A Simple Technique for Digital Imaging of Live and Preserved Small Fish Specimens

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ABSTRACT The present methodological paper presents a simple technique for digital imaging of small fish specimens using conventional flatbed scanners. Preparing such scanners with a plasticine pool enables fish specimens to be scanned under submerged conditions in water, ethanol or glycerine, depending on whether they are alive or preserved. This technique relies on a quickly prepared, less complicated setup than in photography and provides the opportunity to gain digital images of small fish in the laboratory as well as—with some restrictions—in the field. Lateral scans can be easily made of live specimens after narcotisation or of preserved fish. The scanning method yielded very high-quality images of near-live colors of live fish and of preserved coloration. Images had good contrast, sharpness and illumination, minimal or no shadows and very high resolutions when scanned on high-quality scanners. Depth of field in images was good for fishes of less than 20 cm length and less than 2 cm body width. The method is recommended for applications where digital images are required for body shape analyses, such as geometric morphometric approaches, for qualitative or quantitative analyses of coloration patterns, for fish (re-)identification, and as a basis for illustrations or for publication in electronic sources or print media.

Photographic documentations of live and preserved fish specimens are very important in ichthyology and have a wide field of applications. Fish images are used in species' descriptions, taxonomical issues, morphology and for fish identification. They usually rely on photographic techniques. Emery and Winterbottom (1980) presented a technique for fish photography using vertical aquaria in the field to obtain near-live color photos. Holm (1988) improved this technique but presented a highly complex setup for taking pictures of differently sized fishes. Most photographic techniques for application in ichthyology were published in the 1980s but little updated information is available from the last decade, the time of rapid advances in digital camera technology. Digital photography has considerably eased scientific photographic applications. High-quality digital photos are useful for morphology, presentations, exhibition on the internet, and for the submission to scientific journals or publication in books or field guides. They can be easily processed on a PC and also be used for modern applications such as geometric morphometric approaches, which require numerous digital images for quantitative morphological analyses (e.g. Kassam et al., 2003). Even with the broad use of digital cameras, however, some physical constraints of photography remain, and the technical setups necessary to gain high-quality images are still complex. The need for sufficient light and the avoidance of shadows requires highly efficient strobe systems or high-power lamps and variable setups in laboratory photography (Holm, 1988). The treatment of curved preserved specimens is a further common problem in photographic techniques. Thus, many changes in the technical setup and time-consuming handling may be necessary to accurately document a large series of specimens of different sizes, shapes and/or colors. Documentation of live and preserved fishes may require different setups as well (Rinne and Jakle, 1981; Holm, 1988). Image distortion, a lack of depth of field and sufficient resolution, especially in macro-photography of tiny specimens (<2 cm), are other problems that can only be minimized by using technically advanced, high-resolution cameras. However, such cameras are expensive and the technical setups require specific expertise and photographic skills. Here, we present an alternative technique that is simple, cheap and very efficient for several applications. We expect it to be useful for museum curators, taxonomists, fish morphologists, illustrators, book authors and any researcher who needs to take digital images of live or preserved fish specimens. This technique is not based on cameras but on conventional

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flatbed scanners, which can be specifically adapted. Suitable scanners are present in most laboratories. Such scanners have a largely unknown potential for yielding high-quality images after a quick adaptation of the usual scanner setup. This approach combines the direct creation of digital images of fishes with submersion of the specimen. This has proved to be optimal also in photographic techniques (Smith and Smith, 1975; Emery and Winterbottom, 1980). It is not meant to replace photographic techniques but merely to provide an alternative method, one that is more time-efficient, has many applications in scientific research, and can even provide better quality.

Material and Methods

Scanner setup The most important prerequisite for obtaining good digital images of fish from a flatbed scanner is scanning the specimens while they are submerged, without any additional layer between the scanner glass and the specimen. The specimens are scanned in horizontal, lateral position under submerged conditions. This is achieved by preparing a "pool" on the scanner glass (Fig. 1) that can be filled with various transparent liquids such as water, ethanol or glycerol. All three have been successfully tested. When using fresh water, the water should be allowed to settle beforehand to eliminate bubbles, which can stick to the scanner glass and to the specimen and mar the image. Water should be taken from the tap one day before or stirred for several hours before use. We preferred plasticine to form the pool walls because it adheres very well to the scanner glass and its soft consistency is ideal to attach different accessories necessary to position the specimen. Plasticine is formed into a thick rope and then bent into a rectangular form (Fig. 1). The pool is positioned close to the start position of the scanner lamp to minimize scanning times. The plasticine must be pressed tightly against the scanner glass to prevent leakage. Depending on the type of plasticine used, warming to about 40°C can facilitate manipulation and attachment. Prior to filling, a test scan can reveal whether the plasticine is optimally attached (grooves can lead to leakage). The standard cover unit of the scanner can be replaced with any kind of paper or plastic. We recommend a stiff, thin plate of opaque plastic. The height of the pool should be sufficient to ensure complete submergence of the specimen. Partial emersion of the specimen or immersion of the cover should be avoided: this causes reflections and/or image distortion. A pool height of about 3 cm is usually sufficient because the method is geared to specimens of less

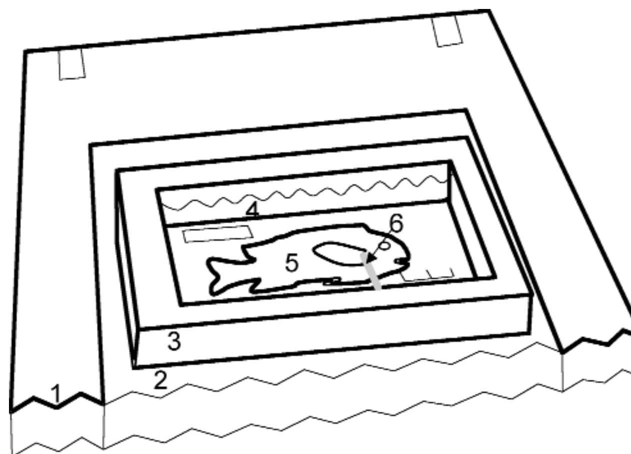

Fig. 1.
Scanner setup for digital imaging of live (narcotised) or preserved fish specimens. 1: flatbed scanner (only upper half shown); 2: scanner glass; 3: plasticine pool; 4: liquid (water, ethanol or glycerine); 5: specimen; 6: fixation rod. Label and ruler are indicated on the scanner glass. Cover unit of the scanner is removed and background cover on pool is not shown.

than 2 cm body width (see below). Deeper pools increase the distance between the cover and the submerged specimen. A short plastic ruler or scale should be placed beside the specimen on the scanner glass and be visible on the image for subsequent size calibrations. Individual water- or ethanol-proof labels placed beside each specimen help avoid confusing the images.

Specific experiments and fish groups used The following scanners were tested: HP ScanJets 6200C, 4070, 4890, Epson Perfection 1250 Photo, 4870 Photo and 4990 Photo. This method was initiated during a study of littoral fish assemblages in the Gulf of Trieste (Piran, Slovenia) in the summer of 2000. Since then, it has been used for various purposes on different groups of small fishes, alive and preserved. The present account relies on test scans and working images of hundreds of living and preserved gobies, blennies, triplefins and clingfishes (size range: 10 mm to 10 cm total length) and cichlids (size range: 5 to 15 cm total length). A special test was carried out on one of the scanners (Epson Perfection 4870 Photo) to estimate image distortion and depth of field. The change of image size with distance from the scanner glass was measured by a system of rulers placed at different distances from the scanner glass. Six small rulers (each 2 cm long, 2 mm thick and divided into 10-mm units) were placed on each other on the scanner glass, yielding 2-mm steps (0-10 mm). This setup was then scanned in fresh-water, 70% ethanol and pure glycerol. The rulers were scanned in the horizontal (perpendicular to the movement direction of the scanner lamp) and vertical (parallel to lamp

movement direction) axis of the scanner. We scanned fishes both with and without background cover, using paper or plastic that was white, light and dark grey, black, and light colors of green, blue and yellow.

Treatment of specimens Near-live coloration of fish can be documented by preparing specimens as indicated in Emery and Winterbottom (1980). Another technique is to scan them after narcotisation. Anaesthetization with clove oil was found to work best because fish quickly lose their equilibrium and can be preserved or can easily recover afterwards. For narcotisation we recommend following the protocols of Munday and Wilson (1997) and Griffiths (2000). Narcotised fish can be transferred into the water-filled scanner pool (Fig. 1). It is also recommended to add some clove oil to the water in the pool in order to prevent premature recovery. Fish can be positioned with appropriately sized steel needles, metal wires, or thin rods of glass or transparent plastic (the two latter being less visible on the scan). These aids are inserted into the plasticine and gently press the fish against the glass. This facilitates a lateral positioning. Most fish were successfully positioned by attaching them at the pectoral fin (Fig. 1). The median and pectoral fins can be erected and expanded with forceps. We observed no mortalities in dozens of trials on fishes narcotised according to the above protocols. Cichlids of about 10 cm total length successfully recovered even after a 30-min loss of equilibrium (C. Bauer, pers. comm.). The typically short scanning procedures (1 or 2 min) mean that live fish can be scanned after clove oil narcotisation without irreversible effects. The fish should be released back into aquaria or into aerated water immediately afterwards to ensure optimal recovery. For preservation, we killed fish by an overdose of anesthetic after the scanning process.

For safety reasons, specimens preserved in formalin should be rinsed with fresh water before being scanned. Specimens preserved in ethanol can be either rinsed in water or immediately scanned in ethanol of the same concentration as the specimen is preserved in. The latter approach saves time and is better for specimens that should not be subjected to varying ethanol concentration (e.g., type material).

The technique was also tested for scanning entire cleared and stained fish specimens (mainly small gobiids, 1 to 10 cm total length), which were stored in glycerol after performing standard clearing protocols (Potthoff, 1984). Since glycerol is exchanged very slowly by any other kind of liquid, such specimens should be scanned in the same glycerol as they are stored in. This saves time, minimizes shimmering effects and, most importantly, avoids damaging the specimen itself. One problem with scanning specimens in glycerol is the very low viscosity of this liquid; it therefore takes longer for the specimen to reach its final position in the setup. Especially small and light specimens may take about one minute before they stop sinking. During this time, scans will yield images out of focus, although the specimen's position can be determined by a series of preview scans.

Scanning process An initial scan provides a quick preview of the final image and enables adjustments. Most scanner programs also provide the opportunity of saving specific pre-adjustments for automatic future adaptation or at least allow quick manual adaptations of illumination, contrast, sharpness, resolution, and color of the image based on a preview. Clearly, every type of scanner will need some adjustment. We recommend restricting the size of the image to a frame closely bounding the specimen and including only the label and ruler. This minimizes image file size and scanning time while maximizing contrasts and illumination. Placing the ruler and the label too close to the specimen can create shadows near the specimen's outline. Depending on the purpose of the image and on specimen size, scans were performed at resolutions of between 300 and 4800 dpi. Scans of 4800 dpi were only possible on the three high resolution scanners (HP ScanJet 4890, Epson Perfection 4870 and 4990 Photo).

Results

Advantages of the present technique The most important advantages of the present technique compared to photographic techniques are cited below.

1) Simple and cheap technical setup: Most conventional scanners are now available at low prices, and even cheap scanners can yield satisfactory results. More advanced scanners, however, improve the limits of resolution and scanning speeds. Material costs (plasticine, accessories) are negligible. The setup can be prepared in a few minutes and the scanners quickly reverted for traditional purposes. No damage to the scanners occurred, although great care should be taken to avoid breaking the scanner glass when pressing the plasticine against it. Since most laboratories are equipped with suitable scanners, visiting scientists need not transport complicated equipment. Deployment in the field is only possible if AC power (a small generator or a powerful car battery with an AC transformer is sufficient) is available.

2) Simple treatment of specimens: Specimens can be very easily manipulated, positioned and straightened. Small and highly compressed specimens (such as gobies of the genus

Gobiodon) are especially easily positioned laterally. A major advantage is that the specimen can be manipulated/attached from its side away from the viewer (accessories only visible outside the specimen outline, Fig. 2). Curved specimens can usually be straightened, for example, by spanning a stiff metal wire or plastic rod from one side of the plasticine pool to the other or crosswise, pressing the specimen against the scanner glass. Usually the left side of fish is documented (unless damaged). Specimens with the left side being concave are more easily treated than those that are concave on their right side: it is easier to flatten out a specimen by pressing its body center towards the scanner glass than by pressing its head and tail simultaneously.

3) Fast image control: Position and expected image quality can be quickly controlled by using the scanner's preview mode. This is especially helpful for very small specimens.

4) Minimal image distortion: Although scanners lack a lens system, minimal image distortion will occur. Thus, measured using the graded ruler system, objects appeared smaller with distance from the scanner glass. However, this image distortion occurs in only one axis of the scanner—the horizontal (perpendicular to the scanning direction). In this axis, image size decreased for 1% in 2 and 4 mm distance, 2% at 6 and 8 mm, and 3% at 10 mm from the scanner glass, while in the vertical axis, no size decrease was measured. These values were the same for water, ethanol and glycerol.

5) Quality and processing of images: In a scanner, illumination and focus is automatic. Adjustments of light intensity and contrast may be necessary depending on the brightness of the specimen and the background color. Image quality is high (Fig. 2), and resolutions will be higher than in digital cameras. A small specimen (e.g., 2.5 cm total length) scanned at a resolution of 4800 dpi in 4:3 format will yield an image of 17 megapixels. This is beyond the resolution of even the most advanced digital cameras. However, most applications do not require such high resolutions, and even 2 cm specimens yielded sufficient resolutions if scanned at 3200 dpi. Resolution can be significantly reduced when specimen size increases. The tests on background colors yielded very different results. Shadows disappear only if a black or dark grey background is used or when the pool remains uncovered; this is only recommended for light-colored (e.g., preserved) specimens without transparent fins. Although we used light paper for the colored background, the colors were usually still too intense and disturbed the coloration of the specimen (e.g., transparent fins took on the background color). Best results for contrasts were attained with light grey or white background, although

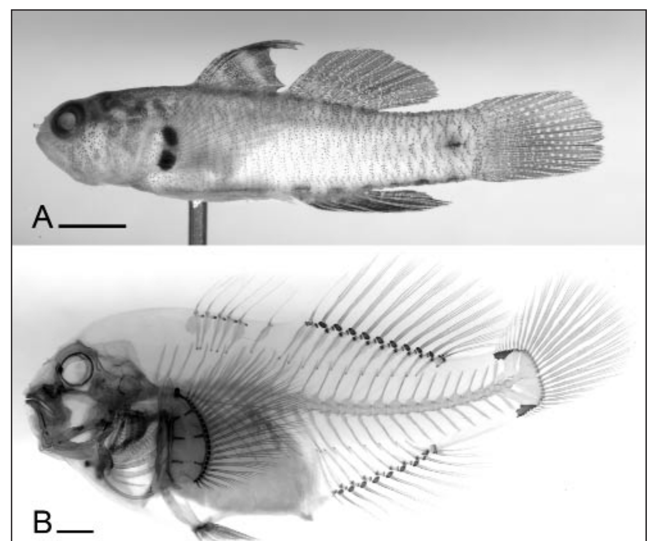

Fig. 2.
Examples of fish specimens scanned on a flatbed scanner under submerged condition. A: ethanol preserved *Eviota distigma* (13 mm SL); scanned at a resolution of 4800 dpi. B: cleared and stained *Gobiodon histrio* (29 mm SL; resolution: 2400 dpi). Ruler and labels are not shown. Both figures are raw scanner outputs and still show fixing accessories. Scale bar = 2mm. [Ed. note: Images are printed here at 300 dpi.]

the latter especially will yield shadows of various intensities. If the background is placed more than 1 cm away from the specimen, then the white will appear as light grey and become darker as the distance is increased. At the same time, this will slightly reduce shadows but worsen the contrast to the background. Although glass or transparent plastic rods are less visible and create weaker shadows than metal objects, they are typically less suited to flatten out curved (preserved) specimens. Illumination (minimal shadows and equal contrast) was best on the HP scanners used and on the Epson Perfection 1250 Photo, but sharpness was better on the two high-resolution Epson scanners. Thus, we recommend HP for images for presentation, and Epson for working images. If both good sharpness and good illumination is required, the latter should be used and images subsequently edited. Background colors can be changed by photo-editing software because the contrasts between specimens and background are usually very good. This also removes remnants of the accessories in the image. Image processing is simple because the digital images are sent directly from the scanner to the computer and saved on the hard disk for further treatment.

Application limitations The present technique has certain limitations. The key difficulties and suggested ameliorations are listed below:

1) Maximum specimen size: The size of conventional scanners (A4 format) means that only fish of max. about 20

cm total length fit into a pool on the scanner glass. We have no experience with larger scanners, which may enable the portrayal of larger specimens.

2) Maximum fish body width: Our tests revealed a good depth of field and little image distortion for up to 4 mm from the scanner glass and acceptable results at 8 mm distance. Parts of specimens more distant than 10 mm will lack sharpness. However, such scans may still yield satisfying results. The method is optimal for specimens narrower than 2 cm, resulting in a sharp picture of parts lying in the midsagittal plane such as the median fins. The midsagittal plane is most distant (half of the body width) from the scanner glass when fish are scanned in lateral position. The technique will thus work best for compressed fish body shapes. Depressed forms are difficult to accurately place in lateral position and will lack a sharp midsagittal plane if they are more than 2 cm wide.

3) Scanning time and related biases of image quality: A key disadvantage versus photography is the long time the image needs to be produced. Scanning large specimens at a high resolution may take a considerable time, during which several events can yield unsatisfactory results. Living fish may recover from anesthetization and start to move. This can be avoided by adding anesthetics to the water in the pool. Outside vibrations can affect the focus. This can be avoided by operating the machine in a secure place and by minimizing the scanning resolution to fit the purpose of the image. Moreover, low resolution reduces image file size and speeds up processing. Resolutions of 3200 dpi down to only 300 dpi or less are recommended for specimens between, e.g., 2 and 20 cm total length. Only very high-quality images of tiny specimens require 4800 dpi (Fig. 2A).

4) Not all scanners are suitable: Although inexpensive scanners yielded acceptable results for several applications, only the larger, deep-bodied scanner models were optimal. Ultra-thin scanners provided no depth of field and cannot be used. Clearly, our selection of scanners was limited to those already present in different laboratories. We assume that most larger, more advanced scanners are suitable, although image quality differences will no doubt be present.

Discussion

The present method proved to be very efficient in obtaining high-quality images of living or preserved rather small fish specimens in comparably short times and at low costs. Scans of submerged specimens yield well-illuminated images, good colors and no reflections on the specimen or the background. They compare favorably to high-quality photos of specimens placed in liquid (Herler, unpub. data). Photographic setups designed for dry specimens may yield poor colors, less contrast and reflections; they also include the risk of negatively affecting the specimen (desiccation or varying concentrations of the preservative).

The advantages of the scanning technique are that it uses conventional scanners, is easy to set up, and requires no special technical skills (versus photographic techniques). Another advantage is the simple control afforded by quick scan previews on the computer screen; the specimen can immediately be re-positioned. Final images immediately go to computer hard disks and are ready for further use. In our experience, the live coloration of fish in narcotised condition was more realistic than in fishes photographed in aquaria. Fish kept in aquaria often exhibited fright colorations (e.g., gobiids) or displayed subdominant coloration due to the presence of other (dominant) conspecifics (e.g., cichlids). Photo aquaria often stress live fishes and yield unnatural colors. In most cases, narcotisation yielded coloration very close to that observed in the field in undisturbed specimens. Short-term adaptive color patterns, such as fright or subdominant coloration, disappeared after narcotisation, and near-live coloration was re-established in several cases. We successfully used this method for discriminating small and similarly colored cryptobenthic fish species such as *Millerigobius macrocephalus* and *Zebrus zebrus* (unpub. data). The technique is thus useful for demonstrating biologically relevant color patterns and will help in quantifying inter- and intraspecific variation, describing male courtship coloration, or even in individual (photo-)identification of specimens. For studying variation, a series of small fishes, for example, can be scanned simultaneously and comparisons made directly on a single image. This may also facilitate the pre-selection of variables in coloration, and analyses can be performed on the same image. The lateral line system was also visible in many specimens, including superficial head neuromasts and pore canals in gobies and clingfishes. The opportunity to gain detailed data on such taxonomically important morphological features without killing the fishes may support rapid species identification. Similarly, scale counts and fin ray counts were successfully performed on many of these images. A key advantage in scanning preserved specimens is the opportunity to straighten them out from behind. This allows even long-term preserved (e.g., type) specimens from museum collections to be included in specific analyses, such as geometric morphometric approaches, without damaging them.

Comparable scans from photocopiers yielded worse results because of higher variation in measurements, especially between the two scanning axes and different models (M. Králík, pers. comm.). Concerning variation of replicate scans, flatbed scanners yielded better results than digital cameras or manual measurements (M. Králík, pers. comm.). However, when taking flatbed scanner images of larger (e.g., >10 cm) fishes with a less compressed body, for studies that require high accuracy (such as geometric morphometrics), the user might consider to correct for image distortion. This is especially important when different scanners are used for a certain dataset. Most important is the scanning direction, which must remain constant (Urbanová et al., 2006; M. Králík, pers. comm.).

Although the present paper describes the application on fishes, this technique may well be successfully used for other groups of organisms, including entire specimens or parts of plants and animals. Submerged objects in a size range of 1-20 cm and with a two-dimensional shape will yield best results. The present technique, but without using an immersion liquid, was already applied by other researchers for bone and finger length measurements (Urbanová et al., 2006; M. Králík, pers. comm.) and color patterns in flowers (V. Barca, pers. comm.). We would appreciate also the responses of other researchers, technicians or other operators who use this method. This will increase our knowledge about its applicability and allow us to provide updated information and potentially improved techniques in the near future.

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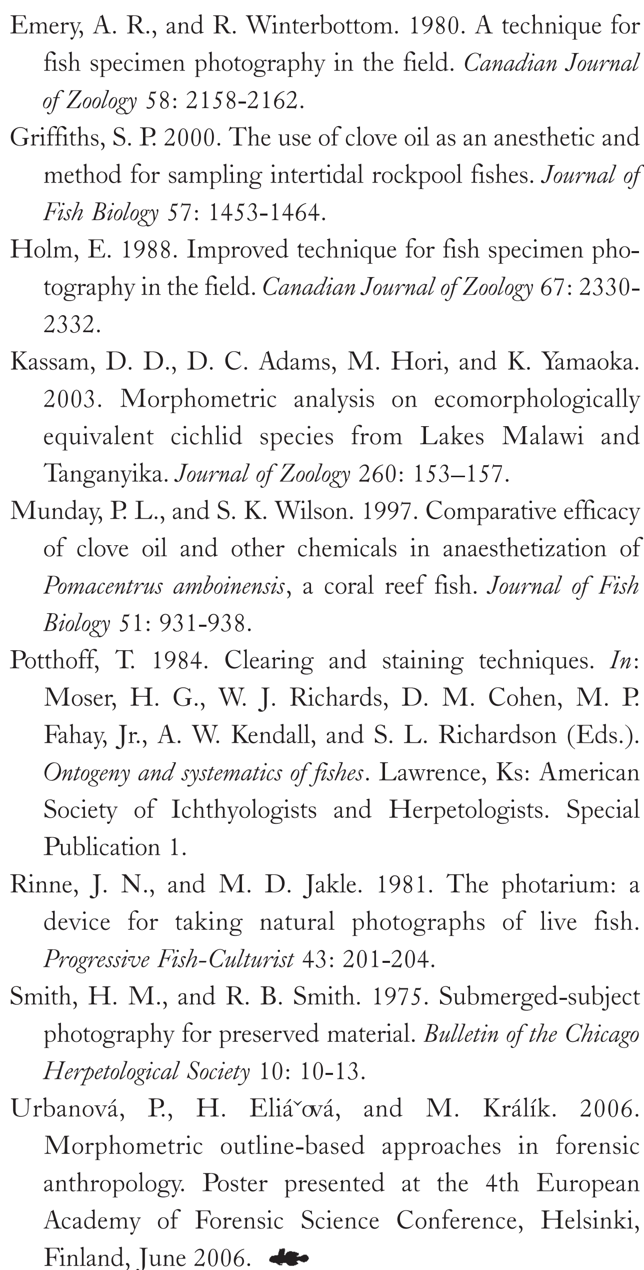

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