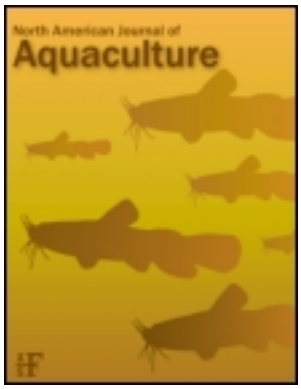

North American Journal of Aquaculture
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/unaj20

Fish Handling and Ultrasound Procedures for Viewing the Ovary of Submersed, Nonanesthetized, Unrestrained Channel Catfish

Amy M. Guitreau [a c], Bruce E. Eilts [b], Noel D. Novelo [a] & Terrence R. Tiersch [a]

[a] Aquaculture Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, 2410 Ben Hur Road, Baton Rouge, Louisiana, 70820, USA

[b] School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana, 70803, USA

[c] Woman's Hospital, 9000 Airline Highwa, Baton Rouge, Louisiana, 70815, USA

Available online: 04 Apr 2012

To cite this article: Amy M. Guitreau, Bruce E. Eilts, Noel D. Novelo & Terrence R. Tiersch (2012): Fish Handling and Ultrasound Procedures for Viewing the Ovary of Submersed, Nonanesthetized, Unrestrained Channel Catfish, North American Journal of Aquaculture, 74:2, 182-187

To link to this article: http://dx.doi.org/10.1080/15222055.2012.655852

North American Journal of Aquaculture 74:182–187, 2012

ISSN: 1522-2055 print / 1548-8454 online
DOI: 10.1080/15222055.2012.655852

ARTICLE

Fish Handling and Ultrasound Procedures for Viewing the Ovary of Submersed, Nonanesthetized, Unrestrained Channel Catfish

Amy M. Guitreau[1]
Aquaculture Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, 2410 Ben Hur Road, Baton Rouge, Louisiana 70820, USA

Bruce E. Eilts
School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana 70803, USA

Noel D. Novelo and Terrence R. Tiersch*
Aquaculture Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, 2410 Ben Hur Road, Baton Rouge, Louisiana 70820, USA

Abstract

This study addressed the development of rapid, straightforward, and minimally stressful procedures for the ultrasound imaging of ovaries of channel catfish *Ictalurus punctatus* in a commercial hatchery setting. The objectives were to (1) describe the ultrasound imaging equipment and settings used, (2) describe the fish handling procedures during imaging, and (3) illustrate image orientation with respect to the physical positioning of the probe and the catfish. Ultrasound images of the ovaries of channel catfish were recorded as digital video recordings (audio video interleave format) and as still images (ultrasound image format files). This study integrated the use of nonanesthetized, submersed fish within a recirculating tank system or portable container and a submersed waterproof probe, which enabled us to use water as a transmission medium for ultrasound. This allowed us to image the fish in ventral recumbency (upright swimming position) without using a physical restraint in the tank system, or by positioning the fish in the portable container by adjusting the position of its caudal peduncle with one hand and that of the probe with the other hand. The ease of using this technique allows it to be employed as a systematic method for fish handling under laboratory and hatchery conditions. The detailed ultrasound imaging procedures and instrument control settings reported can be used in future testing, improvement, and standardization of procedures for viewing ovaries in channel catfish and potentially other species.

The channel catfish *Ictalurus punctatus* industry in the United States increased steadily from 35,000 metric tons in 1980 to 300,000 metric tons in 2003; however, production has been in decline over the past few years owing to a number of reasons, including the high price of feed resulting from the high price of the corn and soybean meal components, increased imports of catfish, and a decrease of water acreage in production (Hanson and Sites 2011). In response to these challenges, the culture of hybrid catfish (female channel catfish × male blue catfish *I. furcatus*) has been suggested as a means to improve commercial

*Corresponding author: ttiersch@agcenter.lsu.edu
[1]Present address: Woman's Hospital, 9000 Airline Highway, Baton Rouge, Louisiana 70815, USA.
Received March 11, 2011; accepted May 26, 2011

production (Argue et al. 2003; Waldbieser and Bosworth 2008). This hybrid is considered valuable because of economically important production traits such as increased disease resistance, survival, and growth rate compared with channel catfish (Giudice 1966; Ligeon et al. 2004).

Traditional spawning techniques for large-scale commercial production of channel catfish are based on natural spawning in earthen ponds, with a male-to-female ratio of between 1:2 and 2:3 (Kelly 2004), but this is not applicable for hybrid production because of behavioral and physiological reproductive barriers between the species (Tave and Smitherman 1982; Avery et al. 2005). Commercial production of hybrids, therefore, relies on artificial spawning, which includes the identification of channel catfish females in reproductive condition, use of hormones to induce ovulation, manual collection of oocytes, and artificial fertilization with blue catfish sperm.

Noninvasive methods for evaluating oocyte maturity and spawning condition of female channel catfish have included external visual inspection for a soft, distended abdomen, and a swollen, red urogenital orifice (Clemens and Sneed 1957; Sneed and Clemens 1960), observation of active spawning behavior (Bates and Tiersch 1998; Lang and Tiersch 2007; Phelps et al. 2007), and measurement of degree-day heating requirements for spawning in ponds (Pawiroredjo et al. 2008). Physically invasive methods for evaluating oocyte maturity and readiness to spawn of channel catfish have included ovarian catherization (Markmann and Doroshov 1983), measurement of gonadosomatic index (MacKenzie et al. 1989), germinal vesicle visualization (Stoeckel 2000), and measurement of serum estrogen, testosterone, vitellogenin, and cathepsins (Barrero et al. 2007).

These techniques (with the exception of external morphology inspection) are applicable mostly to research, and they involve lengthy, specialized, technical procedures that have limited application for gonadal assessment in commercial production. Invasive techniques provide accurate biological data of gonadal condition, but they typically involve a time delay in assessment, extensive fish handling (which may result in death) (Blythe et al. 1994), or potential disruption of ovulation (Phelps et al. 2007), or they may jeopardize gamete quality and quantity (Newman et al. 2008), which is detrimental for commercial production. On the other hand, ultrasound imaging techniques provide a real-time portal for direct, rapid viewing of the gonads and bypass the limitations of invasive techniques.

Ultrasound imaging has been used to study the reproductive biology of two catfishes, the endangered Neosho madtom *Noturus placidus* (Bryan et al. 2005) and the African catfish *Clarias gariepinus* (Laszlo et al. 2008). It has also previously been used to estimate fillet yield in channel catfish (Bosworth et al. 2001). A rapid, efficient procedure that minimizes fish handling and is potentially less stressful, would be beneficial for noninvasive and direct viewing of the ovary of channel catfish broodstock females, especially for commercially relevant use in hatchery-level production. This study describes handling procedures and imaging techniques for screening of broodstock females in a commercial hatchery setting. The objectives were to (1) describe the ultrasound imaging equipment and settings used, (2) describe fish handling procedures during imaging, and (3) illustrate image orientation with respect to the physical positioning of the probe and the catfish. This combination of fish handling and ultrasound imaging procedures offers a practical approach to application of ultrasound imaging for use in the hatchery.

METHODS

Ultrasound imaging equipment and settings used.—Ultrasound images were obtained in 2005 by means of a portable laptop ultrasound unit, TelaVet 1000 (Classic Medical, Tequesta, Florida) with a multifrequency, waterproof probe (model LV7.5/60/96Z). The settings for acquiring the image were provided by the built-in features of the probe model and the TelaVet software–user interface. The probe was capable of producing linear array images, a range of ultrasound emission frequencies (5–8 MHz), and a range of ultrasound penetration depths (3–15 cm). The main software control settings used were: real-time B-Mode echo display, with probe frequency set at 5 MHz, ultrasound penetration depths set at 80 and 110 mm, power (acoustic power control of ultrasound beam) set at 100%, overall gain control (causes uniform amplification of returning echoes) set at 100%, reject control (alters threshold for stronger or weaker range of returning echoes) set at 0, and time-gain compensation (TGC) controls (adjusts the gain at specific depths) set at 0 (TGC control 1), 27 (TGC control 2), 55 (TGC control 3), 82 (TGC control 4), and 110 mm (TGC control 5).

Experimental fish.—Adult broodstock were obtained from a commercial channel catfish producer (Haring's Fish Farm, Wisner, Louisiana). Ultrasound images of ovaries of broodstock ($n = 72$ fish, 1.4 ± 0.3 kg in weight [mean $\pm$ SD] and 50 ± 4 cm in length) were viewed and recorded (as videos) before and after spawning during February–April 2005 for fish conditioned in warmwater ponds (Lang et al. 2003; Lang and Tiersch 2007) at the Aquaculture Research Station of the Louisiana State University Agricultural Center.

Fish handling and scanning procedures used.—All fish in this study remained completely submersed at least 2–3 cm below the water surface in 80-L fiberglass tanks during ultrasound examination, or in a portable, 49-L cooler (52 qt; Igloo, Sportsman) filled with about 25 L of water. The fish maintained an upright swimming position (ventral recumbency) used in the natural environment. They were not physically restrained or anesthetized during the ultrasound procedure in the tanks, which were aerated and maintained a constant inflow and outflow of water (Figure 1). Fish were transferred to and from the portable container by using dip nets or baskets. The size of the container limited movement, and fish were positioned by adjusting its caudal peduncle so that images of the ovaries could be obtained. The water of the portable container was exchanged after imaging 15–30 fish. The acquisition of images from individual fish

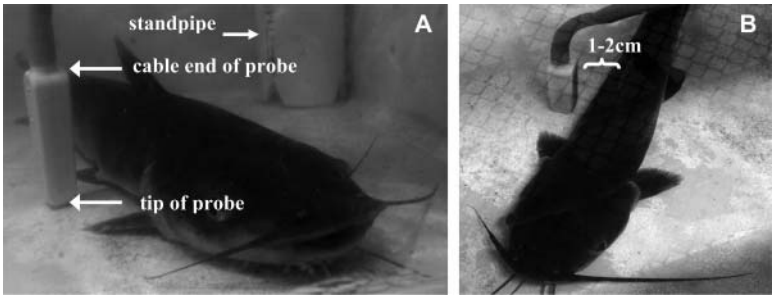

FIGURE 1. The scanning procedure used for channel catfish in 35 × 58 × 50-cm tanks (28 cm standpipe height, 80 L volume) in a recirculating system. Although the fish were free to swim, they had limited space (no more than two catfish were held in each tank at one time), and therefore a duration of less than1 min was needed to obtain ultrasound images of the ovaries. The fish were not restrained physically (nor held by the caudal peduncle) or chemically (i.e., no anesthesia). Ultrasound images of ovaries were obtained from unrestrained channel catfish completely submerged in water, by positioning the probe next to the lateral side of the fish with the free end (tip) of the probe towards the ventral surface of the fish and the cable end of the probe (connected to the ultrasound machine) towards the dorsal surface, and with the fish in ventral recumbency. The probe was positioned 1–2 cm from the fish (B) to allow water to act as a conductor for the ultrasound (5 MHz).

was accomplished by two operators (one to position the fish and probe, the other to use the TelaVet software–user interface to capture and label files).

Position of the probe and scanning region.—The left lateral aspect of the body cavity was scanned between the pectoral and pelvic fins, and the ultrasound images were recorded at 1–2 cm anterior to the base of the pelvic fin and posterior to the dorsal fin (Figure 1). The probe was positioned vertically with the free end towards the ventral surface of the fish, and the cable end (providing connection to the ultrasound unit) towards the dorsal surface of the fish (Figure 1). During the entire procedure (<1 min), the probe was completely submersed in water, which provided the sole transmission medium for ultrasound images displayed on the monitor. Digital videos of ultrasound images of ovaries were stored as audio video interleave (AVI) files in the laptop computer and still images were captured from videos and stored as ultrasound image format (USI) files.

RESULTS AND DISCUSSION

Fish Handling and Ultrasound Examination Procedures for Viewing Channel Catfish Ovaries

Ultrasound images of one or both ovaries from each channel catfish were captured by using the handling and ultrasound examination procedures as described in this report, and digital still images (Figures 2, 3) were captured from video images. The videos were obtained with an ultrasound system (2 kg), which communicated with the laptop computer (2 kg) through the USB 2.0 port. The laptop ultrasound system provided a software interface for ultrasound-imaging controls and storage capacity was limited only by the memory size of the hard drive (60 gigabytes) installed on the laptop. The features of the laptop ultrasound system made this ultrasound unit useful, especially because it was compact, lightweight, and enabled the users to directly save a large number of images to the laptop computer. Video images (904 video images, duration of 23 ± 14 s (mean ± SD), with an average electronic storage size per video of 3 megabytes) in AVI format were available for storage in compressed and uncompressed formats, and it was possible to capture still images in "Freeze" mode (display of still echo-image on the monitor) in Windows bitmap (BMP) and USI format.

The labeling of the probe and fish depicted in Figure 1 correspond to the probe and fish positions shown in the subsequent ultrasound images (Figures 2, 3). The top of the ultrasound images (Figure 2, arrow A–B; Figure 3, line A–B) corresponds to the submersed position of the probe alongside the fish, as illustrated in Figure 1. The space between the probe position and the skin of the catfish (C in Figures 2, 3) corresponds to the space occupied by water (the ultrasound transmission medium), which made possible the detailed visualization of internal structures that showed the ovary. The ovary closest to the probe (the upper region of the scan labeled left ovary, Figure 2) was always clearly visible and immediately identified in fish before and after strip spawning (Figure 3). The ovaries were discernible from surrounding structures in the ultrasound images, which included a partial view of the skin, musculature, vertebrae, ovaries, and oocytes.

The ultrasound images captured before oocytes were manually stripped provided a view of round, extended ovaries with advanced development, which dominated the body cavity and in which individual oocytes were clearly visible (females 1 and 2, Figure 3). The ultrasound images after eggs were collected provided a view of a fish with an irregularly shaped contour, a concave abdomen, and irregularly shaped ovaries that were partially (female 1, Figure 3) or completely (female 2, Figure 3) devoid of oocytes.

By using these fish handling and imaging procedures ultrasound images of channel catfish ovaries were rapidly obtained and made possible without physical or chemical restraint (Figure 1) while the fish was maintained in water. No additional steps, such as covering the probe with a plastic sleeve or applying ultrasound imaging gel to the probe for ultrasound

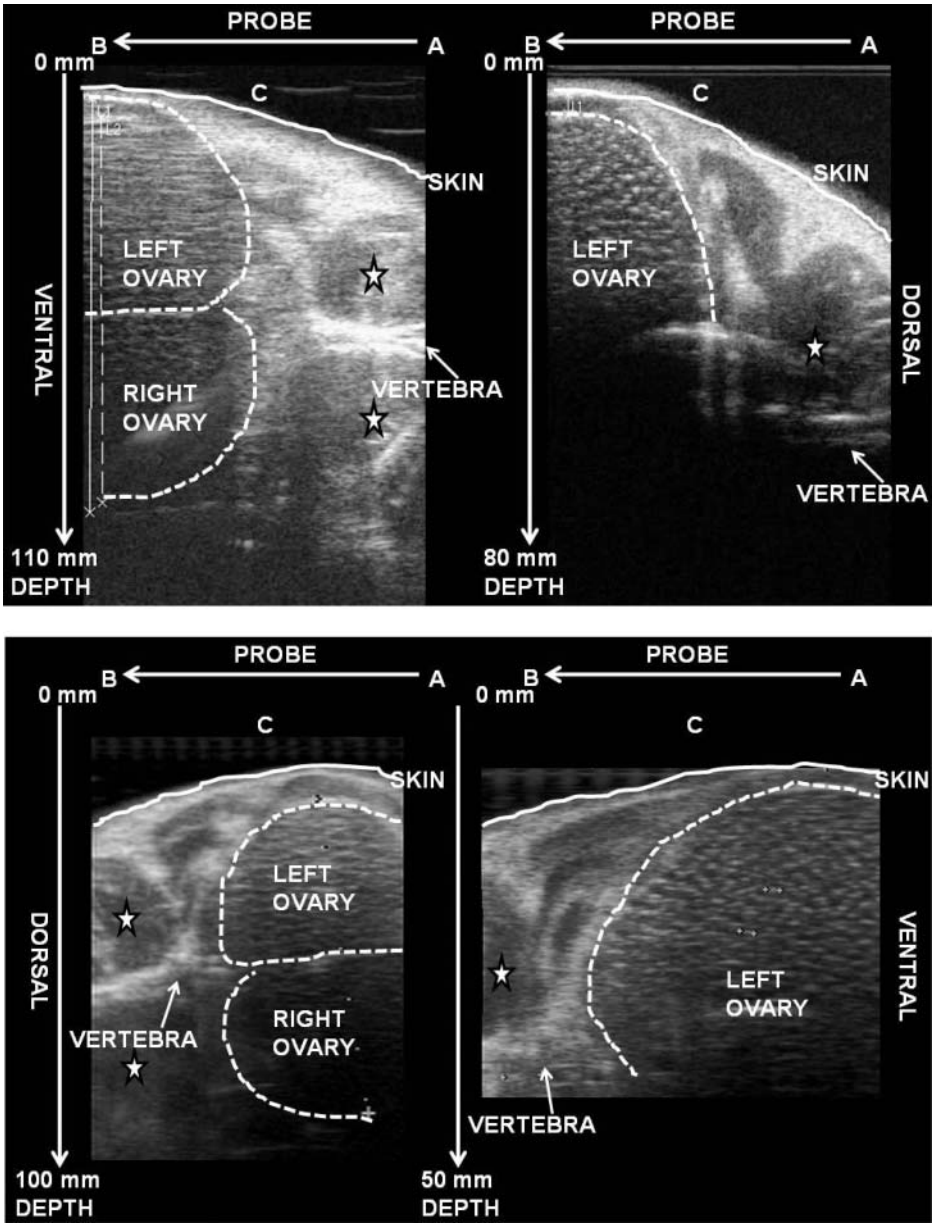

FIGURE 2. Ultrasound images as seen in the display monitor, representing the orientation of the probe in relation to a cross section of channel catfish ovaries and surrounding tissues. The arrow A–B represents the surface of the probe (positioned on the left lateral surface of the fish) where the ultrasound was emitted in a linear array. Point A was the position of the cable end of the probe (Figure 1, with the cable connecting to the ultrasound unit), corresponding to the dorsal surface of the fish. Point B was the position of the free end of the probe corresponding to the ventral surface of the fish. The physical orientation of probe and the fish in Figure 1 remained the same, but the scanning direction control (software control) enabled the user to change the display image orientation with the ventral side of the fish on either the left (upper panel) or right side (lower panel) of the image. The standard orientation of the near-field view (portion of the image close to the probe) as it appears during ultrasound examination was at the top of the image. Ultrasound images of one (left ovary; 5 MHz and 80 mm depth) or both ovaries (5 MHz and 110 mm depth) were recorded for each fish ($n = 72$ adult catfish) as videos ($n = 906$ files) saved in audio video interleave (AVI) format, and still images were obtained from these videos and saved as ultrasound image (USI) format files. The star represents the muscle bundle adjacent to the vertebrae.

transmission, were needed. The ability to obtain still images and videos in less than1 min per fish in a portable container has direct application in field conditions where catfish may be scanned beside the pond immediately after seining or in a hatchery holding facility.

The procedures for this study integrated ultrasound technology with practical approaches, provided specific control settings, detailed handling procedures for use with unrestrained, unanesthetized, submersed catfish, and used water as a transmission medium for the ultrasound emitted from a submersed,

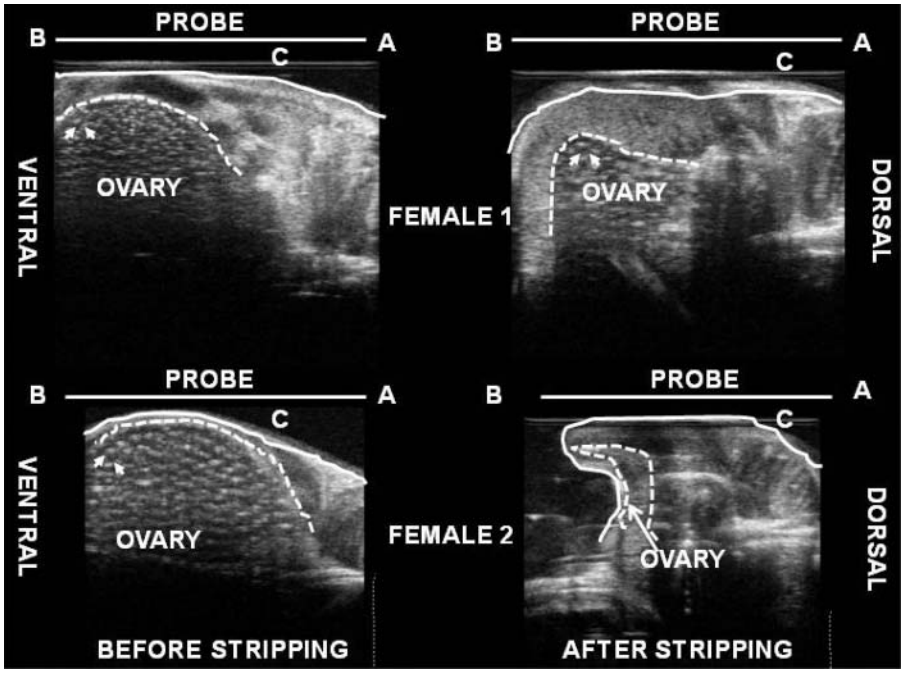

FIGURE 3. Ultrasound images of ovarian cross sections depicting the left ovary (closest to the probe) of channel catfish before and after eggs were manually collected (strip-spawning). The ultrasound images of females before they were stripped of eggs (left panels) revealed a fully extended ovary, clearly visible oocytes (arrowheads), and a sharp smooth delineation of the periphery of the ovary. The ultrasound image of female 1 (upper panels) after eggs were manually collected revealed residual oocytes (arrowheads) in the ovary. The ultrasound image of female 2 (bottom right of lower panel) after eggs were stripped revealed a shrunken, greatly reduced ovary, irregular indentations on the partial contour of the periphery of the ovary, and no visible oocytes.

waterproof probe (without the use of ultrasound gel). The utility of these ultrasound imaging procedures was validated by initial corroboration of ultrasound images and corresponding histological profiles of channel catfish ovarian development (Novelo et al. 2011). Most previous studies have removed fish from the water or restrained or anesthetized them (reviewed elsewhere by Novelo and Tiersch 2012, this issue), and procedures of this type have limited potential compared with the minimal handling and high throughput provided by the method described herein. The ultrasound imaging and fish handling procedures developed for viewing catfish ovaries could be used for a wide variety of species. The ultrasound unit used in this work was capable of basic gray-scale imaging, and thus expensive ultrasound units are not required for applying this technology. Future studies will address the development of an ultrasound imaging classification system for assessing ovarian development in channel catfish, with particular utility in hybrid production.

ACKNOWLEDGMENTS

We thank T. Campbell, W. Burnside, P. Pawirodejo, R. McLellan, H. DiMaggio, J. Bland, B. Brooks, B. Baumgardener, A. Raymond, and C. Ashley for assistance during data collection and preparation of the manuscript. We thank M. Saltz for photography, and M. Christensen, R. Cuevas-Uribe, J. Daly, and R. Romaire for critical review. This study was supported in part by funding from the U.S. Department of Agriculture (USDA), Cooperative State Research, Education, and Extension Service, and the USDA, Small Business Innovative Research program. This manuscript was approved for publication by the Director of the Louisiana Agricultural Experiment Station as number 2011-244-5687.

REFERENCES

Argue, B. J., Z. Liu, and R. A. Dunham. 2003. Dress-out and fillet yields of channel catfish, *Ictalurus punctatus,* blue catfish, *Ictalurus furcatus*, and their F_1, F_2 and backcross hybrids. Aquaculture 228:81–90.

Avery, J., J. Steeby, B. G. Bosworth, and B. C. Small. 2005. Producing hybrid catfish fry: workshop manual. U.S. Department of Agriculture, Aquaculture Research Station Catfish Genetics Research Unit and National Warmwater Aquaculture Center, Mississippi State University, Stoneville.

Barrero, M., B. C. Small, L. R. D'Abramo, L. A. Hanson, and A. M. Kelly. 2007. Comparison of estradiol, testosterone, vitellogenin and cathepsin profiles among young adult channel catfish (*Ictalurus punctatus*) females from four selectively bred strains. Aquaculture 264:390–397.

Bates, M. C., and T. R. Tiersch. 1998. Preliminary studies of artificial spawning of channel catfish as male-female pairs or all-female groups in recirculating systems. Journal of the World Aquaculture Society 29:325–334.

Blythe, B., L. A. Helfrich, W. E. Beal, B. Bosworth, and G. S. Libey. 1994. Determination of sex and maturational status of striped bass (*Morone saxatilis*) using ultrasonic imaging. Aquaculture 125:175–184.

Bosworth, B. G., M. Holland, and B. L. Brazil. 2001. Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. Journal of Animal Science 79:1483–1490.

Bryan, J. L., M. L. Wildhaber, and D. B. Noltie. 2005. Examining Neosho madtom reproductive biology using ultrasound and artificial photothermal cycles. North American Journal of Aquaculture 67:221–230.

Clemens, H. P., and K. E. Sneed. 1957. The spawning behavior of the channel catfish *Ictalurus punctatus*. U.S. Fish and Wildlife Service Special Scientific Report Fisheries 219.

Giudice, J. J. 1966. Growth of a blue × channel catfish hybrid as compared to its parent species. Progressive Fish-Culturist 28:142–145.

Hanson, T. R., and D. Sites. 2011. 2010 U.S. catfish database. Department of Agriculture Economics, Information Report 2011–01, Mississippi State University, Mississippi State. Available: www.aces.edu/dept/fisheries/aquaculture/catfish-database/catfish-2010.php. (April 2011).

Kelly, A. M. 2004. Broodfish management. Pages 129–144 *in* C. S. Tucker and J. A. Hargreaves, editors. Biology and culture of channel catfish, 1st edition. Elsevier, Amsterdam.

Lang, R. P., R. P. Romaire, and T. R. Tiersch. 2003. Induction of early spawning of channel catfish in heated earthen ponds. North American Journal of Aquaculture 65:73–81.

Lang, R. P., and T. R. Tiersch. 2007. Early out-of-season induced spawning of channel catfish *Ictalurus punctatus* (Rafinesque) conditioned in heated earthen ponds. Aquaculture Research 38:498–507.

Laszlo, K., H. Csaba, H. Arpad, G. Janos, U. Bela, and L. K. Katalin. 2008. Use of ultrasonography in the description of sexual cycle of African catfish. Magyar Allatorvosok Lapja 130:475–480.

Ligeon, C., R. A. Dunham, C. Jolly, J. Crews, B. Argue, Z. Liu, R. Yant, J. Benfrey, and F. Gagalac. 2004. Economics of production of channel catfish, *Ictalurus punctatus,* female × blue catfish, *I. furcatus*, male hybrid fingerlings and foodfish. Aquaculture Economics and Management 8:253–267.

MacKenzie, D. S., P. Thomas, and S. M. Farrar. 1989. Seasonal changes in thyroid and reproductive steroid hormones in female channel catfish (*Ictalurus punctatus*) in pond culture. Aquaculture 78:63–80.

Markmann, C., and S. I. Doroshov. 1983. Ovarian catheterization of the channel catfish, *Ictalurus punctatus*. Aquaculture 35:163–169.

Newman, D. M., P. L. Jones, and B. A. Ingram. 2008. Sexing accuracy and indicators of maturation status in captive Murray cod *Maccullochella peelii peelii* using non-invasive ultrasonic imagery. Aquaculture 279:113–119.

Novelo, N., D. Kuenz, C. C. Green, and T. R. Tiersch. 2011. Ultrasonic monitoring of channel catfish ovarian development. Pages 134–144 *in* T. R. Tiersch and C. C. Green, editors. Cryopreservation in aquatic species, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana.

Novelo, N. D., and T. R. Tiersch. 2012. A review of the use of ultrasonography in fish reproduction. North American Journal of Aquaculture 74:169–181.

Pawiroredjo, P., J. Lamoureux, S. G. Hall, and T. R. Tiersch. 2008. Degree-days as a tool to determine the heating requirement for channel catfish spawning in earthen ponds. North American Journal of Aquaculture 70:328–337.

Phelps, R. P., R. Hastey, A. Pendetar, L. Linley, N. Papanikos, and R. A. Dunham. 2007. Effects of temperature on the induced spawning of channel catfish and the production of channel × blue catfish hybrid fry. Aquaculture 273:80–86.

Sneed, K. E., and H. P. Clemens. 1960. Use of fish pituitaries to induce spawning in channel catfish. U.S. Fish and Wildlife Service Special Scientific Report Fisheries 329.

Stoeckel, J. N. 2000. A method for viewing the germinal vesicle in oocytes of commercial catfishes. North American Journal of Aquaculture 62: 240–247.

Tave, D., and R. O. Smitherman. 1982. Spawning success of reciprocal hybrid pairings between blue and channel catfishes with and without hormone injection. Progressive Fish-Culturist 44:73–74.

Waldbieser, G. C., and B. G. Bosworth. 2008. Utilization of a rapid DNA-based assay for molecular verification of channel catfish, blue catfish, F_1 hybrids, and backcross offspring at several life stages. North American Journal of Aquaculture 70:388–395.