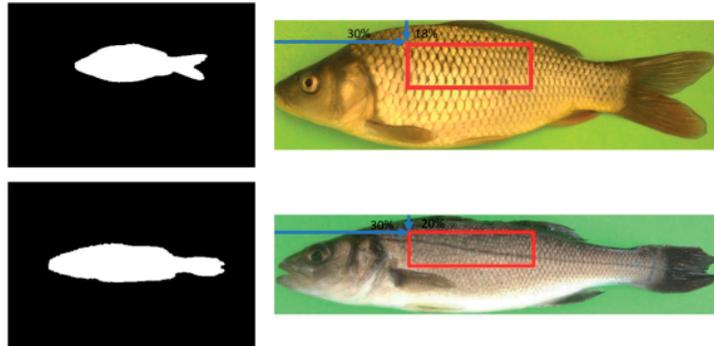


Figure 4. Identification scheme. (Left images), fish detected in the original image (black and white mask) what is the output of the fish detection procedure; (Right images), ROI (ROI(LL) for seabass (bottom image) and ROI(I) for carp (upper image) localized in the fish bounding box by the defined percentage of fish width and height.

The height and width of the fish object bounding box was then used for specific ROI localization. Each ROI was defined as a rectangle with a relative location to the fish bounding box to eliminate scaling and fish growth. Examples of ROI localization are shown in Figure 4.

2.3.2. European Seabass ROIs

Four different ROIs (different parts of the body) were used to identify seabass individuals (Figure 5). ROI(LL) refers to the part of the fish body covering the lateral line and some parts of the body with scales in the top-left corner, encompassing 30% of the fish's length (FL) from the left border and 20% of the fish's height (FH) from the top border (see Figure 4). The right bottom corner was 59% of the FL from the left border and 53% of the FH from the top border. ROI(O) refers to the region of the body that covers the operculum. The top-left corner was 19% of the FL from the left border and 24% of the FH from the top border. The right bottom corner was 34% of the FL from the left border and 52% of the FH from the top border. ROI(HLL) was half of the ROI(LL). The top-left corner was 40% of the FL from the left border and 20% of the FH from the top border. The right bottom corner was 50% of the FL from the left border and 53% of the FH from the top border. ROI(S) covers only scales under the lateral line. The top-left corner was 41% of the FL from the left border and 48% of the FH from the top border. The right bottom corner was 51% of the FL from the left border and 67% of the FH from the top border.

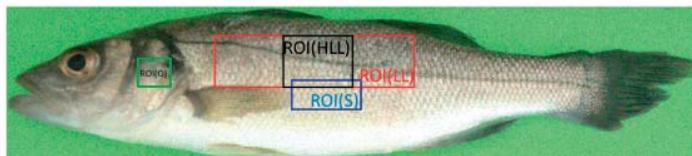


Figure 5. Different seabass ROIs that were used for identification.

2.3.3. Common Carp ROIs

For carp identification, we used three different ROIs (Figure 6). ROI(I) covers the body of the fish from the side (only the scales). The top-left corner is 30% of the fish's length (FL) from the left border and 18% of the fish's height (FH) from the top border (see Figure 4).

The right-bottom corner is 58% of the FL from the left border and 52% of the FH from the top border. ROI2 refers to the same part of the body but longer and wider. The top-left corner is 27% of the fish's length (FL) from the left border and 23% of the fish's height (FH) from the top border. The right-bottom corner is 60% of the FL from the left border and 62% of the FH from the top border. ROI3 is the operculum part of the carp. The top-left corner is 12% of the fish's length (FL) from the left border and 43% of the fish's height (FH) from the top border. The right-bottom corner is 20% of the FL from the left border and 61% of the FH from the top border.

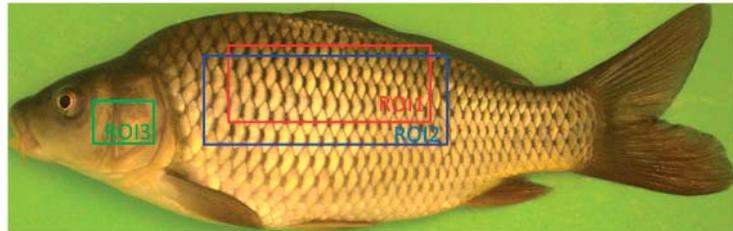


Figure 6. Position of ROIs for carp identification.

All the ROIs were converted to feature vectors using the histogram of oriented gradients (HOG) descriptor [32], which offered the best accuracy in our previous experiments (Bekkozhaeva, 2021). In general, the descriptor codes the edges in the image. All ROIs were transformed into rectangles of different sizes (64×64 , 128×128 , 64×128 , and 128×64) to facilitate efficient calculation of the HOG and achieve image-scale compensation. The best results were achieved with a size of 64×64 pixels for all combinations, except ROI2 for carp when using the 01 * 02 combination, where the size was 64×128 . Two different settings of HOG were tested (cell sizes of two and four). The best results were achieved with a cell size of two for the majority of combinations, but some of them provided better accuracy with a cell size of four. The ROIs for carp data were as follows: ROI1, combinations 01 * 03 and 01 * 04; and ROI2, combinations 01 * 04, 03 * 04. Due to possible errors in localizing the ROIs on the fish body, the scanning approach was used to find the best match for the two ROI subsets. Only the subset of ROI without a border area was used for similarity measurements. Different sizes of borders (10, 16, 20, and 30 pixels) were tested. The best results were achieved with a border size of 10 pixels. The similarity between the two ROIs was measured using the similarity of the HOG features. HOG1 was calculated from the subset of the first ROI1 (reference fish). The subset was ROI1 without the borders of size, Off_x and Off_y (see Figure 7).

HOG2 was calculated from a subset of ROI2 (unknown fish). This subset is defined by window scanning over ROI2. The similarity between HOG1 and all HOG2 feature vectors was calculated as the Euclidean length of the difference between the two HOG vectors. The minimal value of the similarity was then used along with the similarity between ROI1 and ROI2.

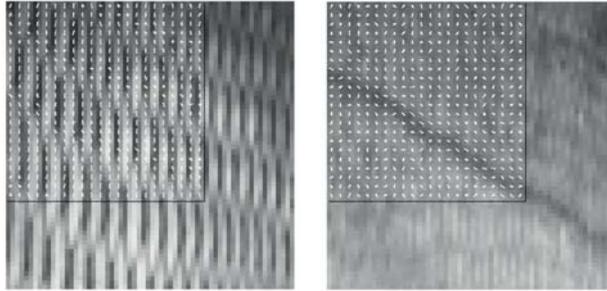


Figure 7. HOG visualization for ROIs. The left image is the ROI1 for carp resized to 64 by 64 pixels, where the white holes are the scales of the fish, and black lines are the connecting parts of the scales. The right image represents the ROI(LL) for seabass resized to 64 by 64 pixels, where the black line is the lateral line. The rectangle in the left-top corner of both images visualizes the HOG gradients used for pattern parametrization. The gradients are tiny white lines, which correspond to the orientation of the edges in the image. For common carp it follows the shape of the scale and for the seabass it follows the lateral line.

2.3.4. Identification Procedure

The fish identification task was performed for classification in the closed group (with a known number of individuals), where the number of classes corresponded to the number of individual fish in each experiment (300 sea bass and 32 carp in the short-term scenario and 32 sea bass and 32 carp fish in the long-term scenario). Classification was performed separately for both the short-term and long-term data sets. The nearest-neighbor classifier was used to classify the fish, while the similarity between ROIs was used as the measure.

Short-term experiment: Each fish was represented by 6 to 11 ROIs of the images from one data set. One randomly selected ROI was used as the unknown fish, and the rest were used as representative ROIs (database) of the fish. The unknown ROI of each fish was compared with all representative images of all fish in the database. The unknown fish was classified as the most similar fish in the database. The same process was applied to each data set separately. In total, 300 seabass and 32 carp (classes) were used.

Long-term experiment: Each fish was represented by 6 to 11 ROIs of the images from one data set. Images of one fish from the first data set were compared with images of all fish from the second data set. This process was repeated for all fish from the first data set. For carp, this procedure was repeated for all combinations of the four data sets. Three images of the fish from one data set were used as the reference set, and three images of the fish from the other data set were used as the test images. The process used the same similarity measure used for the short-term data set. A test of pattern uniqueness was conducted for the first and second data sets separately for the seabass, as well as for the first, second, third, and fourth data sets, using the same approach described for the short-term classification of 32 fish (32 seabass and 32 carp classes, separately). The results of the classification were confusion matrices describing the similarity between all combinations of individual fish.

Manual (human-based) identification for the long-term experiment involved exploring the skin patterns via human observation. Five randomly selected fish were chosen for identification. One image of each fish from the first data set and one image of each fish from the last data set were introduced to two people. Both individuals performed the identification independently.

3. Results

3.1. Seabass Identification Results

Short-term (ST) (300 fish) and long-term (LT) (32 fish in two data sets) experiments were performed to identify seabass individuals. The results of the identification are presented in Table 1.

Table 1. Short- and long-term experiment results for seabass identification.

Region of Interest	Accuracy for ST (300 Fish)	Accuracy for LT (32 Fish)
ROI (LL)	100%	100%
ROI (O)	91.66%	40.62%
ROI (HLL)	98.66%	96.87%
ROI (S)	98.66%	93.75%

According to the results in Table 1, the best accuracy was observed for ROI (LL), which presented 100% accuracy for both experiments (short-term and long-term). ROI (HLL) and the ROI (S) had the same accuracy (98.66%) for the short-term experiment, but ROI (HLL) had higher accuracy in the long-term experiment (96.87% compared to 93.75% for ROI (S)). The lowest accuracy was obtained for ROI (O). In the short-term experiment, the accuracy for the operculum part was 91.66% (ROI (O)) and in the long-term experiment results, the accuracy was 40.62%.

The results of manual identification (human-based) were 100% accurate for both experiments (long- and short-term).

3.2. Common Carp Identification Results

Results of individual carp identification are presented in Table 2.

Table 2. Results of identification for the short- and long-term experiments.

Data Collection	01			02			03			04		
	ROI1	ROI2	ROI3	ROI1	ROI2	ROI3	ROI1	ROI2	ROI3	ROI1	ROI2	ROI3
01	100	100	100	80.64	80.64	77.41	100	96.77	64.51	90.32	83.87	32.25
02				100	100	100	80.64	80.64	67.74	70.41	80.64	29.03
03							100	100	100	100	100	87.09
04										100	100	100

For the one-day data set (short-term experiment), the accuracy was 100% for all data sets and all three ROIs, which means that each fish featured unique scale formations and could be identified as an individual between at least 32 individual fish.

For the long-term experiment, identification between the first and second data sets resulted in 80.64% accuracy for ROI1 and ROI2 and 77.41% accuracy for ROI3. Combined, the first and the third data sets presented 100% accuracy for ROI1, 96.77% accuracy for ROI2, and 64.51% for ROI3. Identification with the first and fourth data sets provided 90.32%, 83.87%, and 32.25% accuracy for ROI1, ROI2, and ROI3, respectively. Identification using the second and third data sets provided 80.64% accuracy for ROI1 and ROI2 and 67.74% accuracy for ROI3. The second data and fourth data sets presented 70.41% accuracy for ROI1, 80.64% for ROI2, and 29.03% for ROI3 (the lowest accuracy was observed for ROI3 (and all ROIs) among all tested combinations). The identification accuracy for the third and fourth data sets was 100% for ROI1 and ROI2 and 87.09% for ROI3.

The best accuracy among the defined ROIs was observed for ROI1 and ROI2, which covered the largest part of the body containing the scales. ROI3 (operculum) provided lower accuracy for all combination between the four data sets. A comparison of the results for

ROI1 and ROI2 showed that ROIs with scale patterns offered the best accuracy. Moreover, based on the results, no large difference was observed based on the part of the selected region, as ROI1 and ROI2 offered almost the same accuracy (90.32% vs. 83.87, 70.41% vs. 80.64%) for all corresponding combinations among the four data sets.

The method was implemented in Matlab and tested on HP Pro book laptop (Intel (R) Core (TM) i5-6300U CPU @ 2.40 GHz, 8 GB RAM). The processing time of identification of one fish is 1.5 s on the dataset of 300 fish individuals. The HOG features for each fish in the database were precomputed to speed up the process of identification.

4. Discussion

The aim of this study was to test if the image-based individual-fish-identification approach could also be used for fish without obvious patterns on the body. Obvious body patterns include dot patterns on salmonids and stripe patterns on Sumatra Barb [27,29]. For this study, we selected common carp and sea bass as representative fish without obvious patterns. We tested different parts of the fish body to identify individual fish within close groups of fish, to explore which patterns (part of the body) offer the best classification power and sufficient stability over time for use in long-term identification.

4.1. Seabass

Seabass identification obtained 100% accuracy for both short-term identification and long-term identification, which means that the patterns on the body enabled individual identification of this species. In the event that there is no obvious pattern on a seabass's body, the shape of the lateral line on the fish together with the texture of the fish's scales should be sufficiently unique. ROI (LL) provided the best classification accuracy. Here, the lateral line itself had strong classification power, and the size of the region was the largest among all chosen regions (Figure 8).

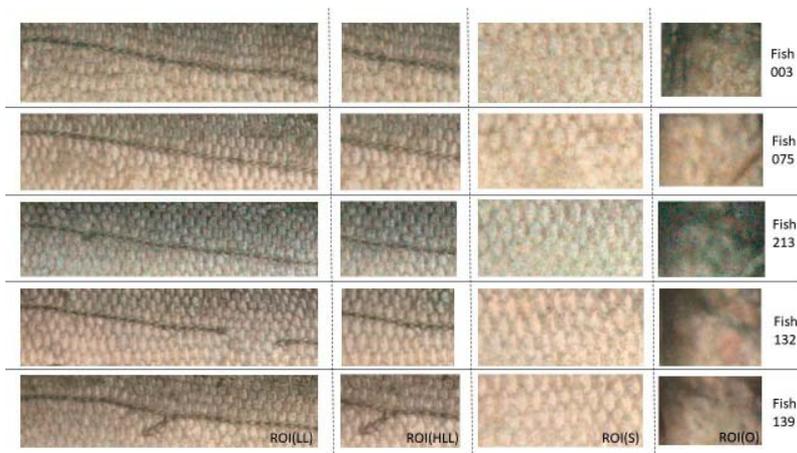


Figure 8. Differences in ROIs for different fish (seabass).

The best results were obtained using ROI(LL), where the region of the fish body covered the lateral line and offered high classification power. ROI (HLL) also had high accuracy (98.66%). This region covered the middle part (1/3) of the lateral line of ROI (LL). The accuracy, however, decreased for both the short- and long-term experiments. The most probable reason for this decrease is a lack of detail. The lateral line's unique shape was confirmed by the human-based identification, where the two observers were able to

correctly identify five fish using the shape of the lateral line. We obtained lower accuracy of 98.66% for short-term identification and 93.75% for long-term identification under ROI (S). This region covered only the part of fish body (with scales and without the lateral line). The accuracy was still high enough to use this method for identification. We thus proved that seabass scale formation is unique and stable over at least a two-month period. ROI (O) covered the operculum part and provided the lowest identification accuracy (91.66% for the short-term and 40.62% for the long-term experiments). The operculum patterns also differed among the fish, but the long-term stability was very low. A manual assessment of operculum patterns confirmed this observation. The operculum pattern thus has smaller classification power than the lateral line and scales. The best accuracy was offered by ROI (LL), which covered the largest part of the lateral line and scales among all the ROIs. ROI (LL) was the maximum possible size that could be effectively used for identification. The ROI could not be extended to the tail part as the tail is too flexible and changes shape during swimming. Furthermore, the part of the fish close to the head could not be used as it is often covered by the pectoral fin. The size of the operculum (ROI (O)) is likely too small to provide sufficient detail for correct identification and not stable enough for the long-term identification of individuals. The identification results reveal that the chosen region of interest clearly influences the accuracy of identification. The size of the chosen region was also found to be important.

Using the shape of the lateral line for identification is not novel. Sfakianakis et al. [33] used the shape of the lateral line and scale shape to identify two different species (among wild and farmed sea bass and gilthead sea bream). The scale patterns were also previously used to identify species [34]. We likewise demonstrated that the shape of the lateral line and formation of scales are useful for identifying individuals in close groups of fish (sea cage, tank) of the same species.

As shown by the experimental data (Figure 8), the scales on the lateral line regenerate differently to the original scales. Some scales grow, while other scales attempt to compensate for the hole lacking scales by distributing themselves around the nearest area. Scales of the lateral line generally do not regenerate and are different to those on other parts of the body [35].

4.2. Carp

The short-term identification for carp was 100% accurate under all three ROIs. The accuracy of long-term identification varied from 100% to 29% depending on the ROI and combination of data sets. The best long-term identification accuracy was higher than 80% for all combinations of data sets and ROI2.

The accuracy of the combinations of data sets 01 and 02 was lower (80.64%) than that for the combination of 01 and 03 (100%), and the accuracy for the combination of 01 and 04 was higher (90%) than that for 02 and 04 (70%) (for ROI1). We expected that the identification accuracy would decrease with an increase in time between data collections, corresponding to the changes in the scale patterns influencing identification accuracy. The results, however, corresponded more strongly to issues with the data collection itself and localization of the ROI on the fish's body. This method was able to 100% correctly identify fish between sessions with a delay of two months, but only 80% accurately for one-month delays.

The identification accuracy of ROI3 (operculum) clearly decreased from approximately 80% for one-month-delayed data sets to approximately 30% for three-month-delayed data sets. These results correspond to the changes in the operculum area from the perspective of texture-feature extraction. The contrast in the edges of ROI3 was much lower than the contrast in the edges for the scales of ROI1 and ROI 2, which is likely the reason for the decrease in accuracy.

Based on the results of the experiments with both seabass and carp, the accuracy of the method is strongly dependent on many factors, such as the type of the scale (ctenoid or cycloid), the scale from which the ROI is extracted, the localization of the ROI, the extracted

and chosen features, the sizes of the ROIs, and the conditions of the experiment, which all impact the quality of the data.

There are three main aspects influencing individual-fish identification: the scale pattern itself, the proper localization of the ROI, and the image quality.

The scale pattern is affected by the geographic location of the fish [36], which means that the conditions of fish cultivation (abiotic factors such as temperature, light conditions, etc.) also affect scale appearance and skin development. The recovery of lost scales influences identification as the growth of a scale begins not in the scale's center, but closer to its edge [37].

The individual identification of fish based on scales has not been well studied. Scales of fish have been used to identify species since the 1900s [38]. In subsequent years, scale-based identification of fish has become popular not only for species identification but also for population identification [12,39]. Furthermore, descriptors for the shape of the scale have been developed [40,41]. Based on this concept, Ibañez et al. [39] developed geometric morphometric methods (GMMs) to identify two Mugilidae species using their scales. Another study [42] tested the identification of larvae from two species based on melanophore patterns. The authors examined the distribution patterns of melanophores in various larvae of bullet tuna (*Auxis rochei*) and frigate tuna (*Auxis thazard*). Bräger [43] tested the landmark-based geometric morphometric method to identify two species from mixed samples. Only one study [22] explored the identification of individuals of one species without obvious patterns. The authors used five common carps for individual identification by human experts and obtained 100% accuracy for the identification of five individuals. Our approach shows that fully automated identification is possible for a high number of fish (300 seabass and 32 carp). For seabass, long-term image-based identification was found to be possible with 100% accuracy and could thus serve as a substitute for invasive fish tagging. For common carp, short-term identification is possible (100% accuracy), but long-term identification (approximately 80%) cannot provide accurate identification. In a future study, we intend to improve this approach for carp as it is obvious from the data set that carp scales have unique patterns.

Another approach used to identify fish species is deep learning. There is a first attempt for this task by Pedersen [24]. Brown trout (a commercially important fish species) was used in this study. Their method was able to identify individual fish with 94.6% precision and 7.6% recall. However, the limitation of the study is the low number of individuals (39 individuals). Brown trout is the species with a clearly visible dot pattern on the body. Furthermore, the identification was conducted only for short term data. No experiments with the long-term data were performed. The deep learning based methods are promising, especially in the task of fish detection combined with identification. Villon et al. [18] explored the fish identification of coral reef fish species using HOG features combined with support vector machine (SVM) and deep learning. The results for deep learning ($T = 98\%$) were more robust than those for the HOG and SVM approaches (below 49%). In 2018, Villon et al. [17] used the deep learning method to achieve accurate and fast identification of coral reef fish (20 fish sp.) in underwater images. The rate of correct identification was 94.9%, which was greater than the rate of correct human-based identification (89.3%). Yusup et al. [44] used the deep learning method for real-time reef-fish identification (24 species of reef fish), with the highest percentage of detection accuracy observed for *Holacanthus tricolor* (90.70%).

However, all present studies focused on species identification did not include long-term identification. No study which deals with the identification of fish without an obvious pattern on the fish body was published and there is no study presenting real-time identification of the fish without an obvious pattern on the body.

Our study used 300 individual fish for identification in the short-term experiment. The number of fish in the cultivation tank varied from tens to thousands of individuals. The accuracy of 100%, which was achieved for both species, shows that the studied patterns are unique and could be used for a larger number of individual fish. However, the exact limits of the patterns among seabass and carp must be studied in a larger sample to confirm this

observation. The long-term experiment covered a four-month cultivation period for carp and a two-month period for seabass. The standard cultivation periods for both species, however, are longer. No significant distortions in the lateral lines or scale patterns were observed for seabass; we expect that only scale loss or bodily injury would affect long-term identification accuracy. For the long-term identification of carp, the main issue was the data collection procedure and the localization of the ROI. Carp have larger scales, meaning that scale loss can more strongly affect identification accuracy for carp. Larger (mature) fish were used in our experiment when a stable scale pattern was expected. Continuous identification from larva to maturity could be problematic due to development of the fish and changes in the scale patterns. To solve this problem, the identification process could be divided into the periods of fish cultivation with stable short-term patterns. The main limitation of the present approach is that each fish was photographed out of water under anesthesia. As we demonstrated in a previous paper [27], underwater imaging of non-anesthetized fish is also possible for image-based identification. Studying the application of this method to identification under real conditions is our future research goal.

In the future, individual identification based on fish scale patterns could be used for individualized fish treatment. Such a system could continuously identify individuals in the tank/sea cage swimming around the camera and provide information for other systems to ensure fish welfare, growth, and disease analysis. In this way, we could continuously monitor changes in the states of individual fish. Continuously monitoring and updating the images of the fish would eliminate the problems of changes in each fish's appearance. Individual identification is currently used mainly for research purposes, where the number of individual fish is low. The future potential of individual identification lies in the automation of intensive commercial fish production. Such an application is presently impossible, but the market continues to seek solutions for precision fish farming.

The computational time (1.5 s per fish) of the approach is sufficient for real-time processing in the sea cages or fish tank as the detection rate of the fish will be low (one fish per 10–30 s in the case of high fish density). The identification procedure needs to work with the image of the fish almost perpendicular to the camera plane and therefore the time between fish detection will be longer than the identification itself.

Our future plan is to use this approach and apply to the real time condition. The next step is to conduct the fish detection and identification under real time conditions for the tank monitoring.

This method could be immediately used for research purposes as a substitute for invasive fish tagging. Standard fish sampling includes implantation of the tag into the fish and reading this tag each time a fish is sampled to identify the fish. The present non-invasive approach is instead performed out of the water under controlled conditions.

5. Conclusions, Limitations, and Future Research

The present study demonstrated that the identification of fish individuals without obvious patterns on the body from the same species and within a close group (i.e., a tank, cage, or aquarium—in our case, in a tank with a limited number of fish) can be done fully automatically based on images of the fish using image processing methods. We tested our approach on the commercially important fish species of seabass and common carp. Accuracy of 100% was obtained for the short-term identification of both species, and 100% and 80% accuracy were obtained for the long-term identification of seabass and carp, respectively. Different parts of the body, mainly parts featuring scales and the patterns on the operculum of the fish, were tested for identification. The study showed that the chosen patterns can be used for long-term identification, except for patterns on the operculum. The uniqueness of scale patterns was proven, together with their stability during the cultivation period (in our case, the longest period was four months). Our approach reveals that even a fish without obvious patterns on its body (only scale body) could be used for the automatic non-invasive identification of individual fish. Photo identification is thus a possible substitute for commonly used invasive fish tagging identification methods.

The approach we have used has several limitations that need to be solved in future studies. Such limitations include the position of the ROI, and to cut the ROI precisely enough so as to get the best identification accuracy is still challenging. We tested a maximum of 300 fish individuals, whereas in the tank, the number of fish is much higher. The present approach was tested on two different fish species to demonstrate the generalizability of the method. This approach could be used for the real-time identification of individuals under real conditions in a tank/sea cage, which could be helpful in precision fish farming for controlling and documenting the fish-growth process. Studying this application will be the next step in our complex research. A future study is to test this approach to the real time monitoring of the individual fish in tank, and increase the number of the fish individuals to a thousand.

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CHAPTER 5

TOWARDS FISH INDIVIDUALITY-BASED AQUACULTURE

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Towards Fish Individuality-Based Aquaculture

Rudolf Schraml , Heinz Hofbauer, Ehsaneddin Jalilian , Dinara Bekkozhayeva, Mohammadmehdi Saberioon , Petr Cisar, and Andreas Uhl 

Abstract—By bringing concepts of precision farming to intensive aquaculture fish production, it can be optimized to be more sustainable while focusing on fish welfare criteria. This requires a shift from mass to smart production and to consider each fish as an individual. Therefore, it is required to be able to identify each fish in a tank or sea cage. In this article, we prove the feasibility of fish identification using the iris as a biometric characteristic. Based on a new dataset, captured in a controlled out of water environment: 1) a fully automated iris recognition system is presented and utilized for the experiments and 2) the distinctiveness and the stability of the iris pattern of Atlantic salmon (*Salmo salar*) is assessed. Results prove the distinctiveness, which indicates that the iris pattern of Atlantic salmon is suited for biometric identification. However, the iris pattern has a low stability, which means it changes over time. Due to frequent interaction of fish and system, usually multiple times a day during feeding, there is ample opportunity to keep the biometric template up-to-date, which makes the lack of long-term stability a nonissue. It can be concluded that a biometric fish identification system is feasible, with the precondition that biometric templates of each fish are periodically updated to combat the low stability.

Index Terms—Fish iris identification, precision fish farming (PFF).

I. INTRODUCTION

THE PRODUCTION requirement of aquaculture in the last 30 years has risen steeply and continues to do so. The edible fish consumption per capita is rising and outpaces the naturally occurring fish population, making this consumption sustainable only through aquaculture production. This trend will not decline and aquaculture production plays a crucial

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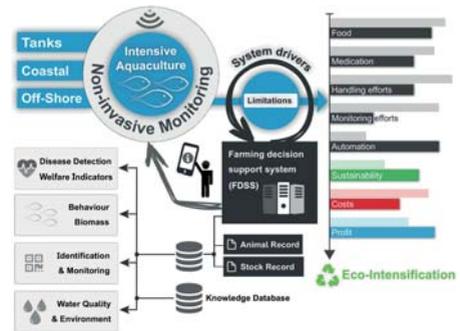


Fig. 1. Farming decision support system (FDSS).

role to ensure sustainable development in economic, social, and environmental terms [1].

For intensive aquaculture, the fish is cultivated in tanks or sea cages. An increase in production can often only be achieved through a higher density of fish. This exacerbates problems in the management of disease and health of the fish. Optimization of fish production, therefore, also requires an improvement of fish welfare. Toward precision fish farming (PFF) control-engineering principles are applied to fish production, thereby improving the farmer's ability to monitor, control, and document biological processes [2]. The move from mass to smart production allows application of control-engineering principles to individual fish instead of the population as a whole. It is all about data, which are collected, analyzed, and exchanged almost in real time, allowing for medication or removal of individual fish as well as the optimization of yield per fish. Smart production requires that data are assigned or linked to a set of objects or single (living) objects in the production. Data and information enable to improve and/or completely rethink well-established processes.

Further, regarding intensive aquaculture considering each fish as an individual, requires noninvasive monitoring to set up a farming decision support system (FDSS). This type of smart fish farming as envisioned by a FDSS relies on the identification of individual fish. Fig. 1 illustrates our vision for such a system that follows the paradigm of ecological intensification. This system enables to assign information about fish traits such length, weight, sex and maturity, and fish skin color during different growth stages to the corresponding animal or stock record, to monitor growth status for better management [3]. Common ways for individual identification of fish are invasive

methods relying on tagging and marking [4]. Invasive methods may cause technical as well as health and animal behavioral problems amplifying a problem we want to solve. Even currently available noninvasive approaches (e.g., external colorants) may cause behavioral alteration and pose health risks, which require to take care of welfare issues [5]. Furthermore, invasive identification is time consuming and incurs a substantial cost. *To avoid these problems* and additional cost, it would be optimal to be able to have a noninvasive and contact free identification method.

For this article, and the envisioned FDSS, the focus is on noninvasive fish identification using biometric characteristics of the fish body. Specifically, we will evaluate the suitability of the iris for this purpose, since it is always visible (due to lack of eyelids), permanent (as opposed to skin patterns e.g., [6]) and has a good track record for humans and other animals (e.g., for cow identification [7]).

The rest of this article is organized as follows. First, in Section II, a review on related work is presented, followed by the main contributions of this article. Section III introduces the computation and matching of fish iris codes (FICs). The experimental setup and evaluation are presented in Section IV, and finally, Section V concludes this article.

II. RELATED WORK AND CONTRIBUTIONS

Literature on fish identification can be categorized based on 1) the direction from which the fish and the biometric characteristic is captured: lateral, dorsal, or ventral and 2) based on the utilized feature extraction/matching approach, e.g., skin pattern or shape features. Although there exists plenty of research, only a few approaches make use of machine vision methods.

In the works of [8]–[10], the identification of different fish species was examined on the basis of lateral images. The regions, utilized for biometric feature extraction, were selected manually. For Patagonia catfish identification in [8] skin pattern spots were marked manually (position and size) and three reference points set the region of interest (ROI). For 45 fish, which were captured 14 times for 254 days, a Rank-1 identification accuracy of 96% was reported. Similarly, for Atlantic salmon identification in [9] spots were marked manually and utilized for a specific matching algorithm, requiring at least three spots. At the age of 12 months most fish showed less than three spots and 17 out of the 20 remaining fish were identified correctly. For lionfish identification in [10], three different ROIs were selected in which speeded up robust features (SURF) keypoints are detected, computed, and used for matching. For the best body part (flank) and 48 individuals, captured at one point in time, the authors report a Rank-1 identification accuracy of 68%.

In [11], [12] dorsal head view images were assessed as biometric characteristic. For Chinook salmon identification in [11], the ROI was marked manually, the spot pattern was binarized and the spot centroid coordinates were used as biometric features. Results show 100% identification accuracy for fish that developed a pattern, which was only the case for 42% of all fish (=295 fish captured seven times over 251 days). Castillo *et al.* [12] used a reverse image search engine to assess delta smelt identification based on three manually selected ROIs. Fish were captured at three points in time and for the fusion of the two

best areas, an identification rate of 94% for adjacent sessions and 59.2% between the first and the last session was reported.

In [5], naked-eye and computer-assisted identification of armored catfish based on ventral images, captured in laboratory and field conditions, were evaluated. The computer-assisted approach is based on scale invariant feature transform key points. ROIs were selected manually and results for 120 comparisons from the laboratory and 224 comparisons from the field data showed an identification accuracy (Rank-1) of 82.2% and 93.8%, respectively. These prior works have following two major shortcomings.

- 1) Manual annotation of the ROI and/or the utilized biometric information/pattern is required. Such an approach is well-suited for small-scale experiments, but it is not applicable on a large scale, i.e., for intensive aquaculture and the envisioned FDSS. For example, Dala-Corte *et al.* [5] reported that for 225 comparisons, 17 min were required for computer assisted identification.
- 2) Related literature has shown that the skin pattern is not universal; some fish do not form them and are not stable once formed. That is, the assessed skin patterns change over time and some fish showed no pattern at all or only formed them at some later stage of growth. This can even vary for minimal divergence from a base strain of fish; for example, [6] showed that some Zebrafish mutations show no more pattern at all.

Regarding these shortcomings, we will look at iris patterns in Atlantic salmon as member of the Salmonidae family. All members of this family have eyes and are lidless, making the iris a universal trait. The basic layout of the iris biometric toolchain known from human iris biometric identification will be used (and be described later). While this solution sounds reasonable, the following has been evaluated in order to see if the iris is a usable biometric characteristic.

- 1) *Localization and Orientation of the Iris*: To establish fully automated fish identification, it is required to detect the iris region automatically and to rotationally prealign each iris preliminary to feature extraction and matching. Hence, for the Atlantic salmon iris, a segmentation approach is introduced, and a set of rotational prealignment strategies is tested.
- 2) *Stability*: The lifespan of an intensive aquaculture fish is short, but the fish grows rapidly within this timespan. Thus, another contribution of this article is to evaluate the stability of the Atlantic salmon iris pattern, i.e., if and how the pattern changes over time.
- 3) *Automatic Iris Recognition System*: In contrast to other works in this field, the evaluation is done using state-of-the-art biometric system evaluation protocols and metrics. Regarding fish iris image processing and biometric identification a fully automated system will be assessed.
- 4) *R³ Research Principles*: Replicability, Reproducibility and Reusability. In order to repeat, improve or develop new methods for fish iris biometry a database is required. Thus, we make public the acquired database of fish iris images (see Section IV-A) including source code and libraries at a GitHub repository.¹

¹[Online]. Available: <https://github.com/rschraml/fishid>

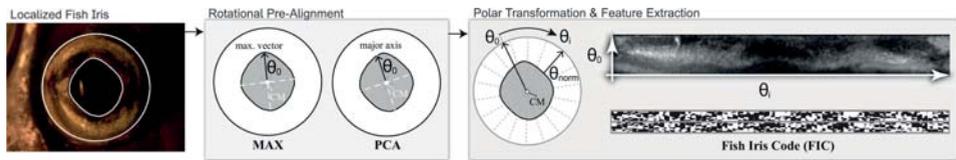


Fig. 2. Illustration of the pipeline to generate the fish iris code (FIC) from a segmented image.

To sum up: Our contribution is a state-of-the-art based fish iris identification system based on a universal trait. However, we note that the main objective of this article is to assess the basic feasibility of such a system and that the experimental evaluation is based on fish iris images acquired in a controlled out of water environment.

III. FISH IRIS CODES

The first step in the biometric toolchain is to acquire an iris image for which a fish iris code (FIC) is computed in four consecutive steps (see Fig. 2): iris segmentation, rotational prealignment, iris normalization, and feature extraction.

A. Fish Iris Anatomy

The anatomy of fish eyes is similar to the human eye anatomy on a basic level. Considering the human eye, we are looking at the stroma, a fibrovascular layer connecting the sphincter (for closing the iris) and dilation (for opening the iris) muscles or the eye. The layer consists of fibers (fibro-), some running in a circular pattern, but mostly radially mixed with nerves and blood vessels (-vascular). In addition to the fibres, the dilation muscle also runs along the radial axis. The formation of the fibres in the stroma is different for individuals and stable over time, which makes it a perfect candidate for biometric recognition of humans. If the stroma contains pigments, it appears dark and the structures are not apparently visible. To counteract this, the human iris is captured with near-infrared cameras where the pigmentation does not interfere with image acquisition.

For fish there are differences pertaining the iris, which are not uniform over classes of fish. Iris of different fish species can differ in terms of muscle, shape, and function, which leads to a noncircular iris pattern, for example. As such the usability of the iris for fish identification has to be judged for different fish classes and species. For salmon, the iris is nonfunctional in that it does not open or close to moderate light, i.e., it does not exhibit a photometric response. Instead, the salmon uses retinomotor movement of photoreceptors and retinal pigmentation to change the light exposure of rods and cones [13], [14]. The iris is well-formed and prominent despite its vestigial function. It is an extension of the epithelial pigment layer of the retina (which is used to moderate illumination) [15]. The pupillary opening shows rounded diamonds shape (see Fig. 2).

B. Fish Iris Segmentation

For iris recognition the pupillary boundary, i.e., between pupil and iris, and the limbic boundary, i.e., between iris and sclera (the

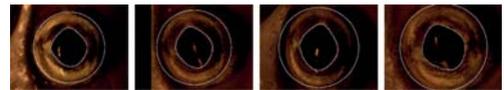


Fig. 3. CNN-based segmentation results for fish #0F571E captured in four time delayed sessions. As shown, the iris is growing significantly from Session 1 to 4, accompanied by changes in the iris pattern.

white of the eye in humans), need to be detected. This allows 1) to segment the ROI containing the biometric information and 2) to polar transform this ROI to an uniform rectangular representation. Traditional human iris segmentation approaches are not well-suited as they often rely on the circular shape of the human iris. For example, we mention the segmentation approaches contrast-adjusted Hough transform (CAHT) [16] and weighted adaptive Hough and ellipsopolar transform (WAHT) [17]. Preliminary experiments using a traditional morphological-based segmentation approach led to poor results, which are not worth to be considered. However, recent research showed segmentation approaches based on convolutional neural networks (CNN), which are well-suited for human iris segmentation. For instance Hofbauer *et al.* [18] showed that a CNN-based semantic segmentation approach outperforms traditional approaches like CAHT in case of low quality databases. Based on this insight, the inapplicability of traditional iris segmentation methods and the insufficient results with the tested morphological approach a CNN-based semantic segmentation approach, requiring groundtruth data, has been envisioned. Thus, for all images in the utilized database the pupil (=inner boundaries shown in Fig. 3) was detected in a semiautomated manner. The black pixels of the pupil where clustered, holes where filled and the boundaries were corrected manually to avoid under/over segmentation. The limbic boundary (=outer boundary) was approximated based on the pupillary boundary. Basically, by a circle the center of which is defined as the pupil center of mass (CM). The radius is $2 \times$ larger as the mean distance between the CM to pupillary boundary vector lengths. The semiautomated estimated pupillary boundary and approximated limbic boundary are supposed to bound the groundtruth for the iris.

CNNs are a multilayered class of artificial neural networks that gained great success in resolving many key computer vision challenges such as visual segmentation. The network architecture we used to segment the fish pupil is identical to the “SegNet-Basic” fully convolutional encoder–decoder network [19]. The network’s encoder architecture is organized in four stocks, containing a set of blocks. Each block comprises a convolutional layer, a batch normalization layer, a ReLU layer,

and a pool layer with kernel size of 2×2 and stride 2. The corresponding decoder architecture, likewise, is organized in four stocks of blocks, whose layers are similar to those of the encoder blocks, except that here each block includes an up-sampling layer. The decoder network ends up to a soft-max layer, which generates the final segmentation map. The network implementation is realized in the Caffe deep-learning framework. As ground-truth data the semiautomated segmented pupils were utilized. In order to perform, the segmentation on all available images in the database and yet keep the training and testing separate, we used the twofold training scheme. In particular, we divided the whole database into two equal parts, and used one part as our testing data and the other one as our training data. Then, we switched the training and testing folds, and so we obtained the pupillary boundary for each iris image in the database. The limbic boundary was approximated in the same way as for the semiautomated segmentation. Exemplary results are shown in Fig. 3.

C. Rotational Prealignment and Polar Transformation

During matching of two FICs rotation compensation can be performed by comparing shifted versions of the FICs. However, the available fish iris data shows exceptionally strong rotational differences between images of the same iris (see Fig. 3). Compensating for such large angular differences is too slow. The goal of rotational prealignment preliminary to feature extraction is to reduce the rotational differences to an extent where they can be compensated in the matching phase without undue loss of speed. For this article two different prealignment strategies (PCA, MAX) have been implemented which are assessed in the experimental evaluation (see Fig. 2). Both strategies rely on the observation that the fish pupil is not circular and thus it is assumed that a prealignment vector (Θ_0) can be determined. For the first strategy, principal component analysis (PCA) is applied to the points of the pupillary area, which leads to two perpendicular eigenvectors giving the major axes of the pupillary. The dominant axis is then used as prealignment vector. For MAX the pupillary boundary is first smoothed with a Gaussian filter and the vector with the maximum CM to pupillary boundary distance is utilized as prealignment vector (Θ_0). In the experiments, it was observed that for both approaches it happens that for iris images captured at different dates the prealignment can lead to 90° flipped versions.

D. Normalized Polar Transformation

Features are extracted from a normalized iris texture. Note that no image enhancement has been applied to the iris texture. The iris is polar transformed using Daugman's rubber-sheet model [20], this is in essence an unrolling of the iris texture, and stretching to a uniform size. This normalization corrects two factors which can lead to a different iris texture area: 1) The distance and angle between the camera and iris can vary, which introduced a scale change and geometric distortion; and 2) as the fish grows, so does the skeletal and soft tissue, including the eye. The polar transformation on the other hand allows for a rotation of the eye to be expressed as a horizontal shift, which is much easier to compute. Such a rotation can happen due to a

rotation of the fish in the water or of the eyeball in the eye-socket. For our normalized polar transformation, Θ_0 (calculated in prior steps) is used as initial vector used to unroll the iris into the polar domain which is positioned on the left edge of the transformed fish iris (see Fig. 2). For normalization each pixel in the polar image is stretched according to the length of Θ_{norm} , which is specified as the largest pupillary to limbic boundary vector. For the transformation bicubic interpolation is applied.

E. Feature Extraction

For feature extraction and matching of FICs, we use the open University of Salzburg Iris Toolkit (USIT) [21]. A note on transfer learning and domain specific improvement: To transfer knowledge from one domain (human iris) to another (fish iris), we simply used the USIT methods as is to see what does work and what does not. Specifically, the one-dimensional (1-D)-Log-Gabor [16] based feature extraction worked very well and we kept that as is, the segmentation on the other hand did not work at all, mostly due to a difference in the shape of the iris and periocular tissue, so most of our attempts to improve the knowledge transfer fell into this part (=feature extraction) and the polar transformation of the iris biometric toolchain.

1-D local Gabor features are extracted from a number of 1-D signals. To generate the 1-D signals from the texture, we first split the texture into horizontal bands with a height of roughly 8% of the distance from pupillary to limbic boundary. Then, the remaining verticality is removed by averaging the values for each horizontal position. This combination of information along the radial axis counteracts sampling artifacts due to resolution and different pupillary dilations. Since the outer boundary is only an approximation we will not use the outermost parts (about 20%) in the comparison since they might contain scleral or noneye textures. The Gabor filter used has a real and an imaginary component, which roughly equate to an edge (change in signal) and a line (constant signal) filter. This relates to radial edges and lines features in the unrolled image.

Note: To reduce the size of the FIC, we only use the signs of the line and edge filters, which represent the absence of lines and edges, respectively.

IV. EXPERIMENTS AND RESULTS

A. Salmon Iris Image Database (SIIDB)

SIIDB was captured 2018 by the authors within the AquaExcel2020 TNA project AE050006, FISHID. SIIDB is hosted at <https://github.com/rschraml/fishid>. For image acquisition 330 adult Atlantic Salmon (~1kg, 42–46 cm length) were selected initially. The cultivation period is usually between 12 to 18 months in tanks and between 12 to 24 months in sea cages. For iris image acquisition the USB microscope Dino-Lite AM3113T (no additional light) was utilized. A spacer [see Fig. 4(a)] was utilized to keep the distance, roughly constant. Each fish was anesthetized [see Fig. 4(b)] and one iris (head showing to the left) was captured several times (8–16 \times) with minor rotations caused by movements of the fish. Unusable images due to blur of focus problems were removed. The database is subdivided into a short-term (ST) and a long term (LT) dataset. A schematic

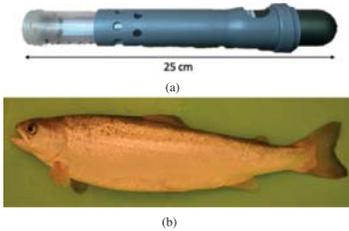


Fig. 4. SSIDB: Utilized sensor and exemplary lateral image of an Atlantic salmon fish from the LT dataset. (a) Dino-Lite AM3113T with spacer. (b) Fish with ID #0F571E –Session 1.

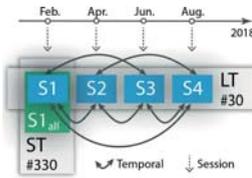


Fig. 5. Testset structure overview.

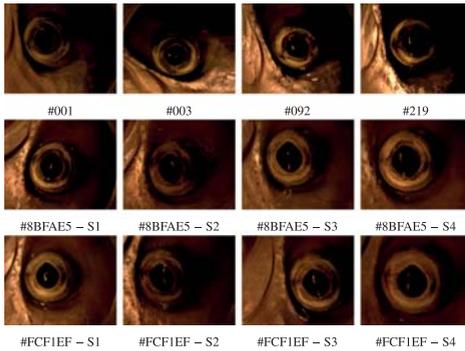


Fig. 6. Exemplary iris images of the ST (row 1) and LT dataset (row 2 and 3).

overview of the database structure is illustrated in Fig. 5. The ST dataset are composed of iris images from 330 different salmon fish, which were captured within one week. For the LT dataset, a subset consisting of 30 fish from Session 1 (S1) was captured again in three subsequent sessions (S2,S3,S4) with approximately two months time span in between. Exemplary iris images for four different fish of the ST dataset and two fish of the LT dataset are depicted in Fig. 6.

B. Experimental Setup

For all fish iris images in the LT and ST dataset FICs were computed for different rotational prealignment strategies,

which results in a set of configurations (MAX, PCA, MAX_{OPT}, PCA_{OPT}) as described in Section III-C.

Furthermore, two additional configurations based on PCA and MAX were used, utilizing four FICs per iris image. One FIC is the same as for regular PCA and MAX and the other three have a 90°, 180°, and 270° rotational offset from the first. These configurations are denoted as PCA_{ROT} and MAX_{ROT}. The goal is to avoid errors caused due to 90° rotated versions of the same fish iris. During matching the best match (=highest similarity) between the four FICs of each iris is determined and used as matching score (MS). One baseline configuration (NO) is computed without applying rotational prealignment. All configurations were computed for semiautomated (GT) and CNN segmented (CNN) fish irides in SIIDB.

For each configuration and all combinations of FICs MSs are computed. MSs which are computed between FICs from the same session are denoted as session MSs and MSs computed between FICs from different sessions as temporal MSs (see Fig. 5). Session MSs are computed for the ST dataset together with the data of S1 from the LT dataset. The corresponding score distribution (SD) is denoted as S1_{all}. Furthermore, session MSs are computed for the different sessions of the LT dataset, which results in four different SDs denoted S1, S2, S3, and S4, respectively. Temporal MSs are computed between the different sessions of the LT dataset that leads to six different comparisons: S1 ↔ S2, S2 ↔ S3, S3 ↔ S4, S1 ↔ S3, S2 ↔ S4, and S1 ↔ S4. Note that each session and temporal SD is further subdivided into an intra and interclass SD, which correspond to the genuine and impostor SDs in biometrics [22]. Genuines are MSs computed between FICs from the same fish and impostor MSs are computed between FICs from different fish.

a) Fish Iris Distinctiveness and Stability: The results for ST and LT evaluations present an insight into the distinctiveness (same session performance) and stability (change over time) of the Atlantic salmon fish iris. Both are quality criteria of a biometric characteristic. Distinctiveness is the main prerequisite and expresses that the biometric characteristic enables the distinction between different individuals. Stability is crucial for the robustness of a biometric system and expresses that the biometric characteristic does not change or vary over time. Intrinsic changes mainly result from ageing. Extrinsic changes are caused by different acquisition conditions, e.g., light or position (rotation, tilt, and camera distance) of the fish.

In the following, we experimentally assess fish iris distinctiveness and stability. The session SDs enable to draw conclusions on the distinctiveness of the fish iris and the temporal SDs enable to assess fish iris stability. Furthermore, results for semiautomated and CNN-based segmentation enable to draw conclusions on the theoretical performance, as well as for a fully automated biometric system.

C. Results and Discussion

The experimental evaluation is done in four steps. 1) It is assessed how much rotation is in the data. Since rotation negatively influences the MSs we need to ascertain if rotational prealignment is required or if rotation compensation in the

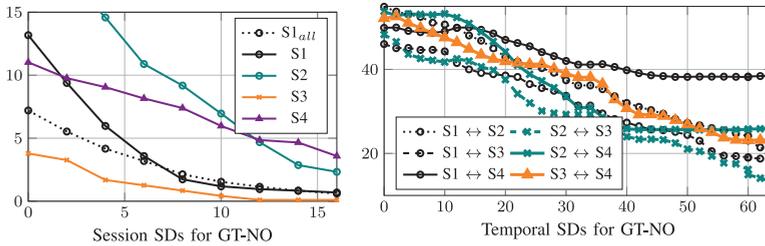


Fig. 7. EERs for different rotation compensation shifting values [X-axis: Rotation compensation in \pm Bit, Y-Axis: EER in %].

matching stage is sufficient (Section IV-C1). Thus, rotational differences in the session and temporal SDs are assessed by comparing the results of the baseline configurations where no rotational prealignment (NO) is applied. 2) We assess the basic suitability of the different rotational prealignment strategies by analyzing the verification performances for the temporal and session SDs (Section IV-C2). 3) Identification performance results are presented. Results for the temporal and session SDs reflect real world scenarios in terms of repeated identification with no time delay and varying time delays for tracking and monitoring of a fish (Section IV-C3). 4) Finally, the presented results are contrasted with the results presented in related literature.

1) **Rotation Compensation Performance:** In order to get an impression of the rotation, which is contained in the LT and ST dataset an analysis of the verification performances of NO for the session and temporal SDs is performed. For verification performance evaluation the equal error rate (EER) is a general benchmark. Basically, the question is if shifting during matching is sufficient to overcome rotational variations, i.e., to show the need for rotational prealignment. To avoid side effects caused by segmentation errors the semiautomated segmented fish irides (GT) were utilized.

It is expected that with an increasing shifting value the EER decreases until a lower boundary is reached. Therefore, the shifting value in the matching stage is varied from 0 to 16 for the session SDs and from 0 to 64 (stepsize 2) for the temporal SDs and it is assessed how the EERs change. A shifting value of 1 corresponds to a rotation of $360^\circ/512 = 0.7^\circ$, where 512 is the width of the polar transformed and normalized iris. This means that the maximum amount of rotation, in case of the temporal SDs, which has been compensated for is $\pm 44.8^\circ$.

The charts in Fig. 7 show the EERs achieved for different shifting values and the different session- and temporal SDs, respectively. For the session SDs rotation compensation in the matching stage is sufficient to achieve good performances (EERs < 4%) with a shifting value set to 16. Even with a lower shifting value of 8 EERs below 9% are achieved. However, rotation compensation is required to attain acceptable EERs for the temporal SDs. The difference between the session and temporal SDs can be attributed to the data acquisition. Within a session the rotational variation for the iris images of a fish were nominal and mainly caused by body movements of the fish. For each new acquisition session each fish was once again positioned on a table, which leads to stronger rotational differences in the

temporal SDs. For the temporal SDs in the right chart of Fig. 7, it is obvious that this shift-based rotation compensation is not sufficient to overcome the rotational variations. Even with very high shifting values no acceptable EERs are achieved. Whereas for the session SDs a shifting value of 16 is suited to achieve EERs below 4%, for the temporal SDs all EERs stay over 39%. While it would be possible to use a higher shift-based rotation compensation this affects the outcome in terms of timeliness, i.e., matching would take longer, as well as in performance since interclass FIC matches are also improved, see [23] for research on this topic as pertaining to the human iris. Based on these results, it can be concluded that for fish iris images captured at different dates (as present in the LT dataset) rotational prealignment is required, in addition to rotation compensation in the matching stage. This finding also applies to data recorded in a realistic application, since this will result in different rotations of the iris from the same fish.

The low EERs (< 4%) for the session SDs already give a first evidence that the fish iris shows a high distinctiveness, i.e., it enables to discriminate between fish in the individual sessions ($S_{all} = 330$ fish). On the other hand, the temporal SD EERs are affected by external variations (i.e., rotational variations) and it is not possible to draw conclusions on the stability of the fish iris.

2) **Rotational Prealignment and Verification Performance Analysis:** The verification performances, expressed as EERs, for the different rotational prealignment strategies as well as the session- and temporal SDs enable to draw first conclusions on the stability. The results allow to determine to which degree the verification performance is affected by intrinsic changes of the fish iris and if prealignment is suited to overcome extrinsic changes, i.e., rotational variations. Also, it is not clear how the results for the session SDs, which show less rotational variations, are affected by rotational prealignment. Again, all results were computed for the semiautomated segmented fish irides to avoid side effects. Results for CNN-based segmentation enable to investigate the feasibility of a fully automated fish identification system and how it impacts the verification performances.

Results are summarized in Table I. Based on the insights of the rotation compensation analysis all EERs are computed with shifting values 16 and 32. It is not clear if a shifting value of 32 always improves the EER. Basically, a higher shifting value increases the chance to find the correct rotational alignment of two FICs from the same fish, but it also increases the risk of

TABLE I
VERIFICATION PERFORMANCES (EERs [%]) FOR THE SESSION AND TEMPORAL SDs, DIFFERENT ROTATIONAL PREALIGNMENT CONFIGURATIONS, ROTATION COMPENSATION SHIFTING VALUES 16/32 AND FOR SEMIAUTOMATED (GT) AND CNN SEGMENTED (CNN) FISH IRIDES

Segment.	Config.	Session SDs (ST)					Temporal SDs (LT)					
		S1 _{all}	S1	S2	S3	S4	S1↔S2	S2↔S3	S3↔S4	S1↔S3	S2↔S4	S1↔S4
SHIFT 16												
GT	NO	0.65	0.71	2.52	0.15	3.91	/	/	/	/	+	-
	PCA	0.92	1.03	0.29	0.19	*	/	11.69	/	/	+	-
	MAX	3.94	0.45	0.21	0.06	*	15.52	10.32	/	15.42	29.28	-
	PCA _{ROT}	*	*	*	*	*	/	12.81	/	/	+	-
	MAX _{ROT}	*	*	*	*	*	14.96	9.87	19.6	15.96	24.44	32.56
CNN	NO	0.62	0.96	2.9	1.43	*	/	/	/	/	+	-
	PCA	0.52	0.77	1.13	1.39	4.92	/	12.73	/	/	+	-
	MAX	1.14	0.4	1.3	1.2	*	15.52	10.89	/	16.73	26.31	-
	PCA _{ROT}	*	*	*	*	*	/	12.92	/	/	+	-
	MAX _{ROT}	*	*	*	*	*	15.52	11.46	19.7	16.85	24.85	33.01
SHIFT 32												
GT	NO	0.21	0.41	0.0	0.06	1.04	/	/	/	/	+	-
	PCA	0.27	0.47	0.02	0.02	2.94	17.58	9.71	18.21	19.23	+	-
	MAX	4.11	0.48	0.01	0.05	*	15.67	9.72	/	15.83	28.84	-
	PCA _{ROT}	*	*	*	*	*	18.4	10.21	19.41	18.69	29.83	-
	MAX _{ROT}	*	*	*	*	*	14.6	10.17	17.24	15.15	23.54	34.02
CNN	NO	0.17	0.45	1.09	1.44	2.04	/	/	/	/	+	-
	PCA	0.18	0.37	1.09	1.41	1.95	17.87	11.15	19.55	/	+	-
	MAX	1.11	0.35	1.17	1.25	*	15.46	11.59	/	16.4	26.43	-
	PCA _{ROT}	*	*	*	*	*	17.65	11.79	19.65	/	+	-
	MAX _{ROT}	*	*	*	*	*	14.58	10.86	18.87	15.98	24.58	33.77

Irrelevant EERs are replaced as follows: Session SDs EERs worse than 5% are replaced by a star (*). Green colored results signalize all EERs < 1% in the session SD results. For the first four columns in the temporal SDs EERs worse than 20% are replaced by a slash (/). For the S2↔S4 EERs results worse than 30% and for S2↔S4 EERs worse than 35% are replaced by a plus (+) and minus (-), respectively. For all temporal SDs yellow colored results highlight EERs < 10%.

finding a rotational alignment of two FICs from different fish at which they are more similar to each other.

Results for GT and NO show that for the session SDs a shifting value of 16 is sufficient to achieve acceptable EERs < 4%, which improves to EERs < 1.04% when shifting with a value of 32. As already stated, this indicates the distinctiveness of the salmon fish iris pattern. Fortunately, the EERs for the CNN results of NO (SHIFT 16 and 32) are close to the GT EERs, which indicates that the employed CNN segmentation performs well and enables to set up a fully automated system.

When considering the temporal EERs for NO (GT&CNN) two assumptions can be made: 1) as already concluded in Section IV-C1 there is more rotational variation in the temporal SDs compared to the session SDs and 2) the salmon fish iris definitely changes over time. The first assertion is shown by comparing the NO temporal SD results (GT&CNN) to all others where rotational prealignment, as well as a shift of 16, is applied. In contrast to the session SDs the EERs of the temporal SDs improve when applying rotational prealignment. This means that in case of the session SDs, which contain only little rotational variations, some of the rotational prealignment strategies add rotation to the data (EERs increase) and for the temporal SDs the majority of strategies reduce rotational variations significantly, i.e., the EERs decrease.

Results also show that for all prealignment strategies the higher shifting value 32 improves the EERs for the majority of results. Another interpretation of the results is that the current prealignment is future work and should be improved. Due to the good performance of the CNN-based segmentation most of

the results are similar to the GT results. Thus, all subsequent conclusions hold for GT as well as for CNN. For the session SDs, S1, and S4 the results for SHIFT 16 and SHIFT 32 show that PCA performs better than MAX. For S2 and S3 there is no significant difference.

Contrary to the session SDs, for the temporal SDs MAX significantly outperforms PCA, especially when considering the SHIFT 16 EERs. Fig. 8(a), and (b) illustrates the cumulative MS distribution functions (CDF) for the different intraclass temporal SDs of MAX and PCA (GT), respectively. Furthermore, the interclass CDF computed over all temporal SDs (GT) is shown. The CDF of a SD gives the probability that a certain MS exists, which is less or equal to that MS. The CDFs of certain intraclass SDs and the interclass SD are used to observe their overlap and to draw conclusions about their separability. It is easy to see that compared to PCA for MAX the intraclass CDFs shift away from the interclass CDF. However, there still remains an intersection with the interclass CDF for all temporal CDFs where S4 is involved. This is also reflected by the high EERs achieved for all temporal SDs, which indicates that the salmon iris pattern changed from S3 to S4. This is further substantiated by the fact that for the session SDs and S4 with SHIFT 32 and NO (GT) an EER of 1.04% is achieved. Thus, it is very likely that the high EERs for all temporal SDs with S4 are caused by internal variations of the iris, i.e., growth of the fish eye and changing iris pattern.

Considering MAX_{ROT} and PCA_{ROT} the session SDs show that the EERs (see Table I) increase significantly compared to NO. Note that EERs worse than 5% are replaced by a star (*) in the

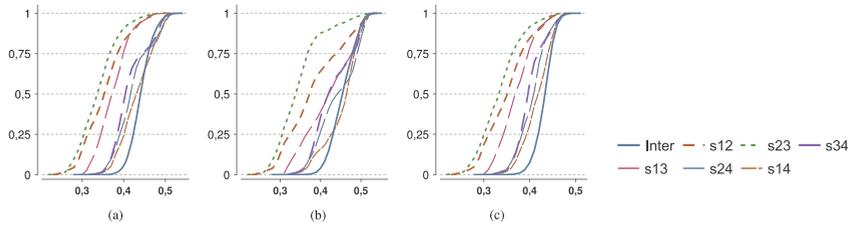


Fig. 8. Intra-/Interclass CDFs of the temporal SDs and selected rotational prealignment strategies (GT, SHIFT 16) [X-Axis: MS, Y-Axis: Cumulative Probability]. (a) MAX. (b) PCA. (c) MAX_{ROT}.

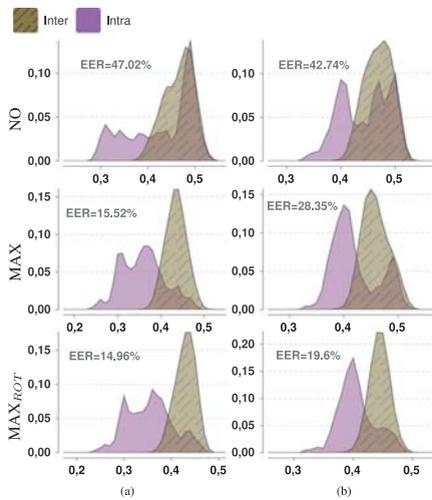


Fig. 9. Intra-/Interclass distribution charts for selected temporal SDs and selected rotational pre-alignment strategies (GT, SHIFT 16). [X-Axis: MS, Y-Axis: Probability]. (a) S1↔S2. (b) S3↔S4.

table. An explanation for this effect is that four FICs per iris and additional shifting significantly increases the risk of finding rotational alignments where the iris of different fish are similar to each other. However, the MAX_{ROT} EERs for the temporal SDs are superior to all other results. This is independent of the shifting value, confirming the assumption that if the rotational prealignment works further shift based compensation beyond what is required for a single session is not needed. Interestingly, PCA_{ROT} is not suited to improve the verification performances of the temporal SDs. The corresponding intraclass CDFs for the temporal SDs of MAX_{ROT} (GT, SHIFT 16) are shown in Fig. 8(c). Compared to the MAX CDFs in Fig. 8(a) it is obvious that the intersection of the intraclass CDFs with S4 and the interclass CDF decreases. Finally, Fig. 9 enables to compare the intraclass and interclass SDs for the temporal SDs S1↔S2 and S3↔S4 (GT, SHIFT 16) computed with NO, MAX and

MAX_{ROT}. For NO the charts illustrate that rotational misalignment causes an overlap of intraclass SDs with the interclass SDs. Considering MAX this overlap is significantly reduced by rotational prealignment and rotation compensation. For MAX there still is a high overlap of the interclass and interclass SD, which is reduced when applying MAX_{ROT} for rotational prealignment.

3) *Identification and Real-World Scenario Performances:* By considering the identification performances for the session and temporal SDs first conclusions on the feasibility of salmon fish iris identification in a real-world scenario can be drawn. Hence, the CNN-based segmented fish irides were utilized for the identification performance experiments.

Basically, session SDs indicate the feasibility of ST identification and temporal SDs show the performance for LT identification. Identification performances are assessed based on the Rank-1 recognition rate (RR). In Figs. 10 and 11, the Rank-1 RR for the rotational prealignment strategies and the session and temporal SDs are summarized, respectively. The temporal SDs results are comparable to the verification results for SHIFT 16 and the general statements are the same. Summarized, PCA performs better than MAX and MAX_{ROT} and PCA_{ROT} improves the performance for S4 slightly. With PCA, except for S4, all Rank-1 RRs are higher than ~98.5%. The best performance for S4 is achieved with PCA_{ROT} showing a Rank-1 RR close to ~96%.

Results confirm that the salmon fish iris is highly distinctive and enables ST fish identification. However, same as for the verification results the identification performances for the temporal SDs again show that intrinsic variations, i.e., aging, cause decreasing Rank-1 RRs. Again, the best performances are achieved with MAX and MAX_{ROT}. The best performance is shown for the temporal SD S2↔S3 with ~80% followed by S1↔S2 and S1↔S3. Again, this indicates that the iris changed significantly from the S3 to S4. Even S1↔S3 with ~65% is better than ~50% achieved for S3↔S4 with a shorter time-span between the acquisition sessions. Together with the verification performance results, it can be concluded that the robustness of fish iris biometrics suffers from a missing LT stability of the fish iris. However, the S1↔S2, S2↔S3, and S1↔S3 results indicate that identification in a real-world scenario is feasible but the system needs to consider this issue by updating the biometric templates of each fish (FIC) in short periods. Especially, at an

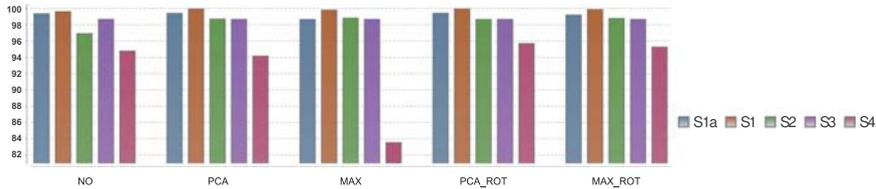


Fig. 10. Session SDs (CNN, SHIFT 16) – Identification performance evaluation [Y-axis: Rank-1 recognition rate (RR) %].

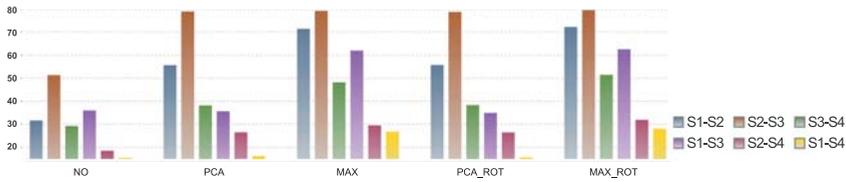


Fig. 11. Temporal SDs (CNN, SHIFT 16) – Identification performance evaluation [Y-axis: Rank-1 RR %].

age over 6 months this becomes crucial as the pattern changes significantly at this age.

This is also an interesting result with regard to the biometry of the human iris, since the human iris shows ageing effects, although the severity of the impact is controversial (see [24]). The fish under study have now also shown an ageing effect, which can much more readily be observed and researched owing to the faster life cycle of the Atlantic salmon.

4) *Comparison to Related Literature*: Finally, the Atlantic fish iris identification results are compared and discussed with the literature presented in Section II. Different to the low stability of the Atlantic salmon iris, the results for Patagonian catfish in [8] showed that the lateral skin spot pattern has a high distinctiveness as well as LT stability. A direct comparison of the results is not feasible, as the approach in [8] relies on I³S [25], which is a computer-aided photo identification application for underwater animals. With the help of this software, three reference points and all spots in each lateral image were annotated manually and the software performed the matching. If the authors achieve similar results in the future with an automated method, the approach would have great potential in terms of distinctiveness and stability.

If the skin pattern is used as a characteristic it is often not clear if it is present for all fish of the same species and if this pattern is present at all ages. The results for Atlantic salmon identification in [9], which are based on the lateral operculum pattern indicate the nonsuitability as a biometric characteristic because some fish showed no pattern or it disappeared. Similarly, in [11], the absence of the dorsal head view pattern of Chinook salmon for a large amount of individuals has been reported.

The results presented by [12] for delta smelt identification based on dorsal head view images are comparable to ours in terms of stability. Even if the pattern was localized manually, results for automated matching indicated that the pattern changes over time and matured fish show more distinctive patterns. On

the contrary, our results show that the distinctiveness of Atlantic salmon based on the iris pattern could get a little worse with older age. A comparison regarding the distinctiveness is not possible because the fish sample size was smaller and no results for one point in time (=session SDs in this article) were presented.

Compared to our session SD results the experiments for armored catfish identification using ventral images [5] and lionfish identification using lateral images [10] showed poorer recognition accuracies, although manual localization was performed.

It can be concluded, that the suitability of the skin pattern as a biometric characteristic must be examined closely, same as for the iris pattern. In the future approaches with automated skin pattern localization should be sought by the community.

The basic advantage of the iris is that most fish species show a visible iris pattern which is likely suited as a biometric characteristic to set up a FDSS. Additionally, as shown in this article the iris pattern can be localized automatically which enables automated identification.

V. CONCLUSION

Fish identification is a basic tool required to move from mass to smart production in intensive aquaculture. Noninvasive methods are fast, cheap, and beneficial for fish welfare. Biometric approaches based on the individuality of the skin pattern lack of visible patterns in general and missing patterns in various life phases of a single fish. Therefore, this article demonstrated the principal feasibility of Atlantic salmon fish identification using iris images as biometric characteristic. Distinctiveness and stability of the salmon fish iris were assessed based on a ST and LT dataset.

Results for 330 different fish in the ST dataset showed that the fish iris is highly distinctive. For all subsets in the ST dataset identification rates of over 95% could be achieved. The stability of the fish iris was assessed based on the LT dataset.

Due to different rotational alignments between iris images of the same fish captured at different points in time a set of rotational prealignment strategies were applied and evaluated. Experiments showed that rotation compensation in the matching stage, even with a high shifting value, is not sufficient to achieve acceptable EERs. The best results for the LT dataset were achieved with the rotational prealignment strategy MAX, which uses the maximum length pupillary CM to boundary vector for alignment. An additional improvement could be achieved by enrolling four 90° rotated templates of each iris (MAX_{ROT}), reducing errors caused by rotational prealignment resulting in at most 45° rotational error in iris images.

Results showed that the verification performances decrease with an increasing time span between the different acquisition sessions. Interestingly, results for the first two (S1↔S2 = 14.96%) and the last two successive sessions (S3↔S4 = 19.6%) sessions are worse than for the middle sessions (S2↔S3 = 9.87%). This leads to two main conclusions: 1) The salmon fish iris shows a weak stability, i.e., due to ageing (=size and pattern changes). 2) The variations caused from ageing from month 2 to 4 and 6 to 8 are much stronger than in-between from month 4 to 6.

Results achieved with semiautomated segmented fish irides were compared to those computed with a fully automated CNN-based approach. The results showed that automated segmentation is possible and comparable to that achieved with the semiautomated segmented irides. This was crucial in order to establish a fully automated fish identification system. Additionally, for a real-world scenario the identification performance of the LT dataset is of relevance and the identification rates for MAX_{ROT} on the different subsets vary between 28% and 80%. Based on the missing stability of the salmon fish iris and the accuracies for the successive subsets S1↔S2 = 72.00%, S2↔S3 = 80.00% and S3↔S4 = 51.00% the following conclusion can be made: Salmon fish iris identification is feasible in a real-world scenario with the precondition that the biometric template of each fish in the database of the biometric system is updated periodically, especially when the fish gets older than 6 months. In human biometrics this is referred to as adaptive biometric systems.

A. Future Work

It was not feasible to consider the impact and change of pigmentation with age in this article. The change in pigmentation can be disregarded for short time spans. However, given the decrease in identification performance between image acquisition sessions that are further apart in time, this may be the reason for the decrease.

Future work needs to consider a realistic environment, i.e., underwater iris images of swimming fish. For example, fish could be forced to pass through a narrative tube with their lateral side to the camera at a relatively constant distance similar to what explained in [26] and [27]. In order to compensate for differences between iris images from different sessions future experiments should consider iris image preprocessing.

Furthermore, the use of near-infrared imaging could improve the identification performance since the iris is likely pigmented

given that it is an extension of the epithelial layer. It is known that the speed of adaptation and the pigmentation of the epithelial layer changes, stronger pigmentation with increasing age [15]. The impact on the pigmentation of the iris is unknown but is likely to happen. Independent of visible light or near infrared imaging, an appropriate illumination as common in human iris imaging needs to be considered. However, special care must be taken to ensure that the lighting does not pose any health risks or impacts fish welfare.

Finally, the use of other or additional biometric performance metrics should be considered in future work. The use of other metrics will depend in particular on the respective application or the focus of the investigation.

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CHAPTER 6

GENERAL DISCUSSION

ENGLISH SUMMARY

CZECH SUMMARY

ACKNOWLEDGEMENTS

LIST OF PUBLICATIONS

TRAINING AND SUPERVISION PLAN DURING STUDY

CURRICULUM VITAE

General discussion

To develop non-invasive image-based identification of the fish individuals based on a fish appearance, we systematically performed four studies to prove our approach for different fish species to understand the pattern's stability during the time. In total, we tested the method for four fish species and the most extended period of six months.

The first study (Chapter 2) aimed to test the possibilities and limitations of the non-invasive individual identification on a small number of fish individuals. In this study, the identification of the individuals of Sumatra barb *Puntigrus tetrazona* was made. Sumatra barb is an ornamental fish with black vertical stripes on the body we used for the identification procedure. We used a small number of fish (43) in this experiment and collected two data sets with a delay of two months. We have obtained promising results (100% accuracy – short-term identification and 96% accuracy for two months of identification) from this experiment which led us to continue the research. The experiment's outcome was that identification based on fish appearance is possible.

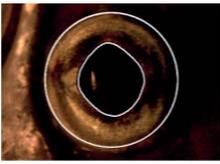
The next step was identifying the Atlantic salmon, where we increased the number of the fish individuals (compared to the Sumatra barb experiment). We used 330/30 fish (which is the highest number of individuals in all existing fish individuals identification studies), and the duration of the experiment was extended to six months (4 data collection). For the identification procedure were used different visible models of the patterns such as the iris of the fisheye and dot patterns on the fish body (side view). Data were collected under two conditions, fish out of the water and fish in the aquarium, to test how the condition affects the accuracy of the results. Three different approaches (including state of the art CNN based approach) were used during those experiments.

Furthermore, different regions of interest (ROIs) were tested to achieve the best results. The best results (100% accuracy) were obtained using the position of dots on fish skin for identification. The outcome of this study was that the dot pattern on Atlantic salmon *Salmo salar* is unique and useful for identification. It is stable for the long-term, and there is no significant decrease in accuracy between out of the water and under water fish images data collection. These experiments are described in Chapters 3 and 5.

The final step of individual fish identification based on an image was the identification of the fish species that have no apparent patterns on a body (only scales on the body). Common carp *Cyprinus carpio* and European seabass *Dicentrarchus labrax* were used in our last experiment (Chapter 4) as a representative of these species. For the identification, different ROIs were used with different identification accuracy. The primary outcome of this study was that the identification is possible also for fish species without obvious skin patterns with 100% accuracy.

During the complex study, two types (from the point of view of time) of experiments were done. The short-term experiments for testing the uniqueness of the patterns, and the long-term experiments to test the stability of the pattern during the time. Table 1 summarizes all ROI used for all fish species for identification. Table 2 and 3 contains the summary results of the experiments for all fish species. Table 2 contains the information about the short-term studies, and table 3 includes information on the long-term experiment results and conditions.

Table 1. Summary table with the visualizations of ROIs of the fish.

Species	Name of the ROI	Description	ROI visualization
Sumarta barb	ROI1	one stripe only	
	ROI2	two middle stripes	
	ROIW	The ROI covering the whole fish body	
	ROIH	from head to tail	
Atlantic salmon	ROI	Manual localization of dots on ROI	
	Dots location	Examples of data for dots localization CNN training	
	Pattern on eye	Localized fish eye	
European seabass	ROI(LL)	Lateral line	
	ROI(HLL)	Half of the lateral line	
	ROI(S)	A region with scales only	
	ROI(O)	An operculum part	

Common carp	ROI1	covers the body of the fish from the side (only the scales)	
	ROI2	refers to the same part of the body (ROI1) but longer and wider	
	ROI3	the operculum part of the carp	

Table 2. Summary table with the primary information and accuracies for all fish species for the short-term experiments.

Species	No of the fish	Data collection conditions	Parametrization	Type of the patterns	ID of data collection	ROI	Accuracy, %	
Sumatra barb	43	aquarium	HOG	Black vertical body stripes	1	ROI1	96	
						ROI2	100	
						ROIW	96	
						ROI1	96	
	27					2	ROI1	96
							ROI2	100
							ROIW	96
							ROI1	96
Atlantic salmon	330 /30	out of the water in the photo tent	HOG	Dot pattern	1		100	
						ROI	2	100
							3	100
							4	100
		Aquarium	CNN	Dots	1			100
						ROI	2	100
							3	100
							4	100
	Microscope camera out of the water	Fish iris code +CNN	iris of the eye	1	Pattern on eye			95

European seabass	300	out of the water	HOG	Lateral line	ROI(LL)	100
				Part of the lateral line	ROI(HLL)	98.66
				Scale	ROI(S)	98.66
				Operculum	ROI(O)	91.66
Common carp	32	out of the water into the photo tent	HOG	scale	ROI1	100
					ROI2	100
				Operculum	ROI3	100
				Scale	ROI1	100
					ROI2	100
				Operculum	ROI3	100
				Scale	ROI1	100
					ROI2	100
				Operculum	ROI3	100
				Scale	ROI1	100
					ROI2	100
				Operculum	ROI3	100

The main outcome of the four connected studies is that the identification of fish individuals based on the images of skin patterns is possible with 100% accuracy (for the sample 330 fish). Therefore, non-invasive identification can be used as a substitute for invasive fish tagging. The other outcome is that the skin pattern is sufficiently unique and stable for a long-term period (at least six months). The fish with and without obvious skin patterns can be identified using the introduced methodology, which is based on the HOG descriptor, and which is general to be applied to other fish species. The main challenge is to adapt the methodology to the freely swimming fish under the real conditions of fish production units.

A very limited number of studies deal with non-invasive automatic fish identification. The main focus of image-based fish identification is species identification (Strachan et al., 1990; Hsiao et al., 2014; Saitoh et al., 2015). Our approach can be compared with just a few studies dealing with individual identification of the same species (Huntingford et al., 2013; Hirsch and Eckmann, 2015; Stien et al., 2017; Al-Jubouri et al., 2018; Navarro et al., 2018). All those studies deal with a very limited number of fish (from 5 to 92 fish) and a limited time period (maximum is a 6-week duration). An only extended study from (Stien et al., 2017) has 246 fish individuals and two data collection in ten months period. But they manually made the identification of only 30 fish individuals in one group. Such as a study of *Danio rerio* identification of five individual fish by Al-Jubouri et al., 2018. In this research, they developed the identification procedure for five individuals of zebrafish using the HSV (hue, saturation, value) colour model. The accuracy was 99% for the short-term identification. This is the only fully automatic study. The other studies were done manually or semi-automatically. A more detailed comparison of our study to the others is in the published papers.

Stripes on the body were used in the research of Hirsch and Eckmann 2015 where they had used individuals of Eurasian perch (*Perca fluviatilis*). There were six groups of eight fish (larvae) in each group. Scale pattern was also used to identify common carp in the study by Huntingford et al., 2013. The matching accuracy was 95.76% for 15 individuals. As it was mentioned before, in the literature already have been done studies about identification of

species (Ibáñez and Gallardo-Cabello, 2005; Saitoh et al., 2015; Shafait et al., 2016; Ibáñez et al., 2020; Sato et al., 2020). But all those research deals with species identification. The popular approach for the identification of animals is deep learning (Villon et al., 2018, 2016). But till now, this method has been used to identify the fish species only. Individual identification is a more challenging task. Only several papers deal with the identification of individual fish within one specie. Stien et al. (2017) have worked on the non-invasive identification of individuals who used consistent melanophore spot patterns on Atlantic salmon (*Salmo salar*). They tested the long-term stability of these patterns during the time. They used semi-automated computer-based identification. They worked with 246 individuals and two data collection periods recorded over ten months of cultivation. The study proved the stability of the salmon dot pattern with 93% accuracy, but the number of individuals used in one identification task was 30. Through the studies, it was proven that the long-term non-invasive individual identification of the fish using image processing methods is successful and could be used as a substitute for the tagging methods, which are harmful and stressful for the fish (Ombredane et al., 1998; Castillo et al., 2019; Pine et al., 2003; Andrews, 2004).

In comparison to the existing studies, our study is the first systematic attempt to automatically identify individual fish using the skin patterns with a significantly higher number of fish (compared to current studies) and long-term pattern stability testing. The contribution of our studies beyond the state-of-the-art is that we proved that the skin patterns can be used for fully automatic individuals identification and that the method can also be used for fish species without obvious dot or stripe patterns. Our method for salmon dots detection and identification based on the dots is unique and outperformed the existing state-of-the-art methods. Our work opened new possibilities in the field of aquaculture, especially in personalized aquaculture.

In our studies, we tested different patterns parametrizations (HOG, LBP, CNN, BS, HP) and different classification methods for four fish species with different appearances. Various data collection conditions were tested during the experiments to explore the limitations of the approach. The data were collected out of the water (to have high-quality images for testing the approach without additional noise) and underwater to test our methods under the more realistic conditions. A different number of individuals were used for the particular species. The smallest group of fish started with the 27 fish individuals (Sumatra barb), and the biggest group of the fish was 330 individuals (Atlantic salmon). The 330 individuals are a representative number for standard 2 m² tanks used at the aquaculture facilities. To be able to upscale the method for large tanks or sea cages, we would need to test the method with a higher number of fish individuals (thousands).

Table 3. Obtained accuracies for all fish species for the long-term experiments.

Species	No of the fish	Data collection conditions	Duration of the experiment	Parametrization	Combination of data collection	ROI	Accuracy, %						
Sumatra barb	27	aquarium	Two months	HOG	1*2	ROI1	80						
						ROI2	88						
						ROIW	80						
						ROI1	80						
Atlantic salmon	30	out of the water into the photo tent		HOG	1*2	ROI	100						
							83.3						
							36.6						
							100						
							53.3						
							93.3						
							83.3						
							73.3						
		Aquarium	Six months			CNN	1*2	Dots location	70				
									100				
									100				
									100				
									100				
									100				
									72				
									65				
Microcamera out of the water				Fish iris code +CNN	1*2	Eye pattern	80						
European seabass	32	out of the water	1 month	HOG	1*2	ROI(LL)	100						
						ROI(HLL)	96.87						
						ROI(S)	93.75						
						ROI(O)	40.62						
						ROI1	80.64						
						ROI2	80.64						
						ROI3	80.64						
						ROI1	100						
						ROI2	96.77						
						ROI3	64.51						
						Common carp	32	out of the water into the photo tent	4 months	HOG	1*4	ROI1	90.32
												ROI2	83.87
ROI3	32.25												
ROI1	80.64												
ROI2	80.64												
ROI3	67.74												
ROI1	70.41												
ROI2	80.64												
ROI3	29.03												
3*4	ROI1	100											
	ROI2	100											
	ROI3	87.09											

We were interested what factors mainly influence the accuracy of the identification. Based on tables 2 and 3, we can see the changes in identification accuracy based on the data collection conditions, used parametrization and selected region of interest. The first and most important factor is the extraction of the ROI. The area we choose for the identification will play a central role in the robustness of the long-term identification. It can be seen from the

results of identification for most of the tested fish. Identification procedures using different ROIs were tested for all four fish species. All those species show different identification accuracy results for the different ROIs. For the Sumatra barb the best accuracy had the ROI2; it is the part of the body covering two middle black stripes on the fish body. We experimented with only one stripe and almost full length of the body. Both had lower accuracy than two stripes. The reason is that one stripe does not contain enough information, and the full body length is deformed during the fish movement. Also, accuracy results for the seabass have similar results. The best accuracy has ROI11 (100%), and less accuracy has ROIHLL (96.87%). Here is just the part of the lateral line covered, which is already influenced by the accuracy and robustness of the results.

Further, different visible models of the pattern on a fish body were tested. Black vertical stripes, dot patterns, dots localization on the body, scale patterns, lateral lines and even the operculum were tested to see which part of the body is the best for the individual identification. Each fish species has 100% accuracy, at least for one visible pattern. Atlantic salmon and common carp accuracy results are 100% for all tested ROIs (exception is the eye results for salmon). The Sumatra barb results were influenced by data collection; they are the worst mainly because of the quality of the images. According to the results of the tables 2 and 3, the uniqueness of the visible pattern on the fish body is proven. We can conclude that for the accuracy of the results, the uniqueness of the pattern is significant for the quality of the data and the chosen part of the body. Another factor which can affect the accuracy is chosen pattern on the body itself. The dots on the salmon body and stripes on the Sumatra barb body was unique and stable. But the lateral line and scale pattern had a high accuracy (100% for the lateral line and 93.75% for the scale pattern) which was surprising. So, we can conclude that those patterns are stable and contains enough information for the identification. The accuracy of the carp' operculum is not stable, and the best accuracy has the combination of 3*4 data collections (87.09%). Moreover, the accuracy for the operculum of the seabass is 40.62% which shows that this part is not stable for the long-term identification procedure. Concerning the eye pattern of salmon, the best accuracy has the combination of 2*3 data collection, which has 80.00%. According to the results for the European seabass and common carp operculum part and salmon eye, those parts cannot be used for the long-term perspective of individual identification.

We have tested different texture-based and shape parametrization methods. From the obtained results, the best descriptor was texture descriptors HOG (Dalal and Triggs, 2005). This approach shows the highest accuracy results for the Sumatra barb, seabass and common carp. The advantage of the descriptor is that the approach can be generalized to other fish species. HOG is not based on the description of the particular part of the skin pattern, and that is the reason why it can be generally used. For salmon, we tested two approaches. The first one was based on the general HOG descriptor. The second one was based on the localization of the skin dot using CNN approach, and the mutual dots' positions were used for identification. The results show that the dots based approach was higher accuracy than HOGs because it parametrizes the particular information about the dots' positions. HOG descriptor also got acceptable results of identification, but the advantage is that it can also be used for other species. The recommendation is therefore, to use general parametrization instead of parametrization specific for the fish species.

The approaches based on a convolutional neural network are very powerful and can outperform the classical image processing methods in many areas. Therefore, we also tested this approach for our identification. The testing was done on the salmon data (skin dot pattern). We used an end-to-end CNN based approach for parametrization of the dot pattern and for classification into 330 classes of the individuals. The approach was based on triplet

loss function. The identification accuracy was much lower (maximum 55%) than the accuracy obtained by HOG approach. The problem was mainly the limited number of training images. We plan to do more experiments with CNN based identification, but the general problem of CNN based identification is that it can be used for the skin patterns it was trained for. It cannot be easily adapted to the new fish skin pattern without re-training the network. In comparison the HOG based approach can be easily adapted to new fish skin patterns.

Our introduced method is not yet perfect and has several limitations. The method is sensitive to the localization of the ROI. This part is the weakest part of the approach, so we had to find different ways to extract the ROI. The solution to this problem is the use of CNN based fish detection and the ROI. CNN is the best candidate for real-time and real conditions ROI detection. We plan to implement this solution in our future research.

Another limitation is the age and size of the fish. We tested our approach only with the mature fish (one year and older). We did not test our method with the juveniles. It means that we cannot be sure that we can identify the individuals from their early growing stages. But with the Atlantic salmon experiment, which was the longest data collection period, we can see that photo identification of fish individuals works even if new dots on the body appear during the time. We suggest that it's enough to collect the data and then repeat the procedure for no longer than one month for identification. At the same time, new dots will not influence classification accuracy.

But even with several limitations, our studies confirmed the possibility of automatic the image-based fish individual identification, which has a long-term impact on the aquaculture. Our approach enables us to substitute the commonly used invasive fish identification, which has several disadvantages (Rácz et al., 2021)(time-consuming, harmful, and stressful for the fish) and is not beneficial for the farmers. There will be no need in the future to tag the fish, catch them for the tagging, all the time saw them check the tags and IDs, which will stress fish and waste the farmer's time (Andrews, 2004). Also, the tag loss will not be a problem (Bolland et al., 2009). The video recording system will take a picture of the fish, automatically identify individuals, and update the information during the cultivation time.

Such beneficial advantages will help the farmers control, monitor and document all cultivation stages of the fish. Automatization of the cultivation process can save the time, humans' work power, control the feeding process as well as the quantity of feed (Zhou et al., 2018), which helps to maintain the quality of water (Pautsina et al., 2015); in terms of fish welfare which is essential to keep the water quality in the appropriate level (Hook et al., 2019).

To support the development of image-based individual identification, all our experimental data are available to the public in an open-access dataset. To open the data, we use our internal bioWes platform.

This study was the initial step in the automatic, real conditions of individual fish identification. Future research is needed to implement it on fish farms. Our future work is to identify the individuals in real-time and real cultivation conditions where the camera will record and identify the individuals.

Conclusion

This thesis includes four publications where we highlighted the non-invasive individual fish identification. Our study confirms that identifying the individual fish is possible from the images, an alternative to the invasive fish tagging method.

During this study we have identified individual fish in the close group of fish (aquarium, tank). We have tested different patterns (body patterns – dots, stripes; iris of the eye, lateral

line, scale patterns, scale formation) on a fish body of a different fish species such as Sumatra barb, Atlantic salmon, European seabass and common carp.

We have developed the method for automatic fish individual identification from the images. Furthermore, we have proved automatic concept of non-invasive individual fish identification from images. And stability of the chosen patterns during the cultivation period was tested and proved.

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English summary

Increasing consumption of fish and fish products motivates the farmers to increase their production. Rising fish production is possible by using automatization in the industry. Today, automatization is widely used in different stages of fish production, such as feeding, welfare monitoring, etc. It is a precision fish farming concept principle. The concept allows the farmers to control, monitor and document all stages of fish cultivation. One of the tools for automation is to provide individualised information about fish by individuals identification. This could help to get precise information about the individual and not about the whole fish school only. Getting the information in time is crucial to predict the early stage of the disease, control feeding and fish welfare, and possible fish sorting.

Nowadays, individual fish identification is made by tagging. Tagging is an invasive method of fish identification that can cause injuries to fish and stress to them, leading to increased mortality. Tagging is a time consuming and expensive identification way. The disadvantages of this method are obvious. To solve those problems, identification from the images could be used. Image-based fish individual identification is an excellent alternative. It is cheap, fast and not stressful to the fish. Fish identification from images is widely used in species identification. But not many studies deal with the identification of individual fish. All described reasons motivate us to work toward individual fish identification from the images as a substitute for fish tagging.

We have done complex research with different fish species, different data collection conditions, and long-term perspective identification. In Chapter 2, we tried the first attempt to automatically identify individuals of ornamental fish Sumatra barb *Puntigrus tetrazona* in an aquarium. Fish were freely moved in an aquarium with water; the green background was used to do the fish segmentation. Totally 43 individuals were used in this experiment. Identification accuracy was 100% and supported us to continue with the next step experiment.

The next step was to increase the number of photographed fish to 330 fish. We used the commercially important Atlantic salmon *Salmo salar* in this study. We have tested different visible patterns on the fish body, such as dots on the body (Chapter 3) and the iris of the eye (Chapter 5). The duration of the experiment was six months. In this study, different data collection conditions were tested. Images of fish underwater in an aquarium and out of the water in a photo tent were taken. For the pictures of the fisheye, we used a micro-camera. The best results obtained from those experiments were 100% accuracy for the dot approach and HOG parametrization methods and 95% for fisheye data.

The last experiment in this dissertation (Chapter 4) was done to prove that the fish species which have no obvious pattern on the body, such as Sumatra barb (black vertical stripes) and Atlantic salmon (dots on the body), could be identified non-invasively from the images. European seabass *Dicentrarchus labrax* and common carp *Cyprinus carpio* were used in this study to prove this idea. Totally 300 seabass and 32 carp were photographed out of the water to get high-quality data. Together with the short-term experiment, we collected long-term data (two months for seabass and four months for carp). Different parts of the body were tested for identification (lateral line, scale pattern, operculum). Surprisingly, the identification results were high enough (100% for both species, even for the long term experiments) to conclude that the photo-identification works with species without an obvious pattern on a body.

The conclusion supported by the results of the experiments is that the automatic photo-identification of individual fish is possible using machine vision. Data processing and identification procedure were fully automated. The approach works for species with and without an obvious pattern in the body (Sumatra barb, Atlantic salmon, European seabass and common carp), and it is useful for long term individual identification. The method can be used as a substitute for invasive fish tagging.

Czech summary

Zvyšující se spotřeba ryb a rybích výrobků motivuje farmáře ke zvýšení jejich produkce. Zvyšování produkce ryb je možné pomocí automatizace, která zajišťuje snížení nákladů a otevírá nové možnosti produkce. Dnes je automatizace široce používána v různých fázích produkce ryb, jako je krmení, sledování welfare atd. Jde o princip přesného chovu ryb, který umožňuje farmářům kontrolovat, monitorovat a dokumentovat všechny fáze chovu ryb. Jedním z možných způsobů automatizace je monitorování individuálních ryb místo celého hejna, jak je tomu doposud. To by mohlo pomoci rychleji získat přesné informace o aktuálním stavu jednotlivců. Získání individuálních informací je zásadní pro předvídaní raného stadia onemocnění, kontrolu krmení a dobrých životních podmínek ryb a možné třídění ryb. Individualizace je možná díky identifikaci jednotlivých jedinců.

Nejpoužívanější metodou identifikace ryb je jejich značkování. Značkování je invazivní metoda identifikace ryb, která může rybám způsobit zranění a stres, což vede k zvýšení jejich úmrtnosti. Invazivní značkování je časově náročný a nákladný způsob identifikace. Nevýhody této metody jsou zřejmé. Možnou alternativou invazivní identifikace je neinvazivní identifikace z digitálních fotografií ryb. Jedná se o levné a rychlé řešení, které ryby nestresuje. Tento postup je používán při klasifikaci jednotlivých druhů. Identifikaci jednotlivých ryb se ale zabývá pouze pár studií. Popsané výhody nás motivovali k práci na individuální identifikaci ryb ze snímků jako náhradě za značkování ryb.

Provedli jsme sérii experimentů, které dohromady tvoří komplexní výzkum ověřující identifikaci jednotlivců pro různé druhy ryb, různé podmínky sběru dat a dlouhodobou možnost identifikace založenou na obrázcích. První experiment je popsán v kapitole 2, kdy jsme provedli test identifikace jedinců okrasných ryb Sumatra barb *Puntigrus tetrazona* v akváriu. Ryby se volně pohybovaly v akváriu s vodou a bylo použito zelené pozadí k segmentaci ryb. V tomto experimentu bylo použito celkem 43 jedinců. Úspěšnost identifikace byla 100% a prokázala použitelnost navržené metody pro další vývoj.

Dalším krokem bylo zvýšení počtu ryb na 330 a otestování dlouhodobé stability vzorů použitých pro identifikaci. V této studii jsme použili komerčně významný druh ryb: lososa atlantického *Salmo salar*. Testovali jsme různé viditelné vzory na těle ryb, jako jsou tečky na těle (kapitola 3) a oční duhovka (kapitola 5). Obrázky vzorů byly fotografovány po dobu šesti měsíců pro ověření jejich stability. V této studii byly testovány různé podmínky sběru dat. Byly pořízeny snímky ryb pod vodou a mimo vodu ve foto stanu pro stanovení vlivu kvality dat na úspěšnost identifikace. Vytvořili jsme dvě metody pro identifikaci. Specializovaná metoda pro parametrizaci polohy teček na těle dosáhla dlouhodobé úspěšnosti 100%. Obecná metoda využívající parametrizaci textury dosáhla úspěšnosti 95%. Stabilita vzoru v oku se ukázala jako velmi nízká pro dlouhodobou identifikaci.

Poslední experiment našeho výzkumu (kapitola 4) byl proveden s cílem otestovat, zda je možné použít vytvořenou metodu pro druhy ryb, které nemají na těle žádný zjevný vzor (tečky či pruhy). K prokázání této hypotézy byl v této studii použit mořský okoun *Dicentrarchus labrax* a kapr obecný *Cyprinus carpio*. Celkem bylo vyfotografováno 300 mořských okounů a 32 kaprů mimo vodu. Společně s krátkodobým experimentem jsme shromáždili dlouhodobá data (dva měsíce pro mořského okouna a čtyři měsíce pro kapra). Pro identifikaci byly testovány různé části těla (boční čára, vzor šupin, skřele). Úspěšnost identifikace byla pro oba druhy 100% a bylo prokázáno, že metodu je možné využít i pro druhy bez zjevného vzoru. Pro okouna byla nositelem unikátnosti postranní čára a pro kapra to byl vzor samotných šupin.

V rámci našeho výzkumu byla vytvořena automatická metoda pro detekci a identifikaci ryb využívající vzory na těle ryb pro jednoznačnou identifikaci. Metoda funguje se 100% úspěšností jak pro druhy ryb s jasnými vzory na těle, tak pro druhy bez těchto vzorů. Prokázali jsme i dlouhodobou stabilitu těchto vzorů pro identifikaci jednotlivců stejného druhu. Vytvořená metoda může být použita jako náhrada aktuálně používané invazivní metody značkování ryb. Všechna data získaná v rámci experimentů jsou nabízena v rámci otevřeného přístupu pro další rozvoj této oblasti výzkumu.

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- The Ministry of Education, Youth and Sports of the Czech Republic, The CENAKVA Centre Development [No.CZ.1.05/2.1.00/19.0380]
- HORIZON 2020, AQUAEXCEL2020 project (652831), AQUAEXCEL 2020 – Aquaculture infrastructures for excellence in European fish research
- University of South Bohemia in České Budějovice, GAJU 013/2019/Z, New technologies for aquaculture monitoring with the respect to the water organism welfare.

List of publications

Peer-reviewed journals with IF

- Bekkozhayeva, D.**, Císař, P., 2022. Image-based automatic individual identification of fish without obvious patterns on the body (scale pattern). *Applied Sciences* 12, 5401. (IF 2021 = 2.838)
- Mamilov, N., Sharakhmetov, S., Amirbekova, F., **Bekkozhayeva, D.**, Sapargaliyeva, N., Kegenova, G., Tanybayeva, A., Abilkasimov, K., 2022. Past, current and future of fish diversity in the Alakol lakes (Central Asia: Kazakhstan). *Diversity* 14, 11. (IF 2021 = 3.029)
- Bekkozhayeva, D.**, Saberioon, M., Cisar, P., 2021. Automatic individual non-invasive photo-identification of fish (Sumatra barb *Puntigrus tetrazona*) using visible patterns on a body. *Aquaculture International* 29, 1481–1493. (IF 2020 = 2.953)
- Cisar, P., **Bekkozhayeva, D.**, Movchan, O., Saberioon, M., Schraml, R., 2021. Computer vision based individual fish identification using skin dot pattern. *Scientific Reports* 11, 16904. (IF 2021 = 4.996)
- Schraml, R., Hofbauer, H., Jalilian, E., **Bekkozhayeva, D.**, Saberioon, M., Cisar, P., Uhl, A., 2020. Towards fish individuality-based aquaculture. *IEEE Transactions on Industrial Informatics* 17, 4356–4366. (IF 2020 = 10.215)

Peer-reviewed journals without IF

- Bekkozhayeva, D.**, Saberioon, M., Císař, P., 2019. Image based individual identification of Sumatra barb (*Puntigrus tetrazona*). In: Rojas, I., Valenzuela, O., Rojas, F., Ortuño, F. (Eds), *Bioinformatics and Biomedical Engineering. Lecture Notes in Computer Science* 11465, 7th International Work-Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2019, Granada, Spain, 116–119. (SJR 2018 = 0.283)
- Bozhynov, V., Souček, P., Bárta, A., Urbanová, P., **Bekkozhayeva, D.**, 2019. Dependency Model for Visible Aquaphotomics. In: Rojas, I., Valenzuela, O., Rojas, F., Ortuño, F. (Eds), *Bioinformatics and Biomedical Engineering. Lecture Notes in Computer Science* 11465, 7th International Work-Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2019, Granada, Spain, 105–115. (SJR 2018 = 0.283)
- Urban, J., **Bekkozhayeva, D.**, Bozhynov, V., Bárta, A., Urbanová, P., Souček, P., 2019. Zařízení pro standardizované fotografování živých objektů, zejména ryb. Užitiný vzor 32990. Úřad průmyslového vlastnictví, Praha.
- Urbanová, P., Bozhynov, V., **Bekkozhayeva, D.**, Císař, P., Železný, M., 2019. Pipeline for electron microscopy images processing. In: Rojas, I., Valenzuela, O., Rojas, F., Ortuño, F. (Eds), *Bioinformatics and Biomedical Engineering. Lecture Notes in Computer Science* 11465, 7th International Work-Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2019, Granada, Spain, 142–153. (SJR 2018 = 0.283)

Abstracts and conference proceeding

- Bekkozhayeva, D.**, 2021. Image based individual fish identification. In: USB conference of doctoral students “Over the horizon and for mutual acquaintance”. Ceske Budejovice, Czech Republic. November 3–4, 2021. (Oral presentation)
- Bekkozhayeva, D.**, Císař, P., 2021. Image based automatic individual identification of fish without obvious pattern on a body (scale pattern). In: EAS (eds.), *Aquaculture Europe 2021 Abstract*, Madeira, Portugal, October 4–7, 2021. (Oral presentation)

- Císař, P., **Bekkozhayeva, D.**, Movchan O., 2021. Fish individual identification – Can we substitute invasive tagging? In: EAS (Eds), Aquaculture Europe 2021 Abstract, Madeira, Portugal, October 4–7, 2021. (Abstract)
- Movchan O., Císař, P., **Bekkozhayeva, D.**, 2021. Deep learning fish in tank behaviour monitoring. In: EAS (Eds), Aquaculture Europe 2021 Abstract, Madeira, Portugal, October 4–7, 2021. (Abstract)
- Bekkozhayeva, D.**, Saberioon, M., Císař, P., 2019. Image based individual identification of Sumatra barb (*Puntigrus tetrazona*). 7th International Work-Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2019, Granada, Spain, May 8–10, 2019. (Oral presentation)
- Bekkozhayeva, D.**, Saberioon, M., Císař, P., 2019. Individual photo – identification of fish with visible skin patterns (Sumatra barb *Puntigrus tetrazona*) using computer vision. In: EAS (Eds), Aquaculture Europe 2019 Abstract, Berlin, Germany, October 7–10, 2019. (Oral presentation)
- Císař, P., **Bekkozhayeva, D.**, 2019. Computer vision based fish individual identification as an alternative to fish tagging. 7th International Work-Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2019, Granada, Spain, May 8–10, 2019.
- Bárta, A., Souček, P., Bozhynov, V., Urbanová, P., **Bekkozhayeva, D.**, 2018. Trends in online biomonitoring. 6th International Work-Conference on Bioinformatics and Biomedical Engineering (IWBBIO2018), Granada, Spain. (Abstract)
- Bozhynov, V., Soucek, P., Barta, A., Urbanova, P., **Bekkozhayeva, D.**, 2018. Visible aquaphotomics spectrophotometry for aquaculture systems. 6th International Work-Conference on Bioinformatics and Biomedical Engineering (IWBBIO2018), Granada, Spain. (Abstract)
- Urbanova, P., Cyran, N., Soucek, P., Barta, A., Bozhynov, V., **Bekkozhayeva, D.**, Cisar, P., Zelezny, M., 2018. Unsupervised parametrization of nano-objects in electron microscopy. 6th International Work-Conference on Bioinformatics and Biomedical Engineering (IWBBIO2018), Granada, Spain. (Abstract)

Projects

Non-invasive fish identification – scale patterns. TNA projects. PID:AE140007.

Training and supervision plan during study

Name	Dinara Bekkozhayeva
Research department	2017–2022 – Laboratory of Signal and Image Processing, Institute of Complex Systems of FFPW USB
Supervisor	Jan Urban Ph.D.
Period	23 rd October 2017 untill 14 th September 2022
Ph.D. courses	Year
Basic of scientific communication	2018
English language	2018
Biostatistics	2019
Ichthyology and fish taxonomy	2019
Pond aquaculture	2019
Applied hydrobiology	2019
Scientific seminars	Year
Seminar days of FFPW	2018
Seminar days of FFPW	2019
Seminar days of FFPW	2020
Seminar days of ICS, Nove Hradý	2020
Seminar days of FFPW	2021
International conferences	Year
Bekkozhayeva, D. , Saberioon, M., Císař, P., 2019. Image based individual identification of Sumatra barb (<i>Puntigrus tetrazona</i>). In: Rojas, I., Valenzuela, O., Rojas, F., Ortuño, F. (Eds), Bioinformatics and Biomedical Engineering. Lecture Notes in Computer Science 11465, 7 th International Work-Conference on Bioinformatics and Biomedical Engineering, IWBBIO 2019, Granada, Spain, 116–119. (Oral presentation)	2019
Bekkozhayeva, D. , Saberioon, M., Císař, P., 2019. Individual photo – identification of fish with visible skin patterns (Sumatra barb <i>Puntigrus tetrazona</i>) using computer vision. In: EAS (Eds), Aquaculture Europe 2019 Abstract, Berlin, Germany, October 7–10, 2019. (Oral presentation)	2019
Bekkozhayeva, D. , Císař, P., 2021. Image based automatic individual identification of fish without obvious pattern on a body (scale pattern). In: EAS (Eds), Aquaculture Europe 2021 Abstract, Madeira, Portugal, October 4–7, 2021. (Oral presentation)	2021
Foreign stays during Ph.D. study at RIFCH and FFPW	Year
Prof. Andreas Uhl, Computer science department, University of Salzburg, Salzburg, Austria Data processing of Atlantic salmon, extraction of the feature vectors and classification from two different experiments: short-term experiment – for proving the uniqueness of the visible features, and long-term experiment – for proving the stability of patterns (features) during cultivation period.	2019
Stavros Chatzifotis Ph.D., Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Center for Marine Research, Thalassocosmos, Gournes Pediados, Heraklion, Crete, GREECE Data collection (images) of sea bass, for short-term experiment – for proving the uniqueness of the visible features.	2019

Pedagogical activities	Year
• Participation on the realization of the workshop focused on the bioinformatics with the lectures „The Kinetics of Biological Control” given by Prof. Peter Ruoff and „Big Data Cybernetics” given by Prof. Harald Martens	2017
• Leadership of Daria Zabelina and Anastasiia Rozhyna summer school project entitled Non – invasive individual fish identification based on scale patterns.	2018
• The collaboration on the supervision of Ph.D. students Oleksandr Movchan and Oleksandr Mashchenko for the specific task.	2020
• The collaboration on the supervision of Ph.D. student Oleksandr Movchan for the specific task.	2021
• Helping in the summer school project entitled “Fish photo identification demonstration system – real conditions”. Project leader is Petr Cisar, Magdaléna Kůtová is a student. Teaching how the identification of the individual fish from the images works.	2021

Curriculum vitae**PERSONAL INFORMATION**

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**EDUCATION**

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2014–2016 M.Sc., Faculty of Biology and Biotechnology, al-Farabi Kazakh National University,
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PROFESSIONAL EXPERIENCE

07/2016–09/2017 Junior researcher, Research Institute of Biology and Biotechnology Problems,
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01/2015–06/2016 Trainee – researcher, Research Institute of Biology and Biotechnology
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TRAINING

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