1 Potential sources of intra-population variation in painted turtle

2 (*Chrysemys picta*) hatchling overwintering strategy

3

4 **Julia L. Riley[1,2], Glenn J. Tattersall[3], and Jacqueline D. Litzgus[1,*]**

5

6 [1]Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury,

7 Ontario, P3E 2C6, Canada.

8 [2]Current address: Department of Biological Sciences, Macquarie University, Sydney,

9 New South Wales, 2109, Australia.

10 [3]Department of Biological Sciences, Brock University, St. Catharines, Ontario, L2S 3A1,

11 Canada.

12

13 *Author for correspondence (jlitzgus@laurentian.ca)

ABSTRACT

Many temperate animals spend half their lives in a non-active, overwintering state, and multiple adaptations have evolved to enable winter survival. One notable vertebrate model is *Chrysemys picta* whose hatchlings display dichotomous overwintering strategies: some hatchlings spend their first winter aquatically after nest emergence in fall, while others overwinter terrestrially within their natal nest with subsequent spring emergence. Occurrence of these strategies varies among populations and temporally within populations; however, factors that determine the strategy employed by a nest in nature are unknown. We examined potential factors that influence intra-population variation in *C. picta* hatchling overwintering strategy over two winters in Algonquin Park, Ontario. We found that environmental factors may be a trigger for hatchling overwintering strategy: fall-emerging nests were sloped towards the water and were surrounded by a relatively higher percentage of bare ground compared to spring-emerging nests. Fall-emerging hatchlings were also relatively smaller. Overwintering strategy was not associated with clutch oviposition sequence, or mammalian or avian predation attempts. Instead, fall emergence from the nest was associated with the direct mortality threat of predation by Sarcophagid fly larvae. Body condition and righting response, measured as proxies of hatchling fitness, did not differ between overwintering strategies. Costs and benefits of overwintering aquatically versus terrestrially in hatchling *C. picta* are largely unknown, and have the potential to affect population dynamics. Understanding winter survival has great implications for turtle ecology, thus we emphasize future research areas on dichotomous overwintering strategies in temperate hatchling turtles.

INTRODUCTION

Factors influencing population dynamics are well-studied in animal ecology and conservation. Many environmental factors impact population dynamics because they directly influence reproduction and survival, especially during particular seasons (Aars and Ims, 2002). For temperate animals, winter is a severe energetic challenge that greatly influences both survival and the subsequent year's reproductive output (Sendor and Simon, 2003). Winter adaptations include behavioural means of avoiding low temperatures (e.g., migration, habitat selection), and physiological changes that permit survival at low temperatures (e.g., biochemical adjustments, reduced metabolic rate; Marchand, 2013). Many species combine adaptations for an overwintering strategy that effectively promotes winter survival. Juvenile life-stages experience lower survival, particularly within the first year of life, than adults; this trend is consistent across juvenile endotherms (small mammals, Muchlinski, 1998; Sendor and Simon, 2003; birds, Peach et al., 2003; rodents, Marchand, 2013) and ectotherms (salmonids, Huusko et al., 2007; amphibians, Tester and Breckenridge, 1964; Resetarits, 1986; McCafferya and Maxellb, 2010; snakes, Viitanen, 1967; Parker and Brown, 1980; Macartney, 1985; Brentcharlan, 1989; turtles, Ultsch, 2006). Overwintering survival greatly impacts juvenile recruitment and can, in turn, affect population dynamics. For example, in *Vipera vipera,* winter climate primarily affects population growth/decline through its effects on juvenile survival (Altwegg et al., 2005). Winter exerts substantial selection pressures on overwintering strategies that optimize survival.

Interestingly, some animals exhibit intraspecific variation in overwintering strategy, particularly with respect to biochemical adjustments used to survive freezing temperatures. For example, many invertebrates vary in their ability to tolerate ice formation within body tissues (i.e., freeze tolerance) and their ability to lower the temperature of cellular fluid below its freezing point (e.g., supercooling, Baust et al., 1979; Baust and Lee, 1981; Tanaka 1997; Lombardero et al., 2000). Also, *Hyla versicolor* differs in its capacity for freeze tolerance along a latitudinal gradient (Costanzo et al., 1992). Many turtles during the hatchling life-stage (the first year of life) exhibit behavioural and physiological variation in overwintering strategies and can spend winter in either aquatic or terrestrial sites (Ultsch 2006, Gibbons 2013, Lovich et al. in press). Hatchlings that overwinter terrestrially either avoid freezing temperatures by burrowing below the frostline (e.g*., Terrepene ornata*; Ultsch, 2006), or remain within their nest cavity

68 (above the frostline) and survive by either supercooling or freeze tolerance (e.g., *Chrysemys*

69 *picta*, *Graptemys geographica*; Costanzo et al., 2008). Although an aquatic environment buffers

70 turtles from freezing temperatures because of water's high thermal buffering capacity (Costanzo

71 et al. 1992), overwintering terrestrially within the nest is thought to be the preferred strategy for

72 turtle hatchlings (Gibbons, 2013; Lovich et al. in press).

73   One field-based question that has yet to be answered is why, within a single turtle

74 population, is overwintering strategy variable? Two main hypotheses have been proposed to

75 explain variability in hatchling turtle overwinter strategy; firstly that overwintering in the nest is

76 a passive response to environmental conditions that hamper fall emergence, and secondly that

77 overwintering strategy is a plastic response to nest environmental factors and increases offspring

78 fitness. The first hypothesis stems from the ideas that hatchlings may be (1) unable to emerge in

79 the fall because they cannot penetrate encrusted overlying soil, (2) clutches laid later in the

80 season may not emerge because they require additional developmental time, and/or (3) fall

81 emergence is prevented by the cool weather that reduces mobility of hatchlings (Obbard and

82 Brooks, 1981; Tinkle et al., 1981; DePari, 1996; Blouin-Demers et al., 2000; Parren and Rice,

83 2004). The second hypothesis asserts that in-nest overwintering confers benefits that increase

84 survival. Larger hatchling size confers greater overwintering survival (Mitchell et al., 2014),

85 perhaps because larger hatchlings have more liver and carcass mass, the tissues used by

86 hatchlings to support energetic costs of overwintering (Muir et al., 2013). In contrast, Costanzo

87 et al. (2004) theorized that smaller hatchlings might supercool more readily, and thus survive

88 lower sub-freezing temperatures, because the likelihood of lethal freezing increases with fluid

89 volume. Nest environment also affects hatchling overwinter survival; for example, winter

90 mortality is greatest when temperatures are lowest and soil moisture is highest (Storey et al.,

91 1988; Costanzo et al., 2000, 2001, 2004). Fall emergence could be to avoid a direct mortality

92 threat; for instance, nest infestation by Sarcophagid fly larvae may stimulate emergence

93 (Warkentin, 1995; Bolton et al., 2008; Spencer and Janzen, 2011). Finally, overwintering in-nest

94 may provide hatchlings with additional time to develop in a lower risk environment (Costanzo et

95 al., 2008).

96   The purpose of our study was to elucidate why overwintering strategy used by hatchlings

97 varies within a population using the model species, *C. picta*. Generally, a clutch of eggs is

98 oviposited in the spring or summer, and the eggs hatch 89-99 days later (Ernst and Lovich,

99 2009). *C. picta* hatchlings may emerge from the nest in the fall and are assumed to move to

100 aquatic sites, or they remain in the nest throughout the winter and emerge the following spring

101 (Hartweg, 1944; Cagle, 1954; Woolverton, 1961; Gibbons and Nelson 1978; Churchill and

102 Storey, 1992; Costanzo et al., 2008). The relative frequency of these two overwintering strategies

103 varies temporally and geographically (Costanzo et al., 2008; Gibbons, 2013; Lovich et al., in

104 press), but fall emergence and subsequent assumed aquatic overwintering has been documented

105 throughout the range of C. *picta* (Finneran, 1948; Ernst, 1971; Christiansen and Gallaway, 1984;

106 St. Clair and Gregory, 1990; DePari 1996; Rozycki, 1998; Waye and Gillies, 1999; Pappas et al.,

107 2000; Costanzo et al., 2004; Carroll and Ultsch, 2007). In order to examine variation in

108 overwintering strategy, our study had multiple objectives:

109   1) To explore if variation in overwintering strategy can be explained by nest environmental

110   factors, concentrating on factors that have been highlighted in previous studies: heat units

111   accumulated over incubation (Storey et al., 1988), vegetative cover, nest oxygen

112   availability (Costanzo et al., 2001; Rafferty and Reina, 2012), oviposition date, and soil

113   moisture (Costanzo et al., 2000, 2001), organic content (Costanzo et al., 1998) and texture

114   qualities (Packard and Packard, 1997; Costanzo et al., 1998).

115   2) To determine if overwintering strategy benefitted hatchlings. We tested whether

116   mammalian/avian predators or predatory Sarcophagid fly (*Tripanurga importuna*) larvae

117   triggered hatchlings to emerge. We compared body size of hatchlings before overwintering

118   to determine if hatchling size differed between strategies. Also, we compared proxies for

119   hatchling fitness (body condition and righting response) prior to overwintering between

120   strategies to determine if hatchlings that stayed in-nest would benefit from a longer

121   developmental time.

122   3) To examine risks associated with overwintering aquatically versus terrestrially by

123   comparing winter temperature and available oxygen between marshes and nests. We

124   predicted that winter temperatures in marshes would be higher than in nests, but that winter

125   oxygen would be higher in nests than in marshes.

126   4) Finally, we undertook a preliminary investigation of maternal influence on overwintering

127   strategy by examining whether the strategy was the same between multiple clutches from

128   the same female (Friebele and Swarth, 2005).

129

130   **RESULTS**

131   **Observational data**

132   In 2010-11, 2 nests emerged in fall and 23 overwintered in-nest (5 were excavated in fall). In

133   2011-12, 16 nests emerged in fall and 20 overwintered in-nest (6 were excavated in fall). Mean

134   hatching success differed significantly between fall and spring-emerging clutches (W_1=322,

135   P=0.02), with higher hatching success in fall (84 ± 5%) compared to spring-emerging clutches

136   (61 ± 6%). Overwintering success of hatched individuals in spring-emerging clutches was 67 ±

137   8%.

138   We found that of hatchlings tracked post-emergence, 45% (38/85) reached water within 24

139   hours post-release. The other turtles moved towards grass and woody brush piles away from the

140   nearest water body (43%), and 12% were not found.

141   Hatchlings emerged from nests that accumulated more heat units (ºD) over incubation than

142   nests wherein hatchlings remained (Table 1). Hatchlings emerged from nests that were warmer

143   over winter compared to nests in which hatchings remained (Table 2; t_{17}=2.11, P<0.01). The

144   lowest winter temperature within a nest was -4.9ºC (Table 2). Nests from which hatchlings

145   emerged in fall spent on average 500 ± 142 hours below -0.6ºC (freezing point of body fluids)

146   and 28 ± 21 hours below -4ºC (limit of freeze-tolerance). Nests in which hatchlings overwintered

147   spent on average 940 ± 185 hours below -0.6ºC and 11 ± 6 hours below -4ºC. The first principal

148   component for soil characteristics did not differ between fall and spring emerging nests

149   (t_{32}=1.74, P=0.09). The nests contained low organic content (mean = 2.1 ± 0.3%), and consisted

150   of predominately sandy soil (means: 22 ± 2% gravel, 13 ± 1% coarse sand, 39 ± 2% sand, and 17

151   ± 1% fines).

152

153   **Nest environment and overwinter strategy**

154   The model that had the lowest AIC_c score (30.36) included the two environmental variables: nest

155   slope and bare ground 1 m^2 around the nest (R^2_N=0.41; Table 3). Hatchling painted turtles were

156 more likely to emerge from nests surrounded by bare ground (β=-0.51, *LCL*=-1.21, *UCL*=0.03)

157 and sloped towards water (β=-0.17, *LCL*=-0.39, *UCL*=-0.03).

158 Overwintering strategy did not differ significantly between first and second clutches

159 (*P*=0.59). Thirty percent of first clutches emerged in the fall and 70% overwintered in the nest,

160 and similarly 20% of second clutches emerged in fall and 80% overwintered in-nest.

161

Potential benefits to hatchlings

163 The number of mammalian/avian predation attempts did not affect overwinter strategy (*P*=0.95).

164 Mean number of predation attempts on a clutch was 0.3 ± 0.2 per nest for fall-emerging nests,

165 and 0.3 ± 0.1 per nest for clutches that overwintered in-nest. Sarcophagid fly larvae nest

166 infestation was significantly related to hatchling overwinter strategy (*P*=0.04); 50% of fall-

167 emerging nests were infested with fly larvae, whereas 23% of spring-emerging nests had

168 evidence of fly larvae infestation (Table 4).

169 Body condition of hatchlings was not different among those that emerged in the fall, those

170 that were excavated in the fall but would have overwintered in-nest, and spring-emerging

171 hatchlings ($F_{2, 49}$=1.52, *P*=0.47). Hatchlings with a smaller carapace length were more likely to

172 emerge from nests in fall (Wald's z-statistic=2.35, *P*=0.02, R^2_N=0.21). In the fall, hatchlings

173 from nests that emerged had a mean carapace length of 26.21 ± 0.25 mm, and hatchlings that

174 would have overwintered but were excavated had a carapace length of 26.66 ± 0.46 mm (Fig. 1).

175 Spring-emerging hatchlings had shorter carapaces (mean = 25.18 ± 0.52 mm) post-overwintering

176 when compared to hatchlings in the fall pre-overwintering ($F_{2, 48}$=5.83, *P*<0.01; Fig. 1).

177 Hatchlings with a smaller mass were also more likely to emerge from nests in fall (Wald's z-

178 statistic=1.94, *P*=0.05, R^2_N=0.13). In the fall, hatchlings from emerged nests weighed 4.58 ± 0.50

179 g, and hatchlings from excavated nests weighed 4.59 ± 0.61 g. Mass was not different between

180 fall and spring-emerging hatchings ($F_{2, 48}$=2.59, *P*=0.09); spring-emerging hatchlings had a mean

181 mass of 4.24 ± 0.52 g.

182 Latency period (LP) of hatchlings did not differ among sampling periods ($F_{2, 49}$=1.80,

183 *P*=0.41). Mean LP of fall-emerging hatchlings was 110 ± 31 s, and of hatchlings that were

184 excavated in the fall was 115 ± 27 s. Mean LP of spring-emerging hatchlings post-overwintering

185 was 121 ± 27 s. The second measurement in righting response, righting period (RP), also did not

186 differ among sampling categories ($F_{2, 49}$=0.40, P=0.82). Mean RP of fall-emerging hatchlings

187 was 13 ± 7 s, of hatchling that were excavated in fall was 16 ± 6 s, and of spring-emerging

188 hatchlings was 26 ± 9 s.

189

190 **Maternal influence on overwintering strategy**

191 From 2010-2012, there were 6 females for which we sampled two clutches during a nesting

192 season. Out of these 6 double clutches, 4 (67%) exhibited the same overwintering strategy.

193

194 **Comparison between aquatic and terrestrial overwintering sites**

195 Winter temperatures differed between putative hibernation sites in marshes and nest cavities

196 (t_{53}=2.01, P<0.01). The lowest marsh temperature ranged from -11.9 to 2.8ºC, and mean marsh

197 temperature was 3.7 ± 0.2ºC. The lowest in-nest temperature ranged from -4.9 to -1.7ºC, and

198 mean temperature was 2.4 ± 0.1ºC. Dissolved oxygen in marshes ranged from 2.1 to 95% air

199 saturation from September 2011 until March 2012, and mean dissolved oxygen was $37 \pm 4\%$ air

200 saturation. Available oxygen in nests over the same time period ranged from 20.6 to 21.2%, and

201 mean nest oxygen available during winter was $21.0 \pm 0.01\%$.

202

203 **DISCUSSION**

204 **Observational findings**

205 Hatching success was higher in fall-emerging nests than in spring-emerging nests, suggesting

206 that conditions in the nest are not optimal for development and that overwinter strategy may be a

207 passive response to a subpar nest environment. Winter survival in-nest depends on many factors,

208 including nest temperature. In our study, nest temperatures fell below -4ºC during winter (in 9

209 out of 19 nests), and overwintering success of spring-emerging clutches was $67 \pm 8\%$.

210 Hatchlings remained overwinter in cooler nests when compared to nests from which

211 hatchlings emerged. Also, clutches that overwintered in-nest experienced less time below the

212 temperature limit of freeze-tolerance (< -4ºC), below which they may have had to employ

213 supercooling to survive. Due to our field study limitations, the exact physiological mechanism

214 hatchlings used to survive overwinter in our study is unknown. The four nests in which

215 hatchlings remained overwinter that fell below -4ºC experienced 0–100% overwintering success.

216 Perhaps hatchlings are remaining in nests that keep them cool with a concomitant reduced

217 metabolic rate overwinter, while still at temperatures above the limit for freeze tolerance.

218 Paterson et al. (2012) also found evidence that hatchling *Emydoidea blandingii* and *Glyptemys*

219 *insculpta* selected overwintering sites with cooler temperatures than random plots. This begs the

220 question, how might hatchlings in the fall sense the future temperature conditions in their nest?

221 Are hatchlings using another, closely-related environmental variable to assess future nest

222 temperatures?

223   Tracking hatchlings post-emergence in the fall revealed that approximately half the

224 hatchlings successfully reached aquatic sites within 24 hours. The other hatchlings moved

225 towards grass and woody brush piles away from the nearest water body, and buried themselves

226 2-5 cm into the leaf litter. It is important to note that we tracked hatchlings for a limited time

227 period (24 hours), and their movements post-tracking are unknown. Hatchling *C. picta* natural

228 post-emergence movements have not been previously studied (Ultsch, 2006), and it has been

229 assumed that all fall-emerging hatchlings move directly towards water (Costanzo et al., 1995).

230 Our findings suggest there may be more variability in post-emergence movements. Warner and

231 Mitchell (2013) examined *C. picta* post-emergence movements within a small arena and found

232 that hatchlings tended to move directly towards water. In contrast, Congdon et al. (2011) found

233 that naïve hatchling *C. picta* within an arena oriented towards nearby, open, illuminated horizons

234 regardless of whether the environment was aquatic or not. Post-emergence in the fall, the other

235 half of tracked hatchlings buried themselves in terrestrial sites. Similarly, terrestrial

236 overwintering after fall emergence has been observed in *Malaclemys terrapin* (Muldoon and

237 Burke, 2012), another species with dichotomous hatchling overwintering strategies (Baker et al.,

238 2006). Interestingly, there was also evidence that fall-emerging hatchlings utilized autumn to

239 increase their energy reserve: one of the fall-emerging hatchlings was observed actively foraging

240 in water for aquatic insects less than 7 hours post-emergence. Painted turtle hatchlings may

241 emerge in the fall to build up needed energy reserves for winter survival. Our findings highlight

242 that hatchling post-emergence movements and overwintering behaviours remain largely

243 unknown, even in a well-studied model study species such as *C. picta*.

244

245 **Nest environment and overwintering strategy**

246   Our observational findings suggest that nest environmental factors may influence overwintering

247   strategy, but these findings were not conclusively supported by our statistical modelling. We

248   found some evidence that overwintering strategy is influenced by vegetation and slope of the

249   ground surface at the nest; although the effect strength of both environmental variables was

250   weak; Nagelkerke's R-squared value (R^2_N=0.41) suggests that these two variables explain a little

251   less than half of the variability in overwintering strategy. There were 7 other models within 2

252   delta AIC_c of the model with the lowest AIC_c, and these other models included additional

253   environmental variables: average percent oxygen, oviposition date, total heat units, and nest soil

254   principle component 1 (Table 3). Of these 8 models within 2 delta AIC_c of each other, the one

255   with the highest R^2_N included vegetation and slope of the nest, and also oviposition date and the

256   total heat units accumulated over incubation (R^2_N =0.60). Our limited sample size restricts the

257   conclusions that we can derive from our models because our statistical power was limited. But

258   overall, the models suggest that environmental variables may affect overwintering strategy in

259   painted turtles. In contrast, Friebele and Swarth (2005) found that nest environment did not

260   influence overwintering strategy in *Pseudemys rubriventris* hatchlings; however, they did not

261   examine the same environmental variables used in our study.

262   As nest slope towards water increased, hatchlings were more likely to emerge in fall. One

263   explanation for this relationship is that as slope increases it approaches the critical angle of

264   repose, which is the steepest angle at which a material can be before it collapses (Graf, 1984;

265   Jaeger et al., 1989). Thus, it would be beneficial for hatchlings to leave nests at steeper slopes

266   that are unstable and could increase mortality. The angle of repose for sand is 35°, yet the

267   steepest nest slope in our study was 22° so the risk of collapse was low. Furthermore, turtle nest

268   collapse appears rare: sea turtle (*Chelonia mydas*) nest collapse, due to sand textural

269   characteristics, has been reported to cause hatchling mortality (Mann, 1977), and one *Glyptemys*

270   *insculpta* nest, on a very steep slope, collapsed during incubation (Riley pers. obs.). Nest

271   collapse may cause hatchlings to emerge from steeply-sloped nests, but it is more likely that

272   slope is related to other environment factors.

273   Steeper slopes may increase the temperature of nests because of enhanced solar radiation

274   absorbance (Schwarzkopf and Brooks, 1987; Wood and Bjorndal, 2000). Warmer nests increase

275   developmental rate and allow hatchlings to complete development by an earlier date. Once

276   development is completed, hatchlings may need to emerge from nests because warm nests might

277   reach lethally high temperatures, and hatchlings may experience high metabolic rates, causing

278   them to quickly consuming their yolk reserves and exit nests to forage prior to winter (Muir et

279   al., 2013). Our models suggest that fall-emerging hatchlings are from nests that accumulated

280   more heat units over incubation (i.e., spent more time within optimal developmental

281   temperatures, Holt, 2000). Nest temperatures, stability, and slope of the nest may be inter-related

282   variables, but how hatchlings can perceive nest slope or future temperature post-hatching while

283   within the nest remains unknown.

284   Our models also suggest that hatchlings emerge from nests surrounded by bare ground.

285   Less vegetation above a nest can result in lower temperatures during winter; without plants and

286   leaf litter to trap an insulating layer of air between the snow and ground, nest temperatures

287   become colder. Although it is logical to directly relate nest environment to its effects on

288   hatchlings, environment may also affect presence of organisms that in turn affect hatchlings

289   (e.g., predatory fly larvae). Sarcophagid flies lay their eggs in sandy areas, and larvae burrow

290   through sand into a nest (Bolton et al., 2008). Sarcophagid flies may avoid laying eggs in turtle

291   nests with high levels of vegetation, and our study shows that hatchlings emerge from nests with

292   Sarcophagid larvae (discussed below). Are environmental factors directly triggering hatchling

293   response, or indirectly affecting overwintering strategy by altering presence of predatory flies?

294   Nest environment is complex, with many inter-related factors potentially influencing

295   development of hatchling turtles. More research is required to understand how hatchlings sense

296   their nest environment. Our discussion about environmental factors that influence overwintering

297   strategy is mainly speculative because of the weak effect the environmental variables we

298   measured had on overwintering strategy, yet we hope our discussion inspires avenues of

299   subsequent research.

300

301   **Potential benefits to hatchlings**

302   Fall emergence appears to lower the risk of predation by Sarcophagid fly larvae. Sarcophagid

303   larvae opportunistically prey upon turtle eggs and hatchlings (Iverson and Perry, 1994; Smith,

304   2002; Bolton et al., 2008), and fly larvae cause direct mortality of hatchlings (Gillingwater,

305   2001). In contrast, disturbance by avian and mammalian predators did not appear to trigger

306   hatchling emergence (but note that our nests were caged, and nest predator behaviour around

307   uncaged and caged nests may differ, but see Riley and Litzgus, 2013). Overall, variation in

308   overwintering strategy may be related to avoidance of a direct mortality threat from Sarcophagid

309   fly larvae. Overwintering in-nest is thought to be the *de facto* strategy (Gibbons, 2013), and

310   perhaps predation by fly larvae has triggered a plastic response of fall emergence and a

311   subsequent alternative terrestrial or aquatic overwintering strategy.

312   Fall-emerging hatchlings were smaller than hatchlings that overwintered in-nest. This

313   finding is in contrast to the idea that smaller hatchlings should remain in-nest because they likely

314   have increased supercooling abilities (Costanzo et al., 2004). Ice nucleation occurs when water

315   molecules form ice in an organism (Rasmussen and MacKenzie, 1973), and probability of ice

316   nucleation increases with fluid volume (Vali, 1995). Our findings are supported by a recent study

317   by Mitchell et al. (2014) that found survival overwinter was higher for larger hatchlings. There

318   may be a size threshold at which hatchlings leave the nest in the fall to forage to build up

319   energetic reserves prior to overwintering. Proxies for hatchling fitness (body condition and

320   righting response) were not related to hatchling overwintering strategy, which contradicts the

321   hypothesis that overwintering in-nest may be a *de facto* response to a lower developmental state

322   (Costanzo et al., 2008).

323

Maternal influence on overwintering strategy

325   There may be some degree of parental influence on overwinter strategy, but overwintering

326   strategy was not consistently associated with maternal identity. In our study, 4 out of 6 females'

327   nest pairs exhibited the same overwinter strategy. In contrast, *P. rubriventris* nests oviposited by

328   the same female did not exhibit the same overwinter strategy (Freibele and Swarth, 2005);

329   however, their sample size was small (N=2). Perhaps *C. picta* overwintering strategy is

330   paternally influenced; if clutches oviposited by the same mother have different fathers, their

331   overwintering strategy may differ. Paternity was unknown in our study; however, *C. picta* first

332   and second clutches can share paternity 97.5% of the time (McGuire et al., 2011). Overall, the

333   low sample size (N=6) limits the inferences we can make regarding genetic influence on

334   overwintering strategy.

335

Comparison between aquatic and terrestrial overwintering environments

Putative aquatic overwintering sites had higher winter temperatures than terrestrial nests. In Indiana, aquatic overwintering sites also experienced higher temperatures than nests (Costanzo et al., 2008). Winter temperatures were colder but more stable in natal nests. Marshes experienced more fluctuations in winter temperatures thus exposing hatchlings to potentially deleterious environmental extremes. In our study, some aquatic overwintering sites reached lethal minimum temperatures, but others did not even reach freezing temperatures during winter (Table 2). Exposure to temperature extremes increases mortality either by exposure to freezing temperatures or as a result of increased metabolic rate causing energy depletion and death (Greaves and Litzgus, 2007; Edge et al., 2009; Muir et al. 2013). Thus, in regards to temperature, natal nests pose less of a winter survival risk to hatchling *C. picta*.

More oxygen was available in nests than in marshes. Dissolved oxygen in marshes ranged from close to anaerobic (0% air saturation) to normoxic levels (above 80% air saturation); however, on average marsh oxygen was close to hypoxic levels (below 30% air saturation). Winter oxygen concentration in nests was consistently similar to the oxygen concentration of air (21.0% oxygen); thus, gas exchange is likely not a challenge to terrestrially overwintering hatchling turtles that breathe with their lungs. In contrast, gas exchange in aquatic sites presents a challenge during winter, especially because hatchlings have a lower anoxia tolerance compared to adults (Packard and Packard, 2004; Reese et al., 2004), and must rely on less-efficient cutaneous routes of gas exchange. Lower anoxia tolerance in hatchlings may itself be a driving factor in the prevalence of terrestrial overwintering by hatchling turtles (Gibbons, 2013).

CONCLUSION

Overwintering strategy of hatchling *C. picta* may be influenced by nest environmental factors. Smaller hatchlings were more likely to emerge from the nest in the fall, which may indicate that they use the fall to increase energy reserves prior to overwintering. Hatchlings were significantly smaller post-overwintering, which indicates that overwintering terrestrially may influence future survival. Yet, fitness consequences of dichotomous overwintering strategies are unknown, and more research is needed to understand the costs of overwintering aquatically versus terrestrially for hatchling turtles. Most notably, Sarcophagid fly larvae may be stimulating fall emergence,

13

366 such that hatchling overwintering strategy is associated with predator avoidance. Our study was a

367 first step in elucidating the factors that cause within-population variation in a turtle species with

368 a dichotomous hatchling overwinter strategy.

369

MATERIALS AND METHODS

Study area

372 The two study sites are in Algonquin Provincial Park, Ontario, Canada. The western site is

373 located along the Highway 60 corridor west of the Wildlife Research Station (WRS, 45º 35' N,

374 78º 30' W). The eastern site is 60 km northeast of the first (45º 87' N, -77º 77' W). Habitat at

375 both sites consists of a mosaic of water bodies within forest (Edge et al., 2010). Elevations at the

376 western site are higher (*ca*. 585 m above sea level) than at the eastern site (*ca.* 150 m above sea

377 level), and consequently the first site experiences a colder and wetter climate (Ontario Ministry

378 of Natural Resources, 1998). Nest data were pooled from both sites to address our study

379 objectives.

380

Field sampling

382 *Nest Site Monitoring*

383 Monitoring of nesting sites began when females started exhibiting nesting behaviours, and

384 occurred from 20 May to 20 June in 2010, and from 5 June to 4 July in 2011. Nest sites were

385 searched by researchers on foot from dawn (~5:00) to about 10:00, and in the afternoon from

386 dusk (~17:00) until nesting activity ceased. Both first and second clutches were sampled; this

387 allowed examination of maternal effects and whether overwinter strategy depends on oviposition

388 date. Nest site monitoring ceased when three days elapsed continuously without nesting

389 behaviours.

390   Nests were excavated after females completed oviposition. As eggs were removed, they

391 were numbered using a pencil to ensure they were returned to the nest in the same order and

392 orientation, and then placed in moist vermiculite in a plastic bin. After eggs were removed, nest

393 cavities were filled with excavated nest cavity soil to prevent desiccation. Eggs were transported

394 in a vehicle to a field lab at the western site, and measured in the field at the eastern site. Mass

395 and dimensions of eggs were measured for a long-term study (R.J. Brooks, University of Guelph

396 and J.D. Litzgus, Laurentian University). Measuring occurred within 24 hours post-oviposition,

397 prior to the vitteline membrane adhering to the shell surface (Yntema, 1968; Rafferty and Reina,

398 2012), ensuring no trauma to developing embryos (Samson et al., 2007).

399 Eggs were reburied in the original nest cavity, with a waterproofed temperature data logger

400 that recorded temperature hourly in the centre. Data loggers were either an iButton[®] (accuracy of

401 ± 1ºC or 0.5ºC; Thermochron DS1921G; Dallas Semiconductor, Sunnyvale, CA), or a HOBO

402 Stowaway[®] (accuracy of ± 0.2ºC; TidbiT TBI32-05+37; Onset Computer Corp., Bourne, MA).

403 Data logger types and the waterproofing methods did not differ in temperature readings (Roznik

404 and Alford, 2012; our data: $F_{3, 2480}$=2.01, P=0.94). Also, in 2011, a golf Wiffle[®] ball (4 cm

405 diameter, Wiffle Ball Inc., Shelton, CT) with 30 cm of tubing (Tygon®, R-3603, Fisher

406 Scientific, Whitby, ON) extending into the middle of the Wiffle[®] ball was buried in the centre of

407 the nest cavity with tubing extending out of the ground. A two-way stop cock closed the tube

408 from the environment. This system was used to measure oxygen in the nest (see below). Finally,

409 each nest was covered by a wire cage to prevent depredation. Cages were made of 1 cm mesh

410 hardware cloth, and were open-bottom cubes with dimensions of 30 x 30 x 40 cm with 8 cm

411 flaps (Riley and Litzgus, 2013).

412

413 *Nest Environment Monitoring*

414 In 2010, once per month from oviposition to October, and on the day of emergence,

415 environmental variables were measured. In 2011, environmental variables were measured bi-

416 weekly from oviposition to October, on the day of emergence, and monthly during winter

417 (October to April). Environmental measurements were collected on: vegetation cover, soil

418 moisture, nest slope, and oxygen. Vegetation cover was estimated by placing a 1-m^2 quadrat on

419 the ground with the nest in the middle; percentage of bare ground, herbaceous plants, woody

420 plants, and leaf litter within the quadrat were visually estimated (Wilson, 1998). Soil samples

421 (~150 g) were collected 30 cm away from the nest at the same depth; the sample was held in a

422 sealed glass bottle until measurement of soil moisture. Slope of the ground above the nest was

423 measured using a level with a rotating vial (Fatmax® Xtreme Torpedo Level, 43-609, Stanley

424 Tools Canada, Oakville, ON) angled towards the closest water body.

425   Nest oxygen was measured by drawing air out of the nest though the Wiffle® ball and tube

426   system, and then through an oxygen sensor (S102 Flow-through Oxygen Sensor, Quibit Systems

427   Inc., Kingston, ON) using a DC air pump (Garrett et al., 2010). The oxygen sensor was

428   calibrated using ambient air prior to each measurement. Using volume of the average nest cavity

429   (32 cm^3), Wiffle® ball (17 cm^3), and tubing (4 cm^3), and the rate at which the DC air pump

430   pulled oxygen from the nest (176 cm^3/min), we estimated that the time for oxygen in the nest to

431   reach the sensor was approximately 20 sec. Thus, the oxygen measurement at 30 sec was used in

432   analysis, as we wanted to examine steady-state values.

433

434   *Monitoring Nest Predator Interactions*

435   Nests were surveyed daily during nesting and hatching seasons for depredation attempts, and

436   weekly in July and August (Burke et al., 1998; Kolbe and Janzen, 2002). A 'depredation attempt'

437   was recorded if substrate was cleared away from around the nest cage, and/or the nest cage was

438   unearthed. After recording a depredation attempt, soil was replaced so that multiple attempts

439   could be recorded (Riley and Litzgus, 2014).

440

441   *Hatching Season Monitoring*

442   Hatchlings were collected and sampled during three periods: 1) natural fall emergence, 2)

443   excavated in late fall from nests in which they would have remained overwinter, and 3) natural

444   spring emergence. The demarcations of each period are described below.

445   Known incubation duration for painted turtles (Ernst and Lovich, 2009) was used to

446   estimate emergence time at which time daily monitoring for fall emergence began. When a nest

447   emerged, hatchlings and un-hatched eggs were collected and the presence or absence of

448   Sarcophagid fly larvae within the nest was recorded (Chidester, 1915; Bolton et al., 2008). Data

449   loggers were left in nests to record winter temperatures (see below).

450   A proportion of the un-emerged nests (N=11/61, 5 nests in 2010 and 6 in 2011) were

451   excavated in late fall, and the hatchlings and un-hatched eggs were collected. Excavation

452   occurred after daily mean air temperature dropped below 5ºC for three consecutive days. In

453   2010, nests were excavated from 1 to 4 October, and in 2011 nests were excavated from 2 to 7

454   October. These hatchlings, if not excavated, would have likely overwintered in-nest as

455 temperatures had become too cool for emergence. These hatchlings were used in comparisons of

456 body size and condition, as well as righting response (see below) between fall-emerging

457 hatchlings and hatchlings preparing to overwinter in-nest.

458   In spring 2011, nests were monitored daily for spring emergence after mean air

459 temperatures rose above freezing consistently (late-April to early-May). In spring 2012, nests

460 were excavated from 6-10 April. Hatchlings and un-hatched eggs were collected, and probable

461 cause and time of death for any dead hatchlings was noted - whether death occurred during

462 incubation/overwinter and was due to freezing/energy depletion or destruction by roots and/or fly

463 larvae. Hatchlings that died during winter due to freezing/energy depletion had little trauma and

464 were not decomposed. Hatchlings that died during incubation because of plant roots were

465 covered in root masses, and hatchlings that died because of fly larvae predation consisted of only

466 skin and scutes. Hatchlings that died during incubation also were more decomposed (e.g., scutes

467 flaking off, body parts rotten).

468

469 *Hatchling Variables Measured*

470 Hatchling midline carapace and plastron lengths were measured to the nearest 0.01 mm (3148,

471 Traceable Digital Calipers, Control Company, Friendswood, TX). Hatchling mass was measured

472 using a digital scale (SP202, Scout Pro, Ohaus Corporation, Pine Brook, NJ) to the nearest 0.1 g.

473   Each hatchling underwent a righting test, which consisted of placing each hatchling on its

474 carapace on a cloth-covered board (30 x 15 cm) and waiting for the turtle to flip over onto its

475 plastron. Two variables were measured: (1) latency period (LP) which is time from placement

476 until the first righting attempt, and (2) righting period (RP) which is time from first righting

477 attempt until successful righting (Rasmussen and Litzgus, 2010; Riley and Litzgus, 2013).

478 Temperature in the lab was recorded for each trial (range 17 to 27ºC). Each trial was recorded

479 with a digital camera (Photosmart R742, Hewlett-Packard Development Company, Mississauga,

480 ON), and LP and RP were scored from the videos. Turtles that cannot right themselves are more

481 likely to be predated, to desiccate and/or drown (Finkler and Claussen, 1997). Thus, righting

482 ability is considered to represent a hatchling's future survival (Freedberg et al., 2004; Delmas et

483 al., 2007). Hatchlings were processed within 24 hours, and released at their nests.

484   During release, we examined if hatchling *C. picta* move to aquatic overwintering sites after

485   fall emergence (Costanzo et al., 1995). In 2011, before release, fall-emerging hatchlings (N=85)

486   were dusted with ultraviolet light-activated fluorescent powder (UV Phosphorus Powder,

487   Singapore). We avoided contact with eyes, mouth, nostrils and cloaca (Stapp et al., 1994). At

488   night, hatchling trails were tracked using hand-held UV lamps (Raytech Raytector 5, Model R5-

489   FLS-2, Middletown, CT), and we recorded whether trails led towards or away from water during

490   this first 24 hours.

491

492   *Winter Environmental Monitoring*

493   Nest and aquatic overwintering environments were monitored from October 2011 to April 2012.

494   Nest oxygen (using the method described above) was measured monthly. Temperature loggers

495   recorded data hourly at a nest depth of 10 cm. Aquatic overwintering environment was

496   monitored over the same period; firstly, by measuring dissolved oxygen (DO) content in the

497   marshes (with overwintering adult turtles) adjacent to nests. DO was measured at a standardized

498   water depth of 60 cm using a DO meter (accuracy of ± 2% air saturation; YSI 556 MPS, YSI

499   Inc., Yellow Springs, OH). Data loggers recorded hourly temperatures at a depth of 10 cm within

500   the marsh substrate at putative hatchling overwintering sites, and some known adult

501   overwintering sites. Previous observations indicate that turtle hatchlings overwinter at a substrate

502   depth of 2-15 cm within water bodies (Ultsch et al., 2007; Paterson et al., 2012). In total, 44 data

503   loggers were placed in 12 marshes; from each marsh, 2-5 data loggers were retrieved in April

504   2012.

505

506   **Soil analyses**

507   Soil samples were used for several analyses: grain size, moisture and organic content. About 5 g

508   of soil collected at oviposition and each environmental measurement period was weighed and

509   then dried in an oven at 65ºC within 24 hours post-collection. Dried samples were weighed and

510   mass lost was recorded as soil moisture content. The remaining soil samples were transported to

511   Laurentian University, Sudbury, ON and air-dried. For organic content analysis, empty crucibles

512   were first weighed and then dried samples were sieved using a # 25 sieve, and ~5 g of soil was

513   placed in each crucible. Crucibles were then re-weighed and placed in a muffle furnace. The

514 muffle furnace program consisted of increasing temperature by 0.7ºC/min to a temperature of

515 150ºC, then temperature remained at 150ºC for half an hour. The temperature was then increased

516 by 0.3ºC/min to 450ºC where it remained for 2 hours. After 30 hours elapsed, crucibles were

517 removed, cooled in a desiccator, and weighed a final time. The weight lost was recorded as

518 organic content (Hughes et al., 2009).

519 Sieve analysis was used to determine soil grain size. The sieve sizes used were #8, #16,

520 #30, #50, #100, and #200. Sieves were weighed individually, and placed in a stack. About 50 g

521 of an air-dried soil sample was placed at the top of the stack, and put on a sieve shaker for 10

522 min. Sieves were individually weighed again to determine the amount of soil retained in each

523 (Soil Survey Division Staff, 1993; Hughes et al., 2009). The percentage of soil in each sieve out

524 of the total amount of soil in the stack was calculated, and the Canadian Soil Survey Committee

525 system was used to classify soil types: gravel ($\geq$ 2 mm grain diameter, percentage in #8 sieve),

526 coarse sand (0.5–2 mm grain diameter, percentage in #16 and #30 sieves), sand (0.15–0.5 mm

527 grain diameter, percentage in #50, #100, and #200 sieves), and fines ($\leq$ 0.075 mm grain

528 diameter, percentage that had gone through the #200 sieve).

529

530 **Data handling and analyses**

531 *Summary Statistics*

532 To assess if oviposition date affected overwinter strategy, nesting dates were coded in annual

533 numeric sequence (Wilimovsky, 1990). Hatching success (%) was calculated as the number of

534 hatched eggs divided by the number of eggs laid. Hatchlings that were depredated by fly larvae

535 or roots during incubation were included as hatched in this calculation. Overwintering success

536 (%) was calculated as the number of hatchlings alive after winter divided by the number of live

537 hatchlings in the nest pre-overwintering. Hatching and overwintering success were compared

538 between hatchlings from fall and spring-emerging nests using a Mann-Whitney-Wilcoxon test

539 (Gotelli and Ellison, 2004).

540 Nest temperature data were used to calculate total heat units (^{o}D): a variable that relates

541 both mean nest temperature and variation to embryonic development (Holt, 2000). Degree days

542 (^{o}D) are the number of heat units accumulated over 24 hours above a threshold temperature (T_{o}).

543 Below the threshold temperature, no development takes place, but above it development occurs

544 (Holt, 2000). The threshold temperature (T_o) for painted turtles is 14°C (Les et al., 2007). The

545 equation uses hourly temperatures (T_0, T_1, T_2,… T_{23}) to calculate heat units above a threshold

546 temperature (T_o; Holt, 2000).

547

548 $$HU = [((T_0+T_1)/2)+((T_1+T_2)/2)+…+((T_{22}+T_{23})/2)]/(24-T_o) \qquad (1)$$

549

550   Mean, minimum, and maximum nest temperatures, as well as the amount of time a nest

551 spent below -0.6°C (freezing point of body fluids for hatchlings; Packard and Packard, 2004) and

552 -4°C (lower limit for freeze tolerance; Storey et al., 1988) from October until April was

553 calculated to understand the nest environment to which hatchlings were exposed.

554

555 *Statistical Analyses*

556 All statistical analyses were performed in R (R Development Core Team, 2011). All data are

557 shown as means $\pm$ 1 standard error. A significance level of $\alpha=0.05$ and 95% confidence limits

558 were utilized for all tests.

559   Environmental variables measured throughout incubation were averaged for each nest. A

560 principle component analysis (PCA) was used to transform 6 soil variables (percentage of soil

561 within each soil texture class, organic content, and moisture) into one principal component that

562 summarized 54% of variation within these variables (Manly, 2005). The maximal multivariate

563 logistic regression model examined the relationship between turtle overwinter strategy (a binary

564 response variable: spring=0, or fall emergence=1) and 8 environmental variables () considering

565 main effects only (due to our low sample size; N=22 nests), and was fit using glm (Logan, 2010).

566 Variables were standardized (by subtracting each variable by its mean divided by 1 standard

567 deviation) for use in the models. Model ranking with AIC_c and averaging was completed using

568 the R packages glmulti (Calcagno and de Mazancourt, 2010) and MuMIn (Barton, 2009). To

569 assess model fit, we used Nagelkerke's R-square (R^2_N; Nagelkerke, 1991), which is fitted by

570 maximum likelihoods, and generalizes traditional linear regression R^2 to situations where

571 residual variance is not adequately defined (Nakagawa and Schielzeth, 2013). R^2_N is not sensitive

572 to the number of variables in the model, so AIC_C is used to identify the model that accounts for

573 the most variation in the data with the fewest variables.

574   Data on clutch oviposition sequence (i.e., first or second clutch for an individual mother)

575   are count data (N=61); thus, a Fisher's Exact test was used to examine relationships between

576   oviposition sequence and overwinter strategy (Gotelli and Ellison, 2004).

577   Predator presence, hatchling size, body condition and righting response were analyzed to

578   determine if overwintering strategy might provide benefits to hatchlings (N=61 nests).

579   Mammalian/avian predation attempts and Sarcophagid fly larvae presence data are count data, so

580   data were analyzed using Fisher's Exact tests (Gotelli and Ellison, 2004). Hatchling variables

581   were averaged across hatchlings for each clutch (Davy et al., 2014). Size, body condition and

582   righting response were compared among hatchling sampling periods: fall-emerging hatchlings,

583   hatchlings that were excavated in the fall, and spring-emerging. Assumptions of normality and

584   heterogeneity of variance were verified, and data were transformed for normality as needed (see

585   below). Carapace length and mass were compared among hatchling sampling periods using an

586   ANOVA, and an *a posteriori* Tukey HSD that adjusted for multiple comparisons was used to

587   identify differences among sample means (Logan, 2010). A logistic regression, fit using glm

588   (Logan, 2010), examined the relationship between overwintering strategy (a binary response

589   variable: spring=0, or fall emergence=1) and hatchling size (carapace length and mass). To

590   examine body condition, a linear mixed effects model (LMEM) of mass against the fixed effects

591   of carapace length and hatchling sampling period, and the random effect of mother's identity (to

592   control for genetic effects) was used (Garcia-Berthou, 2001; Litzgus et al., 2008; Riley and

593   Litzgus, 2013). Righting response variables (LP and RP) were transformed using log $(y+1)$ to

594   ensure normality. LP and RP were compared among hatchling sampling periods using LMEMs

595   that included the fixed covariate of trial temperature which is linearly related to ectotherm

596   performance (Hutchison et al., 1966), and the random effect of mother's identity. LMEMs were

597   performed using the R package lme4 (Bates, 2010). If a significant interaction was found in the

598   LMEMs it was reported; if no significant interaction was found, only main effects were tested

599   and reported.

600   To determine risks associated with aquatic versus terrestrial overwintering, winter

601   temperatures were compared between marshes and nests using an unequal-variance Student's t-

602   test. Nest temperature data were averaged from October 2011 until April 2012. Oxygen in-nest,

603   and DO in marshes was averaged from September 2011 until April 2012. Oxygen data were not

604 compared statistically, as oxygen was measured in different media (water in marshes versus air

605 in nests). Instead, oxygen data are described and compared qualitatively.

606

Acknowledgements

613

Competing interests

615 The authors declare no competing financial interests.

616

Author contributions

618 J.L.R., G.J.T. and J.D.L. conceived and designed the experiments. J.L.R. performed the

619 experiments and analyzed the data. J.L.R., G.J.T. and J.D.L. wrote the manuscript.

620

Funding

627

References

Aars, J. and Ims, R. A. (2002). Intrinsic and climatic determinants of population demography: the winter dynamics of tundra voles. *Ecology* **83,** 3449-3456.

Altwegg, R., Dummermuth, S., Anholt, B. R., Flatt, T. and Benton, T. 2005. Winter weather affects Asp Viper, *Vipera aspis*, population dynamics through susceptible juveniles. *Oikos* **110,** 55-66.

Baker, P. J., Costanzo, J. P., Herlands, R., Wood, R. C. and Lee, Jr, R. E. (2006). Inoculative freezing promotes winter survival in hatchling diamondback terrapin, *Malaclemys terrapin. Can. J. Zool.* **84,** 116-124.

Barton, K. (2009). MuMIn: multi-model inference. R package, version 0.12.2. Available at: http://r-forge.r-project.org/projects/mumin/ .

Bates, D. (2010). Linear mixed model implementation in lme4. Package lme4 vignette, available at: http://cran.r-project.org/web/packages/lme4/vignettes/Implementation.pdf

Baust, J. G., Grandee, R. and Condon, G. (1979). The diversity of overwintering strategies utilized by separate populations of gall insects. *Physiol. Zool.* **52**, 572-580.

Baust, J. G. and Lee, R. E. Jr. (1981). Divergent mechanisms of frost-hardiness in two populations of the gall fly, *Eurosta solidaginsis. J. Insect Physiol.* **27**, 485-490.

Blouin-Demers, G., Prior, K. A. and Weatherhead, P. J. (2000). Patterns of variation in spring emergence by black rat snakes (*Elaphe obsoleta obsoleta*). *Herpetologica* **56,** 175-188.

Bolton, S. M., Marshall, S. A. and Brooks, R. J. (2008). Opportunistic exploitation of turtles eggs by *Tripanurga importuna* (Walker) (Diptera: Sarcophagidae). *Can. J. Zool.* **86**, 151-160.

Brentcharlan, M. (1989) Size and winter survivorship in neonatal western rattlesnakes (*Crotalus viridis*). *Can. J. Zool.* **67,** 1620-1625.

Burke, V. J., Rathbun, S. L., Bodies, J. and Gibbons, J. W. (1998). Effect of density on predation rate for turtle nests in a complex landscape. *Oikos* **83,** 3-11.

Cagle, F. R. (1954). Observations on the life cycles of painted turtles (Genus *Chrysemys*). *Am. Midl. Nat.* **52**, 225-235.

657 **Calcagno, V., and de Mazancourt, C.** (2010). glmulti: an R package for easy automated model

658   selection with (generalized) linear models. *J. Stat. Software* **34,** 1-29.

659 **Carroll, D. M. and Ultsch, G. R.** (2007). Emergence season and survival in the nest of

660   hatchling turtles in southcentral New Hampshire. *Northeast. Nat.* **2**, 307-310.

661 **Chidester, F. E.** (1915). Sarcophagid larvae from the painted turtle. *J. Parasitol.* **2**, 48-49.

662 **Christiansen, J. L. and Gallaway, B. J.** (1984). Raccoon removal, nesting success, and

663   hatchling emergence in Iowa turtles with special reference to *Kinosternon flavescens*.

664   *Southwest. Nat.* **29,** 343-348.

665 **Churchill, T. A. and Storey, K.B.** (1992). Natural freezing survival by painted turtles

666   *Chrysemys picta marginata* and *C. picta belli*. *Am. J. Physiol.* **262,** R530-R537.

667 **Congdon, J. D., Pappas, M., Brecke, B. and Capps, J.** (2011). Conservation implications of

668   initial orientation of naïve hatchling snapping turtles (*Chelydra serpentina*) and painted

669   turtles (*Chrysemys picta belli*) dispersing from experimental nests. *Chel. Conserv. Biol.* **10,**

670   42-53.

671 **Costanzo, J. P., Wright, M. F. and Lee Jr., R. E.** (1992). Freeze tolerance as an overwintering

672   adaptation in Cope's grey treefrog (*Hyla chrysoscelis*). *Copeia* **1992,** 565-569.

673 **Costanzo, J. P., Iverson, J. B., Wright, M. F. and Lee Jr., R. E.** (1995). Cold hardiness and

674   overwintering strategies of hatchlings in an assemblage of northern turtles. *Ecology* **76,**

675   1772-1785.

676 **Costanzo, J. P., Litzgus, J. D., Iverson, J. B. and Lee Jr., R. E.** (1998). Soil hydric

677   characteristics and environmental ice nuclei influence supercooling capacity of hatchling

678   painted turtles *Chrysemys picta*. *J. Exp. Biol.* **201,** 3105-3112.

679 **Costanzo J. P., Litzgus, J. D., Iverson, J. B. and Lee Jr., R.E.** (2000). Ice nuclei in soil

680   compromise cold hardiness of hatchling painted turtles, *Chrysemys picta*. *Ecology* **81,** 346-

681   360.

682 **Costanzo J. P., Litzgus, J. D., Larson, J. L., Iverson, J. B. and Lee Jr., R. E.** (2001).

683   Characteristics of nest soil, but not geographic origin, influence cold hardiness of hatchling

684   painted turtles. *J. Thermal Biol.* **26,** 65-73.

685 **Costanzo, J. P., Dinklebacker, S. A., Iverson, J. B. and Lee Jr., R. E.** (2004). Physiological
686   ecology of overwintering in the hatchling painted turtle: multiple-scale variation in
687   response to environmental stress. *Physiol. Biochem. Zool.* **77,** 74-99.

688 **Costanzo, J. P., Lee Jr., R. E. and Ultsch, G. R.** (2008). Physiological ecology of
689   overwintering in hatchling turtles. *J. Exp. Zool.* **309A,** 297-379.

690 **Davy, C. M., Paterson, J.E., Leifso, A. E.** (2014). When righting is wrong: performance
691   measures require rank repeatability for estimates of individual fitness. *An. Behav.* **93,** 15-
692   23.

693 **Delmas, V., Baudry, E., Girondot, M. and Prevot-Juilliard, A.** (2007). The righting response
694   as a fitness index in freshwater turtles. *Biol. J. Linn. Soc.* **91,** 99-109.

695 **DePari, J. A.** (1996). Overwintering in the nest chamber by hatchling painted turtles, *Chrysemys*
696   *picta*, in northern New Jersey. *Chel. Conserv. Biol.* **2,** 5-12.

697 **Edge, C. B., Steinberg, B. D., Brooks, R. J. and Litzgus, J. D.** (2009). Temperature and site
698   selection by Blanding's turtles (*Emydoidea blandingii*) during hibernation near the species'
699   northern range limit. *Can. J. Zool.* **87,** 825-834.

700 **Edge, C. B., Steinberg, B. D., Brooks, R. J. and Litzgus, J. D.** (2010). Habitat selection by
701   Blanding's turtles (*Emydoidea blandingii*) in a relatively pristine landscape. *Écoscience* **17**,
702   90-99.

703 **Ernst, C. H.** (1971). Population dynamics and activity cycles of *Chrysemys picta* in southeastern
704   Pennsylvania. *J. Herpetol.* **5,** 151-160.

705 **Ernst, C. H. and Lovich, J. E.** (2009). Turtles of the United States and Canada, Second Edition.
706   Baltimore: The Johns Hopkins University Press.

707 **Finkler, M. S. and Claussen, D. L.** (1997). Use of the tail in terrestrial locomotor activities of
708   juvenile *Chelydra serpentina*. *Copeia* **1997,** 884-887.

709 **Finneran, L. C.** (1948). Reptiles of Branford, Connecticut. *Herpetologica* **4,** 123-132.

710 **Freedberg, S., Stumpf, A. L., Ewert, M. A. and Nelson, C. E.** (2004). Developmental
711   environment has long-lasting effects on behavioural performance in two turtles with
712   environmental sex determination. *Evol. Ecol. Res.* **6,** 739-747.

713 **Friebele, E. and Swarth, C. W.** (2005). Potential factors determining hatchling emergence
714   patterns in red-bellied turtles (*Pseudemys rubriventris*). In *Proceedings of the 2005 Joint*

Meeting of Ichthyologists and Herpetologists, Tampa, FL, 6-11 July 2005. Tampa: American Society of Ichthyologists and Herpetologists.

Garcia-Berthou, E. (2001). On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *J. An. Ecol.* **70,** 708-711.

Garrett, K., Wallace, B. P., Garners, J. and Pladino, F. V. (2010). Variations in leatherback turtle nest environments: consequences for hatching success. *End. Species Res.* **11,** 147-155.

Graf, W. H. (1984). Hydraulics of sediment transport. New York: Mcgraw-Hill Book Company.

Gibbons, J. W. (2013). A long-term perspective of delayed emergence (aka overwintering) in hatchling turtles: some they do and some they don't, and some you just can't tell. *J. Herpetol.* **47,** 203-214.

Gibbons, J. W. and Nelson, D. H. (1978). The evolutionary significance of delayed emergence from the nest by hatchling turtles. *Evolution* **32,** 297-303.

Gillingwater, S. D. (2001). A selective herpetofaunal survey, inventory, and biological research study of Rondeau Provincial Park. Unpublished report to Rondeau Provincial Park, Morpeth, Ontario.

Gotelli, N. J. and Ellison, A. M. (2004). A primer of ecological statistics. Sunderland: Sinauer Associates, Inc.

Greaves, W. F. and Litzgus, J. D. (2007). Overwintering ecology of wood turtles (*Glyptemys insculpta*) at the species' northern range limit. *J. Herpetol.* **41,** 32-40.

Hartweg, N. (1944). Spring emergence of painted turtle hatchlings. *Copeia* **1944,** 20-22.

Holt, S. M. (2000). Development and evaluation of a model for turtle embryonic growth. M.Sc. thesis, University of Guelph, Guelph, Ontario.

Hughes, G. N., Greaves, W. F. and Litzgus, J. D. (2009). Nest-site selection by wood turtles (*Glyptemys insculpta*) in a thermally limited environment. *Northeast. Nat.* **16**, 321-338.

Hutchison, V. H., Vinegar, A. and Kosh, R. J. (1966). Critical thermal maxima in turtles. *Herpetologica* **22,** 31-41.

Huusko, A., Greenberg, L., Stickler, M., Linnansaari, T. Nykänen, M., Vehanen, T. Koljonen, S., Louhi, P. and Alfredsen, K. (2007). Life in the ice lane: the winter ecology of stream salmonids. *River Res. Appl.* **23**, 469-491.

745 **Iverson, J. B. and Perry, R. E.** (1994). Sarcophagid fly parasitoidism on developing turtle
746   eggs. *Herpetol. Rev.* **25,** 50-51.

747 **Jaeger, H. M., Liu, C. and Nagel, S. R.** (1989). Relaxation at the angle of repose. *Phys. Rev.*
748   *Lett.* **62,** 40-43.

749 **Kolbe, J. J. and Janzen, F. J.** (2002). Spatial and temporal dynamic of turtle nest predation:
750   edge effects. *Oikos* **99,** 538-544.

751 **Les, H. L., Paitz, R. T. and Bowden, R. M.** (2007). Experimental test of the effects of
752   fluctuating incubation temperatures on hatchling phenotype. *J. Exp. Zool.* **307A,** 274-280.

753 **Litzgus, J. D., Bolton, F. and Schulte-Hostedde, A. I.** (2008). Reproductive output depends on
754   body condition in spotted turtles (*Clemmys guttata*). *Copeia* **2008,** 86-92.

755 **Logan, M.** (2010). Biostatistical design and analysis using R, a practical guide. Chichester:
756   Wiley-Blackwell.

757 **Lombardero, M. A., Ayres, M. P., Ayres, B. D. and Reeve, J. D.** (2000). Cold tolerance of
758   four species of bark beetle (Coleoptera: Scolytidae) in North America. *Environ. Entomol.*
759   **29,** 421-432.

760 **Lovich, J. E., Ernst, C. H., Ernst, E. M. and Riley J. L.** (in press). A 21 year study of seasonal
761   and interspecific variation of hatchling emergence in a Nearctic freshwater turtle
762   community: to overwinter or not to overwinter*? Herpetol. Monogr*.

763 **Macartney, J. M.** (1985). The ecology of the northern Pacific rattlesnake, *Crotalus viridis*
764   *oreganus*, in British Columbia. M.Sc. thesis, University of Victoria, Victoria, British
765   Columbia.

766 **Manly, B. F. J.** (2005). Multivariate statistical methods, a primer. New York: Chapman &
767   Hall/CRC Press.

768 **Mann, T. M.** (1977). Impact of developed coastline on nesting and hatchling sea turtles in
769   southeastern Florida. Unpubl. M.Sc. thesis, Florida Atlantic University, Boca Raton,
770   Florida.

771 **Marchand, P. J.** (2013). Life in the cold: an introduction to winter ecology, 4th edition. New
772   Hampshire: University Press of New England.

773 **McCafferya, R. M. and Maxellb, B. A.** (2010) Decreased winter severity increases viability of
774   a montane frog population. *PNAS*, **107**, 8644-8649.

775   **McGuire, J. M., Congdon, J. D., Scribner, K. T. and Capps, J. D.** (2011). Variation in female

776   reproductive quality and reproductive success of male midland painted turtles (*Chrysemys*

777   *picta marginata*). *Can. J. Zool.* **89,** 1136-1145.

778   **Muchlinski, A. E.** (1988). Population attributes related to the life-history strategy of hibernating

779   *Zapus hudsonius*. *J. Mammal*. **69,** 860-865.

780   **Muir, T. J., Dishong, B. D., Lee Jr, R. E. and Costanzo, J. P.** (2013). Energy use and

781   management of energy reserves in hatchling turtles (*Chrysemys picta*) exposed to variable

782   winter conditions. *J. Thermal Biol*. **38,** 324-330.

783   **Muldoon, K. A. and Burke, R. L.** (2012) Movements, overwintering, and mortality of hatchling

784   Diamond-backed terrapins (*Malaclemys terrapin*) at Jamaica Bay, New York. *Can. J. Zool.*

785   **90**, 651-662.

786   **Nakagawa, S., and Schielzeth, H.** (2013). A general and simple method for obtaining R^2 from

787   generalized linear mixed effects models. *Methods Ecol. Evol.* **4,** 133-142.

788   **Nagelkerke, N. J. D.** (1991). A note on a general definition of the coefficient of determination.

789   *Biometrika* **78**, 691-692.

790   **Obbard, M. E. and Brooks, R. J.** (1981). Fate of overwintered clutches of the common

791   snapping turtle (*Chelydra serpentina*) in Algonquin Park, Ontario. *Can. Field-Nat.* **95,**

792   350-352.

793   **Ontario Ministry of Natural Resources.** (1998). Algonquin Provincial Park management plan.

794   Ottawa: Queen's Printer for Ontario.

795   **Parker, W. S. and Brown W. S.** (1980). Comparative ecology of two colubrid snakes,

796   *Masticophis t. taeniatus* and *Pituophis melanoleucas deserticola*, in northern Utah. *Milw.*

797   *Public Mus. Contrib. Biol. Geol.* **7,** 1- 104.

798   **Paterson, J. E., Steinberg, B. D. and Litzgus, J. D.** (2012). Revealing a cryptic life-history

799   stage: differences in habitat selection and survivorship between hatchlings of two turtle

800   species at risk (*Glyptemys insculpta* and *Emydoidea blandingii*). *Wildl. Res*. **39,** 408-418.

801   **Packard, G. C. and Packard, M. J.** (1997). Type of soil affects survival by overwintering

802   hatchlings of the painted turtle. *J. Thermal Biol*. **22,** 53-58.

803   **Packard, G. C. and Packard, M. J.** (2004). To freeze or not to freeze: adaptations for

804   overwintering by hatchlings of the North American painted turtle. *J. Exp. Biol.* **207,** 2897-

805   2906.

806   **Pappas, M. J., Brecke, B. J. and Congdon, J. D.** (2000). The Blanding's turtles (*Emydoidea*

807   *blandingii*) of Weaver Dunes, Minnesota. *Chel. Conserv. Biol.* **3**, 557-568.

808   **Parren, S. G. and Rice, M. A.** (2004). Terrestrial overwintering of hatchling turtles in Vermont

809   nests. *Northeast. Nat.* **11,** 229-233.

810   **Peach, W. J., Siriwardena, G. M. and Gregory, R. D.** 2003. Long-term changes in over-winter

811   survival rates explain the decline of reed buntings, *Emberiza schoeniclus*, in Britain. *J.*

812   *Appl. Ecol.* **36**, 798-899.

813   **Rafferty, A. R. and Reina, R. D.** (2012). Arrested embryonic development: a review of

814   strategies to delay hatching in egg-laying reptiles. *J. Comp. Physiol. B: Biochem. Syst.*

815   *Environ. Physiol.* **279,** 2299-2308.

816   **Rasmussen, M. L. and Litzgus, J. L.** (2010). Patterns of maternal investment in spotted turtles

817   (*Clemmys guttata*): Implications of trade-offs, scales of analyses, and incubation substrates.

818   *Écoscience* **17,** 47-58.

819   **Rasmussen, D.H. and Mackenzie, A. P.** (1973). Clustering in supercooled water. *J. Chem.*

820   *Physics* **59,** 5003-5013.

821   **Reese, S. A., Ultsch, G. R. and Jackson, D. C.** (2004). Lactate accumulation, glycogen

822   depletion, and shell composition of hatchling turtles under simulated aquatic hibernation. *J.*

823   *Exp. Biol.* **207,** 2889-2895.

824   **Resetarits, W. J. Jr.** (1986). Ecology of cave use by the frog, *Rana palustris*. *Am. Midl. Nat.*

825   **116**, 256-266.

826   **Riley, J. L. and Litzgus, J. D.** (2013). Evaluation of predator-exclusion cages used in turtle

827   conservation: cost analysis and effects on nest environment and proxies of hatchling

828   fitness. *Wildl. Res.* **40**, 499-511.

829   **Riley, J. L. and Litzgus, J. D.** (2014). Cues used by predators to detect freshwater turtle nests

830   persist late into incubation. *Can. Field-Nat.* 128: 179-188.

831   **Roznik, E. A. and Alford, R. A.** (2012). Does waterproofing Thermochron iButton dataloggers

832   influence temperature readings? *J. Thermal Biol.* **37,** 260-264.

833   **Rozycki, C. B.** (1998). Reproductive and nesting ecology of the painted turtle (*Chrysemys picta*)

834   in Acadia National Park, Mount Desert Island, Maine. M.Sc. thesis, Bard College,

835   Annandale-On Hudson, New York, NY.

836   **Samson, J., Hughes, E. J. and Brooks, R. J.** (2007). Excavation is a nondeleterious method for

837   obtaining fecundity and morphometric data from small-sized eggs of freshwater turtles.

838   *Chel. Conserv. Biol.* **6,** 255-259.

839   **Schwarzkopf, L. and Brooks, R. J.** (1987). Nest-site selection and offspring sex ratio in painted

840   turtles*, Chrysemys picta*. *Copeia* **1987,** 53-61.

841   **Sendor, T. and Simon, M.** (2003). Population dynamics of the pipistrelle bat: effect of sex, age,

842   and winter weather on seasonal survival. *J. An. Ecol.* **72,** 308-320.

843   **Soil Survey Division Staff.** (1993). Soil Survey Manual. Washington: Soil Conservation

844   Service, US Department of Agriculture Handbook 18.

845   **Smith, K. A.** (2002). Demography and spatial ecology of wood turtles (*Clemmys insculpta*) in

846   Algonquin Provincial Park. M.Sc. thesis, University of Guelph, Guelph, Ontario.

847   **Spencer, R. and Janzen, F. J.** (2011). Hatching behaviour in turtles. *Integr. Comp. Biol.* **51,**

848   100-110.

849   **St. Clair, R. C. and Gregory, P. T.** (1990). Factors affecting the northern range limit of painted

850   turtles (*Chrysemys picta*): winter acidosis or freezing? *Copeia* **1990,** 1083-1089.

851   **Stapp, P., Young, J. K., VandeWounde, S. and Van Horne, B.** (1994). An evaluation of the

852   pathological effects of fluorescent powder on deer mice (*Peromyscus maniculatus). J.*

853   *Mammal.* **75,** 704-709.

854   **Storey K. B, Storey, J. M., Brooks, S. P. J., Churchill, T. A. and Brooks, R. J.** (1988).

855   Hatchling turtles survive freezing during winter hibernation. *PNAS* **85,** 8350-8354.

856   **Tanaka, K.** (1997). Evolutionary relationship between diapause and cold hardiness in the house

857   spider, *Achaearanea tepidariorum* (Araneae: Theridiidae). *J. Insect Physiol.* **43**, 271-274.

858   **Tester, J. R. and Breckenridge, W. J.** (1964). Populations dynamics of the Manitoba toad,

859   *Bufo hemiophrys*, in northwestern Minnesota*. Ecology* **45**, 592-601.

860   **Tinkle D. W., Congdon, J. D. and Rosen, P. C.** (1981). Nesting frequency and success:

861   implications for the demography of painted turtles. *Ecology* **62,** 1426-1432*.*

862 **Ultsch, G. R.** (2006). The ecology of overwintering among turtles: where turtles overwinter and
863   its consequences. *Biol. Rev.* **81,** 339-367.

864 **Ultsch, G. R., Draud, M. and Wicklow, B.** (2007). Post-emergence movements and
865   overwintering of snapping turtle*, Chelydra serpentina,* hatchlings in New York and New
866   Hampshire. *Can. Field-Nat.* **121,** 178-181.

867 **Vali, G.** (1995). Principles of ice nucleation. Pp. 1-28 in R.E. Lee, Jr., G. J. Warren, and L.V.
868   Gusta, eds. Biological Ice Nucleation and its Applications. St. Paul: American
869   Phytopathological Society.

870 **Viitanen, P.** 1967. Hibernation and seasonal movements of the viper, *Vipera berus berus* (L.), in
871   southern Finland. *Ann. Zool. Fenn.* **4,** 472-546.

872 **Warner, D. A. and Mitchell, T. S.** (2013). Does maternal oviposition site influence offspring
873   dispersal to suitable habitat? *Oecologia* **172,** 679-688.

874 **Warkentin, K. M.** (1995). Adaptive plasticity in hatching age: a response to predation risk
875   trade-offs. *PNAS* **92,** 3507-3510.

876 **Waye H. L. and Gillies, C.** (1999). *Chrysemys picta bellii* (Western painted turtle). Early
877   emergence. *Herpetol. Rev.* **30,** 94-95.

878 **Wilimovsky, N. J.** (1990). Misuses of the term 'Julian Day'. *Trans. Am. Fish. Soc.* **119**: 162.

879 **Wilson, D. S.** (1998). Nest-site selection: microhabitat variation and its effects on the survival of
880   turtle embryos. *Ecology* **79,** 1884-1892.

881 **Wood, D. W. and Bjorndal, K. A.** (2000). Relation of temperature, moisture, salinity, and slope
882   to nest site selection in loggerhead sea turtles. *Copeia* **2000,** 119-119.

883 **Woolverton, E.** (1961). Winter survival of hatchling painted turtles in northern Minnesota.
884   *Copeia* **1963,** 569-570.

885 **Yntema, C. L.** (1968). A series of stages in the embryonic development of *Chelydra serpentina*.
886   *J. Morphol.* **125,** 219-251.

887

888 **Table 1. Environmental variables measured during the summer of 2011 that were**
889 **included in the logistic regression model to examine whether environment**
890 **influences overwintering strategy used by painted turtle (*Chrysemys picta*)**
891 **hatchlings.** Mean ± standard error (SE) of each environmental variable for fall and
892 spring-emerging nests (non-standardized values).

Variable	Description	Mean ± SE	
		Fall-emerging	Spring-emerging
date.laid	Date of oviposition translated to date during the calendar year	166 ± 8	165 ± 8
nest.depth	Depth to bottom of nest cavity (cm)	9.6 ± 1.6	10.0 ± 1.3
total.hatch	Number of hatchlings within a clutch	7 ± 2	6 ± 2
total.heat.units	Heat units (ºD) calculated using hourly trapezoid method	3958.3 ± 414.5	3846.0 ± 417.4
avg.oxy	Percent oxygen in nest cavity measured in July, August and September	21.0 ± 0.1	21.1 ± 0.1
slope	Slope (°) of ground surface at nest. Negative values indicate a downward slope towards water, and positive values indicate an upward slope towards water.	-5.3 ± 8.7	2.3 ± 7.7
soil.pc1	First principal component for nest soil moisture and texture characteristics from PCA.	-1.2 ± 2.2	-0.3 ± 2.1
avg.bare.grnd	Percent bare ground in 1 m^2 quadrat around nest measured in June, July, August and September	73.5 ± 9.7	80.6 ± 13.4

893

894 **Table 2. Thermal environment of painted turtle (*Chrysemys picta*) nests (N=19)**

895 **from October to April 2010-2011 and 2011-2012.** Mean ± standard error (SE) nest

896 temperature, the range of minimum and maximum temperatures, and the number of

897 nests that spent time below -0.6ºC (freezing point of body fluids for hatchling turtles) and

898 -4ºC (lower limit for freeze tolerance) summarized for fall-emerging and spring-emerging

899 nests.

900

Thermal Characteristics (ºC)	Fall-emerging (N=6)	Spring-emerging (N=13)
Mean temperature	2.82 ± 0.16	1.91 ± 0.13
Minimum temperature range	-4.84 to -2.33	-4.91 to 1.67
Maximum temperature range	20.68 to 38.27	15.78 to 25.42
Percentage of nests below -0.6ºC	100	83
Percentage of nests below -4ºC	100	31

901

902 **Table 3. Top ten multiple logistic regression models that examined how environmental variables affect**

903 **overwintering strategy in hatchling painted turtles (*Chrysemys picta*).** The models were identified by their AIC_c

904 scores. The model parameters, number of parameters (*k*), log-likelihood of parameters (*LL*), Nagelkerke's R-squared

905 (R^2_N), AIC_c, delta AIC_c, and model weights (*wi*) are all shown.

906

Model	*k*	*LL*	R^2_N	AIC_c	Delta AIC_c	*wi*
avg.bare.grnd + nest.slope	2	-11.55	0.41	30.36	0	0.18
soil.pc1 + avg.bare.grnd + nest.slope	3	-10.29	0.51	30.81	0.45	0.14
avg.oxy + nest.slope	2	-11.93	0.38	31.12	0.76	0.12
nest.slope	1	-13.27	0.26	31.14	0.79	0.12
avg.bare.grnd + total.heat.units + nest.slope + date.laid	4	-8.92	0.60	31.38	1.02	0.11
avg.bare.grnd + avg.oxy + nest.slope	3	-10.82	0.47	31.86	1.5	0.08
soil.pc1 + nest.slope	2	-12.31	0.35	31.89	1.53	0.08
avg.bare.grnd + total.heat.units + nest.slope	3	-10.93	0.46	32.08	1.73	0.08
total.heat.units + nest.slope	2	-12.88	0.30	33.02	2.67	0.05
(null)	0	-15.75	0	33.68	3.32	0.03

Table 4. Number of spring and fall-emerging painted turtle (*Chrysemys picta*) nests with Sarcophagid fly larvae present in the nest cavity. Larvae presence was significantly related to hatchling overwintering strategy (*P*=0.04).

Presence of Sarcophagid Fly Larvae	Fall-emerging Nests (N=18)	Spring-emerging Nests (N=43)
Present	9	10
Not Present	9	33
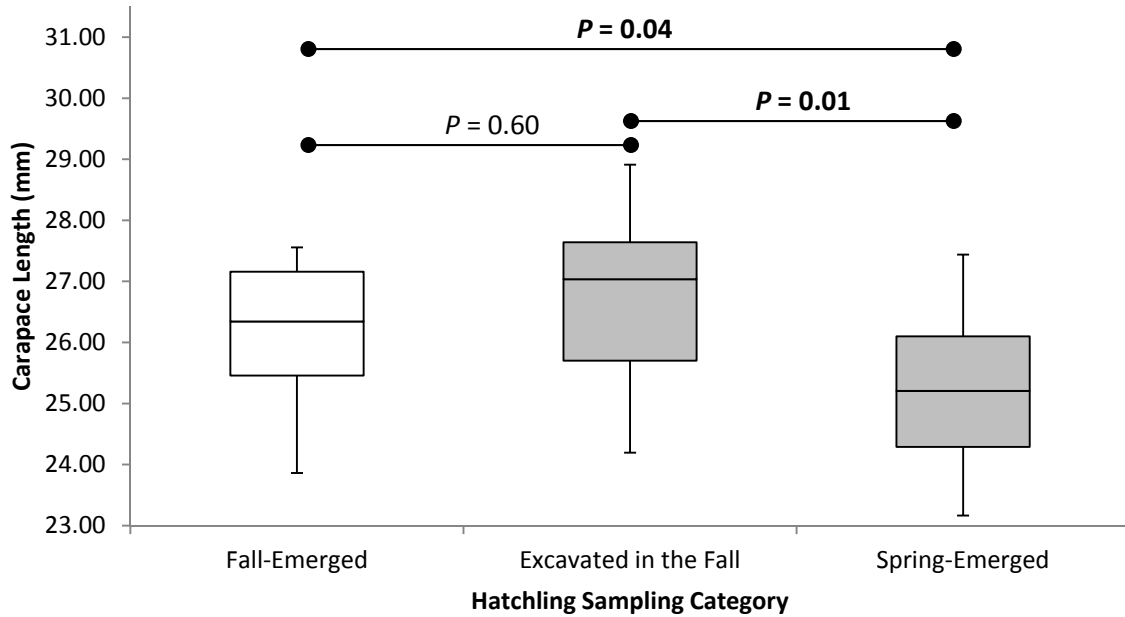
913

Fig. 1. Carapace lengths of hatchling painted turtles (*Chrysemys picta)* that emerged in the fall (white box-plot) and of hatchlings that overwintered in-nest (grey box-plots) split into two groups: hatchlings excavated from nests in the fall, and those that overwintered in-nest and were measured after natural emergence in the spring. Carapace length of hatchlings influenced overwintering strategy. In the fall, small hatchlings were more likely to emerge from their nests (Wald's z-statistic=2.35, *P*=0.02, R^2_N=0.21). Post-overwintering, spring-emerging hatchlings were significantly smaller than hatchlings in the fall preparing to overwinter in-nest ($F_{2, 48}$=5.83, *P*<0.01; Tukey HSD values displayed on the figure). This indicates that overwintering had consequences that could impact hatchling survival.