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A Low-Cost, Efficient, and Precise Technique to Quantify Key Life Cycle Events in Nests of Oviparous Reptiles

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ABSTRACT.—Phenological timing is of central interest to evolutionary ecologists because it is associated with fitness, but there has been limited study in animal groups with relatively secretive habits such as reptiles. This is especially true for the timing of hatchling behavior in wild reptile nests, likely attributable to few noninvasive methods for estimating parameters associated with egg hatching. We show that tri-axial accelerometers, small data loggers that measure rotation and inclination, can accurately quantify hatchling movement in wild reptile nests. In June 2018, we deployed an accelerometer in each of five freshly laid Snapping Turtle (*Chelydra serpentina*) nests in Algonquin Provincial Park, Ontario, Canada. In September 2018, nests were visited once daily to quantify the timing of hatchling emergence. The accelerometers worked as expected: there was statistically significant correspondence between the timing of accelerometer rotation in the nest (caused by movement of the hatchlings) and the timing of hatchling emergence. Furthermore, the number of hatchlings emerging from a nest was strongly and significantly correlated with the extent of accelerometer displacement. Our new technique allows new types of phenological data to be collected. It requires minimal effort and financial investment and thus is accessible to a broad range of research programs.

The timing of key life cycle events, such as oviposition, hatching, and emergence from a nest, is strongly linked to individual fitness (Rowe and Ludwig, 1991; Rowe et al., 1994; Einum and Fleming, 2000; Edge et al., 2017), and quantifying variation in reproductive phenology has long been a key goal in ecology and evolutionary biology (Janzen, 1967). The past 2 decades in particular have seen an explosion of interest in quantifying phenological timing as rapid climate warming has led to a globally cohesive signature of phenological advance in terrestrial and aquatic systems (Menzel et al., 2006; Poloczanska et al., 2013). Yet, where phenological data do exist, observations are typically confined to traits that are readily observable, such as the timing of flowering, breeding, or egg laying. For more secretive taxa such as reptiles, collection of phenological data is largely confined to reproductive traits of adults (but see Janzen et al., 2018), because major life cycle events associated with the eggs (e.g., nest depredation) and hatchlings (e.g., egg hatching and nest emergence) occur at less predictable times of year than egg laying.

The behavior of hatchling turtles within cryptic nests remains enigmatic, because it is difficult to observe directly. For example, relatively little is known about whether hatchling turtles emerge from nests synchronously (Baker et al., 2010, 2013; Santos et al., 2016), or about the cues that cause hatchlings to emerge from nests in the first place (Spencer and Janzen, 2011). Currently, there are few noninvasive methods of estimating hatching time and hatchling behavior within nests. For example, camera traps can be useful in estimating the timing of nest emergence of hatchling turtles (Doody and Georges, 2000), but it may be expensive and logistically difficult to implement camera traps in some field situations. The goal of the present study is therefore to present a simple method that can be used to estimate timing of the key life cycle events during the early lives of reptiles.

The recent advent of tri-axial accelerometers has been embraced by behavioral ecologists. These small data loggers measure acceleration and angular displacement on three axes (X, Y, and Z), storing measurements at intervals from <1 sec to several hours apart, thereby allowing quantification of spatial orientation of the loggers, and by extension the animals, in three dimensions. To date, tri-axial accelerometers have been used primarily to quantify movement patterns of large animals (Shepard et al., 2008; Moreau et al., 2009; Mitani et al., 2010; Naito et al., 2013) as well as patterns of parental care behavior (egg turning) in birds (Shaffer et al., 2014). A cost comparison is provided in Table 1. Here, we show that tri-axial accelerometers allow the timing of movements within wild nests of oviparous reptiles to be accurately quantified and that 1) movement within nests closely precedes physical emergence from nests by hatchlings and 2) the extent of accelerometer displacement is strongly correlated with the number of hatchlings emerging from the nest. By extension, tri-axial accelerometers allow researchers to infer hatching dates, emergence dates, or both and hatchling survival, without being present at the study site.

MATERIALS AND METHODS

The present report is part of a long-term study (1972–present) on Snapping Turtles residing near Lake Sasejewun, Algonquin Provincial Park (Ontario, Canada). Clutch size averages 37 eggs per nest in the focal population (Rollinson, Litzgus, and Brooks, unpubl. data). Hatchling turtles in this population must emerge from nests in the current season before the arrival of winter, because hatchlings that overwinter in the nest have negligible survival (Obbard and Brooks, 1981).

Each year, the Lake Sasajewun dam is monitored from May to June for nesting female Snapping Turtles. Females nest undisturbed, and upon completion of nesting, eggs are subsequently excavated from the nest. As eggs are removed, they are sequentially numbered, as a reverse proxy for the order in which they were laid. Care is taken to preserve the original nest cavity. The eggs are then brought to a field laboratory where diameter is measured (to the nearest 0.1 mm) and mass is recorded (to the nearest 0.1 g) before being returned to their original nest within 48 h. Eggs are then returned to their original

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ACCELEROMETERS IN TURTLE NESTS

TABLE 1. Cost and accuracy comparison of compact, battery powered, tri-axial accelerometer models.

Model	Manufacturer	Accuracy (g)	Approximate cost (USD\$)
HOBO Pendant G Data Logger	Onset, Massachusetts, USA	$\pm 0.075 \\ \pm 2 \\ \pm 0.1$	83
X2-2 High Sensitivity Accelerometer	Gulf Coast Data Concepts, LLC, Mississippi, USA		150
ORI400-D3GT	Little Leonardo, Tokyo, Japan		3,000

nest cavity in reverse order of removal, which approximately preserves each egg's original depth.

Data loggers.—We used HOBO Pendant G tri-axial acceleration Data Loggers (Onset Computer Corporation, Pocasset, Massachusetts, USA; Fig. 1A), although other types of tri-axial accelerometers are available (Shepard et al., 2008; Mitani et al., 2010; Naito et al., 2013; Table 1). Pendant loggers are small (58 mm \times 33 mm \times 23 mm), lightweight (18 g), inexpensive (USD\$83.00), and record both acceleration and inclination through measurement of an analog signal in each of their three axes, although only inclination was used in the present study. Each Pendant logger has a memory of 64 kB, allowing up to 21,800 three-dimensional data points at intervals of <1 sec up to intervals up to 18 h, programmed by the user. We programmed the read interval to be 15 min.

Field Study.-In 2018, one HOBO Pendant G data logger was placed in each of five haphazardly chosen Snapping Turtle nests, from 16 June 2018 (day of year 167) and 23 June 2018 (day of year 174), to capture variation in lay date and subsequent hatch timing. Upon reburial of eggs, as mentioned above, the logger was placed on the topmost eggs of each nest. Each logger was placed in a visually consistent orientation in each nest (X \approx 82°, Y \approx 67°, Z \approx 163°), to minimize error associated with different initial orientation in different nests (Fig. 2). Substrate was placed on top of the eggs and loggers, reburying the eggs, according to standard protocol. Each nest was then protected from predators by surrounding the nest with a square cage (approximately 30 cm \times 30 cm \times 30 cm, Fig. 1B,C) with wire mesh (1 cm \times 1 cm); cages had an exit hole to allow hatchling escape without researcher interference. Wire mesh nest cages do not interfere with natural thermal characteristics of the nests (Riley and Litzgus, 2013). Three of the five nests were in sandy substrate near the water's edge (Fig. 1B), and the remaining two nests were in tight-packed gravel substrate at the top of the dam near the edge of a lightly travelled road (Fig. 1C).

Because the timing of hatchling emergence is difficult to predict, we sampled 10 nests without tri-axial accelerometers on 30-31 August 2018, before any hatchlings had emerged, to assess hatch readiness. Of these 10 nests, one contained several hatchlings in the uppermost eggs, one nest had one hatchling, and one nest had a pipped egg; the remaining nests had no hatchlings. A patrol was then initiated on 2 September 2018 to monitor the status of all nests. The patrols were strictly observational, and all nests were visited once daily and visually examined for traces of hatchlings. A nest was considered "emerged" if a hatchling was observed in the cage, if clear traces of hatchlings were present (such as clear hatchling footprints in sand), or if an exit hole was observed (Fig. 1B); thus, the "observed date of hatchling emergence" hereafter refers to the first date in which hatchlings (or signs of hatchlings) were observed.

On 13–14 October 2018, each of the five nests was excavated, and the number of nonviable embryos (e.g., early developmental failure) and moribund hatchlings within the nest (e.g., birth deformities) was determined. To estimate the number of

hatchlings that emerged from each nest, we subtracted the number of failed embryos and hatchlings remaining in the nest in October from the original clutch size. Notably, temperature at our study site is generally very cool by mid-October, and our presumption is that in the typical year hatchings are unlikely to emerge this late in the season.

Accelerometer data from the field, namely, the inclination of the X, Y, and Z planes (in degrees), were plotted for the duration of embryonic development, from egg laying in June until 30 September 2018. Our assumption was that nothing other than movement of hatchlings should affect the inclination of accelerometers. Therefore, a sudden change in inclination, preceded and followed by prolonged period of stable inclination, should signify hatchling movement, allowing us to infer egg hatching, hatchling emergence, or both from the nest. We therefore analyzed the variance in inclination over 24-h periods (equivalent to 96 data reads), with the expectation that maximum variance in inclination will coincide with observed hatchling emergence. Because accelerometer data are expressed in degrees, we used circular statistics, leveraging the package 'circular' in R (Lund et al., 2017). To estimate maximum variance in inclination, we used the *rollapply* function within the zoo package (Zeileis et al., 2018) in the R statistical environment (R Core Team, 2013) to calculate the rolling circular variance across all 24-h periods, from June until 30 September 2018 (see the Appendix 1 for R code). The time point at which the maximum variance was observed for each orientation plane provided an estimate of maximum hatchling movement, expressed in fractions of a day (e.g., day of year 245.7). When the X and Y planes provided different estimates for the timing of maximum hatchling movement, we took the average date.

Armed with the value of maximum accelerometer variance for each nest, as well as the date on which the maximum variance was observed, we used linear regression to estimate the relationship between 1) observed day of emergence and day of emergence estimated from maximum accelerometer variance. Here, we expected that the observed and estimated date of hatchling emergence would be positively associated and that the slope of the regression would not be different from 1.0. Next, we tested whether accelerometer disturbance is proportional to the number of hatchlings emerging from a nest. We used linear regression and predicted that 2) mean accelerometer variance (across all relevant axes) and maximum accelerometer variance (i.e., on the most variable axis) would be positively associated with the number of hatchlings emerging from a nest.

Lab Study.—Between 23 October and 26 October 2018, we performed a retrospective lab study to evaluate the precision of the Pendant G, specifically when placed in the initial orientation we used in the field study, as well as in a few other haphazardly selected orientations. The motivation of the lab study was not to explore the relative precision of every possible position of the Pendant G; this is unnecessary because the precision conditioned on orientation may vary within batches of Pendant G loggers that are purchased and will likely vary among different models of tri-



FIG. 1. (A) Pendant G data logger. (B) Nest cage on the Sasajewun dam (Algonquin Provincial Park, Ontario, Canada) on a nest in a sandy soil with clear emergence hole forged by hatchling Snapping Turtles (*C. serpentina*). (C) A nest in rocky soil on the shoulder of a lightly traveled road.

axial accelerometers. Thus, we aimed to estimate whether the initial placement of the logger that we chose in the field (X $\approx 82^{\circ}$, $Y \approx 67^{\circ}$, $Z \approx 163^{\circ}$, Fig. 2A,B) was associated with relatively low precision of inclination estimates along any axis and whether other initial orientations that we did not choose in the field might have increased the precision of inclination estimates in the field. We filled a single box (30 cm \times 20 cm \times 7 cm) with dry vermiculite and placed the box on a level surface. Next, all loggers were placed in approximately equal orientations on the surface of the vermiculite, programmed to read at 15-min intervals. The starting orientation of all data loggers [Fig. 2, "Wild (in lab)"] represented approximately the same initial orientation as is the field study. The data loggers were rotated to a new (and approximately equal) position each day at 0900 h and 1600 h. We subsequently calculated and compared the circular standard deviation of the inclination across orientations, ultimately identifying whether the relative precision of the loggers (estimated by the SD) varied as a function of their orientation.

RESULTS

Variation in X inclination and Y inclination, calculated across sequential 24-h periods (96 data reads per 24 h), appeared negligible for all nests during embryonic development, except just before observed hatchling emergence (Fig. 3). However, variation in inclination seemed relatively pronounced along the Z axis in a majority of nests, even during periods of presumed stasis of the accelerometer (Fig. 3). Pronounced variation in Z inclination appeared to result from estimation error (accelerometer precision) conditioned on the particular orientation of the accelerometer along the Z axis that we chose to use in the field. This was confirmed by our lab study that showed that the initial orientation that we chose in the field resulted in relatively low precision of Z inclination estimates (Fig. 2A,B). In fact, precision appeared to vary in the X, Y, and Z axes depending on the orientation of the logger, with particular positions resulting in very high precision (Fig. 2A,B). Therefore, we inferred that variation in Z inclination in the field was relatively uninformative for deducing movement of hatchlings, and Z inclination is ignored.

The maximum observed variance in inclination in the field (i.e., the inferred date of maximum hatchling movement) was highly correlated between the X axis and the Y axis ($r^2 = 0.99$, n = 5, df = 3, P = 0.001; Fig. 4A), suggesting that maximum rotation was occurring at nearly identical times along both axes. The date at which maximum variation was observed was averaged across the X inclination and Y inclination to produce a single date upon which maximum hatchling movement was inferred. The mean date of maximum inclination variance was strongly and significantly correlated with observed date of hatchling emergence (i.e., the date on which hatchlings or signs of hatchlings were first observed at a nest [$r^2 = 0.98$, n = 5, df = 3, P = 0.001]; Fig. 4B). The estimated date of maximal hatchling movement within the nest occurred an average of 1.72 d before observed emergence from the nest (range = 0.37-4.4 d). Notably, four of the five nests emerged within 2 d of maximum hatchling movement within the nest (1.1 \pm 0.65 d [mean \pm SD]). The remaining nest, which was the first-laid nest of the nesting season and the largest clutch in our sample (n = 40eggs), emerged 4.4 d after maximum hatchling movement (Fig. 3C).

Finally, the estimated number of hatchlings that emerged from a nest was strongly and significantly correlated with



FIG. 2. Mean inclination (A) and inclination error (expressed as SD; B) in wild Snapping Turtle (*C. serpentina*) nests from Algonquin Provincial Park (Ontario, Canada) between days 175 and 235 (i.e., when no hatchling movement occurred) and in a laboratory study where the orientation of each of five Pendant G loggers was rotated twice daily. Circles are values from one Pendant G logger; thick

absolute maximal inclination variance (expressed as the maximum SD observed on either the X or Y axis) ($r^2 = 0.95$, n = 5, df = 3, P = 0.005; Fig. 4C). Similarly, the estimated number of emerged hatchlings was significantly correlated with mean absolute maximal inclination variance (i.e., the mean maximum SD averaged across the X and Y axes) (Mean SD = $0.0120 \times$ hatchlings – 0.118, $r^2 = 0.98$, n = 5, df = 3, P < 0.001). Therefore, a relatively large maximum change in accelerometer orientation over a 24-h period was very closely associated ($r^2 \ge 0.95$) with a relatively large number of hatchlings emerging from the nest.

DISCUSSION

The present study demonstrates that tri-axial accelerometers can be used to detect the movement of hatchlings within wild reptile nests and that movement coincides with hatching and subsequently emergence timing. This is evidenced from a clear association between variation in inclination of the accelerometer and emergence behavior of hatchlings.

A surprising result of the present study was that the extent of accelerometer variation was strongly and significantly associated with the number of hatchlings that emerged from the nest. This result has two main implications. First, there is likely a minimum clutch size for which the signal-to-noise ratio will remain strong enough to detect hatchling movement, an implication that can also be deduced from first principles. It therefore remains to be tested whether the technique proposed herein would work for smaller bodied species with small clutch sizes. Second, there appears to be some degree of proportionality between accelerometer displacement and biologically relevant parameters within the nest. As such, accelerometer data may be useful not only for determining point estimates of behavior (e.g., a single day of maximal movement, or emergence) but also for deducing whether emergence occurs over a short period or a protracted period (Spencer and Janzen, 2011) and for estimating number of hatchlings successfully emerging from the nest. Any extensions of accelerometer data beyond that quantified herein would however have to be tested.

To accurately detect movement within a nest, the signal-tonoise ratio of the accelerometer must be high, particularly before egg hatching. For the model of accelerometer we used in the present study (the Pendant G), we found that only two of the three axes were consistently informative with respect to hatchling movement; the Z axis was relatively imprecise (Fig. 3). It is nevertheless encouraging that this problem arose only because of the particular accelerometer orientation we chose to use in our field study, because our laboratory study demonstrated that the precision of inclination estimates varies with the orientation of the accelerometer (Fig. 2B). Notably, our laboratory study was performed retrospectively to explore potential reasons why Z inclination appeared unreliable in the field, yet our laboratory study suggests that particular accelerometer orientations result in relatively high precision along all axes (Fig. 2). We therefore recommend that studies intending to apply the method presented herein first test the

horizonal lines are the means of all five loggers for each orientation. Orientation values (i.e., 1, 2, 3 on the X axis) represent a small sample of orientations tested in the lab, presented herein to demonstrate that estimation error in the accelerometers varies with orientation. "Wild (in lab)" represents the approximate orientation used in the wild. Note that "Wild (in lab)" and "Wild" have approximately the same inclination values (A) and similarly large estimation errors along the Z axis (B).



FIG. 3. (A, C, E, G, I) Inclination of accelerometers (along the X, Y, and Z axes) in nests of wild Snapping Turtles (*C. serpentina*) from Algonquin Provincial Park (Ontario, Canada) from egg reburial to hatchling emergence. (B, D, F, H, J) Rolling variance (expressed in SD, scaled to a maximum of 1.0) of inclination calculated across all consecutive 24-h periods in each nest. Sample size (*n*) is the number of hatchlings emerged from each nest, and dashed line is the observed day of hatchling emergence. Panels on the right correspond to panels on the left-hand side of figure (e.g., A corresponds to B).



FIG. 4. Relationships between inclination variance and characteristics associated with hatching for Snapping Turtle (*C. serpentina*) embryos from Algonquin Provincial Park (Ontario, Canada), with each circle representing data from a single accelerometer or nest. (A) The 24-h period in which maximum variance in inclination was observed closely was similar on the X axes and Y axes. (B) Observed day of hatchling emergence from the nest closely followed the mean day of maximum inclination variance in the nest. (C) Maximum inclination variance (expressed as the SD) recorded over a 24-h period (either on the X or Y axis) was greater when more hatchlings emerged from the nest.

Downloaded From: https://bioone.org/journals/Journal-of-Herpetology on 21 Nov 2020 Terms of Use: https://bioone.org/terms-of-use Access provided by University of Toronto precision of the accelerometer in a variety of positions before field deployment. This is important, because the method proposed herein is useful only if the accelerometer estimates a static orientation for the period before hatchling movement. Prospective study can therefore establish which axes are relatively precise given a particular orientation, and hence which initial orientation may be more likely to maximize the signal-to-noise ratio in the nest. In any event, even though we chose a suboptimal orientation in the field, we were readily able to detect hatchling movement within the nest.

Finally, it seems unlikely that weather or substrate composition had a strong effect on variation in accelerometer inclination. For example, on day 205, the site was deluged by 34.9 mm of torrential rain in less than 24 h, and a similar phenomenon occurred on day 229, with 34.1 mm of rain. In neither case was there any signature in the inclination of the accelerometers (Fig. 3). This is perhaps not surprising, because the accelerometers were buried roughly 7–10 cm into the soil, trapped in the same position. Furthermore, two of the five nests were buried in relatively rocky soil (Fig. 1C), and although it is difficult to draw firm conclusions from a small sample, the signal of hatchling movement did not appear to be impeded by the rocky soil. For example, the second-largest signal (estimated from maximum inclination variance) was detected in a nest in rocky soil (Fig. 31). Thus, we tentatively suggest that accelerometers will work well in both sandy and rocky soils, although further testing is warranted. Conceivably, the size of the accelerometer (18 g) could allow it to affect thermal properties of the nest (Ackerman and Lott, 2004), especially if placed in the middle of the embryos rather than immediately above the embryos as in the present study.

In sum, we provide compelling evidence that tri-axial accelerometers can be used to precisely quantify the timing of hatchling movement in wild reptile nests and that peak displacement of accelerometers closely corresponds with emergence behavior of hatchlings. Our methods may be particularly interesting for use in species with highly plastic emergence times, such as Painted Turtles (Chrysemys picta) and Northern Map Turtles (Graptemys geographica), that can delay emergence for several months. Furthermore, there appears to be proportionality between the extent of axial displacement and biologically relevant properties of the nest, such as the number of hatchlings emerging, so there may be scope to use accelerometers to estimate characteristics other than simple timing of emergence. Finally, the use of accelerometers within wild vertebrate nests has largely been ignored to date (but see Shaffer et al., 2014), but much may be learned from accelerometer data within the nest. For example, nests found excavated toward the end of embryonic development could have been depredated before hatchling emergence, or hatchling emergence itself could have alerted scent-oriented predators to excavate the nest postemergence (Riley and Litzgus, 2014). Accelerometers could help determine whether predation or emergence occurred first as well as the timing of nest depredation more generally, and by extension predator behavior.

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APPENDIX 1:

R CODE FOR CIRCULAR STATISTICS USED IN THIS STUDY

Rolling window to calculate circular standard deviation. Orientation measures of X,Y,Z axes taken every 15mins. We wish to find the standard deviation of orientation for each day (i.e, each set of 96 measures).

library(zoo)

library(circular)

data<-read.csv("RollingAllNest.csv")#this dataset is available from njal.rollinson@gmail.com

use the roll apply function to calculate the mean rolling Julian date

rolling.mean.date<-rollapply(data\$Julian, 96, mean)

use a loop to calculate the circular standard deviation for each 24hour period.

assign the data as a circular object in degrees

data\$Q10X<-circular(data\$Q10X,units="degrees")

data\$Q10Y<-circular(data\$Q10Y,units="degrees")

data\$Q10Z<-circular(data\$Q10Z,units="degrees")

N<-9504 # length of dataset

window<-96 # size of the rolling window

X axis

rolling.sd.X<-rep(0,N) # empty vector to store the results in

for (i in 1:N) {

if (i<window) {

rolling.sd.X[i]=NA # if i is less than the window size assign as NA, because the first rolling window will fit at i=96 (window length)

} else {

rolling.sd.X[i] = sd.circular(data\$Q10X[(i-window):i]) # set as column you wish calculate circular standard deviation for

}

rolling.sd.X<-rolling.sd.X[-c(1:window-1)] # remove the NA's (95)

Y axis

rolling.sd.Y<-rep(0,N)

for (i in 1:N) {

if (i<window) {

rolling.sd.Y[i]=NA
} else {
rolling.sd.Y[i] = sd.circular(data\$Q10Y[(i-window):i])
}
rolling.sd.Y<-rolling.sd.Y[-c(1:window-1)]
Z axis
rolling.sd.Z<-rep(0,N)
for (i in 1:N) {
if (i<window) {
rolling.sd.Z[i]=NA
} else {
rolling.sd.Z[i] = sd.circular(data\$Q10Z[(i-window):i])
}
rolling.sd.Z[-c(1:window-1)]</pre>

plot the results against the rolling mean Julian date
plot(rolling.mean.date,rolling.sd.X, ylim=(c(0,0.2)),
xlim=(c(178,270)), type = "1")

plot(rolling.mean.date,rolling.sd.Y, ylim=(c(0,0.2)), xlim=(c(178,270)), type = "1") plot(colling mean date rolling of 7, ylim=(c(0,0.2))

plot(rolling.mean.date,rolling.sd.Z, ylim=(c(0,0.2)), xlim=(c(178,270)), type = "1")

Below is a second method to approach the data with. You can use the rollapply function to calculate circular standard deviation if you change the orientation from degrees to radians. Sometimes using sd.circular() within rollapply() throws up warning messages saying the object has been coerced into type circular. This is okay. # E.g.

assign the data to a circular object as before

data\$Q10X<-circular(data\$Q10X,units="degrees")

then convert to radians

 $data \$Q10X_radian <-conversion.circular(data \$Q10X)$

use rollapply on measurements in radians

rolling.sd.X.radians<-rollapply(data\$Q10X_radian, 96, sd.circular)

plot this against rolling mean date as before

plot(rolling.mean.date,rolling.sd.X.radians, ylim=(c(0,0.15)), xlim=(c(178,270)), type = "1")