UNIVERSITY OF WATERLOO

Monitoring mudpuppy behaviours in an artificial den context

Observations of mudpuppy denning activities and other behaviours

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THESIS PART I

Preface

This document is a part one of a two-part thesis series. Its focus is on the denning behaviour of mudpuppies, *Necturus maculosus*, in a lab setting and using artificial dens. Appendix 1 contains supplementary research on the rate and process of healing an injured mudpuppy, *N. maculosus*, in a lab setting.

For information on the potential to expand the range of the endangered mudpuppy mussel, *Simpsonaias ambigua*, by using artificial dens in Kemptville Creek please see thesis part two.

Abstract

Four juvenile mudpuppies were observed from April to June 2011 in the Wet Lab of the University of Waterloo. Their denning habits and general behaviours were recorded and notes from observations on mudpuppy healing process are also included. The primary purpose for is to examine the potential for conserving the mudpuppy mussel, *Simpsonaias ambigua*, by better understanding its mudpuppy host. The specimens studied did not show any signs of den territoriality or aggression, and were often highly mobile when more than one den option was available. Artificial dens may not be a suitable option for developing a monitoring program as the species may tend to change locations on a daily basis.

Acknowledgements

Discussions with and resources from David Lawrie (Toronto and Region Conservation Authority) and Bob Johnson (Toronto Zoo) were influential in the early stages of thesis development. I would like to thank Dr. Todd Morris, Research Scientist at Fisheries and Oceans Canada, for his continued support and his contribution of resources during the early stages of developing this thesis. Dr. Susan Sykes in the Office of Research Ethics at the University of Waterloo has been very helpful in recommending on-campus resources and in assisting me to complete all necessary ethics materials quickly and successfully. Scott Smithers and Art Timmerman, Ministry of Natural Resources, were also extremely instrumental in acquiring the necessary permits and helping to complete ethics requirements. Ryan Martin, a Technician at the University of Waterloo Wet Lab, has also been instrumental in securing lab space and resources. Perhaps most instrumental of my field contacts were Dr. Frederick Schueler and Ms. Aleta Karstad of the Bishops Mills Natural History Centre near Oxford Mills in South Eastern Ontario. I thank them for their time, resources, and for capturing and transporting the mudpuppies from Kemptville Creek to Toronto. Finally, I am grateful for my advisor, Professor Stephen Murphy, for actively committing to my thesis and related processes throughout the Winter and Spring 2011 terms.

Contents

List of Tables and Figures

Introduction

The mudpuppy, *Necturus maculosus*, is believed to be fairly widespread in Ontario and is not of concern due to apparently stable and even increasing populations (Hammerson, 2004; SARA, 2010; COSWEIC, 2010). Even so, little is known about the species' territory, the extent of its mobility or its interaction with its parasite, the mudpuppy mussel, *Simpsonaias ambigua* (Morris, 2011: Pers. Comm.). One issue that prevents us from better understanding the host is they are highly mobile and we have yet to identify signs of its den or shelter (Schueler and Karstad, 2011: Pers. Comm.). Because we don't know where the shelters are, it is nearly impossible to monitor *N. maculosus* populations or activities at any life stage.

Necturus maculosus is a host to the only mussel we have which uses a non-fish host. The mudpuppy mussel, *Simpsonaias ambigua*, is listed in Canada as endangered under both the *Committee on the Status of Endangered Wildlife in Canada* (COSEWIC) and the *Species at Risk Act* (SARA). The only known population exists along a 50-km stretch of the East Sydenham River in South-Western Ontario (Morris and Burridge, 2006: 23). The purpose of this study is to assess whether we can increase the distribution potential of the mudpuppy mussel, *Simpsonaias ambigua*, via the range of the host mudpuppy, *Necturus maculosus*. The primary objective is to find a more effective way to monitor *N. maculosus* by creating artificial dens that can be tagged and observed in the field; observations in the lab will note whether wild-caught *N. maculosus* actively use and accept the artificial dens. Secondary objectives include observing individual behaviours and group interaction.

One of the few things we know about the *Necturus-Simpsonaias* relationship is that *S. ambigua* uses *N. maculosus* as a host to carry its young, called glochidia (DFO, 2008). The glochidia are carried around on the *N. maculosus'* gills (see similar example in Figure 1) until they mature, at which point they fall off (see similar example in Figure 2) and attach themselves to surfaces at that location. Because *N. maculosus* spend most of their time in a den – either for breeding, hunting, or temporary shelter purposes, the glochidia are usually found in these areas. One problem with monitoring *S. ambigua* is that locating the dens of its host is difficult (Morris, 20011; Schueler and Karstad, 2011: Pers Comm.). Thus, if *N. maculosus* accept artificial dens created by researchers and drop *Simpsonaias ambigua* glochidia into these areas, both species – *N. maculosus* and *S. ambigua* – can then be monitored by conservation authorities.

Figure 1: Glochidia of the pearly mussel, *Lampsilis higginsii*, on preserved fish gills (Bernhart, 2006).

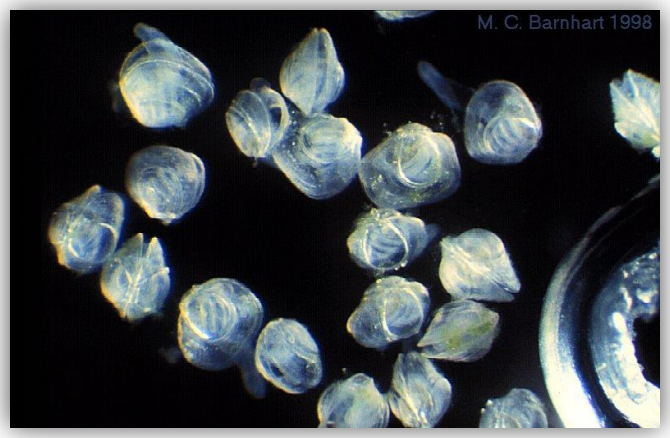

Figure 2: Early juvenile pearly mussels, *Lampsilis higginsii*, recently dropped from host. Size comparison: object at lower right corner is a standard office staple (Bernhart, 2006).

Literature reviewed

There is little peer-reviewed research on the mudpuppy, *N. maculosus*, and virtually no published work on its denning behaviour – especially regarding artificial dens. Much of the background research was compiled from personal communication with those in the field as well as documents provided by those directly involved in the species' conservation. Furthermore, the relationship *N. maculosus* has with *S. ambigua* in general is little-understood despite being vital to the life cycle of the endangered mussel. A study by McDaniel et al (2009) was able to confirm that at least part of each species' territories overlapped for the duration of the study. The researchers also noted a high females-to-males ratio at most sites of the Sydenham where *Simpsonaias* can also be found, with one region believed to be an expanse of nesting grounds. Whether their territories overlap completely, and for all or part of each species' life cycle, remain to be studied.

According to observations by Dr. Schueler and Ms. Karstad of the Bishop Mills Natural History Centre, wild dens are not uniformly shaped inside and the roof is approximately 2-3cm from the riverbed. The majority of *Necturus* located in the Creek are found under large, boulder-like rocks which can be nearly a meter across and several inches deep. I have not found any other information disputing these observations, nor is there much confirming information regarding specific details of the dens. Descriptions from notable sources such as the Department of Fisheries and Oceans Canada, National Geographic, and various others only state that they are often found – sometimes denning – beneath 'large rocks' in various sand, mud, and gravel substrates.

Defining terms

As discussed with Dr. Morris, artificial dens can be defined as a shelter put together by humans but which is meant to imitate natural conditions as closely as possible. Since the glochidia could potentially be released anywhere the host spends a lot of time, temporary shelters include any commonly shelters that are not used for breeding or rearing purposes. The

parasite-host relationship is temporary or specific to one part of *S. ambigua'* life cycle, and is not believed to harm the host (Lembcke, 2005).

Methodology

Four wild-caught *N. maculosus* were caught from Kemptville Creek about an hour from Ottawa in South-Eastern Ontario. This site was chosen for a number of reasons. It is one of the best-monitored sites for *N. maculosus* populations in Ontario, has a healthy mussel population, and is fairly clean as far as water systems in urban and agricultural areas go. The geological gradient of Kemptville Creek is similar to that of the East Sydenham, so perhaps the habitat and specimens of this region would be more representative of their counterparts in the East Sydenham. The sample is defined by availability of wild individuals, although juvenile to adult females are preferred as they would be the best candidates for a *Necturus-Simpsonaias* monitoring program. Long-handled dip nets were used to capture specimens, and a large bin with ice and water from the Creek was used for transporting them.

Necturus were housed at the University of Waterloo Wet Lab, which has lights on dimmers and is always connected to fresh, untreated well water. There are four 180 gallon holding tanks (Figure 3) on a 12-hour light cycle from 0700-1900. The bases of the tanks are about 42 inches^2 and the heights are around 26 inches. They are each hooked up to a hose and tap, an airline with air stone and a drain. Water is constantly circulating and 100% of the water is changed approximately every hour. The tanks sit about a foot and a half off the ground on cinder blocks.

Figure 3: Tanks 1-4 in the Wet Lab.

Necturus were fed feeder minnows and earthworms purchased from a trusted pet store and bait shop, respectively. The dens were created using natural slate and river rocks, selected to mimic known den sites and natural habitats more closely but also to allow for proper maintenance. Perhaps one of the most important limitations to consider is that *Necturus* populations, although widespread, are often sparse and not well known. This limits the availability of wild specimens, and thus decreases the sample size. The only regularly monitored population is the winter-active mass found in Kemptville Creek.

Four different den designs were tested during individual studies. The following table describes the dens of each tank, according to tank number:

Table 1: Den designs per tank (according to tank #).

#	Den design	Other item
1	**Low open** den, current hitting **entrance** and going into/through den	Large cinder block
2	**Low sheltered** den, current hitting **rear** and going around den	Large cinder block
3	**High open** den, current hitting **entrance** and going into/through den	Flat yellow brick
4	**High sheltered** den, current hitting **rear** and going around den	Flat yellow brick

And the following are examples of a low open (Figure 4) and a high closed (Figure 5) den:

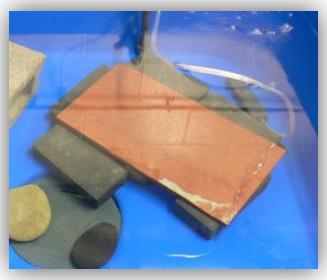

Figure 4: Low open den; black bricks are flat in tank and water current flows into den.

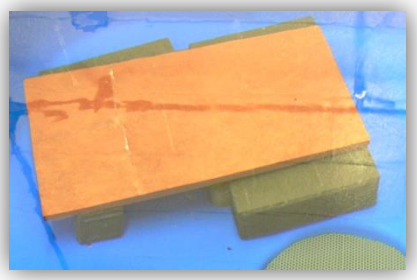

Figure 5: High closed den; black bricks are on their side and water current flows around den.

Three dens and an additional shelter were tested during the group study (Figure 6). The first den, Den 1, was open to the current which flowed right through it. Den 2 was more secure with a short wall at the opening, and two sides – 2A facing the current, and 2B facing away from the current, sheltered. Den 3A and B were both sheltered from the current and had only a short wall in the middle which the *Necturus* were able to move around. The greenery, a plastic aquarium plant with mesh/grid base for planting in gravel, served as an additional shelter.

Figure 6: Community tank with views of closed (left) and open (right) dens.

Results

Each *N. maculosus* was in a tank for 10 days before being rotated onto the next tank, until each specimen had been in every tank. At this point all the specimens were combined into one community tank for the group study, which lasted 21 days. The lab was checked one to two times daily, with most observations occurring during the once-a-day routine maintenance check. The time at which the tanks were checked was unscheduled and so it varied each day. One important note is that one *N. maculosus* arrived at the lab with an injury that likely occurred during transport. A record of the healing process for this specimen can be found in Appendix 1.

Individual observations

Individuals were almost always in or under their dens when checked, regardless of which tank they were in or at what time. The time at which they were least likely to be in the den was after 4pm and especially after 8pm when it was dark both in and out of the lab. In tall dens the specimens went into the den for shelter, whereas in short dens the specimens used both the den interior as well as the gap between the flat brick at the front entrance and the drain or bottom of the tank. Even within the dens, the specimens in general – whether living in tall or short dens – would often seek shelter between, under, or flat against the bricks, and sometimes even between the flat den roof and the brick walls.

Though *Necturus maculosus* are known to be nocturnal, specimens accepted food which was dropped in at nearly any time of day. However, whether this behaviour is completely natural or not is unclear since specimens were usually disturbed during routine checks before or during feeding, and so their normal activity routine would have been disturbed to begin with. Also, less mobile food (i.e. earthworms) was often dropped near den entrances so the specimens did not need to emerge from their dark shelter in order to eat. Most hunting routines required the specimen to sit as still as possible, usually sheltered or at least camouflaged with rocks, then slowly crawling towards the prey once it was near enough to assess and grab. Attack was sudden and nearly always effective.

Specimens readily used all den designs, often posturing themselves so they would rest in the curve of each den, usually touching the den walls, despite having more than ample room to rest outstretched. Some were even found loosely curled in the darkest and most sheltered corner. When moved from one tank to another the specimens were almost always disoriented, swimming first to the place where the old den was then swimming somewhat rigorously around the tank before quickly slipping into the new den. Interestingly, when specimens swam around the tank, they nearly always swam along with the current.

Community observations

All specimens were combined into a single tank for the last 21 days of the study, with about 4tbsp aquarium salt added to the tank every few days. While there were some dens – 2R (right side of Den 2) and the greenery – were almost always occupied, the specimens moved freely from den to den and did not make any attempts to ward of other individuals or to establish themselves in one location. In fact, specimens could be seen 2-3 on top of each other

most of the time, especially in the greenery. Feedings did not appear competitive; *N. maculosus* simply attacked whatever food was near to their mouths and did not bother to move off to the next meal.

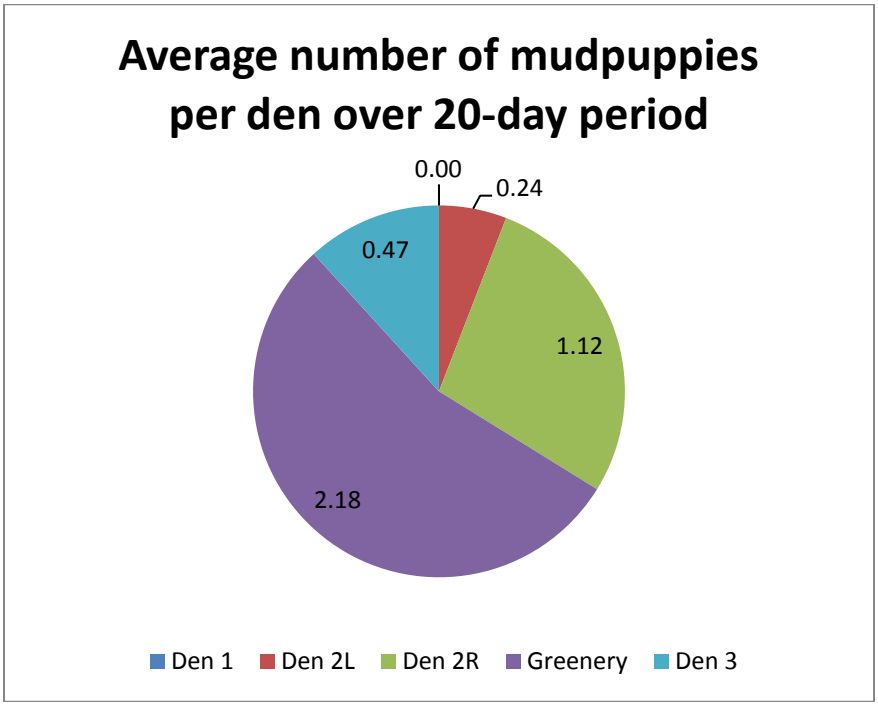

Figure 7: Average number of mudpuppies in each den over 20 days in the community tank. For full dataset, see Appendix 2 – Den use over 20 days in community tank.

Discussion

An important factor to consider with the observed behaviour is that all four *N. maculosus* were similarly-aged juveniles, and so behavioural results may not be representative of the whole population. This might be an area to consider furthering the research: assessing any differences in behaviour between the observed lab specimens and their wild counterparts – of any age – in the wild. When comparing the types of dens used, results would be similar whether we looked at data from the individual or from the community observations (Figure 7 and Appendix 2). Community Den 2 – both the left and right sides – was the most similar to the dens provided in individual studies and was the second most used den in the tank, after the greenery. Whether this style of den is preferred or if it was simply most familiar is unknown.

Individual observations

As specimens were often found pressed up against surfaces or in the tightest spaces – especially between the flat brick and the drain – there may be a preference for thick, broad surfaces with tight spaces underneath as opposed to roomier dens where they have room to move around. This is supported by observations by Dr. Schueler and Ms. Karstad, who monitor *N. maculosus* populations regularly throughout the winter. Most specimens found at

Kemptville Creek are located under large, nearly immovable rocks in spaces which are no more than a few centimetres all around.

N. maculosus does not appear to be territorial, aside from the breeding season when females are known to establish nests and safeguard their eggs (Lembcke, 2005). One *N. maculosus* was even observed to grab a minnow from within the den, and so they likely do not discriminate between eating and resting areas. In addition, many of the observations can confirm that *N. maculosus* will use a space they rest in for hunting and even consuming food that was caught elsewhere, as happened quite often with all four specimens. This is especially true when prey is too large to eat all at once; specimens were often observed to eat some of the prey in the area it was caught, and then once it was secure they would often move into their dens and slowly consume the meal over a course what was seen to be up to an hour.

Community observations

In the wild each individual would spend much of its time searching for food and would be unlikely to pass up an opportunity. However, specimens in the lab are fed well and a few times per week – enough such that they no longer even wish to even swim around. In essence, they get lazy – or conservative, depending on how you look at it – although from my observations they are not nearly as lethargic as their distant relative the axolotl (*Ambystoma mexicanum*). As such, competition between individuals during the community study was non-existent.

No territoriality or aggression of any kind was observed throughout the study. Communal living is likely a survival mechanism inherent to younger specimens of similar age and similar behaviour can be found in many species, aquatic and terrestrial. Some reasons for this may be that hunting and defence against predators are often more successful in groups than alone. However, this being said, the lack of territoriality could signify that artificial dens of any kind – no matter how closely they mimic real sites – may not be a successful monitoring technique as the species appears to be fairly mobile. In fact, if the results of this study are found to be representative of the whole population, even natural den sites which are known to house *N. maculosus* for long periods of time may become vacant at any time. This would complicate any monitoring program attempted in this context.

Conclusion

The four juvenile *Necturus maculosus* provided consistent results throughout the study with regards to denning and related behaviours. When provided with one den option each specimen spent most of their time in the same relative area of the den, however when multiple dens were available in the community tank the specimens changed locations and dens almost daily. No territories were established in the community nor were there any signs of aggression. In the case of using artificial dens for developing *N. maculosus* and *S. ambigua* – mudpuppy mussel – monitoring programs, the lack of strict territory and apparently high mobility of the species may induce poor results.

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APPENDICES

APPENDIX 1 – Healing record of *Necturus maculosus* in a lab

BACKGROUND

Elaine was notified of the capture of four juvenile mudpuppies, *Necturus maculosus* on 7 March 2011. No food was offered between the capture and the delivery to the lab. The mudpuppies were received by Elaine at the University of Waterloo wet lab at around 15:00 on Thursday 14 April 2011, and each mudpuppy was assigned a letter to make record-keeping more effective. Details about the intended research can be found in the main thesis paper (Thesis part II). Mudpuppies were held in their own 180-gallon tanks with a constant flow and drainage of well water. Water temperature was at 12°C and lights were on dimmers and timers on a 12-hour cycle. The exchange rate of water is estimated at 5-6 changes per hour depending on tank and water flow (estimate provided by lab Technician based on a flow of ⅓ to ½ of full flow capacity).

DESCRIPTION OF MUDPUPPY A UPON ARRIVAL

When mudpuppy A arrived, and for the first few days, it was thin and could not swim well. It was roughly 9.5” long from tip to tip, 1” wide and 0.75” high at the middle. The mudpuppy was injured on arrival; the skin looked almost normal except for a few inconspicuous red patches near the wound, which is why the collectors may not have noticed a wound during their keep. Periodic salt baths were provided to keep the area clean and to help the healing process. Hagen Nutrafin Freshwater Aquarium Salt was used to provide salt baths, which were 10-15 minutes each. Salt baths were not done daily to reduce stress from handling. About 2L of water was used in each bath. When and how the injury occurred is unknown, although speculations include either an abrasion on the air stone before travelling (speculation of the collectors) or an injury – likely a bite mark – from another mudpuppy during transportation to the lab.

SALT BATH RECORDS

Salt baths used water from the mudpuppy’s tank with aquarium salt added to it. The following table outlines the salt bath schedule over the three weeks of treatment:

Date (2011)	Tank	Time of bath	Strength of bath	Length of bath
Tuesday 19 April	1	15:20h	1 tbsp/L	15 minutes
Wednesday 20 April	1	16:50h	1 tbsp/L	15 minutes
Thursday 21 April	1	14:20h	1 tbsp/L	10 minutes
Tuesday 26 April*	1 to 4*	20:45h	0.75 tbsp/L	12 minutes
Wednesday 27 April	4	11:30h	0.75 tbsp/L	10 minutes
Thursday 28 April	4	14:30h	0.75 tbsp/L	10 minutes
Tuesday 3 May	4	10:10h	0.5 tbsp/L	10 minutes

Wednesday 4 May	4	09:30h	0.5 tbsp/L	10 minutes
Thursday 5 May	4 to 3*	14:15h	0.5 tbsp/L	10 minutes

* All mudpuppies rotated; Mudpuppy A transferred from one tank to another after salt bath.

INJURY DESCRIPTIONS AND OBSERVATIONS DURING SALT BATH PERIOD

Upon arrival, the injury was roughly 1" long and 0.25" at its widest, and it was located on the upper right side of the animal from between its hind legs to part-way down the base of the tail. It consisted of the red patches surrounding a long flap of otherwise normal-looking skin, which was attached at the centre of the wound but which was loose near the edges – particularly on the left side of the mudpuppy. The mudpuppy spent the first few days barely moving.

After the mudpuppy's bath on April 20th, it wandered around seeming disoriented and unable to find its way back to its den. It was, however, stronger and able to direct itself and managed to catch a worm on its own. Dead skin hanging off the injured area began to peel off in pieces, exposing some still open areas of the wound. Edges of the wound were beginning to heal but the center was still raw/tender.

The wound reopened slightly on the 21st as last bits of loose skin came off in the dip net just before the salt bath. Mudpuppy A had more energy and was much feistier than before. When returned to the tank it proved to be a stronger swimmer as well, and more oriented with the tank; it went straight to its nook under one of the bricks.

By 27th April the wound closed fairly well, although the top skin had not replaced itself yet – raw flesh was still visible. Red patches near wound had not changed much since arrival, as they had only faded in intensity slightly but were still as present as they were on the 14th. A complete visual check – including underbelly – of the mudpuppy was done today and the rest of the amphibian appears to be in good health: the skin was smooth, gill and tail sizes were healthy, colouration was proper and physical strength and activity had increased. Mudpuppy A was hunting for itself sufficiently.

On April 28th the mudpuppy was somewhat disoriented when replaced back into tank, but since it was recently moved into tank 4 this was a similar pattern to the first few days of salt baths. Instead of sitting relatively still in the pail, the mudpuppy now swam all around and practically jumped out of the water from time to time. It was more active in the tank as well.

On May 3rd flesh was still visible but completely sealed and skin around edges was beginning to move in to cover the wound. Mudpuppy A moved around well in the pail and

instead of tossing around in the current as in the beginning, the mudpuppy was able to direct itself right to the bottom of the tank after its bath. When returned back to the tank, Mudpuppy A did not return to den. Rather, it hid near/under some rocks near the den, mostly over the drain.

INJURY DESCRIPTIONS AND OBSERVATIONS AFTER SALT BATH PERIOD

The wound was completely sealed by May 30th and a thin layer of translucent skin had covered the area. All mudpuppies were combined into Tank 1, known as the community tank (see Appendix 2), and Mudpuppy A hid immediately under greenery. Mudpuppy A did not have a problem with other mudpuppies, and appeared to be in a physical condition that would not affect or be affected by others living in a community setting. June 20th was the last day the mudpuppies were in the lab. Mudpuppy A had been living in the community for 21 days without any further injury from the other Necturus.

GILL AND BODY CONDITION THROUGHOUT STUDY

Gill condition, measured in part by size, has fluctuated slightly between bath periods. Overall, gills have grown fuller since the baths, likely to adapt to the daily salt content which makes it more difficult to breathe. Gill colour changes very quickly (i.e. on an hourly basis) and may signify feelings of stress or aggression – the transition from 'flight' to 'fight', possibly from feeling cornered in a small, bright, coverless area like the bath pail. Other reasons for changes in gill colour are hormones (including mating instincts, increased state of awareness in situations like hunting, etc) and increased blood flow (could overlap with other causes). After moving into Tank 3 on 5th May, gills thinned out by about 50% since 3rd May (1 week). This is likely due to increased oxygen from the air stone and no more salt baths.

The mudpuppy generally maintained a healthy 'average' weight, although there were some dates where either the researcher or the assistant recorded the mudpuppy looked leaner. Although the same amount of food was offered the mudpuppy was less inclined to eat, and so fewer feedings occurred to prevent food from being wasted. Fluctuations of body width and height were minor at less than 0.5cm and mudpuppy remained relatively active even when body appeared leaner. The following table shows body measurements taken six days after arrival and the day all the mudpuppies were combined into the same tank, as well as the difference in measurements (gains or losses):

Mudpuppy A	**20 April**	**30 May**	***Difference***
Length (nose to tip of tail)	9.5"	8.5"	*-1"*
Width	1"	1"	*None*
Height	0.75"	0.75"	*None*

MUDPUPPY A FEEDING SCHEDULE (14 April – 30 May)

Date fed	**What was fed?** [all foods live]	**How fed?**
14 April	1 minnow	In tank
18 April	3 minnows	In tank
19 April	1 minnow	By hand
20 April	1 earthworm	In tank
26 April	1 earthworm	In salt bath
29 April	2 earthworms	In tank
2 May	1 earthworm	In tank
4 May	1 earthworm	In tank
9 May	1 earthworm, 5 minnows	In tank
11 May	1 earthworm injected with 0.75cc vita supplement	In tank
27 May	1 earthworm	In tank

FINAL COMMENTS

The injury sustained by Mudpuppy A was a relatively deep injury, and despite mudpuppies being known for their healing abilities the healing process took several weeks. In the first month, during which time the wound was flushed and healed, progress was rapid. After the closing of the wound, when risk of infection or infecting others had past, the mudpuppy was slow to grow skin back over the fleshy area. A normal-coloured top-skin did not grow over the wound while the mudpuppy was in the lab.

All mudpuppies were released at Kemptville Creek on Friday 22 July 2011. For three weeks prior to the release all four mudpuppies lived in the same bin at water temperatures at roughly 18-20°C with only one feeding of minnows in week two and one final feeding of goldfish the day before release. At the time of release, Mudpuppy A showed no signs of weaker disposition relative to the other three specimens. The wound had not gotten worse since being combined with the other specimens in a smaller space, nor did the others show any signs of ill-effects from Mudpuppy A.

Although other mudpuppies gained weight as measured through body length, width, and height, Mudpuppy A only maintained its height and width and actually lost an inch off its length (likely off the tail). Observations in other research have shown that tail thinning results from not enough feeding or nutrition, and so feedings should be increased at this sign (Lembcke, 2005). As Mudpuppy A ate as much as two other mudpuppies did, I suspect the loss of body length was due in part to the stress from recurring salt baths as well as energy and nutrients being used by the body to heal its wound.

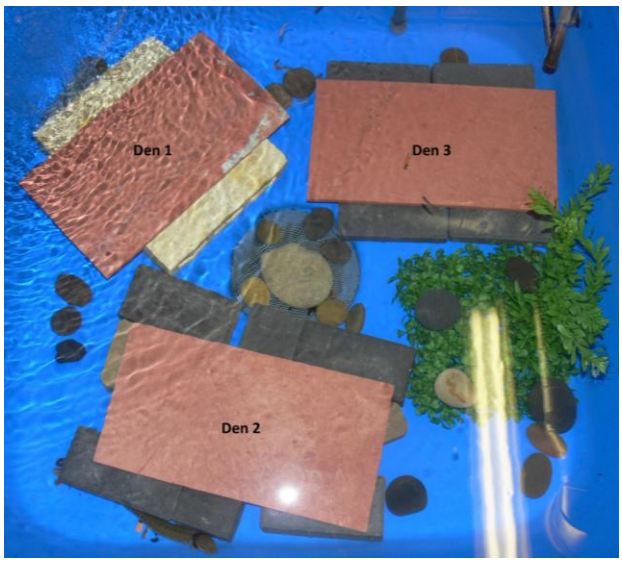
I had expected the final stages of the healing process to be even more rapid than the earlier ones since less intensive healing was necessary, however one explanation could be that preventing infection by closing the wound was a greater priority to the mudpuppy's system than was creating a uniform colour over the closed wound. The mudpuppy's overall health and condition has improved greatly since the first week of treatment, and it remained one of the strongest swimmers in the group.

Moments before releasing the mudpuppies into the creek, Mudpuppy A was observed to have some of its regular pattern over parts of the wound, which now appeared to be more of a scar than an injury. Upon release, Mudpuppy A aptly carried itself across the bedrock and swam straight towards some cracks, which eventually led it to a crevice under the bedrock shelves at the right bank of the creek.

REFERENCES

Lembcke, Peter. (2005). Necturus maculosus. Caudata Culture. Retrieved from http://www.caudata.org/cc/species/Necturus/N_maculosus.shtml.

Images of community tank



Images of injury throughout observation period

20th April, 1700

21st April, 1425

27th April, 1145
(image edited for easier viewing; was about 20% less vivid in reality)

3rd May, 1015

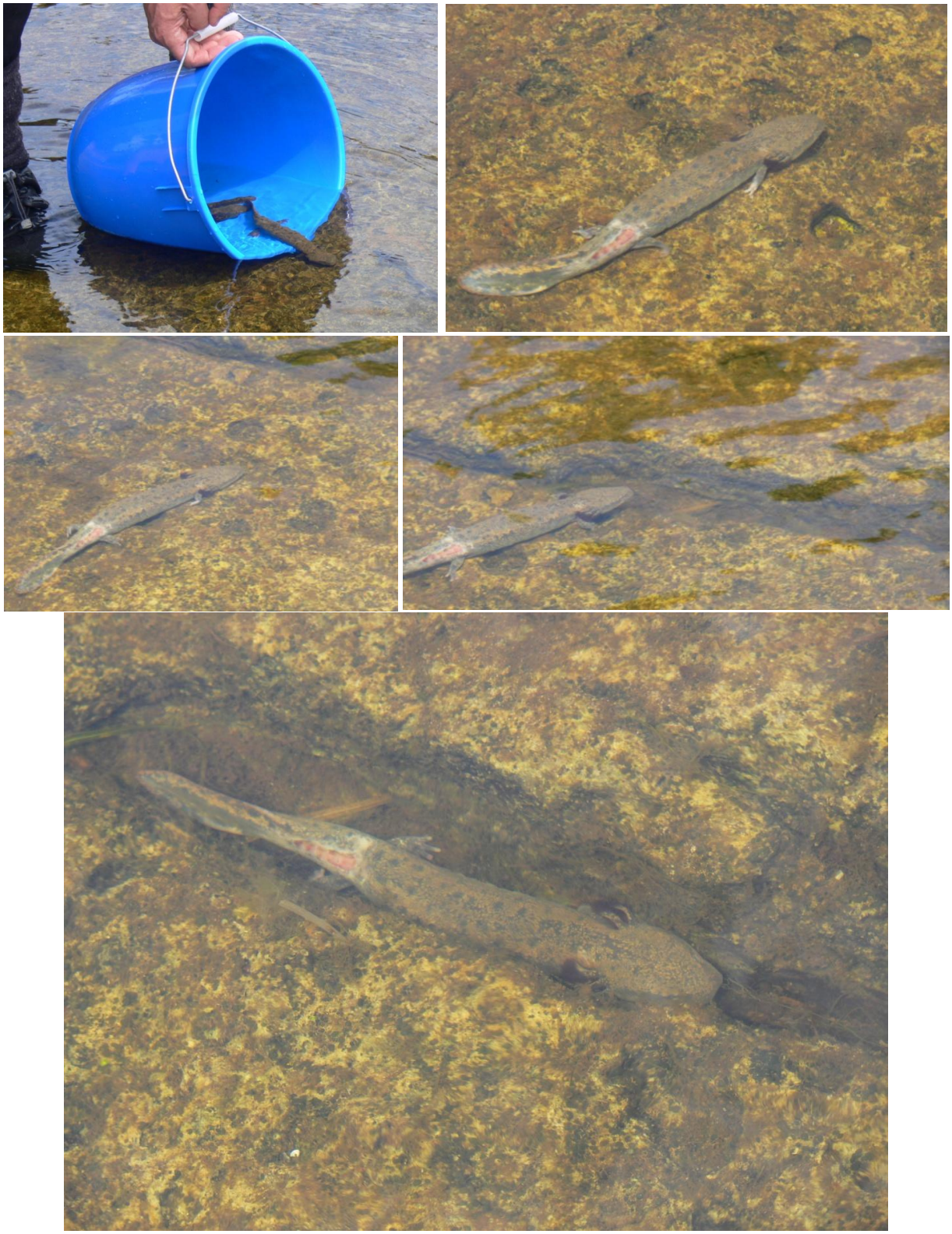
10th May, 1335

30th May, 1820

Images of gill and body condition throughout study

	20th April	
	Left: 3rd May Right: 21st April	
	Left: 10th May Right: 27th April	
3rd May		

Images of Mudpuppy A during release



APPENDIX 2 – Den use over 20 days in community tank

The following is a chart depicting the number of mudpuppies observed to be in each den throughout the community study. When comparing the types of dens, results would be similar if the same chart were created from individual data. Den 2 (Left or Right) was the most similar to the dens provided in individual studies and was the second most used den in the tank, after the greenery.