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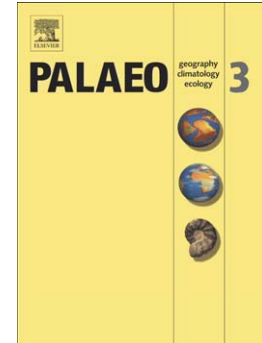
Revising and refining the bryozoan zs-MART seasonality proxy

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Revising and refining the bryozoan zs-MART seasonality proxy**H.L.O. McClelland^{a,b,d,*}, P.D. Taylor^b, A. O’Dea^c and B. Okamura^a**^a *Department of Life Sciences, Natural History Museum, London. SW7 5BD, UK*^b *Department of Earth Sciences, Natural History Museum, London. SW7 5BD, UK*^c *Smithsonian Tropical Research Institute, PO Box 0843-03092 Balboa, Panama*^d *Department of Earth Sciences, University of Oxford, South Parks Rd, Oxford, OX1 3AN, UK***Email address for corresponding author: harrym@earth.ox.ac.uk***Keywords**MART, palaeoseasonality, temperature size rule, zooid variability, *Acanthodesia***Highlights**

- **A revised version of the zs-MART equation is presented**
- **Zooid size variability correlates with seasonality, but predictive power is poor**
- **Previous conclusions based on zs-MART estimates are critically appraised**
- **Position within bifurcating rows in *Acanthodesia* systematically affects zooid size**
- **Externally controlled cyclical patterns in zooid size are attributed to seasonality**

Abstract

The zs-MART (zooid-size derived mean annual range of temperature) seasonality proxy is based on temperature-related variation in the sizes of zooids found in colonies of cheilostome bryozoans. It has been used in a number of studies for inferring palaeoseasonality. Here we present a revised version of the equation for the zs-MART proxy based on an appropriate regression analysis, and an estimate of the error associated with predicted values. We use this improved version to recalculate data used in studies that have employed the zs-MART proxy and evaluate their conclusions in the light of the revised estimates for seasonal regimes. In order to investigate trans-colony zooid-size profiles in zooid size, length and width, 1195 zooids from 40 transects through 19 fossil and Recent specimens of the malacostegine cheilostome bryozoan *Acanthodesia* were measured using a stereomicroscope with eyepiece graticule. The position of zooids within bifurcating rows of *Acanthodesia* colonies is found to influence zooid size, and this exemplifies potential problems for studies based on zooid-size transects and random sampling of zooids. Our demonstration that oscillatory signals in zooid size, possibly attributable to seasonal temperature regimes, can be detected whilst accounting for other sources of variation confirms that a more sophisticated zs-MART proxy may prove useful for constraining parameters in ocean models addressing questions about past climate. However, such a proxy will require more parameters than originally described along with larger raw datasets.

1. Introduction

The *zs*-MART (zoooid-size derived mean annual range of temperature) proxy is used to estimate palaeoseasonality based on morphometric analyses of cheilostome bryozoans (Bryozoa: Gymnolaemata: Cheilostomata). The approach, developed by O’Dea and Okamura (2000), derives from the empirically established inverse relationship between zoooid size and ambient water temperature (reviewed in Okamura et al., 2011). The temperature-size rule (see below) provides a theoretical basis for the *zs*-MART proxy. Cheilostome bryozoans are suited for this type of analysis because their zoooids have determinate growth and they secrete calcium carbonate skeletal walls. These features confer permanent records of the size of the zoooid when budded, and therefore relative ambient temperature at the time of formation. Colony growth is directional and often perennial, and occurs through the incremental addition of zoooids, therefore allowing temporal changes in ambient water temperature to be recorded. Cheilostome bryozoans originated in the Late Jurassic (Taylor, 1988) and are common in shallow marine communities, so can be used to probe climates over a vast interval of geological time.

1.1. The temperature-size rule

The temperature-size rule (TSR; Atkinson, 1994) describes the inverse relationship between ambient temperature and organismal size as an ecophenotypic response among ectotherms. The TSR parallels Bergmann’s law, which applies to homeothermic animals (Van Voorhies, 1996; Blackburn et al., 1999; Angilletta and Dunham, 2003), and is well established as a result of extensive empirical studies (e.g.

O'Dea and Okamura, 1999; Atkinson et al., 2006; Ghosh et al., 2013), albeit with a few notable exceptions (e.g. Mousseau, 1997; Aguilar-Alberola and Mesquita-Joanes, 2014). Although an unequivocal universal mechanism behind the TSR remains elusive, hypotheses based on adaptation (see Kingsolver and Huey, 2008 for review) and phenotypic plasticity (see Atkinson et al., 2006 for review) have been proposed. Two drivers of the phenomenon are currently favoured. The first is based on the putative differential effect of temperature on rates of development and growth (van der Have and De Jong, 1996; Walters, 2006; Kingsolver and Huey, 2008), with higher temperatures promoting fast growth but even faster development and resulting in a mature organism of small size. The second involves differential rates of oxygen diffusion and of metabolic processes involving oxygen (Atkinson, 2006). The existence of a universal mechanism for the TSR in all ectotherms is unlikely particularly since multi- and unicellular organisms appear to exhibit fundamentally different responses to temperature despite ultimately obeying the TSR (Forster et al., 2011; Ghosh et al., 2013).

1.2. The z_s -MART proxy

The TSR is applicable to bryozoan morphometry at the scale of zooids (Menon, 1972; O'Dea and Okamura, 1999; O'Dea and Okamura, 2000a, b, c; O'Dea and Jackson, 2002; Hunter and Hughes, 1994; O'Dea, 2005; Atkinson, 2006; O'Dea et al., 2007a, b), although bryozoan autozoid area is likely also controlled by a multitude of factors including salinity, nutrients, substrate topography and biological interactions such as disease and predation. Autozooids (feeding zooids) are studied because, unlike heterozooids, their morphology is not specialised (e.g. for reproduction or defence)

and, being generally more numerous, they offer greater replication. According to the TSR, the magnitude of variation in ambient temperature experienced during the lifetime of the colony should, to some degree, influence the magnitude of intracolony variation in zooid size. The measures of variation used in the zs-MART proxy equation of O’Dea and Okamura (2000) are the coefficient of variation of zooid area (CV_{ZA}) and the mean annual range of temperature (MART), defined as the difference between mean summer and mean winter temperatures. A normalised measure of variance was used to enable comparison between species with zooids of different average size. O’Dea and Okamura (2000) undertook an analysis of five wild colonies each of 29 cheilostome species collected across 16 localities representing a range of obs-MART values (2.1 – 11.0 °C). The equation they derived describing the observed linear relationship is:

$$MART = -3 + 0.745CV_{ZA} \quad (1)$$

where CV_{ZA} is the coefficient of variation (100 x Standard Deviation/Mean) of zooid area, which is approximated by the product of zooid length and width (O’Dea and Okamura, 2000).

2. Revisiting the zs-MART proxy

O’Dea and Okamura (2000) measured Recent cheilostome bryozoans from a number of localities experiencing a range of known annual temperature ranges. Five specimens per species per locality were measured and averaged. Observed MART (obs-MART) values were taken from various sources in the literature. However, the

large range in CV_{ZA} values for each locality is a characteristic of the dataset that was not considered in the original regression analysis. How variance in these data introduces uncertainties in zs -MART estimates is outlined below.

2.1 Regression

In the original equation of O’Dea and Okamura (2000) (Equation 1), the relationship sought is that between the measured coefficient of variation of zooid size in a colony (CV_{ZA}) and the obs -MART experienced by that colony. During construction of the original dataset, O’Dea and Okamura assumed that the error in obs -MART is negligible, and that all colonies from a single locality experienced the same obs -MART. This assumption relies on accurate estimates of obs -MART. If there is a true underlying linear relationship between the two variables, the large range of species-averaged CV_{ZA} values for each value of obs -MART should be normally distributed about the regression line. The best approximation of the relationship between these variables is therefore obtained via an ordinary least squares linear regression (OLS), which minimizes the distance of points from the regression line in the CV_{ZA} axis only. A total least squares (or major axis) regression places too much weight on deviations parallel to the obs -MART axis.

With obs -MART on the X-axis and CV_{ZA} on the Y-axis, the preferred regression is a Y on X least squares linear regression. The original equation (Equation 1) was derived using an X on Y least-squares linear regression, and is therefore incorrect. The extent of the disagreement between resultant regression lines is shown in Fig. 1. Although figure 2 of O’Dea and Okamura (2000) displays a linear regression line that appears to approximate the revised line (Fig. 1), the equation

presented in the same paper (Equation 1) and used in all subsequent research (Table 1) lies outside of the 95% confidence interval of the revised regression and has a steeper gradient.

2.2 Quantifying uncertainty in *zs*-MART values

There are three components that contribute to the uncertainty in *zs*-MART values generated by Equation 1: (i) the confidence limits of the position of individual points (error bars in Fig. 1); (ii) the 95% confidence interval of the regression (CI, Fig. 2A, 2C); and (iii) the 95% prediction interval (PI, Fig. 2A, 2C). The relative importance of these components are evaluated below:

2.2.1 Position of individual points

The value of CV_{ZA} for each colony is a function of the standard deviation of sampled zooid areas. The CV_{ZA} of a sample is a robust estimator of normalised population variance when the sample size is large enough that values approximate a normal distribution. The confidence in the value of CV_{ZA} is itself a function of the standard deviation and increases at higher values of CV_{ZA} . It is reduced by a factor of \sqrt{n} , where n = number of repetitions. The formula used to calculate the uncertainty in CV_{ZA} (error bars in Fig. 1A and 1B) is based on the uncertainty in the standard deviation for a small sample (taken from Skeskin 2007). Error propagation formulae were used to adapt it for coefficient of variation giving:

$$\sigma(\sigma_{ZA}) = CV_{ZA} \sqrt{\frac{1}{2(n-1)} + \left(\frac{\sigma_{ZA}}{\mu_{ZA}}\right)^2 - \frac{r\sqrt{2}}{\mu_{ZA}\sqrt{n-1}}}$$

(2)

where n = number of zooids, μ_{ZA} = zooid area mean, σ_{ZA} = standard deviation of zooid areas, r = correlation coefficient of μ_{ZA} and σ_{ZA} and CV_{ZA} = coefficient of variation of zooid areas. In the original compilation of data, O'Dea and Okamura implicitly assumed that the uncertainty in obs-MART for each locality is negligible. Although questionable, the validity of this assumption is not addressed here. For the derivation of Equation 1, with 20 zooids measured and five replicate colonies, this uncertainty is significantly lower than that due to the PI (see below). Length and width measurements of zooids that contribute to the value of CV_{ZA} are conservatively accurate to typically ~5%. This is a smaller source of uncertainty than is introduced by the estimation of population CV_{ZA} from a sample of 20 zooids (Equation 2).

2.2.2 95% confidence interval (CI)

The CI is a measure of the uncertainty in the relationship between the given variables. When rearranged such that it is in the form of Equation 1, the revised regression (Equation 3) has a 95% probability of having a gradient between 1.4 and 0.79 and an obs-MART-axis intercept between -9 and -3. This is shown by the shaded region around the regression line in Figure 2C. The original regression line lies outside of this 95% CI.

2.2.3 95% prediction interval (PI)

Where residuals are normally distributed around the regression line (as demonstrated in Fig. 2B) the PI represents the envelope that contains 95% of points centred on the regression line (Fig. 2A, C). When using the derived equation to generate

corresponding values of an output variable from an input variable, the PI is the largest source of uncertainty.

The standard deviation of the residuals about the regression line, and therefore the PI, is dramatically reduced when values are averaged for each species at each locality. The PI for the species-averaged regression is ~ 4 in the CV_{ZA} axis with a corresponding value in the obs-MART axis of $\sim 4^{\circ}\text{C}$ (Fig 2C). This large uncertainty in zs-MART values for a given CV_{ZA} is due to variance in zooid size that is unexplained by temperature.

2.3 Revised equation for zs-MART proxy

Using an appropriate ordinary least squares regression (see section 2.1) and adopting the relevant significant figures for coefficients and errors (see section 2.2), the revised equation relating obs-MART and CV_{ZA} is:

$$MART^{\circ} = CV_{ZA} - 6 \pm 4 \quad (3)$$

The uncertainty in zs-MART values is dominantly determined by the PI of the regression. This is $\pm 4^{\circ}\text{C}$ when based on location means of five replicate colonies per species. If no replicates are used, the PI, and hence the uncertainty, is $\pm 6^{\circ}\text{C}$ (Fig. 2A, B). Although the relationship between obs-MART and CV_{ZA} is well constrained, the scatter of the data about this line is large, and hence the predictive power is poor. Equation 3 is valid only over the observed range of values ($MART = 2\text{-}12^{\circ}\text{C}$). Zs-MART values close to the edge of this range should be treated with caution and extrapolation is strongly discouraged.

2.4 Implications for previous work

Since its publication (O’Dea and Okamura, 2000), the zs-MART proxy has been cited or employed in numerous studies without revision (e.g. Taylor, 2005; Knowles et al., 2009; Williams et al., 2009; Okamura et al., 2011 for reviews). Table 1 reports zs-MART values from the literature, adjusted zs-MART values according to Equation 3 and revised conclusions based on the adjusted values. The studies whose original conclusions are not compromised are those based on differences in zs-MART values of greater than 4°C. The zs-MART proxy in its current state is therefore less powerful than previously believed.

3. Improving the zs-MART proxy

Equation 3 generates values for zs-MART with a large associated uncertainty. In order to explore sources of variation in CV_{ZA} that vary systematically across a colony, and might therefore be elucidated through a non-random selection of zooids, a detailed study of zooid size variation along transects was undertaken. The skeletally simple malacostegine cheilostome *Acanthodesia* was chosen because it lacks zooidal polymorphism, is characterised by colonies that can attain large sizes, and has clearly delineated zooidal margins suitable for zooid-area analysis (Knowles et al., 2009).

A total of 1195 zooids were measured from 40 transects through 19 fossil and Recent specimens. Fossil specimens were borrowed from the collections of the Department of Earth Sciences, Natural History Museum, London. Specimens were from three North American localities: 1) Armuelles Formation ~ 0.04 Ma (Late

Pleistocene) collected by B. Okamura & P. D. Taylor in March 2011 from the Burica Peninsula in the Gulf of Chiriqui; 2) San Diego Formation ~ 3 – 1.5Ma (Late Pliocene – Early Pleistocene) loaned by the San Diego Museum; 3) Imperial Formation (mid-Pliocene) collected by S. Kidwell in 1998 from the Camel Head Member, southern California. Recent material was collected from the field in 2007 by A. Ostrovsky et al. from Veracruz Beach in the Gulf of Panama, from within the intertidal zone. In total, the material studied probably represents between 3 and 5 different species. Although the species-level taxonomy of *Acanthodesia* is difficult and in need of revision, all material used in this study may be reliably referred to this genus.

As cheilostome bryozoan colonies grow, new zooids bud at the distal ends of existing rows (e.g. Goldwasser et al., 1989). In *Acanthodesia* it is relatively easy to identify rows comprising successions of parent and offspring zooids, unlike some cheilostome taxa where tessellation patterns of zooids within colonies can complicate identification of parent-offspring relationships (Hayward, 1980). A single row is here defined as a series of distally budded autozooids between successive row bifurcations (Fig. 3).

3.1 Data collection

Colonies of *Acanthodesia* were chosen such that they were large enough to assume growth for >1 year (Knowles et al., 2009), there was at least one proximal-distal transect of >20 measureable zooids, and for which the effects of substratum irregularity were minimal. Selected specimens were first cleaned using a soft brush, water and an ultrasonic bath. Some Recent bryozoans were bleached, removing

organic matter to facilitate measurement. Each zooid was allocated a unique identifier and a value for:

<i>Generation (G)</i>	A zooid's <i>generation</i> describes its relative generational position within the colony. (The ancestrula was not visible in many specimens, necessitating the use of relative generational positions. These can readily be inferred since the direction of colony growth is apparent.)
<i>Position (P)</i>	A zooid's <i>position</i> refers to its position within a row (e.g. 1-5 in Fig. 3).
<i>Length (L)</i>	Maximum zooid length, parallel to row axis.
<i>Width (W)</i>	Maximum zooid width, perpendicular to row axis.

Length and width measurements were on the order of 100 μ m and accurate to $\pm 5\mu$ m. These were made using a stereomicroscope with an eyepiece graticule. Zooids were measured only if they met the criteria of both O'Dea and Okamura (2000) and Knowles et al. (2009). Only ontogenetically mature autozooids (the ancestrula and early astogenetic stages were avoided) that were free from visible damage or distortion (including teratology) were measured. The first and last zooids of each row (Fig. 3 for definition) were not measured because they tend to be visibly different in size from those in the middle of rows. Specimens were rotated such that the measurements were made in the plane perpendicular to the line of sight, avoiding underestimation due to foreshortening. Measurements were only made through the right-hand eyepiece in order to avoid parallax errors.

3.2. Zooid-length profiles

Transects consist of proximal-distal series of contiguous zooids. Each zooid is budded from its proximal neighbour, so a zooid-size profile along a transect represents a pseudotime series. As zooid size has a temperature controlled component according to the TSR, any colony that has grown for >1 year should exhibit some degree of seasonally induced oscillation in its zooid-size profiles. If this is not observed, it is unlikely that the variation in zooid size is dominated by a seasonally variable parameter such as temperature, and may be due to a number of other factors including packing effects (Hageman et al, 2002). Zooid width and area are strongly influenced by position (Section 3.3), so in order to reduce non-temperature related sources of variance, zooid length, which is least strongly influenced by position, is used as the size variable.

Transects across a colony were examined using a similar protocol to O’Dea and Jackson (2002). Transects in the Recent material were chosen to finish at an active growing edge. Recent colonies with the shiny cuticle intact and opercula visible in mature zooids behind the growing edge are inferred to have been collected while still alive.

3.2.1 Growth rates

Unfortunately there exists no published information on growth rates in *Acanthodesia* from southern California or Central America, and from our knowledge there are no unpublished data either. Indeed, the only data on growth rates in this genus comes from the study of Swami & Karandi (1987) in the nearshore waters around Bombay,

India. These authors reported that colonies of a species they identified as *Acanthodesia* sp. attained diameters of between 0.22 and 2.87 cm after 30 days, depending on month of immersion. This is equivalent to a linear growth rate of 132–172 mm per year. However, it would be very unwise to apply this growth rate to the Panamanian *Acanthodesia* used in the current study given the major variations in growth rates known to exist between bryozoan species and across different environments. For example, the sheet-like bryozoans studied from various European localities by Kuklinski et al. (2012) showed linear growth rates ranging over two orders of magnitude

3.2.2 Extracting the oscillatory signal

The signals generated in zooid length profiles that exhibit oscillations were separated into a *trend* component and a *cyclic* component using a Hodrick-Prescott filter (Equation 3), which is widely used in the field of macroeconomics for extracting the amplitude of annual cyclicality from data with a longer-term trend (Hodrick and Prescott, 1997). Minimising Equation 3 gives the *trend* component of length of each zooid for a given smoothing parameter. The difference between this long term trend and the original data is the *cyclic* component.

$$\underbrace{\sum_{g=1}^n (L_g - T_g)^2}_A + \lambda \underbrace{\sum_{g=2}^{n-1} \left[\overbrace{(T_{g+1} - T_g)}^{\alpha} - \overbrace{(T_g - T_{g-1})}^{\beta} \right]^2}_B$$

(4)

where g = zooid generation, L_g = zooid length at generation, g , T_g = trend component of zooid length at generation, g , λ = smoothing parameter, and n = number of zooids in a transect.

Part A of Equation 4 fits the trend to the data and part B determines the smoothness of the fit. α and β represent the gradient of the trend line either side of the zooid at generation, g . The smoothing parameter (λ) was varied in order to alter the sensitivity of the trend component to variability in the data, with the aim of separating inter-annual and intra-annual variations respectively into the *trend* and *cyclic* components. Through trial and error, a value of $\lambda=1000$ was chosen as optimum, by assuming that the observed period of oscillation represents one year. If this assumption is valid, and if the dominant control of zooid size is temperature, the amplitude of the *cyclic* component should be approximately proportional to the MART experienced.

3.2.3 Characterising the oscillatory signal

Where oscillatory signals are observed in zooid-length profiles, validating their authenticity as responses to external stimuli is required. The protocols developed for this are described below and include: (i) comparison with timing of bifurcations, (ii) comparison with parallel transects, and (iii) concordance of multiple moving average size windows. This last condition is required if moving averages are used to smooth data when noise in a dataset obscures a genuine signal. Adjacent points in such a moving-average smoothed dataset all share constituent values, so they plot closer to one another simply because of their proximity. It is therefore not unusual for oscillatory patterns to appear in smoothed data when none are present – this is the Slutsky-Yule effect (Slutsky, 1937). The phantom patterns generated are *spurious*

cycles. This effect is of extreme importance when studying time series datasets, especially when the period of expected oscillation is unknown. Oscillatory signals must not be interpreted until they are firmly corroborated by independent evidence.

i. Comparison with timing of bifurcations

Zooid length is influenced by position within the row. Bifurcations should therefore not coincide with oscillations if the signal is real and not an artefact of intra-row variation.

ii. Comparison with parallel transects

Parallel transects are chosen such that they are close enough to each other to be unambiguously contemporaneous, but far enough apart to be assumed that zooid sizes do not influence one another. If the oscillatory signal is externally controlled, parallel contemporaneous transects should contain the same signal. The protocol used here is therefore to examine oscillations that appear in at least two parallel transects (those that do not are deemed spurious).

iii. Multiple moving average size windows

Data in this study were smoothed with 3, 5 and 7 point moving averages. The protocol used here is to consider oscillations that do not appear in all three as spurious.

If oscillations do represent temperature seasonality, they should also be repeated with a regular period when growth rates are similar year after year. Although it is impractical to make this a constraint for the given data, spurious cycles are not constrained by period, so oscillations that are not repeated with a regular period are treated with caution.

3.2.4 Results

Of the 19 specimens studied, five exhibited oscillatory signals in their zooid length profiles that passed the above criteria. The data collected from two colonies (Fig. 4 and Fig. S1) provide examples of zooidal growth that is probably determined by an external stimulus. Assuming that the dominant component of variation is due to temperature (though this is impossible to determine without independent verification), it is envisioned that the amplitude of the cyclic (intra-annual) component of these oscillations could be used to predict the magnitude of seasonal temperature variation and form the basis of a new *zs*-MART proxy. Values generated thus however would relate to ambient water temperature, and would require knowledge of the depth habitat of the relevant bryozoan species to make a reasonable estimate of sea surface temperature. This approach would also require thorough calibration either through *in vivo* controlled experiments (e.g. O’Dea et al., 2007b), varying only temperature, or through use of an established temperature proxy such as Mg/Ca or oxygen isotopes in fossil or Recent material (e.g. O’Dea, 2005; Key et al., 2012, in press).

3.3. Elucidating the effect of position

Oscillations observed in many zooid area profiles correlate with bifurcating rows (e.g. Fig. S2). To quantitatively elucidate this artefact, zooid areas, widths and lengths were normalised relative to the colony average, and these normalised values averaged for each position (P) within the row across all specimens. Linear least-squares regressions of average normalised zooid area, width and length against the natural logarithm of position ($\ln(P)$), resulted in strong positive linear correlations (Fig. 5). The relative magnitude of the effect of position is greatest on zooid area, second most on zooid

width and least on zooid length. Although there is a large scatter in the raw data, these are real underlying relationships, which form a source of variation that was previously unexplained. The effect of increasing zooid size with position was also observed in at least three other species (*Watersipora* sp., *Cryptosula pallasiana* and *Thalamoporella californica*; McClelland unpublished data), and may be very widespread.

It is possible to remove the systematic position-related error from zooid area data in *Acanthodesia*, although the relationship is unconstrained beyond zooid number 10 in a row. The equation describing the solid black line in Fig. 5 relating average normalised zooid area (Z_n) and position (P) is:

$$Z_n = 0.96 \ln(P) - 1.5 \quad (5)$$

The correction for the area of the i^{th} zooid at position P is therefore given by:

$$Z_{P=0i} = Z_{Ai} - \sigma_{ZA} 0.96 \ln(P) \quad (6)$$

where σ_{ZA} is the standard deviation of zooid size in the host colony, P is the position within the row, Z_{Ai} is the area of the i^{th} zooid and $Z_{P=0i}$ is the corrected area for the i^{th} zooid, incorporating the correction for position and assuming all zooids are at position 0.

Equation 5 is derived only from, and is therefore only valid for, members of the genus *Acanthodesia*. Increasing zooid size along a row was also observed in the Recent ascophorans *Watersipora* sp. and *Cryptosula pallasiana*, in the anascan *Thalamoporella californica* and in some cupuladriids (McClelland, unpublished data).

Equation 6 generates values for zooid area, giving values as if zooids are all at the 0th position within the row. In any future derivation of the zs-MART proxy, zooid areas should be corrected for positional bias using this equation (for *Acanthodesia*) or an equivalent generated specifically for the studied taxa. Additionally, only zooids of position 2 to n , where n is the position at which the relationship breaks down, should be used for analysis.

3.4 Discussion

3.4.1 Positional bias and bifurcation effects

This study has shown that in addition to the packing effects described by Hageman et al (2002), the position of a zooid within a row can have a systematic effect on its size, superimposed on other external factors such as the ambient temperature. An oscillatory signal observed in a zooid area profile, where periodic increases in zooid size coincide with ascending zooid position, could therefore potentially be an artefact (e.g. Fig. S2). This calls into question the reliability of growth rate analyses of free-living cupuladriid bryozoans (O’Dea and Jackson, 2002) and erect colonies (O’Dea and Okamura, 2000b; O’Dea 2005) because all these studies ignored the effect of zooid position within a row.

The growth rates estimated by O’Dea and Jackson (2002) however, were subsequently corroborated by direct measures of cupuladriid growth (O’Dea, 2006) and the isotopic composition of their skeletons (Key et al., 2012, in press). Oscillations in zooid size observed in *Flustra foliacea* (O’Dea and Okamura, 2000b) were correlated with seasonally-produced growth check lines and oscillations in zooid size in *Pentapora foliacea* shadowed isotopic patterns caused by seasonal variation in

sea water temperature (O’Dea, 2005). Implicitly, measurable oscillatory signals can be responses to seasonal variation in temperature, but without positional data, it is impossible to separate the direct effect of temperature from an indirect influence of temperature on growth patterns and bifurcation frequency.

Different species and colony forms have different row bifurcation frequencies. Forms with lower bifurcation frequency and hence longer row length will have an inherently higher variation in zooid size and therefore *zs*-MART. The inflated variance in zooid size due to the positional effect is removed when data are transformed using Equation 6, although series of rows whose intra-row zooid size gradients differ from the average described by Equation 5 will still exhibit artificial oscillation. The *zs*-MART proxy based on the random sampling of zooids will be similarly compromised by the effects of bifurcation, so the value of correcting for zooidal position using Equation 6 lies in removing a known source of variance from the dataset prior to analysis.

*3.4.2 The future of the *zs*-MART proxy*

O’Dea and Jackson (2002, p. 91) concluded that the amplitude of oscillation in zooid area profiles could not be extracted and interpreted as temperature variability because “the amount of noise within the data limits the quality of any derived information”, stating further that the *zs*-MART proxy is sufficient for that purpose. If the data are too noisy for seasonal temperature variation to be observed however, then values generated by Equation 1 or Equation 3 should be treated with similar scepticism.

The error involved in the revised form reported here (Equation 3) limits this proxy to distinguishing environmental regimes where the difference in seasonality is large ($>4^{\circ}\text{C}$), or to broadly constraining computational models. Nevertheless, there is

certainly potential for developing an improved proxy with the same theoretical basis and which accounts for error. Such future derivations of the zs-MART proxy should include taxon-specific corrections for zooidal position. Thus, zooid size profiling should be undertaken as a preliminary to analysis, and species that lack evidence of genuine externally controlled oscillatory variations in zooid size should be discarded.

Certain features of cheilostome bryozoans make them highly attractive as proxies for palaeoclimate inference (Okamura et al., 2011). Firstly, analysis using the zs-MART proxy is cheap, requiring only a stereomicroscope and relatively little training; palaeoseasonality proxies such as oxygen isotope and Mg/Ca profiling through biogenic carbonate of long-lived molluscs and forams (e.g. Bougeois et al., 2014; Evans et al. 2013), or similar analyses of multiple individual short-lived forams (e.g. Scroxton et al., 2011), are powerful but expensive. Secondly, the distinct individual modules (zooids) of bryozoans are produced iteratively throughout the lifetime of the colony. Although this confers confounding positional effects, it also enables inferences of both intra- and interannual environmental variation, whilst controlling for genetic effects, since all modules are genetically identical barring some mutation. Such information is not readily available from short-lived, unitary organisms that are commonly used as proxies (e.g. foraminifers and ostracodes). Thirdly, many cheilostomes exhibit polymorphism, producing zooids specialised for feeding, reproduction and defence, which can be related to the life history of that colony (e.g. see O’Dea et al., 2011). The zs-MART proxy can inform on the environment experienced by an individual colony, and in combination can directly correlate life history responses with environmental regimes. Cheilostome bryozoans are the only post-Palaeozoic colonial invertebrates that can provide such insights due to a unique set of traits: observed adherence to the temperature size rule, intracolony

polymorphism, the production of carbonate skeletons and determinate zooid sizes (Okamura et al. 2011).

4. Conclusions

1. The biological relationship between the observed mean annual range of temperature (obs-MART), and the coefficient of variation (CV_{ZA}) in cheilostome bryozoan zooid size is not disputed but the original equation (Equation 1; O’Dea and Okamura, 2000) is incorrect and should be replaced by:

$$MART^c = CV_{ZA} - 6 \pm 4$$

2. The revised equation (above) has a large margin of error ($\pm 4^\circ\text{C}$) and is therefore useful only for comparing environments with a difference in MART greater than 4°C . If specimens are not species-averaged, this error is $\pm 6^\circ\text{C}$.

3. The zs-MART proxy is in need of refinement, as there are systematic errors in the approach. In correcting for zooid position within a row, some of this systematic error will be removed, but the numerical correction must be developed on a taxon-by-taxon basis. In *Acanthodesia*, average normalised zooid area (Z_n) is related to position (P) within a row via the equation:

$$Z_n = 0.96 \ln(P) - 1.5$$

4. Some transects through *Acanthodesia* colonies appear to exhibit an oscillatory signal in zooid length, which is suggested to reflect annual temperature variation.

Whether this represents a real signal remains to be tested through established temperature proxies such as oxygen isotopes or Mg/Ca ratios.

5. Acknowledgements

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Fig. 1. Least squares linear regressions between CV_{ZA} and MART. CV_{ZA} is the average value per species, with each species represented by at least 5 colonies. Error bars are errors in species average CV_{ZA} . (A) The original equation was generated using an “X-on-Y” least squares linear regression. The horizontal lines between points and the regression line are the distances whose sum of squares was minimized in order to generate the line. (B) The revised equation is generated using a “Y-on-X” least squares linear regression. The vertical lines between points and the regression line are the distances whose sum of squares was minimized in order to generate the line. The formula used to calculate the error in CV_{ZA} (error bars in Fig. 1A and 1B) is based on the error in the standard deviation for a small sample (taken from Skeskin 2007).

Fig. 2. (A) Least-squares linear regression of coefficient of variation of zooid size. Points represent colonies. (B) Histogram of residuals about least-squares linear regression line for colonies. Dotted black line describes the approximated normal distribution – hence $1.96\sigma_R$ is the 95% prediction interval. (C) Least-squares linear regression of zooid size. Points represent averages per species. Each point is an average of at least 5 colonies. PI_{MART} is the relevant error in the MART axis, and is directly proportional to PI_{CV} , with the constant of proportionality = the gradient of the regression line. CV_{ZA} = coefficient of variation of zooid area. MART = Measured mean annual range of temperature. CI = confidence interval for equation of line. PI = prediction interval for data points. σ_R = standard deviation of residuals. See text for discussion.

Fig 3. Scanning electron microscopy (SEM) image of *Acanthodesia* sp. showing a complete row of autozooids. As pictured, growth is from right to left, with “1” being the first budded autozooid of the new row and “5” the last. Scale bar, bottom right = 500 μ m.

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Fig 4. Top two panels provide data from two transects (A and B) from specimen 25. Growing edge identified and defined arbitrarily as zooid generation 100 in both transects. Dotted vertical lines represent inferred times of growth based on correlation of cool periods with zooid-length maxima and warm periods with zooid-length minima. Evidence that oscillations represent a genuine environmental signal derives from: comparison between parallel transects (25A and 25B); comparison of bifurcations with the oscillatory pattern (note that bifurcations as inferred from data on zooid position within row and that these do not coincide with oscillations); coherence of different moving averages (for 3-, 5- and 7-point moving averages). See text for discussion. Bottom: Local temperature data from the locality for 7 years preceding collection. Living specimens collected *in situ* in October 2007 at Veracruz Beach in the Gulf of Panama by A. Ostrovsky et al.

Fig. 5. Logarithmic relationship between average normalised zooid area (Z_n ; circles and solid line), average normalised zooid width (W_n ; triangles and dotted line), average normalised zooid length (L_n ; squares and dashed line) and position (P) within a row (as defined in Fig. 3). Zooid dimensions were normalised (minus mean and divided by standard deviation) relative to their host colonies in order to elucidate the relationship relevant for all samples of *Acanthodesia* studied. Equations of linear least squared regressions: $Z_n = 0.96\ln(P) - 1.5$ ($r^2 = 0.995$, $p < 0.001$), $W_n = 0.81\ln(P) - 1.3$ ($r^2 = 0.98$, $p < 0.001$), $L_n = 0.56\ln(P) - 0.88$ ($r^2 = 0.93$, $p < 0.001$).

Table 1. Existing zs-MART estimates from the literature, employing the original equation (Equation 1), are altered using the revised equation from this study (Equation 2). The validity of conclusions is evaluated.

Locality (Time period)	Reference	Previous zs-MART estimate(s)	Additional information given	Revised zs-MART estimate(s)	Conclusion affected by revision?	Conclusions
“Faluns”, NW France (Early Miocene)	O’Dea and Okamura 2000	5.4°C	Typical modern MART = 8°C	5.3 ± 4°C	Yes	Miocene zs-MART estimate is within error of the modern analogue, the Bay of Biscay.
Coralline Crag, UK (Mid-Pliocene)	O’Dea and Okamura 2000	8.1 - 8.6°C	Modern MART = 11.5° (Lee & Ramster, 1981)*	8.9 – 9.6 ± 4°C	Yes	Pliocene zs-MART estimate is within error of the modern value for southern England. * Modern MART value quoted differs to that of Knowles et al. (2009).
- Panama – Pacific (Recent)	O’Dea & Jackson 2002	8.4°C	- Gulf of Panama – Upwelling.	9.3 ± 4°C	No	The upwelling and non-upwelling sides of Panama experience high and low seasonality respectively, which is reflected in the zs-MART estimates.
- Panama - Caribbean (Recent)		3.1°C	- San Blas Islands – Non-upwelling	2.2 ± 4°C		
- Panama – Pacific (Recent)	O’Dea 2003	7.7°C	- Gulf of Panama – Upwelling.	8.4 ± 4°C	No	The upwelling and non-upwelling sides of Panama experience high and low seasonality respectively, which is reflected in the zs-MART estimates.
- Panama - Caribbean (Recent)		3.1°C	- San Blas Islands – Non-upwelling	2.4 ± 4°C		
Panama – Caribbean (Miocene - Recent)	O’Dea et al. 2007	Pacific-profile: 6.6°C Caribbean-profile: 3.1°C	- “Pacific profile” = Upwelling. - “Caribbean profile” = Non-upwelling	6.9 ± 4°C 2.3 ± 4°C	No	Seasonality in the Caribbean decreases at around 4Ma. This is interpreted as a transition from an upwelling to non-upwelling regime coincident with, and due to, the closure of the Isthmus of Panama.
North Atlantic (Pliocene)	Knowles et al. 2009	4.97 – 6.54°C 7.41 °C 4.37°C 8.08 - 8.60°C	Modern MART values (Levitus & Boyer,1994): - 7.0 – 9.0 °C - 9.5°C - 1.0 °C - 4.0 – 5.0 °C*	4.7 – 6.8 ± 4°C 8.0 ± 4°C 3.9 ± 4°C 8.9 – 9.6 ± 4°C	- Yes - Yes - Yes - No	Pliocene zs-MART estimates- - are not significantly higher than today in Caribbean Panama - are not significantly lower than today in US Coastal Plain or in Florida - from Corraline Crag are significantly higher than modern southern England. * Modern MART value quoted differs to that of O’Dea and Okamura (2000).
Skomer Island, UK Lleyn Peninsula, UK (Recent)	Knowles et al. 2010	6.9°C 6.8°C	- δ ¹⁸ O range: 5.6 °C - δ ¹⁸ O range: 6.4 °C Data logger (both): 7.8 °C	7.3 °C 7.2 °C	N/A	Revised zs-MART estimates are closer to the measured annual temperature range, but the error is much larger than oxygen isotopes.
Weddell Sea (Early Pliocene)	Clark et al. 2010	4.3 – 10.3°C (6.6 – 7.7°C)	Modern MART = 2 °C	3.8 – 11.9 ± 4°C (6.9 – 8.4 ± 4°C)	Yes (No)	Large spread of zs-MART values, many within error of modern value, but the group of numbers given most credence (in brackets) are significantly higher.
Nye Kløv, Denmark (prior to K/T boundary)	O’Dea et al. 2011	Species 1: 1.5 → 6°C Species 2: 0.5 → 5°C	Corresponding change in avicularian density and mean zooid size through section.	0.0 → 6.1 ± 4°C -1.3 → 4.7 ± 4°C	No (Yes)	The magnitude of the increase in zs-MART prior to the K/T boundary is significant in both species, and is mirrored by other proxies. (Some of the corrected MART values for species 2 are negative and

					are therefore too low to be real.)
Various localities and time periods. Central and southern North America, Miocene to Recent.	Okamura et al. 2013	Shapes of frequency distributions of zs-MART values are used to form arguments about the upwelling regime.	N/A		The shapes of frequency distributions of zs-MART values are independent of the absolute values. When the large error associated with each zs-MART value is included in the analysis however, confidence decreases in the shape of the distribution.

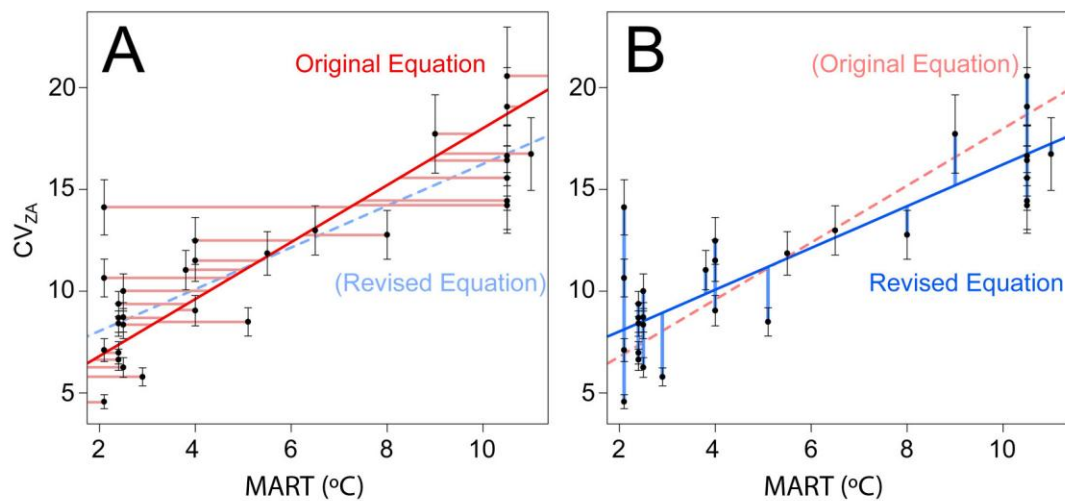


Figure 1

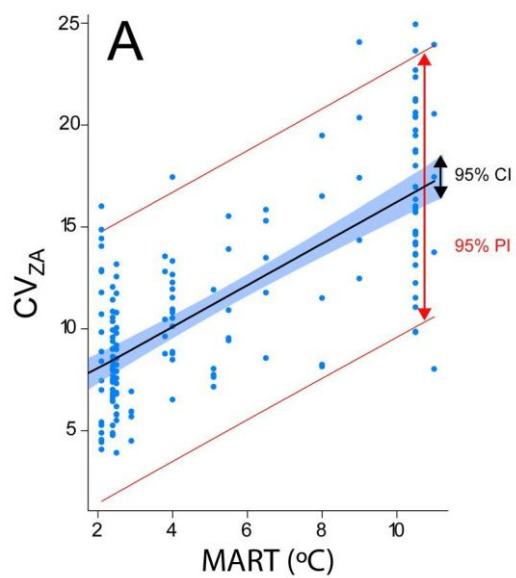


Figure 2A

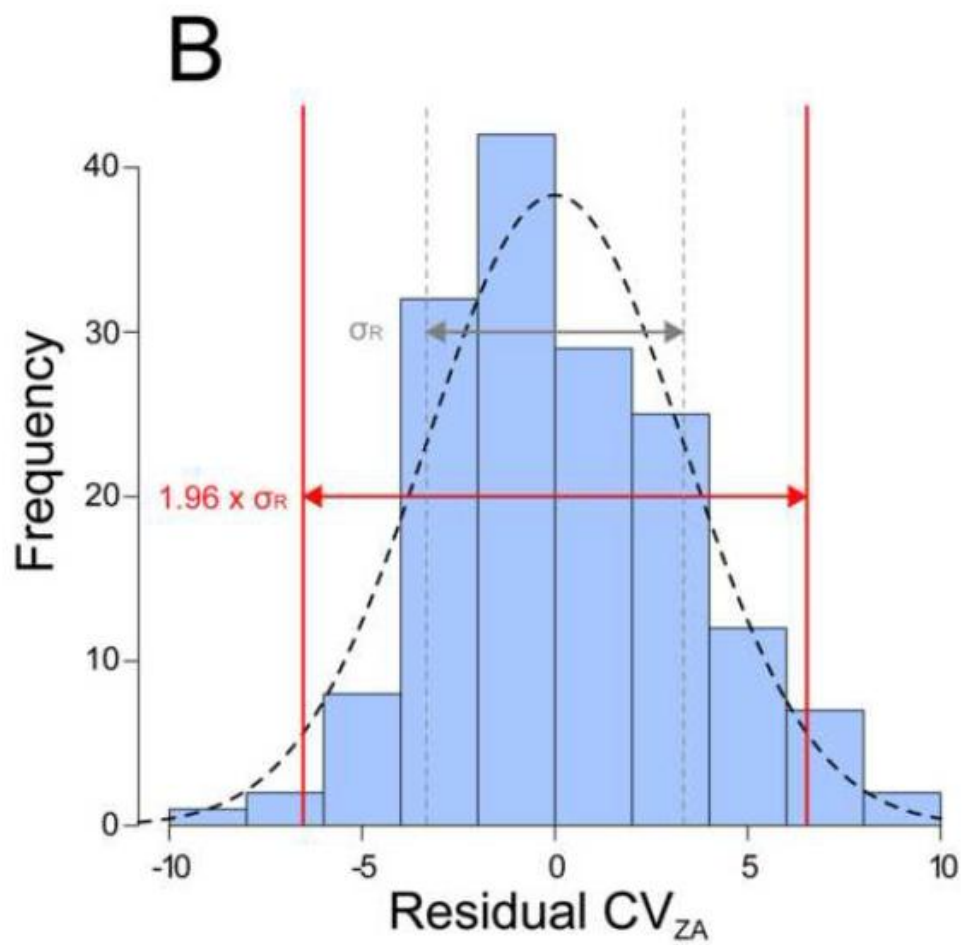


Figure 2B

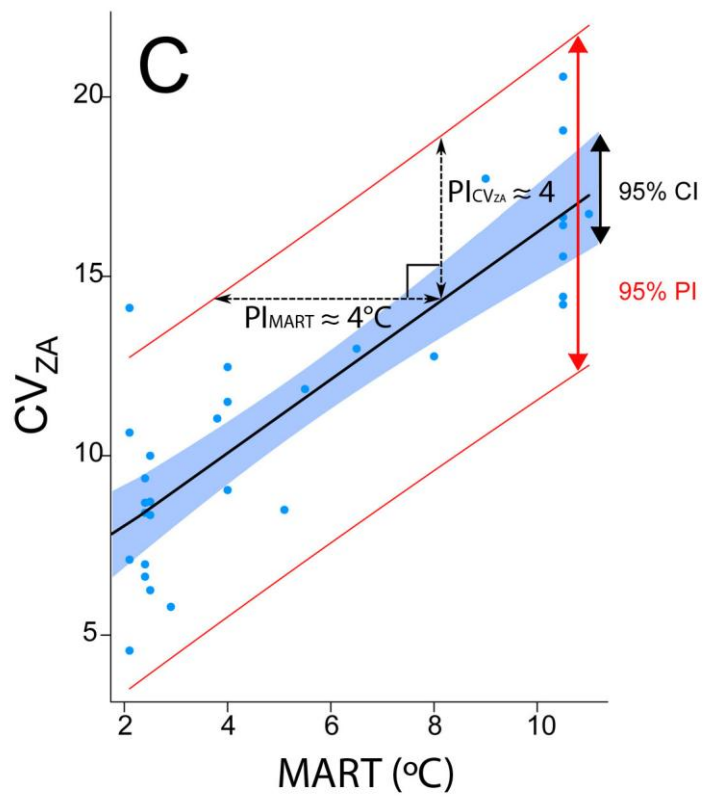


Figure 2C

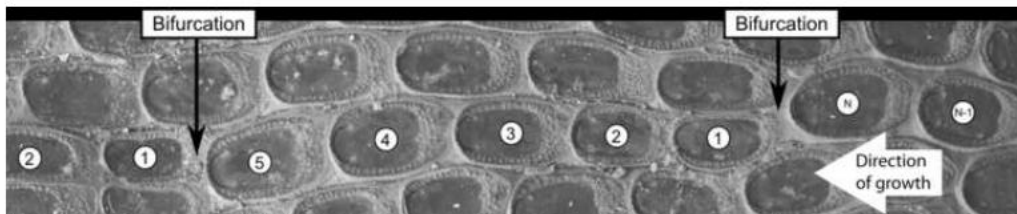


Figure 3

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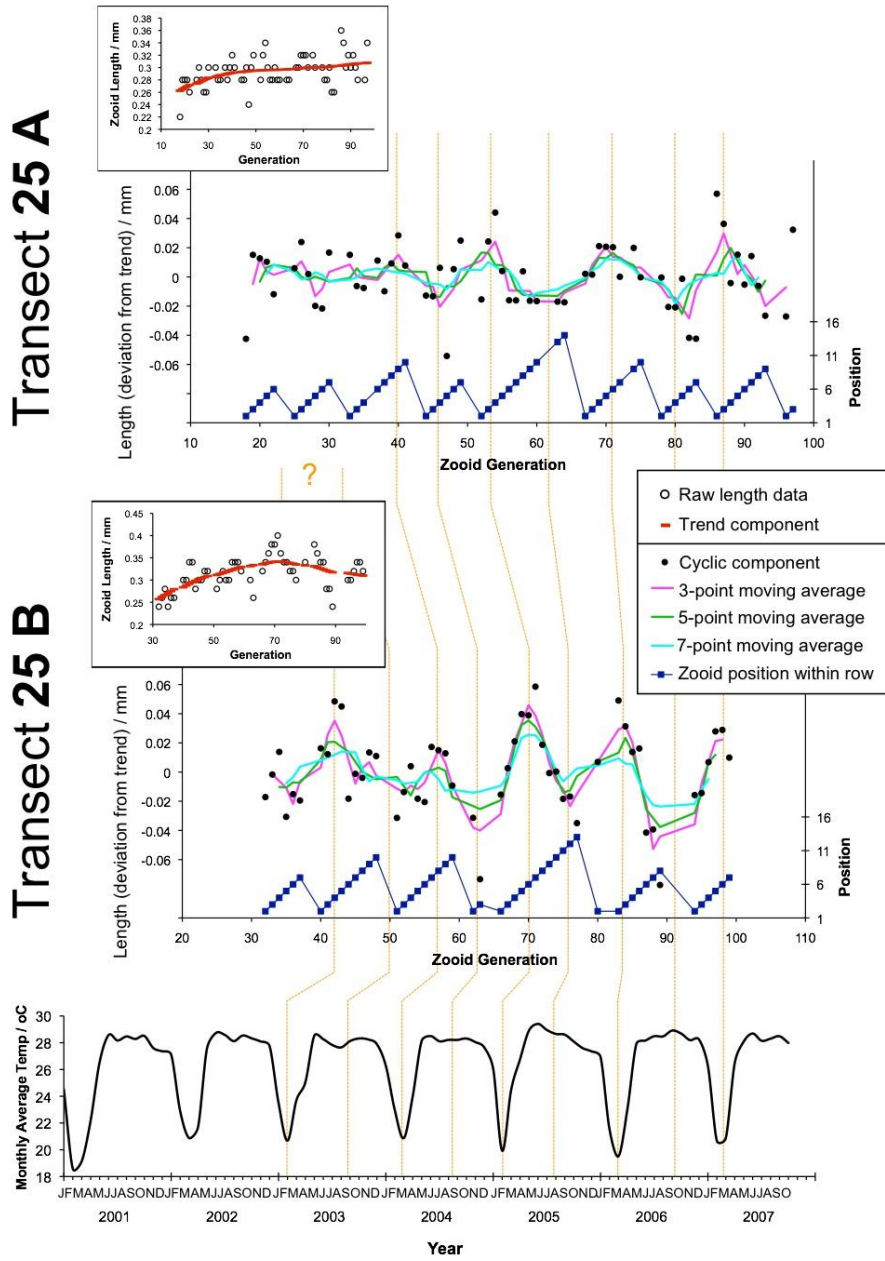


Figure 4

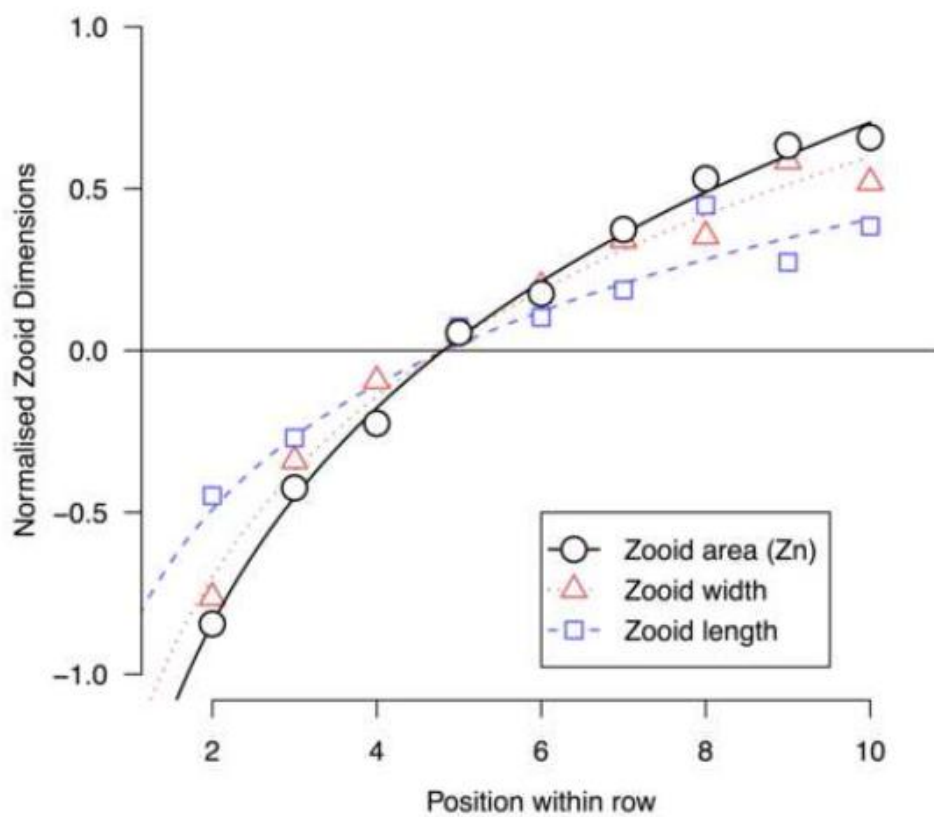


Figure 5