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Ecological Correlates of Alligator Snapping Turtle Bite Performance

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ECOLOGICAL CORRELATES OF ALLIGATOR SNAPPING TURTLE BITE PERFORMANCE

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Ashley Herrin Gagnon

July 2021

ECOLOGICAL CORRELATES OF ALLIGATOR SNAPPING TURTLE BITE PERFORMANCE

Biology

Missouri State University, July 2021

Master of Science

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ABSTRACT

The Alligator Snapping Turtle (*Macrochelys temminckii*) is one of many turtle species facing conservation challenges. Nearly extirpated in the 1980s, it is currently the subject of a head-start initiative, of which, any lasting behavioral or physiological effects were—until now—unknown! To evaluate the ability of captive-reared individuals to excel in natural habitats, and to foresee any future research or conservation challenges regarding this animal, I explored a suite of variables that influence bite performance and behaviors including captive or free-ranging status, and environmental conditions including body temperature and season. My results indicated that free-ranging *M. temminckii* outperform those residing in captivity, and that captive individuals housed in outdoor ponds outperform their counterparts housed indoors. Further, I found free-ranging individuals more willing to engage in bite behaviors, a trend that significantly impacted maximum bite force among test groups. I also found that aspects of bite performance including bite velocity, acceleration, and duration all improve with increases in temperature, and that seasonality significantly influences jaw and lunge movements to an extent that was previously unanticipated, resulting in greater performance at identical temperatures, in the summer than in the Winter. Bite force was relatively robust to temperature and season; but it was maximized at higher temperatures and in the winter. These results indicate that time spent outdoors while in captivity should be maximized to support optimal performance upon release, that rigorous standardization of temperature is required in studies of ectotherm performance, and that care must be taken in selecting the time of year in which temperature studies are conducted.

KEYWORDS: acceleration, behavior, bite force, free-ranging, head-start, kinematics, latency, Oklahoma, scaling, velocity

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OVERVIEW

Turtles are among the most threatened vertebrates worldwide (Hoffmann et al., 2010; Rhodin et al., 2018), and their influence on the ecosystems that they inhabit is disproportionately large, especially at the high measures of biomass that this group historically achieved (Iverson, 1982). With threats including habitat destruction, commercial exploitation, and climate change driving the decline of hundreds of turtle species worldwide (Gibbons et al., 2000), it is likely that we will witness a loss of critical ecosystem services if these animals are not preserved. Services at risk include stabilization of trophic systems and their energy flow, mineral cycling, seed dispersal, and the efficient breakdown of decaying matter (Lovich et al., 2019).

Macrochelys temminckii, the Alligator Snapping Turtle, is one such species of conservation concern, and is a secretive animal that serves dual roles as both a top-order predator and detritivore in Gulf of Mexico-draining river systems across the southern United States (Pritchard, 2006). As long-lived animals with high embryonic and juvenile mortality, turtles are particularly vulnerable to harvest at any age, and *M. temminckii* is no exception (Wilbur and Morin, 1988; Brooks et al., 1991; Congdon et al., 1993, 1994; Heppell, 1998; Reed et al., 2002; Dreslik et al., 2017). Nearly extirpated in the 1980s, *M. temminckii* was previously considered for Endangered Species Status yet remains data deficient for listing (USFWS, 1991). It is currently protected from harvest and possession in nearly every state where it occurs by state-level regulations (Reed et al., 2002). It is a Tier I Species of Greatest Conservation Need in Oklahoma (ODWC, 2016) and is classified as Vulnerable by the IUCN Red List (TFTSG, 1996).

Conservation efforts targeting *M. temminckii* are influenced by the top-down role that predatory turtles serve in their ecosystems and seek to prevent devastating trophic cascades that would result from their loss (Lovich et al., 2019). Translocations, a suite of conservation strategies widely used for the augmentation of decimated populations of wildlife, involve the release of animals into areas they occupied prior to extirpation (IUCN/SCC, 2013). Head-starting is a translocation strategy that is commonly used for turtles and involves the propagation and rearing of juvenile animals in captivity to a size that will reduce predation risk following release into areas where populations are declining (Siegel and Dodd, 2000). In 1999, a head-start program began at Tishomingo National Fish Hatchery (TNFH) in southern Oklahoma to return *M. temminckii* to rivers where it was historically abundant (Moore et al., 2013).

Components of the program at TNFH include ponds that house brood stock, an indoor facility where hand-collected eggs are incubated and juveniles reared, as well as additional ponds that allow for juvenile and subadult turtles to adjust to outdoor conditions for at least one year before release. At present, rivers in Oklahoma that host head-started populations of *M. temminckii* include the Washita, Caney, Neosho, and Verdigris, which are all key to future dispersal of this species into other parts of its historic range (Riedle et al., 2008).

While translocations are often the most realistic option for species recovery, they are expensive in terms of funding and labor and have a high risk of failure (Jule et al., 2008). For this reason, translocation projects must be conducted as efficiently as possible and be continuously evaluated for areas of improvement. The streamlining of these conservation processes will only increase in importance as more species become reliant on extensive management in the future (Berger-Tal et al., 2019). A review of mammalian carnivore reintroductions found that projects involving captive-reared animals are less likely to succeed than projects involving wild-caught individuals (Jule et al., 2008), and anomalies in behavior are the most frequently reported impediment to successful species reintroduction (Berger-Tal et al., 2019). These behavioral syndromes sometimes include a deadly naivety to predators, conspecifics, and even humans due to habituation during the rearing process (Jule et al., 2008; Berger-Tal et al., 2019). In a study of head-started *Cryptobranchus alleganiensi*s, juvenile Hellbenders required predator-recognition training to increase post-release survival (Crane and Mathis, 2011).

Captivity can also result in lasting physiological syndromes that impact the ability of an organism to function in its ecosystem via ecologically relevant tasks (Arnold, 1983). An example of this can be seen in a study of *Alligator mississippiensis* that found differences in skull morphology between wild and long-term captive American Alligators (Erickson et al., 2004). Bite performance is the most relevant measure of performance for turtles (Herrel et al., 2002), as their ability to defend themselves, feed, and compete with conspecifics is reliant on the ability of their built-in weapons to do damage to opponents of any kind (Lappin et al., 2006). For this reason, any change to the skull architecture or musculature of *M. temminckii* risks hampering the danger of its powerful jaws and razor-sharp beak to competitors and prey alike.

There is preliminary evidence of successful survival and growth among head-started individuals in Oklahoma rivers (Moore et al., 2013; Anthony et al., 2015). Formerly head-started free-ranging *M. temminckii* showed significant increases in growth and a high rate of survival in older individuals (Anthony et al., 2015). In a comparison of captive and head-started *M. temminckii* from the same clutch, there was no difference in dimensional growth, but wild individuals gained weight more quickly than similar animals from the same cohort that remained in captivity (Moore et al., 2013). Due to a lack of research on the inconsistencies of performance between captive and wild turtles, and because bite force is a relevant performance measure for turtles (Herrel et al., 2002; Herrel and O'Reilly, 2006), I selected it as a tool to evaluate the performance of captive and head-started *M. temminckii* in the wild.

In Chapter 1, I compared bite performance in the form of maximum bite force among captive and wild *M. temminckii.* This process began with identification of the best time of day for testing as well as the preferred positioning of bite equipment to maximize performance. I then established standardized procedures to ensure maximal performance across turtles of varying sizes and housing conditions. My first priority was to identify whether captive *M. temminckii* underperformed head-started free-ranging turtles. My second research question explored the role of various bite behaviors including latency to bite and aggressive display in these two groups of turtles. Results of this chapter will potentially shape the future of the head-start program at Tishomingo National Fish Hatchery as well as inform other reptile head-start projects.

In Chapter 2, I investigated the acute and persistent effects of temperature on bite performance in captive *M. temminckii* at two different times of the year because temperature affects all chemical reactions and can have important consequences for muscle performance (Anderson and Deban, 2010). To explore these effects, I evaluated several bite performance measures, including jaw velocity, acceleration, and bite duration across a range of ecologically and seasonal relevant temperatures. In addition to influencing muscle function and speed in ectotherms, temperature differences can also affect willingness to bite (Vervust et al., 2011). As a result, my second objective was to evaluate the effects of temperature on behaviors such as latency to bite and extent of aggressive displays. Results from this chapter will potentially reaffirm the importance of standardizing temperature in performance studies of ectotherms, as well as reveal the ability of *M. temminckii* to perform under adverse conditions encountered within its native range.

BITE PERFORMANCE OF CAPTIVE ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*) IMPROVES AFTER REINTRODUCTION

Abstract

Alligator Snapping Turtles (*Macrochelys temminckii*) possess unique head morphology that suggests strong natural selection for bite performance, which likely influences foraging and prey selection, as well as the outcomes of intrasexual aggressive encounters, mating, and defense against predators. Therefore, bite performance has the potential to directly and indirectly impact fitness. In this study, I assessed the effects of captivity on bite force by comparing the performance of captive and reintroduced *M. temminckii*. On average, free-ranging *M. temminckii* bite with greater force than do individuals residing in captivity, and captive individuals housed under semi-natural conditions in outdoor ponds outperformed those housed indoors. Further, I found that free-ranging *M. temminckii* released into different river systems performed comparably and required less provocation than captives to display gaping and biting behavior. It remains to be determined whether the observed performance differences were more strongly influenced by physiological limitations on muscle performance or by behavioral variation in motivation to bite with maximum force.

Introduction

Reintroduction biology encompasses a suite of tools that are widely used for the augmentation of imperiled populations of fauna and flora. Captive propagation paired with reintroduction represents one such strategy, and often also involves head-starting animals in captivity until they achieve a size that will reduce predation risk following release (Siegel and

Dodd, 2000; IUCN/SCC, 2013). Although head-starting often provides a clear benefit by reducing post-release mortality rates, captivity may result in lasting behavioral or physiological syndromes that negatively impact animals' performance in the wild (Jule et al., 2008; Crane and Mathis, 2011). For example, a review of reintroductions of mammals found that conservation efforts using captive-reared animals were less likely to succeed than those that translocated wild-caught individuals, with anomalies in behavior being the most frequently documented obstacle to success. Common behavioral syndromes included a deadly naivety to predators and humans, as well as stunted interactions with conspecifics (Jule et al., 2008; Berger-Tal et al., 2019). In some cases, experiments have demonstrated that behavioral syndromes can be muted and survival improved by training individuals to recognize and avoid predators (Crane and Mathis, 2011). Altered phenotypes of the muscles and skeleton are chief among physiological syndromes that can afflict post-release survival (Waddington, 1975; Travis, 1994; Schlichting and Pigliucci, 1998; Erickson et al., 2004). Captivity-induced deleterious traits can be influenced by a variety of variables including diet, temperature regime, enclosure, opportunities for social interactions with conspecifics, and opportunities to learn to identify and avoid potential predators (Frye, 1981; Arnold and Peterson, 1990; Donoghue and Langenberg, 1996; Lane, 1996).

A head-start program was launched in 1999 in southeastern Oklahoma to aid the recovery of Alligator Snapping Turtle (*Macrochelys temminckii*) populations that had been decimated by trapping and habitat degradation (Moore et al., 2013). The program, established at Tishomingo National Fish Hatchery (TNFH), includes brood stock maintained in large semi-natural ponds, indoor facilities where eggs are incubated and juveniles are reared, and ponds that function as soft-release enclosures for juveniles, allowing young animals to acclimate to outdoor conditions before release. Head-started *M. temminckii* have been reintroduced in Oklahoma, Illinois, Louisiana, and Tennessee, and these reintroduction sites also provide opportunities for dispersal into other states that lie within the species' historic range (Riedle et al., 2008). However, reintroduction success is dependent on the condition of the stock that is released. To understand potential long-term effects of captivity on the success of this reintroduction program, it is important to consider the impact of captivity on an animal's performance and its ability to perform ecologically relevant tasks (Arnold, 1983; Jule et al., 2008).

Bite force is commonly measured to assess performance in a variety of taxa, and when compared among relevant groups, can be used to address ecological questions (Herrel et al., 1999; Herrel et al., 2002; Erickson et al., 2004; Vanhooydonck et al., 2005). Few species exhibit evidence of as strong selection for bite performance as has *M. temminckii*; megacephaly, beak morphology, size of the masseter muscles, and propensity to maintain a face-forward defensive stance when threatened all suggest that *M. temminckii* has undergone strong selective pressure for bite performance, with any reduction of the skull architecture, musculature, or defensive behavior likely being deleterious to survival (Herrel et al., 2002; Pritchard, 2006).

To detect any adverse effects of captivity on bite performance in *M. temminckii,* I compared bite force among animals in captivity with free-ranging individuals that had been released from the head-start facility into rivers. I assessed several aspects of bite performance, including maximum force, repeatability of bite performance, latency to gape or bite, and aggressive displays prior to snapping. I hypothesized that (1) captive *M. temminckii* would underperform their free-ranging counterparts, and (2) that captive turtles housed indoors would exhibit lower maximum bite force than turtles housed outdoors. I also hypothesized (3) that captive turtles would display greater latencies to gape, bite, or otherwise signal aggression than free-ranging turtles, and (4) that proclivity to display aggression or bite with minimal provocation would correlate with higher bite force measurements.

Materials and Methods

Acquisition of Specimens. I measured the maximum bite force of 126 captive *M. temminckii* housed at TNFH during the summer of 2019. Of these, 82 resided indoors in large plastic raceways and 44 resided outdoors in ponds. To ensure that I tested a range of sizes, I selected turtles in groups of 5–15 from indoor tanks where they were segregated by year class, as well as opportunistically from four outdoor ponds where I captured individuals by hand. All turtles were sexually immature and ranged 60–8,150 g mass and 52–305 mm straight midline carapace length. Year classes of these subjects ranged between 2005 and 2017, resulting in an average age at time of study of 4.5 yr. with a standard deviation of ± 2.4 yr.

In 2020, I also measured the bite performance of 61 free-ranging *M. temminckii* that had been head-started at TNFH but were released between the years of 2008 and 2015. At the time of study, the mean amount of time spent free ranging was 8.3 yr. with a standard deviation of ± 2.0 yr. Of these individuals, 31 were trapped in the Caney River and 30 in the Verdigris River at locations in northern Oklahoma. I trapped *M. temminckii* using hoop nets that were baited with frozen invasive carp. Traps were set in the afternoon and then checked and subsequently moved to new locations the following day (Moore et al., 2013, Anthony et al., 2015, Hollender, 2019). I housed *M. temminckii* in water-filled plastic totes that were kept in shade for the duration of testing, and returned each turtle to its original location of capture within 72 h. Subjects consisted of sexually mature and immature individuals and ranged between 1,350–17,400 g mass and 177–444 mm straight midline carapace length. Year classes of these subjects ranged between 2004 and 2012, resulting in an average age at time of study of 12.5 yr. with a standard deviation of ± 2.0 yr.

Equipment. I measured bite force with a force transducer connected to a charge amplifier (type 9313AA2 transducer and type 5995 charge amplifier; Kistler, Winterthur, Switzerland). The transducer was housed between adjustable bite plates, a design modified from Pfaller et al. (2009). I matched each subject to one of three sizes of stainless-steel bite plates, with assignments to different plates determined by head length. With few exceptions, bite plates were covered with 3-mm thick leather pads to provide friction and prevent injuries (Losos et al., 2002; Lappin et al., 2014). For the smallest test subjects, I padded the bite plates with layers of painter's tape because leather was too thick to accommodate their small gapes. I calibrated the force transducer by suspending weights from the bite plates and regressing nominal force on mass and then converting the units to Newtons. The hanging weights were positioned on the top plate at the same point at which the tip of Alligator Snapping Turtles' beaks typically made contact (Lappin et al., 2014). During all trials, I set the charge amplifier to a sensor sensitivity of 0.09 pC/Mechanical Unit and a range of 200 K Mechanical Units.

Morphometrics. Prior to testing, I measured body mass, straight carapace length, head length, head width, and head height. I took all dimensional measurements parallel to the coronal and sagittal planes of the body with dial calipers, and in the case of large specimens, forestry calipers. I measured straight carapace length from the center of the nuchal scute to the pygal notch, head length from the tip of the snout to the top of the parietal scale, head width across the widest part of the skull, posterior to the orbits and anterior to the tympanum, and head height at the tallest point of the skull which encompassed the lowest point on the mandibles and top of the parietal crest (Fig. 1).

Bite Trials. Each performance trial consisted of three replicate measures of bite force, with 30 min rest between bites. During rest intervals, I housed turtles separately in plastic containers filled to ~20 cm with water. I recorded the quality of each bite as either “Good” or “Poor” based upon visual observations of where the beak struck the bite plate, the angle of the head relative to the bite plate, and a qualitative assessment of the turtle’s apparent motivation to bite. Bites were excluded from the data set when a turtle delivered multiple snaps on the sensor in rapid succession, and I recorded the presence of aggressive displays over the course of each bite trial including shell-raising (Fig. 2), lunging, and snapping. When presented with the bite force apparatus, most turtles responded quickly and without additional provocation, but in cases of refusal to immediately gape or bite, I lightly tapped them on the head to elicit a defensive response and recorded latency to gape and latency to bite for each turtle as either “Immediate” or “Required tapping.”

To standardize data collection, I matched three sizes of stainless-steel bite plates to turtles of differing head lengths. I used small plates for turtles with head lengths 30–49 mm, medium plates for head lengths of 50–74 mm, and large plates for head lengths ≥ 75 mm. This reduced chance of injury from contact with the back of the plates and ensured that turtles interacted with a surface area of contact proportional to their head dimensions. Plates were equipped with short metal stops at their base, preventing turtles from biting too far back on the meter while also guiding the beak tips to a predictable point of contact at the back of the bite plates. Based on preliminary experiments that determined optimal test conditions, I conducted tests between the hours of 0800 and 2000, and standardized bite plate distance at 0.25x head height (Appendix B; Appendix C). To target this ratio of head height to plate distance, I started at a head height of 30–

49 mm and plate distance of 7.5 mm, and moved plates apart 2.5 mm for every head height increase of 10 mm.

Data Analyses. In assessing the repeatability of bite force performance, I first excluded bites scored as ‘Poor’. Prior to analysis, I $\log_{10}$-transformed measurements of force and morphometrics to improve the distribution and homogeneity of variance among groups. I conducted analysis of covariance (ANCOVA) tests to detect differences in maximum bite force among captive and free-ranging groups. In these analyses, I again included body mass as a covariate to adjust for variation in body size. I first compared maximum bite force among the two groups of captive turtles with location status (indoor versus outdoor) included as a main effect, and then compared the two groups of free-ranging turtles from the Caney and Verdigris Rivers in the same way. Upon determining differences between indoor versus outdoor captives and between free-ranging turtles inhabiting different river systems, I further analyzed differences between captive and free-ranging turtles by conducting an ANCOVA on lumped captive and lumped wild individuals. I then performed a linear regression between body size and maximum bite force by plotting maximum bite force of individuals (SigmaPlot 11.0, Systat Software, Inc.). I also investigated scaling of relevant morphometrics and bite force in Microsoft Excel 2012 (Build 13530.20376, Microsoft Store).

To test the relationship between latency to gape or bite and captive versus wild status, I performed a chi-square contingency analysis using Past 4.04 (Hammer et al. 2001) with the sums of turtles from each group that required tapping versus did not require tapping when administering their strongest bite. To test the relationship between latency to gape or bite and resulting maximum bite force, I performed a three-factor analysis of variance (ANOVA) with mass as a covariate, location as a main effect, and willingness to gape or bite as a response variable. To test for differences in frequency of aggressive displays between groups of captive and wild individuals, I again conducted a chi-squared contingency analysis with turtles that exhibited aggressive behavior assigned a score of 1 and all others assigned a score of 0. I then used a three-factor ANOVA to explore the relationship between aggressive display and resulting maximum bite force, again with mass as a covariate, location as a main effect, and aggression score as a response variable.

Except where indicated otherwise, analyses were performed in R-Studio (Version 1.3.1093, PBC; R Studio Team 2020). This study was approved by the Missouri State University Institutional Animal Care and Use Committee on 06/2019 and received Approval #19-014 (See Appendix A). It was also conducted with permission from the Oklahoma Department of Wildlife Conservation (permit no. 19734530).

Results

Captive vs Free-ranging. I recorded 534 good quality bites from 188 specimens, with bite force ranging 5.8–334.1 N for captives, and 69.4–814.5 N for free-ranging individuals (Table 1.). Overall, there was a positive relationship between size and maximum bite force; however, this relationship scaled differently among the groups tested (Table 2.). Maximum bite force scaled to body mass with slopes of 0.68 for indoor captives, 0.75 for outdoor captives, and 0.81 for free-ranging individuals. Maximum bite force scaled to carapace length with slopes of 1.96 for indoor captive, 2.35 for outdoor captive, and 2.28 for free-ranging individuals. Finally, head dimensions scaled to bite force with slopes ranging from 0.65–0.78 for indoor captives, 2.09–2.35 for outdoor captives, and 2.61–3.03 for free-ranging individuals.

There was a significant interaction between body mass and location among indoor- and outdoor-housed captive turtles (interaction: $F_{1,122} = 8.54$, $P = 0.004$), and while body mass strongly predicted bite force, the resulting maximum bite forces of those housed outdoors were slightly more sensitive to variations in body size than those housed indoors (Fig. 3A). There was no significant interaction between body mass and location among free-ranging *M. temminckii* inhabiting different river systems ($F_{1,58} = 0.74$, $P = 0.394$; Fig. 3B). Upon removal of this interaction, body mass predicted bite force ($F_{1,59} = 186.37$, $P < 0.0001$) and there was no significant effect of location ($F_{1,59} = 1.64$, $P = 0.205$). In a comparison of bite force of all captive versus all free-ranging *M. temminckii*, there was no significant interaction between body mass and location ($F_{1,184} = 0.64$, $P = 0.425$), and upon removal of the interaction term, body mass strongly predicted bite force ($F_{1,185} = 4{,}403.95$, $P < 0.0001$). However, free-ranging head-started *M. temminckii* bit with greater force than captive individuals at all sizes ($F_{1,185} = 33.06$, $P < 0.0001$; Fig. 4). Untransformed bite force data regressed on body mass indicated that free-ranging *M. temminckii* bit with approximately 49.6 N more force than captives.

Latency to Bite and Behavior. There was a significant difference in willingness to gape between captive and free-ranging turtles ($\chi^2_3 = 86.09$, $P < 0.0001$), with 86% of captive and 16% of free-ranging turtles requiring tapping to elicit a defensive gape (Fig. 5). Upon gaping, there was not a significant difference in willingness to bite between the groups ($\chi^2_3 = 3.00$, $P = 0.392$). In a comparison of latency to gape or bite and resulting maximum bite force, willingness to gape did not have a significant impact on the maximum bite force of captive and free-ranging turtles ($F_{1,180} = 2.97$, $P = 0.087$), but willingness to bite did ($F_{1,177} = 11.03$, $P < 0.001$). In a comparison of frequency of aggressive displays prior to biting between captive and free-ranging individuals, there was a significant difference in number of turtles that displayed pre-bite aggression among the four groups ($\chi^2_3 = 7.84$, $P = 0.049$). Captive turtles housed indoors had the highest tendency to display aggression prior to biting (71%), followed by turtles from the Caney River (53%), captive turtles reared outdoors (52%), and turtles from the Verdigris River (47%). Importantly, the display of pre-bite aggression was not a significant predictor of maximum bite force for any of the groups ($F_{1,183} = 1.31$, $P = 0.255$).

Discussion

All of the turtles included in my study originated in the same captive breeding and head-start program, yet those that had been released and were free-ranging exhibited both an enhanced willingness to gape and stronger bite force than those that remained in captivity. Moreover, among those in captivity, individuals that were housed outdoors snapped with greater force than those reared indoors under less natural conditions. Interestingly, at smaller body sizes bite force was similar between indoor- and outdoor-housed turtles, but differences in bite performance increased at larger sizes. This pattern suggests that bite performance is, at least temporarily, negatively impacted by prolonged captivity and suggests that duration of time in captivity should be balanced between achieving body sizes that reduce predation and limiting deleterious effects of captivity on bite performance.

It is possible that differences in latencies to gape and bite between captive and free-ranging turtles were influenced by frequent interactions with people while in captivity (Berger-Tal et al., 2019). Although many indoor- and outdoor-reared captives required increased tapping to elicit a bite, few free-ranging individuals required as much encouragement, and were more likely to skip pre-bite aggressive behaviors such as snapping, lunging, or shell-raising. Alternatively, these behaviors could be tools used by less physically fit individuals to decrease chances of a physical altercation with conspecifics by looking more formidable, a phenomenon that has been observed in lizards (Lappin et al., 2006). The similarity in frequency of pre-bite aggressive displays between captive turtles reared outdoors and the two populations of free-ranging turtles could suggest that time spent acclimating to the outdoors in ponds aids in preparing individuals to succeed in the wild. A future investigation of wild-hatched *M. temminckii* could provide more insight into lasting effects of captivity on bite performance.

Even more so than captives that occupied outdoor ponds, individuals in my study that were reared indoors at the time I conducted my study exhibited poor bite force performance; differences in diet offer one possible explanation for this pattern. Indoors, these turtles primarily subsist on a diet of fish-based food pellets which, although crunchy when dry, quickly become soft after absorbing water. Because of this, and the fact that these small pellets can generally be consumed whole, foraging likely provides little opportunity to develop strong masseter muscles that, when warranted, could produce a forceful bite. This explanation is supported by evidence that megacephaly is a phenotypically plastic trait that is sometimes enhanced in aquatic turtles when individuals forage upon hard-bodied prey (Iverson, 2020). Furthermore, differences in head size among species and between sexes of several species of turtle has been attributed to differences in diet, with groups that consume a more durophagous diet exhibiting broader heads than those that primarily target softer prey items (Lindeman, 2000, 2006).

In addition to consuming a diet of soft, easy-to-swallow food, *M. temminckii* that were reared indoors had little incentive to move about in comparison to those housed outdoors or that were free-ranging in rivers. This, in conjunction with complete protection from predators, eliminated most scenarios that might encourage a strong bite, or even an overall well-toned musculature. It is also possible that the relatively weaker bites of this group were, in part or whole, a product of reduced motivation to bite with maximum force. In comparison to *M. temminckii* inhabiting ponds or rivers, the indoor group regularly interacted with hatchery personnel; these repeated interactions may have reduced individuals' defensive posture and contributed to submaximal motivation to bite.

Overall, bite force scaled to mass predicably and in a pattern similar to that observed in other species of aquatic turtle (Pfaller et al., 2009). However, free-ranging *M. temminckii* exhibited greater bite forces for their size, as evidenced by elevated y-intercepts in plots of bite force relative to mass, but also followed a steeper slope indicating that incremental increases in body size resulted in larger increases in bite force among free-ranging turtles. Because free-ranging turtles were larger, on average, than those in captivity, this pattern could simply reflect an ontogenetic shift in development of skull musculature, as has been observed in other species (Pfaller et al., 2011). Alternatively, it is possible that captive-born *M. temminckii* achieve lower-than-normal bite force capabilities in captivity and thus, upon release into natural systems, start out at a performance disadvantage. Following release, environmental conditions may conspire to provide the physical challenges that captivity lacked, and as a result individuals transition from being relatively poor performers to performing at a level that would be typical of true wild specimens of comparable body sizes. This scenario is consistent with the fact that the captives that were maintained indoors gave bites that scaled to head morphology at smaller-than-predicted scaling exponents, whereas those that were free-ranging tended to scale to steeper-than-predicted slopes.

In comparison to those living indoors in raceways, captive turtles living in outdoor ponds may have benefited physically from greater space to move about, access to natural forage that likely included prey items spanning a wide range of body hardness, and fewer interactions with hatchery personnel. These factors may have increased physical fitness, reduced stress, and—with time—reduced complacency during interactions with people. In comparison to free-ranging conspecifics, on the other hand, having protection from most potential predators may have limited pond-dwelling captives' behavioral defensive response. My results suggest that the increase in bite force observed in free-ranging turtles may at least partially result from "post-release behavior modification," the concept that the more time a captive-reared animal spends in the wild, the better adapted it will become to its new habitat (Berger-Tal and Saltz, 2014).

Implications. *Macrochelys temminckii* have declined throughout their range, and reestablishing extirpated populations with captive-reared animals may play a critical role in the species' long-term survival. Preventing further declines is important not just because *M. temminckii* add to the biodiversity of systems in which they occur but also because they, like many turtles, perform important ecosystem services (Lovich et al., 2019). In particular, *M. temminckii* functions as a top-order predator, a benthic scavenger, and a disperser of seeds (Pritchard, 2006; Elbers and Moll, 2011). Any environment in which they occurred historically is likely detrimentally affected by their absence.

Results of previous studies of growth and survival of *M. temminckii* in the Caney River demonstrate that captive-reared *M. temminckii* thrive upon reintroduction in at least some systems (Anthony et al., 2015). However, it is critical in any reintroduction context that organisms that are released be in as near-optimal condition as possible (Berger-Tal et al., 2019). The comparatively poor bite performance of *M. temminckii* housed indoors could be used to infer that animals in these conditions should be offered a wider range of food items that vary in hardness. Alternatively, I propose that a simpler solution—and one that is already implemented at TNFH—is to minimize the amount of time housed indoors and, even more importantly, ensure that all individuals spend an extended period in outdoor ponds prior to reintroduction into natural systems. Optimizing bite performance prior to reintroduction has the potential to expand the available prey base and reduce threats of predation, both key aspects of survival in a new environment.

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Table 1. Medians and ranges—in parentheses—of morphometric measurements, and maximum bite force by group of *Macrochelys temminckii*.

Variable	Captive	Free-ranging
Individuals tested (n)	126	61
Mass (g)	526 (60–8,150)	5375 (1,350–17,400)
Carapace length (mm)	125.2 (52.8–305.0)	278.1 (176.9–444.0)
Head length (mm)	45.3 (23.4–107.6)	92.3 (65.8–128.2)
Head width (mm)	38.8 (20.2–102.8)	89.5 (59.6–128.6)
Head height (mm)	31.1 (15.2–80.2)	68.0 (44.4–95.9)
Maximum bite force (N)	30.7 (5.8–334.1)	284.7 (69.4–814.5)

Table 2. Regressions of body mass and relevant morphometrics against bite force for indoor captive, outdoor captive, and free-ranging *Macrochelys temminckii*. *Bold are slopes that differ from expected allometric scaling relationships. In the comparison of bite force and mass, I expected a slope of 0.66, and for all other comparisons I expected a slope of 2.0.

		R^2	Intercept	Slope	Confidence limits
Indoor captive	Body mass	0.82	-0.35	0.68	0.61–0.75
	Carapace length	0.82	-2.59	1.96	1.75–2.16
	Head length	0.15	0.14	**0.78**	0.36–1.19
	Head width	0.17	0.19	**0.78**	0.40–1.16
	Head height	0.14	0.45	**0.65**	0.30–1.01
Outdoor captive	Body mass	0.92	-0.45	**0.75**	0.68–0.81
	Carapace length	0.94	-3.40	**2.35**	2.17–2.55
	Head length	0.81	-2.22	2.35	1.99–2.70
	Head width	0.77	-1.70	2.12	1.76–2.48
	Head height	0.77	-1.45	2.09	1.74–2.45
Free-ranging	Body mass	0.75	-0.55	**0.81**	0.69–0.93
	Carapace length	0.62	-3.13	2.28	1.82–2.74
	Head length	0.72	-3.38	**2.97**	2.49–3.45
	Head width	0.79	-3.44	**3.03**	2.63–3.43
	Head height	0.75	-2.33	**2.61**	2.22–3.00

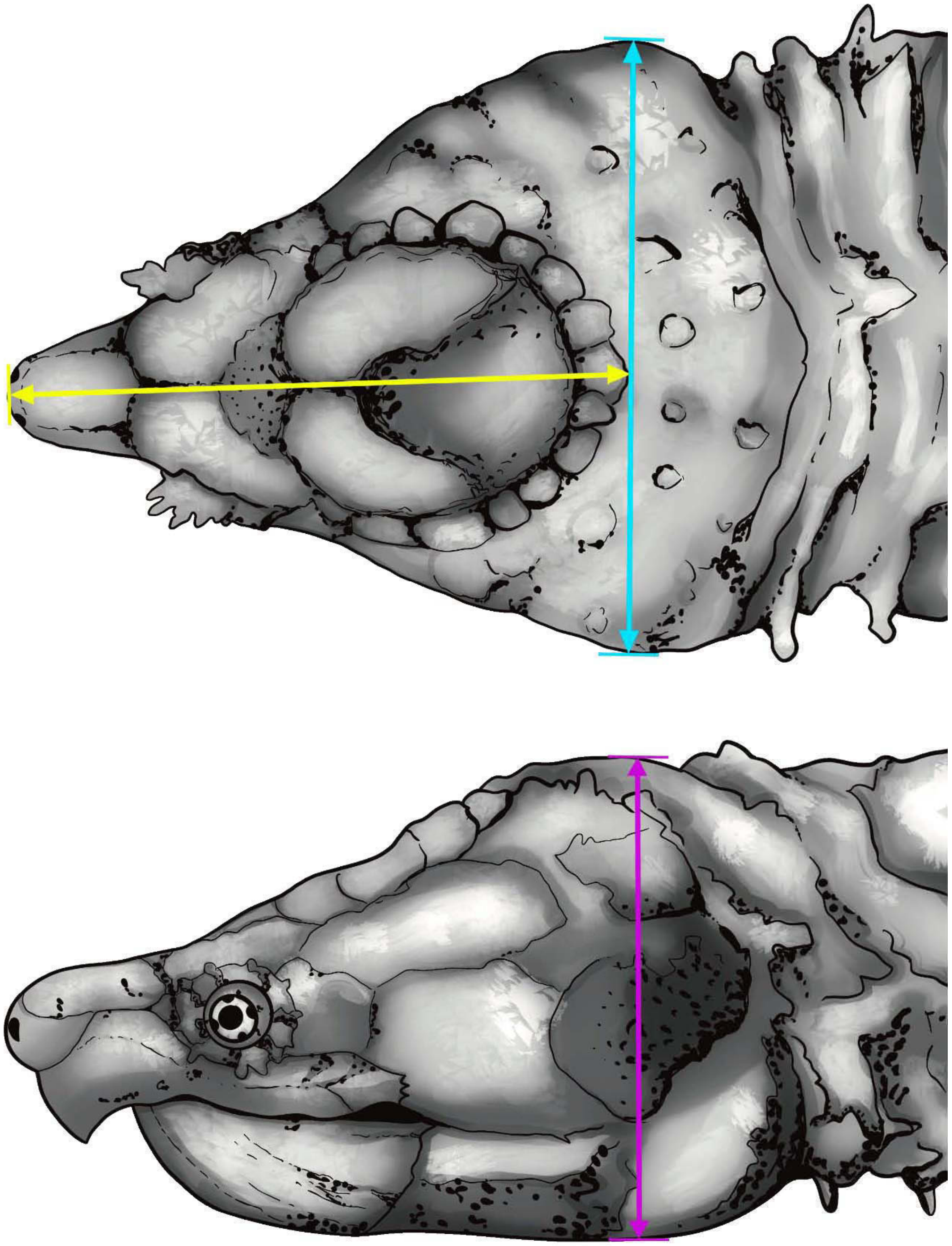

Fig. 1. Anatomical locations of head dimension measurements of *Macrochelys temminckii*, including head length (yellow), head width (teal), and head height (purple).

Fig. 2. A juvenile *Macrochelys temminckii* demonstrating a defensive posture that includes shell-raising and displaying a defensive gape.

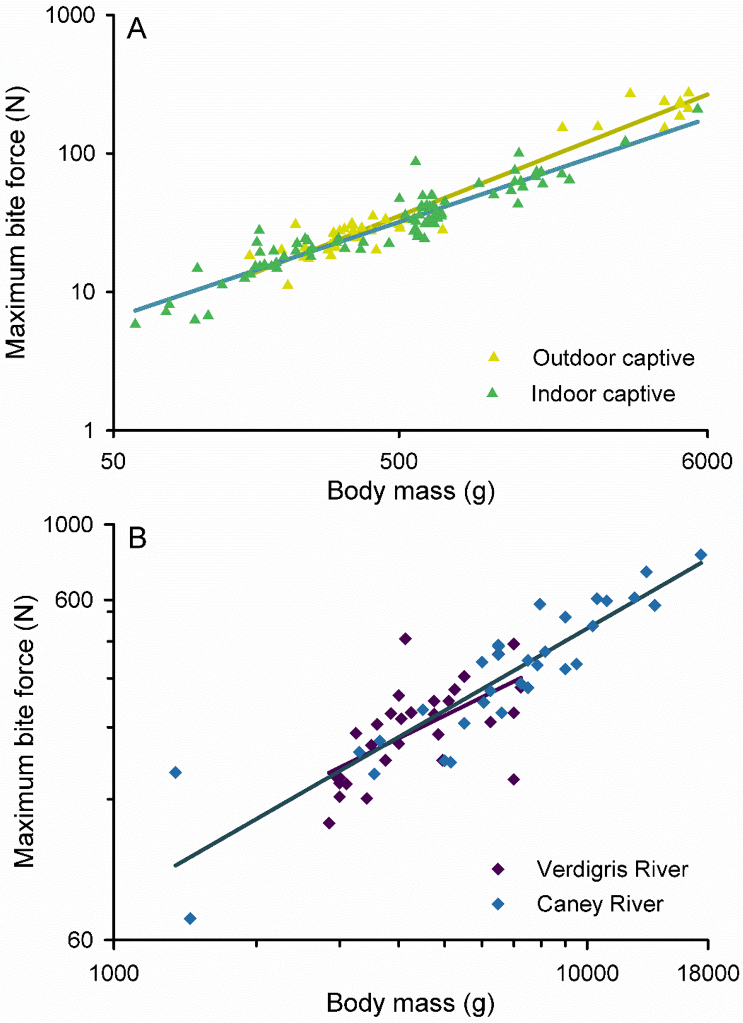

Fig. 3. Maximum bite force of (A) captive-reared *Macrochelys temminckii* reared indoors and outdoors, and (B) head-started, free-ranging *Macrochelys temminckii* inhabiting the Caney and Verdigris rivers in northern Oklahoma. Note that both axes are $\log_{10}$-transformed. Regression line statistics are as follows: outdoor captive ($y = 0.81x - 0.64$, $r^2 = 0.95$, $P < 0.0001$), indoor captive ($y = 0.69x - 0.37$, $r^2 = 0.88$, $P < 0.0001$), Verdigris River ($y = 0.66x - 0.03$, $r^2 = 0.40$, $P < 0.001$), Caney River ($y = 0.80x - 0.51$, $r^2 = 0.81$, $P < 0.0001$).

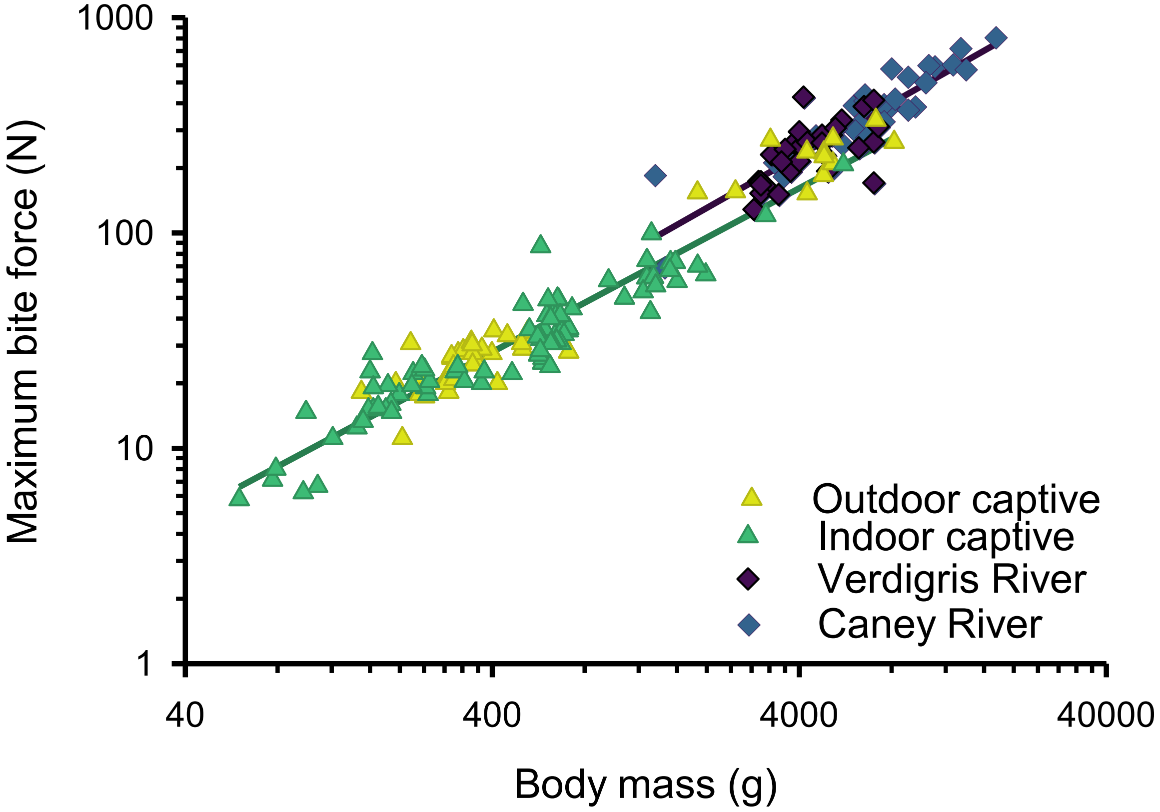

Fig. 4. Relationship between bite force and body mass of captive and free-ranging *Macrochelys temminckii*. Note that both axes are $\log_{10}$-transformed. Equations for regression lines were: captive ($y = 0.76x - 0.53$, $r^2 = 0.91$, $P < 0.0001$), free-ranging ($y = 0.81x - 0.55$, $r^2 = 0.75$, $P < 0.0001$).

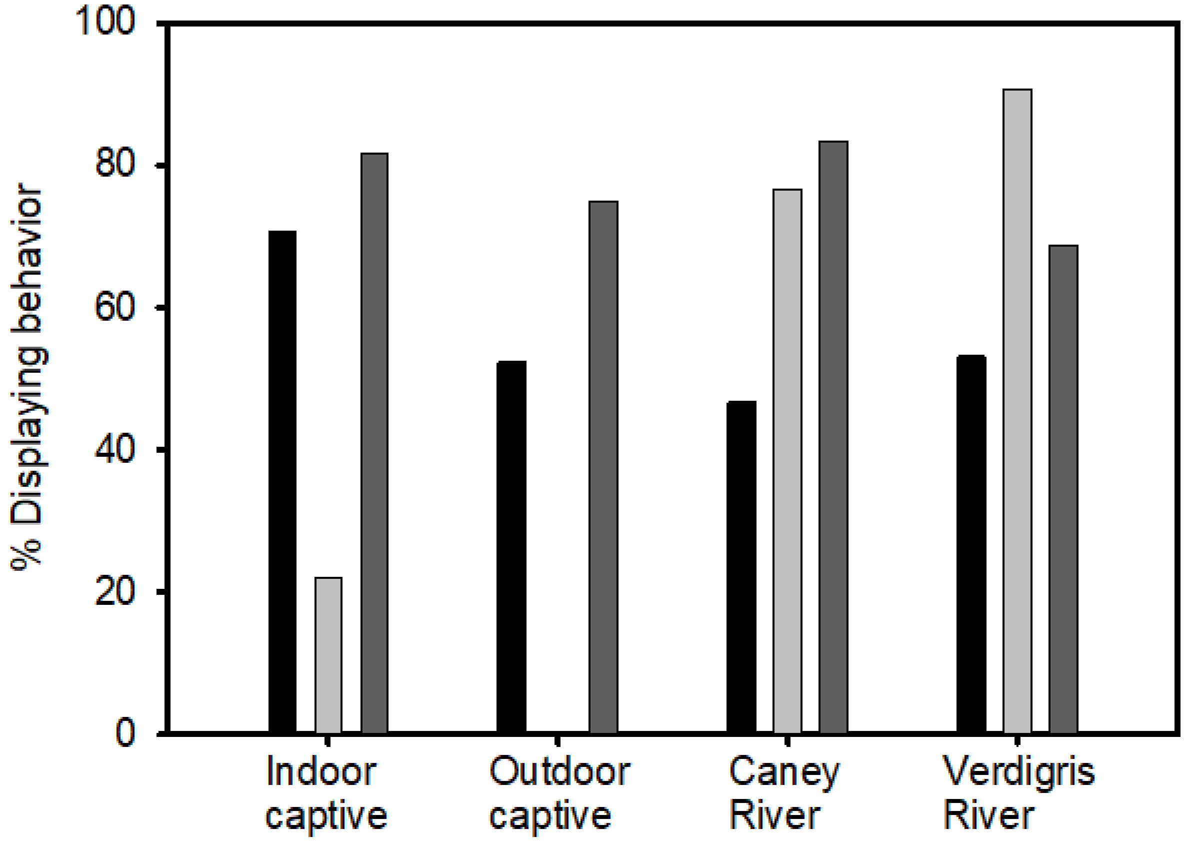

Fig. 5. Frequency of *Macrochelys temminckii* bites by group which required no provocation to elicit a gape or bite, and frequencies of pre-bite aggression displays by group including lunging, shell-raising, or snapping. Pre-bite aggression is denoted by black, immediate gape by light gray, and immediate bite by dark gray.

ACUTE AND SEASONAL EFFECTS OF TEMPERATURE ON BITE PERFORMANCE OF ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*)

Abstract

1. Environmental temperature influences nearly every aspect of ectotherms' biology, particularly in environments where temperature fluctuates widely on daily and seasonal scales. I evaluated the effect of temperature on the bite performance (muscle force and speed) and willingness to bite (central nervous system integration of and response to environmental stimuli) of Alligator Snapping Turtles (*Macrochelys temminckii*), a species that inhabits temperate latitudes in the southeastern United States.
2. Using a force transducer and high-speed videography, I recorded bite force and bite kinematics including bite duration, jaw velocity and acceleration, and lunge velocity and acceleration for 21 captive subadult *M. temminckii*. I also recorded bite behaviors, including willingness to gape and bite, and pre-bite aggressive displays. I conducted trials at 5, 15, and 25 °C, and repeated my measurements in summer and winter, when ambient water temperatures were 6 °C and 25 °C, respectively, to evaluate effects of seasonality.
3. Maximum bite force varied significantly with temperature, albeit not to an extent likely to be biologically relevant.
4. All bite kinematics varied significantly across at least one of the testing temperatures within seasons, with individuals performing maximally at 25 °C.
5. Willingness to bite was more effected by temperature than willingness to gape, with subjects requiring more provocation to gape and bite at 5 °C than at higher temperatures, and displaying fewer pre-bite aggressive behaviors.

6. There was little evidence of seasonal acclimation on kinematics including jaw velocity, jaw acceleration, and lunge acceleration, with higher temperatures in summer often yielding maximal performance and measurements at lower temperature varying little between seasons.
7. My findings support the need for rigorous standardization of temperature in studies of ectotherm performance and suggest that care must be taken in selecting the time of year in which temperature studies are conducted.

Introduction

Temperature affects rates of chemical reactions to varying degrees, a phenomenon that has important implications for rates of physiological and behavioral processes in organisms (Huey, 1982; Cossins & Bowler, 1987; Hochachka & Somero, 2002). Through its influence on metabolic rate, nerve conduction, and muscle dynamics, temperature is integral to whole-organism performance in conducting ecologically relevant tasks (Arnold, 1983; Huey & Kingsolver, 1989). The effects of variable environmental temperatures are most consequential for ectotherms, as methods of modulating body temperature are more limited than for endotherms (Angilletta & Dunham, 2003), and can influence both heart rate (Hochscheid et al., 2002), and speed of movement (Van Damme et al., 1991; Claussen et al., 2002; Herrel et al., 2007). A 10 °C drop in temperature can reduce rates of muscle contraction in ectotherms by half (Bennett, 1985), and in studies of lizards, fish, and frogs, this can equate to an approximately 33% decrease in performance (Huey & Bennet, 1987; Rome, 1990; Anderson & Deban, 2010). Because this relationship can be applied in either direction (Herrel et al., 2007), environmental temperatures have the potential to either constrain or enhance locomotion, and related performance measures such as striking or biting in interactions between predators, prey, and conspecifics (Weetman et al., 1998; Forsman, 1999; Wintzer & Motta, 2004; Whitford et al., 2020b).

Although low temperatures can be deleterious to survival of ectotherms (Anderson & Deban, 2010), the ability of muscles to generate force is generally less dependent on temperature than their speed of activation (Bennett, 1985; Herrel et al., 2007). Additionally, dynamic movements, including the generation of bite force, tends to be independent of significant thermal effects across a range of temperatures, even if dynamic behaviors such as locomotion are not (Herrel et al., 2007; Deban & Richardson, 2011). In some cases, behaviors vary in motivation and help to determine the magnitude of the effect temperature will have on the kinematics of performance. Defensive strikes provide an example; animals' motivation to avoid harm from predators or angry conspecifics often evokes efforts that generate greater raw power, cover greater distances, reach higher velocities, and activate a larger proportion of muscles than do predatory strikes (LaDuc et al., 2002; Moon et al., 2019; Burns & Penning, 2021).

Some animals blunt the effects of temperature on muscle performance by employing elastic recoil mechanisms, a means of temporally isolating the contraction of a muscle from the movement itself that allows for quick and consistent release of kinetic energy without depending on warm temperatures to maximize velocity or acceleration (Anderson & Deban, 2010; Deban & Richardson, 2011; Deban & Scales, 2016). Additionally, some aquatic ectotherms benefit from acclimating to seasonal water temperatures, allowing for comparable physiological function and performance at different temperature settings by compensating for chronic environmental conditions (Hazel & Prosser, 1974; Ligon et al., 2012).

Because they seldom run, jump, or really do much of anything involving maximum effort, bite performance is arguably the most important measure of performance that can be applied to turtles (Herrel et al., 2002). This is particularly true for the Alligator Snapping Turtle (*Macrochelys temminckii*), a species whose common name and dominant physical characteristics suggest the importance of bite performance. As is typical of turtles that inhabit temperate climates, *M. temminckii* forgo feeding during cold winter months; however, bite performance likely remains relevant into late autumn and in early spring when water temperatures are low but males remain active, likely engaging in intrasexual aggression and courtship (Pritchard, 2006; Vervust et al., 2011).

Here I use a combination of high-speed videography and bite force measurements to assess acute and acclimatizational effects of temperature on the bite performance of *M. temminckii*. I recorded bite duration, jaw velocity and acceleration, lunge velocity and acceleration, maximum bite force, and associated bite behaviors at 5, 15, and 25 °C, and conducted trials in summer and winter when ambient water temperatures were 6 and 25 °C respectively at the time of removal from ponds. I hypothesized that (H1) bite force would not vary significantly across testing temperatures or seasons, that (H2) temperature would correlate positively with velocity and acceleration of bite kinematics across temperatures, that (H3) temperature would correlate positively with motivation to bite, as indicated by latency to gape, latency to bite, and displays of pre-bite aggression, and that (H4) seasonal acclimatizational effects would result in similar temperature-specific performance of kinematic variables in winter and summer.

Materials and Methods

Equipment and Software. I measured maximum bite force with a force transducer (Type 9313AA2; Kistler, Winterthur, Switzerland) and charge amplifier (Type 5995; Kistler, Winterthur, Switzerland) contained in a customized housing. During all trials, sensor sensitivity was set to 0.09 pC/Mechanical Units, and range set to 200 K Mechanical Units. Prior to testing, I calibrated the system by hanging weights from the back of the bite plates at the point of contact for the tips of *M. temminckii* beaks and regressing nominal force on mass. I matched subjects to one of two sizes of stainless-steel bite plate relative to their head length, which were then padded with 3-mm thick leather.

I recorded trials using a high-speed camera (Chronos, Kron Technologies, Burnaby, BC, Canada) set to record 1,057 fps, and later used footage to determine bite duration, maximum jaw velocity and acceleration, and maximum lunge velocity and acceleration in the software packages Templo and Vicon Motus 10 (Contemplas GmbH, Kempten, Germany). To standardize positioning of subjects, I placed individuals on an elevated platform and maintained a consistent distance from test subjects to the camera. Additionally, a 38-mm diameter sphere was positioned on the midline of the carapace and posterior to the head to serve as a size standard. Using an infrared thermometer*,* I recorded the water temperature of each turtle's tote prior to testing to confirm it was within 5 °C of the target temperature for each trial.

Bite Trials. Integrating aspects of published methods, I began testing the day after capture and ran trials between the hours of 08:00 and 21:00 (Herrel et al., 2001; Herrel et al., 2002; Herrel & O'Reilly, 2006; Pfaller et al., 2009; Vervust et al., 2011). During winter and summer measurement periods, subjects underwent three defensive bite trials at 5, 15, and 25 °C (Figure 1). I randomly assigned test subjects to one of three groups and started each group in a separate environmental chamber set to one of the three testing temperatures. Turtles were housed at their target temperature overnight and tested at that temperature the following day before being moved to an environmental chamber set at another temperature and held overnight.

Trials at each temperature consisted of three defensive bites per turtle, each involving contact with the bite force apparatus and a simultaneous recording via high-speed videography. Between bites, I allowed at least 30 minutes of rest, during which I returned subjects to their respective environmental chambers. In addition to immediately documenting resulting force, I also scored the quality of each bite as either “Good” or “Poor” based upon visual observations of where the beak struck the bite plate and the presence of aggressive displays over the course of each bite, including shell raising, lunging, or snapping. When presented with the bite force apparatus, most turtles responded quickly and without additional provocation, but in cases where any refused to immediately gape or bite, I gently tapped them on the head to elicit a defensive response and scored latency to gape and latency to bite for each turtle as either “Immediate” or “Required tapping.”

To standardize data collection and control for bite placement, I size-matched each turtle to one of two different bite plates. I used small bite plates for turtles with head lengths of 50–74 mm, and large bite plates for turtles with head lengths ≥ 75 mm. This reduced the chance of injury to the subjects that could result from contact with the back of the plates and ensured that turtles interacted with a surface area of contact proportional to their head dimensions. I equipped plates with short metal stops at their base that prevented turtles from biting too far back on the apparatus while also guiding the beak tips to a consistent point of contact near the back of the bite plates. To control for gape angle at contact, I standardized the spacing between the two bite plates to be within 5 mm of 0.25x head height (Appendix C).

Video Analysis. I recorded a total of 378 videos over the course of my study, from which I calculated kinematics of interest. I calibrated all measurements using a 38-mm diameter size standard placed in the frame of each video and began analysis at the first frame showing detectable motion of the lower jaw. After digitizing the top and bottom tips of the beak I advanced frame-by-frame through both points of contact and recorded the instant of contact for both tips. I defined gape using the beak tips and the back of the crease of the jaw, and in each video, I also digitized the tip of the snout to measure lunge distance (Figure 2). I defined bite duration as the shortest time elapsed for each individual between the first movement of the lower jaw and the point where both mandible and maxilla touched the bite plates. For each individual, I defined maximum jaw velocity as the single highest maximum velocity (instantaneous velocity) generated across all digitized videos for each temperature setting and season. I defined maximum jaw acceleration as the single highest maximum acceleration (instantaneous acceleration) reached across all digitized videos for each temperature setting and season. I calculated lunge kinematics using only turtles that noticeably lunged as opposed to simply closing their mandible with no extension of the neck. I calculated maximum lunge velocity and acceleration using the distance travelled by the point of each turtle's snout from the first sign of forward motion until the point where forward movement stopped. I calculated all maximum kinematics by incorporating the single highest frame-by-frame calculation from within any movement. Though unreported in this chapter, I also measured lunge distance, and lunge duration, average values for all bite force and kinematic variables, as well as their temperature sensitivities (Appendix D; Appendix E; Appendix F).

Statistical analysis. Of the 378 videos captured, I processed 368 videos containing bites recorded as "Good" quality from individuals which were captured in both test seasons. Of the processed videos, only the maximum measurements of kinematic performance for each turtle in each season went on to be used in statistical analyses. These included the fastest accelerations, shortest bite durations, and maximum bite forces gleaned from the set of three measurements conducted at each temperature. This left a sample size of 126 measurements per force and kinematic variable, used in a series of repeated measures Analysis of Variance (RM-ANOVA) tests to detect differences in kinematics across temperatures and seasons. I calculated RM-ANOVA tests in R-Studio (Version 1.3.1093, PBC), using individual ID as a factor and "winter" and "summer" as categorical descriptions of the seasons (R Studio Team, 2020). I then used partial models and Tukey's Honestly Significant Difference tests to further explore the effects of season and temperature.

I used a full factorial model and log-transformed measurements to test for interactions between temperature and season on maximum bite force, and a partial model to determine significant predictors of bite performance in instances where interactions were not significant. To detect differences in bite behaviors between temperature settings and across seasons including willingness to gape or bite, and display of pre-bite aggression, I performed a series of chi-square tests with the sums of turtles from both seasons and in each group that required tapping to gape or bite in their highest measurement of bite force, or that displayed pre-bite aggression in any of their three bites for a single trial (Hammer et al., 2001). While the combined data points from both seasons were not independent of each other due to repeated measures from each individual, I first checked for differences across temperature settings in each season separately before combining them to explore for potential interactions. Whenever it was inappropriate to conduct chi-square contingency analysis due to small frequencies, I instead described relationships quantitatively, or analyzed the ratios of animals that did not display the behavior. Finally, I calculated mean and median Q10 values for all kinematics and bite force variables across an increase from 5–25 °C to assess the temperature sensitivity of each performance measure. I compared actual Q10 values to conceptual thresholds of temperature dependence using means and 1-tailed, 1-sample t-tests when data were normal, and medians and 1-tailed Wilcoxon tests when data were not. I defined the lower and upper limits of Q10 values representing thermal independence as 0.5 and 1.5, testing variables with increasing rates against the latter and variables with decreasing rates against the former (Deban & Richardson, 2011).

This study was approved by the Missouri State University Institutional Animal Care and Use Committee on 06/2019 and received Approval #19-014 (See Appendix A).

Results

Overview. In December 2019 I hand-captured and tested a sample of 21 captive, subadult *M. temminckii* housed in an outdoor pond at Tishomingo National Fish Hatchery (TNFH), and in September 2020, I recaptured and retested the same 21 individuals. Winter and summer pond temperatures at the time trials were conducted were 6 and 25 °C, respectively, resulting in prior acclimatization to the lowest and highest test temperatures. All individuals tested were sexually immature and hatched between the years 2002 and 2011. They ranged 1.74–8.39 kg in mass, and 18.8–31.5 cm in straight carapace length (Appendix G).

Upon presentation of the bite force apparatus, *M. temminckii* typically responded with a defensive gape, followed by target tracking with their eyes and sometimes a twitch of the tongue in preparation to strike. Lunges were characterized by a rapid strike toward the target that included closure of the eyes and the lower jaw, followed by contact with both beak tips and continued momentum until the rest of the body caught up, usually resulting in a forward pitch of the limbs (Figure 3). Jaw closing velocities and accelerations were typically highest at the beginning of the bite sequence, with acceleration peaking approximately 0.01 ms sooner than velocity (Figure 4A). Lunge velocity and acceleration also increased at this time, but lunge velocity plateaued through the rest of the bite sequence while acceleration undulated in a series of successively smaller peaks until completion of the bite (Figure 4B).

Bite Force Performance. Maximum bite force ranged from 84.52–482.19 N, and in a full factorial model, there was no significant interaction between temperature and season ($F_{2,100} = 1.32$, $P = 0.27$; Figure 5). After removing the interaction term from the model, temperature ($F_{2,102} = 12.21$, $P < 0.0001$) and season ($F_{1,102} = 7.73$, $P < 0.01$) were both significant predictors of bite force. Bites at 5 °C generated significantly less force than at 15 °C ($P < 0.01$) or 25 °C ($P < 0.0001$), which did not differ significantly from one another ($P = 0.12$). Across measurement temperatures, bite forces were higher in winter than in summer (Appendix H; $P = 0.01$; Table 1).

Bite Kinematics. Analysis of 126 samples of maximal performance for each individual revealed that kinematic measurements and maximum bite force vary across temperature settings and seasons, and in most cases subjects bit faster at warmer temperatures and in the summer (Table 1). An exception to this was often at 5 °C, where turtles bit either the same or faster in the winter than they did at the same temperature in the summer.

Minimum bite duration ranged from 7.5–178 ms and there was no significant interaction between temperature and season in a full model ($F_{2,100} = 0.76$, $P = 0.47$). In a partial model, temperature was a significant predictor of bite force ($F_{2,102} = 43.19$, $P < 0.0001$) but season was not ($F_{1,102} = 0.40$, $P = 0.53$), and across measurement temperatures, bites got significantly shorter with increases in temperature (all $P < 0.01$; Figure 6; Table 1). Maximum jaw velocity ranged from 0.147–4.667 m/s. There was a significant interaction between temperature and season

($F_{2,100} = 7.5$, $P < 0.0009$), and velocity increased significantly with temperature at all settings (Figure 7; $P < 0.0001$). I detected no significant difference between the performance of turtles in either season at 5 °C ($P = 0.83$), but at 15 °C ($P < 0.01$) and 25 °C ($P < 0.004$), they bit with significantly higher maximum jaw velocities in the summer than in the winter (Table 1). Maximum jaw acceleration ranged from 11.82–675 m/s^2 and there was no interaction between temperature and season in a full factorial model (Figure 8; $F_{2,100} = 0.42$, $P = 0.66$). In a partial model, season was not a significant factor ($F_{1,102} = 1.13$, $P = 0.29$), but temperature was ($F_{2,102} = 93.70$, $P < 0.0001$), and maximum jaw acceleration increased significantly with each temperature setting (all $P < 0.0001$; Table 1).

Maximum lunge velocity ranged from 0.13–2.636 m/s and there was no significant interaction between temperature and season in a full factorial model ($F_{2,79} = 2.33$, $P = 0.10$) (Figure 9). Season was not a significant factor in a partial model ($F_{1,81} = 2.02$, $P = 0.16$), but temperature was ($F_{2,81} = 78.33$, $P < 0.0001$), and velocities increased significantly with each temperature setting (all $P < 0.0001$; Table 1). Maximum lunge acceleration ranged from 3.97–535.76 m/s^2, and there was a significant interaction between temperature and season in a full factorial model ($F_{2,79} = 3.4$, $P < 0.04$; Figure 10). While lunges in either season were not different from one another at 5 °C ($P = 1.0$), turtles lunged significantly faster in the summer than in the winter at 15 °C and 25 °C (both $P < 0.002$). Maximum lunge acceleration increased significantly with each temperature setting in the summer (all $P < 0.01$), but in the winter, bites at 5 °C and 15 °C were not significantly different ($P = 0.62$; Table 1).

Sensitivity of Performance to Temperature. Mean Q10 values for all variables from 5–25 °C ranged from 0.3–47.6 in the summer and from 0.3–22.5 in the winter. All variables displayed thermal dependence (all $P > 0.05$), and for some variables including maximum jaw velocity and acceleration, and maximum lunge velocity and acceleration, dependence was highest in the summer trials (Table 2).

Bite behavior. Alligator Snapping Turtles significantly differed in willingness to bite among test temperatures during the summer trials ($\chi^2_2 = 14.13$, $P < 0.0008$), requiring greater percentages of tapping to elicit a defensive bite at 5 °C (86%) than at higher temperatures (both ≤ 38%). They did not otherwise vary significantly between seasons in bite behaviors including willingness to bite ($\chi^2_2 = 5.53$, $P = 0.06$), willingness to gape ($\chi^2_2 = 1.44$, $P = 0.49$), or pre-bite aggression ($\chi^2_2 = 2.83$, $P = 0.24$). Similarly, during summer trials they did not vary significantly among temperatures in bite behaviors including willingness to gape ($\chi^2_2 = 3.63$, $P = 0.16$) or pre-bite aggression ($\chi^2_2 = 14.13$, $P = 0.38$). Winter trials did not yield frequencies of tapping or pre-bite aggressive behaviors large enough for chi-square analysis, however little tapping was necessary to elicit gape or bite behaviors in these trials (all percentages of tapping ≤ 5%), and instances of pre-bite aggressive behavior were highest at 25 °C (67%) and less at lower temperatures (both ≤ 5%).

Discussion

Overview. My finding that maximum bite force was relatively robust to the effects of temperature, and the positive relationship witnessed between all other measurements and temperature were expected due to physiological patterns in ectotherms (Weetman et al., 1998; Forsman, 1999; Wintzer & Motta, 2004; Whitford et al., 2020b), however, the significant interactions between kinematic performance variables and season were unprecedented and stand to be further explored. In general, kinematic performance was hindered at 5 °C, no matter the season, with greater seasonal differences for some variables becoming apparent at greater temperatures.

These findings suggest that the relationship between temperature, season, and bite performance is complex in *M. temminckii* and that chronic exposure to pond temperatures does not appear to result in acclimatization effects that provide comparable performance across summer and winter trials. Further, these findings should be thoughtfully considered when designing future performance studies, and when planning conservation efforts in the face of global climate change. Rising seasonal temperatures will potentially drive the interactions of ectothermic predators and their prey into the future, as previously observed in coral reef fish which displayed higher rates of prey capture and attack with a 3 °C increase in temperature (Dell et al., 2014; Allan et al., 2015). For conservation initiatives such as the head-start program operated out of TNFH, performance studies provide a chance to evaluate the viability of captive-reared individuals prior to or after release, and my experiments justify the need to strictly standardize both the temperature settings, and the season of study.

Effect of Seasonality and Acclimation on Performance. The kinematics most influenced by seasonality included maximum jaw velocity and maximum lunge acceleration, both of which interacted significantly with season in a full factorial model, and at higher temperatures, saw significantly greater performance in the summer than in the winter. Another variable determined to have season as a significant predictor of performance included maximum bite force. The formerly mentioned variables did not vary significantly across seasons at 5 °C but did at 15 °C and 25 °C. Despite a lack of significant difference in performance at 5 °C, maximum lunge velocity saw higher mean values at this temperature in the summer than in the winter (Table 1).

My Q10 values further revealed a trend toward increased temperature sensitivity in summer for several variables, although high summer Q10 values for maximum lunge velocity and acceleration can likely be attributed to small sample sizes. I suspect that my findings indicate a lack of acclimatization to chronic temperatures present in the outdoor holding pond at TNFH, and that while these animals are quite capable of defending themselves at cold temperatures, and in the winter, they lack sophisticated methods of acclimatization including extra calcium pumps or changes in enzymes that other species of ectotherms frequently employ to maintain comparable performance across chronic, or seasonal environmental changes (Hazel & Prosser, 1974; Nelson et al., 2003). A similar lack of acclimatization ability was previously observed in the metabolic rates of *M. temminckii* (Ligon & Lovern, 2009), and could possibly be related to a lack of genetic diversity within river systems native to this species of conservation concern (Roman et al., 1999).

Effect of Temperature on Bite Force. Of all the performance variables I tested, maximum bite force appeared the most resilient to temperature's acute and seasonal effects. Though maximum bite force varied at 5 °C within seasons, it is possible that this difference is not biologically significant. Further, it appeared that overall, subjects performed maximally in the winter, perhaps because individuals were already adjusted to cold environmental temperatures. Although not determined to significantly differ from either threshold of temperature dependence, the close grouping of my data, and lack of variance could lend to the detection of statistical differences where there would otherwise be none. If this was the case, it could provide additional support that rapid movements often display a surprising consistency across temperature (Anderson & Deban, 2010; Deban & Scales, 2016). Ours also reflect results from other relevant studies involving lizards (*Trapelus pallida*) and salamanders (*Hydromantes* *playcephalus*) that a muscle's ability to generate force is less dependent on temperature than its speed of activation (Bennett, 1985; Herrel et al., 2007; Deban & Richardson, 2011).

Effect of Temperature on Bite Kinematics. Within both seasons of my study, I found the performance of each kinematic variable to increase significantly with temperature at one or more settings, a trend that remains consistent across a variety of ectotherms in similar studies. In particular, my findings that performance of jaw acceleration, jaw velocity, and bite duration increase significantly with temperature were similar to those made by Vervust et al. in their 2011 study of the Common Snapping Turtle (*Chelydra serpentina*). Other findings akin to my own include those from a study of viperid snakes which found a significant increase in strike acceleration with temperature in both field and captive experiments, as well as those from other studies of defensive strikes in snakes which found higher velocities and accelerations at warmer body temperatures (Rowe & Owings, 1990; Stepp-Bolling, 2012; Whitford et al., 2020; Whitford et al., 2020b). Despite coinciding with my findings that strike acceleration increases with temperature, the study by Whitford et al. in 2020 (b) varied in its conclusion that maximum strike velocity does not vary across a wide range of testing temperatures 5–35°C. Similarly, my findings that maximum lunge accelerations were significantly higher at multiple temperature settings between and within seasons, and that maximum lunge velocities were significantly different at all temperatures within seasons, varied from those of Vervust et al. (2011), that strike velocity and acceleration of *C. serpentina* were not affected by temperature. Both cited studies suggested elastic recoil mechanisms as a potential explanation for temperature independence in some kinematics, however, my own results suggest that these structures may not exist in *M. temminckii* and—along with a low potential for acclimatization to seasonal or chronic temperatures—could influence its need to hibernate during the coldest months of the year (Pritchard, 2006; Vervust et al., 2011).

My calculated Q10 values indicated significant thermal dependence for all kinematic variables (Deban & Richardson, 2011). Additionally, the twofold—if not greater—increase I witnessed in Q10 values of the variables jaw velocity, jaw acceleration, lunge velocity, and lunge acceleration were consistent with previous findings that the performance of muscle-driven movements double with each 10°C increase in temperature (Bennett, 1985; Herrel et al., 2007). It is possible that the extreme Q10 values associated with acceleration were due to trials varying in quality, and perhaps not representative of biological reality.

Effect of Temperature and Season on Bite Behaviors. My results suggest that willingness to gape in *M. temminckii* was not greatly influenced by temperature, but willingness to bite was. For either variable, subjects required more tapping to elicit a response at 5 °C than nearly any other temperature, and in the winter, subjects did not require as much provocation in the form of tapping as during the summer. Interestingly, this trend also held true for pre-bite aggressive behaviors in both trials of my study, with a majority of individuals displaying aggression at 25 °C or 15 °C and fewer displaying aggression at 5 °C. There were dramatically fewer turtles displaying aggressive behaviors at lower temperatures in the winter than during the summer. My findings could be due to resilience of subjects to challenging conditions during winter months, and perhaps an enhancement of aggressive and defensive behavior during summer and at warmer temperatures. My findings varied from those in a study of *C. Serpentina*, primarily because none of my subjects went unresponsive below 15 °C, and secondarily because I witnessed differences in willingness to bite at low and high temperatures while they did not (Vervust et al., 2011).

Concluding Thoughts. While all individuals in my study were captive and experienced identical conditions within their outdoor enclosure, results of my study in Chapter 1 study suggest that captive *M. temminckii* underperform free-ranging specimens in bite force, and a study of prey capture in rattlesnakes found a similar relationship in the form of reduced bite velocity in captive specimens when compared with wild ones (LadDuc, 2002). As suggested by Whitford et al. it is possible that animals underperform in situations including predatory strikes, in exchange for increased accuracy, and in some species, larger individuals scale so that their strike performance is higher than in smaller individuals due to negative allometry between head and body size (Herrel et al., 2011; Penning et al., 2019; Whitford et al., 2020b). While my results surely reveal aspects of temperature's effects on this species, they should not be assumed for wild individuals without further testing. Another aspect to consider, is though conducting this kind of study in captivity is common, the sudden confinement within adjustment bins and environmental chambers likely had an impact on the motivation and stress of subjects, potentially impacting their performance in ways that are not consistent with defensive strikes in this turtle's natural, aquatic environment (Vervust et al., 2011; Whitford et al., 2019). As suggested in a study by Ligon and Lovern in 2009, *M. temminckii* appears to have a positive correlation between physiological processes and temperature, but does not necessarily compensate for cold chronic temperatures through acclimatization. This ability of *M. temminckii* to tolerate a variety of environmental conditions is likely what allows this species to occupy such a wide geographic range and use movement within the water column to optimize its efforts when necessary (Riedle et al., 2006). Regardless, we must ensure that future performance studies involving *M. temminckii* involve careful standardization of temperature, and that future conservation efforts incorporate the effects of seasonality and of global climate change into their planning.

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TABLE 1 Bite performance results for Alligator Snapping Turtles (*Macrochelys temminckii*) measured at three temperatures and repeated in summer and winter. Values are mean ± 1 SE

	Summer			Winter		
Variable	5 °C	15 °C	25 °C	5 °C	15 °C	25 °C
Maximum bite force	26.0 ± 14.7*	248.9 ± 15.1	254.6 ± 14.7	246.9 ± 17.0*	252.2 ± 15.8	269.0 ± 16.4
Minimum Bite duration	67.4 ± 7.0*	33.2 ± 7.6*	19.4 ± 3.1*	58.3 ± 3.8*	34.0 ± 1.9*	20.5 ± 0.9*
Maximum jaw velocity	0.5 ± 0.1*	1.9 ± 0.1*†	3.20 ± 0.2*†	0.7 ± 0.0*	1.5 ± 0.1*†	2.7 ± 0.1*†
Maximum jaw acceleration	55.9 ± 6.0*	203.5 ± 23.4*	377.3 ± 34.8*	90.9 ± 9.6*	198.9 ± 19.1*	407.8 ± 33.4*
Maximum lunge velocity	0.4 ± 0.1*	1.1 ± 0.1*	1.5 ± 0.2*	0.4 ± 0.0*	0.7 ± 0.1*	1.6 ± 0.1*
Maximum lunge acceleration	58.5 ± 23.9*	184.7 ± 15.1*†	305.5 ± 21.9*†	49.0 ± 10.0	80.7 ± 12.3†	195.6 ± 17.0*†

* Indicates significant differences ($P \leq 0.05$) among temperatures within a season, † indicates significant differences between seasons at a given temperature, and bold font indicates significant interactions between temperature and season. See text for explanations of interactions.

TABLE 2 Means, standard deviations, and medians of Q10 values for kinematic and bite force measurements for *Macrochelys temminckii* across a temperature range of 5–25 °C and during summer and winter trials respectively. *P*-values indicate thermal dependence based upon 1-sample t-tests and mean Q10 values where data was normal—summer maximum bite force, maximum jaw velocity, and maximum lunge velocity—and Wilcoxon tests and median Q10 values where data was not normal—winter maximum bite force, minimum bite duration, and maximum jaw acceleration. Q10 thresholds for temperature dependence were primarily 1.5, but in the case of decreasing rates—minimum bite duration—I used a threshold of 0.5.

	Summer	5–25 °C			Winter	5–25 °C		
Variable	Mean	SD	Median	*p*-value	Mean	SD	Median	*p*-value
Maximum bite force	1.2	± 0.2	1.2	$P < 0.0001$	1.1	± 0.2	1.1	$P < 0.001$
Minimum bite duration	0.3	± 0.2	0.2	$P < 0.01$	0.3	± 0.2	0.3	$P < 0.0001$
Maximum jaw velocity	11.6	± 7.8	9.4	$P < 0.0001$	5.1	± 1.7	4.9	$P < 0.0001$
Maximum jaw acceleration	15.2	± 25.9	8.0	$P < 0.0001$	10.8	± 12.2	6.0	$P < 0.001$
Maximum lunge velocity	7.3	± 4.5	6.9	$P < 0.045$	6.2	± 3.3	6.0	$P < 0.0001$
Maximum lunge acceleration	47.6	± 82.3	6.8	$P < 0.03$	22.5	± 47.7	7.3	$P < 0.001$

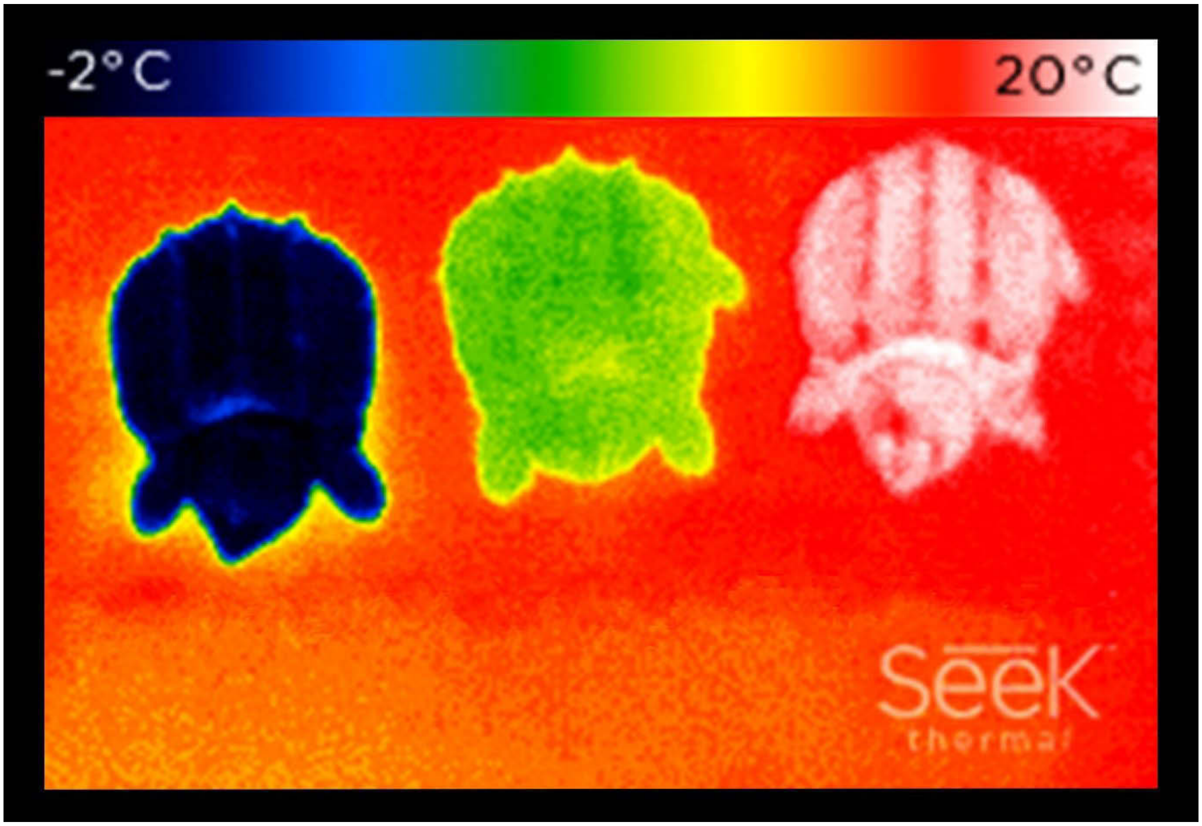


FIGURE 1 Thermal image of a series of *Macrochelys temminckii* test subjects at each of the three testing temperatures 5 °C, 15 °C, and 25 ° C

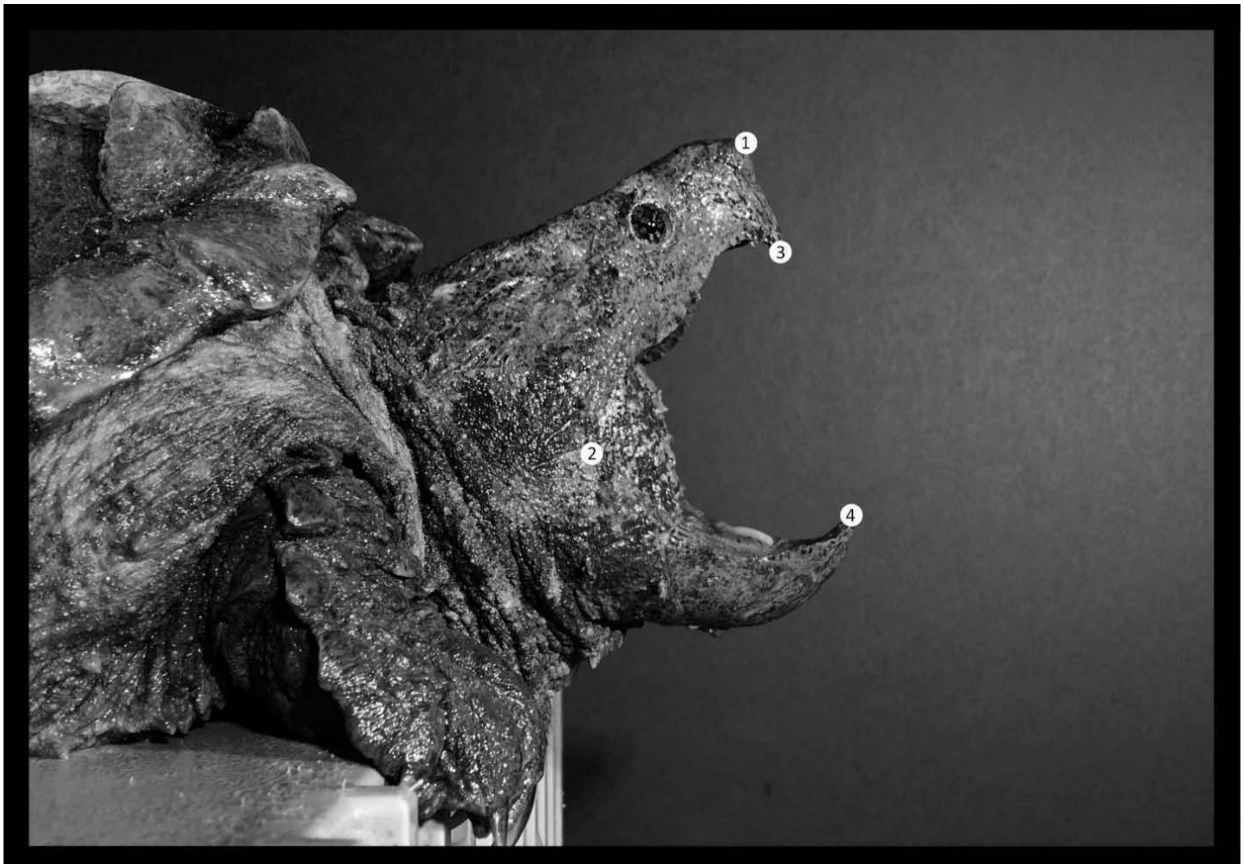


FIGURE 2 Digitized points for bite kinematic generation in *Macrochelys temminckii*. Point 1 represents the tip of snout, point 2 the jaw crease, point 3 the top beak-tip, and point 4 the bottom beak tip

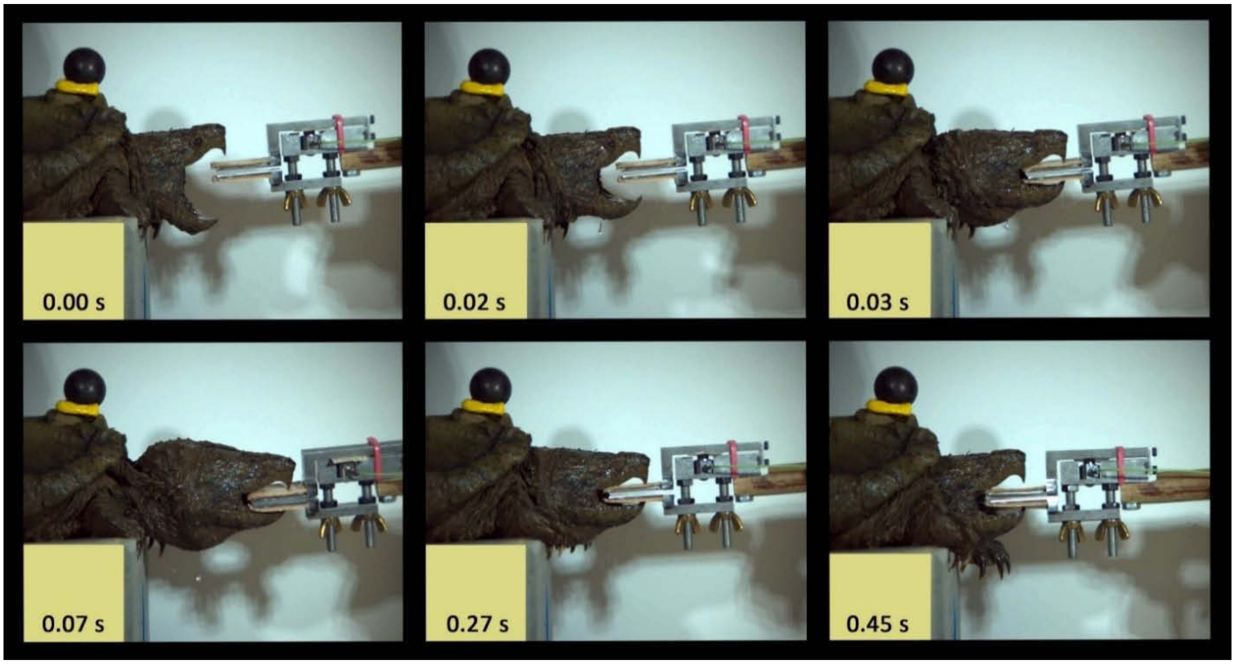
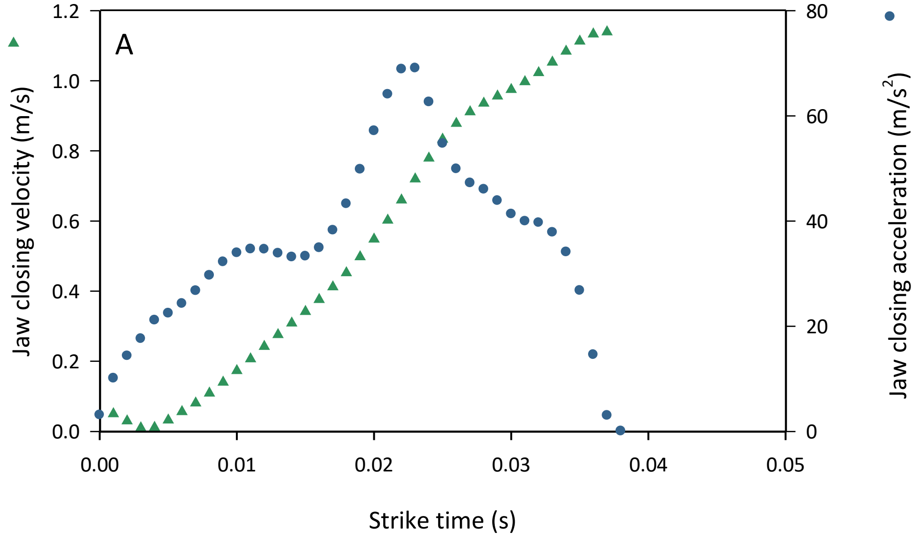


FIGURE 3 Major stages of a bite by *Macrochelys temminckii*, including target tracking, striking/lunging, contact by both beak tips with target, full extension, retraction, and release. Visible atop the carapace is a 38-mm size standard.



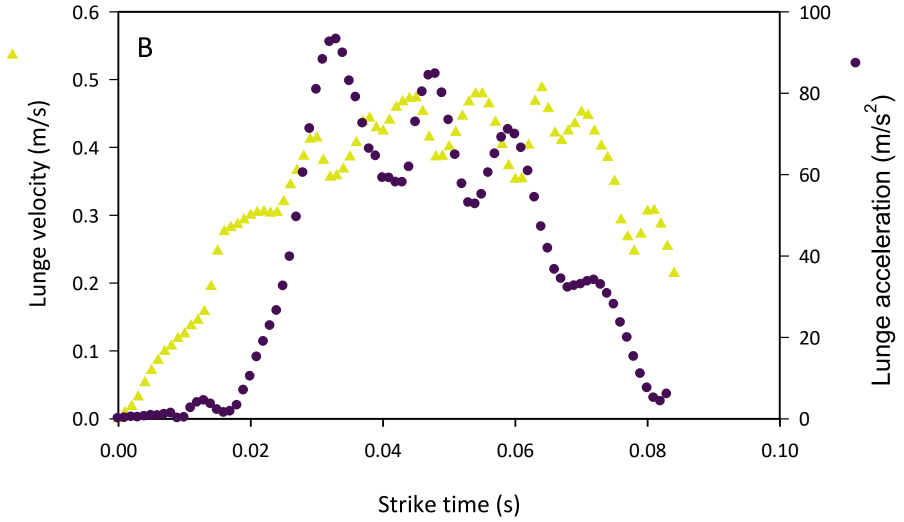


FIGURE 4 Patterns of jaw closing velocity and acceleration (A), and lunge velocity and acceleration with strike time in a sample *Macrochelys temminckii* bite

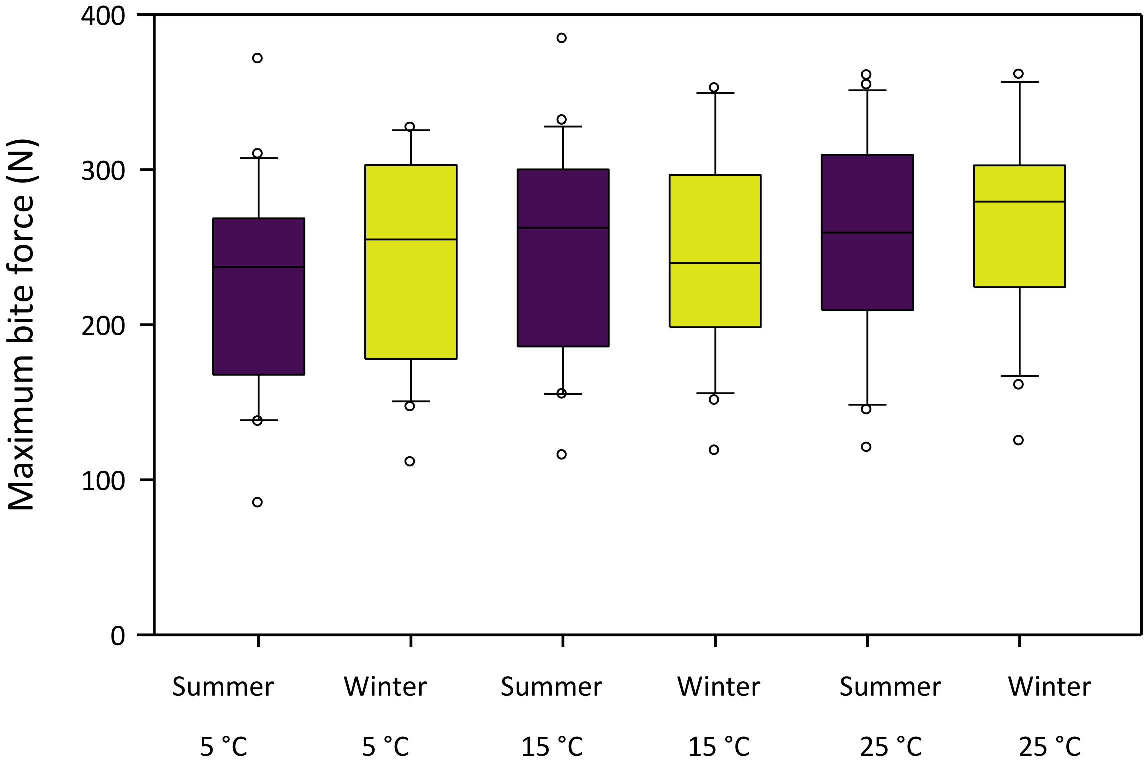


FIGURE 5 Maximum bite force achieved by *Macrochelys temminckii* across temperature settings and seasons

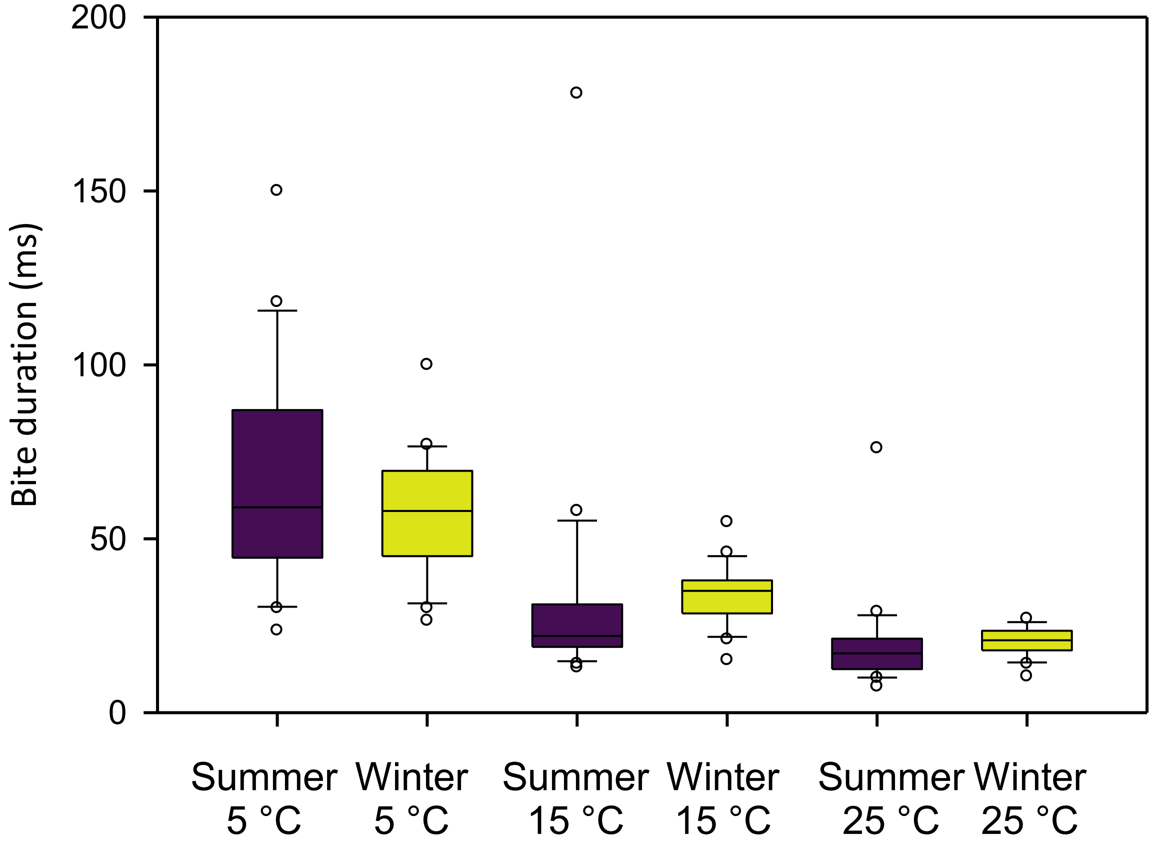


FIGURE 6 Bite duration of *Macrochelys temminckii* across temperature settings and seasons

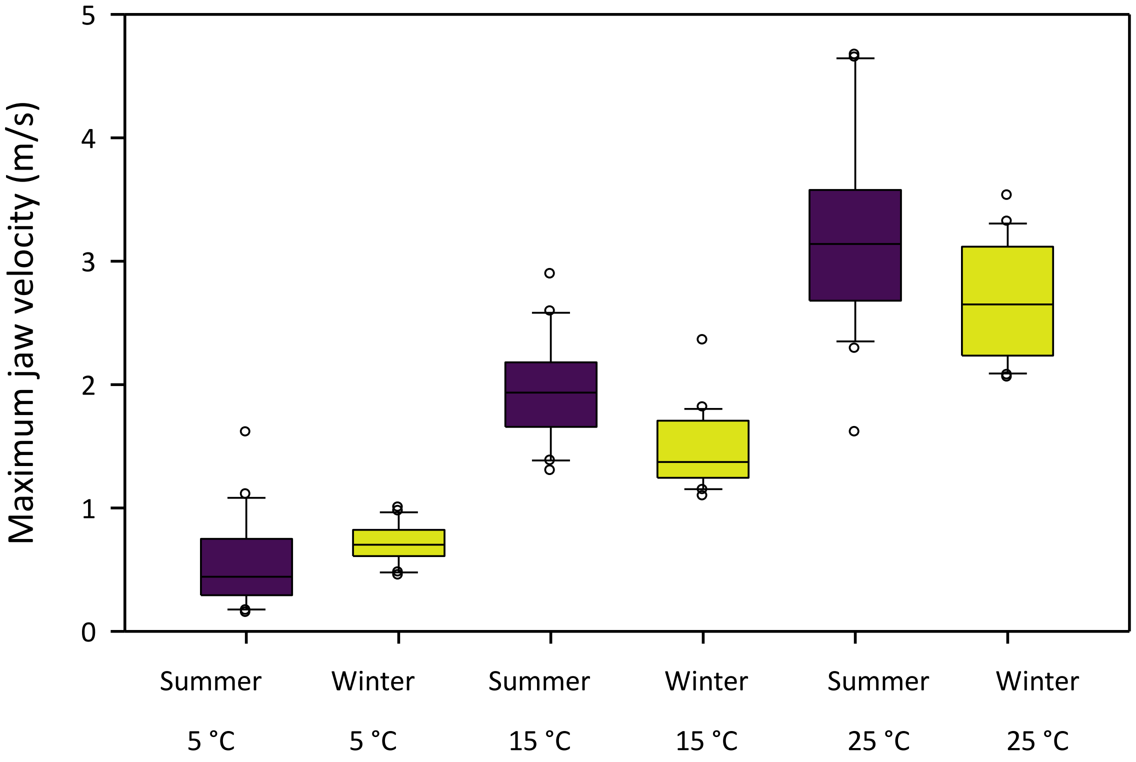


FIGURE 7 Maximum jaw velocity of *Macrochelys temminckii* across temperature settings and seasons

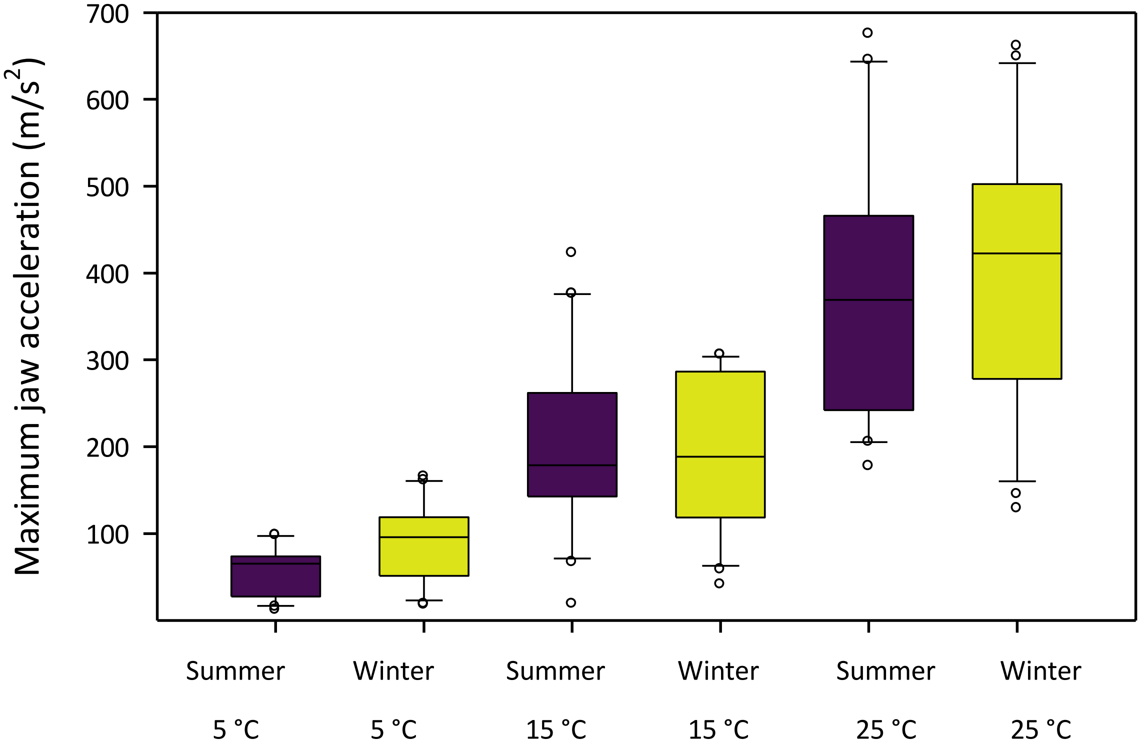


FIGURE 8 Maximum jaw acceleration of *Macrochelys temminckii* across temperature settings and seasons

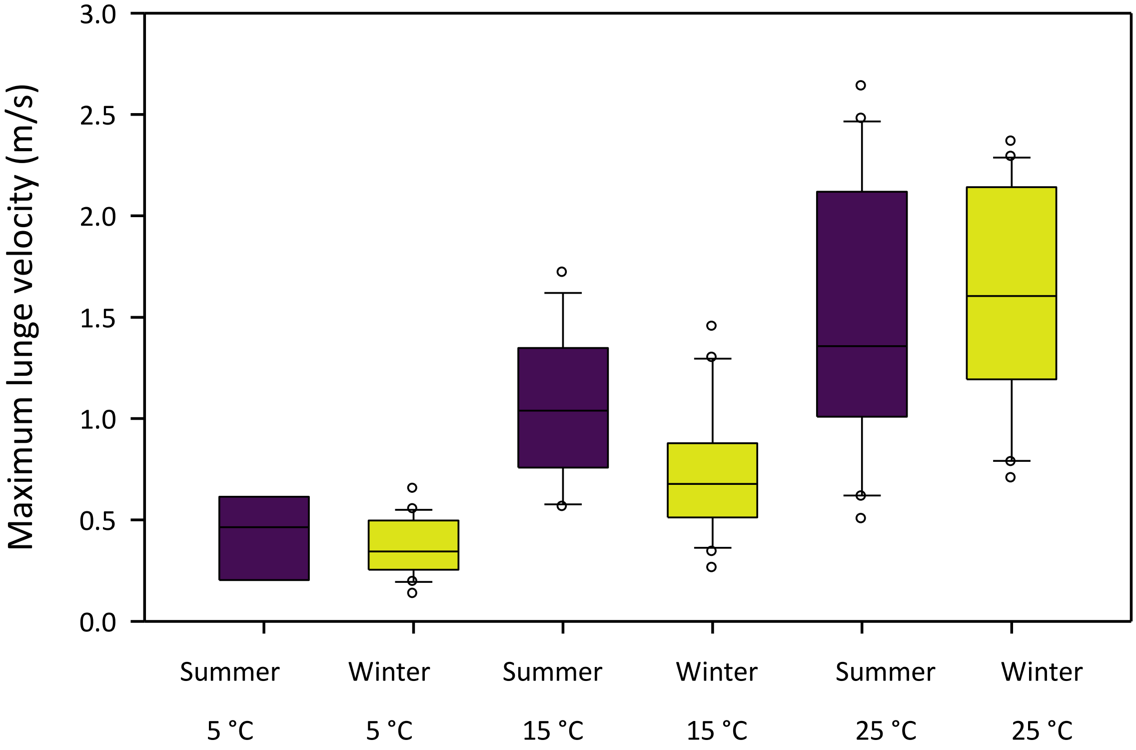


FIGURE 9 Maximum lunge velocity of *Macrochelys temminckii* across temperature settings and seasons

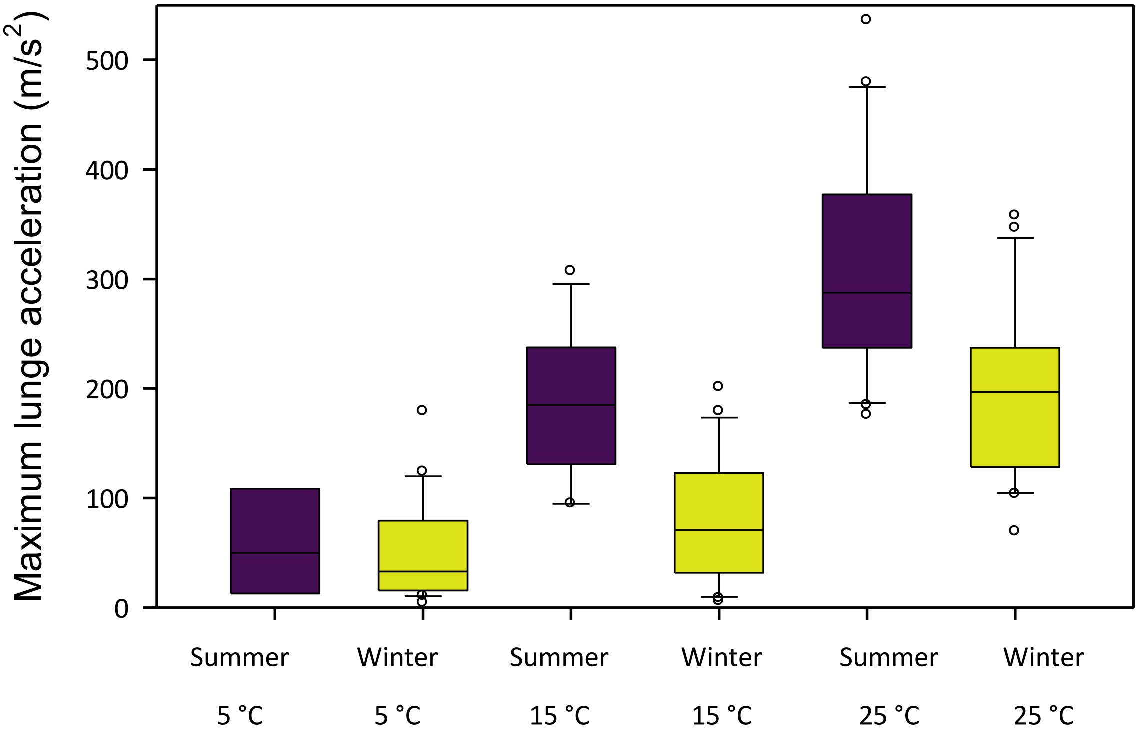


FIGURE 10 Maximum lunge acceleration of *Macrochelys temminckii* across temperature settings and seasons

SUMMARY

The number of species that could benefit from translocation efforts and extensive post-introduction management will likely increase with time, and with that unfortunate increase, so will the need to maximize the efficiency of head-start initiatives (Berger-Tal et al., 2019). With this in mind, studies of performance are valuable tools for determining the ability of an animal to complete ecologically relevant tasks upon release into the wild and must be conducted rigorously to ensure standardization and accuracy among measurements (Arnold, 1983). Unfortunately, captive breeding programs can result in diminished performance due to myriad factors, reinforcing the need for facilities that mimic natural conditions and for distinction between captive and free-ranging individuals in studies of performance (Erickson et al., 2004; Crane and Mathis, 2011).

In my first chapter, I determined that the bite performance of *M. temminckii* varies not only with free-ranging or captive status, but also with indoor and outdoor housing within captivity. While there was no significant difference in maximum bite force among free-ranging, head-started individuals on the Caney and Verdigris Rivers in Northern Oklahoma, both groups outperformed captive animals, and captives housed outdoors in ponds performed significantly greater than those housed indoors in raceways. Additionally, bite behaviors such as latency to gape and bite varied by test group, with free-ranging individuals requiring far less provocation to snap than conspecifics in captivity. Whether underperformance by captive individuals is due to diet, exercise, frequent exposure to caretakers, or other confounding variables, the results of my study suggest that time in captivity prior to release should prioritize optimizing growth and performance, both of which benefit from time in naturalistic outdoor housing.

In my second chapter, I determined that the bite performance of *M. temminckii* is also at the whim of temperature—and in some cases—season. As expected, there was a positive relationship between temperature and kinematic variables including bite duration, acceleration, and velocity, and lunge acceleration and velocity. Kinematic variables that exhibited significant interactions between temperature and season included maximum jaw velocity and maximum lunge acceleration, with higher temperatures in summer producing better performance than in winter. Maximum bite force increased significantly with temperature, and in a partial model, differed significantly between summer and winter. In contrast to other variables that I measured, performance was greater in the winter than summer. With evidence of the complex relationship between bite performance, temperature, and season in mind, standardization for these variables must be thoughtfully considered in future performance studies involving *M. temminckii*, or other ectotherms.

Finally, turtles provide valuable ecosystem services, for example, *M. temminckii* are top-order predators in river systems they inhabit (Pritchard, 2006; Lovich et al., 2019). While promoting the return of a once-decimated species is no simple task, there is early evidence of successful growth and survival in some rivers where head-started individuals were released, and with slight adjustments to conditions fostered by the head-start initiative at Tishomingo National Fish Hatchery, the condition of released animals could be maximized (Anthony et al., 2015). The ability of this species to maintain defensive performance across a wide range of temperatures encountered across its native range, will likely further aid in its future conservation.

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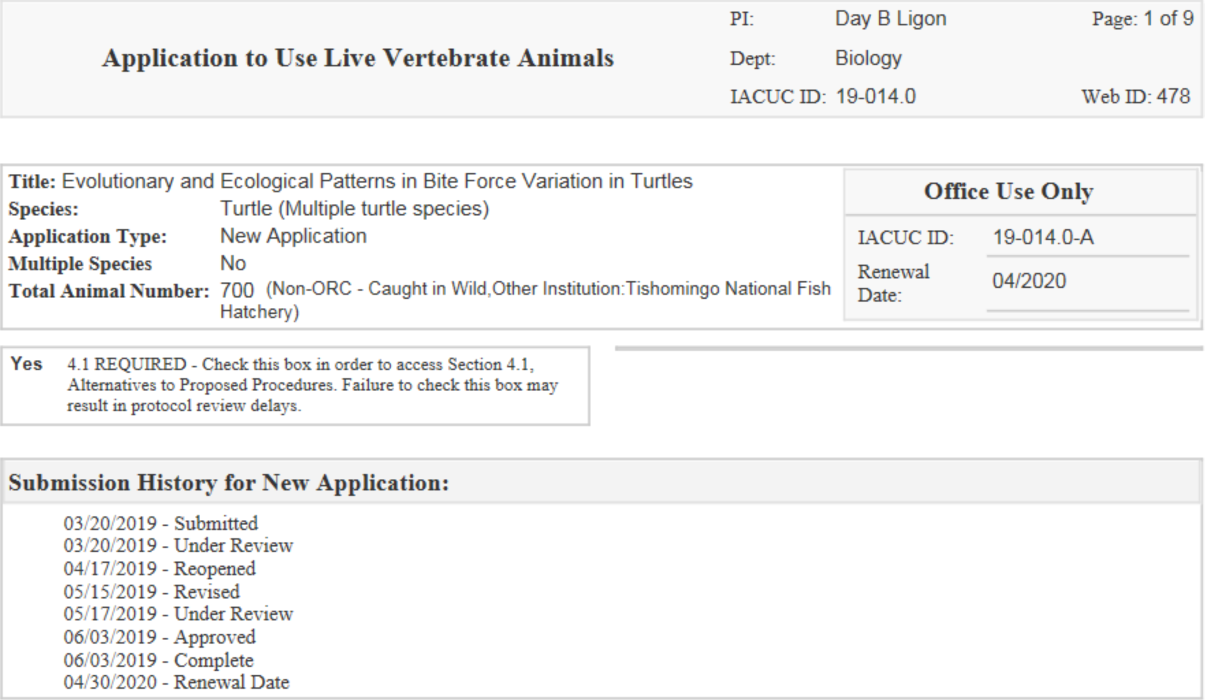
APPENDICES

Appendix A. All research methods were approved by the Missouri State University Institutional Animal Care and Use Committee in June of 2019 (see protocol 19-014 below).



Application to Use Live Vertebrate Animals	PI: Day B Ligon		Page: 1 of 9
	Dept: Biology		
	IACUC ID: 19-014.0		Web ID: 478

Title: Evolutionary and Ecological Patterns in Bite Force Variation in Turtles

Species:	Turtle (Multiple turtle species)
Application Type:	New Application
Multiple Species	No
Total Animal Number:	700 (Non-ORC - Caught in Wild,Other Institution:Tishomingo National Fish Hatchery)

Office Use Only

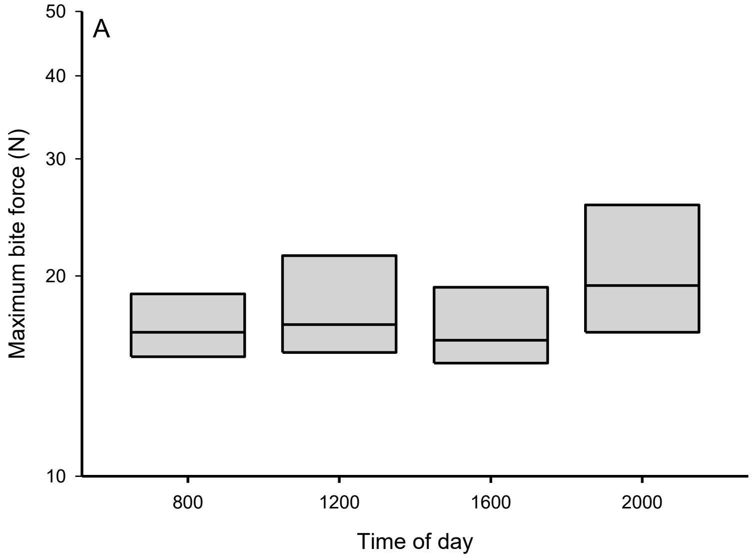
IACUC ID:	19-014.0-A
Renewal Date:	04/2020

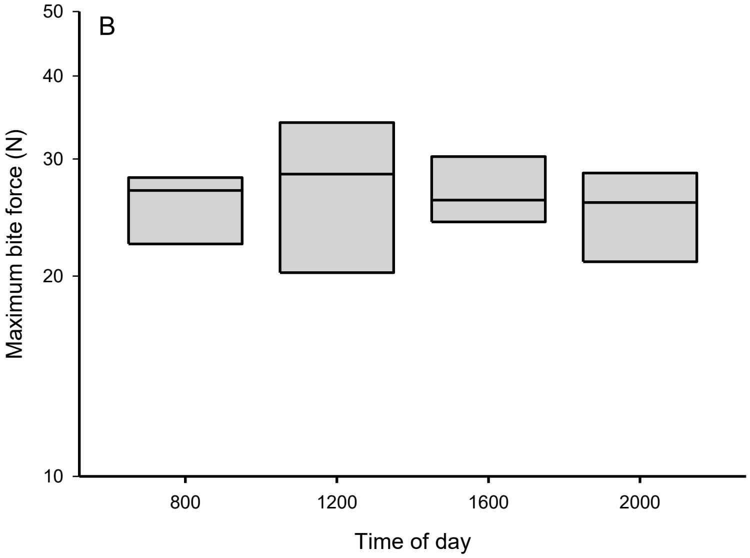
Yes 4.1 REQUIRED - Check this box in order to access Section 4.1, Alternatives to Proposed Procedures. Failure to check this box may result in protocol review delays.

Submission History for New Application:

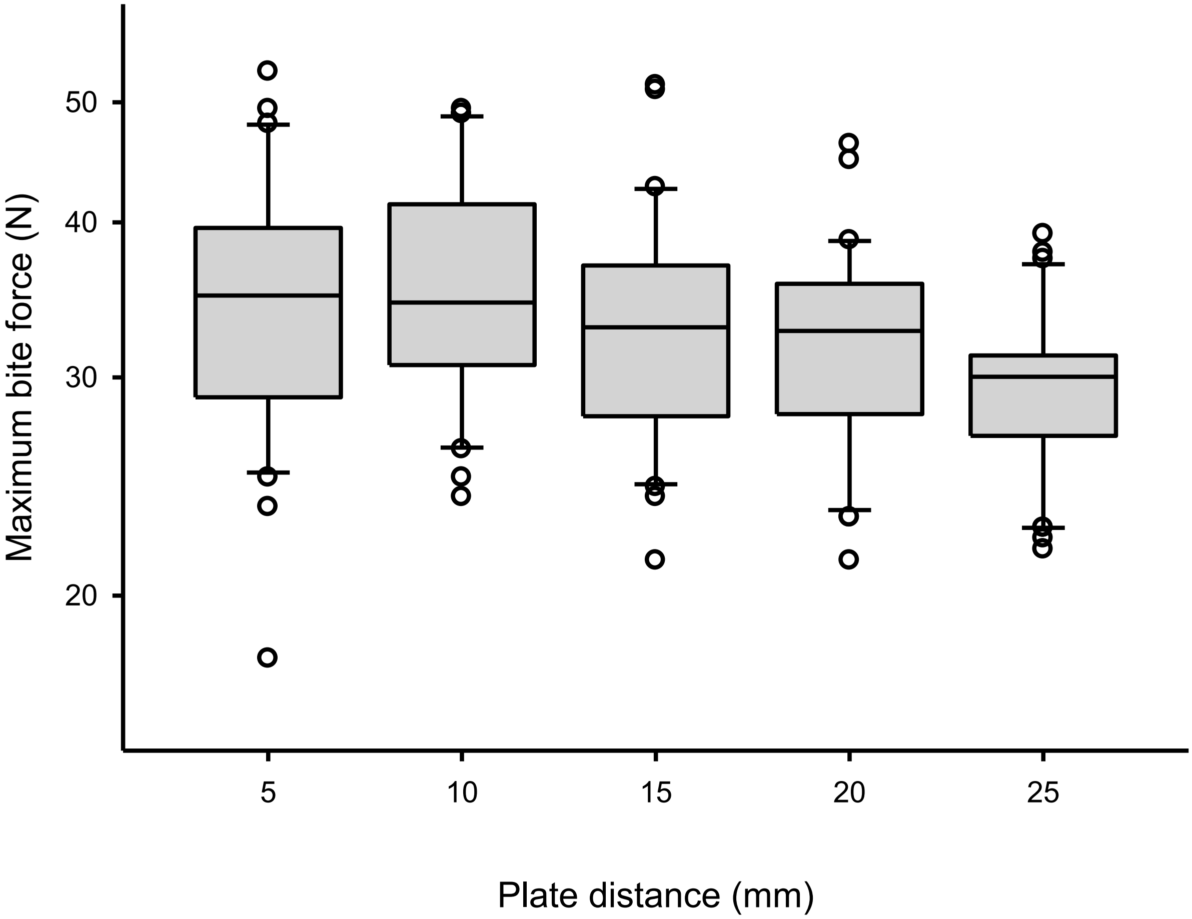
03/20/2019 - Submitted
03/20/2019 - Under Review
04/17/2019 - Reopened
05/15/2019 - Revised
05/17/2019 - Under Review
06/03/2019 - Approved
06/03/2019 - Complete
04/30/2020 - Renewal Date

Appendix B. To evaluate times of peak performance for indoor and outdoor *Macrochelys temminckii*, a sample of 16 indoor turtles were sampled from TNFH ranging 158–247 g body mass, 20–25 mm head height, and 30–37 mm head length, and a sample of 16 outdoor turtles ranging from 217–448 g body mass, 25–32 mm head height, and 37–45 mm head length. In two separate experiments, individuals were randomly assigned to groups of four and tested each group at either 08:00, 12:00, 16:00, or 20:00 h. For all trials, bite plates were set at the spacing that was closest to 0.25x each individual's head height. After using a full factorial model on the time-of-day results for indoor and outdoor captives to test for interactions between time of day and housing conditions, and a partial factorial model to determine the significance of time of day on maximum bite force, time of day was determined to have no effect on maximum bite force of captive turtles and there was no significant interaction between housing conditions and time of day ($F_{3,24} = 0.80$, $P = 0.508$). After removal of the interaction term, the effect of time of day on maximum bite force remained nonsignificant ($F_{3,27} = 0.30$, $P = 0.829$).





Appendix C. To determine ideal bite plate distances necessary to produce maximum bite performance of *Macrochelys temminckii*, a sample of 30 indoor captive turtles were sampled at the TNFH facility ranging from 30–35 mm in head height, and 40–50 mm in head length. Turtles were randomly divided into 10 groups of 3 individuals to undergo bite force trials at plate distances of 5, 10, 15, 20, and 25 mm, which were approximately 0.17, 0.33, 0.50, 0.67, and 0.83x head height, and over the span of nine days, were tested at all spacing settings, staggering the starting time for each group to minimize possible effects of fatigue. Three bite force measurements were recorded at each plate setting, and individuals were allowed at least 30 min rest between trials and before starting trials at the next greater plate distance. Subjects were tested at no more than three plate settings per day. Using a repeated-measures analysis of variance (ANOVA) with body mass included as a covariate, and a Tukey's honestly significant difference test, bite force was determined to be inversely proportional with bite plate spacing ($F_{4,116} = 7.80$, $P < 0.0001$). The two narrowest plate settings—corresponding with 0.17 and 0.33x head height—resulted in significantly greater bite forces than the widest setting at 0.83x head height (Tukey's HSD $P < 0.012$), while the other widths did not (Tukey's HSD $P > 0.169$).



Appendix D. Average bite performance, maximum lunge distance, and minimum lunge duration results for *Macrochelys temminckii* measured at three temperatures and repeated in summer and winter. Values are mean ± 1 SE. Average kinematics were calculated by dividing the sum of all frame-by-frame calculations by the total number of involved frames.

	Summer			Winter		
Variable	5 °C	15 °C	25 °C	5 °C	15 °C	25 °C
Mean jaw velocity	0.3 ± 0.0*	1.1 ± 0.1*†	1.8 ± 0.1*†	0.4 ±0.0*	0.8 ± 0.0*†	1.4 ± 0.1*†
Mean jaw acceleration	22.5 ± 2.7*	99.9 ± 6.7*†	201.9 ± 12.7*†	25.3 ± 2.6*	70.4 ± 6.7*†	150.9 ± 5.7*†
Maximum lunge distance	2.25 ± 0.3*	4.7 ± 0.4	4.6 ± 0.4	3.3 ± 0.3*	5.0 ± 0.4	4.9 ± 0.3
Minimum lunge duration	166.58 ± 31.2*	107.41 ± 3.5*	78.27 ± 3.5*	213.34 ± 19.0*	138.76 ± 11.4*	86.71 ± 3.3*
Mean lunge velocity	0.2 ± 0.1*	0.4 ± 0.0*	0.7 ± 0.1*	0.2 ± 0.0*	0.4 ± 0.0*	0.7 ± 0.0*
Mean lunge acceleration	17.4 ± 6.6*	55.6 ± 4.3*†	88.8 ± 6.4*†	16.0 ± 3.3	25.6 ± 3.8†	61.5 ± 5.2*†

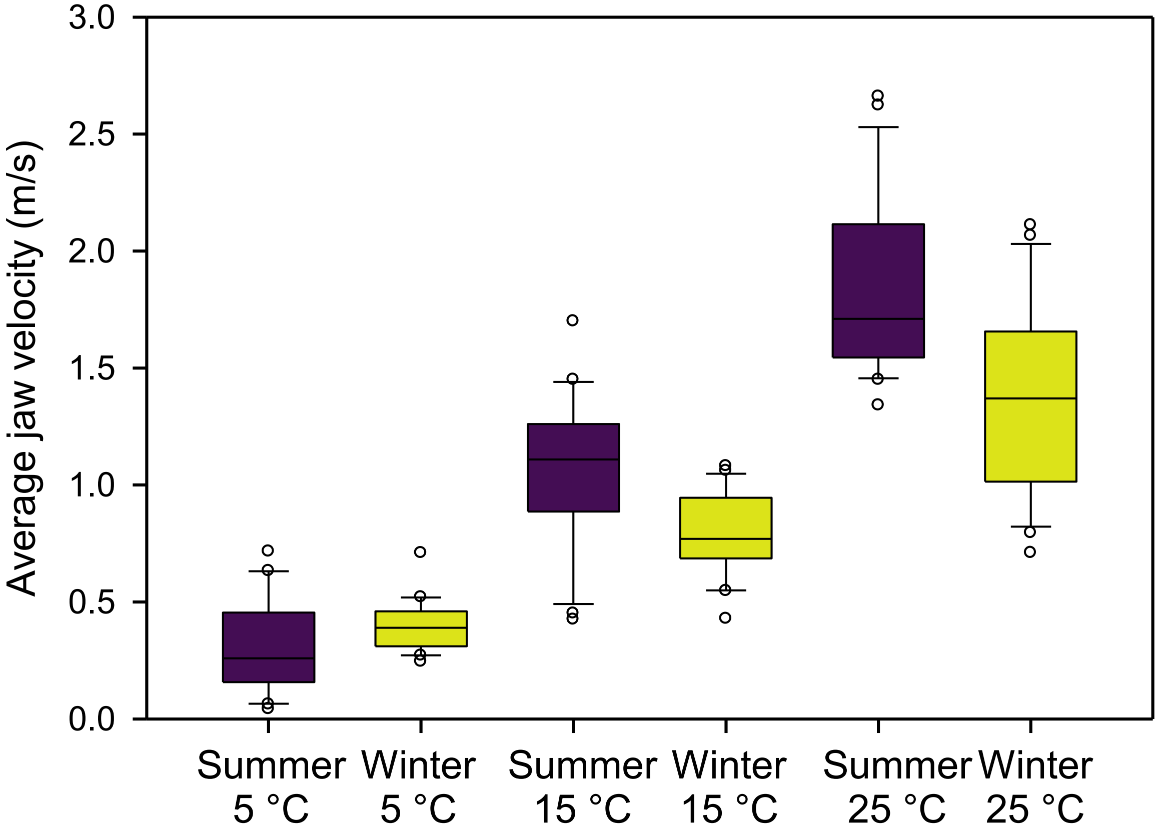
* Indicates significant differences ($P \leq 0.05$) among temperatures within a season, † indicates significant differences between seasons at a given temperature, and bold font indicates significant interactions between temperature and season in a full factorial model. See additional appendices below for further explanation of interactions.

Appendix E. Means, standard deviations, and medians of Q10 values for bite kinematic measurements of *Macrochelys temminckii* across a temperature range of 5–25 °C and during summer and winter trials respectively. *P*-values indicate thermal independence/dependence based upon 1-sample t-tests and mean Q10 values where data was normal—summer maximum lunge distance, summer minimum lunge duration, and average lunge velocity—and Wilcoxon tests and median Q10 values where data was not normal—average jaw velocity, average jaw acceleration, winter average lunge velocity, and average lunge acceleration. Q10 thresholds for temperature dependence were primarily 1.5, but in the case of decreasing rates—minimum lunge duration—a threshold of 0.5 was used. Variables that are not significantly temperature dependent include maximum lunge distance, summer minimum lunge duration, and summer average lunge acceleration, although summer results for these variables may be driven by small sample sizes.

	Summer	5–25 °C			Winter	5–25 °C		
Variable	Mean	SD	Median	*p*-value	Mean	SD	Median	*p*-value
Average jaw velocity	13.3	± 11.1	8.4	$P < 0.0001$	4.4	± 1.5	4.0	$P < 0.0001$
Average jaw acceleration	22.8	± 27.9	12.5	$P < 0.0001$	10.9	± 8.3	9.1	$P < 0.0001$
Maximum lunge distance	3.3	± 2.6	2.4	$P > 0.17$	2.0	± 1.2	1.7	$P > 0.07$
Minimum lunge duration	0.5	± 0.5	0.4	$P > 0.46$	0.4	± 0.2	0.4	$P < 0.04$
Average lunge velocity	12.1	± 8.7	11.6	$P < 0.026$	5.5	± 3.2	5.1	$P < 0.001$
Average lunge acceleration	37.2	± 58.1	16.8	$P > 0.06$	12.5	± 10.3	11.5	$P < 0.0001$

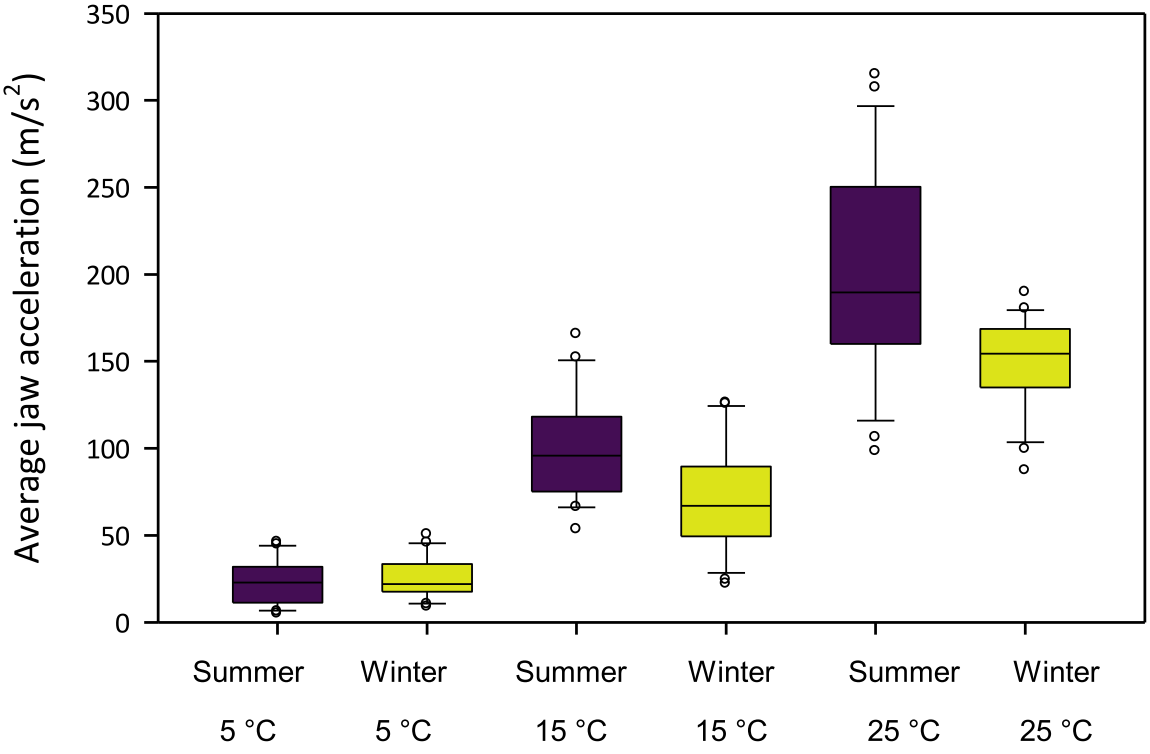
Appendix F. Average jaw velocity (F-1), average jaw acceleration (F-2), maximum lunge distance (F-3), minimum lunge duration (F-4), average lunge velocity (F-5), and average lunge acceleration (F-6) achieved by *Macrochelys temminckii* across temperature settings and seasons. Further details for statistical analyses are reported in text.

1. Average jaw velocity in m/s, defined as the highest average velocity reached by the mandible during the entire motion of the bite across all digitized videos for each temperature setting and season, and ranging from 0.042–2.66 m/s. There was a significant interaction between temperature and season ($F_{2,100} = 13.0$, $P < 0.0001$) in a full factorial model, and velocity increased with temperature in both seasons ($P < 0.0001$). There was no significant difference between the performance of turtles in either season at 5 °C ($P = 0.81$), but at 15 °C ($P < 0.008$) and 25 °C ($P < 0.0001$) they bit with significantly higher average jaw velocities in the summer than in the winter (Appendix D).



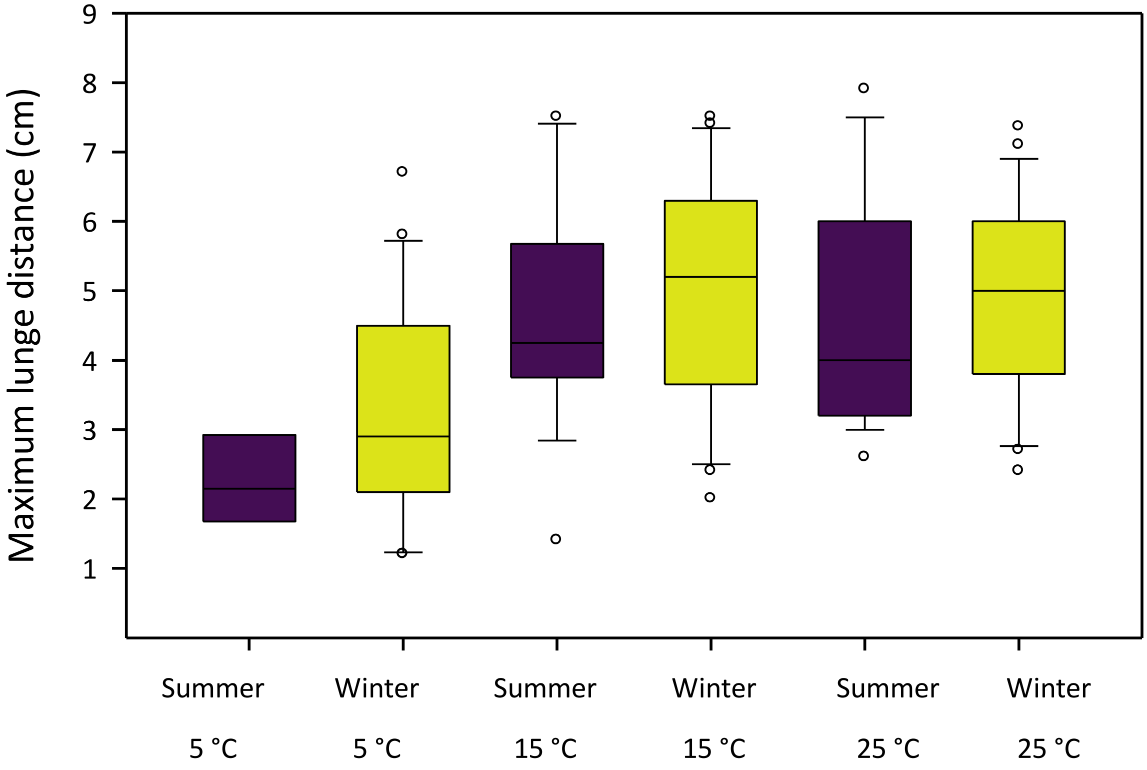
Appendix F continued.

2. Average jaw acceleration in m/s^2, defined as the highest average acceleration achieved by the mandible during the entire motion of the bite across all digitized videos for each temperature setting and season, and ranging from 4.78–314.82 m/s^2. There was a significant interaction between temperature and season ($F_{2,100} = 7.4$, $P < 0.001$) in a full factorial model, and acceleration increased with temperature in both seasons ($P < 0.0001$). There was no significant difference in the performance of turtles in either season at 5 °C ($P = 1.0$), but at 15 °C ($P < 0.04$) and 25 °C ($P < 0.0001$), they bit with significantly higher average jaw accelerations in the summer than in the winter (Appendix D).



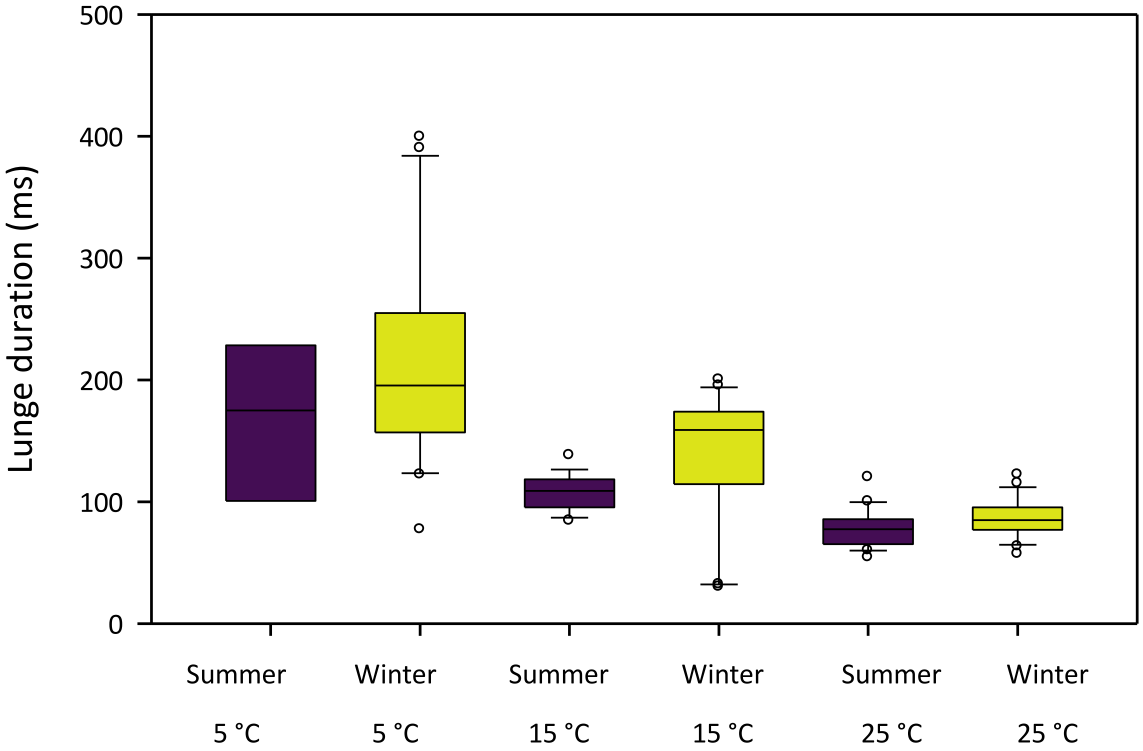
Appendix F continued.

3. Maximum lunge distance, defined as the single longest lunge coming from any of the digitized videos displaying lunge behavior and derived from the distance traveled by the tip of the nose from first forward movement to last forward movement. It ranged from 1.2–7.9 cm and there was no significant interaction between temperature and season in a full factorial model ($F_{2,77} = 2.05$, $P = 0.14$). In a partial model, season was not a significant factor ($F_{1,79} = 2.59$, $P = 0.11$), but temperature was ($F_{2,79} = 17.39$, $P < 0.0001$), and lunges at 5 °C were significantly shorter than those at 15 °C and 25 °C (Appendix D; $P < 0.0001$).



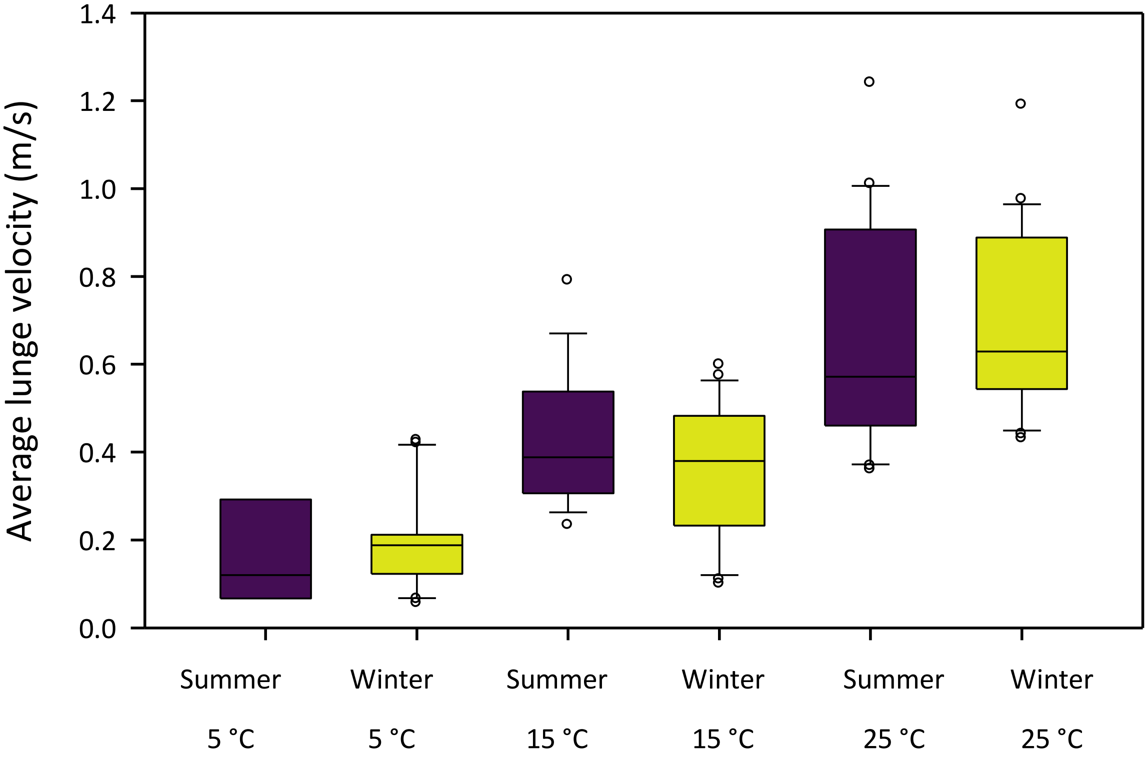
Appendix F continued.

4. Minimum lunge duration, defined as the single shortest lunge duration from any digitized videos displaying lunge behavior and quantified as the duration of the lunge. It ranged from 30.0–399.2 ms and there was not an interaction between temperature and season in a full factorial model ($F_{2,79} = 0.20$, $P = 0.41$). In a partial model, both season ($F_{1,81} = 6.51$, $P > 0.01$) and temperature ($F_{2,81} = 53.61$, $P < 0.0001$) were significant factors, and lunge duration decreased significantly as temperature increased at all temperatures (all $P < 0.01$; Appendix D).



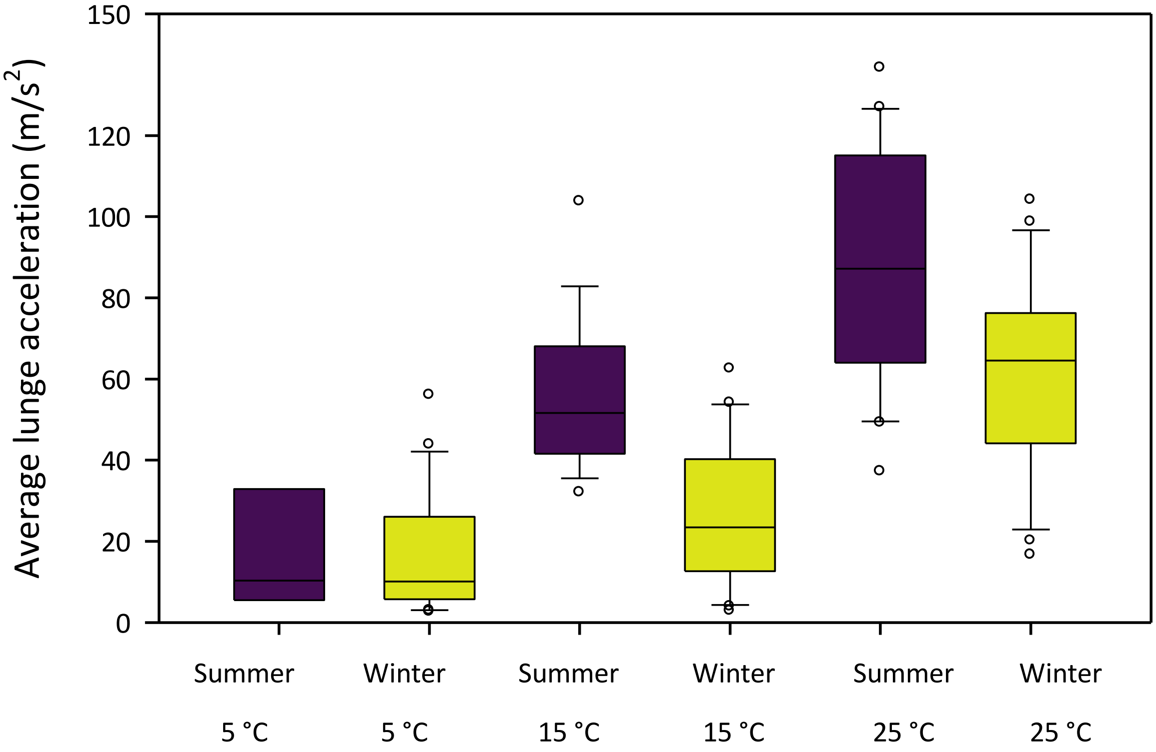
Appendix F continued.

5. Average lunge velocity in m/s, defined as the highest average velocity reached by the point of each turtle's snout during the entire motion of the lunge across all digitized videos for each temperature setting and season. It ranged from 0.06–1.24 m/s, and in a full factorial model there was no significant interaction between temperature and season ($F_{2,79} = 1.71$, $P = 0.19$). In a partial model, season was not a significant factor ($F_{1,81} = 0.04$, $P = 0.84$), but temperature was ($F_{2,81} = 88.7$, $P < 0.0001$), and velocities increased significantly with each temperature setting (Appendix D; both $P < 0.0001$).



Appendix F continued.

6. Average lunge acceleration in m/s, defined as the highest average acceleration reached by the point of each turtle's snout during the entire motion of the lunge across all digitized videos for each temperature setting and season. It ranged from 1.96–136.6 m/s^2, and there was a significant interaction between temperature and season in a full factorial model ($F_{2,79} = 3.7$, $P < 0.03$). Average lunge acceleration increased significantly with each temperature setting in the summer (all $P < 0.004$), but in the winter, bites at 5 °C and 15 °C were not significantly different from each other ($P = 0.68$). Lunges in either season at 5 °C were not different from one another ($P = 1.00$), but turtles lunging at 15 °C and 25 °C had significantly higher accelerations in the summer than in the winter (Appendix D; both $P < 0.0005$).



Appendix G. Medians and ranges of body mass, straight carapace length, head length, head width, and head height of *Macrochelys temminckii* during summer and winter trials respectively. Details for measurement methods are reported in text.

Variable	Summer	Winter
Body Mass (g)	5,174 (1,736–8,385)	4,995 (1,770–8,310)
Carapace Length (mm)	273.8 (188.4–314.8)	266.1 (188.2–310.5)
Head Length (mm)	94.7 (73.1–109.6)	93.8 (73.8–108.2)
Head Width (mm)	89.6 (66.34–108.4)	86.8 (64.7–105.1)
Head Height (mm)	66.3 (49.8–80.7)	68.4 (48.2–80.7)

Appendix H. Log_{10}-transformed maximum bite force measurements of *Macrochelys temminckii* during summer and winter trials respectively. Winter forces were significantly higher than those recorded in the summer ($P < 0.01$).