# Colonization and usage of an artificial urban wetland complex by freshwater turtles

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Conservation authorities invest heavily in the restoration and/or creation of wetlands to counteract the destruction of habitat caused by urbanization. Monitoring the colonization of these new wetlands is critical to an adaptive management process. We conducted a turtle mark-recapture survey in a 250 ha artificially created wetland complex in a large North American city (Toronto, Ontario). We found that two of Ontario's eight native turtle species (Snapping turtle, *Chelydra serpentina,* and Midland Painted turtle, *Chrysemys picta marginata*) were abundant and both were confirmed nesting. The Blanding's turtle (*Emydoidea blandingii*) was present but not well established. Species richness and turtle density were not equally distributed throughout the wetland complex. We noted Snapping turtles almost exclusively populated one water body, while other areas of the wetland had a varying representation of both species. The sex ratios of both Snapping and Midland Painted turtles were 1:1. We tracked the movement of Snapping and Blanding's turtles and found that most turtles explored at least two water bodies in the park, that females explored more water bodies than males, and that 95% of turtles showed fidelity to individual overwintering wetlands. We performed DNA analysis of two Blanding's turtles found in the created wetlands and could not assign these turtles to any known profiled populations. The genetic data suggest that the turtles probably belong to a remnant local population. We discuss the implications of our results for connectivity of artificial wetlands and the importance of the whole wetland complex to this turtle assemblage.

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## Abstract

Conservation authorities invest heavily in the restoration and/or creation of wetlands to counteract the destruction of habitat caused by urbanization. Monitoring the colonization of these new wetlands is critical to an adaptive management process. We conducted a turtle mark-recapture survey in a 250 ha artificially created wetland complex in a large North American city (Toronto, Ontario). We found that two of Ontario's eight native turtle species (Snapping turtle, *Chelydra serpentina,* and Midland Painted turtle, *Chrysemys picta marginata*) were abundant and both were confirmed nesting. The Blanding's turtle (*Emydoidea blandingii*) was present but not well established. Species richness and turtle density were not equally distributed throughout the wetland complex. We noted Snapping turtles almost exclusively populated one water body, while other areas of the wetland had a varying representation of both species. The sex ratios of both Snapping and Midland Painted turtles were 1:1. We tracked the movement of Snapping and Blanding's turtles and found that most turtles explored at least two water bodies in the park, that females explored more water bodies than males, and that 95% of turtles showed fidelity to individual overwintering wetlands. We performed DNA analysis of two Blanding's turtles found in the created wetlands and could not assign these turtles to any known profiled populations. The genetic data suggest that the turtles probably belong to a remnant local population. We discuss the implications of our results for connectivity of artificial wetlands and the importance of the whole wetland complex to this turtle assemblage.

# 1 Introduction

3      Turtles play an important role in wetlands as omnivores, scavengers, predators, food sources, and
4  nutrient recyclers, and some turtles are considered keystone species (Paine 1995). Turtle biomass can be
5  very high in comparison to other vertebrates, measuring up to 877 kg/ha in one temperate wetland
6  (Congdon et al. 1986). Turtle populations are in decline in North America due to the loss and degradation
7  of habitat, invasive species, pollution, disease, climate change and overexploitation (Ernst & Lovich
8  2009). Road mortality is also a significant threat to several species (Beaudry et al. 2008) and is linked to
9  reduced gene flow and male-skewed sex ratios (Gibbs & Steen 2005).

10     Ontario has eight native species of turtles, and seven have been documented in Lake Ontario or
11  its tributaries: Midland Painted (*Chrysemys picta marginata*), Common Snapping (*Chelydra serpentina*),
12  Northern Map (*Graptemys geographica*), Spiny Softshell (*Apalone spinifera spinifera*), Eastern Musk
13  (*Sternotherus odoratus*), Spotted (*Clemmys guttata*) and Blanding's (*Emydoidea blandingii*). A seven-
14  year synoptic trapping study concluded in 2010 at 16 sites along the Lake Ontario's shore found only four
15  native turtle species present (Midland Painted, Snapping, Blanding's and Map). Low water quality and
16  lack of submergent vegetation was likely to blame for the absence of Eastern Musk (DeCatanzaro &
17  Chow-Fraser 2010). The Spotted turtle is mostly found on the northern shore of Lake Erie, in the
18  Georgian Bay and in a few scattered locations in southwest Ontario.  Habitat loss restricts the Ontario
19  distribution of the Spiny Softshell to a few disjunct populations located in south western Ontario.

20     Habitat loss due to urbanisation disproportionately affects wetland habitat, and is therefore
21  critical to replace (Dudgeon et al. 2006; Mitsch & Gosselink 2000). Wetlands provide important
22  ecosystem services and are critical to preserve and restore (Bolund & Hunhammar 1999; Woodward &
23  Wui 2001; Zedler & Kercher 2005b). Wetlands represent approximately 14% of Canada's land area, but
24  development has reclaimed, contaminated or degraded their ecological functions in urban centres (Price &
25  Waddington 2000).

26     Wetlands provide valuable ecosystem services such as regulating water quantity, minimizing the
27  impact of floods and storm water (Woodward & Wui 2001; Zedler & Kercher 2005a).  Thus, the
28  conservation, mitigation and/or restoration of wetlands is a major priority for the Government of Ontario
29  and Ontario conservation authorities (Ontario Ministry of Natural Resources and Forestry 2017).
30  Restoration ecology has focused a great deal on restoring and creating wetlands in urban areas to
31  compensate for the rapid loss of wetland habitat due to urbanisation, yet restored wetlands consistently

32    underperform reference wetlands on many ecological indicators (Maron et al. 2012; Suding 2011; Zedler

33    & Callaway 1999). Restored wetlands can take > 100 years to return to reference levels after restoration,

34    or may become permanently altered by novel environmental conditions and never return to a reference

35    state, especially in colder climates (Moreno-Mateos et al. 2012). Colonization of new wetlands by

36    wildlife is dependent on the quality of the habitat, its age, size and its connectivity to source populations

37    (Cosentino et al. 2010; Kadoya et al. 2004; Ruhi et al. 2009; Ruhi et al. 2012). As a major urban wetland

38    conservation organization, Toronto and Region Conservation Authority (TRCA) has dedicated a large

39    proportion of its annual C$40 million budget to monitoring, protecting and restoring wetlands in Canada's

40    largest urban centre. The aim of this study was to investigate if turtles had colonized these artificial

41    wetlands, to what extent, and for any Blanding's turtles, from what wild population are they most likely

42    derived.  Our secondary goal was to test if turtles that had recolonized restored wetlands were evenly

43    distributed within the complex, and if species richness and distribution was similar to what could be

44    expected in a natural Lake Ontario coastal wetland. We hope that monitoring and documenting the extent

45    of any colonization will serve to gauge the success of these particular wetland creation efforts and inform

46    other wetland creation or restoration projects.

47    ## Methods

48

49    **Study site**:

50    Tommy Thompson Park- Toronto, Ontario (43º37'4"N 79º20'33"W)

51         The City of Toronto, Canada's largest metropolitan area, is built on a series of watersheds that cut

52    along a north-south axis ending in Lake Ontario. Toronto is historically rich in wetland habitat that has

53    been gradually lost due to urbanisation. In the 1960s, the Toronto Port Authority built a 5 km long

54    breakwater (the Leslie Street Spit) into Lake Ontario to create additional lands for port infrastructure in

55    conjunction with the opening of the St. Lawrence Seaway. As the Spit grew in size, native and non-native

56    plants began to colonize the land, and it became an important stopover habitat for migrating birds. By

57    1991, large colonies of cormorants, gulls and herons nested at the Spit.

58         TRCA was tasked to develop and implement a master plan for a public park on the Spit, leading

59    to the creation of Tommy Thompson Park (TTP). One component has been natural area enhancement, and

60    TRCA has focused efforts on creating a varied wetland complex, to improve aquatic habitat including

61    features such as aquatic vegetation and structural habitat (logs, root balls, stones, gravel beds, sandy

62    beaches, etc.). TRCA continues to tweak wetland design at TTP using an iterative adaptive management

63    process that relies on active monitoring.

64    **Description and History of the individual water bodies at TTP:**

65          At TTP, there are three types of created water bodies forming the wetland complex: ponds,

66    embayments and cells (Figure 1). Ponds are small and unconnected stand-alone bodies of water. There are

67    two main ponds in the wetland complex, Triangle and Goldfish ponds.  Goldfish Pond is the smallest

68    pond and was accidentally created through asphalt disposal in the late 1970s.  It measures approximately

69    500 m$^2$ (0.05 ha). TRCA has not undertaken any restoration projects in Goldfish Pond and it has been

70    allowed to naturalize without any intervention. Triangle Pond was constructed between 1975 and 1978 as

71    a trial confined disposal facility and it covers 0.8 ha.  TRCA capped the facility in 1997 and subsequently

72    created a wetland by adding structural habitat (large stones, rocky shoals, woody materials), as well as

73    aquatic and riparian vegetation in 1998.

74          The TTP embayments were created as coastal wetlands.  Embayment A covers 6.25 ha and has

75    benefited from two restoration projects; a) 2004-2005 to create spawning shoals on the east side of the

76    embayment; b) 2009 to create backwater refuge and coastal wetland along the west and south shorelines.

77    Embayment B covers 19 ha and has received two restoration projects; a) 1995-1997 to create Northern

78    pike (*Esox lucius*) spawning channels in the sheltered backwater area to the east of the embayment, as

79    well as structural habitat in the main embayment; b) 2011 to improve and enhance the coastal wetland

80    conditions along the shoreline of the main embayment. Embayment C covers 24 ha and has seen three

81    restoration projects; a) 1996-1998 to create structural, wetland and riparian habitat on the west portion of

82    the embayment; b) 1998 to create pike spawning channels on the southeast shoreline; c) 2010 to improve

83    coastal wetland habitat along the east shoreline along with additional structural habitat enhancements

84    throughout the embayment.  Embayment D covers 7 ha. In 2012-2014 a coastal wetland restoration

85    project isolated the embayment from the lake through the construction of a berm and the installation of a

86    fish and water level control structure. Embayments A, B and C are open to the lake, with backwaters and

87    fish spawning channels, while Embayment D is connected to the lake via a fish gate with 7 cm vertical

88    grates, impeding the travel of large invasive common carp (*Cyprinus carpio*) into the wetlands (French et

89    al. 1999).

90          Cells were created as confined disposal facilities to store dredged material from the lower Don

91    River and Keating Channel and eventually capped and transformed into wetlands. Cells are also

92    connected to Lake Ontario via fish gates. Cell-1 was filled to capacity in 1985 and capped.  TRCA created

93    11 ha of terrestrial and aquatic habitat in Cell-1, including a 7.7 ha coastal hemi-marsh between 2003-

94    2006. A fish and water level control structure was installed in 2012. Cell-2 covers 10 ha and was filled to

95    capacity in 1997.  Cell 2 was capped and a coastal hemi-marsh wetland was created in 2015-2017 by

96    adding structural habitat and vegetation.  TRCA expected to install fish and water level control structures

97    in late 2017-18 and to plant aquatic vegetation into 2018. Cell-3 measures 32 ha and is still accepting

98    dredged material. TRCA does not expect to transform it into a wetland for some time, and hence this cell

99    is currently devoid of emergent vegetation or structural habitat suitable for turtles.

100    INSERT FIGURE 1 HERE

101    **Survey methods**:

102        We studied the turtle population using three main methods: a capture-mark-recapture (CMR)

103    methodology, very high frequency (VHF) tracking, and shore-based visual basking surveys.

104    Capture-Mark-Recapture:

105        We conducted a CMR study in 2016 and 2017, trapping turtles using hoop nets, basking traps or

106    seine nets. CMR (Lecren 1965) is a robust method to determine population densities and trends (Pollock

107    et al. 1990). We used one metre diameter- three-ring hoop nets (no.15 net with 6.25 cm mesh from

108    Champlin Net Company, Jonesville, LA), basking traps (manufactured in-house, varying in size from 1

109    $m^2$  to 2 $m^2$, all with 6.25 cm mesh size) and a 30 m seine net (manufactured in-house, with 6.25 cm

110    mesh). The size of the mesh precluded capturing very small turtles or juveniles, so our demographic

111    information is skewed towards adults.  Juveniles were captured opportunistically by hand. We baited the

112    hoop traps with a variety of food, including frozen fish, canned cat food, canned sardines, and frozen

113    chicken, depending on availability. Bait was placed in perforated bags, hung near the back of the traps at

114    such a height that the bait was in the water. The trap was three-quarters submerged in water, in order to

115    always have air available to trapped animals. Captured turtles were removed from the traps and processed

116    on shore, where we recorded weight with a digital scale, body morphology with digital vernier calipers

117    (plastron length, straight carapace length, carapace width, body depth, precloacal length, foreclaw length)

118    and sex (if possible).  For Midland Painted turtles, we identified males based on the elongated foreclaws

119    and long tail, with the cloaca situated well beyond the limit of the carapace, compared to shorter

120    foreclaws and tails in females. For Snapping turtles, we used the relative length of the posterior lobe vs.

121    the base – cloaca tail measurement, and by gentle rocking to encourage penile eversion. For Blanding's

122    turtles, males have a concave plastron and longer tail.  We notched the marginal scutes of the shells to

123    give each turtle a unique identifying number (Cagle, 1939). After processing, we returned turtles to the

124    general area of the capture trap. We trapped for 25 days during the months of June and July 2016 and for

125    24 days in May and June 2017, using up to 20 hoop traps (13 traps in 2016 and 20 traps in 2017), 4

126    basking traps and one seine net (2016 only). The seine net was only used one day in 2016 (one snapping

127    turtle captured) and was unavailable in 2017 and the basking traps had little success in 2016 and were not

128    used in 2017.

129    We trapped in each water body for a one to two-week period, depending on water body size and success

130    of trapping. Using a CMR protocol in successive years also allowed us to observe turtles moving from

131    one water body to another. As part of our invasive species protocol, we removed all captured Red-Eared

132    Sliders and transferred them to an adoption centre (Little RES Q, Pefferlaw, ON).

133           We used previously recorded locations of visually surveyed turtles to decide in which water body

134    we should trap. Staff and volunteers had reported many turtle sightings in both ponds, in all embayments

135    and in Cells 1 and 2. Although Cell-2 had previously recorded turtle activity, it was under further

136    restoration during the time of this study and was unsuitable for trapping. Consequently, we trapped in

137    seven distinct water bodies (see Table 1): Goldfish pond (2016/17), Triangle pond (2016/17), Cell-1

138    (2016/17), Embayments A (2016), B (2016), C (2017) and D (2016/17). Wetlands that produced very few

139    turtles in 2016 were not re-sampled in 2017.

140    INSERT TABLE 1 HERE

141    Statistics:

142    1.  Population estimates: We applied the Chapman correction for small sample sizes to a simple two-

143        sample Lincoln-Petersen index to estimate population size (Chapman 1951). We estimated the

144        population size for the two main turtle species at TTP where $N=[(K+1)(n+1)]/(k+1)$; $N$ is the

145        estimated population, n is the individuals captured in 2016, K is the captured population in 2017,

146        and k is the population recaptured in 2017 that was marked from a previous capture.

147    2.  Turtle sizes: Straight-line carapace length is reported using the mean ± 1 standard deviation, with

148        ranges reported as minimum-maximum.

149    3.  Species representation: We tested for uniformity of individual species distribution through the

150        various water bodies using a Chi-Square test.

151    4.  Sex ratio: We performed a binomial test (nonparametric two-sided) to determine if the sex ratio

152        of the two main turtle species was unbiased. The resulting probability, $p$, is two-tailed and is

153        significant at 95% confidence level if the resulting $p$ is smaller than 0.025 or greater than 0.975.

154    VHF tracking:

155           We affixed a 12 g VHF transmitter (model R185, Advanced Telemetry Systems, Isanti, MN) to

156    certain individuals of turtle species considered endangered or species at risk in Ontario (Blanding's and

157    Snapping turtles). Using a water epoxy, we glued the transmitters to the back of the carapace, generally

158    offset to one side, with the antenna trailing. We only attached transmitters to turtles > 300 g (transmitter

159    weight <5% turtle body weight). ). In 2016, we aimed to get an even number of males and females and we

160    able to track eight male and eight female Snapping turtles and three female Blanding's turtles. After an

161    initial data investigation, we noticed that the female Snapping turtles were travelling greater distances

162    than the males. Therefore, in 2017 we became more interested in the movement of females, and

163    consequently added new transmitters only to new female Snapping turtles. We also removed the old VHF

164    transmitters (most of which had depleted their battery power) from any recaptured male Snapping turtles.

165    As a result, in 2017, we replaced the transmitters on all three female Blanding's turtles, on four recaptured

166    female Snapping turtles, added five new female Snapping turtles to the study, and removed old, non-

167    functional transmitters from three recaptured male Snapping turtles.  We continued to track the remaining

168    five male Snapping turtles until we lost their VHF signals.

169           We tracked each individual turtle to a specific water body on a weekly basis during the active

170    period and once monthly over the winter. Hibernacula locations were determined in the fall by locating

171    turtles that had buried themselves under vegetation, logs, stumps or in the mud and reconfirming these

172    locations in the winter. The weekly time period will necessarily underestimate the movement of turtles

173    throughout the wetland complex but we used it as a baseline indication of the minimum habitat use and of

174    exploratory movement at the study site.

175    Visual surveys:

176           Staff and volunteers at the park also conducted regular visual surveys (using binoculars) of

177    basking turtles from shore and reported results back to the authors. Visual surveys offer a continuous data

178    set starting in 2004. Staff and volunteers also reported opportunistic observations of turtle nests

179    throughout the 2016 - 2017 study period.

180    DNA Analysis:

181           We collected blood samples from the Blanding's turtle by caudal venipuncture and stored these

182    samples on FTA cards (Whatman Inc., Little Chalfont, UK), for later DNA analysis to understand the

183    likely source of these individuals, which could have re-colonized TTP from a nearby population or

184    introduced to the site by humans after collection at a distant source site.  DNA was extracted using

185    standard methods for nucleated blood (Smith and Burgoyne, 2004) and genotyped using 11 microsatellite

186    loci. These included loci GmuB08, GmuD16, GmuD21, GmuD28, GmuD55, GmuD87, GmuD88,

187    GmuD93, GmuD107, and GmuD121 (King & Julian 2004), and loci Eb17 and Eb19 (Osentoski et al.

188    2002). Polymerase chain reaction (PCR) followed the recipe and thermocycling parameters detailed in

189     Davy et al. (2014). We ran the TPP samples alongside a positive control DNA from a Blanding's turtle

190     genotyped previously in Davy et al. (2014) to ensure that the microsatellite genotypes obtained were

191     accurate and comparable to existing genotype data. The new genotypes were compared to an existing

192     database for Ontario Blanding's turtles (Davy et al. 2014) and used Geneclass2 (Piry et al. 2004) to assign

193     or exclude populations of origin for the new samples. We used Bayesian methods of assignment (Rannala

194     & Mountain 1997), and Monte-Carlo resampling simulation algorithm (Paetkau et al. 2004), with 1,000

195     simulated individuals and $\alpha = 0.05$, and then ran the analysis a second time using frequency-based

196     assignment (Paetkau et al. 2004).

197     Permits:

198     This study was conducted with the approval of York University's Animal Care Committee

199     (YUACC#2016-16W- and #2017-16W-R1) and under the Ontario Ministry of Natural Resources

200     (Wildlife Scientific Collector's Authorization numbers # 1083601 and 1085922) and Endangered Species

201     Act Permit for Species Protection or Recovery (AU-B-007-16 and AU-B-006-17).

202     **Results**

203

204     Trapping surveys:

205     We captured 126 turtles (97 new and 29 recaptured) over 496 trap-days (catch-per-unit-effort,

206     CPUE, 25.4%) in 2016 and 103 turtles (50 new and 53 recaptured) over 514 trap-days (CPUE 20.0%) in

207     2017 (Table 2). Three of the four expected native turtle species were represented in our trapping: Midland

208     Painted, Snapping, Blanding's and the introduced Red-Eared Slider (see Table 2). Based on the trapping

209     results we estimated the number of individual Midland Painted turtles within TTP at 140 turtles ± 11 (n =

210     69, K = 71, k = 35) and the number of Snapping turtles at 35 turtles ± 3 (n = 20, K = 24, k = 14).  Neither

211     Midland Painted turtles (df = 6, n = 105) =143.067, $p<0.001$) or Snapping turtles (df = 7, n = 30) =23.867,

212     $p<0.001$) were uniformly distributed across the individual capture locations. The proportion of Midland

213     Painted to Snapping turtles in the whole wetland complex was 3.5:1 but varied by individual wetlands

214     from 1:12 to 28:1. The male:female sex ratios of both Midland Painted (45:44, $p = 0.584$) and Snapping

215     turtles (12:15, $p = 0.351$) did not statistically differ from the expected 1:1 ratio if the demographics of

216     each species are taken as a whole over the entire TTP wetland complex. However, Midland Painted turtle

217     sex ratios deviated from the expected 1:1 in two wetlands, where in Triangle Pond was male skewed, but

218    just below significance (1.7:1, *p* = 0.970) and Embayment D was significantly female skewed (1:3.5, p =

219    0.015).

220

221    INSERT TABLE 2 HERE

222

223         We report Midland Painted and Snapping turtle size and mass for the wetland complex and by

224    specific wetland in Table 3 below. We also captured three mature female Blanding's turtles (straight

225    carapace length 215 mm, 241 mm and 230 mm, and mass of 1356 g, 2200 g, 1885 g respectively).  Blood

226    samples were successfully collected from two of these females.

227

228    INSERT TABLE 3 HERE

229

230         We report on turtle density and biomass (all species) as well as species-specific density and

231    biomass for Midland Painted Turtle (MP) and Snapping Turtle (SN) for the most productive wetlands in

232    Table 4. Note that because of the small size of Goldfish pond, density and biomass per hectare are

233    artificially inflated.

234    INSERT TABLE 4 HERE

235

236         Our first method of quantifying movement through the site was based on the locations of

237    recaptured turtles, and provided a minimum value of exploratory movement. Out of the 35 recaptured

238    Midland Painted turtles, 7 (20%) switched water bodies between captures, while only 1 (7%) recaptured

239    Snapping turtle was first captured in a different location.

240         We also captured 52 fish of 10 species as by-catch, including 37 common carp (*Cyprinus carpio*)

241    (see Table 5). Of note, we had not captured common carp in 2016 in Embayment-D, but carp were able to

242    migrate into the embayment in 2017 as a consequence of record high lake levels in spring. In general, we

243    were able to release by-catch unharmed. However, there were a few occasions where fish caught in the

244    mesh of the hoop traps had drowned, or had been mortally injured and/or partially consumed by trapped

245    or nearby Snapping turtles.

246    INSERT TABLE 5 HERE

247

248   Habitat use by Snapping and Blanding's turtles (see Table 6 below)

249         Snapping turtles: We monitored a total of 21 Snapping turtles (8 males and 13 females), and 14 of
250   these turtles (3 males and 11 females) were observed visiting more than one wetland. Of the 11 Snapping
251   turtles that were initially captured in a wetland and then explored other water bodies (8 females and 3
252   males), 10 returned to their initial capture point for overwintering. The mean minimum distance travelled
253   (straight line distance) was 0.5 km (0-2.5 km) for males and 0.76 km (0- 2.6 km) for females. One female
254   that was captured on land (in July 2017) left TTP to overwinter at an island, 1.6 km away. We tracked the
255   turtles to hibernacula from mid-September to end of October (Figure 2). However, a few Snapping turtles
256   in Embayment-D relocated in early November 2016, likely due to sudden drop in the water level caused
257   by the demolition of a beaver dam located inside the fish gate. All of the Snapping turtles overwintered in
258   shallow water (less than 1 m of water above the hibernaculum). Overwintering sites included: under
259   vegetation mats (12), under logs, root balls and stumps (10), in mud flats with no vegetation above (4), at
260   the edge or inside of beaver lodges (2), and under artificial structures (a wooden dock and a concrete
261   slab).

262         Blanding's turtles: We tracked three female Blanding's turtles in 2016 and in 2017. All
263   Blanding's turtles explored at least three wetlands and all three returned to overwinter in the same small
264   pond. The most active Blanding's turtle traveled a minimum distance of 2.9 km over the summer of 2016
265   and 3.2 km in 2017. These turtles overwintered together in both study years, in an area measuring
266   approximately 10 m$^2$ located in the deepest section of Goldfish pond (> 1.5 m deep). The Blanding's
267   turtles returned to their overwintering ponds by mid-September and remained active in that pond until
268   mid-November of each year.

269

270   INSERT TABLE 6 HERE

271

272   INSERT FIGURE 2 HERE

273

274   Visual surveys

275        Opportunistic basking surveys conducted by staff and citizen scientists (Table 7) identified the

276    presence of two further species: a Northern Map turtle (*Graptemys geographica*) in Cell-2 and a

277    suspected Spiny Softshell turtle (*Apalone* cf. *spinifera*) in Cell-1 and in Cell-2.

278    INSERT TABLE 7 HERE

279        Turtle nests were opportunistically discovered throughout the study period, either by observing a

280    nesting turtle or by discovering a nest site that had been depredated.  During our study period, we came

281    across five Snapping turtles actively laying eggs along the gravel shoulder of the main spine road or in

282    gravel side roads and trails. We did not observe any Midland Painted turtles actively laying eggs in a nest.

283    Nesting activity was first observed in mid-June and as late as early August. We also discovered a number

284    of other nest sites by finding dug out holes with scattered empty shells at the surface, presumably

285    depredated. We did not identify any of the depredated nests to species.

286

287    Results of the DNA analysis

288        Microsatellite locus Eb19 did not amplify, and loci GmuD55 and GmuD93 produced genotypes

289    for the positive control that did not match those in the reference data from Davy et al. (2014). These three

290    loci were excluded from further analyses. Neither turtle was strongly assigned to any previously profiled

291    population: probability of assignment was < 0.65 in all cases for the Bayesian analysis, and < 0.45 in all

292    cases for the frequency-based analysis. This result suggests that the two TTP Blanding's turtles originated

293    from a population that was not represented in the reference data (including samples from the proximate

294    Golden Horseshoe population), and that these assignment results should only be used to infer genetic

295    similarity, but not genetic origin. The Bayesian analysis assigned the first individual most strongly to the

296    Kawartha Highlands, and the second most strongly to Parry Sound District (the eastern shore of Georgian

297    Bay).

## Discussion

299

300        Two turtle species, the Midland Painted and the Snapping turtle, have successfully colonized our

301    study site and established breeding populations. Both species are found in Lake Ontario at various sites

302    within a few kilometres from TTP. Turtles were present in all surveyed areas of the TTP wetlands

303    complex and were found in high densities in the two isolated ponds. Elsewhere, Painted turtle densities of

304    greater than 100 individuals per hectare have been reported at a suburban site in North Carolina (Eskew et

305  al. 2010), in New York (Zweifel 1989) and in Nebraska (Iverson et al. 2006). The juveniles of Midland

306  Painted and Snapping turtles were discovered opportunistically, as well many nests and nesting Snapping

307  turtles, which we interpreted as a sign of well-established populations. We attribute the lack of observed

308  nesting Midland Painted turtles to timing of oviposition in the late afternoons or early evenings (Rowe et

309  al. 2005), a time when researchers were not at TTP.

310  Blanding's turtles have started to re-colonize TTP, with sightings recorded since 2007, but remain

311  rare, and the lack of observed males suggests that this colonization remains precarious. The nearest

312  known occurrence of Blanding's turtles is approximately 15 km inland in the Rouge River. Blanding's

313  turtles can travel considerable distances. Females have been documented travelling distances of up to

314  3,479 m during nesting forays and nesting sites have been found as far as 1,850 m away from wetlands

315  (Millar & Blouin-Demers 2011) however the genotype of the TTP samples could not be assigned to any

316  known population, and thus provides no evidence as to how this species arrived at TTP. The single

317  observation of Northern map turtle suggests that this species has only recently begun to colonize TTP.

318  Map turtles generally require high-quality water that supports mollusc prey and are found in clean lakes

319  and rivers. The TTP study site does support some crayfish species (both native and non-native) and snails,

320  so we would expect to eventually find Map turtles in greater numbers. In urban landscapes, introduced

321  exotic species common in the pet trade, such as the Red-Eared Slider turtle, are ubiquitous in easily

322  accessible wetlands and can have detrimental effects upon native turtles (Cadi & Joly 2004). Although we

323  found some adult Red-Eared Slider turtles throughout the wetland complex, we did not find any evidence

324  of juveniles, suggesting that perhaps this exotic population was not successfully nesting. The presence of

325  the Spiny Softshell (*Apalone* cf. *spinifera)* at our site remains enigmatic. Although historically present but

326  rare in Lake Ontario, the native Eastern Spiny Softshell (*Apalone spinifera spinifera*) is now considered

327  locally extirpated in Lake Ontario (COSEWIC 2016). Recent sightings of Softshell turtles in the lake have

328  been attributed to non-native species, likely released.

329  Midland Painted and Snapping turtles are sympatric (Bodie et al. 2000), however, these species

330  have shown some different habitat preferences. At TTP, Midland Painted turtles preferred smaller ponds

331  with little or no fish and abundant shoreline vegetation consistent with past findings (Hughes et al. 2016;

332  Marchand & Litvaitis 2004), whereas Snapping turtles were found in each wetland. Surprisingly,

333  Snapping turtles represented more than 20% of all individuals and 73% of the biomass of all turtles

334  captured at our site. The Midland Painted to Snapping turtle ratio of 3.5:1 was less skewed towards

335  Midland Painted turtles when compared to an 11:1 ratio found from a previous study of multiple sites in

336  Lake Ontario (DeCatanzaro & Chow-Fraser 2010) and a ratio of 8.5:1 at the coastal Dunnville marshes

337  near Lake Erie (Brown 2016). Although the relative preponderance of Snapping turtles at TTP compared

338    to natural sites stands out as unusual, it is not unique in artificial pond systems. A study of artificial ponds

339    in Pennsylvania found an overall 1.3:1 ratio between these species, with some ponds having a majority of

340    Snapping turtles (Hughes et al. 2016). A study of 88 wetlands, refuges and golf courses in upstate New

341    York found a ratio of 1:1.5, with Snapping turtles showing better body condition and more even sex ratios

342    in golf course wetlands than in protected areas (Winchell & Gibbs 2016). The TTP Snapping turtle

343    density of 1.7 individuals per ha (biomass 10 kg per ha) approaches that of other sites in Ontario ranging

344    from 2 to 2.7 turtles per ha (biomass 13 to 18 kg per ha) at shallow artificial lakes, but nowhere near the

345    58 turtles per ha (biomass 313 kg per ha) at a nutrient enriched pond (Galbraith et al. 1988). Cell-1 had

346    many Snapping turtles but was mostly devoid of Midland Painted turtles.  TRCA had documented several

347    sightings of Midland Painted turtles in Cell-1 from 2004 to 2012, but none in 2013 and only one in 2014.

348          Common carp have destructive effects on shallow freshwater ecosystems including reducing

349    aquatic macrophyte cover (Lundholm & Simser 1999) due to the carp's bottom-feeding behaviour

350    (Fletcher et al. 1985; Koehn 2004) and increasing water turbidity (Chow-Fraser 1999). We suspect that

351    the fish gate, equipped with 7cm grate width, in Cell-1 has allowed smaller carp (total length < 50 cm) to

352    access the cell in the spring (French et al. 1999). These carp may have grown over the summer to larger

353    than 50 cm total length thus becoming trapped by the fish gate inside the cell and unable to return to the

354    lake.  The presence of carp on its own does not explain the lack of Midland Painted turtles in Cell-1 given

355    that Midland Painted turtles and carp coexist in Embayment D.  However, the continuous presence of

356    large carp in combination with the clay substrate in Cell 1 that is easily disturbed and suspended

357    (Daviescolley et al. 1992; Harter & Mitsch 2003) could have caused the degradation of the emergent

358    vegetation.  Turbidity, per se, does not affect the ability of Painted turtles to locate prey species (Grosse et

359    al. 2010) but turbidity does negatively affect vegetation growth necessary for omnivorous species such as

360    the Midland Painted turtle (Parkos et al. 2003).

361          Although Snapping turtles are also omnivorous and prefer less turbid waters (Bodie et al. 2000),

362    adults may be better equipped to co-exist in carp-modified environments (Paterson et al. 2012). Snapping

363    turtles will readily feed on fish and evidence from our by-catch suggests that fish are abundant in Cell-1.

364          Safe and accessible terrestrial habitat between wetlands is an important characteristic of

365    successful wetland complexes (Burke & Gibbons 1995; Gibbons 2003). At our site, turtles could move

366    freely through the park, as there was very little vehicular traffic, and they could also use the terrestrial

367    habitat to gain access to a variety of suitable nesting sites. The safety of the terrestrial habitat is a likely

368    contributor to the evenness of the sex ratios and the presence of turtles in all surveyed wetlands. Given the

369    amount of movement we documented between water bodies, we are inclined to consider the population

370    sex ratios at the wetland complex level. The sex ratios of both Midland Painted and Snapping turtles

371   across the whole wetland complex were remarkably even. The evenness of the sex ratio at our site is

372   notable given that turtle sex ratios have been showing a male bias in North America (Gibbs & Steen

373   2005) as well as in Ontario (DeCatanzaro & Chow-Fraser 2010) attributed to the higher risks of vehicle

374   collisions to females searching for nesting sites by the roadside (Aresco 2005; Steen et al. 2006).  Possible

375   sources of observed sex-ratio bias could stem for local climatic conditions (both Midland Painted and

376   Snapping turtles exhibit temperature-dependent sex determination), differential exposure to predators

377   (Marchand & Litvaitis 2004) and capture methods (Ream & Ream 1966).  Dupuis-Desormeaux et al.

378   (2017) found a heavily male-skewed sex ratio in Midland Painted turtles at a nearby urban wetland site

379   (Heart Lake Rd.) with a history of turtle road mortality.  The TTP and Heart Lake Rd. study sites share

380   similar general climatic conditions (microhabitat differences aside), similar predator presence and used

381   the same capture methodology. Two key differences stand out between the TTP and Heart Lake Rd. study

382   sites: differences in traffic volume (fewer than 50 vehicles per day at TTP, mostly dump trucks, versus

383   5000-7500 vehicles per day at Heart Lake Rd.) and differences in the timing of that traffic (7:30 am to

384   4:15 pm, weekdays only at TTP, versus 24 hours per day, seven days per week at Heart Lake Rd.).

385   Therefore the times of highest traffic volume at the TTP site avoided the peak nesting times (early

386   morning or late afternoon) for these two turtles species (Obbard & Brooks 1981; Rowe et al. 2005).  We

387   did not set out to study road mortality versus sex-ratios at TTP, and consequently have no data on TTP

388   road mortality; however given the above described similarities and differences between sites, we are

389   inclined to speculate that the lack of vehicular traffic at TTP contributes to the evenness of the observed

390   sex-ratio.

391        The results of our recapture and our tracking study revealed that Midland Painted, Snapping and

392   Blanding's turtle species visited all of the surveyed water bodies inside the wetland complex.

393   Furthermore, some Snapping and Blanding's turtles explored large portions of the park during the

394   summer yet displayed site fidelity towards their ponds of capture for overwintering.  Nesting site fidelity

395   has been documented in both Blanding's (Standing et al. 1999) and Snapping turtles (Loncke & Obbarde

396   1977) as well as overwintering site fidelity (Brown & Brooks 1994; Innes et al. 2008; Meeks & Ultsch

397   1990). Snapping turtle hibernacula were distributed throughout the wetland complex and we noted three

398   pairs that overwintered in close proximity to each other (two male-female pairs and one female-female

399   pair).  However, we did not find evidence of mass congregation at a communal site as has been reported

400   in some other populations (Meeks & Ultsch 1990). The structure of the hibernacula was similar to what

401   has been reported in past studies at natural wetland sites (Brown & Brooks 1994; Paisley et al. 2009) and

402   in that respect, the overwintering sites at the TTP artificial wetland site mimicked that found in natural

403   ponds.

404        The overland movement of turtles will increase their exposure to predators. At TTP, we have

405    documented many potential turtle predators, including Eastern coyote (*Canis latrans*), raccoon (*Procyon*

406    *lotor*), otter (*Lontra canadensis*) and mink (*Neovison vison*). We noted several Midland Painted turtles

407    with shell markings consistent with predator encounters, including bite marks, puncture wounds, missing

408    carapace pieces, scratch marks and missing limbs. We also discovered many depredated turtle nests.

409    Turtles seem to be colonizing the created wetlands despite the presence of predators, but we would have

410    to monitor survivorship over time to understand exactly how predation impacts population growth at TTP.

411

412    Conclusion

413    The two Blanding's turtles were not strongly assigned to any previously genotyped populations (Davy et

414    al., 2014) based on their microsatellite genotypes, indicating that they belonged either to a remnant

415    population of turtles from the TTP area, or that they had been introduced to TTP from an entirely

416    different area. Of the populations previously profiled by Davy et al. (2014), the genetic profiles of the

417    TTP Blanding's turtles were most similar to the closest candidate populations, which were in the

418    Kawartha Highlands and the east shore of Georgian Bay. Thus the genetic data suggest that the TTP

419    Blanding's turtles probably belong to a remnant local population, although the possibility that they were

420    introduced from elsewhere cannot be completely excluded. This result is most consistent with a

421    colonization of TTP by Blanding's turtle from nearby areas (possibly the Rouge River), and a pattern of

422    genetic isolation by distance between Blanding's turtle populations on the Canadian Shield, and remnant

423    populations near Lake Ontario. Confirming this hypothesis would require genotyping a robust sample of

424    turtles from the Rouge or elsewhere along the north-western shore of Lake Ontario, as this would allow

425    frequency-based analyses of the similarity between the Rouge and TTP individuals. However, collecting

426    sufficient samples from the Rouge would be challenging as the original population apparently declined

427    rapidly following the urbanization of the Greater Toronto Area.

428        We conclude that the artificial wetland complex at TTP has provided adequate habitat to attract

429    four native species and that two of these species are nesting and recruiting juveniles to the TTP

430    populations. Many other factors can influence colonization success, including the connectivity of the

431    surrounding landscape matrix, specific habitat suitability, predator density, and the particular dispersal

432    ecology of neighbouring seed populations. We suggest regularly assessing the progress in the

433    colonization of each individual water body of this artificial wetland complex and of extending the

434    movement study to include specimens from the nearby reference wetlands to better assess colonization

435    vectors.

436

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443

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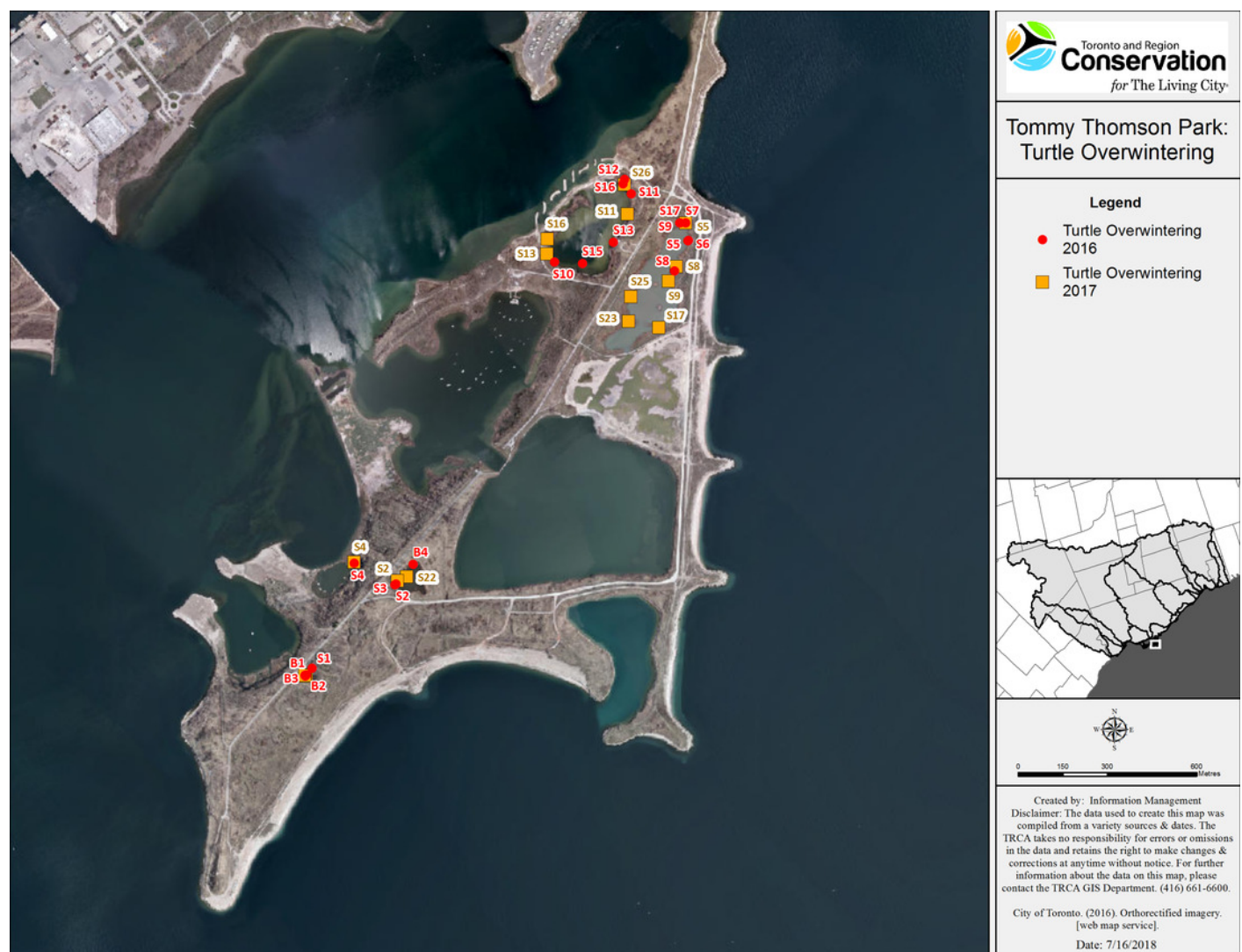
# Figure 1

Map of Tommy Thompson Park, Toronto, Ontario, Canada. TRCA image 2017.

# Figure 2

Map of overwintering locations of tracked Snapping and Blanding's turtles at TTP 2016-17.

Character codes: first letter represent species (B=Blanding's, S=Snapping) and following numeral indicates the specific individual turtle.

Created water bodies at Tommy Thompson Park (TTP), describing type, size, and year of completion.

| Name | Type | Size (ha) | Year completed |
|------|------|-----------|----------------|
| Goldfish | Pond | 0.05 | 1970s |
| Triangle | Pond | 0.8 | 1998 |
| Emb-A | Embayment | 6.25 | 2009 |
| Emb-B | Embayment | 19 | 2011 |
| Emb-C | Embayment | 24 | 2010 |
| Emb-D | Embayment | 7 | 2014 |
| Cell-1 | Cell | 6 | 2012 |

PeerJ

Total number of new turtles captured in each water body surveyed at Tommy Thompson Park, 2016-17.

Each entry represents: total (male, female, unsexed juveniles) and ratio of Midland Painted turtle to Snapping Turtle (MP:SN).

| Species vs Specific Wetland | Midland Painted (*Chrysemys picta marginata*) | Snapping (*Chelydra serpentina*) | Blanding's (*Emydoidea blandingii*)) | Red-Eared Slider (*Trachemys scripta elegans*) | Total number of individual turtles | Ratio of MP: SN |
|---|---|---|---|---|---|---|
| Goldfish Pond | 28 (14,13,1) | 1 (1,0,0) | 3 (0,3,0) | 1 (1,0,0) | 33 | 28:1 |
| Triangle Pond | 49 (26,15,8) | 5 (3,2,0) | 0 | 1 (0,1,0) | 55 | 9.8:1 |
| Cell-1 | 1 (0,1,0) | 12 (4,6,1) | 0 | 4 (2,2,0) | 17 | 1:12 |
| Emb-A | 1 (1,0,0) | 1 (0,0,1) | 0 | 0 | 2 | 1:1 |
| Emb-B | 1 (0,1,0) | 1 (1,0,0) | 0 | 0 | 2 | 1:1 |
| Emb-C | 0 | 1 (0,1,0) | 0 | 0 | 1 | 0:1 |
| Emb-D | 24 (4,14,6) | 6 (3,3,0) | 0 | 3 (2,1,0) | 33 | 4:1 |
| On land | 1 (0,0,1) | 3 (0,3,1) | 0 | 0 | 4 | 1:3 |
| Total | 105 (45,44,16) | 30 (12,15,3) | 3 (0,3,0) | 9 (5,4,0) | 147 | 3.5:1 |

Sex, mean straight carapace length (SCL) and mass of adult Midland Painted Turtle, *Chrysemys picta marginata*, and Common Snapping Turtle, *Chelydra serpentina*, by wetland at the Tommy Thompson Park.

Sample sizes shown in parentheses, means are followed by ± 1 standard deviation.

| Wetland | Painted | | Snapping | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| TTP - sample<br>SCL (mm)<br>Mass (g) | (45)<br>127±12,<br>263± 63 | (44)<br>146±19<br>447±148 | (12)<br>295±66<br>7,265±4,252 | (15)<br>245±53<br>4,108±1,994 |
| Goldfish<br>SCL (mm)<br>Mass (g) | (14)<br>125±12<br>262±59 | (13)<br>149±9<br>482±80 | (1)<br>270<br>3,963 | |
| Triangle<br>SCL (mm)<br>Mass (g) | (26)<br>125±11<br>250±57 | (15)<br>139±21<br>401±162 | (3)<br>368±33<br>11,833±3,085 | (2)<br>292±4<br>5,025±671 |
| Cell-1<br>SCL (mm)<br>Mass (g) | | (1)<br>161<br>532 | (4)<br>319±31<br>9,035±2,269 | (6)<br>246±53<br>4,108±1,993 |
| Emb-D<br>SCL (mm)<br>Mass (g) | (4)<br>138±12<br>327±79 | (14)<br>148±23<br>452±184 | (3)<br>229±18<br>3,200±625 | (3)<br>202±11<br>2,245±321 |
| Emb- C<br>SCL (mm)<br>Mass (g) | | | | (1)<br>171<br>1,200 |
| Emb- B<br>SCL (mm)<br>Mass (g) | | (1)<br>153<br>509 | (1)<br>195<br>1,975 | |
| Emb- A<br>SCL (mm)<br>Mass (g) | (1)<br>139<br>352 | | | |
| On Land<br>SCL (mm)<br>Mass (g) | | | | (3)<br>284±14<br>5,800±435 |

Densities and biomass of captured turtles at selected productive wetlands in the Tommy Thompson Park, Toronto, ON. 2016-17 data.

Entries represent the total for all species including Red-eared sliders and Blanding's, with detail inside brackets for Painted (MPTU) and Snapping turtles (SNTU).

| Selected wetland | Area (ha) | Density-Individual per ha: All Species (MP, SN) | Mass All Species (kg) | Mass: MP, SN (kg) | Biomass: Total (MP, SN) (kg/ha) |
|---|---|---|---|---|---|
| Goldfish Pond | 0.05 | 660 (560, 20) | 22.939 | 9.988, 3.963 | 459 (200, 79) |
| Triangle Pond | 0.8 | 69 (61, 6) | 60.470 | 13.204, 45.505 | 76 (17, 57) |
| Cell-1 | 6.0 | 2.8 (0.2, 2) | 74.637 | 0.532, 68.487 | 12.4 (0.1, 11.4) |
| Embayment D | 7.0 | 4.7 (3.4, 0.9) | 32.886 | 8.403, 21.642 | 4.7 (1.2, 3.1) |
| Total | 13.85 | 10 (7.4, 1.7) | 190.932 | 32.127,139.597 | 13.78 (2.3, 10) |

Incidental fish capture (bycatch) by wetland area at the Tommy Thompson Park.
Number of fish (2016, 2017).

| Common name | Species | Goldfish | Triangle | Cell-1 | Emb-D | Emb-C | Total |
|---|---|---|---|---|---|---|---|
| Common Carp | *Cyprinus carpio* | | | 8,13 | 0, 14 | 1,1 | 9,28 |
| Pumpkinseed | *Lepomis gibbosus* | | | 0,1 | | 0,2 | 0,3 |
| Creek Chub | *Semotilus atromaculatus* | | 0,2 | | | | 0,2 |
| Northern Pike | *Esox lucius* | | | 0,2 | | | 0,2 |
| Brown Bullhead | *Ameiurus nebulosus* | | | 0,1 | | 0,1 | 0,2 |
| Temperate bass | *Morone chrysops* | | | 0,2 | | | 0,2 |
| Largemouth Bass | *Micropterus salmonides* | | | 0,1 | | | 0,1 |
| Gizzard Shad | *Dorosoma cepedianum* | | | 0,1 | | | 0,1 |
| Black Crappie | *Pomoxis nigromaculatus* | 0,1 | | | | | 0,1 |
| Bluegill | *Lepomis macrochirus* | | 0,1 | | | | 0,1 |
| Total | | 0,1 | 0,3 | 8,19 | 0,14 | 1,4 | 9,43 |

Movement of turtles equipped with a VHF transmitter during 2016/2017 at Tommy Thompson Park.

Individual water bodies inside the wetland complex are identified with a two-letter code as such: C1, C2, C3 (Cell#), EA, EB, EC, ED (Embayment A, B, C, or D), G (Goldfish Pond), T (Triangle Pond).

| Species | Notch Number | Sex | Initial capture point | Winter 2016 | Winter 2017 | Wetlands used over study period (some are used multiple times) | Minimum distance covered 2016-2017 | Size estimation of habitat used (ha) |
|---|---|---|---|---|---|---|---|---|
| Blanding's | 1 | F | G | G | G | C1, C2, C3, EA, EB, EC, G, T | 6.1 km | 112 |
| Blanding's | 2 | F | G | G | G | EA, EB, G | 0.8 km | 18 |
| Blanding's | 3 | F | G | G | G | EA, EB, G | 1.7 km | 18 |
| Snapping | 2 | F | T | T | T | EB, T | 0.4 km | 3 |
| Snapping | 5 | F | C1 | C1 | C1 | C1 | 0 km | 6 |
| Snapping | 8 | F | C1 | C1 | C1 | C1, C2 | 1.2 km | 17 |
| Snapping | 9 | F | C1 | C1 | C1 | C1, ED | 0.7 km | 30 |
| Snapping | 11 | F | ED | ED | ED | C1, ED | 0.7 km | 30 |
| Snapping | 12 | F | ED | ED | No signal | ED | 0 km | 8 |
| Snapping | 13 | F | ED | ED | ED | C1, ED | 0.7 km | 30 |
| Snapping | 17 | F | C1 | C1 | C1 | C1, C2 | 0.4 km | 17 |
| Snapping | 22 | F | T | | T | EB, T | 0.6 km | 3 |
| Snapping | 23 | F | C1 | | C1 | C1, ED | 0.7 km | 30 |
| Snapping | 25 | F | Road ED-C1 | | C1 | C1, EC, ED | 1.6 km | |
| Snapping | 26 | F | Road C1 | | ED | C1, ED | 0.3 km | 30 |
| Snapping | 27 | F | Road ED | | Ward Isl. | EA, ED, WI | 2.6 km | 168 |
| Snapping | 1 | M | G | G | Not tracked | G | 0 km | 0.1 |
| Snapping | 3 | M | C1 | T | Not tracked | C1, C2, C3, T | 2.5 km | 60 |
| Snapping | 4 | M | EB | EB | EB | EB | 0 km | 2 |
| Snapping | 6 | M | C1 | C1 | No signal | C1 | 0 km | 6 |
| Snapping | 7 | M | C1 | C1 | Not tracked | C1, ED | 0.7 km | 30 |
| Snapping | 10 | M | ED | ED | No signal | ED | 0 km | 8 |
| Snapping | 15 | M | ED | ED | No signal | EC, ED | 0.8 km | 31 |
| Snapping | 16 | M | ED | ED | ED | ED | 0 km | 8 |

Years of detected turtle species by visual surveys at TTP. 2004-2017 data.

| Species/Wetland | Goldfish | Triangle | Cell-1 | Cell-2 | Emb-D |
|---|---|---|---|---|---|
| Painted | 2004-2017 | 2012-2017 | 2004-2012, 2014 | | 2004-17 |
| Snapping | | | 2012 | | |
| Blanding's | 2007-2017 | | | | |
| Map | | | | 2015, 2016 | |
| Spiny Softshell | | | 2006, 2012, 2015, 2016 | 2017 | |