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An automated vision system for measurement of zebrafish length using low-cost orthogonal web cameras

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ABSTRACT

Fish size inspection is becoming increasingly important in aquaculture applications and research including growth monitoring, an early indication of health problems and others. However, sizing of small fish has not yet been thoroughly explored in the literature due to challenges associated with rapid and unpredictable changes in the swimming direction of such fish. This paper presents a new low-cost computer vision system for estimating the length of small fish length using dual synchronized orthogonal webcams. The contour and location of the fish body are identified through continuous capturing of front and side images of the fish population under study. A mathematical model that accounts for the projection error that is mainly caused by the depth from the camera and light refraction is derived and implemented in this study. An automatic calibration procedure is also suggested to account for light-refraction during the fish length measurements. Similarly, a camera distance calibration is performed experimentally and considered throughout the estimation process of the fish length. The performance of the developed vision system is assessed experimentally using individual free-swimming adult zebrafish. The obtained results for the subjects under test have demonstrated an average estimation error of approximately 1%. Such a relatively high estimation performance demonstrates the validity of the proposed model and compares favourably to the state of the art of small-fish sizing.

1. Introduction

Video measurement and tracking systems have been widely used in aquaculture to measure fish and play a significant role in the enhancement of fish welfare levels. Furthermore, the continuous growth in fish research highlights the need for monitoring devices and procedure as that can acquire relevant information remotely (e.g. Beddow et al., 1996; Lines et al., 2001; Butail and Paley, 2012). Numerous studies on detection and tracking methods of underwater objects have been reported in the literature (e.g. Stien et al., 2007; Duarte et al., 2009; Martinez-de Dios et al., 2003) and using image processing-based methods (e.g. Shi and Karl, 2005; Shiau et al., 2012; Chaturvedi et al., 2013). A comparison between the length-estimates mean from simultaneous underwater visual census diver-operated stereo video measurements for four fish-species was reported (Davis et al., 2015) to examine a technique that quantify and correct for observer bias in individual bias. In this study, the authors emphasized that an improvement in the length-estimation accuracy was achieved using a dive-specific correction factor.

Several computer vision methods have also been found to be particularly effective, however, due to their positive impact in remote monitoring under various operational conditions (e.g. Ruff et al., 1995; White et al., 2006). A 3D-infrared underwater monitoring system has also been proposed by Pautsina et al. (2015), using water's high level of absorption of the near-infrared light range as the main distance indicator as reflected in the brightness of the camera image. Another method was reported in Rizzo et al. (2017), using a paired-laser photogrammetric for fish-length estimation. In this method, a set of digital photographs was taken for each fish individual by using waterproof camera equipped with two parallel lasers that were amounted on both sides of the camera. It was claimed that fish-length estimation was achieved with high accuracy. However, this system requires a precise alignment for the laser beam and is expensive and cumbersome to setup.

Thus, many computer vision systems based on image processing have been proposed for monitoring, tracking and size estimation of free-swimming fish. For example, Martinez-de Dios et al. (2003) reported an automated system for fish sizing based on stereo vision in sea cages with several pre-processing algorithms to compensate for the local variations in light illumination. Costa et al. (2006), meanwhile,

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developed a geometric algorithm with Fourier analysis to outline the fish body and to estimate its size using an underwater imaging system with a dual camera module connected to a portable waterproof PC. It was reported that the accuracy of fish-size estimation was improved by a post-processing procedure based on a neural network for error correction. Costa et al. (2009) also reported a stereo imaging system that utilizes dual-cameras with an optical ranging system to estimate the size of Bluefin Tuna fish remotely. An Artificial Neural Network (ANN) was suggested to recognize the fish species. Furthermore, a single underwater side camera system has also been utilized to measure the size of different fish species (Zion et al., 2007). Another technique for measuring a tuna's snout to fork length (SNFL) in digital images using hand-held camera was reported in (Hsieh et al., 2011). The images were taken on the deck of tuna fishing vessels. As reported, this technique can be efficiently used to estimate length of large stock fish but not free-swimming small fish.

Fish sizing systems have been mainly applied to larger-sized commercial species of fish, with only a few studies and computer vision systems having been reported to address the sizing challenges of smaller fish species. For instance, a manual separation, counting and inspection process for small commercial tropical guppy fish (Poecilia reticulate) has been reported (Karplus et al., 2003, 2005), as has gender classification based on extracting the shape and colour features for the same kind of fish species (Zion et al., 2008). The results of these studies demonstrate that these applications can have a positive effect in terms of reductions in labour cost and other aspects. Another small tropical fish species (zebrafish) has recently emerged as an experimental animal in various aspects of biological studies (e.g. Steenbergen et al., 2011; Stewart et al., 2011; Das et al., 2013), and large numbers of this kind of fish are now commonly used in laboratory experiments. There is, therefore, a demand for new monitoring, tracking, sizing and behavioural analysis techniques applicable to zebrafish (e.g. Al-Jubouri et al., 2014; AlZubi et al., 2016). This is a developing area of research that has not yet been thoroughly explored in the literature, with currently only a limited number of studies investigating behavioural aspects of zebrafish (e.g. Siccardi et al., 2009; Papadakis et al., 2012; Papadakis et al., 2014; Al-Jubouri et al., 2015). None of these studies, however, has tackled the challenges of sizing small fish including the rapid and unpredictable changes in the swimming direction of the fish.

Addressing these urgent needs, here we introduce a new method for estimating the length of free-swimming fish and outline its potential application to small-fish (zebrafish) sizing. A new cost-effective vision system based on dual synchronized orthogonal webcams is designed and developed. The segmented area and location of the fish body are identified through continuous capturing of front and side images of the fish. A mathematical model is proposed for fish-length estimation that accounts for the fish-length projection error. An automatic calibration procedure is also suggested to account for light-refraction during the fish-length measurements. A camera-distance calibration is also assessed experimentally and the performance of the entire sizing system is evaluated using couple of free-swimming zebrafish.

The remaining part of the paper is organized as follows. Section 2 describes the experimental setup; the mathematical model is presented in Section 3; Section 4 describes computer-vision system; Section 5 presents the obtained experimental results that are discussed in Section 6.

2. Materials and methods

The proposed system comprises a transparent-glass swimming container (i.e. water tank), two calibration objects, two web cameras and a computer. A generic laptop is used for data collection, processing and monitoring purposes: Intel R Core™ i5–3320 M CPU @ 2.6 GHz, 8.0 GB RAM, running MATLAB version 2013b (MathWorks, Inc.; Natick, MA, USA) and 64-bit Windows™ 7 operating system.

The main components of this experimental setup are shown in Fig. 1 and are described briefly as follows.

2.1. Water tank

This is made up of 3 mm thick glass with dimensions of 32 × 20 × 24 cm (length, width and height), filled with water to a height of 18 cm. The top of the tank is open and exposed to ambient lighting while its bottom is clear. Two calibration objects (coins with 2.03 cm diameter) are fixed to two adjacent sides of the tank.

2.2. Web cameras

Two generic low cost webcams (TRIXES) with the following specifications are used in this study; video frame size of 640 × 480 pixels (i.e. 300k pixels), 29 frames per second, and a focal length of 3.85 mm are used in this study. These cameras are fixed at the front and side views of the swimming container/tank with different camera-tank distance, which helped capturing a full-vision for both sides of the tank. The front camera is used as the main source of image capturing to measure length of the fish body while the side camera only measures location of the subject. Knowing the camera's depth of focus helps obtaining a clear image but such a specific information is not available in the technical specifications of the low-cost web camera used in this study, thus making calculation of this factor (i.e. the depth of focus) a difficult task. Instead, the depth of focus is assessed experimentally through immersing a coin object in the center of the tank and adjusting the focal point manually until a clear image is obtained. Two snap images for first and last glass surface of the tank are then acquired to evaluate the effect of the obtained focal point on the detection quality of the front camera. Only a slight difference in the image quality was observed between the images taken at the first and last glass surfaces of the tank. The depth of focus for the front camera is therefore considered approximately equal to the depth of the tank.

Two coins are used to calculate the calibration factor (mm/pixel) as calibration objects. Utilisation of these objects helps in obtaining real physical metric of the fish under test as well as estimating the camera-tank distances $Z_{\oslash 1}$ and $Z_{\oslash 2}$ automatically. A similar calibration procedure is adopted for tracking and length estimation by both cameras.

2.3. Test subjects

Two free-swimming adult zebrafish with different lengths; 42 and 45 mm are used in this study. These fish are placed in two tanks with previous mentioned dimensions which filled with room temperature (25 °C) and filtered water. The actual length of fish is measured by using additional small glass tank with 30 × 11 × 17 cm (length, width and height) and a flat piece of white plastic is used to temporary confine the fish under test in a certain desired area closed to the ruler scale, as shown in Fig. 2. The actual fish length can therefore be measured, using a ruler fixed at the front-side of the test tank. It should be mentioned here that this manual measurement is only required for comparison with the fish-length estimation by the proposed vision system.

3. Mathematical modelling

3.1. Challenges and assumptions

The typical inverse proportionality relationship between distance and apparent length is a common phenomenon in vision systems. In vision-based sizing systems, therefore, this phenomenon is considered as a measurement challenge, where the actual length of fish needs to be reconstructed from a measured one that normally does not represent the real physical length. In the present study, the refraction through glass is negligible due to the relatively small thickness of the wall's tank. The effect of water refraction is, however, a significant parameter. The proposed computer-vision system is based on a mathematical