Development and Deployment of a Small Autonomous Underwater Vehicle with Novel Navigation Methods for the Study of Common Snapping Turtle (*Chelydra serpentina*) Hatchling Behavior

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Introduction

Snapping turtles, *Chelydra serpentina*, have long been thought to be a fairly quiet organism, but more recent studies have proven that these intriguing animals have the ability to vocalize through various chirps, clucks, hisses, and clicks. A study found that the vocalizations of hatchling snapping turtles change as they develop in their eggs.[I] In the period right before egg emergence, the hatchlings made simple 'squeak' sounds, whereas after emergence from the egg the hatchlings produced more complex vocalizations. It is thought that this change of complexity in vocalizations serves a cue to the other young that it is time to hatch. This is important because in the same study it was observed that hatchlings who emerged as a group had higher body masses than a single hatchling emerging on its own. The larger body mass hatchlings begin their life with an advantage for survival. Another study resulted in actual recordings of adult snapping turtle vocalizations, though the meaning of the sounds were unknown.[II] The use of AUV's is imperative to this research in order to record and analyze the vocalizations. A study was conducted involving a pair of conductivity sensors mounted on an AUV to track chemical signals released by lobsters.[III] Chemical signals released by aquatic vertebrates are known to be used in conjunction with many behaviors such as avoiding predation, finding food sources, and locating potential mates. The goal of the AUV was to determine the source of these chemical signals for easier tracking of the animal. The development of a similar AUV could be beneficial by interpreting chemical signals released by snapping turtles. Although the amount of research has been increasing with these vocalizations, very little is known about the vocalizations of snapping turtle hatchlings and the role that vocalization plays in turtle behavior. Further studies on this topic, which include audio recordings and autonomous underwater vehicle (AUV) tracking[IV], can help to better understand the dispersal patterns of turtle hatchlings, the interactions between turtles, and other behavioral aspects that may be affected by vocalizations.

Location:

Chelydra serpentina is Wisconsin's largest and heaviest turtle species. They are able live in most aquatic environments within the state, but prefer ponds, lakes, and the backwaters of rivers as these areas provide vegetation for the turtles to hide among. In choosing our location, we researched DNR website articles to find areas within Kenosha County that would provide C. serpentina with a suitable habitat. Our location, located in the New Munster Wildlife Area approximately 37 minutes away from Carthage's campus, has a multitude of ponds in a variety of sizes. Open wetlands constitute 25% of the New Munster Wildlife Area's total area, providing reed grasses in which the turtles can seek shelter or refuge. The oak and lowland woodlands, shallow marshes, open grassland, and agricultural fields in the area would also provide turtles with a relatively contiguous habitat, and the nearby Palmer and New Munster Creeks flowing into the Fox River would provide ample food. We chose two moderately sized ponds in the area to focus our field research on, Pond A and Pond B.

Pond A is located at 42°34'51.1"N 88·12'42.6"W and has an area and depth of approximately 15192 m^2 by ~1-7ft, making it a good location for turtles to inhabit, as well as allowing enough room for the AUV to operate.

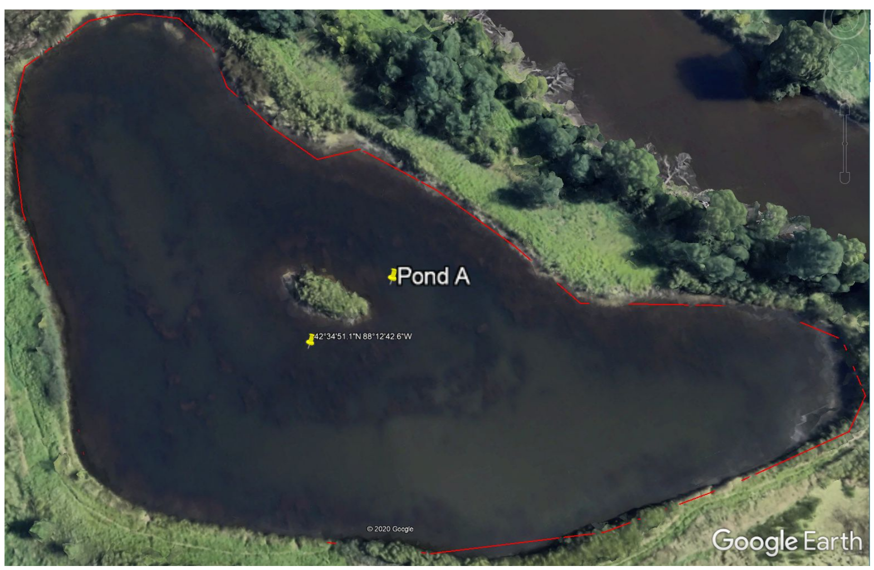

Fig 1. (Satellite image of proposed location 'A')

Pond B is located at 42°34'42.81"N 88°12'30.08"W and has a larger area of 42753 m^2 due to its narrow shape. The depth of this pond is likely similar to Pond A, though it looks slightly shallower in satellite images.

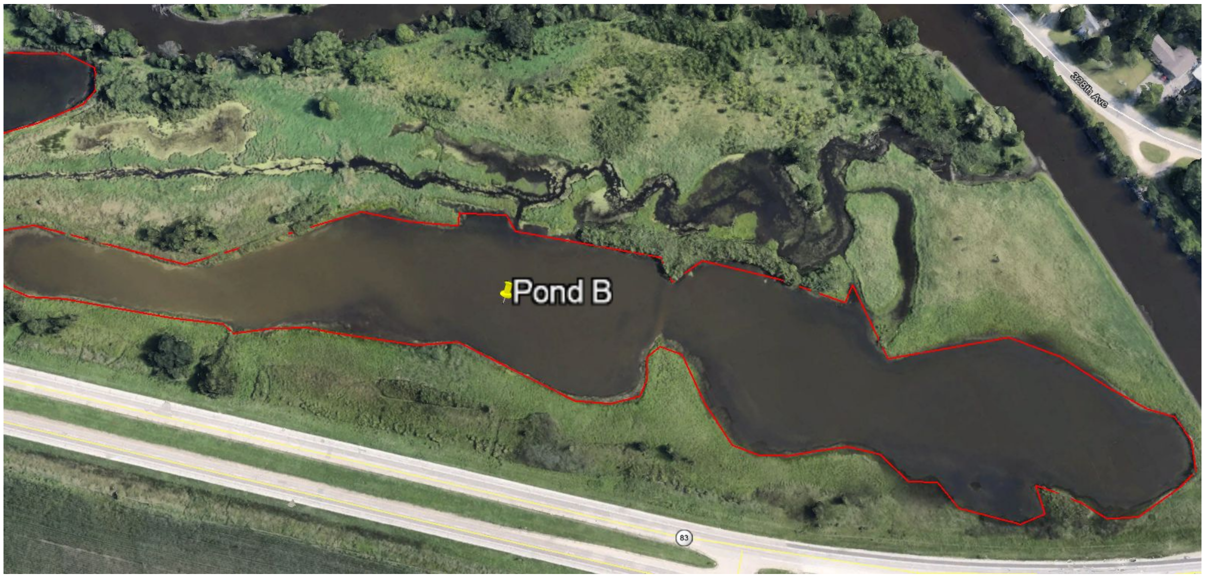

Fig 2. (Satellite image of proposed location 'B')

The New Munster Wildlife Area is also home to many predators of *Chelydra serpentina*, including the largemouth bass, panfish, northern pike, and the great blue heron, which we will need to protect the hatchlings from by using wire mesh fencing. The sites themselves are isolated enough to provide a safe space for research, but close enough to roads or trails that they would not be too difficult to get to. There are also canoe launching sites if shoreline vegetation makes launching the AUV too difficult to do from land. While 12% of New Munster Wildlife Area's cover is forested wetland, the ponds we have selected appear to be relatively open, allowing for easy movement of the AUV within the aquatic environment. During the summer months, the DNR reports these locations to have a high water clarity, making visual navigation of the AUV easier, however, the bottom of ponds are 90% muck which could hinder visibility in the fall.

Specific Aims

Specific Aim 1: Navigate an AUV towards turtle species by detecting chemical secretion gradients in a local body of water. Chemical signals released by C. serpentina or other turtle species may be detectable and navigable with an AUV. The most common compound is likely cholesterol trimethylsilyl ether or a derivative (Ibáñez et al. 2020)[v]. Based on the electrical conductivity, the presence of a signal in the area may be detected. A chemical gradient is key for navigation, otherwise the data will still provide knowledge of chemical signal presence but without a heading.

Aim 1 Hypothesis: The presence of chemical signal components such as cholesterol trimethylsilyl ether - specific to freshwater turtles - can be detected in a gradient via a pair of electrodes to provide heading information in a body of water.

Specific Aim 2: Audio recordings allow for specific detection and understanding of C. serpentina vocalizations. We will take audio recordings in-situ. The audio can be analyzed with a spectrum analysis to isolate Chelonia-like sounds. Isolated sounds can then be compared with known C. serpentina audio for distinct features.

Aim 2 Hypothesis: The recorded audio can be parsed despite background noise to reveal vocalizations specific to C. serpentina outside of captivity.

Specific Aim 3: This is important to test the effectiveness of AUVs for future studies. Some C. serpentina vocalizations are too low for the human ear which is why using effective equipment is important. It is thought that C. serpentina hatchlings produce different sounds before they are hatched. The vocalizations must be recorded post hatching to observe differences in pre hatching vocalizations.

Aim 3 Hypothesis: Pre-hatchling vocalizations will be 20-30 Hz lower in range than post-hatchling vocalizations.

Methods

C. serpentina RFID tagging/AUV involvement:

Materials:

- RFID (PIT) tags, injection tool, tweezers, PIT tag readers, latex/nitrile gloves, and disinfection materials.
    - Approximately 33 PIT tags required based on the median size of a C. serpentina clutch (15 to 50).
    - Since the target of this study is hatchling individuals, small, passive, full-duplex (FDX-B) PIT tags will be used.

        - Biomark MiniHPT8 (Pre-load individual)
        - Mass: 30 mg (±6 mg)
        - Dimensions: 8.2 mm (±0.3 mm) L X 1.4 mm (±0.1 mm) diameter
        - Operating Frequency:134.2 kHz
        - Cost: $0.65 per tag & $43 per MK65 implant gun.

Fig 3. (Comparison of Biomark PIT tags)

    - A 16 gauge (0.165 mm) pre-loaded individual needle and complimentary MK65 implant gun will be used for injection.
    - All Biomark PIT tags are ISO 11784 and 11785 compliant (This specific compliance refers to the international standards that regulate the radio-frequency identification of animals.)
    - Disinfection materials and gloves are required since aseptic technique will be observed for the safety of the animals.
- Mesh wire fence and flags for nesting sites.
- Scale for mass measurement of hatchlings.
- Ruler for length measurements of hatchling carapace.
- Logbook

Design:

Tagging Restrictions:[VI]

- Turtles with recent injuries or that appear ill in any way should not be tagged.
- Turtle Hatchling Size Requirements: Carapace length - 50mm or minimum 7g.
- Both tag size and syringe size should be minimized when implanting small turtles. BioMark and Avid manufacture tags are approximately 8mm long; these are the smallest tags currently available and are injected using a 16 gauge (0.165 mm) needle, and a 15 gauge (0.183 mm) needle, respectively.
- As long as tags are not placed into the body cavity, the majority of veterinarians do not consider PIT tagging to result in increased health risk.

Tagging Procedure:

1. On foot, gravid females may be spotted and tracked back to their nest, noting the date of oviposition. Nest site locations will be recorded, protected with a wire-mesh cage for predators, and physically flagged about 5-6 feet away. Additionally, nest location may be narrowed down, confirmed, or spotted in conjunction with AUV operation limited to the scope of its traversal and sensing.
2. Manual collection of hatchlings at nest site coordinates as soon as they hatch.
3. Measurement of hatchlings to ensure they meet minimum size requirements before PIT tagging.
    a. Researchers have varied in the minimum size of turtle hatchlings that are tagged. Our team will set the minimum at 50mm in carapace length, or 7g.
4. Injection of tag subcutaneously into the hind limb, parallel with the femur.

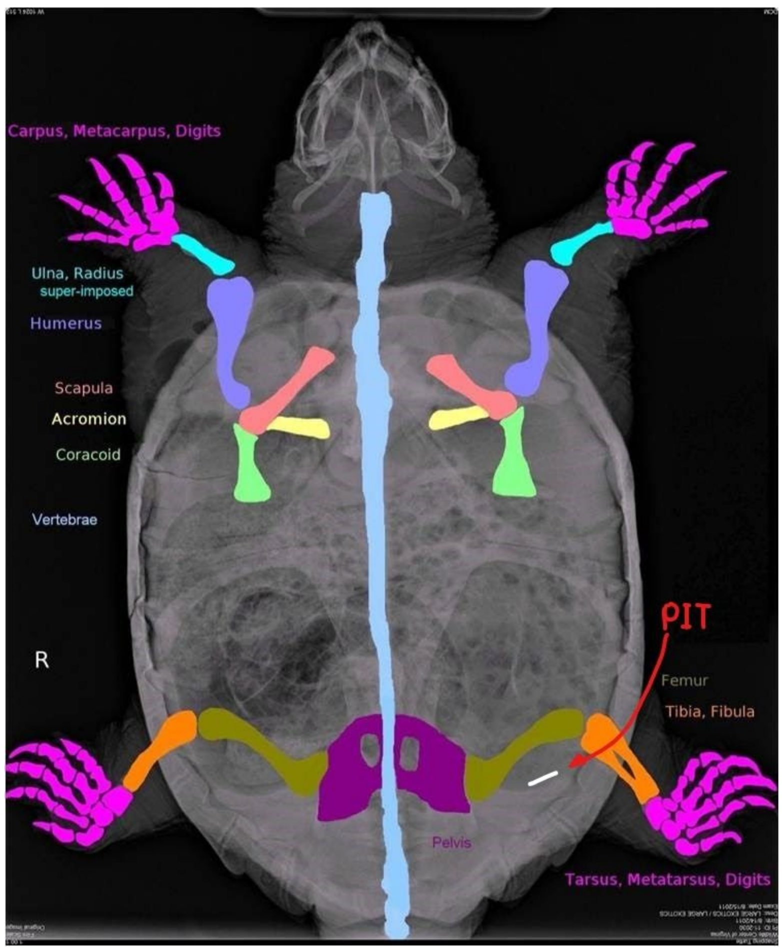

Fig 4. (X-Ray image of C. serpentina with PIT location highlighted)

    a. Dark green colored bones are the femur. The placement will be running parallel to the femur on one of the hind legs.

5. When done correctly, there should be very little or no bleeding after injection. When bleeding does happen, pressure is applied until bleeding has stopped, and that animal is observed for several hours to ensure normal behavior and to make sure it is not injured.
    a. Aseptic technique is observed when handling hatchlings in the field.
6. After injection, the wound should be treated with liquid bandage antiseptic solution. This helps seal the injection site and prevent infection.
7. Following tag injection, hatchling turtles will be directly monitored for a minimum of 20 min and observed up to 24 hours to ensure they are uninjured from the procedure.
8. The animal should be scanned with a PIT reader before being released to ensure the tag is in place and functioning properly.
    a. Proper documentation and logging is observed through the tagging procedure.

Chemical Signals Gradient Detection Methods

Materials:

- AUV equipped with a set of electrodes capable of detecting conductivity levels in surrounding waters.
- Microcontroller and onboard data processing to extrapolate turtle chemical directional and presence information.

Design:

- The most commonly used olfactory-based navigation algorithm is "chemotaxis" , which was introduced by Berg and Brown.[vii] This strategy is based on the detection of a concentration difference between two chemical sensors and a steering mechanism (microcontroller, data processing, and motors in this case) toward the direction of higher concentration with a constant moving speed (Berg & Brown , 1972; Berg, 1993).
- Polarographic electrodes mounted on the AUV comprise the physical design. The majority of the directional determination and quantification of chemical signal concentration is done with software algorithmically. Sensors with more extensive capabilities than conductance, e.g. mass spectrometers, Raman spectrometers, and fluorometers are too bulky and massive for this study.
- After calibration of resistance to known freshwater turtle chemical signals such as trimethylsilyl ether, a gradient can be established between the two electrodes. I.e., electrode 1 appropriate resistance signal > electrode 2 signal == gradient differential. Resistance values will likely be on the order of milliohms (mΩ).

Strategy	*Maintain behavior*	*Reacquisition behavior*	*Parameters*
1. Upwind with wind-relative counterturns	If the searcher has detected odor within the last λ seconds, then the searcher travels straight upwind.	If the searcher has detected odor within the last T_W seconds, but not the last λ seconds, then the searcher travels at an angle $\gamma = \pm 90°$ to upwind. The sign of γ is defined based on the vehicle heading and wind direction to cause the vehicle to reverse its crosswind direction (i.e., counterturn)	$\lambda = 1$, $T_W = 4$, and $\gamma = \pm 90°$
2. Constant crosswind with counterturn	If the searcher is currently sensing odor, it turns toward straight upwind. If the searcher is not currently sensing odor, but has sensed odor within the last λ seconds, then the searcher travels at an angle β with respect to upwind.	If the searcher has detected odor within the last T_W seconds, but not the last λ seconds, then the searcher travels at an angle $\gamma = \pm 90°$ to upwind. The sign of γ is defined as the opposite of the sign of β. This generates counterturning travel and the sign of γ is not sensitive to the measured wind direction or vehicle heading	$\lambda = 1$, $T_W = 4$, $\beta = 35$, and $\gamma = \pm 90°$
3. Progressive crosswind with counterturn	If the searcher has detected odor within the last λ seconds, then it travels at an angle $\beta(t)$ relative to upwind, where t is the time since odor was first detected on the present crosswind traverse of the plume.	If the searcher has detected odor within the last T_W seconds, but not the last λ seconds, then the searcher travels at an angle $\gamma = \pm 90°$ to upwind. The sign of γ is defined as the opposite of the sign of β. This generates counterturning travel and the sign of γ is not sensitive to the measured wind direction or vehicle heading	$\lambda = 1$, $T_W = 4$, $\beta(t)$ described by Eq. 12, and $\gamma = \pm 90°$

Table 1. (Summary of chemotaxis algorithm strategies/parameters explored by Farrell et al. 2005)

Procedure:

1. AUV electronics will record fluctuations of local impedance near sides of AUV to determine a potential chemical gradient.
2. Apply the best algorithm[VIII] (see table 1) to data to extrapolate a heading where the chemical signal plume is likely coming from.
3. Apply heading information to AUV thrust.

Vocal Recordings Methods

Materials:

- AUV
    - Equipped with a set of (at minimum) hydrophones, a camera capable of live and recording, GPS breakout module. Will operate autonomously or via remote control.
        - Cetacean Research Technology SQ26-H1 Portable Underwater Sound Recording System
    - AUV design “Zeno” adapted from J.Gelli et al.[IX]

Design:

- Material = aluminium and carbon fiber

- Dimensions = 1000x800x200mm
- Mass = 35kg
- Operable depth = 100m
- Max longitudinal speed = 4 kn
- Working longitudinal speed = 2 kn
- Battery:
    - Battery life = slightly over 1 hour @~300W
    - Battery module = 4-cell battery, Tattu 22000mAh 14.8V 25C 4S1P Lipo Battery Pack with NA plug
    - Charge time = 1 hour
    - About 40 W for the vital pc, 250 W for the propulsion, 30 W for the communication, emergency devices, navigation, etc.; according to this, about 300 W or more have to be considered, i.e. an energy of 300 Wh per each hour of mission
    - A single module of 325.6Wh is suitable for a mission of about 1 hour
    - Dimensions are 165x90x42mm
    - Has the ability to move on slide guides to easily insert and remove the battery module
- Dimensions = 1000x800x200mm (+225 of antenna)
    - The compact size is advantageous for small lakes/ponds, which is the desired area of study for this research.
    - Concept renderings of design:

 

- Navigation/communication sensors = MEMS IMU, depth sensor, echo sounder, GPS, radio modem, Wifi
    - Optional Doppler Velocity Log
    - Optional single-axis Fiber Optic Gyroscope
- Payload = frontal camera, vertical camera, illuminator LEDs, 2d Forward Looking Sonar, Side Scan Sonar

- Propulsion system = consists of 8 actuators, T200 Blue Robotics (4 lateral thrusters + 4 vertical thrusters)
    - Advantages of having 8 thrusters:
        - Controls roll, pitch, and yaw
        - Guarantees hovering capability
        - Reduces axial dimension of the vehicle
- Limitations:
    - Various factors will influence the chemical signals, such as the time the chemical was released, the amount of chemicals released, and dispersal of chemicals in the aquatic environment. Based on these variables, the AUV may have difficulty determining the source of the chemicals.
    - Battery life is not tremendously long, which will result in multiple battery changes that need to be done during research time blocks. Having multiple batteries, though, will help with efficiently replacing batteries so as to not waste time that can be used for collecting. Also, the similar battery life and charge time is beneficial so that there is no waiting time between interchanging batteries, even if we only have two that are being swapped with one another.
    - The original design of the AUV is white, which may affect turtles' response behaviors when it is nearby. This can influence the data collected, including the vocalizations and chemical signals. In order to avoid this, we can construct the AUV with a darker exterior color, similar to that of the pond in question, so as to blend in as seamlessly as possible.

Procedure:

1. Position hydrophone ~0.5m from bottom of pond
2. Hydrophone sensitivity set to 5Hz-200Hz ± 3 dB for hatchlings and adults
3. Recordings will be taken from 8 am to 12 pm and 4 pm to 8 pm each day and throughout the year. Recording months will go from April through October as this is the mating and nesting season for C. serpentina. Hatchlings will begin to emerge in August.
    a. Recordings can later be parsed/post-processed with their respective spectrograms and cross-reference of known control clips of C. serpentina vocalizations.
    b. Data analysis may reveal correlations between vocalizations and movement/behavior.[x]
    c. If a previously tagged hatchling is not retrieved for up to 3 weeks, the hatchling will be removed from the study, and this will be recorded in a log file. The data that was recorded from that hatchling will be kept until the end of collection from the associated year.

4. While recording, we will monitor the audio in real time to account for any outside disturbances or airborne sounds and adjust the signal to noise ratio to prevent audio distortions and isolate the turtle vocalizations.
    a. While recording, we will turn off the motors in order to ensure there is no interference within the frequency range of hatchlings. The expected frequency range of hatchling snapping turtles is 30-100Hz.
    b. If we want to continue using the AUV while recording vocalizations, we will reduce or increase the speed at which the blades of the thrusters are spinning until it reaches a frequency that is outside of the range of hatchling turtles (frequency can be found by multiplying the number of blades on the thruster by the speed at which they are moving).

Endnotes:

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