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Low-Cost Computer-Assisted Image Analysis for Fisheries Research

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Abstract.—An image analysis system based on the Macintosh personal computer and public domain software (NIH Image, version 1.57) allows capture of digitized images for data analysis. Three techniques for image capture and aquisition of linear and area measurements from digitized images were tested: (1) estimation of egg size, (2) estimation of number of eggs or fry, and (3) measurement of fish length. For estimation of egg size, egg samples were taken by catheterization from two female channel catfish *Ictalurus punctatus*. Egg diameters measured manually (1.82 ± 0.27 and 2.59 ± 0.19 mm) were not significantly different ($P = 0.08$ and 0.20) from the diameters estimated with image analysis (1.92 ± 0.21 and 2.63 ± 0.17 mm). For egg counting, channel catfish eggs were hand-stripped into Hanks' balanced salt solution and divided into seven 15-mL aliquots. The number of eggs was determined manually and images were captured for analysis. The number of eggs determined manually (436 ± 41) was not significantly different ($P = 0.80$) from the number estimated with image analysis (430 ± 43). Also, total lengths were measured manually on 50 fingerling channel catfish and 50 fingerlings of the hybrid channel catfish × black bullhead *Ameiurus melas*. An image of each fish was also recorded for measurement by image analysis. Replicate sets of measurements were made by three individuals with different levels of experience with the computer system. The mean (±SD) of total lengths measured manually for the channel catfish fingerlings (242 ± 33 mm) was not significantly different ($P = 0.37$) from the lengths determined by image analysis (237 ± 33, 235 ± 33, and 231 ± 34 mm). The mean of total lengths measured manually for the hybrids (157 ± 39 mm) was not significantly different ($P = 0.98$) from the lengths measured by image analysis (158 ± 40, 157 ± 39, and 155 ± 39 mm). The availability of public domain software and inexpensive or commonly available input devices make image analysis more accessible to researchers.

Computer-assisted image analysis is used in applications as diverse as analysis of satellite data (Busbey et al. 1992) and characterization of microscopic organisms (Dallai et al. 1991). Until recently, purchasing the required hardware and software to equip a laboratory for image analysis often required an investment of US$60,000 or more (Bauchan and Campbell 1994). Recent systems have lowered this cost into the $15,000–$30,000 range (e.g., Alpha Innotech, San Leandro, California, personal communication). However, these costs can be minimized by the availability of inexpensive personal computers, readily available input devices, and public domain image analysis software.

Established uses of image analysis in fisheries research, such as truss analysis (Wimberger 1993), rely on accurate linear measurements to determine distances between anatomical landmarks (Lutz et al. 1988, Douglas 1993). The resulting data can be used to characterize a species, to highlight differences between stocks or species, or to identify hybrids. Another application of image analysis uses a process known as thresholding, in which objects of interest, such as eggs or fry, are separated from the rest of the image for counting or size determination.

This paper was written with three goals in mind: (1) to describe a low-cost image analysis system based on the Macintosh personal computer and public domain image analysis software; (2) to suggest a variety of inexpensive or commonly available input devices suitable for image analysis; and (3) to demonstrate the utility of image analysis for fisheries data collection and analysis.

Methods

Image analysis system.—The system used in this study is based on a Macintosh LCIII (Apple Computer, Cupertino, California) with a Motorola 68030 microprocessor (25 MHz). The computer was equipped with an internal 80-megabyte (MB) hard drive, an external 340-MB hard drive, a Motorola 68882 floating point unit math coprocessor, 12-MB random access memory (RAM) and a 14-in (35.6-cm) Magnavox RGB color monitor. However, any Macintosh computer with a color or gray scale monitor and 8 MB of RAM would be suitable for this type of analysis.

Digital cameras use a grid or array of light-collecting picture elements, or pixels, in place of conventional film. Resolution in images produced by digital cameras is determined by the number of

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pixels in the array. In this study, two types of digital cameras were used. The first was the Fotoman digital camera (Logitech, Fremont, California), which is capable of capturing digital images in 8-bit gray scale with a resolution of 376 × 240 pixels. In 8-bit gray scale images, each pixel in the image can be any of 256 gradations, with 0 being pure white and 255 being pure black. This camera stores 32 images and was connected via serial cable to the computer through the modem port for downloading of images to the hard drive. Software packaged with the camera, Digital Darkroom (version 2.01, Silicon Beach Software, San Diego, California), stored images from the camera in the standard tagged image file format (TIFF). The second digital camera was the Quicktake 150 (Apple Computer) capable of capturing images in 24-bit color. In a 24-bit image, each pixel can be any of over 16 million colors. This camera stores 32 low-resolution images (320 × 240 pixels) or 16 high-resolution images (640 × 480 pixels) and was connected via serial cable to the computer through the modem or printer port for image downloading. Software packaged with the camera, Photo Flash (Apple Computer) stored images in several standard file formats. All image analysis was carried out with the public domain NIH Image program (version 1.57).[2] All statistical analyses were performed with Data Desk (version 4.2, Data Description, Inc., Ithaca, New York).

Measuring eggs by area determination.—The Quicktake camera was mounted 30 cm above a fluorescent light box (model E2, Laboratory Supplies Company, Inc., Hicksville, New York). An acrylic sheet (such as the type used on overhead projectors) was used to protect the surface of the light box. A clear ruler was positioned to appear at the bottom of the images to set the scale for each image. Two mature pond-raised female channel catfish *Ictalurus punctatus* were catheterized with the method of Markmann and Doroshov (1983) to obtain egg samples. The eggs, which do not form an adhesive mass until after fertilization, were positioned on the light box to ensure that they did not touch one another before images were captured. This allowed NIH Image to provide an egg count and to give the area occupied by each egg. The 24-bit color images were converted to 8-bit gray scale with the Photo Flash software package. The 8-bit images were further modified with the thresholding function of NIH Image (Tagliavini et al. 1993). Thresholding determines a gray intensity value that differentiates objects of interest from the background. In NIH Image, the degree of thresholding is controlled through the look-up-table (LUT; Figure 1). After thresholding, a binary image was created in which all pixels at or above the threshold value were displayed as black and all below were displayed as white (Figure 2). Typically, items placed on a light box and photographed from above will be the darkest objects in the image and thus can be well differentiated by thresholding. A derivation of the standard formula to determine area of a circle was used to determine the diameter of the eggs: $d = [\sqrt{(A/\pi)}] \times 2$, where d = diameter and A = area. The eggs were also measured manually to the nearest 0.5 mm. The diameters determined with image analysis were compared with a two-tailed t-test to those measured manually.

Counting eggs by area determination.—Eggs were hand-stripped from a channel catfish into Hanks' balanced salt solution (Tiersch et al. 1994) in circular 850-mL plastic containers coated with a thin layer of vacuum grease (Dow Corning, Midland, Michigan). Seven 15-mL replicates of eggs were placed in a single layer on the light box, and images were captured. An additional image was prepared with a known number of eggs (150) to serve as a calibration image. The images were thresholded, total area occupied by the eggs was determined from the binary image (Gatlin et al. 1993), and the data exported to a spreadsheet program (Excel, version 4.0, Microsoft, Inc., Seattle, Washington), where it was formatted for analysis. Because the number of eggs is correlated with the area occupied in a digitized image (Figure 3), the following equation was used to estimate the number of eggs in each sample: $E_s = A_s\,(E_c/A_c)$, where E_s = number of eggs in sample image, A_s = area of eggs in sample image (mm^2), E_c = number of eggs in calibration image, and A_c = area of eggs in calibration image (mm^2). The eggs were also counted manually to compare with the values obtained by image analysis. The mean values for image analysis and manual counting were compared by two-tailed t-test. Time required for estimation of number of eggs with image analysis was compared with the time required to manually count the eggs with a one-tailed t-test.

Length measurements.—Fish were anesthetized

[2] NIH Image was written by W. Rasband at the U.S. National Institutes of Health and is available from the Internet by anonymous FTP from zippy.nimh.nih.gov. Complete documentation and an interactive message board devoted to NIH Image are available on the Internet.

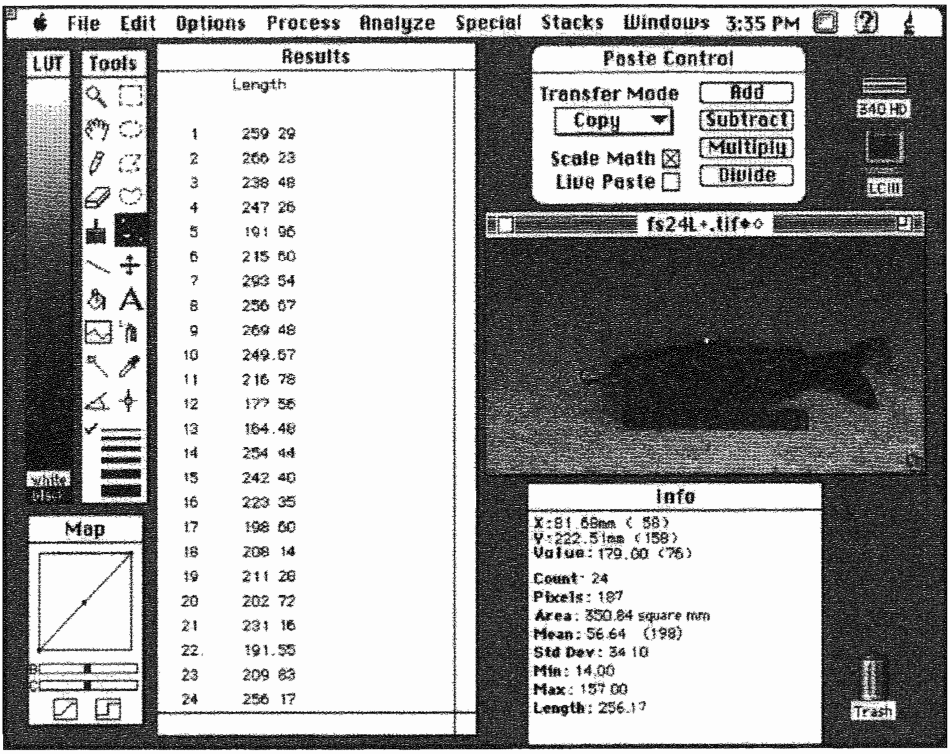

FIGURE 1.—Program display of NIH Image (version 1.57) public domain software configured for measurement of length and compilation of results. The look-up-table (LUT) is used for color selection and thresholding. The tools window contains tools used for object selection and measurement and for basic drawing functions. The map window is used to control brightness and contrast of the current image. The results window stores data from a series of images. The info window contains information on the current image. The active window (black stripes in menu bar) contains the current image. All windows can be moved, resized, and closed to suit the user's needs. Results can be exported to spreadsheet or graphing programs.

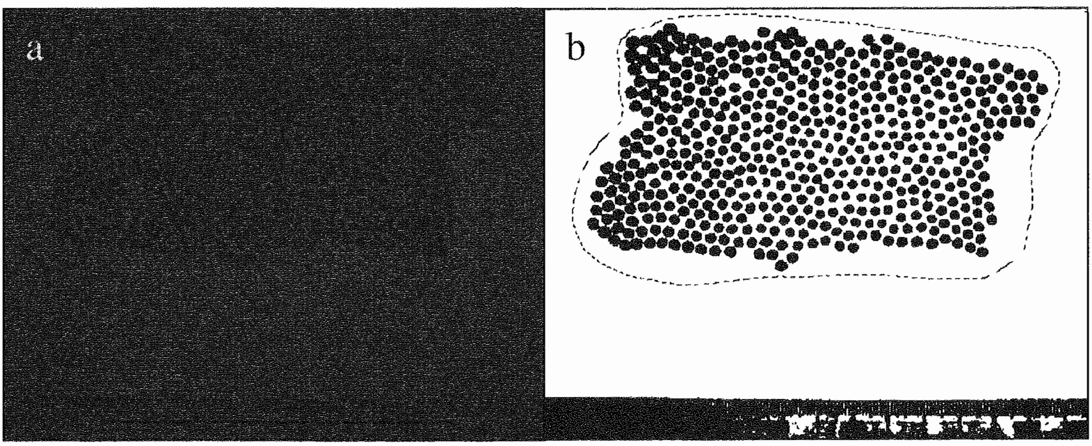

FIGURE 2.—(a) Gray scale image and (b) the resulting thresholded counterpart. The scale is set for each image by drawing a line on the ruler at the bottom of the gray scale image. A region of interest is selected by drawing a circle around the objects to be measured with one of the drawing tools. Following thresholding, only selected objects in the thresholded image are measured.

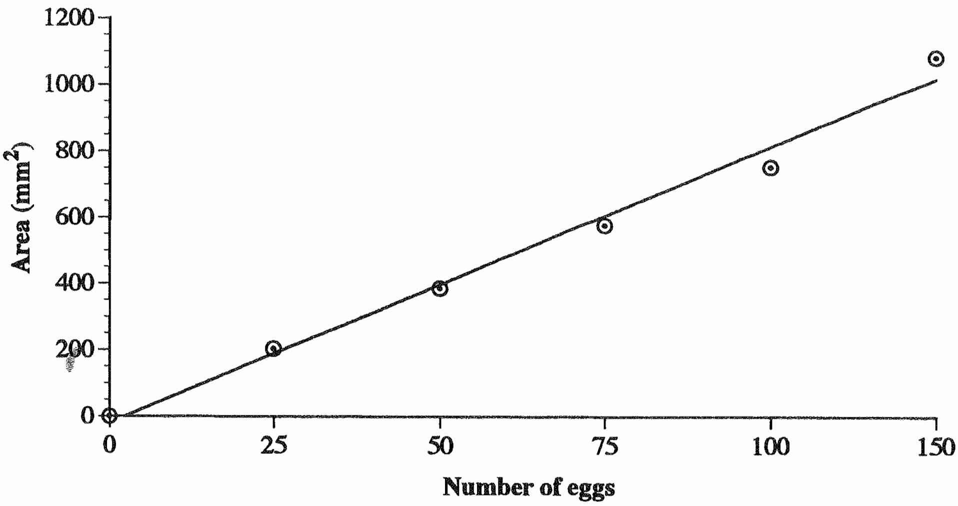

FIGURE 3.—The plot of area and egg number gives a linear relationship (r^2 = 0.98).

with tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, Washington) before they were measured. A standard measuring board (model 153, Wildlife Supply Company, Saginaw, Michigan) was used to manually measure total lengths (TL, to the nearest millimeter) of 50 fingerling channel catfish and 50 fingerlings of the hybrid channel catfish × black bullhead *Ameiurus melas*. A white styrofoam board (1 × 16 × 12 cm) was used as a background for the captured images. The board included a 100-mm reference line and a groove (made with a 2.5-cm-diameter wooden dowel) to ensure the fish would lie flat. An image of each fish was captured with the Fotoman digital camera and downloaded for analysis. The size scale was set for each image by using the reference line, length was measured by drawing a line on the image, and length values were stored in the results window of NIH Image (Figure 1). Length data were exported from NIH Image to a spreadsheet program and formatted for analysis. Statistical significance was set at $P < 0.05$ for all analyses in this study.

Results

Thirty-nine eggs were catheterized from the first female, and the egg diameter that was determined manually (1.82 ± 0.27 mm) was not significantly different (P = 0.08) from that determined with image analysis (1.92 ± 0.21 mm). Fifty-seven eggs were catheterized from the second female, and the diameter that was determined manually (2.59 ± 0.19 mm) was not significantly different (P = 0.20) from that determined with image analysis (2.63 ± 0.17 mm).

The number of eggs counted manually for the seven samples (436 ± 41; range = 100) was not significantly different (P = 0.80) from the number of eggs estimated with image analysis (430 ± 43; range = 109). Time required to count the eggs manually (7.7 ± 1.5 min/replicate) was significantly longer (P = 0.0001) than the time required to estimate the number of eggs by area determination (3.0 ± 0.0 min/replicate).

The lengths measured manually for the channel catfish fingerlings (242 ± 33 mm; mean ± SD) were not significantly different (P = 0.37) from those determined by image analysis (237 ± 33, 235 ± 33, and 231 ± 34 mm). The lengths measured manually for the hybrid catfish (157 ± 39 mm) were not significantly different (P = 0.98) from those determined by image analysis (158 ± 40, 157 ± 39, and 155 ± 39 mm).

Discussion

For hormonally induced spawning, determination of reproductive status of channel catfish females is aided by measurement of egg diameter. Markmann and Doroshov (1983) determined a minimum egg diameter of 2.3 mm for hormonal induction of spawning of channel catfish. With this criterion, the first catheterized female would have been classified as having immature eggs, and the second female would have been classified as having mature eggs (suitable for injection with hormones to induce ovulation).

TABLE 1.—Input devices suitable for use with NIH Image program.

Input device	Advantages	Disadvantages	Cost (US$)
CCD video camera[a]	Real-time image acquisition	Not suitable for field use	3,000–10,000
Camcorder[b]	Suitable for field use, portable	Low-quality images	500–1,000
Internal digitizing board	Image capture from video camera	Must install in computer	800–1,500
External digitizing board	Image capture from video camera	Uses parallel port	200
Slide scanner[c]	Multipurpose, high-quality images	Photographic processing time	1,500–10,000
Flatbed scanner	Multipurpose, commonly available	Photographic processing time[d]	750–3,000
Digital camera	Suitable for field use, portable	Low-quality images	350–10,000
Photo CD[c,e]	High-quality images	Photographic processing time	30[f]

[a] Charge coupled device; requires a digitizing board.
[b] Requires digitizing board.
[c] Requires standard 35-mm slides or negatives.
[d] Only if scanning photographs.
[e] Requires compact disk drive.
[f] Price per 24-exposure roll of film.

Techniques commonly used for counting eggs (e.g., gravimetric and volumetric techniques) work well with relatively large numbers of eggs. However, these techniques are cumbersome and inaccurate for estimating size of replicates for small numbers of eggs (200–500). For example, in this study, even though the same volume was used for each replicate, there was a difference of 100 eggs (minimum = 383 eggs; maximum = 483). When experimental design calls for assignment of treatments to small groups of eggs before fertilization, an image can be recorded and the eggs counted later from the digitized image, reducing the time and handling required for counting. After hatching, fry can be evaluated with the same procedure to determine hatch-out percentage, survival, or growth. For species with adhesive egg masses, images can be recorded after fertilization if the glycoprotein matrix that holds the egg mass together is chemically dissolved to free the eggs (Isaac and Fries 1991). However, care should be taken when chemically treating egg masses because overexposure can weaken the egg membrane and lead to premature hatching of fry (Ringle et al. 1992; our unpublished observations).

Measurements of fish length and weight are common in fisheries field work and in the monitoring of culture species. Carlander (1969, 1977) reported r^2 values between weight and length of at least 0.97. Therefore, the less precise or more time consuming of the two measures can be discarded without the loss of valuable information (Gutreuter and Krzoska 1994). A monitoring system that used length measurement by image analysis without weighing would reduce stress and save time because fish would be handled less. Such a system would be useful with endangered fishes or cultured fishes during sensitive phases of their life cycle. Images could be captured in the field (for example by camcorder) for later analysis (Douglas 1993). Measurement of length directly from a digitized image would provide accurate measurements, reduced input errors, and archivable images for future analysis.

NIH Image uses tools similar to those found in many popular drawing and painting programs available for the Macintosh or Windows platforms (Rasband and Bright 1995). Input devices (hardware required to capture a digitized image for display on a computer screen) account for a major portion of the expense in an image analysis system. A variety of inexpensive or commonly available input devices can be used for image acquisition (Table 1). The input device should be chosen based on the type of work, availability, and affordability. For example, a compromise may be made between the excellent image quality of an expensive charge coupled device (CCD) video camera with digitizing board (Shaw et al. 1995) and the lower image quality of an inexpensive digital camera. More affordable digital cameras with higher resolution are being introduced to the market, but possibly the best value available at this time is use of standard 35-mm slide film in conjunction with Photo CD processing (images on CD ROM). The use of equipment that may be already available as input devices (e.g., flatbed or slide scanners) in combination with public domain software allows access to image analysis at little or no cost.

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