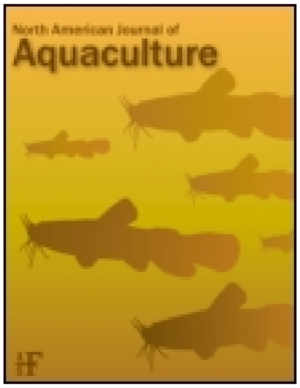

North American Journal of Aquaculture
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/unaj20

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Available online: 04 Apr 2012

To cite this article: Noel D. Novelo & Terrence R. Tiersch (2012): A Review of the Use of Ultrasonography in Fish Reproduction, North American Journal of Aquaculture, 74:2, 169-181

To link to this article: http://dx.doi.org/10.1080/15222055.2012.672370

North American Journal of Aquaculture 74:169–181, 2012

ISSN: 1522-2055 print / 1548-8454 online
DOI: 10.1080/15222055.2012.672370

ARTICLE

A Review of the Use of Ultrasonography in Fish Reproduction

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Abstract
Ultrasound imaging analysis involves development of an effective combination of physical properties, equipment, instrument settings, and protocols. This review focuses on the application of ultrasonography to fish reproduction. The goal was to assemble a comprehensive reference data set to serve as a decision-enabling tool for potential users. The specific objectives were to (1) identify the ultrasound equipment, settings, and procedures used during examination, (2) review the fish handling procedures used during examination, and (3) review current data on sex identification and reproduction indices developed using ultrasonography. The 27 studies selected for inclusion in this review represent 21 fish species. Most (96%) of the studies reported the model name for the ultrasound unit, but only 19% reported the probe model. The most reported probe features were the frequency capability (96% of the studies) and array format (linear, sector, or annular; 81%). The majority of the studies (89%) did not report any of the control settings used. The combinations of handling and ultrasound procedures were variable even within the same species, and the majority (78%) of the studies included a form of restraint. None of the studies simultaneously integrated the use of unrestrained, unanesthetized, submersed fish with a submersed waterproof probe, which would enable the use of water as a transmission medium for ultrasound. Size, life stage, gonadal growth of reproductively active adults, and fish morphology influenced the ability to use ultrasonography for sex identification and the development and application of qualitative and quantitative reproductive indices. The utility of ultrasonography in fish reproduction has been repeatedly validated, and innovative indices for noninvasive use have been developed. However, this review identifies a clear lack of consistency in reporting of instrument settings and handling procedures and provides suggestions for standardizing the use of ultrasonography with aquatic species.

Ultrasonography utilizes technology that interconverts electric and acoustic energy to create an image of internal anatomy, typically as gray-scale images produced for human and veterinary diagnostics. These images are created by different modes of ultrasound echo reception and display, including A-Mode (amplitude mode), B-Mode (brightness mode), Real-Time B-Mode (moving gray-scale images), M-Mode (motion, or time-motion mode), and color modes (introduced by Doppler technology). A-Mode produces one-dimensional displays of echo amplitudes, displayed as spikes on a vertical line. It is used especially for ophthalmic examination and for evaluating the fat and lean portions of meat in animals; it is least frequently used in reproduction studies (Ginther 1995). B-Mode produces two-dimensional gray-scale images, such that the brightness displayed by dots (or pixels) on the echo display screen corresponds to the amplitude of the individual echo signal, and the position of the dot corresponds to the depth of echo origin (Nyland et al. 2002).

Real-Time B-Mode creates a moving, cross-sectional gray-scale image. This, the most commonly used mode for examining the reproductive tract of animals, is composed of multiple B-Mode lines created by an ultrasound beam swept across a triangular (sector) or rectangular (linear) field of view. This yields a triangular- or rectangular-shaped ultrasound image displayed on the monitor (Nyland et al. 2002). The sector format may

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Received March 11, 2011; accepted May 26, 2011

be produced by different probe types such as mechanical sector scanners, or by electronic steering of the ultrasound beam by a probe composed of multiple elements ("arrays"), including curvilinear arrays, curved arrays, and phased arrays (Nyland et al. 2002). The rectangular format is produced by the linear array probe which has multiple, rectangular, piezoelectric elements. The M-mode, an adaptation of the B-Mode, is used for evaluating moving structures such as the heart, and the motion of the dots, or the change in distance between the probe and the reflecting interfaces, is displayed on depth and time axes. The modes utilizing Doppler technology register the motion of blood toward, away, or at an angle to the transducer (probe) to construct color images of flow patterns. Ultrasound imaging technology refers to one or a combination of these modes of ultrasound echo reception and image display.

Ultrasonography, which provides direct, visual access to internal anatomy, has revolutionized access to biological data to monitor and diagnose changes in internal anatomy. This imaging technology was first used in humans in the 1970s to study heart (Gowda et al. 2004), musculoskeletal structures (Kane et al. 2004), and the reproductive system (Jansen and van Os 1989). Ultrasonography also has been widely used for reproductive applications in farmed animals (Medan and Abd El-Aty 2010). In aquatic species, ultrasonography has been used to study reproduction in aquatic mammals, such as the bottlenose dolphin *Tursiops truncatus aduncas* (Brook et al. 2000; Brook 2001; Robeck et al. 2005), the Indo-Pacific humpback dolphin *Sousa chinensis* (Brook et al. 2004), and killer whales *Orcinuc orca* (Robeck et al. 2004); in amphibians, such as the tomato frog *Dyscophus antongili* (Schildger and Triet 2001); and in reptiles, such the American alligator *Alligator mississippiensis* (Lance et al. 2009).

In fish, ultrasonography has been used to study (1) internal structures such as muscle mass in channel catfish *Ictalurus punctatus* (Bosworth et al. 2001), thyroid gland in spotted dogfish *Scyliorhinus stellaris* and small-spotted catshark *S. canicula* (Gridelli et al. 2003), blood flow and vein contractions in the common cuttlefish *Sepia officinalis* (King et al. 2005) and (2) disease, such as liver tumors in zebrafish *Danio rerio* (Goessling et al. 2007), ocular lesions in halibut *Hippoglossus hippoglossus* (Williams et al. 2007), and internal disorders in ornamental common carp *Cyprinus carpio* (Saint-Erne 2010). Ultrasonography has also been used to study fish reproduction for nearly 30 years (Table 1).

The goal of this review was to assemble a comprehensive reference data set to serve as a decision-enabling tool for potential users. The objectives were to identify ultrasound equipment, settings, and procedures used during examination; review fish handling procedures used during examination; and review current data on sex identification and reproduction indices developed using ultrasonography. This review identified a large number of inconsistencies and omissions in reporting of equipment settings, fish handling, and description of the ultrasound imaging procedures that are relevant to reproduction studies using ultrasonography, whether in commercial or public hatcheries, or in field studies. Our review summarizes contributions made by use of ultrasonography in fish reproduction and identifies approaches for improving throughput, fish handling, and reporting of equipment and instrument settings used for imaging. Application of ultrasonography in fish reproduction can be improved by collecting and routinely reporting the information necessary to replicate, standardize, and optimize imaging procedures.

METHODS

Search strategy and selection criteria.—Four databases (afsjournals.org, googlescholar.com, isiknowledge.com, onlinelibrary.wiley.com) were searched for peer-reviewed articles addressing ultrasound technology and reproduction in fish, representative of the literature in scientific journals published in English. The following key words used were in alternative combinations: "ultrasound," "fish," "reproduction," and "imaging." The "References" and the "Times Cited" links for each of the articles listed in the results of the main search query were searched. Based on this scheme, the search was extended into additional links and to the references of publications with titles explicitly stating the use of ultrasonography in fish reproduction. Eight percent (27 publications) of the 327 studies that were initially identified for review were selected based on their direct relevance to ultrasonography in fish reproduction. A key list was created for these studies, including a study identification number, with corresponding species names and references, for use in this review (Table 1).

Data extraction.—Publications were reviewed for the specifics reported on equipment and settings, biological data of fish, fish handling procedures, use of ultrasonography for sex identification, and development and application of reproductive indices. Data were compiled on (1) the ultrasound instrument, probe (transducer), storage device used, and corresponding technical procedures and settings; (2) biological data of fish, including the sample number, the number of male and female fish, age (years), length (cm), weight (g, kg), and reproduction terminology used for identifying life stage and reproductive condition; (3) fish handling procedures before and during ultrasonography; and (4) description of the scanning procedure. An EndNote Library and an Excel spreadsheet detailing the goal, common and scientific names, and corresponding biological, fish handling, and ultrasound technique of each study were compiled. If data on ultrasound practices in fish reproduction were not reported, or explicitly stated, the data were classified as "NR" (not reported).

RESULTS

Ultrasound Unit and Control Settings Used

The model name of the ultrasound unit, along with the probe features reported, were compiled (Table 2). Most (96%) of the studies reported a model name for the ultrasound unit used, and 41% reported the use of portable machines suitable for use

TABLE 1. Studies of the use of ultrasonography in fish reproduction. The key listing includes the study identification number (ID), the common and scientific names (based on Nelson et al. [2004] whenever possible), the goal of the study, and references. Fish ($N = 21$ species) were grouped into two main categories—freshwater (24% of species) and marine and anadromous (76%)—according to family, genus, and species. The studies are listed chronologically for each species within a taxonomic order. The goal of each study is identified as sex identification (SI), the development or application of reproductive indices (RI), or both (SI, RI). Sex identification and reproductive indices were divided into two types of data: data based solely on ultrasonography (SI-1, RI-1) and those derived from both ultrasonography and other methods (SI-2, RI-2).

ID	Species	Goal	Reference
	Freshwater fishes		
1	Stellate sturgeon *Acipenser stellatus*	SI-1, RI-2	Moghim et al. (2002)
2	Shovelnose sturgeon *Scaphirhynchus platorynchus*	SI-1, RI-2	Colombo et al. (2004)
3	Shovelnose sturgeon	SI-2	Wildhaber et al. (2005)
	Pallid sturgeon *S. albus*	SI-2	
4	Shovelnose sturgeon	RI-2	Wildhaber et al. (2007)
5	Shovelnose sturgeon	RI-2	Bryan et al. (2007)
	Pallid sturgeon	RI-2	
6	Neosho madtom *Noturus placidus*	RI-2	Bryan et al. (2005)
7	Murray cod *Maccullochella peelii*	SI-1, RI-2	Newman et al. (2008)
	Marine and anadromous fishes		
8	Pacific herring *Clupea pallasii*	SI-1, RI-2	Bonar et al. (1989)
9	Atlantic cod *Gadus morhua*	SI-2	Karlsen and Holm (1994)
10	Atlantic cod	RI-1	Davie et al. (2003)
11	Atlantic cod	SI-2	McEvoy et al. (2009)
12	Barfin flounder *Verasper moseri*	SI-1	Matsubara et al. (1999)
13	Atlantic halibut *Hippoglossus hippoglossus*	RI-1	Shields et al. (1993)
14	Atlantic halibut	SI-1, RI-2	Martin-Robichaud and Rommens (2001)
	Winter flounder *Pseudopleuronectes americanus*	SI-1, RI-2	
	Yellowtail flounder *Limanda ferruginea*	SI-1, RI-2	
	Haddock *Melanogrammus aeglefinus*	SI-1, RI-2	
15	Haddock	RI-2	Martin-Robichaud and Berlinsky (2004)
16	Atlantic salmon *Salmo salar*	SI-1, RI-2	Mattson (1991)
17	Coho salmon *Oncorhynchus kisutch*	SI-1	Martin et al. (1983)
18	Steelhead[a] *O. mykiss*	SI-2, RI-2	Evans et al. (2004a)
19	Steelhead	RI-2	Evans et al. (2004b)
20	Striped bass *Morone saxatilis*	RI-2	Will et al. (2002)
21	Striped bass	SI-2, RI-2	Blythe et al. (1994a)
	Striped bass × white bass *M. chrysops*	SI-2	
22	Striped bass	SI-2, RI-2	Blythe et al. (1994b)
23	Striped bass	RI-2	Jennings et al. (2005)
24	Red hind *Ephinephelus guttatus*	SI-1, RI-2	Whiteman et al. (2005)
25	Nurse sharks *Ginglymostoma cirratum*	RI-2	Carrier et al. (2003)
26	Broadnose sevengill shark *Notorynchus cepedianus*	RI-1	Daly et al. (2007)
27	Small-spotted catshark *Scyliorhinus canicula*	RI-2	Whittamore et al. (2010)
	Thornback ray *Raja clavata*	RI-2	

[a]Anadromous form of rainbow trout.

outside of the laboratory. However, the majority of the studies (89%) did not report the control settings used for obtaining ultrasound images. Use of the B-mode echo display was reported for four studies (Table 2). Although control settings such as focus depth, output power, and frame rate were reported by 11% of the studies (Table 2), the values for these settings were not reported, except for a single study that reported a specific value for "gain" and two studies that reported a specific value for "power" (Table 2).

The Probe: Features, Frequency Used, and Procedures

The most reported probe features (Table 2) were frequency capability (96% of the studies) and the array (linear, sector, annular) formats (81% of the studies). The majority of the studies (74%) used a linear array, which produces rectangular-shaped images. Fewer studies (11%) used sector-type probes that produce triangular-shaped images, including a mechanical sector probe (study 14), a curvilinear array probe (study 26), and a comparison of the use of curved array and linear array probes

TABLE 2. Compilation of data from publications for ultrasound imaging equipment (ultrasound unit and probe), the storage and formatting of images, and the corresponding technical procedures and settings used in fish reproduction studies. The following are reported for the ultrasound units: model, portability, echo display mode used, and basic control settings used for obtaining images (power settings [control of voltage, and thus the intensity of sound output by the probe], overall gain [causing uniform amplification of all returning echoes regardless of depth of origin], reject settings [causing suppression of returning echoes], and time-gain compensation settings [controlling near to far field amplification of returning echoes]). The probe features include the model, whether it was single or multiple frequency (S/M), the available frequencies (MHz), and the arrangement and shape (array) of the piezoelectric crystal elements, which produced linear (L), annular (A), sector (S), curved (C), and convex sector–linear (C&L) image constructs. The data on probe frequency transmission procedures and settings included whether the probe was covered, whether it was submersed, what frequency setting was utilized, and what medium of ultrasound transmission was used (ultrasound transmission gel or water). The ultrasound image storage device and whether formatting of the ultrasound image was provided were reviewed (Sub = submersed; NR = not reported).

		Ultrasound unit[a]			Probe features				Probe frequency transmission				Ultrasound image	
ID	Species	Model	Mode	Control settings	Model	S/M	MHz	Array[b]	Covered	Sub	MHz used	Medium	Storage device	Format
1	Stellate sturgeon	Pie Medical 200 VET	Yes	NR	Yes	NR	5/7.5	L	Yes	NR	5, 7.5	Water	Yes	NR
2	Shovelnose sturgeon	Sonosite 180 Plus, portable	NR	NR	Yes	NR	5	L	NR	NR	5	Gel	NR	NR
3	Shovelnose and pallid sturgeons	Shimadzu SDU-400 Plus	NR	Gain	NR	NR	7.5	L	Yes	NR	7.5	Gel	NR	NR
		Sonosite 180 Plus, portable	NR	Gain	NR	M	5-10	L	Yes	NR	NR	Gel	NR	NR
4	Shovelnose sturgeon	Shimadzu SDU-400 Plus	NR	NR	NR	NR	7.5	L	Yes	NR	7.5	Gel	NR	NR
		Sonosite 180, portable	NR	NR	NR	M	5-10	L	Yes	NR	NR	Gel	NR	NR
5	Shovelnose and pallid sturgeons	Shimadzu SDU-400 Plus[c]	NR	NR	NR	NR	7.5	L	NR	NR	7.5	NR	NR	NR
6	Neosho madtom	GE LOGIQ 700 Expert[c]	NR	NR	NR	S	8, 13	NR	NR	NR	8, 13	NR	NR	NR
		Shimadzu SDU-400 Plus[c]	NR	NR	NR	NR	7.5	NR	NR	NR	7.5	NR	NR	NR
7	Murray cod	Sonosite 180 Plus	NR	NR	NR	NR	5	L	NR	NR	5	NR	Yes	NR
8	Pacific herring	Unirad EDP 1000 B scanner	NR	NR	NR	NR	5	NR	NR	Yes	5	NR	NR	NR
9	Atlantic cod	Pie Medical Scanner 450 VET	Yes	Power	NR	NR	3.5	L	NR	Yes	3.5	Water	NR	NR
10	Atlantic cod	NR, portable	NR	NR	NR	NR	7.5	NR	NR	NR	7.5	NR	NR	NR
11	Atlantic cod	Aloka SSD 500, portable[c]	NR	NR	NR	NR	7.5	L	NR	NR	7.5	NR	NR	Yes
12	Barfin flounder	Echo Camera SSD-1000[c]	NR	NR	Yes	NR	10	A	NR	NR	10	No gel	NR	NR
13	Atlantic halibut	Aloka model 210DX11[c]	NR	NR	NR	NR	7.5	L	NR	Yes	7.5	Mucus	Yes	VR
14	Atlantic halibut	Ultramark 4 Plus[c]	NR	NR	Yes	M	5, 7.5, 10	S	NR	Yes	5, 7.5	NR	Yes	VR
	Winter and yellowtail flounder	Ultramark 4 Plus[c]	NR	NR	NR	M	5, 7.5, 10	S	NR	Yes	5	NR	Yes	VR
	Haddock	Ultramark 4 Plus[c]	NR	NR	NR	M	5, 7.5, 10	S	NR	Yes	NR	NR	Yes	VR
15	Haddock	Ultramark 4 Plus[c]	NR	NR	NR	NR	NR	NR	NR	NR	5	NR	Yes	VR
16	Atlantic salmon	Pie Medical Scanner 450 VET	NR	Power	NR	NR	3.5	L	NR	NR	3.5	NR	NR	NR
17	Juvenile coho salmon	Xenotec XUC-4[c]	NR	NR	NR	NR	15	NR	NR	Yes	15	Gel	NR	NR
	Adult coho salmon	Advance Technology Laboratories Mark V[c]	NR	NR	NR	NR	5	NR	NR	NR	5	Gel	NR	NR
18	Steelhead[d]	Aloka SSD-500v, portable	NR	NR	NR	NR	7.5	L	NR	NR	7.5	NR	NR	NR
19	Steelhead	Aloka SSD-500v, portable	NR	NR	NR	NR	7.5	L	NR	NR	7.5	NR	Yes	NR
20	Striped bass	Pie Medical Scanner 100LC, portable	NR	NR	NR	M	6/8	L	NR	NR	NR	NR	Yes	VR

TABLE 2. Continued.

		Ultrasound unit[a]			Probe features				Probe frequency transmission				Ultrasound image	
ID	Species	Model	Mode	Control settings	Model	S/M	MHz	Array[b]	Covered	Sub	MHz used	Medium	Storage device	Format
21	Adult striped bass, hybrids[e]	Aloka 500 V	Yes	NR	NR	NR	5	L	NR	NR	5	NR	NR	NR
22	Striped bass	Aloka 500Z	Yes	NR	NR	NR	5	L	NR	NR	5	NR	Yes	VR
23	Striped bass	Pie Medical Scanner LC100, portable	NR	NR	NR NR	M M	3.5/5.0 6.0/8.0	C L	NR NR	NR NR	NR NR	NR NR	Yes Yes	NR NR
24	Red hind	Pie Medical Scanner, portable	NR	NR	NR	M	3.5–5.0	L	NR	NR	NR	NR	Yes	NR
25	Nurse sharks	Pie Medical Scanner, model 200[c]	NR	NR	Yes	NR	3.5	L	NR	NR	3.5	NR	Yes	NR
26	Broadnose sevengill sharks	Aloka SSD500[c]	NR	NR	NR	NR	3.5	C&L	Yes	Yes	3.5	Gel	Yes	NR
27	Small-spotted catshark, thornback ray	Concept MCV, portable[c]	NR	NR	NR	NR	7.5	L	No	Yes	7.5	Seawater	Yes	NR

[a]Names as reported in publications.
[b]L = linear, S = mechanical sector, C&L = convex sector–linear scanner, A = annular, and C = curved.
[c]Manufacturer and address were reported for the model of ultrasound machine or probe used.
[d]Anadromous rainbow trout.
[e]Juvenile and adult striped bass × white bass.

(study 23). Most of the studies (96%) reported either a single ultrasound frequency or multiple frequencies as a probe attribute (Table 2). Five of seven studies that reported multiple frequency values of the probe did not specify the actual frequency used during ultrasound imaging (Table 2).

Ultrasound frequencies used in all the studies reviewed ranged from 3.5 to 15 MHz. The frequencies used for acipenserid fish of 44–150 cm fork length (FL) were 5 and 7.5 MHz; those for salmonid fish of 10–45 cm FL were 3.5, 5, 7.5, and 15 MHz; those for moronid fish of 55–100 cm total length (TL) were 3.5, 5, 6, and 8 MHz; and those for pleuronectid fish were 10 MHz for fish 1–40 TL and 5 and 7.5 MHz for fish 54–120 cm FL. The procedure reported for use of the ultrasound probe and for ultrasound frequency transmission included covering the probe (15% of studies), not covering the probe (4%), completely submerging the probe in water (26%), using water as an ultrasound transmission medium (11%), and using gel as an ultrasound transmission medium (22%).

The Storage Device and Ultrasound Image Format

The type of storage device used for recording images was reported by 48% of the studies reviewed (Table 2). The main storage device was videotape. One study identifying the use of a videotape recorder reported photographing recorded images displayed on the monitor; another reported the use of thermal print images, digitized into Tagged Image Format Files (TIFF). Studies that reported video recording of ultrasound images generally did not report the image format.

Handling Procedures

Handling before ultrasonography.— The holding systems (tanks mostly) were used as containers during ultrasonography in 13 studies for steelhead, striped bass, broadnose sevengill sharks, Atlantic halibut, and haddock (Table 3). Containers other than the holding systems were used for the Neosho madtom, Murray cod, barfin flounder, striped bass, nurse sharks, small spotted catshark, and the thornback ray.

The majority (70%) of studies reported use of live fish during ultrasound imaging, a few studies (22%) reported killing fish as a standard procedure before imaging, and two studies reported killing fish for ease of transportation (study 12) or as a consequence of handling (i.e., air embolism; study 24).

Handling leading up to scanning.—In 63% of the studies fish were completely submersed, including fish that were alive (e.g., broadnose sevengill shark) or dead (e.g., Pacific herring); in one study, fish (Murray cod) were partially submerged. Several (37%) of the studies did not report whether the fish remained submersed in water (Table 3).

The majority (78%) of the studies involved procedures that physically (10 studies) or chemically (15 studies) restrained the fish. Fish categorized as physically restrained included fish that were killed as part of the sampling procedure (shovelnose sturgeon, Murray cod, Pacific herring, barfin flounder, steelhead, small-spotted catshark, and thornback ray), those unintentionally killed as a result of handling (red hind), those sampled during electrofishing (striped bass), and those physically enveloped in a vinyl bag (broadnose sevengill sharks). The fish that were chemically restrained (use of anesthesia) included

TABLE 3. Compilation of data on fish handling procedures, including the holding system (tanks, raceways, or ponds) in laboratory or field, the container used to hold fish during ultrasonography, whether or not the fish was killed before imaging, and whether or not the fish was submersed (Sub) in water, physically restrained (Res), or anesthetized (Anes). Data on fish position (Pos), the external anatomy scanned (scan region), and the duration of the ultrasound imaging procedure (time/fish) are also listed. (yes = reported in the study, NR = not reported in the study.)

ID	Species	Holding system	Container	Killed	Sub	Res	Anes	Pos[a]	Scan region	Time/fish
1	Stellate sturgeon	NR	Tank	No	Yes	NR	Yes	NR	1 cm from the skin, on the lateral sides, between the pectoral and anal fins	<30 s
2	Shovelnose sturgeon	NR	NR	Yes	NR	Yes	Yes	NR	On the left side, above the third and fourth ventral scutes anterior to the pelvic fins	NR
3	Shovelnose sturgeon	Flow-through circular tanks	NR	No	Yes	NR	No	D	Ventral abdominal surface from vent to opercula	NR
	Pallid sturgeon	NR	NR	No	Yes	NR	No	D	Ventral abdominal surface from vent to opercula	NR
4	Shovelnose sturgeon	Flow-through circular tanks	NR	No	Yes	NR	No	D	Ventral abdominal surface from vent to opercula (illustrated) and alongside, ventrally, between the row of belly scutes and the first row of side scutes	NR
5	Shovelnose sturgeon	NR	NR	No	NR	NR	NR	NR	Three equidistant points along the gonad	NR
	Pallid sturgeon	NR	NR	No	NR	NR	NR	NR	Three equidistant points along the gonad	NR
6	Neosho madtom	720-L living stream	A pan of water	No	NR	NR	Yes	D	Ventrally, against the abdomen	NR
7	Murray cod	2500-L circular tanks	100-L bath	Yes	Yes[b]	Yes	Yes	D	Midlateral region, ventral	<1 min
8	Pacific herring	On ice	10-cm-deep water bath	Yes	Yes	Yes	No	L	Anterior to the dorsal fin, and perpendicular to the long axis of the fish	"Within seconds"
9	Atlantic cod	NR	Glass aquarium	No	Yes	NR	Yes	D	Ventral side up, caudal part of abdominal cavity (illustrated)	<30 s
10	Atlantic cod	6.5-m^3 tanks	NR	No	NR	NR	NR	NR	NR	NR
11	Atlantic cod	In large tanks	Yes[c]	No	NR	NR	Yes	NR	Coelomic cavity	20–40 s
12	Barfin flounder	In aquarium	Shallow plastic tray	No, Yes	NR	Yes	No	L	The skin on abdomen, moving laterally (illustrated)	2 min
13	Atlantic halibut	4.5-m-diameter tanks	Holding system	No	Yes	No	No	L	The skin above the ovary, on three positions, and the entire ovary (illustrated)	NR
14	Atlantic halibut	NR	44-L container	No	Yes	NR	Yes	L	On side of fish, flatfish (lying flat), directly posterior to the gut region (illustrated)	NR
	Winter flounder	NR	44-L container	No	Yes	NR	Yes	L	On side of fish, flatfish (lying flat), directly posterior to the gut region (illustrated)	NR
	Yellowtail flounder	NR	44-L container	No	Yes	NR	Yes	L	On side of fish, flatfish (lying flat), directly posterior to the gut region (illustrated)	NR
	Haddock	NR	76-L container	No	Yes	NR	Yes	D	Ventral side up, directly anterior to the urogenital pore (illustrated)	NR
15	Haddock	147-m^3 tanks	Holding system	No	Yes	NR	Yes	NR	NR	NR
16	Atlantic salmon	NR	Tank	No	Yes	NR	Yes	D	Upside down, over the belly, back and forth over the belly	NR
17	Coho salmon	NR	On a rotating platform	No, Yes	Yes	Yes	Yes	NR	At various posterior distances from the gill and ventral distances from the lateral line	NR
18	Steelhead[d]	Temporary 190-L tank	Holding system	No	Yes	NR	Yes	D	Moved anterior or posterior on ventral side of abdomen	NR
19	Steelhead	Temporary 190-L tank	Holding system	No, Yes	NR	Yes	Yes	NR	Along abdominal surface	4 min
20	Striped bass	NR	NR	No	NR	Yes	NR	NR	Oriented probe perpendicular or parallel to fish body until ovary visible	NR

TABLE 3. Continued.

ID	Species	Holding system	Container	Killed	Sub	Res	Anes	Pos[a]	Scan region	Time/fish
21	Striped bass	2200-L tanks; raceways	180-L tank	No	Yes	NR	Yes	V	Midlaterally along length of gonad, at base of pelvic fin, at isthmus of dorsal fin	<1 min
	Hybrids[e]	NR	NR	NR	NR	NR	NR	NR	NR	NR
22	Striped bass	2,200-L tanks; raceways	Tank	No	Yes	NR	Yes	V	Midlaterally along length of gonad, at base of pelvic fin, at isthmus of dorsal fin	NR
23	Striped bass	Holding tanks	Holding tanks	No	NR	NR	No	D	Against the ventral side (abdomen) of the fish (illustrated)	3–4 min
24	Red hind	NR	NR	No, Yes	NR	Yes	NR	NR	Along ventral surface, at four intervals from the vent to the anterior section of the ovary	NR
25	Nurse sharks	Temporary enclosures; pools[f]	1 × 1 × 3 m transport unit	No	Yes	Yes	Yes	D	NR	NR
26	Broadnose sevengill sharks	Oceanarium tanks	Holding system	No	Yes	Yes	No	D	Anterior to the pectoral girdle and finishing at the pelvic girdle (illustrated)	≤10 min
27	Small-spotted catshark	1090 × 610 × 1,020 mm tanks	1,230 × 550 × 260 mm tanks	Yes	Yes	Yes	No	Tilted	Ventral surface, along the abdomen, on ventral left side (illustrated)	NR
	Thornback ray	1091 × 610 × 1,020 mm tanks	1,231 × 550 × 260 mm tanks	Yes	Yes	Yes	No	V	1–3 cm above dorsal surface, in a lateral orientation (illustrated)	NR

[a]V = ventral recumbency (held upright, in swimming position; does not include flatfishes, which were in swimming position but scanned on one side [lateral recumbency]); D = dorsal recumbency (held ventral side up, upside down, inverted, supine position); L = lateral recumbency (left to lie on one side, fish placed on side).

[b]Partially submersed.

[c]In this study fish were transferred to bucket for anesthesia, but whether the bucket was the container used during ultrasonography was not specified.

[d]Anadromous form of rainbow trout.

[e]Juvenile and adult striped bass × white bass.

[f]Temporary enclosures; pools: temporary enclosures in field; indoor (12 m diameter, 1.5 m deep) and outdoor (10 m length, 4.5 m width, 1.5 m deep) pools.

stellate sturgeon, Neosho madtom, Atlantic cod, Atlantic salmon, Atlantic halibut, winter and yellowtail flounders, haddock, steelhead, striped bass, and nurse sharks (Table 3). One study physically and chemically restrained juvenile coho salmon, which were anesthetized and strapped onto a rotating platform. One of the 27 studies (adult Atlantic halibut, 12–25 kg) did not use any chemical or physical restraint, and a few studies (11%) did not use anesthesia because fish (shovelnose sturgeons 65–74 cm TL, 1 kg; striped bass 60–100 TL, 3–19 kg) were docile after being positioned ventral side up.

Handling during scanning.— The majority of the studies (63%) reported the physical positioning of the fish (Table 3). Fish held in dorsal recumbency (in an inverted position) included shovelnose sturgeon, pallid sturgeon, Neosho madtom, Murray cod, haddock, Atlantic salmon, steelhead, striped bass, nurse sharks, and broadnose sevengill sharks. Fish held in ventral recumbency (upright swimming position) included striped bass and thornback ray. Fish held in lateral recumbency (on their side) were mostly flatfishes, except for Pacific herring.

As to the scanning region, 8 studies described the physical position of the probe on the fish by referencing external anatomy (in-text descriptions of the scanned region) and by providing a schematic representation (Table 3). The majority of the studies (59%) described the scanned region of the fish without illustration, or with descriptions ranging from specific anatomical references such as "midlaterally along length of the gonad, at the base of the pelvic fin, at isthmus of dorsal fin," with the fish in ventral recumbency, to general references such as the probe being perpendicular and parallel to the fish body.

For the duration (s, min) of ultrasound examination, 37% of the studies reported an estimate, such as "within seconds," a range (e.g., 3–4 min), or an estimated average time (ranging from <30 s to 10 min) of the ultrasound procedure per fish.

Biological Data

Three taxonomic sub-classes of fish (Chondrostei, Neopterygii, and Elasmobranchii) were studied using ultrasonography for sex identification or development and for application of reproductive indices. The most studied groupings were marine and anadromous fishes, and the most studied species were shovelnose sturgeon, striped bass, and Atlantic cod (Table 1). The goals of these studies ($n = 27$) were to use ultrasonography for sex identification (19%), measurement of reproductive indices (44%), or sex identification and measurement of reproductive indices (37%). Sex identification was accomplished mostly by the sole use of ultrasonography, followed by dissection and gross visual examination of gonads; ultrasonography was also used in combination with other methods such as endoscopy and histology and with mathematical modeling such as fractional analysis. Reproductive indices were developed on the basis of the sole use of ultrasonography, but also by integrating data from ultrasonography with results of other methods such as measurements of gross anatomy or the use of histology-based gonadal development indices.

In total, over 6,434 fish were used in all the studies combined (Table 4). Sixty-three percent (4,078 fish) of the total number was reported as being either male (1,195) or female (2,883). No study reported exclusively on male fish, whereas a third of the studies (30%) were exclusively on females, including haddock, striped bass, and elasmobranch fishes; the majority of the studies (56%) reported imaging of male and female fish. Length differences among species ranged from 10 to 14 cm TL for Neosho madtom to 265 cm TL for broadnose sevengill shark. Life stage–dependent length differences ranged from 54 to 71 cm FL for juvenile Atlantic halibut, 10–25 cm FL for juvenile coho salmon, 35–45 cm FL for adult coho salmon, and 96–120 cm FL for adult Atlantic halibut (Table 4). Species-dependent weight varied between 68 and 94 g for Pacific herring and between 4 and 16 kg for adult striped bass (Table 4). Terminology used for describing the reproductive condition of fish included life stage terminology, such as "juvenile," and "adult"; gonadal maturity terminology, such as "immature," and "mature"; and descriptions of reproductive activity of fish, such as "spawning migration" for shovelnose sturgeon and "spawning aggregation" for red hind (Table 4).

Sex Identification

The ease of sex identification generally increased with an increase in the size of the gonad, which corresponded to the adult life stage, and gonadal maturation of reproductively active fish during their spawning period. For instance, accuracy of sex identification for female stellate sturgeons was 99–100% for fish designated as mature and immature, 96% for male stellate sturgeons designated as mature, but only 76% for male stellate sturgeons designated as immature (Table 2). Overall accuracy of sex identification was 86% for shovelnose sturgeons designated as mature and immature (Table 2), 70% for shovelnose sturgeon, 86% for pallid sturgeon designated as adults, 78% for Baltic cod broodstock of more than 40 cm body length, and 95% for adult striped bass throughout their annual reproductive cycle. Accuracy of sex identification exceeded 90% for Atlantic cod when the gonads were largest, leading to spawning. However, one study on female Atlantic salmon reported that imaging the ovary became increasingly difficult as gonadal maturation advanced.

Ease of differentiating between male and female fish decreased for smaller-sized gonads. This was attributed to early life stages (juveniles, or young adults), to gonadal development during the reproductive cycle (previtellogenic, late vitellogenic, or atretic gonads), or to the morphology of the gonad regardless of life stage or gonadal maturity during a reproductive cycle. For example, the effect of juvenile life stages and the corresponding small size of the gonads on the inability to identify gonads were reported for hybrid bass (striped bass *M. saxatilis* × white bass *M. chrysops*, including age-2 adult males), barfin flounder, and coho salmon (Table 2). Gonads of Atlantic cod were not visible until their gonad growth was distinct (study 9), and the gonads of red hind were reliably identified at the final stages of maturation only in the days immediately before spawning (study 24). Gonads were not identified for "immature" or "spent" female shovelnose sturgeon (study 3), "recovering" male and female Atlantic cod (study 9), and "atrophied" testes of steelhead (study 18). The "recrudescent" ovaries of shovelnose sturgeon were difficult to distinguish from testes (study 2). In some cases, the gonad could not be identified because of the morphology, regardless of life stage (e.g., juvenile, young adult), or the maturity status of reproductively active adults. In the Neosho madtom, for example, the testes of adult males could not be identified from surrounding organs in the ultrasound image (study 6).

Basis of Reproductive Indices

Ultrasonography alone.—Qualitative descriptions of gonadal growth, ovulation, and oocyte maturation were reported for Atlantic halibut monitored using ultrasonography only (study 13). Quantitative reproductive indices based on the sole use of ultrasonography were developed for Neosho madtom (study 5), Atlantic cod (study 10), steelhead (study 18), and broadnose sevengill sharks (study 26). For Neosho madtom, fecundity was calculated from ultrasound images of the ovary. For steelhead, the presence or absence of oocytes in ultrasound images was used to distinguish between prespawn and postspawn females, and a threshold area of 1.25 cm^2 (cross-sectional testis area) was used for separating prespawn from postspawn males (study 18). For broadnose sevengill shark, ultrasonography was used to measure changes in follicle diameter over a period of 1–13 months and to develop a behavior index (study 26).

Ultrasonography combined with other methods.—Descriptions of ultrasound images for different gonadal development stages were developed based on interpretation of structures in the ultrasound image and data derived from other gonadal assessment methods. For example, stellate sturgeon were designated as mature and immature based on previously developed classification stages (Lagler 1978); subsequently, ultrasound images for these mature and immature gonads were reported. For shovelnose sturgeon, data from endoscopic imaging, gross morphology, blood, and gonadal tissue sampling, along with qualitative descriptions of ultrasound images for six gonadal stages, were organized within the framework of histological profiles on gonadal development previously developed by Moos (1978).

The quantitative reproductive indices for shovelnose sturgeon (study 5), striped bass (studies 20, 23), and red hind (study 24) were based on gonad length obtained by using ultrasonography for identifying the anterior margin of gonads and then using a ruler to measure the length of the gonad to the posterior margin (for shovelnose sturgeon) or to the vent (for striped bass and red hind), on the external surface of the fish. These data were combined with the mean cross-sectional gonad area calculated from ultrasound images for estimating ovary volume. Based on

TABLE 4. Compilation of biological data on fish species, including the number sampled (No.), the numbers of males (M) and females (F), age (to the nearest year), length (fork [FL], standard [SL], total [TL], or body length to the nearest centimeter), weight (to the nearest kilogram unless specified otherwise), and the terminology used to describe life stage or reproductive condition. These values provided life stage and gonadal maturity data, which were linked to the results of ultrasonography for sex identification and the development of reproductive indices. In general, size was linked to ease of sex identification, and reproductive indices were developed for adult fish during the spawning season or at different stages of gonadal development during the spawning cycle; NR = not reported in the study.

ID	Species	No.	M	F	Age	Length	Weight	Terminology
1	Stellate sturgeon	249	50	199	6–16	95–150 FL	5–16	Mature, immature
2	Shovelnose sturgeon	51	25	25	NR	44–71 FL	NR	Spawning migration
3	Shovelnose sturgeon	343	183	160	16–19	65–74 TL	1	Adult
	Pallid sturgeon	16	11	3	NR	NR	NR	Adult
4	Shovelnose sturgeon	NR	NR	NR	NR	NR	NR	Adult
5	Shovelnose sturgeon	228	NR	NR	NR	>55 TL	NR	NR
	Pallid sturgeon	16	11	4	NR	NR	12–25	NR
6	Neosho madtom	58	22	36	1–3	10–14 TL	NR	Cyclic spawning condition
7	Murray cod	289	66	223	1–3	NR	1–4	Pubertal transition
		90	25	65	6	NR	6	Reproductively mature adults
8	Pacific herring	176	57	55	NR	16–23 SL	68–94 g	Mature, immature
9	Atlantic cod	788	NR	NR	1–6	NR	1–5	Maturing, nonmaturing
10	Atlantic cod	1,200	NR	NR	1–3	NR	1–3	Immature, mature
11	Baltic cod	32	16	16	NR	44–50 body length	1	Sexually mature
12	Barfin flounder	98	55	43	1–2	1–40 TL	NR	Immature
13	Atlantic halibut	NR	0	NR	NR	NR	12–25	Mature broodstock
14	Atlantic halibut	21	NR	NR	4	54–71 FL	2–5	Juvenile
	Atlantic halibut	NR	NR	NR	NR	96–120 FL	13–28	Mature
	Winter flounder	10	NR	NR	NR	NR	NR	Mature
	Yellowtail flounder	10	NR	NR	NR	NR	NR	Mature
	Haddock	25	0	25	NR	56–73 FL	NR	Mature
15	Haddock	58	0	58	NR	60–65 FL	2	Broodstock
16	Atlantic salmon	79	30	49	NR	NR	3–6	Two sea winter
17	Coho salmon	15	NR	NR	NR	10–25 FL	NR	Juvenile
	Coho salmon	5	NR	NR	NR	35–45 FL	NR	Mature
18	Steelhead[a]	1,353	330	1,023	NR	NR	NR	Adult
19	Steelhead[a]	108+	58+	50+	NR	NR	NR	Adult
20	Striped bass	31	0	31	NR	58–98 TL	4–16	Adult
21	Striped bass	16	8	8	5	55 TL	3	Adult
	Hybrid striped bass[b]	46	12	27	1+	NR	NR	Juveniles
	Hybrid striped bass[b]	20	7	4	2+	NR	≤2	Adult
22	Striped bass	40	20	20	5	55 TL	3	Adult
23	Striped bass	28	0	28	NR	60–100 TL	3–19	NR
24	Red hind	25	NR	NR	NR	28–42 TL	NR	NR
		820	209	611	NR	28–42 TL	NR	Spawning aggregation
25	Nurse sharks	5	0	5	NR	NR	NR	Reproductively active
26	Broadnose sevengill shark	4	0	4	NR	240–265 TL	NR	Sexually mature
27	Small-spotted catshark	77	0	77	NR	38–70 TL	≤1	NR
	Thornback ray	34	0	34	NR	49–85 TL	1–4	NR

[a]Anadromous form of rainbow trout.
[b]Striped bass × white bass.

these imaging measurements, a regression model was developed for predicting ovary volume for striped bass (study 23); fecundity estimates were calculated by integrating ovary volumes with data on oocyte diameter (from ultrasound images) for shovelnose sturgeon, egg enumeration (catheter sampling) for striped bass, and oocyte densities (catheter sampling) for red hind. For striped bass, a threshold ovary size (>30 mm diameter) was developed for characterizing "ripe" females by correlating maximum monthly ovary diameter obtained from ultrasound images to egg diameter obtained from measuring sampled oocytes (catheter) with an ocular micrometer and dissecting microscope; threshold testes size (>20 mm diameter) was determined for spermiating striped bass (study 21). Threshold testes size (1.25 cm^2) was developed for separating prespawn from postspawn steelhead by corroborating ultrasound diagnosis with blood plasma steroid levels (Study 19). Other reproductive indices developed included the following: for shovelnose sturgeon, a volumetric analog of the Gonadosomatic Index [(gonad volume/total body volume) × 100, study 5]; for Murray cod, the Gonad Index[(the cross-sectional gonadal diameter/square root of the body weight) × 100, study 7]; for haddock, the gonadal index [(the area of one ovarian lobe/fork length) × 100, study 14] and the ovarian index [(ovarian area/fork length) × 100, study 15].

DISCUSSION

Equipment (Ultrasound Unit, Probe)

There was great diversity in the size and portability of ultrasound units and probes used in the studies reviewed (Table 2). The prices of those units ranged from US$2,000–9,000 for used or refurbished units (e.g., http://www.sonomahealth.com/sonosite-180plus.html), but specialized machines can cost more than US$100, 000 for use in human medicine (e.g., price listings at http://www.theultrasoundtrader.com/pageinpage/products.cfm). Most studies (96%) reported the model of the ultrasound unit used, but reporting the model of the ultrasound equipment used was inconsistent. For instance, manufacturer information was reported by 44% of the studies; 52% reported only the ultrasound unit model and no manufacturer information; and one study reported the manufacturer information but did not report the model of the ultrasound unit used (Table 2). Furthermore, the probe model was rarely reported, even though the probe determines specific ultrasound features that cannot be changed during the ultrasound procedure (Nyland et al. 2002). For example, the probe may be of a single frequency, which necessitates physically replacing the probe to use another frequency with the ultrasound unit, or a probe may have multiple frequencies, which are adjusted by user interface controls (e.g., software). The probe frequency, in turn, determines the wavelength, the pulse length (axial resolution), the elevation resolution, and the arrangement (array) of the piezoelectric crystals (Nyland et al. 2002). Higher frequencies yield higher resolution, shorter wavelengths, shorter pulses, narrower beam diameters, and less depth penetration into the tissue. Lower frequencies yield lower resolution, longer wavelengths, longer pulses, wider beam diameters, and deeper penetration into the tissue (Ginther 1995). Accordingly, there is a trade-off between imaging depth and image resolution.

Settings

Ultrasound unit.—The features available in the ultrasound unit enable users to select the mode of echo return (e.g., A-mode, B-mode, real-time B-mode). Although the mode of echo display was not reported in most studies, the use of real time B-mode reported by four studies indicated that this gray-scale imaging method (without use of color modes introduced by Doppler technology) was sufficient for assessing reproductive indices or for sex identification in the stellate sturgeon, Atlantic cod, and striped bass, and that it may be adequate for viewing anatomy for sex identification and development of reproductive indices in fish.

Surprisingly few studies (11%) reported the ultrasound control settings, and of these all the studies reported only a single control setting, for instance, only the power or gain used (Table 2). Ultrasound units have a variety of control knobs and sliders that can be manually or electronically adjusted, and the units are constructed in a variety of shapes and user interfaces, making available a suite of adjustments for imaging that are specific to a manufacturer's ultrasound models. Because of this variation in the way the settings are controlled, reporting these settings will include important information. If the manufacturer, model information, and control settings are included in a study, other users of this technology will be able to replicate the imaging reported, particularly novice users who may consider purchasing the same model previously reported for a particular species. At a minimum, three basic types of imaging controls should be reported to ensure the process of replication, evaluation, standardization, and optimization of ultrasonography for reproduction studies among different groups of fish: power (intensity/output), gain and reject, and time-gain compensation (TGC; Nyland et al. 2002). The power control regulates the voltage applied to the piezoelectric crystals within the probe, thus affecting the sound output; the gain and reject controls affect amplification of returning echoes; the TGC controls adjust or compensate for weaker echoes originating from near (near gain), intermediate (slope delay control), or far (far gain) depths of echo origin (Nyland et al. 2002). These settings, at a minimum, must all be reported for a work to be replicated.

Probe.—If the model is identified as a single-frequency probe, most probably that frequency was the one used for imaging. However, if multiple-frequency probes are used, reporting of the actual frequency setting used during ultrasonography is necessary. If the frequency is specified, especially with multiple-frequency probes, users will be able to verify, replicate, or standardize technical procedures for generating images for specific fish or groups of fish. The procedures for using the probe are also relevant because they contain additional steps (e.g., use of gel as an ultrasound transmission medium, or covering the probe) necessary for repetition of studies.

Storage Device and Image Format

The types of storage equipment used for ultrasound image were videotapes, thermal print images, digital camera pictures, and the internal storage capability provided by the user interface of the ultrasound unit. The equipment, and the image formats, which are for the most part dictated by the manufacturer, indicate the procedure of the physical transfer of information and provide information on the potential limitation or utility of the type of format and quality obtained for further image processing and analysis.

Fish Handling

Fish handling is important to the experiment being conducted, and potential effects introduced by handling should be minimized or standardized, to eliminate or account for potential interactions with the variables being studied. In using ultrasonography in fish reproduction, proper handling is critical to reproductive processes, and if ultrasonography is to be justified in its utility as a noninvasive technique, so too should be the associated fish handling techniques. The conditions of the holding system, such as available oxygen and temperature, can accelerate or inhibit reproduction and determine whether the fish being held are dead (as a consequence of no oxygen, for instance) or alive. Equally important is the method of transfer of fish from the holding system to another container, which can be done in different ways depending on fish morphology, or on the techniques of different fish handlers. For instance, the size of the broadnose sevengill shark necessitates leaving the shark within the water and enclosing it with a trap for imaging. For catfishes, physical damage can be avoided by moving the fish quickly in dip nets between the holding system and the container for imaging (e.g., Novelo et al. 2011), rather than holding the caudal peduncle with one hand and the base of the head with the other hand. The potential harm of dropping broodstock fish, which can lead to mortality or disruption of reproduction, can be avoided by transferring with a net and using noninvasive procedures such as ultrasound imaging. Proper reporting of handling conditions and procedures will enable optimization and standardization of techniques that may be adopted by other researchers for similar species.

All of the studies indicated whether the fish were dead or alive during the imaging procedure, but an overview of fish handling showed considerable variability in the procedures used and in the manner in which the procedures were reported. For instance, 13 studies included data on both the fish-holding system in laboratory conditions and the container used in laboratory and field conditions; the rest of the studies reported only the holding system or the container used (Table 3). The data reported varied across the studies for whether or not fish were submersed, physically restrained, or anesthetized; description of the scanned region; and time of ultrasonography procedure per fish (Table 3). For anesthesia procedures, for example, 30% of studies included data on the anesthetic used (chemical name), and the dose; 15% of studies reported only the anesthetic used (chemical name) without dose, and 11% of studies that used anesthesia included data on neither the anesthetic used nor the dose. Studies describing the position of the fish during ultrasound scanning with the probe may or may not have included specific referencing of external anatomy as landmarks for the area scanned, or presented a figure (photograph, or schematic representation) illustrating the physical positioning of the probe and fish. Finally, although 10 studies reported an approximation of the time it took to obtain an ultrasound image per fish, this was the least reported data relevant to fish handling.

Recommendations

Reporting ultrasound equipment and settings.—To move towards standardization of procedures and provide access to information for users (including future potential adopters) of ultrasonography in fish reproduction, consistent reporting is necessary. The most important factors are identifying the equipment and settings used for obtaining ultrasound images. The manufacturer's model name and the manufacturer's contact information (company name and physical address, including internet address if available) should be reported for the ultrasound unit and probe used. Further, although the names used to identify control settings vary among ultrasound units, effort must be made to report the suite of settings that would enable other users of this technology to replicate, test, or optimize technical settings for particular species and life stages (e.g., for juveniles or adults). An additional advantage of manufacturers' preset options in some ultrasound units are that the control settings used for obtaining a particular image are saved in the memory of the unit and can be retrieved for obtaining images of biologically similar animals. Reporting of the ultrasound control settings and values used for generating images will enable users to replicate or experiment with settings reported in the literature, thereby making data collected with this technology available for use by others. This is critical for standardizing procedures among similar species, or within species for juveniles and for adults.

Reporting and improving of fish handling procedures.—The following detailed chronology of fish handling procedures from the point at which the fish is removed from the holding system, through the completion of the ultrasound procedure, was derived from Table 3 and from the studies reviewed. The minimum information necessary for reporting would be as follows: (1) holding system dimensions, water volume, and stocking rate; (2) whether or not the fish were starved (purged) and how long feed was withheld; (3) equipment used to move the fish from the holding system to the container used for ultrasonography (e.g., fish hauler, type of net, baskets, or if fish was moved by hand); (4) whether or not the fish was alive or dead (including time of postmortem diagnosis) before or during ultrasonography; (5) whether or not anesthesia was used, including the chemical name and dose, and how long the anesthetic was effective if the fish was anesthetized; (6) dimensions, or water volume, of container used during ultrasonography (if different from the holding system), and how long the fish was held in this container; (7) whether or not the fish was physically restrained and, if so, how this was done; (8) whether the fish was maintained completely or partially submersed in water, or if it was removed from the water; (9) explicit descriptions of fish position (orientation), that is, in ventral, dorsal, or lateral recumbency; (10) explicit description of the scanning region and procedure, including probe position and movement with respect to standard external anatomical features for the species; (11) an illustration, such as a digital photograph or schematic representation, showing the positioning of fish and probe during the ultrasound procedure; (12) duration of the scanning procedure per fish; (13) equipment used to move the fish from container to a recovery system; and (14) method or equipment for final transport of fish after scanning to the holding system.

Greatest utility would come from fish handling that consistently integrates procedures to minimize stress, such as retaining the fish in water, using the water as an ultrasound transmission medium, and using unrestrained, unanesthetized, submersed fish, such as the procedures recently described for imaging of channel catfish ovaries (Guitreau et al. 2012, this issue). The use of ultrasound imaging in reproduction studies can be elevated to a higher level of noninvasive procedures by reporting and standardizing minimal handling procedures for specific groups of fish, giving special considerations for the biological diversity range illustrated in these studies. If the full utility of ultrasound imaging as a noninvasive tool is to be exploited, it will be necessary to report equipment-specific information, settings, and the detailed handling procedures used before, during, and after ultrasound imaging. Attention should also be given to developing procedures suitable for commercial-scale work outside of the laboratory.

ACKNOWLEDGMENTS

We thank A. Guitreau, B. Eilts, R. Romaire, R. Cuevas-Uribe, E Hu, and D. Kuenz for discussion and review. This study was supported in part by funding from LSU–ACRES Cooperative Research Program, USDA–CSREES, and USDA–SBIR. This manuscript was approved for publication by the Director of the Louisiana Agricultural Experiment Station as number 2010-244-7320.

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