

RECLAMATION

Managing Water in the West

Using Ultrasound as a Tool for Fish Research and Management

Research and Development Office
Science and Technology Program



This document presents research findings. This document does not establish policies for the Bureau of Reclamation. Any mention of trade names or commercial products does not constitute an endorsement.



Mission Statements

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

Cover photo: Applying a 7.5 megahertz linear ultrasound probe to examine a fish (McAuley et al 2010).

Discussion

What is the Problem?

Reclamation's facilities and resource management programs need accurate, non-invasive, non-lethal methods to identify gender, determine reproductive maturation, and assess the body condition of fish in many situations, including:

- **Reclamation fish facilities.** Facilities such as diversions, ladders, and counting stations handle large numbers of fish, many of which are threatened and endangered (T&E) species. Managers and researchers need efficient, easy-to-use, accurate methods to assess the health and body condition of fish being handled to assure that facilities are safe and effective.
- **Habitat restoration.** Managers and researchers restoring habitats need accurate methods to determine if these efforts are benefiting fish and wildlife species.
- **Problem solving at Reclamation facilities.** Managing Reclamation's facilities requires understanding and addressing potential fish issues such as juvenile salmonid predation, developing safe and effective diversion screens and passage facilities, and implementing flows that protect critical fish resources. Managers and researchers need efficient non-invasive, non-lethal assessment technology to address these issues and determine the cost effective solutions to avoid impacts.

Regulatory agencies require Reclamation to use the best available technology, yet often are conservative in their oversight in an attempt to ensure minimal risk to critically imperiled species. Ultrasound can be used in applied research to address concerns such as electrofishing injuries, to determine optimum voltage and wave patterns to minimize injury to threatened and endangered salmon and steelhead species.

What is the Solution?

Ultrasound is a well-developed technology with broad application to Reclamation resource management and research endeavors. Ultrasound has evolved from the bulky, stationary units developed for human medicine in the 1960s to rugged, portable, high resolution units that can be used aboard boats, in adverse field conditions and at fish facilities. These new units were developed in response to requirements of veterinarians for durable, portable, easy to use instruments for use both in the office and on the farm. Aquaculturists seized on the technology early on and have been using this equipment routinely in their hatchery operations since the early 1990s. The first application to fish research was initiated by the University of Washington in

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1980. Early research efforts were conducted on steelhead in the Columbia River in the late 1990s and early 2000s and on declining sturgeon species in the Missouri and Mississippi Rivers also during that same time period.

The advantages of the new generation of ultrasound machines include:

- Analysis and results are rapid, providing more timely information
- Methods are non-invasive and non-lethal
- Machines are portable, rugged, resolution is high
- Analyses are accurate (reductive maturity with > 90% accuracy and gender with > 90% accuracy)
- Analysis can reduce the number of handling events (maturity sorts) from 4 to 2
- Ultrasound can effectively determine body fat and muscle thickness for body condition determination
- Technology can be used with a wide variety of species—from madtoms to sharks, from fish to reptiles to birds and large mammals.

Figure 1 shows the wide variety of probes available and Figure 2 shows a few of the more popular portable ultrasound units. Costs can be relatively inexpensive, depending on the unit selected. For example the Honda Electronics HS101V is approximately \$7,500; the GE LOGIQ 700 is approximately \$13,500. Prices, of course, will change. Other units can be more expensive. Used or refurbished models can result in significant savings and may be a viable alternative.

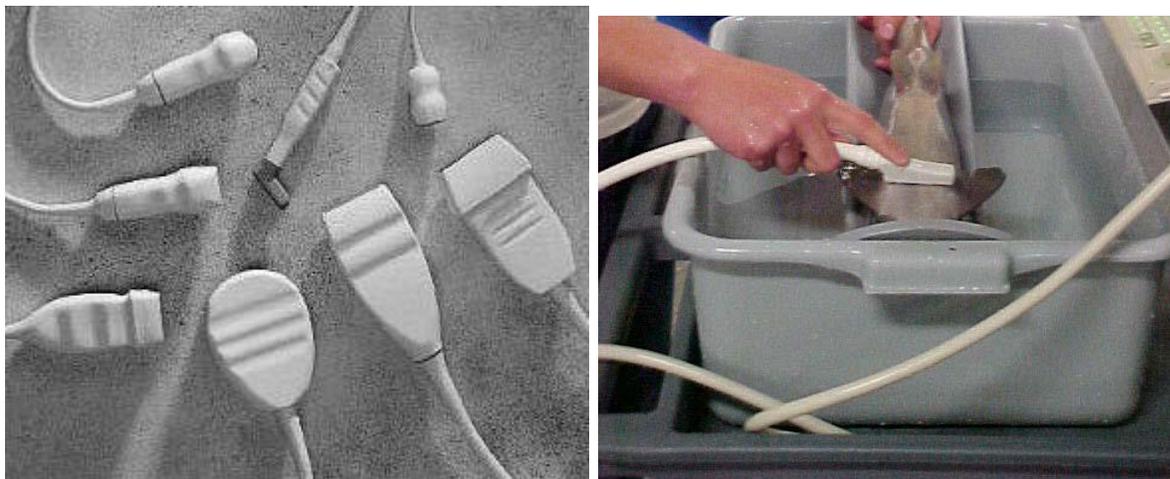


Figure 1. (a) A variety of ultrasound probes are available for specific needs. (b) 7.5 mHz linear probe is most applicable for salmon and steelhead having shallow penetration and good resolution (McAuley et al. 2010).

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Figure 2. A gallery of some portable ultrasound units popular for fish and wildlife applications. Upper left is a Honda HS-101, which was used to capture the ultrasound image, upper right, of a spring chinook egg mass. Middle left is an Ibex Pro, which was used by Oregon Department of Fish and Wildlife to measure body fat in mule deer. Middle right is a SonoSite 180 plus. Lower left is an Aloka 210 S, and lower right is an SIUI CTS-900V.

How Reclamation Researchers and Managers Can Benefit

The following applications for ultrasounds show promise for direct application to Reclamation facilities and operations:

- 1. Gender and reproduction.** Ultrasound imaging is a non-invasive method to determine gender that is more accurate than visual methods. Egg development within the ovary can be measured non-invasively by measuring egg diameter on the screen. Knowing sex and reproductive condition of fish would allow Reclamation to better manage fish facilities (diversions, ladders, hatcheries, counting stations). It provides a good tool for broodstock selection for captive rearing programs.
- 2. Species determinations.** This technique could be used to determine the difference between resident rainbow trout and anadromous steelhead. This will greatly assist in recovery and management efforts for threatened and endangered steelhead stocks.
- 3. Habitat restoration.** Ultrasonic imaging could be used in conjunction with habitat restoration efforts to measure the response of many T&E species in terms of reproductive condition. It is extremely difficult to monitor the response of populations of many T&E species such as sturgeons. Ultrasonic imaging would allow the direct assessment of reproductive stages of sturgeons and other species in the field with minimal impact to the fish. This technology is minimally invasive, yet sufficiently sensitive to allow investigators to track the progress of individual fish through the reproductive cycle and to determine whether spawning has occurred. Fecundity of fish in restored areas can be measured and compared against fish in adjacent areas to determine if environmental cues needed to trigger development and release of eggs are present in newly restored habitats.
- 4. Fish handling.** Reclamation fish facilities routinely handle a large number of fish, including threatened and endangered fish species. Injury assessment is currently done using visual estimates which are often inaccurate, resulting in an underestimation of delayed mortalities. Ultrasound would allow rapid non-invasive non-lethal imaging to detect internal injuries so that the causes of any such injuries can be more readily identified and rectified. Conversely, ultrasonic imaging could be used to demonstrate to regulatory agencies (US Fish & Wildlife Service, NOAA Fisheries) that fish emerging from Reclamation fish facilities are uninjured and in good health.
- 5. Fish predation.** Predation at diversion dams and bypass facilities is a significant source of outmigrating juvenile salmonid losses. Predation studies have been hampered by the need to assess consumption of juvenile salmonids through stomach pumping of piscivorous birds such as cormorants and gulls which is inefficient and often ineffective; or killing fish predator such as smallmouth bass and examination of stomach contents. Ultrasound scanning of birds and fish predators would be non-lethal and efficient and could expand the scope of predation studies, making them more relevant to Reclamation operations.

Literature Review

A Brief Literature Review Relevant to Potential Reclamation Applications

In the late 1990s and early 2000s, a significant effort was expended to adapt portable ultrasound technology to the problem of increasing steelhead spawner survival in the Columbia and Snake Rivers (Evans et al. 2001, Evans and Beaty 2001, Evans et al. 2004, Boggs and Peery 2004, Madson et al 2005). The researchers were able to develop the techniques and skills needed to successfully identify post spawned steelhead (kelts) by imaging egg masses and measuring testis diameters from pre-spawners. This led to the publication of a set of reference images, training and testing of fish culturists to accurately identify stage of spawning, the development of kelt reconditioning programs and improvements in both upstream and downstream survival. More recently NOAA Fisheries is using ultrasound technology at many of its culture and research facilities (McAuley et al 2010). Other fish managers are using ultrasound in their captive broodstock programs for Snake River sockeye (Peterson and Heindel 2010) and threatened spring Chinook (Products Group International, 2010). Hoffnagle et al. (2010) confirmed that ultrasound was a practical method to detect maturing salmon, and thus be able to move them from saltwater to freshwater at a more optimum time as well as to determine gender.

A similar evolution in the use of ultrasound technology occurred with assessments of sturgeon in the Mississippi and Missouri River systems. Early research focused on determining the applicability and utility of ultrasound to sturgeon species (Colombo et al 2004 and Wildhaber et al 2007). Researchers such as Bryan et al. (2007) found that accuracy in gender identification and maturation of gonads varied somewhat but were improved when endoscopic examinations were included. Bryan et al also (2007) tracked gonad characteristics with environmental spawning triggers (a Gonadosomatic Index). Wildhaber et al. 2007 produced a comprehensive reference set of images of shovelnose sturgeon.

A wide variety of fish species have been the subject of ultrasound research and management applications. Novelo and Tiersch (2011) assembled a comprehensive reference data set of ultrasound techniques for research, hatchery and field application in fish reproduction involving gender identification, measuring reproductive indices, and standardizing minimally stressful fish handling and ultrasound techniques for different groups of fishes. Other ultrasound uses include diagnosing fish diseases without resorting to euthanasia (Gumpenberger et al. 2006); determining fecundity, gender identification and state of egg maturity (Achionye-Nzeh and Jimoh 2010, Bryan et al. 2005, Bonar et al. 1989, and Whiteman et al. 2005). Researchers also were able to accurately estimate ovary weight, Gonadosomatic Index, and fecundity in pike and white suckers. Of particular note for rare and endangered species, ultrasound was found to be particularly useful for obtaining data on declining shark species (which formerly were sacrificed to collect such data) including diet, reproductive status, Gonadosomatic Index, and fecundity (Hammerschlag and Sulikowski 2011, Daly et al. 2007).

Ultrasound applications for wildlife species may also be relevant to Reclamation research and management. Portable ultrasound units were used in mule deer studies in Oregon and California to collect information on pregnancy rates, body condition as measured by fat and muscle

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thickness and fetus condition (E.I. Medical 2011 and Monteith et al. 2009). Similarly ultrasound was found to be effective in measuring body fat in free-ranging animals such as raccoons (Stringer et al. 2010). The body condition and reproductive status of virtually any wildlife species can be assessed using portable ultrasound including lizards (Gillman and Wolf 2007 and red knot (Lindstrom et al. 2000).

One important use of ultrasound at Reclamation fish facilities may be its use in determining internal injuries of fish such as salmon and steelhead. Portable ultrasound units—the same units as those being used by fish and wildlife researchers—are being used in the battlefield to visualize internal bleeding (Nations and Browning 2011 and Wills 2011).

Tables 1 through 4 provide an annotated bibliography of the above topics. These tables include the type of ultrasound equipment used and note the websites of portable ultrasound companies.

Table 1. Ultrasound applications for salmon and steelhead.

Literature	Ultrasound Applications for Salmon and Steelhead	Equipment Used
Boggs and Peery 2004	Used ultrasound imagery to distinguish post-spawn steelhead kelts from spring migrating pre-spawn steelhead at the Lower Granite Dam separator.	Aloka SSD-500v ultrasound with 7.5 MHz linear probe
Evans et al. 2001	In a steelhead kelt reconditioning program at the Prosser Fish Hatchery in Prosser, Washington, ultrasound examination ensured that pre-spawn steelhead were not removed from the current spawning population. During the ultrasound examination, a linear probe or transducer was placed against the specimen's abdomen, then moved anterior or posterior to view the ovaries or testes. Assessment of maturation status was based on the size, location, and echogenity of the gonads. Visual examinations were also made and estimates were compared with the ultrasound tests to assess the accuracy of the fish culturists' ability to recognize kelts.	Aloka SSD-500v ultrasound with 7.5-MHz linear probe
Evans et al. 2004	Authors found a high degree of corroboration between ultrasound measurements of gonad size and plasma steroid levels in steelhead, but application of ultrasound imaging may result in some classification error (a few percentage points) if substantial gonad mass is retained in the body cavity after spawning. They found that the use of ultrasound to accurately identify postspawn steelhead is an important first step towards developing effective kelt management practices in the Columbia River basin.	Portable Aloka SSD-500v ultrasound with 7.5-MHz linear probe.
Evans and Beaty 2001	Ultrasound identification techniques, which allow the gonads of the fish to be examined, have been developed specifically for maturation assessment among Columbia Basin steelhead. Developed methods for using ultrasound imaging to distinguish pre-spawn from post-spawn steelhead based on the presence of an egg mass in females and by the cross-sectional diameter of male testes. This technique proved to be highly accurate method for determining maturational status and allowed researchers to gather information on the abundance and general condition of kelts in juvenile bypass facilities.	Aloka SSD-500v ultrasound with 7.5-MHz linear probe
Hoffnagle et al. 2010	Found that ultrasound was more practical than near infrared spectroscopy to improve ability to detect maturing salmon (and thus move them from saltwater to freshwater at a more natural time) and determine gender.	Equipment not specified.
Madson et al. 2005	Authors used ultrasound to distinguish pre-spawn vs. post spawn steelhead at John Day Dam as part of a research project on steelhead iteroparity (repeat spawning) in the Columbia and Snake River system.	Aloka SSD-500v ultrasound with 7.5-MHz linear probe
McAuley et al. 2010	Discussion of ultrasound technology used in 4 captive broodstock and research programs for the threatened Snake River spring Chinook.	Aloka SSD-500v ultrasound with 7.5 MHz linear probe
Peterson and Heindel 2010	Used ultrasound to determine gender on hatchery sockeye adults so that equal sex ratios could be released.	Equipment not specified.
Products Group International 2007	Authors found that using ultrasound in captive broodstock programs to accurately identify gender ensures optimum sex ratios to meet egg production goals and reduces the number of naturally spawning adults taken into captive programs. Ultrasound also significantly reduces observer error.	Honda Electronics Hs-101V with 5.0 MHz linear transducer.

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U.S. Fish and Wildlife Service 2011	Used ultrasound at the Little White Salmon National Fish Hatchery to determine the stage of maturation of naturally spawning White River F1 captive broodstock.	Units not specified.
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Literature	Ultrasound Applications for Sturgeon	Equipment Used
Bryan et al. 2007	Used non-invasive, non-lethal ultrasound to examine reproductive conditions of declining Mississippi River sturgeon species. Found that ultrasound had a mix of accurate measurements as well as over-or under- estimations, but when combined with endoscopic examination provided greater accuracy. Both methods combined can be used to track Gonadosomatic Index in individuals or populations through time, which can be useful to associate gonad characteristics with environmental spawning triggers, or with repeated examinations of individual fish throughout the reproductive cycle.	In the field, used Sonosite 180 Plus with a 5-10 MHZ linear probe. In the lab, used Shimadzu SDU-400 Plus with a 7.5MHZ linear probe.
Colombo et al. 2004	Shovelnose sturgeon were sexed by ultrasound imaging using a portable ultrasonograph with and overall 86% accuracy. Images could be readily identified by any reader with some prior knowledge of sturgeon gonad anatomy. Authors conclude it be an effective noninvasive method for sex determination, and that this portable equipment was suitable for field studies.	Sonosite 180Plus with 38-mm 5-MHZ linear transducer
Heise et al 2009	Authors found that serological measures of reproductive physiology were able to measure reproductive physiology more accurately than ultrasound. However other authors report 90% or better accuracy using ultrasound.	None used - worked with blood plasma samples to test for vitellogenin concentrations and estradiol.
Wildhaber et al. 2007.	Authors conducted monthly sampling of Lower Missouri River shovelnose sturgeon from May 2001 to June 2002 to develop methods for detection of sex and reproductive stage of sturgeons in the field; and produced a comprehensive reference set of images.	In the field used Sonosite 180 Plus with a 5-10 MHZ linear probe. In the lab used Shimadzu SDU-400 Plus with a 7.5MHZ linear probe.

Table 2. Ultrasound applications for sturgeon.

Literature	Ultrasound Applications in Other Fish Species	Equipment Used
Achionye-Nzeh and Jimoh 2010	Ultrasound imaging is a useful tool to determine fecundity in tropical fish, many species of which are difficult to visually assess due to variation in primary and secondary sex characteristics.	SIUI CTS-5000 digital ultrasound with curvilinear probe 3.5 - 5.0 MHZ transducer
Bonar et al. 1989	Investigated the feasibility of using ultrasonic images to rapidly sort Pacific herring by sex and state of maturity and found that these could be done with a high degree of accuracy.	Unirad EDP 1000 abdominal B scanner with 5-MHz transducer (some of the original ultrasound equipment from the 1980s) Has been replaced with newer technology
Bryan et al. 2005	A captive population of the threatened Neosho Madtom was maintained under simulated natural conditions and monitored routinely with ultrasound for reproductive condition. Ultimately 13 out of	Aloka ultrasound machine with 7.5 MHZ linear probe

	41 fish participated in 10 spawnings. Ultrasound was effective and resulted in negligible stress or injury. The use of ultrasound was recommended for future culture of rare, threatened or endangered species.	
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Table 3. Ultrasound applications for other fish species.

Table 3 Ultrasound Applications for Other Fish Species - Continued		
Daly et al. 2007	Authors successfully monitored the growth and regression of follicles in sevengill shark ovaries which allowed the approximation of reproductive stage of these sharks. The ability to follow reproductive events in elasmobranches using ultrasonography is an important step in the application of assisted reproductive technology in these endangered species.	Interson Corporation 3.0-7.5 Mhz probes
Gumpenberger et al. 2006	In the past, most diagnoses of fish diseases were made through euthanasia and necropsy. Ultrasonography allowed the authors to locate and characterize a tumor in a red oscar. X-rays, on the other hand, were not effective in verifying the origin of the tumor.	2d real time B-mode ultrasonography with 8 MHz convex transducer
Hammerschlag & Sulikowski 2011	Top oceanic predators especially large predatory sharks appear to be declining. Lethal sampling remains the most effective and accurate means of gathering diet, reproductive status, age growth and mortality data. The authors make the case that non-lethal methods, including ultrasonography, have potential to obtain these data without killing declining shark species.	Interson Corporation 3.0-7.5 Mhz probes
Macbeth et al. 2011	Authors investigated the feasibility of using field-based portable ultrasonography to accurately estimate ovary weight, Gonadosomatic Index (GSI) and fecundity in large bodied fish species. They found that values for pike were overestimated by 21 to 23%, but that values for white suckers were significantly correlated. Based on the variances for GSI estimations in both species, power analysis indicated that the ultrasound technique could detect a 25% change in GSI using sample sizes of < 20 fish. This study illustrates the utility of portable ultrasonography as a promising non-lethal technique for assessing reproductive endpoints in the field.	GE LOGIQ700 Expert with an 8- or 13-MHz probe or Shimadzu SDU-400 Plus
Novelo and Tiersch 2011	Authors assembled a comprehensive reference data set of ultrasound techniques for research, hatchery and field application in fish reproduction from 23 studies. Study goals were gender identification (22%), measurement of reproductive indices (35%), or both (43%). This paper provides a groundwork for standardizing minimally stressful fish handling and ultrasound techniques for different groups of fishes.	Units not specified.
Whiteman et al. 2005	Ultrasonic imaging used to determine the spawning population structure and develop a fecundity estimation model for a red hind spawning aggregation within the Red Hind Bank Marine Conservation District, St. Thomas, U.S.V.I. Ultrasonic imaging may prove a valuable tool in population assessment for many species and locations in which invasive fish methods are clearly undesirable.	Pie Medical Scanner with 3.5-5.0 Hz linear array probe
Whittamore et al. 2010	Authors verified that ultrasound accurately visualized and measured ovaries, ovarian follicles and egg capsules within oviducts in 2 shark species. In other species, however these structures were difficult to visualize and the authors recommend coupling ultrasound with steroid hormone analysis. The authors stress the importance of seeking non-lethal methods to obtain reproductive data for declining species.	Interson Corporation portable 3.0-7.5 MHz probes

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Table 4. Wildlife ultrasound applications.

Literature	Wildlife Ultrasound Applications	Equipment Used
E.I. Medical 2011	Wildlife veterinarian uses ultrasound in the field to measure body fat depth and to determine pregnancy in mule deer in a project with the Klamath Tribe.	Ibex Pro
Gillman & Wolf 2007	Small size, durable weather-resistant case, long battery life and high imaging resolution of current ultrasound imaging systems make it possible to use this highly accurate non-destructive method for the quantification of reproductive parameters in small animals in the field, in this case lizards. This technology allows individuals to be re-examined over the same season as well as years and can be effective where populations are small and possibly endangered. However there are several factors that affect accuracy of ultrasound imaging. There is a learning curve associated with this method and the experience of the operator, including the ability to correctly identify anatomical structures and effective use of the instrument. In addition there are species specific challenges – such as food in the gut can obscure eggs, compressing eggs during examination can make enumerating them difficult, and in 1 species, intestines overlying the ovary can obscure the eggs.	Sonosite Titan Portable US system & Sonosite Titan L38 5-10 MHZ broadband linear array transducer
Lindstrom et al 2000	Used ultrasound to monitor short-term changes in pectoral muscle size of captive red knots. Pectoral muscle thickness changed rapidly and consistently with body mass changes caused by flight, fasting and fuelling.	Pie 200 ultrasound with 7.5 MHZ linear probe
Monteith et al. 2009	Cal Fish & Game Dept. researchers used ultrasonography to determine body fat as a measure of nutritional condition in mule deer, as well as bicep muscle thickness to measure protein catabolism in animals in poor condition; and pregnancy determination and condition of fetuses in captured females.	Aloka 210 portable ultrasound with 3 & 5 MHZ transducer s
Stringer et al. 2010	Assessment of free-ranging animals is important when evaluating population health and fitness. The study validated ultrasound of body fat is an accurate means of assessing body fat in raccoons and is likely applicable to other mammal species when evaluating the nutritional status of small carnivores.	Sonosite Titan with a C60 5-2MHz transducer

Table 5. Battlefield ultrasound applications relevant to fish and wildlife research and management.

Literature	Battlefield Ultrasound Applications Relevant to Fish & Wildlife Research & Management	Equipment Used
Nations & Browning 2011	Battlefield applications for handheld ultrasound	Various types
Wills 2011	Handheld ultrasound is battle-ready	Sonosite – unspecified portable models

Table 6. Websites of principle portable ultrasound equipment companies.

Websites for Further Information	
Sonosite	http://www.sonosite180ultrasound.com/?gclid=CKTdq_ykv64CFSwCQAodMELfCg
Aloka SSD - 500	http://www.aloka.com/products/view_system.asp?id=9
Ibex Pro	http://www.eimedical.com
CTS 900 V Mfg Siui	http://www.siuiamerica.com/ep2_01_900V.htm
Honda HS-101V	http://www.productsgroup.com/honda_HS-101.htm
GE LOGIQ Ultrasound	http://www.gehealthcare.com/euen/ultrasound/index.html

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