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Development and Evaluation of an Ultrasound Imaging Reproductive Index Based on the Ovarian Cycle of Channel Catfish, *Ictalurus punctatus*

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Abstract

Commercial production of hybrid catfish (female channel catfish *Ictalurus punctatus* × male blue catfish *Ictalurus furcatus*) is reliant on interdependent biological, environmental, and technical procedures. This study addresses one of these critical components – the evaluation of channel catfish ovarian development based on sonographic analysis. The objectives were to: (1) develop a channel catfish ovarian ultrasonography index, (2) test the effect of the spawning trials on fertilization estimates of fish assessed using the ultrasonography index, and (3) evaluate the expected (hypothesized) and observed outcome of the ultrasonography assessments. Seven ovarian morphology classifications were developed based on ultrasonography of 915 channel catfish ($N = 915$ images), and 210 females were selected for spawning. The predictions based on the classifications prior to hormone injection showed a significant effect ($P < 0.002$) on the observed outcomes (viable or nonviable eggs). The probability of correct classification was 0.86–0.89 for Categories 3 (developing), 4 (advanced), and 5 (mature), and 0.93–1.0 for Categories 1 (undeveloped), 2 (underdeveloped), 6 (spawned), and 7 (atretic). The ultrasonography index covered the full range of ovarian dynamics (i.e., recrudescence through spawning). It provided an unobtrusive, direct method of ovarian assessment to work toward improving the efficiency of broodstock selection.

The production of hybrid catfish (channel catfish female *Ictalurus punctatus* × blue catfish male *I. furcatus*) has been pursued in commercial hatcheries to address economic challenges in the US catfish industry by seeking to increase production efficiency in traits such as growth, meat yield, stress tolerance, disease resistance, and food conversion ratio (Giudice 1966; Avery et al. 2005; Arias et al. 2012; Chatakondi 2012; Li et al. 2014). This hybrid production relies on artificial spawning for the collection of unfertilized channel catfish eggs and artificial fertilization with blue catfish sperm because of reproductive constraints that render traditional pond spawning technique impractical for commercial seedstock production (Tave and Smitherman 1982; Avery et al. 2005). Hybrid production involves the manipulation of the multiple complex and interdependent biological processes and environmental factors that include: (1) broodstock age; (2) nutrition; (3) the correct identification of sex; (4) evaluation of channel catfish ovarian development; (5) hormonal injections to induce ovulation; (6) timing of egg collection; (7) constraints involved in the collection of blue catfish testis and sperm (e.g., small testis, poor sperm quality, noncongruent peak spawning times of the two species, and availability of male broodstock); (8) constraints of channel catfish seedstock production as evidenced by 30–50% of the females that have been estimated to spawn in the traditional open-pond method; (9) effect of temperature and handling stress on the physiology of female broodstock, incubated eggs and embryos, and survival of larvae; (10) water quality; (11) disease; and (12) hatchery management practices (Wolters 1993; Small and Bates 2001; Torrans and Lowell 2001; Small et al. 2004; Avery et al.

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2005; Phelps et al. 2007; Sink and Lochman 2008; Hu et al. 2011). Thus, there are numerous and equally critical factors involved in hybrid catfish production, and these must be understood and carefully managed because while they provide the key to successful hybrid seedstock production, they also provide ample sources for error and inefficiencies to be introduced.

This study addresses one of these critical components – the evaluation of channel catfish ovarian development for selection of female broodstock for artificial spawning in hybrid production. The method commonly used for assessing ovarian development in commercial hatcheries is based on expectations associated with the external morphology of fish and ovarian development. If females display "desirable" morphological features (e.g., distension of the abdomen, a reddish swollen papilla), the expectation is that they will likely produce viable eggs (Lee 1991). However, the criteria for selecting broodstock to inject with hormones are subjective, and this varies among workers at different farms (Phelps et al. 2011).

The potential for the use of ultrasonography for assessment of the reproductive state of the ovary with commercially relevant, rapid, and consistent handling and imaging procedures was previously reported for channel catfish (Guitreau et al. 2012). The goal of this study was to initiate the development and evaluation of an ultrasonography approach to ovarian assessment for the selection and management of channel catfish females for artificial spawning in research and commercial hatchery efforts. The objectives were to: (1) develop a channel catfish ovarian ultrasonography index, (2) test the effect of the spawning trials on fertilization estimates of fish assessed using the ultrasonography index, and (3) evaluate the expected (hypothesized) and observed outcome of the ultrasonography assessments. This study provided a novel sonographic perspective on channel catfish ovarian morphology and reproductive stages observed during the annual ovarian cycle through the development, use, and evaluation of a sonographic index for use in assessment of catfish females for hormone-induced spawning.

Materials and Methods

Equipment and Settings

The equipment used was a portable ultrasound unit (Classic TelaVet 1000™ Veterinary Digital Ultrasound Module, Telemed UAB, Vilnius, Lithuania) and a linear probe (model LV7.5/60/96). The ultrasound controls were in real-time B-Mode, with probe frequency set at 8 MHz, transmit control at 10 mm, scanning depth at 80 mm, "dynamic range" (dB) at 56 or 62, power control at 90 or 100%, "overall gain" at 70, zoom at 100%, frame average at 4, reject control at 0, and time-gain compensation controls (horizontal slider control) were adjusted at 0, 20, 40, 60, and 80 mm. Images were recorded in Audio Video Interleave format, ultrasound image format, Windows Bitmap Image format, and Tagged Image Format Files.

Sampling During the Annual Ovarian Reproductive Cycle

In Louisiana, the initiation of vitellogenesis is associated with slow ovarian and oocyte growth during October and November leading to rapid growth in March and April preceding the spawning phase (Trant et al. 1997; Banks et al. 1999). Ultrasound images ($n = 705$ ovarian images, one image/fish) were recorded during six sampling dates of the recrudescence phase of the ovarian cycle of adult (3–5 yr) channel catfish broodstock conditioned for reproduction in earthen ponds at the Louisiana State University Agricultural Center, Aquaculture Research Station in Baton Rouge, Louisiana (Table 1).

Ultrasound images ($n = 210$ ovarian images, one image/fish) were recorded of fish held for reproductive conditioning at ambient temperatures in 12 earthen ponds during the natural spawning phase and of fish in four ponds using established geothermal control protocols of raising the temperature by 2 C/d from the ambient temperature until 28 C was reached (Hall et al. 2002; Lang et al. 2003; Lang and Tiersch 2007) (Tables 2 and 3).

Water temperature of the ponds was recorded in 0.5–1.0-h intervals with data loggers (SK100

TABLE 1. *Ultrasound images (n = 705 images) of channel catfish ovaries (one image/fish) were recorded during six sampling dates in the recrudescence phase of the ovarian cycle.*[a]

Sampling dates	Pond	PS	Fish	TL (cm ± SD)	BW (kg ± SD)	Time (sec ± SD)
October 28, 2008	B6	0.30	108	–	2.4 ± 0.4	47 ± 54
November 05, 2008	B4	0.30	92	64 ± 3	2.6 ± 0.4	32 ± 28
November 14, 2008	M7	0.16	46	61 ± 5	2.2 ± 0.7	35 ± 15
February 03, 2009	H4	0.16	253	63 ± 3	2.5 ± 0.5	54 ± 68
March 13, 2009	H7	0.16	141	61 ± 3	2.2 ± 0.4	55 ± 55
March 25, 2010	B6	0.30	65	61 ± 3	2.0 ± 1.3	75 ± 44

[a]Pond = pond ID; PS = pond size (ha); fish = the number of fish sampled; TL = total length (cm ± SD); BW = body weight (kg ± SD); time = the average time (sec ± SD) between consecutive images recorded.

TABLE 2. *Stocking, egg mass (EM) collection indicating spawning activity, and fish captured by seine for 10 spawning trails during 2008–2010 of channel catfish conditioned for reproduction in heated (H) and ambient (A) temperature ponds.*

	Stocking data							
Trial	Pond	PS	F	M	Date	T	EM	Date of fish capture
I	G2	0.04	30	10	February 22, 2008	H	6	April 04, 2008
II	G6	0.04	30	10	February 29, 2008	A	3	May 26, 2008
III	G7	0.04	27	9	May 13, 2008	A	4	June 02, 2008
IV	R2	0.02	27	9	February 03, 2009	A	6	April 25, 2009
IV	R3	0.02	27	9	February 03, 2009	A	8	April 25, 2009
V	G2	0.04	27	9	March 13, 2009	H	2	May 09, 2009
V	G3	0.04	27	9	March 13, 2009	A	4	May 09, 2009
VI	B3B	0.30	170	–	November 14, 2009	A	–	May 26, 2009
VII	H4	0.16	200	–	November 05, 2009	A	–	June 01, 2009
VII	H7	0.16	310	–	October 28, 2009	A	–	June 01, 2009
VIII	G1	0.04	30	10	February 11, 2010	H	2	March 30, 2010
VIII	G2	0.04	30	10	February 11, 2010	H	4	March 30, 2010
IX	B9	0.30	–	–	–	A	–	June 05, 2010
X	G1	0.04	39	10	May 07, 2010	A	2	May 17, 2010
X	R2	0.02	30	10	March 25, 2010	A	3	May 17, 2010
X	R3	0.02	30	10	March 25, 2010	A	3	May 18, 2010

[a]Spawning cans were removed and a date was selected for fish capture after 10–20% of the females had spawned. Pond = pond ID; PS = pond size (ha); F = females; M = males; date = stocking date; T = thermal conditioning.

Dickson Temperature data loggers; Addison, IL, USA). The temperature data were used to generate degree-day profiles of broodstock ponds by calculating the sum of the daily difference between the mean daily temperature and 21 C (the recommended threshold temperature) from the date of stocking to the date of fish capture (Pawiroredjo et al. 2008). These degree-day profiles were compared to the degree-day guidelines for the onset (57–81 degree-days, 10% spawning), middle (99–129 degree-days, 50% spawning), and conclusion (150–172 degree-days, 90% spawning) of spawning developed for channel catfish broodstock (Pawiroredjo et al. 2008).

Fish Handling and Imaging Procedures

The fish sampled during the recrudescence period (Table 1) were placed into a 2800-L fish hauler provided with compressed oxygen and transported to concrete 1900-L flow-through raceways that held 50–60 fish each. The fish sampled during the spawning trials (Table 3) were placed in a 1200-L fish hauler provided with compressed oxygen prior to ultrasonography. Fish were individually moved using dip nets and fish baskets for weight and length measurements, and placed into a portable 49-L cooler (Sportsman™ 52 Quart, Igloo Products Corp., Katy, TX, USA) half-filled with water (20–25 L). Each fish was gently positioned by

TABLE 3. *Ultrasound images (*n = *210 images) of channel catfish ovaries (one image/fish) were recorded after the fish were captured by seine and before hormone injection.*[a]

Trial	Pond	FC	FS	TL (cm ± SD)	BW (kg ± SD)	Dates
I	G2	39	22	59 ± 4	2.1 ± 0.4	April 4–7, 2008
II	G6	15	7	–	1.9 ± 0.2	May 26–30, 2008
III	G7	27	14	–	1.8 ± 0.5	June 2–6, 2008
IV	R2	22	14	–	2.5 ± 0.4	April 25–29, 2009
IV	R3	22	13	–	2.5 ± 0.4	April 25–29, 2009
V	G2	22	2	–	–	May 9–14, 2009
V	G3	23	8	–	–	May 9–14, 2009
VI	B3B	121	33	61 ± 6	2.2 ± 0.5	May 26–30, 2009
VII	H4	–	–	–	–	June 1–6, 2009
VII	H7	67	18	–	2.5 ± 0.4	June 1–6, 2009
VIII	G1	30	13	–	2.3 ± 0.4	March 30–April 3, 2010
VIII	G2	23	11	–	2.3 ± 0.4	March 30–April 3, 2010
IX	B9	75	21	60 ± 5	2.3 ± 0.5	May 5–10, 2010
X	G1	28	8	62 ± 5	2.6 ± 0.4	May 17–22, 2010
X	R2	32	8	61 ± 4	2.3 ± 0.4	May 17–22, 2010
X	R3	30	18	61 ± 3	2.2 ± 0.3	May 17–22, 2010

[a]Trial = spawning trial; pond = pond ID; FC = the number of fish captured; FS = the number of females selected for hormone injection; TL = total length (cm ± SD); BW = body weight (kg ± SD); dates = the spawning trial dates.

the caudal peduncle in an upright swimming position and the probe was positioned alongside the dorsal fin (Guitreau et al. 2012). The probe was moved toward the ovarian anterior and the posterior on the left side of the fish between the pectoral and the pelvic fins to obtain a general scan and to view the entire ovary. The probe was then held in position alongside the middle of the ovary, the sonographic ovarian morphology was assessed, and one image was recorded for each fish when the ovary was centered in the sonographic field of view, and when the probe was within 0–1 cm of the fish.

The ultrasonography index for evaluating adult channel catfish ovarian development was based on biological and ultrasonography insights integrated into a systematic interpretation approach that included: (1) the use of consistent fish handling and ultrasonography procedures developed previously (Guitreau et al. 2012); (2) knowledge of the location and tissue components of the ovary; (3) observed biological outcomes during artificial spawning; (4) knowledge of the gonadal processes occurring during the ovarian cycle of channel catfish and during artificial spawning (e.g., recrudescence, oocyte maturation, atresia); (5) identification, minimization, and elimination of reverberating ultrasound artifacts created as a consequence of the gas in the airbladder that would otherwise interfere with ovarian imaging; and (6) identification of sonographic anatomical markers such as the skin, muscle, bone, airbladder, and ultrasound artifacts to consistently center, record, and view the ovary (Novelo 2014). The synthesis of these biological and ultrasonography insights informed interpretation of the visibility, relative size, and appearance of the ovary and oocytes, the classification of 915 sonographic images of ovarian development of 915 channel catfish, and the development of a sonographic and descriptive template representative of each category.

The sonographic classification was illustrated with images that were selected from the 705 images recorded during the recrudescence phase and 210 images recorded during the spawning phase of the ovarian cycle of fish selected for the 10 spawning trials. Images recorded in each assessment category in each sampling date were assigned random numbers using the random excel function to provide unbiased and representative illustrations of sonographic assessments. The random numbers generated were sorted from the smallest to highest values, and the first two images were selected for figure illustrations for each category. In all the

illustrated images, the skin of the fish (located next to the position of the probe) was denoted by a solid white line; the periphery of the ovarian cross-section was denoted by a dashed white line; and artifacts caused by movement of the fish, bone (vertebrae), or gas (airbladder) were identified with double-headed arrows.

Artificial Spawning

The number of females selected for hormone injection was 210 (43 in 2008, 88 in 2009, and 79 in 2010), out of a total of 567 fish collected by seine during the spawning trials (Table 3). Fish selected for Trial I were injected with common carp pituitary extract (Stoller Fisheries, Spirit Lake, IA, USA) at a dose of 2 mg/kg on the date of fish collection and with 8 mg/kg 16 h later. Fish selected for Trials II–X were injected with luteinizing hormone-releasing hormone analog at a dose of 20 µg/kg on the date of fish collection and with 80 µg/kg 16–18 h later. Egg release was monitored by applying gentle pressure on the abdomen 20–30 h after hormone injection and every 2 h thereafter. If eggs were readily released, fish were anesthetized with 150–200 ppm of tricaine methanesulfonate (Western Chemical Inc., Ferndale, WA, USA). The eggs were stripped directly into greased food-grade plastic bowls containing modified Hanks' balanced salt solution adjusted to approximately 295 mOsm/kg as measured by a vapor pressure osmometer (Wescor model 5520, Wescor Inc., Logan, UT, USA) (Tiersch et al. 1994; Christensen and Tiersch 1996).

The total egg volume collected was recorded. If clumps were present, they were removed, and that egg volume was recorded. The number of eggs/female was estimated as the average eggs/mL (obtained from counts of two or three 5-mL egg samples) multiplied by the egg volume (mL) collected after removal of any clumps. The egg count of three monolayer samples at the bottom of 100-mL tri-corner plastic beakers (Thermo Fisher Scientific Inc., Suwanee, GA, USA) was used to calculate the average eggs/monolayer for each female. Nine monolayers of eggs were poured into nine beakers for each female, and 0.5 mL of sperm (1×10^9 cells/mL) was added to each monolayer from three males (three replicates per male for each female). Gametes were activated with 10 mL of hatchery water. Additional hatchery water (10 mL) was added after 5 min, and after 10 min, the eggs were transferred for incubation in a recirculating hatching system. Eggs undergoing embryonic development at 30–72 h after gamete activation were counted in each monolayer. The fertilization estimate of each female was calculated as the ratio of the average count of eggs undergoing embryo development divided by the average count of eggs in a monolayer.

Statistical Analysis

The generalized linear mixed model (GLIMMIX) procedure of the Statistical Analysis Software (SAS) system version 9.3 for Microsoft® Windows® (SAS Institute Inc., 2012, Cary, NC, USA) was used to test for differences caused by the spawning trial ($n = 10$ trials) on fertilization estimates of fish evaluated for ovarian development by use of ultrasonography and selected for artificial spawning. The statistical model included the spawning trial as a fixed effect, and all conditions within each spawning trial were included as a single fixed-effect variable, and the fish were combined into a single response variable. The combination of logit link and binomial distribution was used in the model.

The ultrasonography assessments were evaluated based on the expected and observed outcomes of viable or nonviable eggs during artificial spawning. The production of viable eggs was defined as low (<50%) or high (≥50%) fertilization rates based on egg quality criteria defined by Bates and Tiersch (1998). Nonviable eggs was defined as (1) no eggs collection, (2) collection of physically disintegrating eggs, and (iii) collection of eggs with no embryo development (0% fertilization) after 30–72 h of sperm and egg activation. The percent agreement of the observed and expected outcomes (total observed/total injected × 100) was calculated for each category in each trial, and the PROC GLIMMIX procedure in SAS was used to test for differences in the tabulated, expected, and observed outcomes. The expected

outcome, year, and the interaction of expected outcome and year were included as fixed effects in the model, and the observed outcome was the response variable. An error matrix (a cross-tabulation table) of the agreement of the hypothesized (expected) and observed responses was generated based on the model-predicted outcome (i.e., expected outcome) from the previous analysis and the observed biological outcome during the spawning trials to assess the likelihood (0–1.0 probability) of a correct assessment. Differences were considered significant at $P < 0.05$ for all statistical tests.

Results

The Ultrasound Imaging Classification Index

Three main types of echogenic morphology observed during the recrudescence period were designated as Categories 1 (undeveloped), 2 (underdeveloped), and 3 (developing) to denote sequential growth during the slow growth phase of the ovarian cycle (Fig. 1). Category 1 comprised ovaries appearing as an oval mass with a grayish, homogenous echogenic texture (Fig. 1A, B). No oocyte structures were visible. Ovaries appeared small, with visually discernible size variation, and they were identifiable by the increased echogenicity in the ovarian periphery (Fig. 1A, B). Category 2 comprised ovaries with the first visible appearance of oocytes (Fig. 1C, D). Oocytes in ovaries classified as Category 2 appeared as grainy white speckles against a darker background with no individually discernible shape, but collectively they presented a heterogeneous echogenic texture. If the ovary was small, but no oocytes were visible in the image, the ovary was assessed as Category 1. If the ovary was small, but oocytes were visible as speckled structures presenting a heterogeneous appearance, the ovary was assessed as Category 2. Category 2 ovaries were

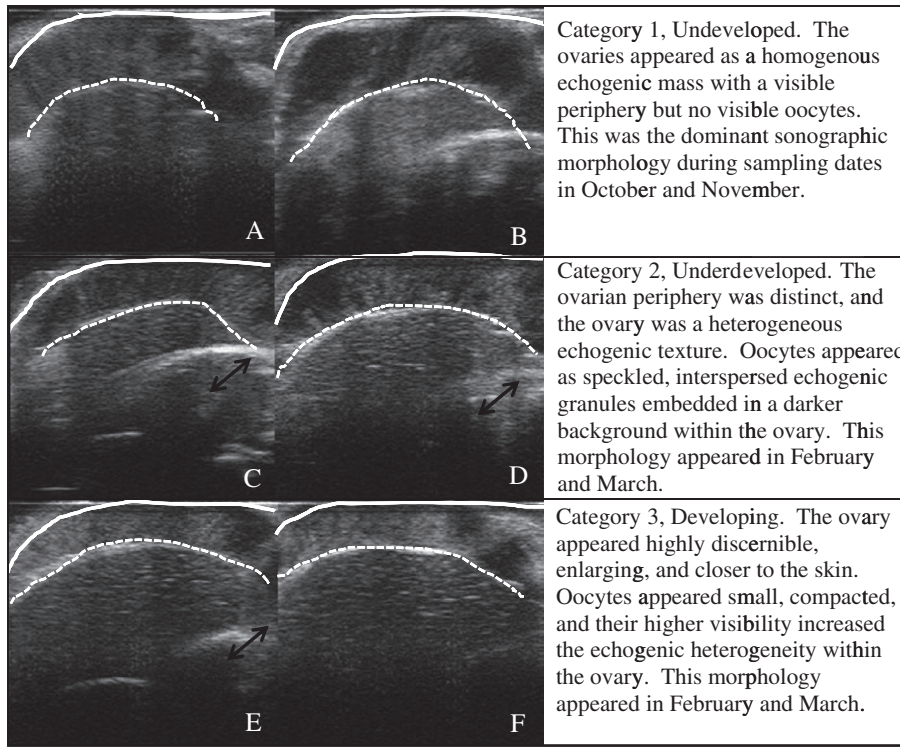

FIGURE 1. *The three main types of echogenic morphology observed during the ovarian recrudescence phase were designated as Categories 1 (undeveloped; A, B), 2 (underdeveloped; C, D), and 3 (developing; E, F). The skin (solid line) and ovarian periphery (dashed line) were demarcated in the paired images representative of these categories.*

in closer proximity to the skin relative to Category 3 ovarian images. Category 3 was defined by increased echogenic heterogeneity within a visibly enlarging ovary containing oocytes that appeared small and compacted (Fig. 1E, F). Category 1 occurred most frequently in October and November. Categories 2 and 3 occurred most frequently in February and March and rarely, or did not occur, in October or November (Fig. 1).

Four main types of echogenic morphology observed during the spawning period of the ovarian cycle were designated as Categories 4 (advanced), 5 (mature), 6 (spawned), and 7 (atretic) (Fig. 2). Category 4, presumably in the fast growth period (late vitellogenesis), presented a larger ovary, and individually distinguishable oocytes (Fig. 2A, B). Category 5, presumably at the end of vitellogenesis and ready for egg release, comprised ovaries of a complex echogenic structure that appeared highly organized (Fig. 2C, D). Individual, enlarged oocytes appeared as bright centripetal structures (caused by the yolk content of the oocytes) surrounded by darker peripheral structures (caused by the cytoplasm and ovarian fluids at the periphery of the oocytes) (Fig. 2C, D). Category 6, presumably in a "spent" state (complete release of eggs), comprised ovaries that appeared small and difficult to distinguish from surrounding tissues, and oocytes were usually not visible (Fig. 2E, F). Category 7 comprised ovaries and oocytes that appeared amorphic and degenerated, with an irregular ovarian echogenic texture (Fig. 2G, H).

The majority of the ovarian sonographic images (56% in 2008, 82% in 2009, and 100% in 2010) were assessed as Categories 1 (undeveloped), 2 (underdeveloped), 3 (developing), 4 (advanced), 5 (mature), 6 (spawned), and 7 (atretic). The remaining images were assigned tentative intermediate numerical categories that included 3.5, 3.75, and 4.5. This exploratory intermediate assessment was discontinued during the establishment of the classification index by development of the criteria and illustrations based on readily discernible morphological changes that were representative of the annual ovarian cycle (Figs. 1, 2).

Accuracy of the Ultrasound Imaging Classification Assessments

There was no significant effect on the mean differences in fertilization caused by the different spawning trials ($P = 0.9349$). The fertilization estimate of catfish females with ovarian development assessed as Categories 1, 2, 3, 4, 5, 6, and 7 ranged from 0 to 0.2 during 2008, from 0 to 0.6 during 2009, and 0 to 0.85 during 2010.

The percent agreement of the predicted (expected) and actual (observed) biological outcomes of artificial spawning was tabulated for each of seven ultrasound imaging classification assessments (Table 4). The expected outcome for Categories 1, 2, 3, 6, and 7 was that no viable eggs would be collected, and the expected outcome for Categories 4 and 5 was that viable eggs would be collected. The average agreement (overall percent average based on the raw tabulation data for the 10 spawning trials) of the expected and observed outcome was 100% accurate for Categories 1, 2, and 6; 91% accurate for Category 3; 29% accurate for Category 4; 45% accurate for Category 5; and 57% accurate for Category 7 (Table 4). Statistical analysis of the tabulated data based on the ultrasonography classifications showed a significant effect ($P < 0.002$) of the expected outcome on the observed outcome. The year (2008, 2009, and 2010) and the interaction between year and expected outcome had no significant effect ($P > 0.05$) on the observed outcome of the seven categories tested.

The error matrix clarified individual relationships of the expected to the observed outcome for the seven ultrasonography classifications by calculating the likelihood (0–1 probability) of correct assessments (i.e., that the expected and observed outcome was the same) for each category. The probability of a correct assessment was 0.99 for Category 1, 0.93 for Category 2, 0.94 for Category 3, 0.86 for Category 4, 0.89 for Category 5, 0.87 for Category 6, and 1.0 for Category 7. Overall, the error matrix indicated that (1) Categories 1, 2, 3, 6, and 7 were the most accurate classifications in predicting the outcome of nonviable egg production and that (2) although Categories 4 and 5 had a high probability of correct

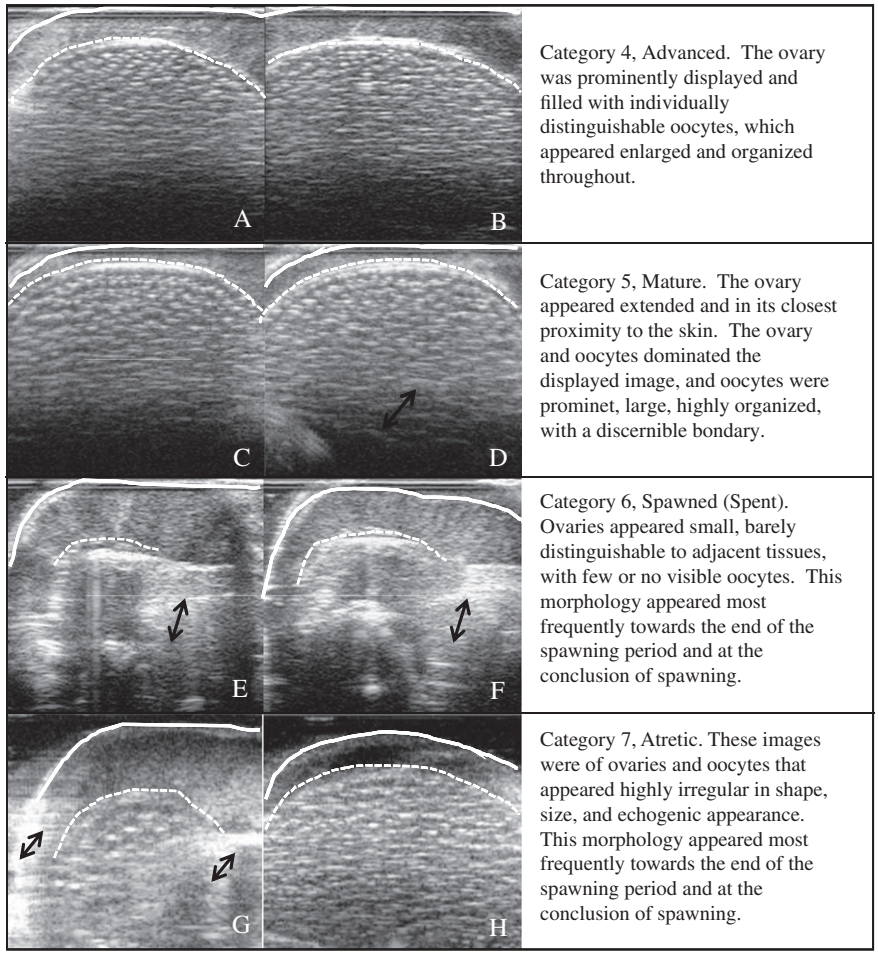

FIGURE 2. *The four main types of echogenic morphology observed during the spawning phase were designated as Categories 4 (advanced; A, B), 5 (mature; C, D), 6 (spawned; E, F), and 7 (atretic; G, H). The skin (solid line) and ovarian periphery (dashed line) were demarcated in the paired images representative of these categories.*

assessments, it was also likely to obtain nonviable eggs.

Discussion

The ultrasound imaging ovarian assessment index was developed based on visually observable echogenic morphologies that captured the continuous growth process and transformations from early to late vitellogenesis (the recrudescence phase), oocyte maturation, ovulation (the spawning phase), and atresia (the regression phase) (Figs. 1, 2). The channel catfish ovarian cycle was selected as the biological framework for the development of an ultrasonography classification index for use in reproductive assessments. Category 1 was representative of fish presumed to be in the initial stages of oocyte development, and therefore the least developed reproductive state. Category 2 was representative of more advanced development than Category 1, but less developed than Category 3, that represented the period of recrudescence prior to fast growth. Categories 4 (advanced) and 5 (mature) were representative of fish that would potentially produce viable eggs. Category 4 was considered to be at a more advanced reproductive state than Category 3 but less advanced than Category 5. Category 5 was presumed to be

TABLE 4. *A total of 175 female channel catfish were assessed using the ultrasonography classification index 1–7 (ultrasound image categorical assessments) of ovarian development and selected for artificial spawning.*[a]

	2008		2009		2010		
Ultrasound imaging categorical assessments	Inj.	Obs.	Inj.	Obs.	Inj.	Obs.	Percent agreement
1. Undeveloped	1	1	–	–	–	–	100
2. Underdeveloped	1	1	1	1	–	–	100
3. Developing	9	9	3	3	9	7	91
4. Advanced	7	3	11	3	24	6	29
5. Mature	5	3	34	13	32	16	45
6. Spawned	1	1	4	4	3	3	100
7. Atretic	–	–	19	8	11	9	57

[a]The hypothesized (expected) outcome for Categories 1, 2, 3, 6, and 7 was that no eggs or nonviable eggs would be collected. The hypothesized (expected) outcome for Categories 4 and 5 was that viable eggs would be collected. The number of fish injected (Inj.) and the number of fish for which the expected and observed outcomes were the same (Obs.) were listed for the spawning trials in 2008, 2009, and 2010. The percent agreement of the observed and expected outcome (total observed/total injected × 100) for the tabulated data was calculated for each assessment.

the most advanced state of ovarian reproductive development leading to oocyte maturation and ovulation. Category 6 was representative of fish in a spent reproductive state, and Category 7 was representative of fish that were visibly undergoing resorption.

Two markedly different ovarian morphologies have been reported to appear during the spawning period: (1) large ovaries with peak gonadosomatic index (GSI) values of prespawn females (9–15%) and (2) small ovaries with the smallest GSI values (0.05–0.06%) of spawned females in April through July (MacKenzie et al. 1989; Banks et al. 1999). In contrast, ovaries observed using ultrasonography during the spawning period in this study included four types (Fig. 2). Three types of large ovarian morphologies were Category 4 (advanced), Category 5 (mature), and Category 7 (atretic). The size of Category 7 ovaries varied from small to large, and large ovaries assessed as atretic presented oocytes that were easily visible, highly irregular in shape, size, and echogenic appearance (indicating a loss of structural integrity). The fourth ovarian morphology identified using ultrasonography was spawned ovaries appearing as a small (difficult to discern) and devoid of oocytes. Although a spent ovary may be atretic, especially if it was recently ovulated and has remnant follicles, two ultrasound imaging categories were created to account for and systematically assess ovaries presumed to be spent (Category 6) or visibly undergoing resorption (Category 7).

Tentative interval categories used early in the development of the ultrasound imaging reproductive index were discontinued with improved understanding of the sonographic view of the ovarian progression and transformations occurring during the annual ovarian cycle, the cumulative experience gained by sonographers, and the definition of categories based on easily recognizable echogenic morphology (Figs. 1, 2). No attempt was made to reassess images of intermediate categories because these were tentative assignments representing 17% of the total ultrasound imaging assessments, and because real-time imaging ensures optimal condition for assessment. For example, artifacts that may be generated during image capture and ultrasonography (by recording of a still image from a video) or artifacts created by internal anatomy (e.g., airbladder gases) may be avoided and eliminated. As a second option, images of ovaries may be assessed in videos that include a complete scan of the ovary, and final positioning of the probe alongside the dorsal fin for 2–3 sec to view and assess the echogenic morphology displayed. The disadvantage of using still images for assessment is that, depending on the way the image was captured or recorded, ultrasound image artifacts may be introduced that could hinder assessment.

Artificial Spawning and Fertilization

Few fish ($n = 3$ total) were injected to induce spawning in Categories 1 and 2 (Table 4) primarily because these fish had small ovaries, no visible oocytes, and these categories occurred less during the spawning periods. No attempt was made to artificially spawn fish in ambient temperature ponds assessed as Categories 1, 2, or 3 in October through March. In general, the estimated fertilization rate of viable eggs was of low quality (i.e., defined as less than 50%) (Bates and Tiersch 1998). The thermal profile data of the ponds indicated that fish in general were held for a longer period of time in ambient and geothermally regulated ponds than was optimal for obtaining broodstock for viable egg production. No fertilization, if any eggs were collected, was expected for Categories 1, 2, 3 (early recrudescence phase), and for Categories 6 and 7 (resorption phase), especially for fish from ponds (e.g., Trial II, Pond G6, 295 dd; Trial V, Pond G2, 358 dd) that greatly surpassed the recommended degree-day guidelines for the conclusion of spawning (150–172 dd) during which 90% of the broodstock were expected to have spawned (Pawiroredjo et al. 2008). This may have reduced overall egg quality, but did not interfere with ultrasound assessments.

Accuracy of the Ultrasound Imaging Classification Assessments

Statistical analysis of the tabulated data (Table 4) showed that there was a strong relationship in the expected and observed outcomes of the ultrasound imaging index. Individual relationships based on the tabulation of the raw data and the error matrix calculations showed that the accuracy of assessments was highest for Categories 1, 2, 3, 6, and 7 that were associated with nonviable egg production. These categories defined a spectrum of ovarian development that identify fish not desirable for hormone injection in commercial hatchery production of hybrids because if hormone injection is administered too early in the season (Categories 1, 2, and 3), when oocytes have not completed vitellogenesis, or if the injection is administered too late (e.g., Categories 6 and 7), the treatment may fail to induce spawning, or eggs of poor quality may be collected. Although Categories 4 and 5 had a 0.86 and 0.89 probability of correct assessment, it was equally likely that nonviable eggs be collected. The results for Category 4 and 5 may be explained by error in assessment; however, the outcome of artificial spawning is a consequence of complex biological and environmental processes, and the outcome of nonviable eggs may have been a consequence of other factors such as the water quality conditions in the egg incubating systems, and lack of timely collection of eggs that may have reduced egg quality. In sum, the tabulated data and the error matrix indicated that the most reliable predictions were based on Categories 1, 2, 3, 6, and 7 and that the outcome for Categories 4 and 5 included the collection of viable and nonviable eggs.

The results obtained in this study have direct application in commercial hatchery production. Selecting a female that is mature and ready for hormone injection is equally important as rejecting a fish that would produce nonviable eggs in order to maximize efficiency of production and decrease costs associated to labor, hormone use, and time. Ultrasonography surpasses the limitations of external examination by providing a highly accurate means of identifying fish that would not spawn, as was demonstrated by the ability to view an enlarged ovary that is atretic (Category 7) that may otherwise be confused as a female that is ready for hormone injection with the sole use of external inspection. This sonographic advantage is especially useful in ponds that may have broodstock with advanced ovarian development, or for assessment of fish toward the end of the spawning season when atretic fish occur more frequently and may not easily be discriminated from fish likely to produce viable eggs. The ultrasound reproductive index may also be used earlier in the spawning season to identify females with ovarian morphology that would likely spawn when the majority of females may not be ready (Categories 1, 2, 3). Every effort was made to document the methods and results in this study so that enough information is provided to replicate the study and for potential use in research, but the overarching intention was

to develop a tool for hatchery use. The suggested models for integration into farm use include (1) training of existing workers and (2) hiring of ultrasound personnel in order for the advantages of this technology to be available to commercial hatcheries. Training and outreach programs need to be developed, and further on-site studies on the use of ultrasonography in commercial-scale hatchery production are needed.

Conclusions

Ultrasound images that provided a direct view of ovarian development in adult channel catfish were observed, interpreted, categorized, and recorded. A reproductive ultrasonography index comprising seven categories was developed based on transformations in the sonographic ovarian morphology observed during the annual ovarian cycle and based on the expected and observed biological outcomes during artificial spawning. The predictions of viable or nonviable egg collection based on the ultrasonography index were in significant agreement to the observed outcome during the spawning trials. The individual relationships of the expected and the observed outcome of each category showed that the most accurate assessments identified fish that would produce nonviable eggs (Categories 1, 2, 3, 6, and 7), and that the outcome for Categories 4 and 5 was variable and produced viable and nonviable eggs.

This study provided a thorough objective analysis of ovarian development and the probability of correct assessments by use of the unprecedented approach of directly viewing sonographic ovarian morphologies throughout the annual reproductive cycle of channel catfish. Images of ovarian development may be obtained and assessed and rapidly (in less than 30 sec/fish) for correctly deciding whether or not to inject females for hormone-induced spawning, and especially for eliminating fish that would not provide viable eggs because they are biologically incapable of it (Categories 1, 2, 3, 6, and 7). This is a significant contribution to improving efforts at successful artificial spawning and to minimizing the costs associated with labor, hormones, and time. Hatchery efforts at the beginning and end of the spawning season would especially benefit from ultrasound evaluation by excluding underripe or atretic individuals, and identifying those fish ready for production of viable eggs.

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