THE DEVELOPMENT OF ULTRASOUND TECHNIQUES FOR SEX IDENTIFICATION IN FISH

BY

NAZOR FRANKLIN NGBOR

A thesis submitted to the
Department of Biology
Mount Allison University
in partial fulfillment of the requirements for the
Bachelor of Science degree with Honours

April 11, 2024

Table of Contents

Abstract

Ultrasonography is a non-invasive technique that can be used to assess the sex and stage of maturity of a fish. The aim of my study was to examine and compare the quality of ultrasonic images captured in different media and resolutions of the ultrasound (US). My overall objective is to help develop protocols to obtain the highest quality ultrasonic images when viewing the internal structures of a fish. The gonad images of Blue Tilapia (*Oreochromis aureus*) and Shortnose Sturgeon (*Acipenser brevirostrum*) were captured in different media (water and gel/air), transducer probe orientation (frontal and Transverse) and placement (lateral and ventral). All images were analysed objectively using the rectangle tool on Fiji/image J to outline region of interest and conducted a fractal analysis “fraclac” in Fiji to generate fractal values that were test statistically using MANOVA.RM and subjectively using a blind survey and reporting my observations. Objectively for Blue Tilapia sample, media and resolution significantly influenced image quality ($p=<0.001$). For media, water and gel were significantly different ($p=0$). For resolution, High resolution RES) and general resolution (GEN) were significantly different ($p<0.001$). Supplementary work was done on shortnose sturgeons, in the two different media (water and gel/air) with only general resolution (GEN). Objective analysis for the shortnose sturgeon sample images shows no detectable difference between media ($p=0.796$). For the subjective approach, forest plots were made to measure heterogeneity of survey responses. For the blue tilapia survey, participants viewed the highest quality image as those captured in water at high resolution (RES). For shortnose sturgeon survey, participants viewed the highest quality image as those captured in gel/air at general resolution (GEN). Based on my observations, there was no relationship between image quality and mean gray values for both blue tilapia and shortnose sturgeon images. This study was conducted using a single ultrasound (Sonosite Edge II). Thus, different results can be obtained using a different ultrasound (US) machine.

Acknowledgements

I would like to begin by thanking God almighty for seeing me throughout my journey at Mount Allison University. I would like to specially thank my supervisor, Dr. Litvak Matthew for his guidance, time, support, dedication, patience, and words of encouragement all through my final year. Thank you, Sir, for providing me with the opportunity to take on my honours research in a field of medicine that I am deeply in love with and hope to pursue in the future. I am indeed grateful. I would also like to thank my second reader, Dr.Vett K.Lloyd for her advice on how to ensure the completion of my image analysis. There is absolutely no way I could have done this without the input of my lab members. Special thanks to all Litvak Lab members, most especially Naomi Meed and Alex Matthew Giroux for helping me during my experimental procedures. I am indeed grateful.

I would love to specially thank my beloved family, Prince Franklin-Ngbor family, for their constant prayers, words of encouragement, support, regular check-ins, and overall enthusiasm towards my honours research. Thank you, mom and dad, for being there for me all through my journey at Mount Allison University. I would specially love to thank my elder sister, Barine Franklin-Ngbor, for being my support system and believing in me. I will forever appreciate you.

Finally, a big thank you to all my friends and well-wishers that made my journey at Mount Allison fun and rememberable.

List of Tables

List of Figures

CHAPTER 1: LITERATURE REVIEW

REVIEW OF ULTRASONOGRAPHY AND ITS IMPORTANCE TO VISUALIZE INTERNAL ORGANS IN FISH.

1.1 GENERAL REVIEW OF THE TECHNIQUE OF ULTRASONOGRAPHY

Ultrasound (US) can be described as any sound that has a frequency above the human audible range (>20 kHz) and requires a medium to propagate, as sound is a mechanical energy (Wilhjelm et al., 2022). Ultrasonography (US) is a diagnostic imaging modality that produces gray-scale images of a specific internal slice of the body of an animal, through the interconversion of electrical energy to acoustic energy, without causing any harm or any kind of modification to the body of an individual (Novelo & Tiersch, 2012; Wilhjelm et al., 2022). The ultrasonic images produced are referred to as tomographic images; *tomo* = Gr. Tome, to cut and graphic = Gr. *Graphein*, to write (Wilhjelm et al., 2022). US is the most commonly used medical imaging modality in clinical practices due to its non-invasive properties, portability, low-cost, non-ionizing nature and real-time feedback (Raina et al., 2023).

Ultrasound technique is effective for diagnosis, emergency medicine, pre- and post-operative assessment, visual assessment of internal anatomy and physiology, monitoring blood flow, examining the reproductive tracts of animals etc. (Novelo & Tiersch, 2012; Raina et al., 2023). With ultrasound, you can examine the elastic and dynamic characteristics of tissues (Harvey et al., 2002). Ultrasound possesses physical wave characteristics which includes frequency, length, and velocity. These waves primarily transmit through bones, scutes, cartilage, soft, tissues and gas in the body of fishes (Abu-Zidan et al., 2011). The different internal structures/tissues in the body of fishes have different levels of reflectivity for ultrasound waves which results in varying levels of tissue brightness. This simply means that some tissues reflect sound waves while others absorb them; this property of US is known as echogenicity.

1.1.2 Echogenicity

Echogenicity is the ability of a sound or an echo to reflect off a surface (Boezaart & Ihnatsenka, 2010). It can also be referred to as the brightness of a tissue (Chebanov & Galich, 2009). Structures with high echogenicity (hyperechogenic structures) reflect sound waves well and are usually represented with lighter/white colours on images (Boezaart & Ihnatsenka, 2010). Examples of hyperechogenic structures includes ligaments, tendons, and gonads (Boezaart & Ihnatsenka, 2010). In contrast, structures with low echogenicity (hypoechogenic structures) reflect little to no sound and appear darker on the screen (Figure 1) (Boezaart & Ihnatsenka, 2010). Examples of hypogenic structures include the cartilage, lymph nodes, fats, fluids (Boezaart & Ihnatsenka, 2010).

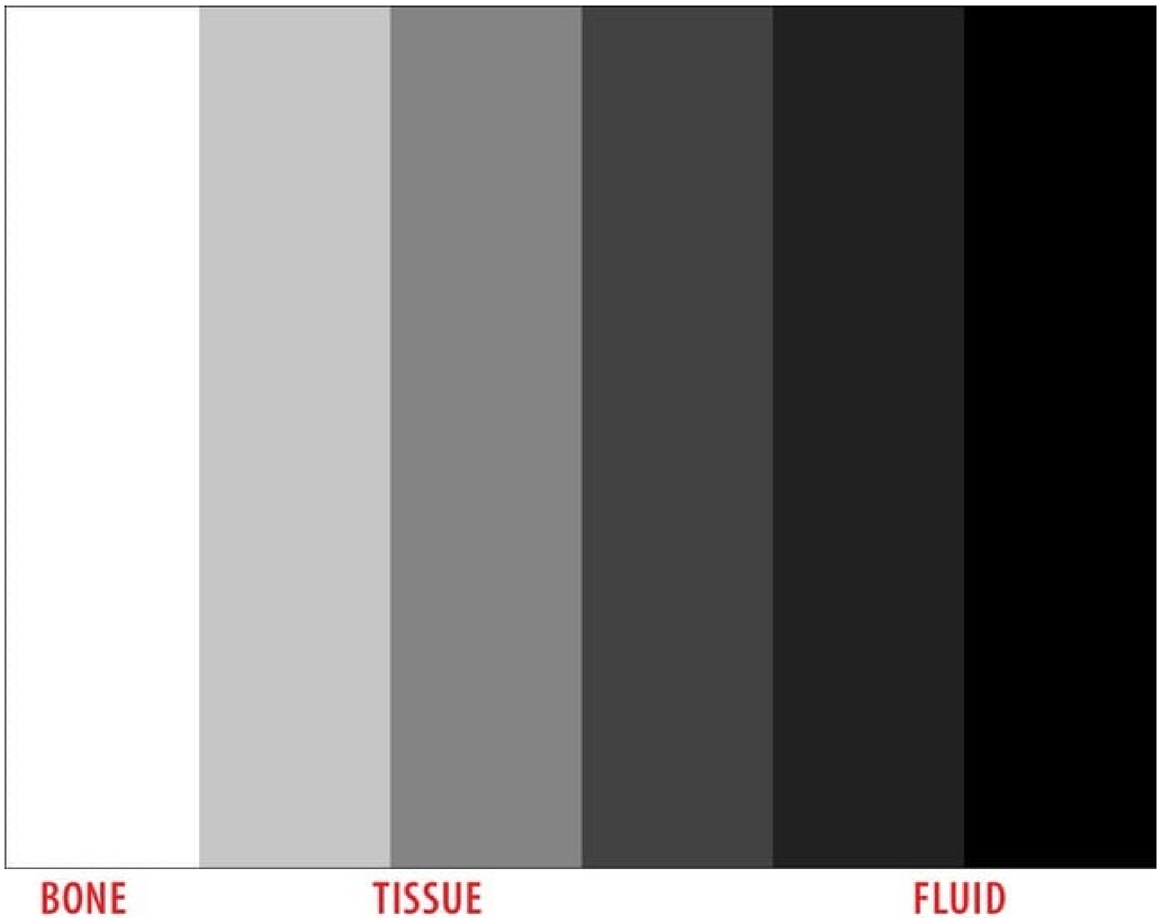

Figure 1. Range of internal structures with varying echogenicity in the body of Fish. Image sourced from https://www.eimedical.com/blog/bid/76503/ultrasound-basics-how-to-read-an-ultrasound-image.

1.2 The physics of ultrasound

1.2.1 Physical Wave characteristics

As stated earlier, sound is a mechanical wave that requires a medium to propagate while causing disturbance in the medium (Powles et al., 2018). Unlike light, that is an electromagnetic wave, sound cannot travel in a vacuum (Lieu, 2010). The medium sound requires to travel can be air, water soft tissues, blood, bone etc. (Lieu, 2010). The **frequency** of sound waves (measured in Hertz) is the number of oscillations or cycles per second (Powles et al., 2018). Although, the frequencies used in medical imaging are much higher, usually between 2-10 megahertz (Powles et al., 2018). The **wavelength (λ),** which is inversely proportional to frequency, is the distance travelled by sound in one cycle (Powles et al., 2018). Shorter wavelengths produce a higher resolution image and improved axial resolution, but less penetration in soft tissues (Powles et al., 2018). As a result, a higher frequency probe (5 MHz) is used in medical imaging for superficial structures like peripheral vessel visualization, and a lower frequency probe (2 MHz) is used for imaging deeper structures like abdominal and pelvic organs (Powles et al., 2018). The **amplitude**, which is the strength or level of sound pressure, can be measured by the height of a sine wave (Lieu, 2010). The **power** of the wave, measured in watts, is proportional to the square of the amplitude (Lieu, 2010). As a result of the quadratic relationship, increasing the amplitude of the sound waves rapidly increases the energy transfer to the patient and theoretically, any adverse bioeffects (Lieu, 2010). Ideally, ultrasound scans should be performed in a low output power or completely in a dark room to allow adequate and complete visualization of the anatomy of the fish. (Lieu, 2010).

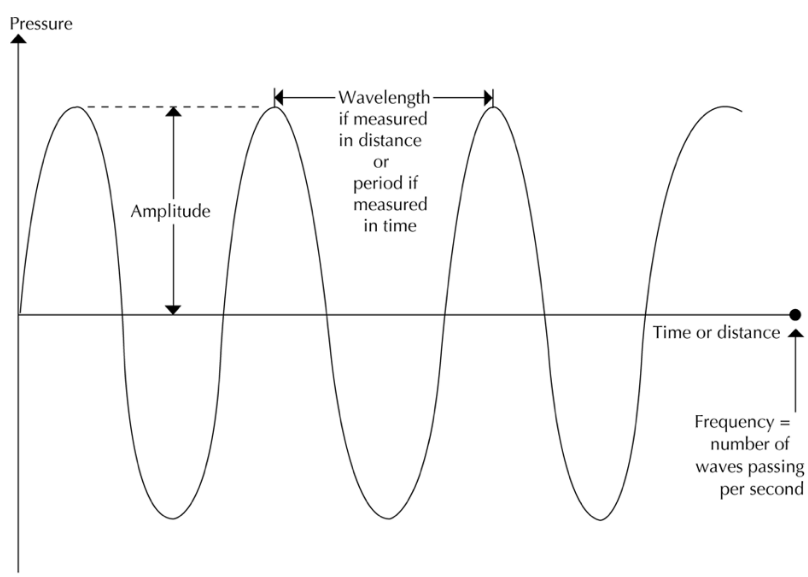

Figure 2. The graphical representation of sound waves as a periodic series of compressions and rarefactions represented by a sine wave (Lieu, 2010).

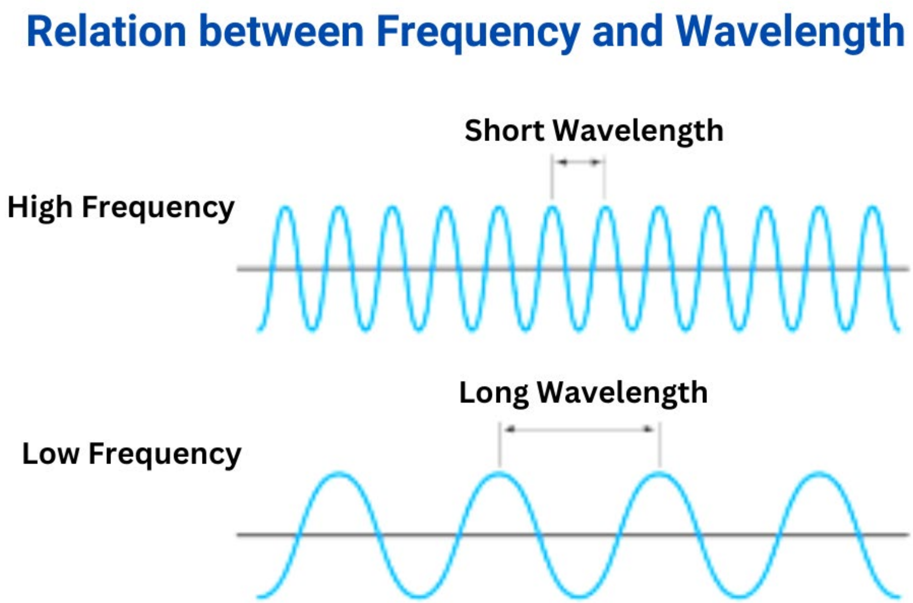

Figure 3. The relationship between frequency and wavelength. Image sourced from https://eduinput.com/relation-between-frequency-and-wavelength/

1.3 The generation of ultrasound

Ultrasound is generated by the ultrasonic transducer, also referred to as probe, which is also responsible for recording echoes produced by the medium (Wilhjelm et al., 2022). For the transducer to produce mechanical vibrations in the megahertz range, it requires a material that can vibrate that fast. Piezoelectric materials have been used for ultrasound probes (Wilhjelm et al., 2022). Piezoelectric crystals are packed into a small enclosure as a 1D array in the transducer as seen in Figure 3 (Wilhjelm et al., 2022). There are two types of probe shapes namely Linear and convex (Wilhjelm et al., 2022). In this study, a linear transducer was used. Ultrasound probes are used to transmit ultrasound energy into the organs of the body and then receive the reflected signal echoes that may be processed into images displayed on the screen monitor of an ultrasound machine (Oglat et al., 2018).

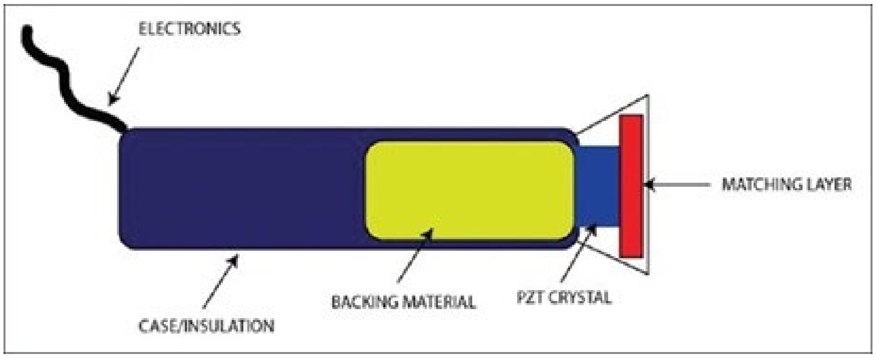

Figure 4. Basic components of ultrasound probe (transducer). Image sourced from (Oglat et al., 2018).

1.4 General application of ultrasonography

The advanced technology of ultrasonic imaging is common in clinical practices and about one-fourth of all medical imaging procedures worldwide are done using this technology (Wells, 2006). With humans, ultrasonography is primarily used in obstetrics and gynecology (Table 1). Other uses in humans includes monitoring blood flow, detection of diseases and tumors in our internal organs, evaluation of bone and muscle injuries etc. (Table 1) (Quarato et al., 2023). Ultrasonography has been used on other mammals including cattle (Shepard et al., 1996), sheep (Berg et al., 1996) and swine (Pinho et al., 2018).

Ultrasonography has also been used on fish. Examples include to diagnose disease in fish e.g., liver tumors in zebrafish (*Danio rerio*) (Goessling et al., 2007), ocular lesions in farmed halibut (*Hippoglossus hippoglossus*) (Williams et al., 2007), to study the internal structures in channel catfish (*Ictalurus punctatus*) (Guitreau et al., 2012), to predict carcass and fillet yield in farm-raised catfish (Bosworth et al., 2001), and to study fish reproduction on over 21 different fish species (Novelo & Tiersch, 2012).

Table 1. Applications of ultrasound across different medical disciplines modified from (Quarato et al., 2023).

Medical Discipline	**Application**
Abdominal	To diagnose the cause of abdominal pain or discomfort; To evaluate liver, gallbladder, bile ducts, pancreas, spleen, and abdominal aorta; To identify occlusions, bleeding, and suspicious lesions in the abdominal organs.
Anaesthesiology	To guide the delivery of local anesthetics to the target area while avoiding structures that might be damaged by the needle; To guide peripheral and central venous catheterization.
Breast	To help the identification and study of breast lumps; To guide biopsy of suspicious breast lesions.
Cardiology	To detect pericardial effusion, cardiac wall motion abnormalities, and vascular stenoses; To estimate ejection fraction (EF) and pulmonary artery pressure (PAP).
Dentistry	To identify carious lesions, tooth fractures or cracks, periodontal bony defects, and maxillofacial fractures;
Endocrinology	To evaluate the thyroid and parathyroid glands; To guide biopsy of suspicious thyroid lesions.
Gynecology	To study the female pelvic organs, mainly the ovaries, fallopian tubes, uterus, bladder, adnexa, and recto-uterine pouch; To help identify malignant and endometriosis lesions.
Musculoskeletal	To study the morphology of joints and muscle fibers;

Medical Discipline	Application
Nephrology	To evaluate the kidney and detect the presence, number, and size of kidney stones;
Obstetrics and Pregnancy	To provide a high-quality two-dimensional image of the embryo or the fetus in real-time; To detect any abnormalities in the growth of the fetus or any other problems associated with the pregnancy.
Ophthalmology	To diagnose ophthalmic diseases, including intraocular or periorbital foreign bodies, globe rupture, lens dislocation, retinal and vitreous detachment and hemorrhage, neoplasm, and vascular pathologies;
Pulmonology	To identify pleural effusion, lung consolidations, and morphological and movement alterations of the hyperechoic pleural line; To guide thoracentesis and percutaneous biopsy of lung lesions.
Urology	To examine the urinary tract for any defects and to identify bladder tumors;

1.5 Blue tilapia introduction

1.5.1 Blue tilapia (*Oreochromis aureus*) Biology

Blue tilapia (*Oreochromis aureus*) is a species of fish in the Cichlidae family (Al-Wan & Mohamed, 2019). Blue tilapia is primarily a fresh and brackish water fish that occurs in streams, rivers, lakes, and ponds (Al-Wan & Mohamed, 2019). They can survive at temperatures down to 8°C. Whereas the lower lethal temperature for most tilapia species is 10-11°C and upper lethal temperature is 42°C (Leonard & Skov, 2022; Pompa & Masser, 1999). In addition to their tolerance for high temperature, they can also survive in high salinity (up to 20 ppt) environment (Pompa & Masser, 1999). They eat a wide range of natural food organisms and can tolerate poor water quality, which makes them ideal for aquaculture. Species of *Oreochromis* are mouth brooders (Al-Wan & Mohamed, 2019). As soon as fertilized eggs are laid in the nest, the adult female immediately picks them up in their mouths and incubate them for several days (Al-Wan & Mohamed, 2019).

1.5.2 Blue tilapia morphology

Blue Tilapia have a laterally compressed, deep body with long dorsal fins (Valikhani et al., 2016). The adult species have bluish-gray sides, and their belly is usually white, except in darker individuals where each scale may comprise of a small dark spot or dusky blotch anteriorly giving the appearance of rows of markings (Valikhani et al., 2016). Breeding males are mostly bluish-gray, with their throats and cheeks being lighter in colour (Valikhani et al., 2016).

1.5.3 Blue tilapia distribution

Blue tilapia is a fresh and brackish water fish that are native species to northern and western Africa, the middle east, central and south America, and south India (Al-Wan & Mohamed, 2019). Blue Tilapia, *Oreochromis aureus,* was first seen in Arvand and Karun River drainages in Khuzestan province, Iran (Pompa & Masser, 1999). Currently, blue tilapias are reared all over the world because the species has the ability to tolerate poor water conditions, eat a wide range of natural food, grow rapidly at warm temperatures, high salinity, and low dissolved oxygen (Pompa & Masser, 1999). Stocking, vegetative control and aquaculture practices have been primarily used to introduced blue tilapia in different water bodies around the world. Escapes or releases of this specie from aquaculture facilities, zoological parks and aquariums is a common occurrence (Al-Wan & Mohamed, 2019).

I.6 Shortnose sturgeon

1.6.1 Shortnose Sturgeon (*Acipenser brevirostrum*) Biology

Shortnose sturgeon is a small, amphidromous North American sturgeon which is a member of the family Acipenseridae. Since 1967, they have listed as endangered in the United States and listed as special concern in Canada in 1980 (COSEWIC, 2015). Their populations have declined drastically due to overfishing, poaching, pollution, and river fragmentation due to damming (Kynard, 1997). Other potential threats include invasive species and global warming (COSEWIC 2015).

External sexual dimorphism is not obvious in shortnose sturgeon (Dadswell et al., 1984). However, some morphological differences in head shapes between males and females have been identified (Balazadeh & Litvak, 2018). Females reach sexual maturity at the age of 18, whereas males do so at the age of 12 on average (COSEWIC, 2015). Shortnose sturgeons are among the five species of sturgeons in Canada. They can grow up to about 120 cm, weigh up to 24 kg and live up to about 60 years (COSEWIC, 2015). One unique feature of shortnose sturgeons is the number of annuli found in the cross sections of the fin spines can be used to estimate their age (Stewart et al., 2015).

1.6.2 Shortnose sturgeon morphology

Shortnose sturgeon, *Acipenser brevirostrum,* has an elongated and fusiform body with no scales (Dadswell et al., 1984). They have scutes in five rows, also known as plates or bucklers: one dorsal (lighter brown), two lateral (yellow tinge), and two ventral (yellow tinge) rows (Dadswell et al., 1984). Young individuals usually have sharp and well-developed scutes, but as they age, they become progressively blunter (Dadswell et al., 1984). They are characterized by an inferior elongated protrusible wide mouth, four barbels anterior to mouth on the rostrum's ventral side, small eyes, and spiracles (COSEWIC 2015).

1.6.3 Shortnose sturgeon distribution

Shortnose sturgeons are found along the east coast of North America from the Saint John River in New Brunswick, Canada, to the St. John rover in Florida (Hall et al., 1991). They do not migrate and have a restricted home range within their river and estuary. Most shortnose sturgeons remain in their natal river (Matsche et al., 2011). They can be found across 25 estuary systems along the east coast of North America (COSEWIC 2015).

1.7 My study

The focus of my research is to determine the efficacy of different ultrasound transducer probe placement methods (water *versus* air), ultrasound resolutions, views, and probe orientation on internal imaging of blue tilapia and shortnose sturgeon. I seek to investigate if the ultrasound immersion in water and placement improves or reduces image quality of the fish internal organs compared to the standard in air/gel method. For my project, I used a SonoSite Edge II portable ultrasound with a linear probe capable of scanning from 6-15MHz. I took ultrasonic images at different resolutions (General resolution, high resolution, and penetration) with the two different ultrasound transducer methods; probe in a gel bag in water *versus* probe directly submerged in water. The latter is a standoff method, as the probe was at a distance(~1cm) away from the fish's body. The ultrasonic images produced from each method was individually labelled and evaluated using both subjective and objective method. For the subjective method, I used a blind survey whereby I paired random ultrasonic images, also known as sonograms, with the same orientation and probe placement but in the different media and asked fellow lab members to choose the image they think has a better quality. For the objective method, I first used the polygon selected tool on image J/Fiji to map out the region of interest and then used the fractal analysis "Fraclac" in image J to generate fractal values, for surface roughness, that was analysed statistically. My research is essential because there is a lack of imaging protocol guidelines for non-invasive techniques like ultrasonography that can help produce high quality images. Ultrasonography is most important as it involves non-destructive techniques that do not cause harm to the fish.

Resources

Abu-Zidan, F., Hefny, A., & Corr, P. (2011). Clinical ultrasound physics. *Journal of Emergencies, Trauma, and Shock*, *4*(4), 501. https://doi.org/10.4103/0974-2700.86646

Al-Wan, S. M., & Mohamed, A.-R. M. (2019). Analysis of the Biological Features of the Blue Tilapia, *Oreochromis aureus* in the Garmat Ali River, Basrah, Iraq. *Asian Journal of Applied Sciences*, *7*(6). https://doi.org/10.24203/ajas.v7i6.6037

Balazadeh, K., & Litvak, M. K. (2018). Using geometric morphometrics for sex determination on adult shortnose sturgeon (Acipenser brevirostrum). *Aquaculture*, *487*, 89–96. https://doi.org/10.1016/j.aquaculture.2017.12.047

Berg, E. P., Neary, M. K., Forrest, J. C., Thomas, D. L., & Kauffman, R. G. (1996). Assessment of lamb carcass composition from live animal measurement of bioelectrical impedance or ultrasonic tissue depths. *Journal of Animal Science*, *74*(11), 2672. https://doi.org/10.2527/1996.74112672x

Boezaart, A., & Ihnatsenka, B. (2010). Ultrasound: Basic understanding and learning the language. *International Journal of Shoulder Surgery*, *4*(3), 55. https://doi.org/10.4103/0973-6042.76960

Bosworth, B. G., Holland, M., & Brazil, B. L. (2001). Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. *Journal of Animal Science*, *79*(6), 1483. https://doi.org/10.2527/2001.7961483x

Chebanov, M. S., & Galich, E. V. (2009). Ultrasound diagnostics for sturgeon broodstock management. *FSGTSR, Krasnodar. Izdatel' Stvo Prosveshenie-Yug*, 116.

COSEWIC. (2015). COSEWIC assessment and status report on the shortnose sturgeon, Acipenser brevirostrum, in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xii + 48 pp.

Dadswell, M. J., Taubert, T. S., Squires, D., Maracette, D., & Buckley, J. (1984). Synopsis of biological data on the shortnose sturgeon, Acipenser brevirostrum LeSueur, 1818. *NOAA Technical Report NMFS 14, National Marine Fisheries Service, Washington, D.C.*

Goessling, W., North, T. E., & Zon, L. I. (2007). Ultrasound biomicroscopy permits in vivo characterization of zebrafish liver tumors. *Nature Methods*, *4*(7), 551–553. https://doi.org/10.1038/nmeth1059

Guitreau, A. M., Eilts, B. E., Novelo, N. D., & Tiersch, T. R. (2012). Fish Handling and Ultrasound Procedures for Viewing the Ovary of Submersed, Nonanesthetized, Unrestrained Channel Catfish. *North American Journal of Aquaculture*, *74*(2), 182–187. https://doi.org/10.1080/15222055.2012.655852

Hall, J. W., Smith, T. I. J., & Lamprecht, S. D. (1991). Movements and Habitats of Shortnose Sturgeon, Acipenser brevirostrum in the Savannah River. *Copeia*, *1991*(3), 695. https://doi.org/10.2307/1446395

Harvey, C. J., Pilcher, J. M., Eckersley, R. J., Blomley, M. J. K., & Cosgrove, D. O. (2002). Advances in Ultrasound. *Clinical Radiology*, *57*(3), 157–177. https://doi.org/10.1053/crad.2001.0918

Kynard, B. (1997). *Life history, latitudinal patterns, and status of the shortnose sturgeon, Acipenser brevirostrum*. https://doi.org/10.1023/A:1007372913578

Leonard, J. N., & Skov, P. V. (2022). Capacity for thermal adaptation in Nile tilapia (Oreochromis niloticus): Effects on oxygen uptake and ventilation. *Journal of Thermal Biology*, *105*, 103206. https://doi.org/10.1016/j.jtherbio.2022.103206

Lieu, D. (2010). Ultrasound Physics and Instrumentation for Pathologists. *Archives of Pathology & Laboratory Medicine*, *134*(10), 1541–1556. https://doi.org/10.5858/2009-0730-RA.1

Matsche, M. A., Bakal, R. S., & Rosemary, K. M. (2011). Use of laparoscopy to determine sex and reproductive status of shortnose sturgeon (Acipenser brevirostrum) and Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus): Laparoscopy to determine sex and reproductive status of sturgeons. *Journal of Applied Ichthyology*, *27*(2), 627–636. https://doi.org/10.1111/j.1439-0426.2011.01679.x

Novelo, N. D., & Tiersch, T. R. (2012). A Review of the Use of Ultrasonography in Fish Reproduction. *North American Journal of Aquaculture*, *74*(2), 169–181. https://doi.org/10.1080/15222055.2012.672370

Oglat, A., Matjafri, M., Suardi, N., Oqlat, M., Abdelrahman, M., & Oqlat, A. (2018). A review of medical doppler ultrasonography of blood flow in general and especially in common carotid artery. *Journal of Medical Ultrasound*, *26*(1), 3. https://doi.org/10.4103/JMU.JMU_11_17

Pinho, R., Camilo, B., Lima, D., Villadiego, F., Vergara, J., Shiomi, H., Cardoso, R., Lopes, P., Guimarães, S., & Guimarães, J. (2018). The use of ultrasonography in the reproductive evaluation of boars. *Reproduction in Domestic Animals*, *53*(2), 393–400. https://doi.org/10.1111/rda.13119

Pompa, T., & Masser, M. (1999). Tilapia: Life History and Biology. *Southern Regional Agriculture Center, 283, 4pp.*

Powles, A. Ej., Martin, D. J., Wells, I. Tp., & Goodwin, C. R. (2018). Physics of ultrasound. *Anaesthesia & Intensive Care Medicine*, *19*(4), 202–205. https://doi.org/10.1016/j.mpaic.2018.01.005

Quarato, C. M. I., Lacedonia, D., Salvemini, M., Tuccari, G., Mastrodonato, G., Villani, R., Fiore, L. A., Scioscia, G., Mirijello, A., Saponara, A., & Sperandeo, M. (2023). A Review on Biological Effects of Ultrasounds: Key Messages for Clinicians. *Diagnostics*, *13*(5), 855. https://doi.org/10.3390/diagnostics13050855

Raina, D., Ntentia, D., Chandrashekhara, S. H., Voyles, R., & Saha, S. K. (2023). Expert-Agnostic Ultrasound Image Quality Assessment using Deep Variational Clustering. *2023 IEEE International Conference on Robotics and Automation (ICRA)*, 2717–2723. https://doi.org/10.1109/ICRA48891.2023.10160435

Shepard, H. H., Green, R. D., Golden, B. L., Hamlin, K. E., Perkins, T. L., & Diles, J. B. (1996). Genetic parameter estimates of live animal ultrasonic measures of retail yield indicators in yearling breeding cattle. *Journal of Animal Science*, *74*(4), 761. https://doi.org/10.2527/1996.744761x

Stewart, N. D., Dadswell, M. J., Leblanc, P., Bradford, R. G., Ceapa, C., & Stokesbury, M. J. W. (2015). Age and Growth of Atlantic Sturgeon from the Saint John River, New Brunswick, Canada. *North American Journal of Fisheries Management*, *35*(2), 364–371. https://doi.org/10.1080/02755947.2015.1011359

Valikhani, H., Abdoli, A., Kiabi, B. H., & Nejat, F. (2016). *First record and distribution of the blue tilapia, Oreochromis aureus (Steindachner, 1864) (Perciformes: Cichlidae) in inland waters of Iran*.

Wells, P. N. T. (2006). Ultrasound imaging. *Physics in Medicine and Biology*, *51*(13), R83–R98. https://doi.org/10.1088/0031-9155/51/13/R06

Wilhjelm, J. E., Kristensson, M., & Andersen, O. T. (2022). *Medical diagnostic ultrasound—Physical principles and imaging*.

Williams, D. L., Goddard, P. J., & Brancker, W. M. (2007). Ultrasonographic examination of ocular lesions in farmed halibut. *The Veterinary Journal*, *173*(2), 456–458. https://doi.org/10.1016/j.tvjl.2005.11.012

CHAPTER 2

2. Introduction

2.1 Review of the ultrasound (US) technique and its importance in Fish

Due to the non-invasive nature of ultrasonography (US), it is widely used a tool in fisheries for research and management. There are numerous advantages of using US technique: it is immediate, and biologically safe/not harmful (Chebanov & Galich, 2009). However, there a few disadvantages of this technique. US machines and probes are expensive, trained personnel is required to efficiently operate the machine and interpret sonograms, lack of units that can handle extensive fieldwork use and ultrasound of high frequency can affect cell viability if placed on the body of the fish for an extended period (Chebanov & Galich, 2009; Chiotti et al., 2016).

Ultrasonography has been employed for many decades to gather information on internal structures in fish. Ultrasonography is mainly used to study reproduction in aquatic animals such as bottlenose dolphin, *Tursiops trancatus aduncas* (Brook, 2001), shovelnose sturgeon, *Scaphirhynchus platorynchus* (Colombo et al., 2004), and killer whales, *Orcinuc orca* (Robeck et al., 2004). However, its application in selective breeding of aquatic species is relatively new and there is no standardized method for recording yield traits for numerous species of economic significance (Rezende et al., 2023).

Tilapias are the second most farmed fish in the world and ultrasonography is an essential tool for accessing meat yield-related phenotypes in Tilapias (Chen et al., 2019; Rezende et al., 2023). Often, breeders eliminate some family members to examine their carcass yield and then go ahead to breed only members of high-yield families to produce seed stock (Gjedrem, 2017). This lethal practice is highly wasteful and impractical. Hence, less invasive techniques like ultrasonography should be promoted. With ultrasonography, the body composition of animals can be accurately evaluated and measured non-lethally for aquaculture production as well as used in breeding programs (Alarcon-Rojo et al., 2019).

Sturgeons are one of the most valuable and most threatened fish species in the world (Moghim et al., 2002). Caviar is a delicacy that consist of salt-cured roe of the family *Acipenseridae* (Tavakoli et al., 2021). As a result of caviar being highly valued and the most expensive aquaculture product in the market, there has been an increase in the demand and production rate of caviar and meat from sturgeons commercially in many countries (Sicuro, 2019; Tavakoli et al., 2021). Therefore, it is critical to the advancement of aquaculture to develop practical and non-lethal methods, like ultrasonography, to determine and identify broodfish sex and maturity stage of sturgeons, most especially in caviar-producing facilities and countries (Moghim et al., 2002). The main objective of most sturgeon facilities is to grow females for caviar production and separate the males early in the production cycle for meat. This can be done using ultrasonography. A fair amount of research has been conducted using the US to sex sturgeons (Table 2). Ultrasonography is a fast and easy technique that can be used for visual inspection of the lipid deposition in sturgeons (Ovissipour et al., 2015). The nutritional quality of caviar can be affected by the fish's lipid content, which serves as both an energy source and essential fatty acids for the development of different tissues, including the gonads and eggs (Czesny et al., 2000). US can also be used for sorting and grading automatization in the meat industry effectively and rapidly (Shiranita et al., 2000).

Table 2. Past research showing the success rate of using ultrasonography and the method of analysis used to determine the sex of sturgeons. SI represent sex identification and RI represents reproductive indices. There are two types of goals: SI-1 & RI-1 are based on only ultrasonography and SI-2 & RI-2 were derived from ultrasonography and other methods.

Species (Common name)	**Species (Scientific name)**	**Methods**	**Probe type**	**Image Analysis method (Subjective/ Objective)**	**Success rate**	**Goal**	**Author and year**
Lake sturgeon	*Acipenser fulvescens*	Ultrasonography	Linear	Subjective	88% (Reader 1) 96% (Reader 2)	SI-1, RI-2	(Chiotti et al., 2016)
Stellate sturgeon	*Acipenser stellatus*	Ultrasonography	Linear	Objective (necropsy)	97-100%	SI-1, RI-2	(Moghim et al., 2002)
Shovelnose sturgeon	*Scaphrihynchus platorynchus*	Ultrasonography	Linear	Subjective	86%	S1-1, RI-2	(Colombo et al., 2004)
Beluga sturgeon	*Huso huso*	Ultrasonography	Linear	Objective	97.5%		(Masoudifard et al., 2011)
Shovelnose	*Scaphrihynchus*	Ultrasonography,	Linear	Objective	59-76%	SI-2	(Wildhaber

sturgeon	*platorynvhus*	Endoscopy, Blood parameters		(Two-way logistic ANCOVA)			et al., 2005)
Pallid sturgeon	*Scaphirhynchus albus*	Ultrasonography, Endoscopy	Linear	Objective (Two-way logistic ANCOVA)	86%	SI-2	(Wildhaber et al., 2005)
Shovelnose sturgeon	*Scaphrihynchus platorynvhus*	Ultrasonography, Endoscopy	unidentified	Objective (Linear model ANOVA and linear regression)	N/A	RI-2	(Bryan et al., 2007)
Pallid sturgeon	*Scaphirhynchus albus*	Ultrasonography, Endoscopy	unidentified	Objective (Linear model ANOVA and linear regression)	N/A	RI-2	(Bryan et al., 2007)
Shovelnose	*Scaphrihynchus*	Ultrasonography,	Linear	N/A	Reproductive	R1-2	(Wildhaber

sturgeon	*platorynvhus*	Endoscopy, blood parameters	stage dependent	et al., 2007)

2.2 Image quality

Like other medical imaging techniques (X-ray), magnetic resonance imaging (MRI), computed tomography (CT)), image quality assurance for ultrasound is also important. US is important because ultrasound diagnosis depends heavily on image quality that is assessed manually by sonographers, thereby limiting its objectivity by making it operator dependent (Sassaroli et al., 2019). The goal is always to achieve the best possible image. The big question is what constitute a good quality image? The simple answer is if structures can be clearly seen and identified on the image then it is a good image. A more complex answer would be it depends on several factors. There are a variety of advanced imaging modes and functions on the ultrasound machine that can be modified to attain a high-quality image. For example, we can manipulate resolution, probe frequency, depth penetration, knobology (gain; auto-gain, far-field and near-field), gray scales, scanning modes, dynamic range (Oglat et al., 2018). In addition to the instrumental settings, image quality also depends on how the sonographer handles the transducer. The echogenicity of a tissue can be affected by how much pressure is applied to the probe (Boezaart & Ihnatsenka, 2010). Ideally, pressure should be applied evenly across the probe to attain the right scanning direction. However, sonographers may deliberately apply more pressure on one side of the transducer to direct US beams in the desired direction. This is referred to as probe tilting or angling (Boezaart & Ihnatsenka, 2010). Sonographers must ensure that the probe is aligned properly to enhance image quality. There is a small orientation marker that shows up at the top right, just beside the image, on the US monitor screen as shown in Figure 5. To identify the appropriate orientation of an image, it is necessary to confirm which side of the probe corresponds to what side of the screen (Boezaart & Ihnatsenka, 2010).

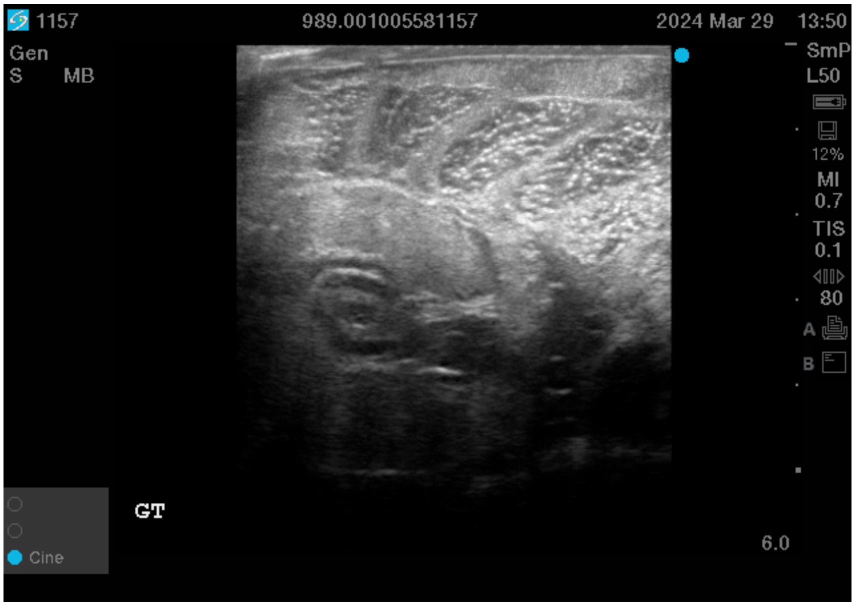

Figure 5. Sonogram of the transverse view of a female shortnose sturgeon captured with the standard gel/ air method. The dot at the top right corner of the image is the orientation marker for the probe.

2.2.1 Trade-off between US resolution and penetrating depth.

The ultrasound machine used in this study has three different resolutions. High resolution (RES) (10-15MHz) has the highest frequencies but lowest penetration depth. As a result, high frequency transducers are preferred for visualizing superficial structures (2-4cm) (Boezaart & Ihnatsenka, 2010). The general (midrange or GEN) resolutions (5-10 MHz) are more suitable for slightly deeper structures (5-6cm) (Boezaart & Ihnatsenka, 2010). Lastly, the low resolution (penetration; PEN) has the lowest frequency (2-5 MHz) and the highest penetration depth (6 cm) which makes it is suitable for viewing deep structures, for example, a proximal sciatic nerve (Boezaart & Ihnatsenka, 2010). Due to the low frequency of the PEN resolution, it produces images with substantially poorer quality than the RES and GEN resolutions. This is one limiting factor of the ultrasound.

2.2.2 Knobology

Knobology is concerned with the knowledge and application of the ultrasound machine settings to achieve the best settings that will produce a high-quality image (Zander et al., 2020). The brightness of an image can be adjusted by alternating the gain function both in near and far field (Zander et al., 2020). In the case of an unbalanced setting, there will be a loss of details due to unused gray scales (Figure 6) (Zander et al., 2020). The US machine used in this study possess an auto-gain setting. However, operators may find that adjusting the gain of the image improves their ability to differentiate amongst the structures displayed on the screen and overall image quality. The amount of gain to use depends on personal preferences (Boezaart & Ihnatsenka, 2010). However, the gain function should not be maxed out as it can blur or wash out structures from the image. Thus, reducing the image quality.

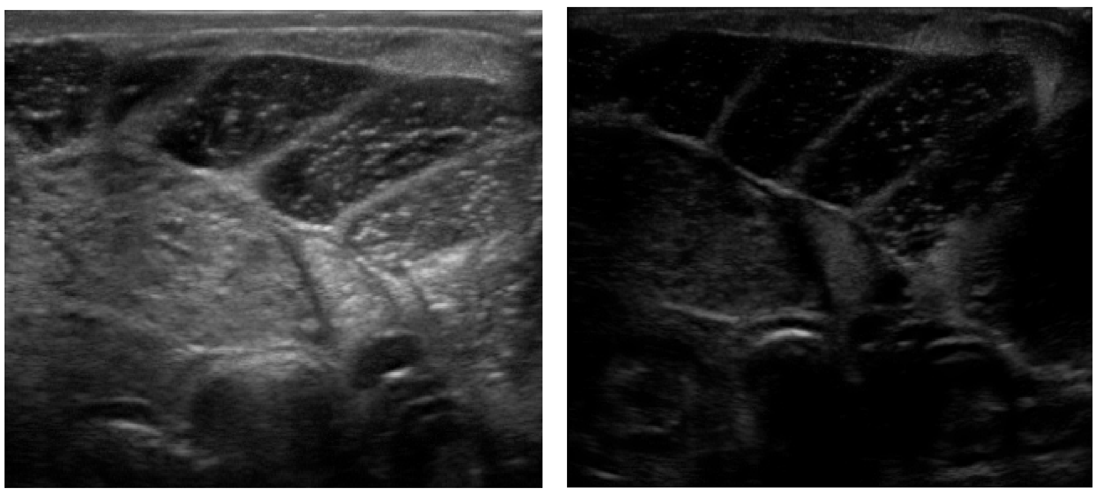

Figure 6. Comparison between an image with good gain function (left) and another with unbalanced gain function (right).

2.3 Ultrasound Transducer application (Transmission medium)

There are various methods of using the ultrasound transducer. One method that is widely adopted by researchers involves using gel as a transmission medium. The gel method involves coating the tip of the probe with ultrasound gel and covering or sealing it tightly, to eliminate air bubbles, with a plastic bag or rubber material then wrapping rubber bands around the probe to keep the plastic bag in place (Figure 7). Once the plastic bag is tightly in place and there is absence of air bubbles then the probe is placed of the body of the fish and scanning begins.

Figure 7. Frontal and transverse scanning images showing ultrasound probe being used with gel in a bag method (Chebanov & Galich, 2009).

The gel in a bag method (Figure 7) can be time consuming, expensive, requires taking the fish out of water and demands extra steps to ensure the absence of air bubbles in the gel, as air bubbles can interfere with the images. Alternatively, emergency medicine make use of direct immersion of US probe in water baths (Figure 8) as the probe is waterproof. The water method is rapid, efficient, straightforward and does not require the use of plastic bags and gel.

Figure 8. Transducer/probe application using water as a transmission medium (Cotton et al., 2024).

2.3.1 Research objective

The aim of my study is to determine the efficacy of different ultrasound probe placement methods (water *versus* air), ultrasound orientation, views, and probe orientation on internal imaging. I hypothesize that image quality is influenced by the transmission medium, probe resolution, probe placement/orientation and potential interactions among these factors. I predict that images taken in water at a high resolution (RES) would have a better quality than images captured in gel at a lower resolution (GEN).

2.4 Method

All images were captured using a SonosSite Edge II portable ultrasound machine (Figure 9) with a linear transducer that has the capacity to scan between the range from 6-15MHz. The ultrasound machine has three different resolutions namely high-resolution (RES, lowest penetration), medium-resolution (GEN, medium penetration) and low-resolution (PEN, highest penetration). Images were captured with the three different resolutions, but PEN images were not analyzed in this study as they had the poorest image quality in the two transmission mediums.

Figure 9. Sonosite Edge II ultrasound with attached to an external monitor.

2.4.1 Capturing blue tilapia images.

Prior to performing the ultrasound scan, fish was fasted for a period of 12- 24 hours to ensure there was no blockage of food or waste during scanning. Fish samples were anesthetized using 150 mg/L of MS-222 and buffered with 300mg/L of baking soda. Just before scanning, all necessary morphometrics were measured and recorded including mass of fish (g), and PIT TAG number and an ultrasound patient file consisting of these measurements. As soon as the fish was anesthetized, we began the ultrasound scan. The ultrasound tank was transparent to allow us to observe the fish. Fish were placed on a horizontal bed. and secured with hoops at both ends, to hold the fish down in the transparent plastic tank containing a lower concentration anesthetic water with an air pump placed in it. For the gel/air method, fish was intubated with a small plastic tube supplied with anesthetic water. The water level was controlled to allow me to use the ultrasound under water or in air when using gel in a bag method. A small air tube, which the higher concentration anesthetic water ran through, was placed in the fish's mouth when the bed was above water level during the gel in a bag method. As shown in Figure 10, during scanning the fish samples were placed on their side (lateral) to capture frontal and transverse view of the internal anatomy and on their back to capture the ventral views (ventral-frontal and ventral-transverse) images of the internal anatomy of the fish showing the gonads with the different resolutions of the ultrasound. After capturing the images, fish samples were first placed in a recovery bath before returning to their tank. A total of 272 imaged were analysed form 17 (4 females and 13 males) blue tilapia samples.

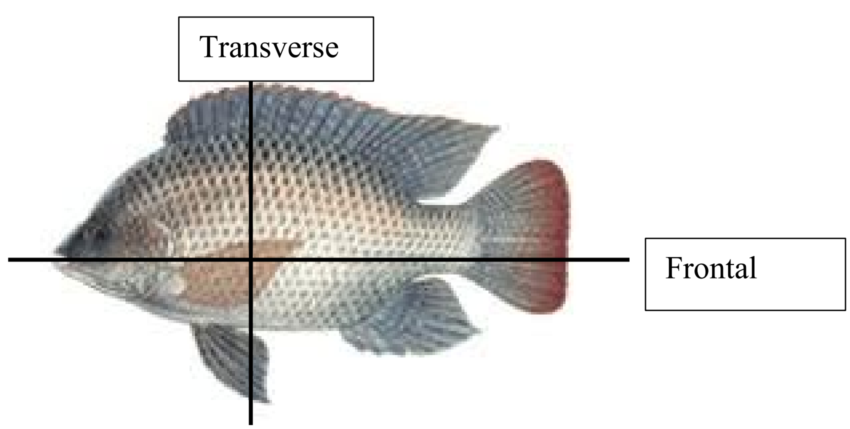

Figure 10. Transverse and frontal view of a blue tilapia. Modified from https://bassonline.com/freshwater-species/blue-tilapia/.

2.4.2 Capturing shortnose sturgeon images.

Supplementary work was done on shortnose sturgeon. The experimental method was similar to that for the blue tilapia (section 2.4.1). The only difference was that images were captured using only general resolution (GEN) because previous work done in our lab found that GEN resolution had a better image quality on shortnose sturgeon. This may be due to GEN having the capacity to penetrate deeper in bigger fishes. Summarizing section 2.4.1, fish samples were fasted, anesthetized with MS-222 (150 mg/L) and baking soda (300mg/L), and scanned at the different orientations (frontal and transverse) and placements (lateral and ventral) as shown in Figure 11.

Figure 11. Shortnose sturgeon (SS) being anesthetized using MS-222 and baking soda (top left), scanning the frontal view of SS in water (top right), and scanning the ventral view of SS using gel in a bag method (bottom photo).

After scanning both species, fish samples were placed in a recovery water (Figure 12) and rapid action mortality predators (RAMP) was performed to evaluate fish health. In total four reflexes were tested including ventilation, orientation, tail grab and body flex (McLean et al., 2016). Once a fish passed all four tests, it was returned to its tank using a fish net.

Figure 12. Shortnose sturgeons in a recovery tank within their tank.

2.4.3 Image analysis in Fiji/image J

All captured Images were analyzed with an open-source imaging software called Fiji (Schindelin et al., 2012). Images were individually labelled with Fish ID and PIT TAG number for each fish. In Fiji, regions of interest (ROI) were outlined using the rectangle selection tool, for both males and female, and measurements under "Analyze" were taken and recorded. Measurements includes mean gray values, standard deviation, area, perimeter, skewness, kurtosis, and slice. Surface roughness was also visualized using surface plots under "Analyze". The region of interest was marked out to exclude out-of-focus aeras and regions with high fat (low echogenicity/ low gain) from biasing the fractal measurements as shown in Figure 13. The captured and selected images were then converted into binary type images (black and white) and region of interest (ROI) was outlined again to perform fractal analysis.

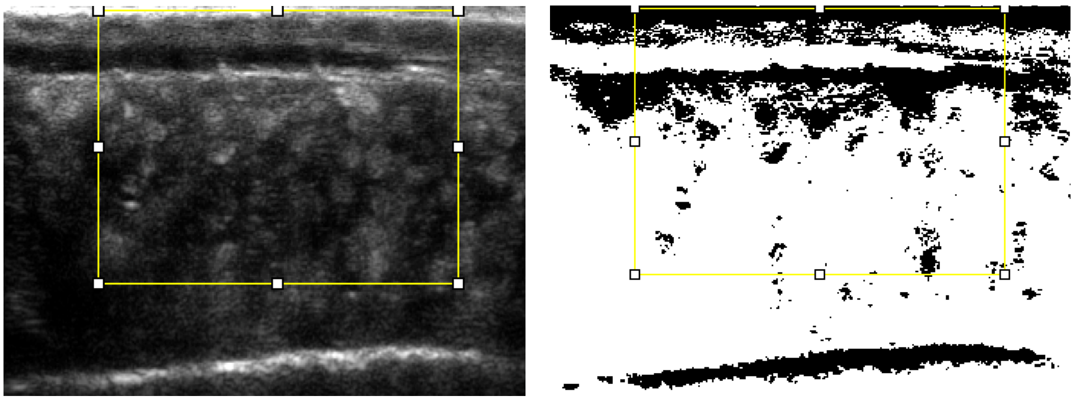

Figure 13. A grayscale image of a frontal view of a female blue tilapia with the region of interest (ROI) outlined (left) and the image converted to a binary-type image (right) with the ROI outlined.

I used the plugin “FracLac” (Karperien, 2013) in Fiji (Schindelin et al., 2012) to generate fractal values including lacunarity, DB (fractal dimension based on box counting) and Dm (mass fractal dimension) that measure for surface roughness. Lacunarity (Λ) refers to both gaps and heterogeneity of an image. With “FracLac”, Lacunarity (Equation 1) is derived by varying pixel density at separate box sizes in both fixed scans and sliding scans (Karperien, 2013). Lacunarity values were derived using equation 1 (Karperien, 2013). In equation 1, M and N represents the length and width in pixels respectively (Karperien, 2013). Equation 2 calculates fractal dimensions (FD) where ε represents box size or scale, g represents grid orientation, and Nr represents the number of overlapping copies. DB (slope of the regression line) is calculated using pixels per box and the number of boxes calculated in grid (Equation 3) (Karperien, 2013). In equation 3, $N\varepsilon$ represents the number of parts at some ε (Karperien, 2013). For Equation 3 and 4, $\lim\varepsilon \to 0$ represents the slope of the regression line μ_ε and ε (Karperien, 2013). DM (equation 4) represents the mass fractal dimension and is calculated by counting the number pixels from within a box on a grid at a given scale. In equation 4, μ_ε represents the mean pixels per box at some ε (Karperien, 2013).

$$L = (\frac{\left(\frac{1}{MN}\right)(\sum_{m=0}^{M=1}\sum_{n=0}^{N-1} I(m,n)^2)}{(\left(\frac{1}{MN}\right)\sum_{k=0}^{M=1}\sum_{l=0}^{N=1} I(k,l)^2)} \text{-1} \tag{1}$$

λε, g = CVε,g2= (σε,g/με,g)^2

$$FD = \frac{\log(N_r)}{\log\left(\frac{1}{r}\right)} \tag{2}$$

$$\mathrm{DB} = \lim \varepsilon \to 0\ [\log N\varepsilon \ / \log \varepsilon] \tag{3}$$

$$\mathrm{DM} = \lim \varepsilon \to 0[\ln \mu\varepsilon \ / \ln \varepsilon] \tag{4}$$

2.4.4 Statistical approach

Values derived from Fiji/Image J were then analyzed using Random factor MANOVA.RM (Friedrich et al., 2019). MANOVA.RM is an available package in R that implements semi-parametric statistical methods based on bootstrapping and permutation approaches that neither assume multivariate normality nor homogeneity of covariances matrices (Friedrich et al., 2019). Data were analyzed using R studio (version 4.3.2) and significance was accessed at 0.05. MANOVA.RM model was made for all five dependent variables mean gray values, skewness of mean gray values, lacunarity, DB (Slope of regression line), Dm (mass fractal dimension)) using the function MANOVA.wide in the package MANOVA.RM (Friedrich et al., 2019). Normality of data was tested and visualized in a multivariate context using multivariate Shapiro-Wilk test in RVAideMemoire package. Homogeneity of covariance matrices was also tested in a multivariate context using Box's M-test (Box, 1949). No data was transformed as MANOVA.RM is robust to violations of assumptions (Friedrich et al., 2019). Correlations among the dependent variables was conducted to evaluate the multi collinearity amongst dependent variables. A perfect correlation among dependent variables will crash MANOVA. The Wald-type statistic (WTS) (Konietschke et al., 2015) for repeated measures using parametric bootstrap (paramBS) p-values was reported in this study. Post-hoc tests were done using SimCI function the multcomp package (Hothorn et al., 2008) to determine individual differences between media and resolutions.

2.4.5 Subjective approach

A blind survey was conducted whereby seven of my lab mates were made to pick one image (A or B) from a randomly selected pair of images with the same resolution but captured in different media (water and gel) that they believe had a better quality. A heterogeneity test of survey responses was measured using forest plots of the odd ratios at 95% confidence interval. The forest plot model was built in such a way that a survey response that is greater than 1 (i.e. falls to the right as shown in Figure 14) means that the images in water at high resolution (RES) had a better image quality than images in gel at a general resolution (GEN). On the other hand, a survey response that is lesser than 1 (i.e. falls to the left as shown in Figure 15) means that the images in gel at general resolution (GEN) had a better image quality than images in water at a high resolution (RES). I also recorded my observations on image quality using mean gray values. I ranked images into 5 categories, with 5 = highest image quality and 1= lowest image quality and made a regression line plot with image quality on the x-axis and mean gray value in the y-axis for both fish species (Figure 26 and 27). I used ANOVA to test the significance of the regression slope.

2.5 Results

2.5.1 Objective results for blue tilapia image

All dependent variables (mean gray values, Lacunarity, DB, Dm and skewness) for blue tilapia sample images were not normal based on multivariate Shapiro-Wilk test (p=2.13e-15). A visual test for normality was conducted and all skewed values were kept in. All dependent variables had unequal homogeneity of variance based on Box's M-test for homogeneity of covariance matrices test (p= 0.000452).

For the blue tilapia samples, there was no significant interaction between media and resolution (p-value=0.575) as shown in table 3. Therefore, the main effects were appropriate to interpret. Both media and resolution had a significant p-value = <0.001 (table 3). Thus, media and resolution had a significant effect on image quality. The post-hoc pairwise comparison between the two media (Water-Gel) had a significant difference (p = 0). The post-hoc pairwise comparison test between the two resolutions (RES-GEN) also had a significant difference (p = 0.04). As shown in Figure 14 and 15, The RES solution had a slightly lower mean gray values than GEN, but RES is more skewed. Both DB and DM, GEN was slightly higher than RES (Figure 16 and 17). Lacunarity between the two resolutions for the different media did not vary a lot (Figure 18).

Table 3. Wald-Type Statistic (WTS) results for blue tilapia image samples.

	Test statistics	df	p-value
Media	28.239	5	<0.001
Resolution	39.751	5	<0.001
Media: Resolution	3.876	5	0.589
Water-gel	N/A	N/A	0
RES-GEN	N/A	N/A	0.041

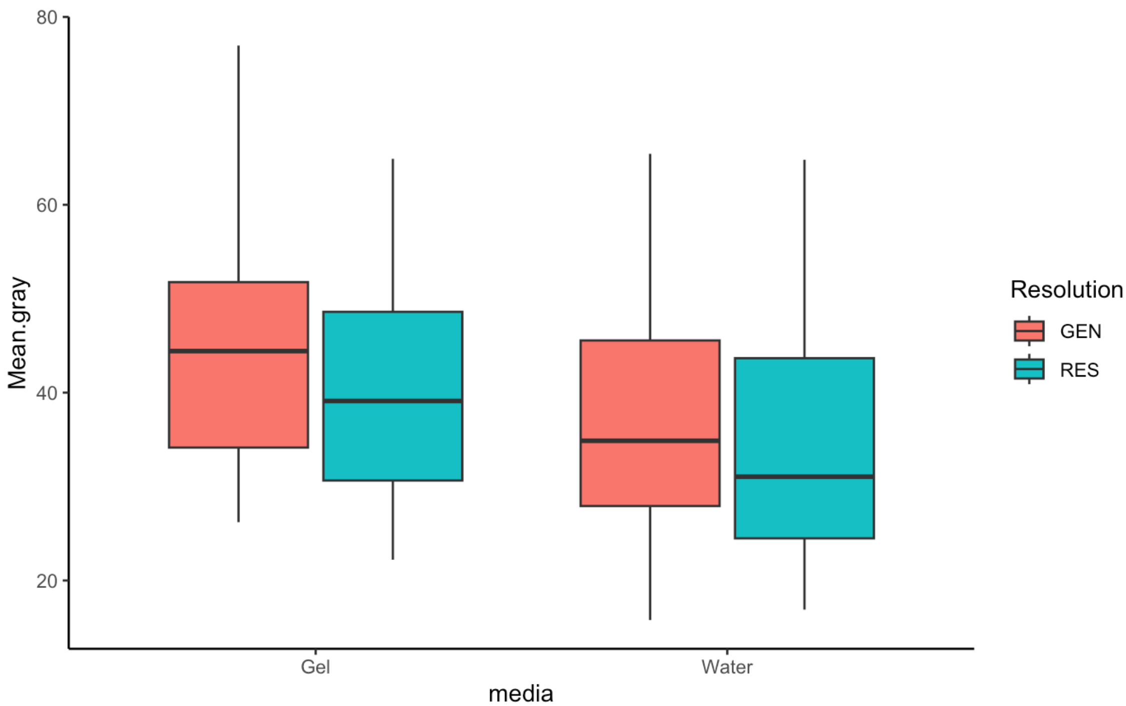

Figure 14. Boxplot showing the relationship between mean gray values (y-axis) of the different resolutions and the media (x-axis) for blue tilapia sample images.

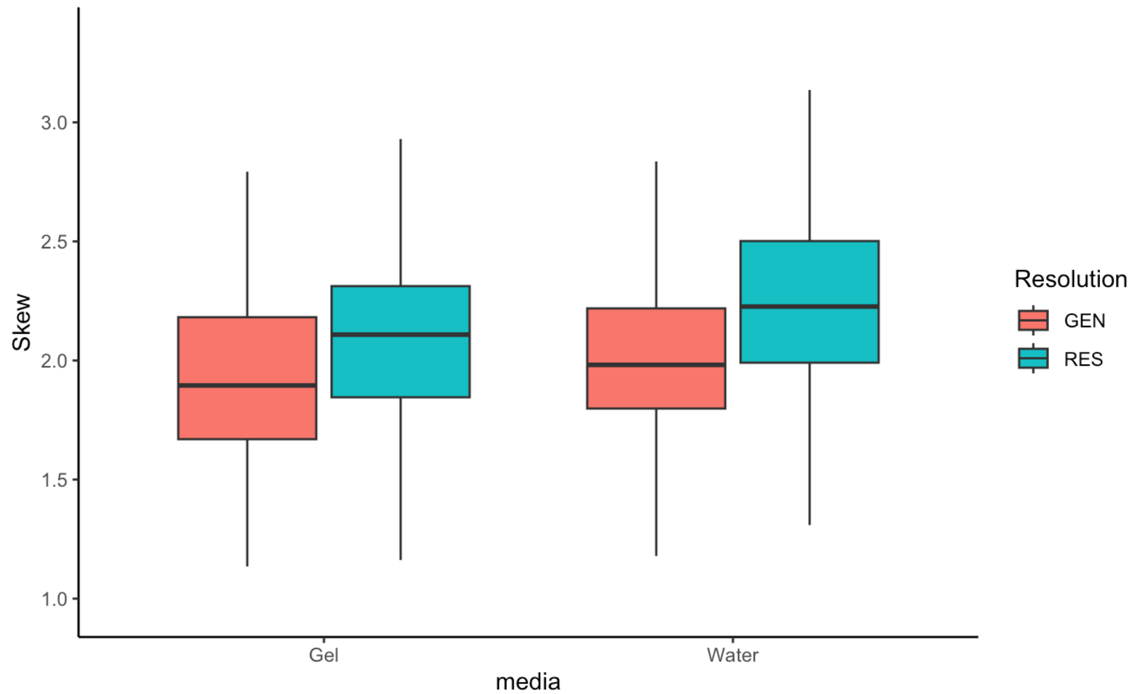

Figure 15. Boxplot showing the relationship between skewness (y-axis) of the different resolutions and the media (x-axis) for blue tilapia sample images.

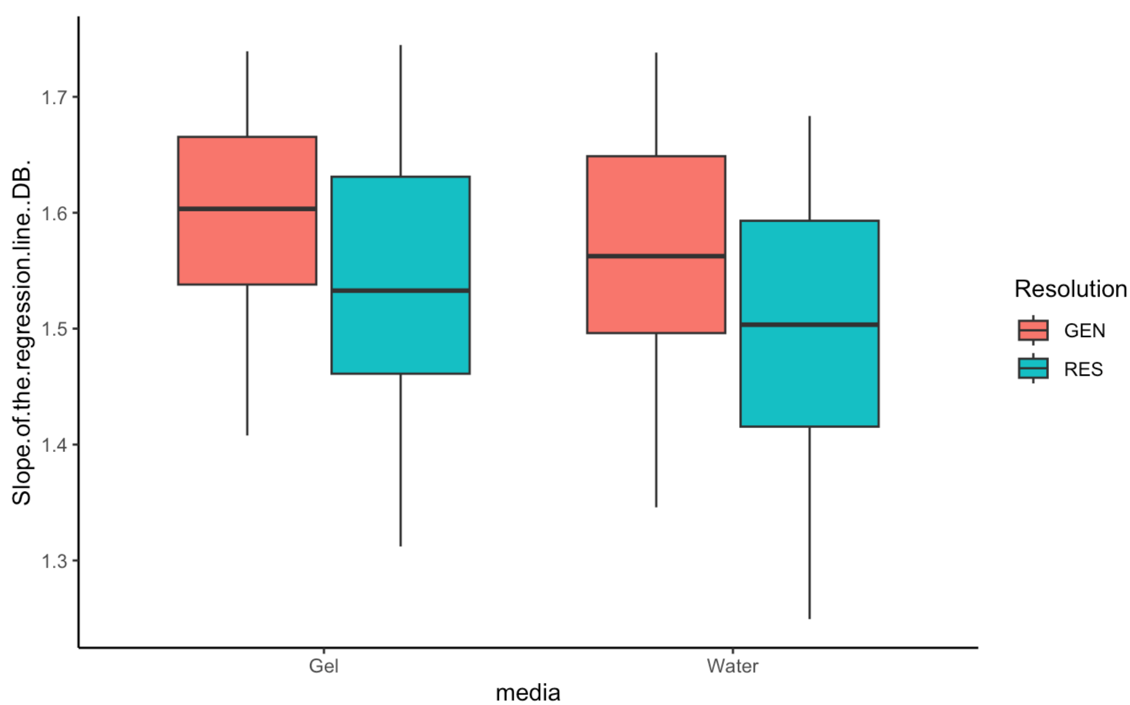

Figure 16. Boxplot showing the relationship between DB (y-axis) of the different resolutions and the media (x-axis) for blue tilapia sample images.

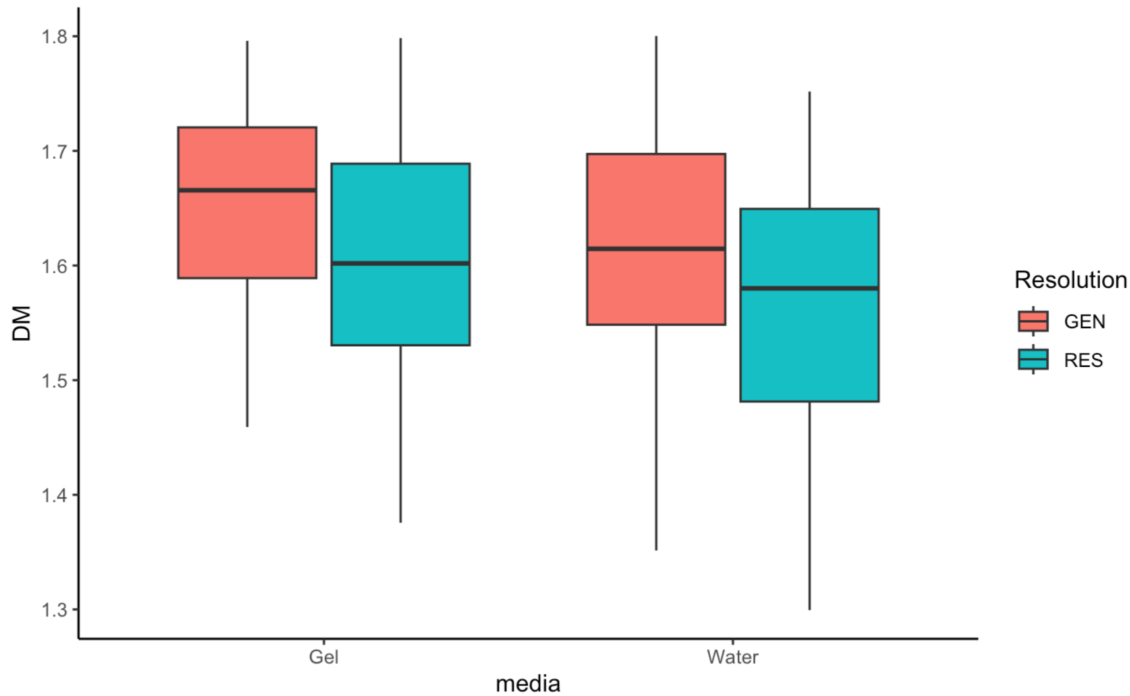

Figure 17. Boxplot showing the relationship between DM (y-axis) of the different resolutions and the media (x-axis) for blue tilapia sample images.

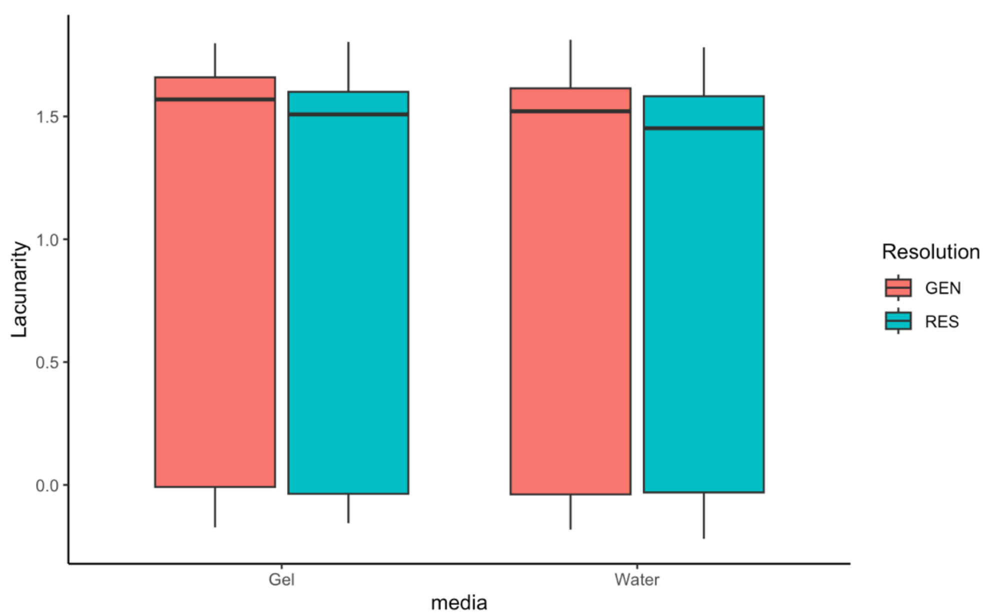

Figure 18. Boxplot showing the relationship between lacunarity (y-axis) of the different resolutions and the media (x-axis) for blue tilapia sample images.

2.5.2 Subjective results for blue tilapia

Figure 19 shows that almost all survey participants picked images captured in water and a high resolution to have a better image quality than in gel at GEN.

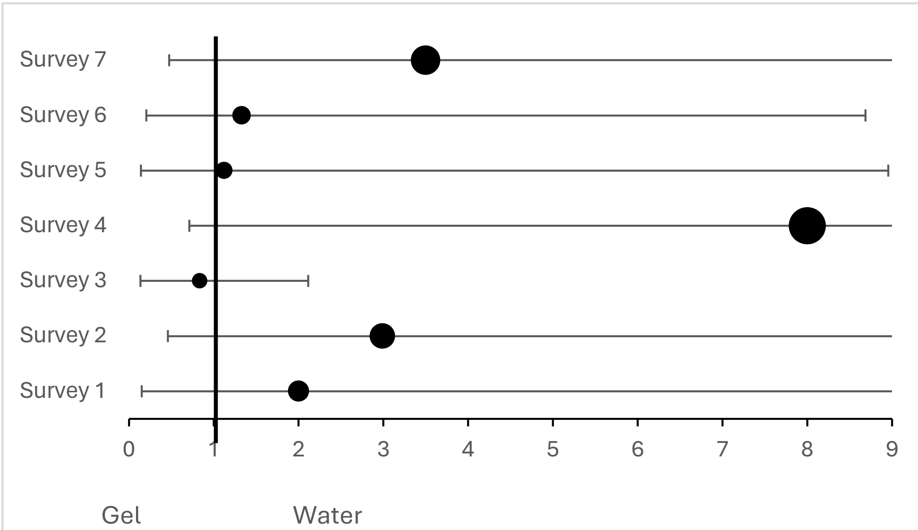

Figure 19. Forest plots of survey responses for blue tilapia image pairs.

Table 4. Parameters used to build the forest plot for blue tilapia images including participants, odd ratios (OR), natural log of odd ratios, lower 95% confidence interval (CI), graph lower 95% CI, upper 95% CI, and graph upper 95% CI.

Participants	Odd ratios	natural log of OR	lower 95% CI	Graph lower 95%	Upper 95% CI	Graph upper 95% CI
Survey 1	2.00	0.69314718	0.149615127	1.850384873	26.7352646	24.7352646
Survey 2	2.99	1.09527339	0.457817913	2.532182087	19.52763259	16.53763259
Survey 3	0.83	-0.1827216	0.134339871	0.698660129	2.113251379	1.280251379
Survey 4	8.00	2.07944154	0.711073194	7.288926806	90.00479913	82.00479913
Survey 5	1.12	0.11332869	0.140076686	0.979923314	8.955094802	7.835094802
Survey 6	1.33	0.28517894	0.203644757	1.126355243	8.686204465	7.356204465
Survey 7	3.5	1.25276297	0.472935424	3.027064576	25.90205633	22.40205633

2.5.3 Objective results for shortnose sturgeon

All dependent variables (mean gray values, gray value skewness, Lacunarity, DB, and Dm) for shortnose sturgeon images were not normal based on multivariate Shapiro-Wilk normality test RVAideMemoire test (p= 8.632e-07). A visual test for normality was conducted and all skewed values were kept in. All dependent variables had unequal homogeneity of variance based on Box's M-test for homogeneity of covariance matrices test (p= 0.02146). Media had no effect on image quality (p-value = 0.796; Table 5). Images captured with gel/air method had a slightly higher mean gray values, but water images were significantly more skewed (Figure 20 and 21). Lacunarity did vary as much (Figure 24).

Table 5. Wald-Type Statistic (WTS) results for shortnose sturgeon sample images.

	Test statistics	df	p-value
Media	2.998	5	0.796

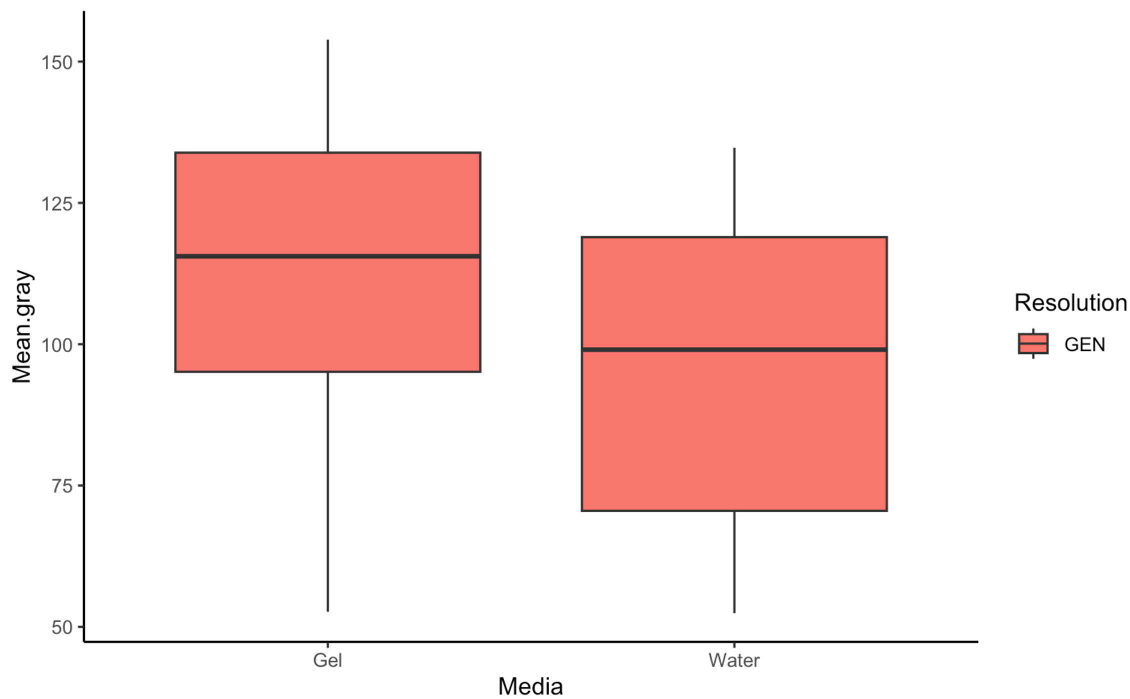

Figure 20. Boxplot showing the relationship between skewness (y-axis) of the different resolutions and the media (x-axis) for blue tilapia sample images.

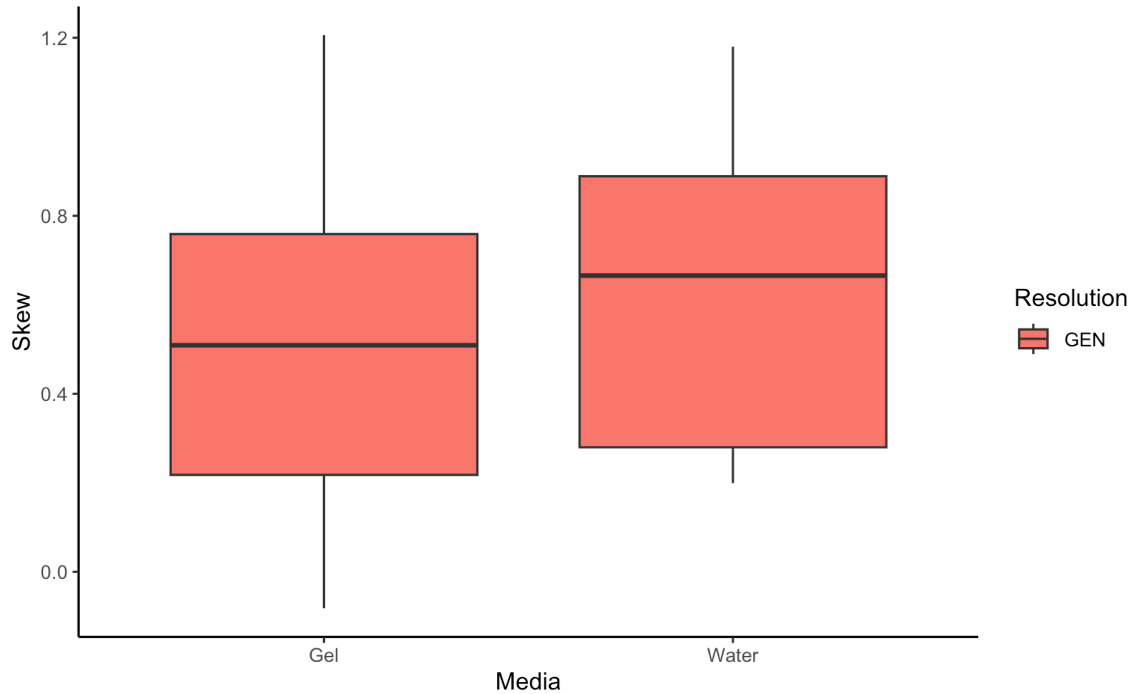

Figure 21. Boxplot showing the relationship between skew (y-axis) of the different resolutions and the media (x-axis) for shortnose sturgeon sample images.

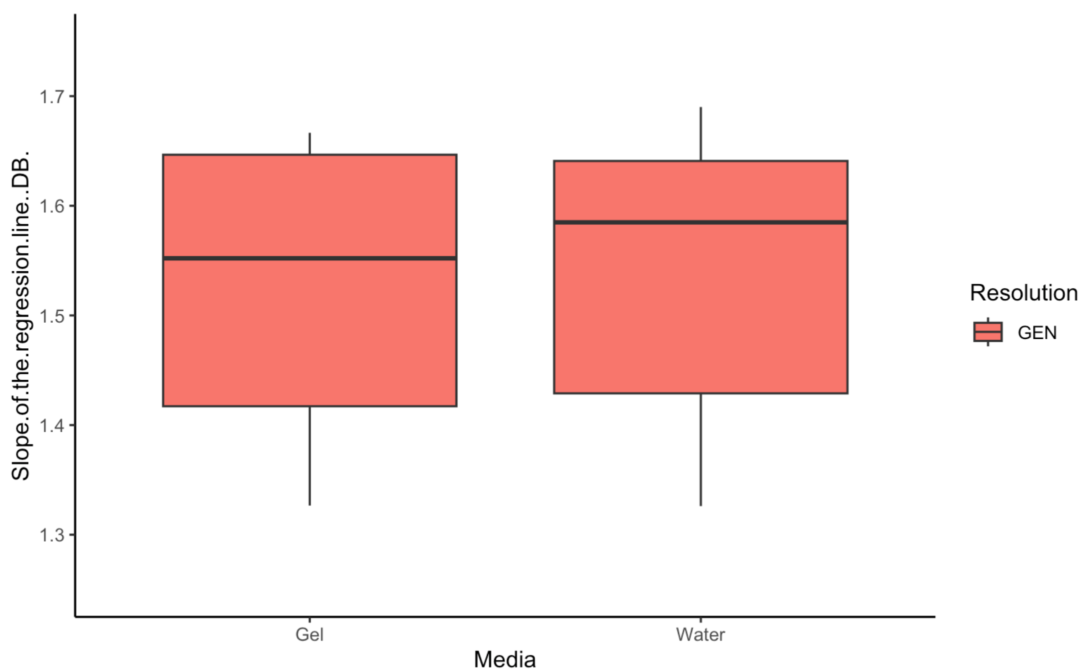

Figure 22. Boxplot showing the relationship between DB (y-axis) of the different resolutions and the media (x-axis) for shortnose sturgeon sample images.

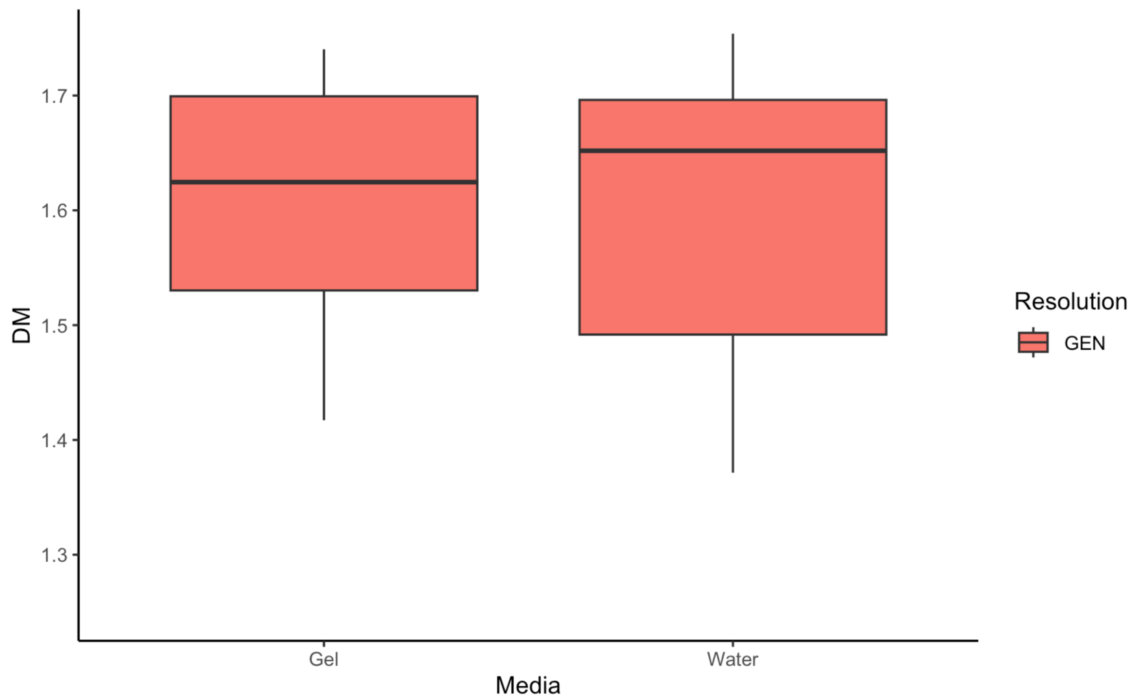

Figure 23. Boxplot showing the relationship between DM (y-axis) of the different resolutions and the media (x-axis) for shortnose sturgeon sample images.

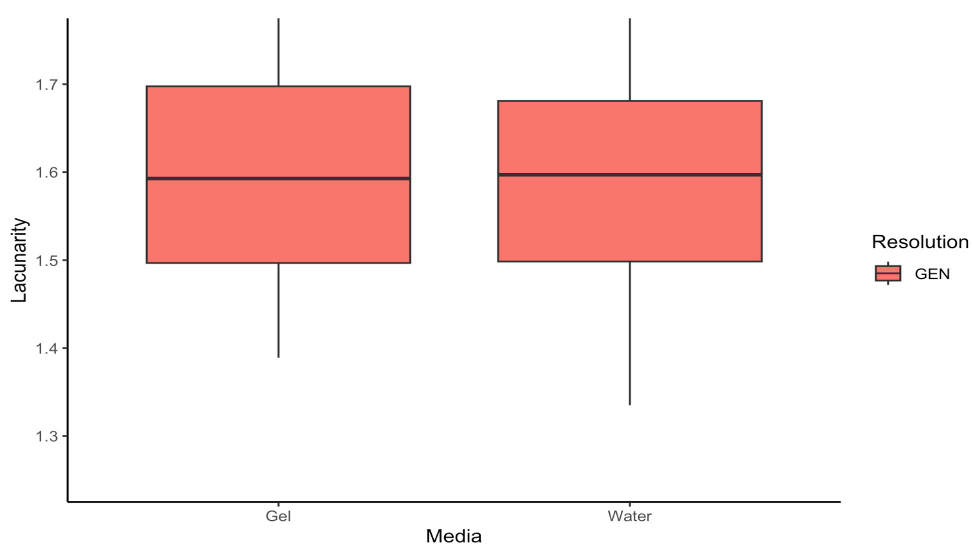

Figure 24. Boxplot showing the relationship between lacunarity (y-axis) of the different resolutions and the media (x-axis) for shortnose sturgeon sample images.

2.5.4 Subjective results for shortnose sturgeon

Figure 25 shows that almost all survey participants picked images captured in gel at general resolution (GEN) to have a better image quality than in water at high resolution (RES).

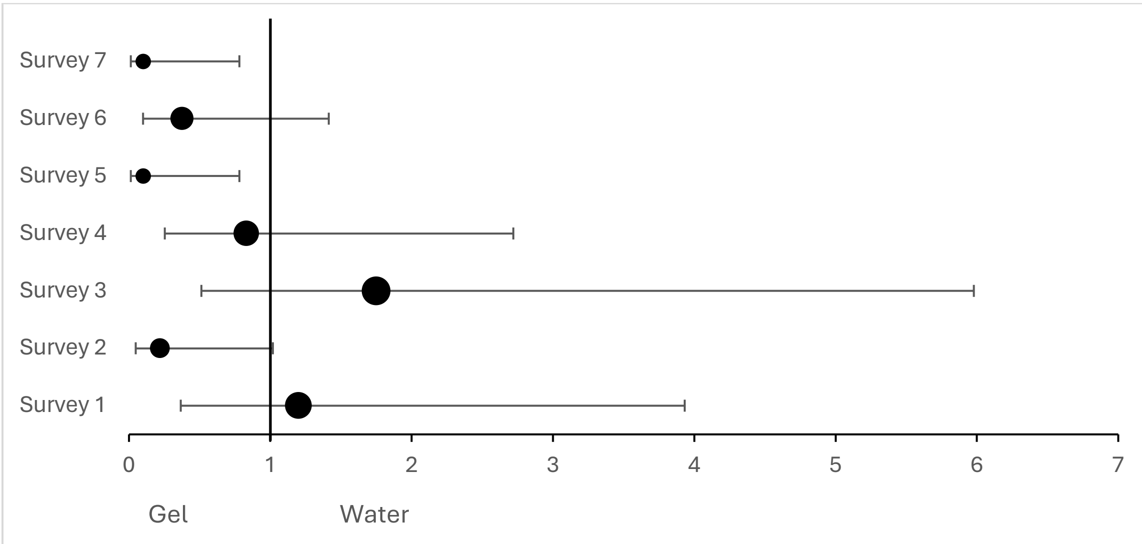

Figure 25. Forest plots of survey responses for shortnose sturgeon image pairs.

Table 5. Parameters used to build the forest plot for shortnose sturgeon images including participants, odd ratios (OR), natural log of odd ratios, lower 95% confidence interval (CI), graph lower 95% CI, upper 95% CI, and graph upper 95% CI.

Participants	Odd ratios	natural log of OR	lower 95% CI	Graph lower 95%	Upper 95% CI	Graph upper 95% CI
Survey 1	1.2	0.18232156	0.366221337	0.833778663	3.932048338	2.732048338
Survey 2	0.22	-1.5141277	0.047533041	0.172466959	1.018239086	0.798239086
Survey 3	1.75	0.55961579	0.512282409	1.237717591	5.978147887	4.228147887
Survey 4	0.83	-0.1863296	0.253303092	0.576696908	2.719666767	1.889666767
Survey 5	0.1	-2.3025851	0.012800764	0.087199236	0.781203382	0.681203382
Survey 6	0.375	-0.9808293	0.099484291	0.275515709	1.41353975	1.03853975
Survey 7	0.1	-2.3025851	0.012800764	0.087199236	0.781203382	0.681203382

2.5.5 My observation

Overall, I observed that the difference in the quality of ultrasonic images produced from the two different media was very distinct. However, I found that there was no correlation between image quality, based on my personal image quality grading, and mean gray values. For both blue tilapia (Figure 26) and shortnose sturgeon (Figure27), I found that some images I viewed to have the highest image quality had similar mean gray values as images I viewed to have the lowest image quality. The regression slope for blue tilapia images was not different than 0 ($p = 0.19242831$; Table 7). The regression slope for shortnose sturgeon images was also not different than 0 ($p = 0.09570588$; Table 9).

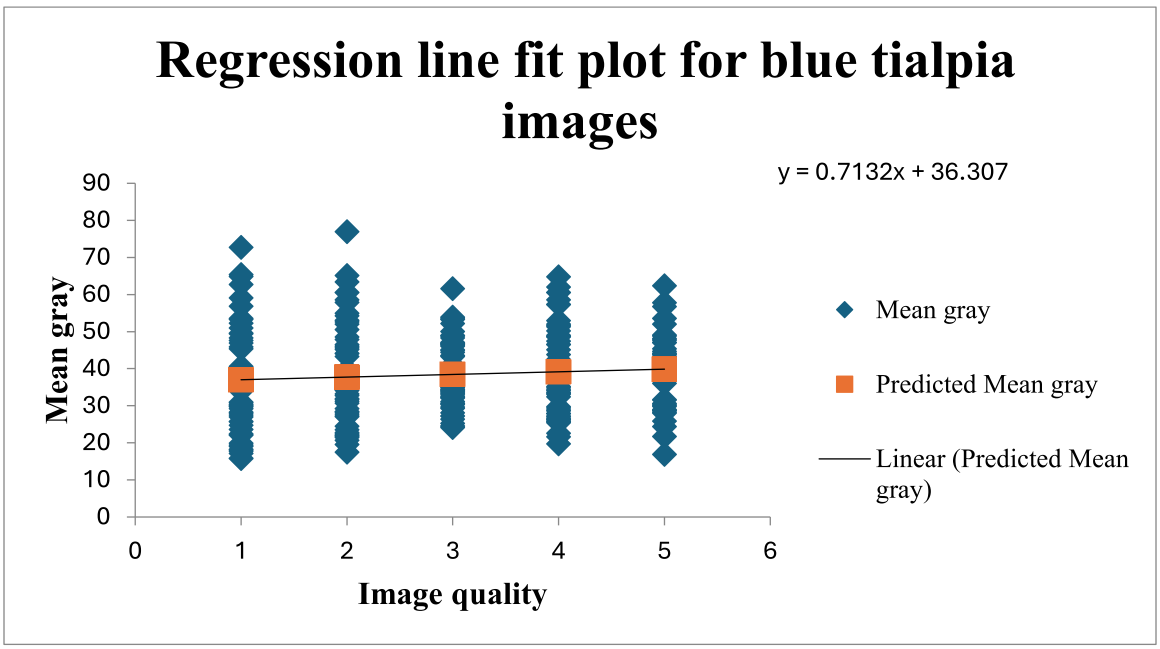

Figure 26. Scatterplot showing the relationship between image quality (y-axis) and average mean gray values (y-axis) for blue tilapia images.

Table 6. Regression statistics summary for blue tilapia images

Regression statistics	
Multiple R	0.07927265
R Square	0.00628415
Adjusted R Square	0.00260372
Standard Error	12.1058835
Observations	272

Table 7. ANOVA results summary for blue tilapia images

	df	**SS**	**MS**	**F**	**Significance F**
Regression	1	250.23107	250.23107	1.70745102	0.19242831
Residual	270	39569.1519	146.552414		
Total	271	39819.383			

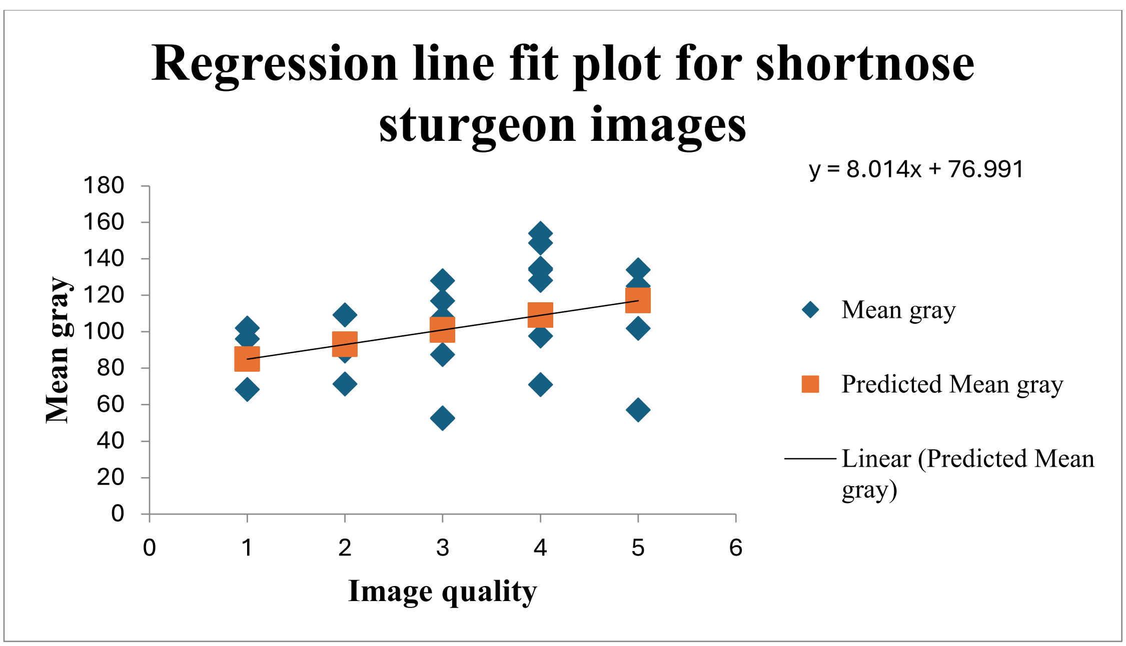

Figure 27. Scatterplot showing the relationship between image quality (y-axis) and average mean gray values (x-axis) for shortnose sturgeon.

Table 8. Regression statistics summary for shortnose sturgeon

Regression statistics	
Multiple R	0.34793144
R Square	0.12105629
Adjusted R Square	0.0811043
Standard Error	28.8737631
Observations	24

Table 9. ANOVA results for Shortnose sturgeon

	df	**SS**	**MS**	**F**	**Significance F**
Regression	1	2526.13034	2526.13034	3.03004428	0.09570588
Residual	22	18341.2724	833.694198		
Total	23	20867.4027			

2.6 Discussion

As mentioned in Sassaroli et al. (2019), quality assessment plays an important role in image analysis. I have shown that using water as a transmission medium while the ultrasound set to the highest resolution (RES) produces a better image quality for small sized fish like blue tilapia. This is as a result of the high resolution having a low penetration depth which is suitable for smaller sized fish like blue tilapia. Any resolution with deeper penetration depth may cause excessive bounce back reflections from the bed holding the holding the fish, consequently, producing a poorer quality image. For shortnose sturgeon, I chose to work with only GEN resolution based on previous work done in our lab my a past graduate student, Balazadeh Keyvan who worked on exploring fractal dimensions on ultrasound of gonad images in shortnose sturgeon. That said, I was able to capture better quality images with gel in a bag versus submerged for shortnose sturgeon. A higher resolution (RES) with lower penetration depth would not the able to see deep structures.

An advantage to my study is that the ultrasound machine is relatively easy to use as anyone that takes the time to understand the manual and instrument settings can use. The ultrasound machine has a number of instrumental settings that can be manipulated to influence image quality. I was fortunate to be able to work on two different fish species that vary in size, morphology, and anatomical structures. It was clear that the US approaches have to be adjusted to match the species under study.

A strength of my work is that I report a detailed experimental method that can assist novel sonographers on the procedures to take prior, during and after scanning. The use of ultrasonography as a non-invasive tool can be advanced by reporting necessary information on handling procedures before, during and after ultrasound imaging (Novelo & Tiersch, 2012). The minimum information necessary for reporting includes holding system, if the fish was anesthetized or not, the anesthetic dose, if they were fasted or not, duration of fasting, whether the fish was physically restrained, detailed descriptions of fish position/orientation (ventral or lateral), duration of scanning period, equipment used to transport the fish, clear description of probe placement (frontal or transverse), an illustration/digital photo showing the positioning of the fish etc. (Novelo & Tiersch, 2012).

The main objective of my study was to provide ultrasound imaging protocols to produce the highest quality image. My study compares the quality of images captured in the standard gel/ air method *versus* images captured with the probe directly immersed in water, different resolutions (RES & GEN), views and probe orientations. The quality of an image is a measure of the perceived degradation of the image (Rangaraju et al., 2012). In this study, I analysed image quality both subjectively and objectively. The subjective assessment of image quality is associated with how a viewer perceives a particular image based on their opinion and preferences (Rangaraju et al., 2012). Human eyes are the ultimate viewer of an image (Rangaraju et al., 2012).

On the other hand, the objective method of image quality assessment is associated with deriving quantitative measures that can be analysed to predict an image quality. The objective analysis can be useful in a variety of applications. Rangaraju et al. (2012) highlights that it allows image quality to be monitored and adjusted dynamically, it can be applied to image processing systems, and benchmark image processing system. In addition, determining image quality objectively is preferable because it eliminates the subjective assessment that may produce inconsistent results. As highlighted in Oglat et al. (2018), there are various advanced imaging modes and instrumental settings that can be altered to attain a better image quality image such as resolution, probe frequency, knobology, grayscales etc. For my study, examined how all these instrumental settings in different media, both objectively and subjectively, influenced image quality.

The standard method of using the ultrasound technique is with the gel in bag method (Colombo et al., 2004). My results suggests that the ultrasound probe can be used submerged in water for smaller sized fish. Both subjectively and objectively, my results suggest that using gel in a bag method is better for larger sized fish. That said, there is a cost to using the bag/gel approach. With the gel in a bag method as seen is Figure 8, the fish are kept out of water. The most important goal is that the fish is surviving in its optimal environment to ensure it is not adversely affected by handling/ultrasonography. In addition, the images captured in water for the shortnose sturgeon were still of high quality. Researchers will have to decide if taking the extra time, expense and potential for fish injury is worth the increase in image quality for the gel/bag ultrasonographic approach. There is a potential that this extra handling could adversely impact their health and reproductive fitness. Based on my experience, I would recommend the water method for all fish sizes as it has also been used in the past for stellate sturgeons as large as 95cm in length (Moghim et al., 2002).

My study is essential because one major limiting factor for developing hatchery-reared broodstocks is the lack of using non-invasive methods for early sex identification and gonad staging (Chebanov & Galich, 2009). Ultrasound is an excellent tool that can be used for this purpose as it is a rapid technique for sexing and identifying maturity stages in fish (Table 2). It has the potential to improve the overall management control of farmed broodstock (Chebanov & Galich, 2009). It is crucial to develop a systematic approach that aims at producing optimal image because the ultrasound technique is commonly used in a wide arrange of medical applications due to its real-time feedback, cost effectiveness, portability, non-ionizing nature, reliability, and non-lethal characteristics (Chebanov & Galich, 2009). My research is significant because it has the potential to improve aquaculture practices and conservation, aid ecological analysis of species status, and overall improve management control of broodstock in fish facilities.

However, there are limitations to the use of US in fish research. For example, US machine and probes are very expensive and fragile: Edge II, used in this study, is over $30K and new probes required for this work are approximately $10K each. Clearly, proper care must be taken while using these expensive systems. The prices of used machine units can range from US$2,000-US$9,000 and is a viable cost saving option (Novelo & Tiersch, 2012). However, specialized US systems can cost over US$100,000 (Novelo & Tiersch, 2012). A second limitation to my study is that the Edge II machine, like most US machines, does not show a quantitative value for the gain control. Consequently, there was a heterogeneity in image quality across images. Ideally, images with the exact same instrumental settings in different media should be compared. Thirdly, the results for shortnose sturgeon may be as a result of a very small sample size as I only captured images of 4 female shortnose sturgeons. Fourthly, images were manually cropped, in Fiji, to outline region of interest (ROI). This resulted to varying area of ROI. Ideally, the region of interest should be the same across images that are being compared.

My research analysed the how image quality of ultrasonic images of the internal anatomy of fish can be influenced by the transmission medium used, the ultrasound probe resolution and probe placements.

Ultrasound images are difficult to interpret due to several factors like shadows, low gain, too high gain, shadows, noise, and poor contrast (Raina et al., 2023). Assessing image quality is made more challenging by the presence of multiple anatomical structures (Raina et al., 2023). For future research, artificial intelligence (AI) ultrasound diagnostic system should be adopted. For AI machine learning, a sonographer is required to just start the scan on the machine. This would help reduce cost of screening and overall reduce the complexity of the system (Huang et al., 2023). This would allow consistent interpretation of ultrasound images as what one sonographer may classify as a good quality image may differ from what another sonographer or radiologists would term a good quality image. With the use of AI machine learning, the subjective approach of interpreting images based on personal bias would be eliminated. Overall, with AI machine learning, ultrasound scanning can be automated and done more quickly. There are a few studies that suggest AI machine learning is more effective, less time-consuming, and more reliable for e.g. Huang et al., 2023.

The importance of my work is not just restricted to just fishes or non-human animals, it can also be useful to clinical medical physicists and technicians in hospital. Having the background knowledge of the ultrasound can influence the instrumental setting they use to scan humans. Overall, improving the efficiency of the ultrasound imaging system in the different medical disciplines it is used.

Resources

Alarcon-Rojo, A. D., Carrillo-Lopez, L. M., Reyes-Villagrana, R., Huerta-Jiménez, M., & Garcia-Galicia, I. A. (2019). Ultrasound and meat quality: A review. *Ultrasonics Sonochemistry*, *55*, 369–382. https://doi.org/10.1016/j.ultsonch.2018.09.016

Boezaart, A., & Ihnatsenka, B. (2010). Ultrasound: Basic understanding and learning the language. *International Journal of Shoulder Surgery*, *4*(3), 55. https://doi.org/10.4103/0973-6042.76960

Box, G. E. P. (1949). A General Distribution Theory for a Class of Likelihood Criteria. *Biometrika*, *36(3/4)*, 317–446. https://www.jstor.org/stable/2332671

Brook, F. M. (2001). *Ultrasonographic imaging of the reproductive organs of the female bottlenose dolphin, Tursiops truncatus aduncas*.

Bryan, B. J. L., Wildhaber, M. L., Papoulias, D. M., DeLonay, A. J., Tillitt, D. E., & Annis, M. L. (2007). Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon. *Journal of Applied Ichthyology*, *23*(4), 411–419. https://doi.org/10.1111/j.1439-0426.2007.00889.x

Chebanov, M. S., & Galich, E. V. (2009). Ultrasound diagnostics for sturgeon broodstock management. *FSGTSR, Krasnodar. Izdatel' Stvo Prosveshenie-Yug*, 116.

Chen, C., Feng, S., Zhou, M., Ji, C., Que, L., & Wang, W. (2019). Development of a structure-switching aptamer-based nanosensor for salicylic acid detection. *Biosensors and Bioelectronics*, *140*, 111342. https://doi.org/10.1016/j.bios.2019.111342

Chiotti, J. A., Boase, J. C., Hondorp, D. W., & Briggs, A. S. (2016). Assigning Sex and Reproductive Stage to Adult Lake Sturgeon using Ultrasonography and Common Morphological Measurements. *North American Journal of Fisheries Management*, *36*(1), 21–29. https://doi.org/10.1080/02755947.2015.1103823

Colombo, R. E., Wills, P. S., & Garvey, J. E. (2004). Use of Ultrasound Imaging to Determine Sex of Shovelnose Sturgeon. *North American Journal of Fisheries Management*, *24*(1), 322–326. https://doi.org/10.1577/M03-016

Cotton, J., Bahner, D., & Prats, M. (2024). The lateral approach water bath: A novel method of ultrasound imaging of the hand. *The American Journal of Emergency Medicine*, *77*, 164–168. https://doi.org/10.1016/j.ajem.2023.11.005

Czesny, S., Dabrowski, K., Christensen, J. E., Van Eenennaam, J., & Doroshov, S. (2000). Discrimination of wild and domestic origin of sturgeon ova based on lipids and fatty acid analysis. *Aquaculture*, *189*(1–2), 145–153. https://doi.org/10.1016/S0044-8486(00)00364-1

Friedrich, S., Konietschke, F., & Pauly, M. (2019). Resampling-Based Analysis of Multivariate Data and Repeated Measures Designs with the R Package MANOVA.RM. *The R Journal*, *11*(2), 380. https://doi.org/10.32614/RJ-2019-051

Gjedrem, T. (2017). Possibility for Improving Carcass Composition and Meat Quality Traits by Selective Breeding. *International Journal of Current Research and Review*. https://doi.org/10.7324/IJCRR.2017.9103

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Huang, X., Qiu, Y., Bao, F., Wang, J., Lin, C., Lin, Y., Wu, J., & Yang, H. (2023). Artificial intelligence breast ultrasound and handheld ultrasound in the BI-RADS categorization of breast lesions: A pilot head to head comparison study in screening program. *Frontiers in Public Health*, *10*, 1098639. https://doi.org/10.3389/fpubh.2022.1098639

Karperien, A. (2013). *FracLac for ImageJ*. https://doi.org/10.13140/2.1.4775.8402

Konietschke, F., Bathke, A. C., Harrar, S. W., & Pauly, M. (2015). Parametric and nonparametric bootstrap methods for general MANOVA. *Journal of Multivariate Analysis*, *140*, 291–301. https://doi.org/10.1016/j.jmva.2015.05.001

Masoudifard, M., Vajhi, A. R., Moghim, M., Nazari, R. M., Naghavi, A. R., & Sohrabnejad, M. (2011). High validity sex determination of three years old cultured beluga sturgeon (Huso huso) using ultrasonography: Beluga high validity sex determination. *Journal of Applied Ichthyology*, *27*(2), 643–647. https://doi.org/10.1111/j.1439-0426.2011.01726.x

McLean, M. F., Hanson, K. C., Cooke, S. J., Hinch, S. G., Patterson, D. A., Nettles, T. L., Litvak, M. K., & Crossin, G. T. (2016). Physiological stress response, reflex impairment and delayed mortality of white sturgeon *Acipenser transmontanus* exposed to simulated fisheries stressors. *Conservation Physiology*, *4*(1), 14. https://doi.org/10.1093/conphys/cow031

Moghim, M., Vajhi, A. R., Veshkini, A., & Masoudifard, M. (2002). Determination of sex and maturity in Acipenser stellatus by using ultrasonography. *Journal of Applied Ichthyology*, *18*(4–6), 325–328. https://doi.org/10.1046/j.1439-0426.2002.00423.x

Novelo, N. D., & Tiersch, T. R. (2012). A Review of the Use of Ultrasonography in Fish Reproduction. *North American Journal of Aquaculture*, *74*(2), 169–181. https://doi.org/10.1080/15222055.2012.672370

Oglat, A., Matjafri, M., Suardi, N., Oqlat, M., Abdelrahman, M., & Oqlat, A. (2018). A review of medical doppler ultrasonography of blood flow in general and especially in common carotid artery. *Journal of Medical Ultrasound*, *26*(1), 3. https://doi.org/10.4103/JMU.JMU_11_17

Ovissipour, M., Al-Qadiri, H. M., Lu, X., Hu, Y., Ross, C. F., Van Eenennaam, J. P., Doroshov, S. I., & Rasco, B. (2015). The effect of white sturgeon (Acipenser transmontanus) ovarian fat deposition on caviar yield and nutritional quality: Introducing image processing method for sturgeon ovary fat determination. *International Aquatic Research*, *7*(4), 263–272. https://doi.org/10.1007/s40071-015-0111-0

Raina, D., Ntentia, D., Chandrashekhara, S. H., Voyles, R., & Saha, S. K. (2023). Expert-Agnostic Ultrasound Image Quality Assessment using Deep Variational Clustering. *2023 IEEE International Conference on Robotics and Automation (ICRA)*, 2717–2723. https://doi.org/10.1109/ICRA48891.2023.10160435

Rangaraju, K. S., Kumar, D. K., & Renumadhavi, C. H. (2012). Review Paper on Quantitative Image Quality Assessment � Medical Ultrasound Images. *International Journal of Engineering Research*, *1*(4).

Rezende, C. E. D., Perazza, C. A., Freitas, R. T. F. D., Hallerman, E., & Hilsdorf, A. W. S. (2023). Use of ultrasonographic imaging for non-invasive carcass yield prediction in Nile tilapia (Oreochromis niloticus). *Aquaculture International*, *31*(5), 2763–2778. https://doi.org/10.1007/s10499-023-01109-4

Robeck, T. R., Steinman, K. J., Gearhart, S., Reidarson, T. R., McBain, J. F., & Monfort, S. L. (2004). Reproductive Physiology and Development of Artificial Insemination Technology in Killer Whales (Orcinus orca)1. *Biology of Reproduction*, *71*(2), 650–660. https://doi.org/10.1095/biolreprod.104.027961

Sassaroli, E., Crake, C., Scorza, A., Kim, D., & Park, M. (2019). Image quality evaluation of ultrasound imaging systems: Advanced B-modes. *Journal of Applied Clinical Medical Physics*, *20*(3), 115–124. https://doi.org/10.1002/acm2.12544

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Shiranita, K., Hayashi, K., Otsubo, A., Miyajima, T., & Takiyama, R. (2000). Grading meat quality by image processing. *Pattern Recognition*.

Sicuro, B. (2019). The future of caviar production on the light of social changes: A new dawn for caviar? *Reviews in Aquaculture*, *11*(1), 204–219. https://doi.org/10.1111/raq.12235

Tavakoli, S., Luo, Y., Regenstein, J. M., Daneshvar, E., Bhatnagar, A., Tan, Y., & Hong, H. (2021). Sturgeon, Caviar, and Caviar Substitutes: From Production, Gastronomy, Nutrition, and Quality Change to Trade and Commercial Mimicry. *Reviews in Fisheries Science & Aquaculture*, *29*(4), 753–768. https://doi.org/10.1080/23308249.2021.1873244

Wildhaber, M. L., Papoulias, D. M., DeLonay, A. J., Tillitt, D. E., Bryan, J. L., & Annis, M. L. (2007). Physical and hormonal examination of Missouri River shovelnose sturgeon reproductive stage: A reference guide. *Journal of Applied Ichthyology*, *23*(4), 382–401. https://doi.org/10.1111/j.1439-0426.2007.00878.x

Wildhaber, M. L., Papoulias, D. M., DeLonay, A. J., Tillitt, D. E., Bryan, J. L., Annis, M. L., & Allert, J. A. (2005). Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. *Journal of Fish Biology*, *67*(1), 114–132. https://doi.org/10.1111/j.0022-1112.2005.00719.x

Zander, D., Hüske, S., Hoffmann, B., Cui, X.-W., Dong, Y., Lim, A., Jenssen, C., Löwe, A., Koch, J. B. H., & Dietrich, C. F. (2020). Ultrasound Image Optimization ("Knobology"): B-Mode. *Ultrasound International Open*, *06*(01), E14–E24. https://doi.org/10.1055/a-1223-1134