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An automatic counting system for transparent pelagic fish eggs based on computer vision

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ABSTRACT

Alive eggs of marine species with pelagic eggs float, while dead eggs usually sink. A non-invasive method for counting the number of floating eggs therefore gives the possibility to track survival throughout experiments. In this paper we present an automatic image analysis method for counting live pelagic eggs of marine fish. Pelagic fish eggs are typically transparent and difficult to detect in images. Current image analysis methods for counting pelagic fish eggs are therefore done on eggs transfixed in a polymer to create contrast between the eggs and the background. This kills the eggs. The main advantage of the presented method is that it is non-invasive and only requires a minimum of handling of the eggs. As case studies we collected images of Atlantic haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) eggs. The eggs in the images were manually counted for verification of the methodology. The average counting error of false positives was 6% and the average counting error of false negatives was 2%. This demonstrates that the method is objective and accurate.

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1. Introduction

Pelagic fish eggs are typically transparent and float near the surface, while eggs that die typically sink (Pelster, 1997). This entails that counting the number of floating eggs may be used to track survival rate of eggs throughout experiments. Traditionally, the quantity of fish eggs is determined either by counting them one by one using a pipette, or by estimating the number of eggs by measuring the total volume or weight and calculate the numbers from established correlations between volume/weight and egg numbers (Simpson, 1951; Davies and Paulik, 1965; Boyar and Clifford, 1967; Dodgshun, 1980; Parrish et al., 1980; Tanasichuk et al., 1985; Witthames and Walker, 1987; Kjesbu, 1989; Musonda, 1999; Gundersen et al., 1999; Hinshaw and Thompson, 2000). Counting using a pipette produces an exact count of the eggs, but requires an experienced operator, that the eggs are dissociated and that the sizes of eggs are just below the diameter of the tube. Counting this way is tedious and time-consuming. Estimating the number of eggs from total sample weight or volume is far less laborious, but also less accurate and in addition requires a measurement of dry egg volume and this is rough treatment of living fish eggs (Browne, 2009). In addition, some automated electronic methods and devices for counting fish egg have been described (Witthames and Walker, 1987). These, however, rely on object singulation and size uniformity which may limit their value for commercial operations (Zion, 2012).

An alternative to the traditional methods of counting eggs is digital image analysis. Pohar and Pen (1983) used photocopying for counting salmonid eggs. Thorsen and Kjesbu (2001) produced images of oocytes fixed in formaldehyde by illuminating them from behind. These oocytes were in the vitellogenic stage where they have a brownish color and therefore easily stand out from a bright background in contrast to fertilized eggs which are transparent. Friedland et al. (2005) developed an image analysis method for scanned images of fish eggs in Petri dishes. This method first transfixes the eggs in a polymer before they are scanned. The method requires that the eggs are loosely distributed and not too close to the sides of the Petri dish. The back part of the Petri dish is painted black and the result of the treatment is an image where the eggs stand out as white discs in front of a black background. The eggs can therefore relatively easily be identified by automatic image analysis algorithms, and hence counted. Mello et al. (2009) developed a

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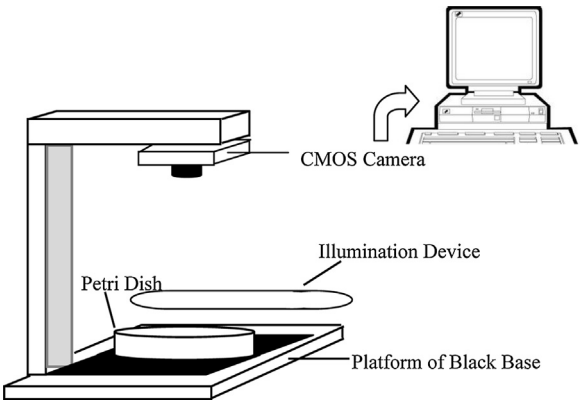

Fig. 1. Schematic outline of the main hardware components of the system. The camera is mounted 160 mm above the Petri dish and the light tube illuminates the Petri dish 130 mm from the side.

semi-automatic method to count the number of eggs in ovitraps images using image processing, particularly color segmentation and mathematical morphology-based non-linear filters.

The above-mentioned image analysis methods use fixated material and can therefore not be applied on living eggs. The main objective of this paper is to develop a non-invasive automatic method to count living eggs by using computer vision technology.

2. Materials and methods

2.1. Eggs and sampling

Haddock eggs were collected on 28 March 2014 from spawning haddock at the Institute of Marine Research's research station in Austevoll, Norway. The eggs were carefully retrieved from egg collection tanks using a 10 ml plastic cylinder with a plankton-net in the bottom (180 μm). The samples were first quickly drained for water and the volume (precision 0.1 ml) measured before the eggs were poured into a Petri dish with some seawater (2–5 ml eggs in each Petri dish, a total of 113 samples). The Petri dishes were photographed, and the eggs immediately transferred to experimental tanks for continued incubation. Egg numbers were also counted manually on 32 photographs. In addition, to test the image analysis method (presented below) with eggs from another marine fish with transparent pelagic eggs, newly fertilized eggs from Atlantic cod were poured into Petri dish with some seawater and photographed (1–5 ml eggs in each Petri dish, a total of 15 samples). The number of eggs counted by means of the image analysis were compared with egg numbers estimated from measured volumes using the calibration from Kjesbu (1989): number of egg per ml (n) = $1222 \times D^{-2.71}$. The average egg diameters (D) of the haddock egg were 1.43 ± 0.06 mm (estimated egg number per ml = 464) and for the cod eggs 1.10 ± 0.02 mm (estimated egg number per ml = 943).

2.2. Automatic counting system

2.2.1. Hardware of system

The hardware of the automatic counting system for transparent fish eggs includes an adjustable bracket, a color digital camera (Canon EOS 650D, Cannon Technologies Corp., Japan), a light tube (38 W, ~50 Hz, l = 290 mm), a Petri dish (∅ = 136 mm, h = 20 mm), and a personal computer (the core of the system is a ThinkPad X230 (Lenovo, China), 2 GB RAM, 500 GB HD, with a window 7 Operating System) (Fig. 1).

The camera is mounted 160 mm directly over the Petri dish, facing downwards. To make the transparent eggs more visible, there

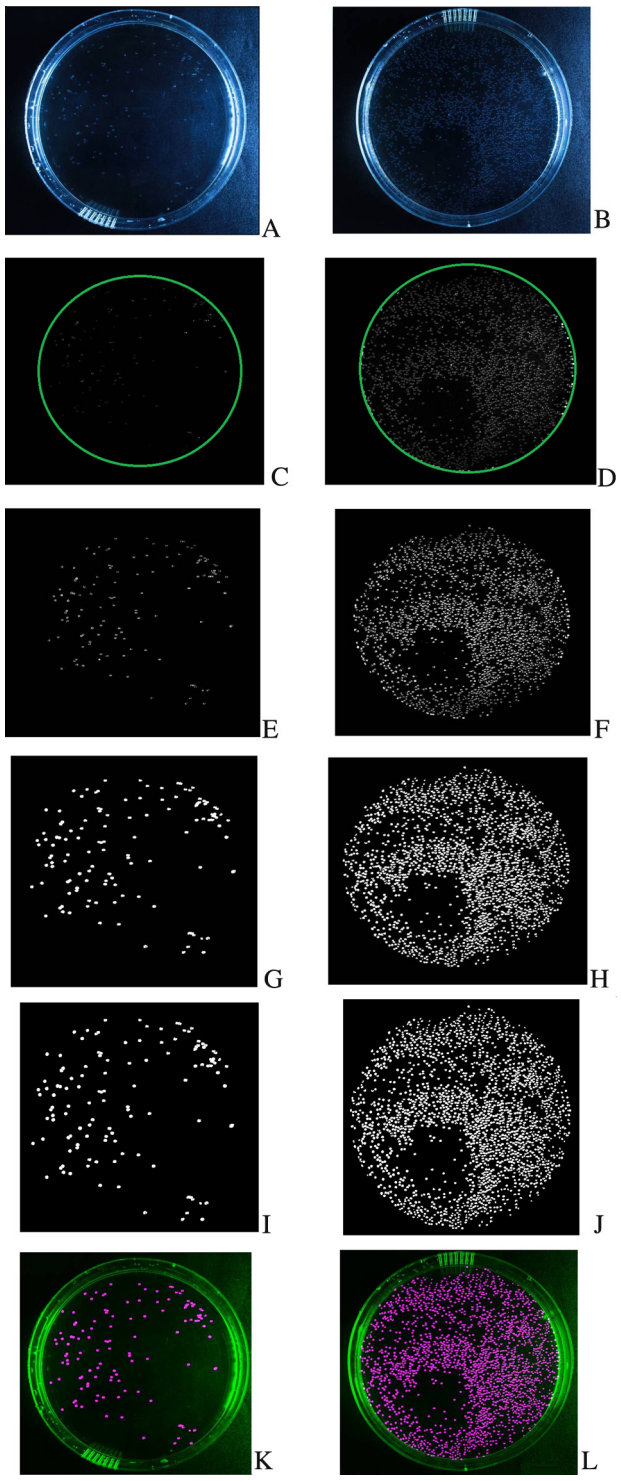

Fig. 2. Original images, result after image pre-processing and result after segmentation of the eggs from the background. (A) Original image of Petri dish with sparse distribution of fish eggs. (B) Original image of Petri dish with dense distribution of fish eggs. (C, D) Gray scale versions of the images in (A) and (B), but where all pixels outside the ROI (indicated by the green circle) have been set to black. (E, F) Results after morphological operations and gamma correction to increase contrast between the eggs and the background and remove noise. (G, H) Results after binarization and morphological operations. (I, J) Results after watershed operation. (K, L) Illustrations of final result.

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