A PHOTOGRAPHIC ATLAS TO THE EARLY DEVELOPMENT OF THE BULLHEAD MINNOW (*PIMEPHALES VIGILAX*)

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

A Photographic Atlas to the Early Development of the Bullhead Minnow (*Pimephales vigilax*). (May 2014)

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The identification of larval fishes is difficult due to their small size and transparent nature. Deciphering between different species placed within the same genus or even family can prove near impossible. Literature currently available on larval fish is focused predominantly on marine species and little information is available that would aid the identification of the larval stages of freshwater species, particularly in the state of Texas. The purpose of this study is to provide a comprehensive photographic atlas to the larval stages of the Bullhead minnow (*Pimephales vigilax*). This guide will facilitate the identification of the larval stages of this common species of minnow throughout Texas (and other states) and will contribute to our knowledge of the larvae of freshwater fishes in general.

DEDICATION

I would like to dedicate this research to my loving parents. Without their continued support and constant push for success, this opportunity would not be possible.

ACKNOWLEDGMENTS

I would like to thank Dr. Kevin Conway for his constant support. Without his apparent passion for fish and his never-ending search for knowledge, I would not have the ambition and strive I personally embody today.

NOMENCLATURE

MYO	Myomere
UFR	Unbranched Fin Ray
BFR	Branched Fin Ray
MELO	Melanocyte
DF	Dorsal Fin
CF	Caudal Fin
PCF	Pectoral Fin
PVF	Pelvic Fin
AF	Anal Fin
LFF	Larval Fin Fold
PAFF	Pre-Anal Fin Fold
SL	Standard Length
LL	Lateral Line

CHAPTER I

INTRODUCTION

In our current day and age, conservation is becoming more and more of an important factor in the natural environment. As human populations grows and interests spread, more and more of our natural resources, including organisms, are being utilized (Foley et al. 2005). Because of this, more pressure is put on individuals within the natural system to survive and reproduce, thus producing an alarmingly large amount of endangered species, more so than in previous years (Dobson et al. 1997). This increase in the number of endangered and threatened species is requiring proper installation of policies and procedures designed to help protect these individuals (Eisner 1995). To effectively create these policies, a sound, scientific knowledge base is needed. This can only be achieved by research and studying the individuals in their natural system and in labs.

When sampling for population distribution, many times large amounts of larval fishes are collected and their proper identification is needed to effectively estimate population size (Bolle et al. 1985). However, as described in a paper from B.C. Victor "a major obstacle to resolving some of the questions concerning the ecology of reef-fish larvae in tropical waters has been the difficulty of identifying these larvae" (Victor 1987). J.M. Miller and Leis stated "…tropical marine fish larvae, particularly reef species, is poorly understood" (Miller and Leis 1976). Araujo-Lima and Oliveira wrote "however, eggs and larvae have been poorly studied in tropical rivers and there are few published accounts of this…" (Araujo-Lima and Oliveira 1998).

Quite clearly, there is a large knowledge gap in the proper means of identifying larval fishes, be it in freshwater or marine systems. Although some taxonomic guides and keys do exist (e.g., Neira et al., 1998; Leis and Carson-Ewart, 2000) the images they depict are hand drawn, lacking fine detail and color, which typically are the defining features when labeling larval fishes. The construction of a photographic guide is needed to allow for proper identification of these fishes. Neira 1998) and (Leis and Carson-Ewart 2000).

Species of minnow found within the state of Texas are very similar in body shape and form, making it extremely difficult to decipher between them, especially earlier developmental stages. Ferguson (1981: 89) stated that "species identification of immature minnows (family Cyprinidae) is often difficult, since distinguishing features are based upon adult characteristics and differences in breeding features between mature males". An effective guide to decipher between species is necessary if they are to be effectively conserved and managed.

The Bullhead Minnow (*Pimephales vigilax*) is a minnow in the family Cypirinidae commonly found throughout Texas. In its larval stages, it can easily be misidentified as either the Red Shiner (*Cyprinella lutrensis*) or the Blacktail Shiner (*Cyprinella venusta*). This species will be the one photographed and described in this paper. This key will allow for the proper identification of *P. vigilax* and with others in the future, proper identification of all Texan minnow species.

CHAPTER II

METHODS

Fish Preparation and Photography

Egg clutches of the Bullhead minnow (*Pimephales vigilax*) were collected in the Navasota River shortly after the spawning season had commenced (late April, 2012). Egg masses were brought back to the lab of Dr. Kevin W. Conway (Department of Wildlife and Fisheries Sciences, Texas A&M) and maintained in aquaria. Upon hatching larvae were collected every daily basis for the first 20 days and then every third day from 21-60 days post hatch. Upon collection larvae were euthanized with an overdose of MS222, placed in a 10% formalin solution for one day and then transferred to 70% ethanol for final storage.

Photography of larval specimens was conducted using the Carl Zeiss Microscopy AxioCam MRc operated using the imaging software AxioVision in conjunction with a high resolution photographic camera mounted on a Zeiss SteReo Discovery V20 microscope.

Prior to photography, specimens were sorted into groups based on their development and size. Once categorized, specimens from each sample group were chosen to be photographed. Criteria for selection included straight orientation in the dorso-ventral plane and complete, in-tact fins. A petri dish with a layer of clear gel into which small entomology mounting needle had been inserted was used to hold specimens steady during photography.

The first image taken is always the dorsal, as the pinning of the fish in this setting does not create visual markings on the fish's exterior than can be seen when the lateral image is taken. Using fine forceps, the fish is pinned lower than the widest point on the fish's body to prevent the image from showing where the pin's entry into the fish is located. The fish must be pinned in a manner that allows it to sit perfectly straight in solution, allowing for equal views of the right and left side of the specimen to be seen.

Once pinned, the petri dish is then centered in the microscopes field of view. The zoom is adjusted to allow for only the fish to be in the image, creating as little empty space as possible. Top maximize this, the fish is setup on a diagonal to allow for the zoom to be the closest and still have the full length of the fish seen in the image. The goosenecks are then adjusted to create a lighting regime that illuminates the fish evenly without casting shadows or creating glares. A combination of adjusting light levels, moving the goosenecks, and using the adjust function is needed to create the optimal light setting.

Once the fish is ready to be photographed, the software is needed to take the image. The software works in a manner that uses multiple images that are in focus at different depths to compile a fully in-focus image of the fish. To create this, the fine adjustment needs to be adjust so that the lowest part visible on the fish is barely in focus. This creates the lower end of the image stack. This depth is then recorded by clicking the lower end limit on the image tab of the software. This is then repeated with the highest point on the fish. The software will generate what it deems is the optimal amount of images to be taken, however this value is usually too high and will take

too long to compile. Typically 12 images are enough to create a highly in-focus image. Once 12 has been replaced for the value in the slices field, the image is ready to be taken.

To take an image, the down arrow is clicked in the image tab to move the camera to the lowest image to be taken. It is important to always take photos from the lowest point to the highest point to allow the small motors to go against gravity. This creates a more stable movement and will prevent the machine and image from shaking. Once lowered, click start and the camera will begin by taking its multiple images. It is also important to remain very still during this time as even the smallest air currents can move the pinned specimen.

Once complete, the images will be displayed playing back and forth. Click the edit button and remove the first image from the slide as this one usually is not in focus from the initial machine movement. Once removed, the remaining 11 images can be compiled. The compiling process will take multiple minutes but upon completion, it will produce a highly in-depth in-focus image of the specimen. A measurement of the standard length of the sample is taken using the measure tool. This process is then repeated with this fish pinned in the dorsal area to produce and image of the lateral side of the fish.

All of these steps are repeated for each individual specimen desired for photography.

Photo Editing and Observation

After saving, photos are saved and exported to Adobe Photoshop C6, where they are processed for editing. First, the canvas of the image is expanded to allow for the image to be full size when

rotated. Next, the image is rotated using the free transform tool to make the image sit horizontal in the field of view.

To allow for the features of the fish to be more viewable, the backgrounds of each image were blacked out using the pen tool. A large tip size using the pencil tool was used to blackout out the large areas of the background, while a small paintbrush tool was used for areas in close proximity to the fish.

Upon completion of all the blacking off the backgrounds, the images were enhanced using specific adjustment tools. The levels of white balance were corrected in a manner which produced a result in which the fins remained transparent but the colors were brightened.

The contrast, sharpness, and color levels were auto adjusted using the features in the tools drop down menu. Once adjustments were completed, the image resolution was doubled and saved as a jpeg.

To complete the process, each image was observed and differences were recorded in the development of each specimen.

CHAPTER III

FINDINGS

The following images (Figures 1.-12.) illustrate the larvae of *Pimephales vigilax* from 4.4-19.5 mm SL in both dorsal and lateral view. In addition to standard length, fin ray counts, and myomere counts, other key morphological and physical features are noted and recorded to aid the identification of this species.

Figure 1. - 4.4 mm SL**:** Yolk sac present with large spherical shape towards front, elongated towards anus; LFF present; predominantly devoid of pigment, very few MELO present on yolk sac and LL; highly reduced PCF, no other fins present; swimmbladder singular; ~34 MYO present.

Figure 2. - 5.0 mm SL: Yolk sac still present, greatly reduced, lacks large spherical bulge near front; LFF still present; more MELO present, found on head, yolk sac, and near CF; still very reduced PCF with no other fins present; pigment found on swimbladder which is still singular; dark pigmentation along ventral line from anus to CF starting to form; ~34 MYO present.

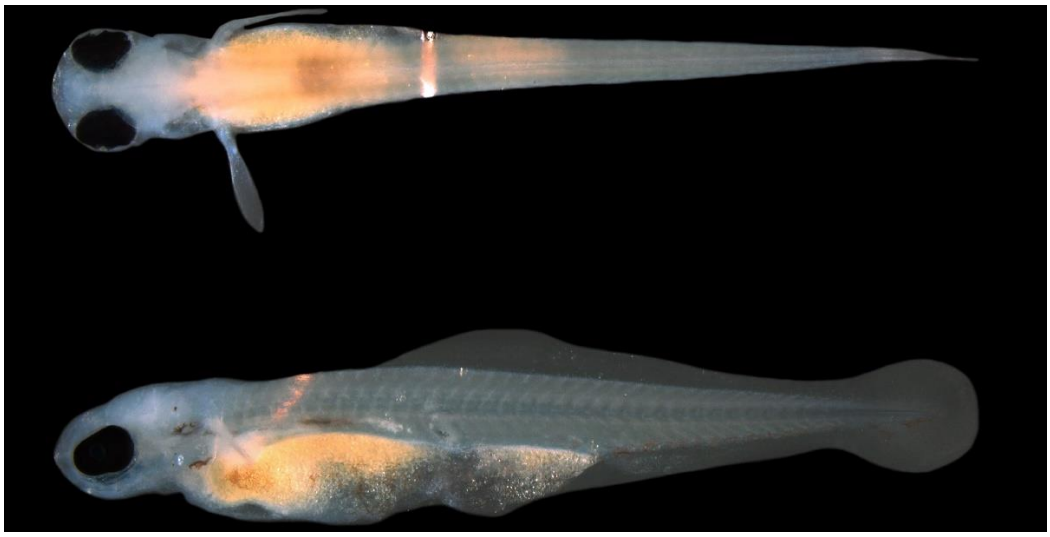

Figure 3. - 5.4 mm SL: Yolk sac still present, yolk more reduced; LFF still present; more separation of swimmbladder; PCF more developed; ~34 MYO present.

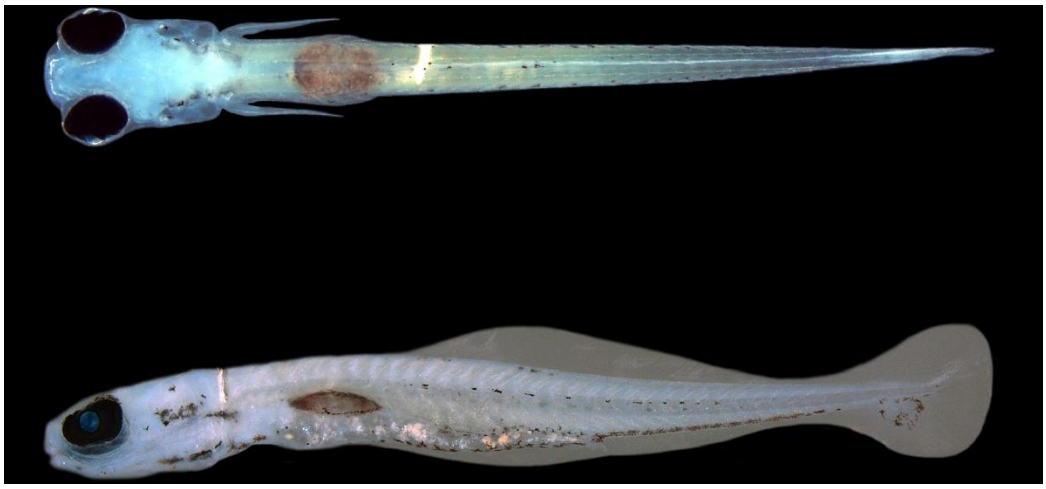

Figure 4. - 5.7 mm SL: Yolk sac absent; LFF still present, greatly reduced; increased MELO along lateral and ventral lines; PCF still only developed fins; swimmbladder has much pigment and is beginning to elongate; ~34 MYO present.

Figure 5. - 6.3 mm SL: pre and PAFF are starting to separate; swimbladder has formed two bulges and is starting to split; MELO present on top of cranium; more pigmentation along LL and ventral line; eyes have developed reflective pigment; fork in CF beginning to develop; very early stages of hypural plate formation; otoliths visibly present in skull; ~ 34 MYO present.

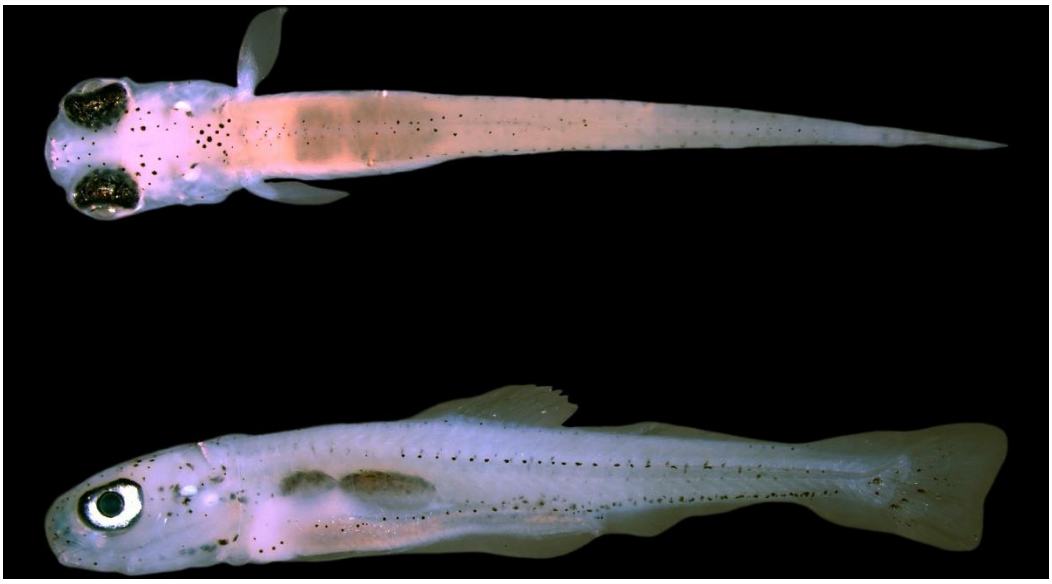

Figure 6. - 7.2 mm SL: LFF very reduced; dorsal fin present with 1 UFR and 5 BFR; swimmbladder close to being completely split; eyes have developed fully reflective layer around pupil; hypural plate still forming; fin rays visible in CF; flexion has occurred; otoliths still visibly present; spot developing on CF; ~34 MYO present.

Figure 7. - 8.1 mm SL: PAFF still present, others reduced; DF, 2 UFR, 7 BFR; swimbladder completely split; hypural plate has developed; 1 UFR, 8 BFR branched on top/bottom of CF; otoliths visible; AF, 1 UFR, 6 BFR branched; spot on CF developing; ~34 MYO present.

Figure 8. - 8.6 mm SL: LFF absent, PAFF still present, very reduced; DF completely developed, 2 UFR, 7 BFR; CF completely developed, 1 UFR, 8 BFR on top/bottom with fork clearly visible; otoliths still visible; AF 1 UFR, 6 BFR; spot on CF clearly visible; guanine developing on visceral cavity; ~34 MYO present.

Figure 9. - 11.2 mm SL: PVF has developed from PAFF, 2 UFR, 6 BFR; AF developed with 2 UFR, 6 BFR; heavy pig. on dorsal line/lateral side below LL; increased guanine on visceral cavity; scales developing from CF forward; otoliths still visible; ~34 MYO present.

Figure 10. - 12.7 mm SL: All fins fully developed; dark stripe along LL; scales still developing from CF forward, scales present up to DF; otoliths no longer visible; dark cleithral streak developed on side of body, posterior to opercular opening; dark area around base of DF; ~34 MYO present.

Figure 11. - 14.20 mm SL; More pigment around base of DF; cleithral streak is darker and longer; large amount of guanine coating visceral cavity; scales present on full body; ~34 MYO.

Figure 12. - 19.5 mm SL: cleithral streak extends up to top of cranium; guanine present on visceral cavity as well as outer scales of mid body and operculum; reticulate pattern present over side of body, formed by pigmentation lining posterior edge of scales; ~34 MYO present.

CHAPTER IV

CONCLUSION

As displayed in the previous images, clear morphological and physical features can be easily seen developing throughout ontogeny, from the early larval stages to the juvenile stage. The yolk sac in no longer present at ~5.5 mm. The larval fin fold is lost at ~8.5 mm with the Pre-anal fin fold lost at ~10 mm. All fins are completely developed by ~12.5 mm. Scales are present at ~11mm and develop from the caudal fin forward. Reticulated pattern becomes present at ~16mm. The individual becomes fully developed by ~20mm.

By logging key developmental features and the lengths at which they occur, identification of wild caught minnow species will become far easier. It is in hope that the information presented in the atlas can be used as a foundation to build similar studies on the larvae of freshwater fishes. With help from other researchers, more minnow species can be added to this atlas effectively allowing one to be able to sample wild larval and juvenile minnow species and accurately identify the individuals within the sample.

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