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Sub-second analysis of fish behavior using a novel computer-vision system

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ABSTRACT

This work presents an advanced version of a previous computer vision system that is appropriate for analyzing more complex fish behavioral traits. The system is capable of long-term recording of fish escape and bite behavior in tanks with excellent sampling accuracy and a minimum number of frames lost. In addition, the system is able to simultaneously record from nine different tanks with nine respective cameras, thereby allowing for specific experimental designs for statistical purposes. The evaluation of the system's operation and capabilities is achieved under specific biological activities in laboratory experimental conditions, with the activities duration similar to the system time characteristics. A sub-second analysis resulted in a detailed description of the escape and bite patterns of sea bream and bass in discrete steps, according to the video sequences. In general, the system was found to be able to assist in performing the behavioral studies of farmed fish. The final cost-effective system is characterized by long recording periods of high sampling accuracy, multiple digital camera acquisitions with high image quality, and state-of-the-art consistency.

1. Introduction

Remote monitoring of fish behavior can potentially provide essential information related to the population status (Huntingford et al., 2006; Johansson et al., 2006; McFarlane et al., 2004; Papadakis et al., 2012; Pinkiewicz et al., 2011) without the requirement of fish handling, which increases the stress conditions. Video technologies (Grubich et al., 2008; Kane et al., 2004; Mueller et al., 2006; Papadakis et al., 2012; Salierno et al., 2008; Stien et al., 2007; Wagget and Buskey, 2007) offer an inexpensive and reliable method to monitor and analyze fish behavior. Behavioral observation of fish activity requires a tremendous amount of recording time to gather multiple measurements of species-specific events. For this purpose, many systems have already been developed, focusing on the estimation of the position (to successfully detect the fish position) of individuals in two dimensions (Delcourt et al., 2013; Kane et al., 2004; Mueller et al., 2006; Papadakis et al., 2012; Pinkiewicz et al., 2011; Stien et al., 2007).

Automated data recording techniques have been developed during the last several years that allow for the remote observation of fish behavior in aquaculture (Pinkiewicz et al., 2011). In laboratory conditions, the developed technology (Duarte et al., 2009; Grubich et al., 2008; Papadakis et al., 2012) successfully recorded and automatically analyzed the frequencies of specific behavioral events (number of events in a specific time period). As a function of the behavioral type, the sampling method is very important, and depends directly on the time scale. A common problem that often arises with these systems is the significant number of lost frames in the video data, due to the long experimental periods that in turn result in a number of lost events. In addition, the instability of the quasi-continuous time sampling prevents the successful analysis of the related steps of each behavioral pattern, which are very short in duration. Furthermore, the number of lost frames in the video recordings has never been an important parameter in behavioral studies and has not being calculated yet, due to the nature of behavioral analysis (the counting of behavioral events).

All previously developed automated methodologies allowed experimenters to save time and, most importantly, to free them from their time-dependent perception and mistakes due to fatigue. These methods are specialized in recording and analyzing specific behavioral traits, primarily focused on the effect of time in many biological aspects, including circadian function and behavioral sequences. Furthermore, these methods are based on the analysis of the majority of dynamic parameters such as speed, acceleration, turning rate and group cohesion variations. In

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contrast, these systems are unable to record over a long time period in combination with a high frame rate and high image quality. These methods are too tedious and manually complex to allow for automatic detection and measurement of short behaviors, such as biting and special flapping of fins, over a long duration.

The objective of this development was to enable recordings over long experimental periods in combination with a high frame rate and high image quality. Enabling such recordings will allow for the accurate and sub-second analysis of the behavioral traits without modifying the possibility for extended video recordings from nine cameras. To achieve this capability, the system was designed to accurately acquire frames in specific time intervals (111 ms) while minimizing the possible frame losses in daily recordings. With this system, it is possible to study the differences in a specific behavioral trait in discrete steps and to observe the factors that vary over long experimental periods (i.e., starvation).

2. Materials and methods

2.1. Hardware design

A computer vision acquisition system was redesigned to operate and record over long experimental periods at a high frame rate and high image quality. Three individual computers are installed, with each one connected to three cameras. Each computer consists of a typical processor (Pentium (R) Dual-core 5300 2.6 GHz, Intel), and 1 TB of HDD storage capacity. The on-board GigE adapter is used, while an additional fire-wire IEEE1394 PCI interface board (Fireboard-Blue, Unibrain) is installed to connect to the 3 digital CCD cameras. All three computers are connected via (USB KVM Switch, CS-64U) to a single monitor, keyboard and mouse. Communication between the three workstations and Internet access is achieved via an Ethernet splitter (D-Link, DGS-1008D, 1 Gbit). The total cost remains very low (1000 € for the cameras, and less than 1000 € for all of the computers and related hardware), providing a computer-vision system that is very inexpensive, reliable and fully capable of operation for laboratory behavior research.

2.2. Software development

Two main applications (acquisition and processing) were developed to assist the system operation. All of the software modules were written via the LabView programming interface, with an additional use of the Vision Development Module (National Instruments).

2.3. Acquisition application

The acquisition application is programed to acquire a single frame from all connected cameras every 111 ms. A multi-cropping algorithm enables the recording only of a pre-selected area of interest (AOI) from each camera view. A new image is then formed that contains only the AOI from the cameras. This approach minimizes the size of the image to be stored to HDD that in combination with a compression algorithm (DivX 4.12, DivX) minimizes data volume. The system records 24 h a day. Each day is saved (at 00:00 h) in a separated video file allowing recording for long experimental periods (i.e., 1 month). In order to reduce any human disturbance during the experiments, a remote control system was developed, via a webserver application installed in each computer. The webserver update settings were initialized to provide one image every 1 s to reduce the processor time requirements and the network capacity.

2.4. Analysis application

Processing and analysis of the video data are achieved by a software application (FVA), which is an advanced version of the algorithms described in (Papadakis et al., 2012). The application is programmed to accept DivX video files, and the analysis is performed in two time intervals (every 111 m and 1 s).

The user initially selects the time periods for analysis. Subsequently, the green color plane was selected because it had the best contrast between the objects and the background. For the first 300 frames of the selected time period, the application calculates the background through an averaging algorithm. Every frame of the video file is then subtracted by the background image, resulting in a new image containing only the moving fish. An object detection algorithm detects the fish and returns the total number of fish found, along with the individual framed fish size and position.

During the analysis, an alarm occurs when the distance of an individual fish from a preset point of interest (POI) is smaller than a critical value (10 pixels). In this case, the user must check whether the extracted frame contains information related to fish activity. The relevant frames are extracted and stored as BMP images in the HDDs of the computers, to a specific location, with a new folder named by the filename of each of the videos.

2.5. Experiments

The aforementioned computer vision system was evaluated under two successive on-going experiments that involved the study of the net-biting activity and escape to the other compartment of the tank for sea bream and sea bass. Both behaviors were selected due to their significance in commercial-scale aquaculture and the potential risk for fish escape. In particular, bite activity was studied in the sea bream, a farmed species that regularly interacts with fairly damaged nets (Papadakis et al., 2013a,b). In addition, because sea bass rarely exhibit any exploratory behavior on the net pen (Papadakis et al., 2013a,b), the present study only focused on the motivation of fish to cross the net tear. Both experiments were performed over 30 days. Such studies require both the monitoring in an appropriate time resolution for the selected behavioral trait analysis and monitoring over long experimental periods.

2.5.1. Experimental setup

The experiments were conducted in nine rectangular cuboid and polyester tanks of 100 L in volume (length: 115 cm, depth: 40 cm, width: 34 cm), filled with artificial seawater (salinity: 38 ppt). The one longest side was replaced by a transparent thick glass (5 mm) that allowed for fish observation throughout the experimental period. All of the tanks were connected to an external biological filter (EHEIM 20 W) for water recycling and an air pump for oxygen saturation (> 85%). A pair of fluorescent tubes (30 W) was installed over the water surface of each tank (50 cm) to control the illumination of the tank (330 ± 7 lux). The photoperiod was set to 12 h L:12 h D. Infrared illumination would be an option to observe fish activity during the 12-h D period. According to the experimental protocol, the tanks were split into two compartments (50:50% of the tank volume) by a removable net pen ($31\,cm \times 28\,cm$, with a 17-mm mesh eye). A tear that was located centrally on the net pen allowed for fish crossings between the compartments of the tank. The tear had an appropriate size (5.1-cm height), based on the average fish height (4.0 ± 0.1 cm). The fish were confined into the left area of the tanks, and then the system initiated the monitoring process.

2.5.2. Experimental fish

A total of 270 fish (2 group $\times$ 135 fish) were used in this study. The fish (15 individuals/tank) were initially left to acclimatize in the tank environment for a period of 7 days; during this time,