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Title: Nacre tablet thickness records formation temperature in modern and fossil shells

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Abstract: Nacre, the iridescent outer lining of pearls and inner lining of many mollusk shells, is composed of periodic, parallel, organic sheets alternating with aragonite (CaCO_3) tablet layers. Nacre tablet thickness (TT) generates both nacre's iridescence and its remarkable resistance to fracture. Despite extensive studies on how nacre forms, the mechanisms controlling TT remain unknown, even though they determine the most conspicuous of nacre's characteristics, visible even to the naked eye. Thermodynamics predicts that temperature (T) will affect both physical and chemical components of biomineralized skeletons. The chemical composition of biominerals is well-established to record environmental parameters, and has therefore been extensively used in paleoclimate studies. The physical structure, however, has been hypothesized but never directly demonstrated to depend on the environment. Here we observe that the physical TT in nacre from modern and fossil shallow-water shells of the bivalves *Pinna* and *Atrina* correlates with T as measured by the carbonate clumped isotope thermometer. Based on the observed TT vs. T correlation, we anticipate that TT will be used as a paleothermometer, useful to estimate paleotemperature in shallow-water paleo-environments. Here we successfully test the proposed new nacre TT thermometer on two Jurassic *Pinna* shells. The increase of TT with T is consistent with greater aragonite growth rate at higher T, and with greater metabolic rate at higher T. Thus, it reveals a complex, T-dependent biophysical mechanism for nacre formation.

Dear Editor Prof. Dr. Heather Stoll:

I thank you very much for your consideration of our manuscript, and agree with the changes suggested by you and the three reviewers. We have made all the changes described below in blue font. In black font is the entire letter from you, interspersed with our responses in blue font. In the manuscript itself the changes are highlighted in yellow. A separate document contains the response to reviewers, also in blue font.

Many thanks and best regards

Pupa Gilbert

Regarding Ms. Ref. No.: EPSL-D-16-00618

Title: Nacre tablet thickness records formation temperature in recent and fossil shells

Earth and Planetary Science Letters

Dear Pupa,

I now have three reviews on your paper. As you will see they overall are supportive of this interesting new dataset and method and support its eventual publication in EPSL. However, from my reading and the comments of Reviewer 3, I find that substantial revision of the text is needed to match the format of EPSL.

Overall, the maintext of the paper is very brief (2569 words) and the supplement very long; yet the maintext word limit for EPSL is 6500 words and up to 10 figures are allowed. Therefore, in order to be most accessible and of interest to a broad audience, I request that you work to make the main text as complete as possible, which will require moving some of the information you have in the supplement currently, into the maintext. It would also be beneficial to move some of the key figures from the supplement to the maintext.

We have made these changes, so the main text now contains 10 figures and is 6478 words long, excluding abstract, acknowledgements, figure captions, and references.

The objective of EPSL is to have the article be as complete and stand-alone as possible because the general reader should be able to get a complete picture from it without having to refer to the supplement. I provide some further guidance below and the reviews are attached. If you are prepared to undertake the work required, I would be pleased to reconsider your paper for publication. Please submit your revision before Nov 03, 2016.

Because there is significant rewriting needed, I consider this a major revision, but essentially no modifications are required in the scientific discussion or interpretations; simply a more complete and more clear explanation of the study in the main text. As you are rewriting look for the best ways to incorporate material from the supplement into the introduction, methods and results and discussion and ways to reorganize so the manuscript is most accessible. The methods section is way too short.

The methods section is now considerably longer. Pages 5-17.

I also think a bit more of the organization and setup of the study should go in the introduction; for example lines 160-167 are really justification of the comparison with clumped isotopes and would be good background for reader in the introduction, and that way the reader would understand why the methods are described for clumped isotopes.

Done, these are now lines 89-96, and are fully integrated into the introduction. Pages 4-5.

It seems like lines 150-157 should be in methods.

We kept it in the main text because it presents a considerable portion of the data. We re-phrased it so it does not read as methods as much. Lines 428-436, page 20.

Please read some recent EPSL papers to get a clearer idea of the format and try to use as much of the word limit as possible to describe this novel and interesting work to the Earth Science community.

Done

Please also evaluate which supplemental figures could be key in the main text;

Done. We now have 10 figures in the main text.

it seems that something showing the bivalve material (such as S5) could be useful, as well as others like S18 and S21 for example.

Done, these 2 figures are now included as Figures 10 and 7B, respectively.

In the introduction I think it is important to be specific about the findings in study of Olson and the new aspects explored in this work; I

find the phrase in line 69-70 very general. The Olson et al. study did not compare a single species at different temperatures, so any efforts to relate temperature to nacre properties were done across multiple species each of which came from a single temperature environment. Mention, this is the first study to compare, within a single genus, the potential environmental controls on tablet thickness.

Done. Lines 63-69, page 3.

I suggest the supplement be used exclusively to give greater details on data or analytical protocols than can be published in the maintext, things that would be of most interest to a specialist with specific interest in the article... a nonspecialist would rarely consult the supplement so it is not the right place to give justifications or general background. Yes, it is the right place for the details of each sample, the museum, etc.

Done. The supplement is now considerably shorter, and only contains details for specialists interested in reproducing the work.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point raised by the reviewers when you submit the revised manuscript.

To submit a revision, please go to <http://ees.elsevier.com/epsl/> and login as an Author.

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When submitting your revised paper, please be sure to include the following items:

Response to Reviewers (mandatory)

This should be a separate file labeled "Response to Reviewers" that carefully addresses, point-by-point, the issues raised in the comments appended below. You should also include a suitable rebuttal to any specific request for change that you have not made. Mention the page, paragraph, and line number of any revisions that are made.

Done

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Done

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Thanks for submitting your exciting study to EPSL.

Yours sincerely,

Heather Stoll, PhD
Editor
Earth and Planetary Science Letters

Response to Reviewers

Reviewers' comments are in black font below, our responses in blue font. Changes in the text are highlighted in yellow.

Reviewer #1:

The authors study the mean nacre tablet thickness and mean formation temperatures in pinnoid specimens from the Jurassic to the Recent, and find a positive correlation. This allows them to propose that nacre thickness can be used as a proxy for paleotemperatures. The study is interesting and technically competent. It is also well written and illustrated. The set of pinnoid specimens from different ages and the account of the preservation state is nice. Although the statistics indicates that the fitting is sound, my concern would have subsided if more data could have been integrated. But I cannot object seriously to the data.

Thank you.

But there is something I would like the authors to comment on. The first idea that comes to my mind after reading the manuscript is that nacre tablets act somehow as growth lines or increments, which are markedly influenced by temperature, as well as by other factors (as the authors also reasonably argue for the tablets). Tablets are, evidently, not growth lines, but this behaviour is very interesting because implies that within nacre, interlamellar membranes and tablets are secreted at different paces, and this is a basic datum for people interested in nacre growth. In view of this I wonder why authors have not checked tablet thickness against palaeotemperatures (from 16°/18° ratios).

This can certainly be done, and will be the subject of a future paper with extensive $\delta^{18}\text{O}$ data.

Second, taking into account the terraced growth of bivalve nacre, nacre and growth lines must be oblique, so that notably thick tablets produced during a particularly warm episode would be staggered in parallel to the growth lines. I would like to see comments on this in a revised version.

There are no data available on this point. Had the nacre transect data worked more reliably, and remained within the acceptable T range, we would have succeeded in doing so, but that is not what happens. See discussion of Px1-1 for example, which yields temperatures 2°-50°C, which are obviously too low and too high, respectively. We would very much like to say something along those lines (pun intended!), but we can't, based on the data. We have tried very hard to form a mechanistic

3D model of how and when each tablet in nacre is formed, but cannot say anything reliable at this time, we can only speculate, and therefore prefer to avoid the entire point and speculation.

Reviewer #2:

This is technically excellent paper - congratulations!

Thank you!

Some weakness is that the correlation between TT and T has not been validated in experimentally raised animals (the same applies to Olson et al. 2012). Tank experiments in which the same modern shells would grow in precisely monitored (and modified during the growth) conditions would dispel any doubts about possible local temperature fluctuations in sampling sites OR that differences in TT may result from genetic differences of the sampled animals (only few shells were examined).

Indeed the reviewer is right. Only tank experiments can completely validate the TT vs. T proxy. These must be done at controlled T, and with all other parameters kept constant, including salinity, pH, nutrients, etc. We have proposed these experiments to the US-Department of Energy, and, if funded, plan to do them for three years. In the meantime, we would like to publish the empirical observation that TT and T are correlated in our subject of study, the *Pinna* and *Atrina* genera in the family Pinnidae.

Another source of evidence that could be provided (but is not) include examination of modern pinnid shells from sites of known different temperature records.

We have done this in the present paper. Perhaps it was confusing to call them “recent” rather than “modern” shells, so we have replaced the word “recent” everywhere in the text, including the title, with the word “modern”, to make it clearer. See title [page 1](#), and most other pages where “modern” is highlighted in yellow.

For example, *Atrina fragilis* in Adriatic Sea typically occurs at 25-50 m depths, but occurrences at 250 m depths are also known (Šimunović et al. 2001: Acta Adriatica 42(1): 49-59), which most likely differ in temperature records.

This is a great idea, and we will do these experiments in the next paper, as this will probably require specific fieldwork to ensure the origin and depth of the specimens. Thank you very much for the excellent

suggestion!

Reviewer #3:

The ms presents new data (Synchrotron PIC and clumped oxygen isotope data, supplemented with EPMA) on Pinna shells in order to establish a link between nacre tablet thickness and seawater temperature as a potential paleo-proxy. The data are original and the approach seems sound. It is an interesting new potential proxy, because it is independent of the chemical composition of the fossil shells, which is ever so often compromised.

Thank you!

My main problem with the manuscript is probably more editorial. I find the way the ms is written and presented rather outside the range of ms submitted to journals in the Earth Science, which is probably not surprising given the background of the first author. The supplementary material (59 pages!) is excessive and makes the impression the actual ms is rather a summary of the supplementary material. I find this a rather worrying development in a number of journals these days that the major part of the evidence seems to be presented in the supplements available only online and separate from the manuscript.

In this ms, the supplementary material seems to be a mix of evidence (text, tables, figures) that are critical for the ms and should really be in the main ms plus evidence that is debatable whether it is necessary to be included at all, even in the supplementary material. EPMA data on aragonite and calcite in the shells is very much standard in the Earth Sciences, I don't see the benefit of including the raw data here (Table S3)

We have considerably shortened the SI, which is now 32 pages long, not 59. We moved many figures to the main text, which now has 10 figures instead of the previous 3, and the methods are much expanded. We also inserted several comments on the data and further explanations in the main text, which we believe add clarity. We removed the raw data of Table 3, which, as the reviewer said, were unnecessary. The new Table S3 is included in the SI, page 32.

similarly, showing the embedded and cut shells does not really contribute at all in my opinion (Fig. S9).

We included these because most people are unfamiliar with the PEEM experiment, and in fact consider PEEM to be a method usable only for conducting samples. This is obviously not the case, and an image convinces the reader more than a 1000 words. Also, in Figure S6 (previously S9) we show that all samples were extracted from the same location in the shell, which was key for this study.

Furthermore, ideally the ms would provide an application of the new thermometer to a fossil site. While I can spot a little bit of this here and there in the ms, I feel this must be done much more prominently to be convincing.

This is another good suggestion, and it will be done as a stand-alone paper by us or by somebody else. Here the use of the nacre TT thermometer on unknown fossils was tested on two Jurassic Pinna shells, and provided reasonable results. We have better explained this now in the abstract and the conclusions. See [lines 41-42, page 2](#), and [lines 534-535, page 25](#).

Including the critical evidence in the ms would strengthen the points the authors want to make (see below). Removing the superfluous supplementary material would focus the ms more. Both are desirable and beneficial.

Thanks, we did this, as described above and below.

As a general note, I feel that in the main ms, the writing style should be edited, some repetitions and jumpiness in structure could be removed.

The paper has been thoroughly edited and we hope that the writing style has been improved.

Abstract

Line 35: How can the wavelength of visible light have an effect on fracture resistance in nacre?

It obviously does not. It is the nacre table thickness that generates both its iridescence and its toughness. We clarified this in [line 28, page 2](#).

Introduction

Line 57: "...and geochemical proxies document climatic variation through the same interval (Zachos et al., 2001)." Add "in these biomineral" after "geochemical proxies"

We added "in various biominerals", because it is not from the same biominerals that typical proxies come from, and that is precisely the point of that sentence: skeletal fossils and foraminiferans come from different environments. [Line 51, page 3](#).

Line 58-63: Why is this relevant? Also, it clearly depends on the scientific questions whether open ocean proxies or continental shelf proxies are important. Shelf proxy archives would probably record more regional and

variable environmental conditions. Why do these need to be reconciled with open ocean proxies at all?

The reviewer is right, temperatures on continental shelves and open ocean are different, and they cannot be reconciled. We did not intend to give the impression that they need to be reconciled. We rephrased so that it is clear that we offer a new proxy for a different environment. **Line 59, page 3.**

Line 69: Cut this sentence, not necessary.

We moved it later so the text is better organized and modified it to: “We selected pinnid bivalves for several reasons.” We did not remove it completely, because we do need to explain why pinnids and the introduction seems the logical place to do so. **Line 70, page 4.**

Line 84: This is a repetition from lines 66ff.

The repetition was eliminated. Line 91 remained the same, but the paragraph at the end of page 3 was changed in response to the Editor’s request. **Line 63-69, page 3.**

Line 97: embedded in what? Why embedded and embedded again?

We clarified the epoxy used, EpoFix **lines 103-104, page 5.** As shown in Figure S6, we embed to protect the flaky fossil shells, then cut out the desired location, then re-embed to have the desired surface of the shell transect at the center of a flat surface, which is necessary for PEEM analysis. We now explain this in **lines 104-105, page 5,** and also in the **caption of Figure S6, SI page 22.**

Line 107: I strongly feel there should be more information in the main ms on the clumped isotope method. Sample size for clumped isotope measurements must be much larger than for the microscopic methods used.

We placed all the clumped isotope methods in main text. See **lines 247-296, pages 11-13.**

Results

Line 117: The information of HOW the lack of diagenesis in these shells is identified using PIC and XAS are only shortly described in the caption to Figure 1, but not in the text. The text needs to state not only that the fossil and recent shells are identical but also how diagenesis would change this.

We have now described this in the text, and most importantly we added Figure 2 to show the effect of diagenesis in the data. Lines 373-403, Pages 18-19 describe diagenesis in detail and explain the observations in Figures 1 and 2.

I am also a bit concerned that PIC only really records a very small area of the shell. XAS can be much more of a bulk method (although I don't think this is the way it is used here), but it must be made much clearer what was done, on which scale

Indeed the reviewer is right that with PIC-mapping we analyze a small region of the shell. We have now clarified that this is a continuous transect of partly overlapping images in each shell, acquired in the same location in all shells: where nacre is thickest, that is, 2 mm posterior to the anterior adductor muscle scar. This is now shown clearly in Figures 4, 5, and 6, and also by the red arrows in Figures 4, S4, S7. It is also explained in the text, lines 428-432, page 20.

and what the evidence for the lack of diagenesis is.

We have clarified in the text that lack of diagenesis is observed as aragonite that is still aragonite and calcite that is still calcite. This was detected in Figure 1 by XANES spectroscopy and in Figure S2 by diffraction. Further evidence is provided by EPMA in Figure S3 and Table S3. Lines 374-403, Pages 18-19. Specifically see “turquoise” in PIC-mapping definition in line 390, and later mentions in lines 392, 420, 742 and EPMA data in Figure S3 and Table S3.

I see from the supplementary material that many overlapping (?) PIC maps were taken per sample, but this is not clear from reading the main ms.

We are now describing in the text that transects were acquired across all shells, always in the same region, which is where nacre is thickest in each shell. We also moved the Figure with all transects into the main text, so readers can't miss it. See new Figures 4, 5, 6 and lines 428-432, page 20.

Line 126: 'still'? meaning what exactly?

They “remain” calcite and aragonite, as now clarified in line 395, page 18.

Line 127: In what way can EPMA (abbreviation not explained in the text, by the way) show excellent preservation? Please present the evidence and describe it here.

We defined the EPMA acronym in [line 396, page 19](#), and clarified that the elemental concentrations measured by EPMA are typical of aragonite and calcite, respectively. [Lines 396-397, page 19](#). In [lines 398-403](#) we provide a summary of these data.

Line 132 ff: This paragraph states that diagenesis was indeed observed (although limited) in many of the samples. I would much appreciate if this was stated more towards the beginning of the chapter on assessment of diagenesis, because it is the honest thing to start with.

We thank the reviewer for this idea. We moved the description of diagenesis to the beginning of the manuscript, and show all the regions that undergo diagenesis in [Figure 2. Lines 374-414, Pages 18-19](#) describe diagenesis in detail and explain the observations in Figures 1 and 2.

Nobody expects fossils to be without any diagenetic change and it is an excellent and sound scientific approach to test the samples for diagenesis, as it is done here.

Thank you!

However, the way it is currently written doesn't give this approach enough credit, but looks rather defensive.

See above.

Secondly: Sample size for clumped isotope measurements must be much larger than for the microscopic methods used. How was diagenesis (and potential contamination from the epoxy resin on that matter) monitored for the large samples used for clumped isotopes?

The detailed clumped isotope methods are now described in the main text. [Lines 247-296, pages 11-13](#). The specific manual separation is described in lines [257-261, page 12](#).

Line 186: Why was this random-sampling method used to arrive at the best fit? Please explain.

Other fit methods do not take into account the experimental error bars, this bootstrapping method does. Additionally, this method provides error bars to the measurement of unknown T, as done here for the Jurassic shells. We now explain this in the text, and include both the conventional fit and the new statistical fit as 2 panels of [Figure 7](#), and describe their differences in the text. [Line 298-360, pages 13-16](#).

Line 191: 'somehow'? I suggest cutting this word

We cut it from “nacre somehow preserves...” which reads now “nacre preserves....”, see [line 479, page 22](#).

Fig. S20: Grey points are very hard to see

We re-plotted the figure with darker and larger grey data points, so they are more visible. Many thanks for yet another great suggestion! See new [Figure S12, SI page 28](#).

Dear Editor Heather Stoll:

A long-standing need in earth sciences is a proxy for ancient temperature that is less sensitive to diagenesis, and therefore more reliable than the current chemical proxies. We solve this problem by presenting a proxy that is unlike all others: physical rather than chemical in nature.

These data demonstrate unambiguously that there is a strong correlation between nacre tablet thickness (TT) and seawater temperature (T) in ancient and modern bivalve shells. This is unprecedented in the scientific literature, but is consistent with the anecdotal evidence that Japanese pearl farmers harvest in winter because pearls have greater luster when TT is smaller. The TT vs. T correlation can be used to measure T in geologic epochs, thus *using a shell as a thermometer*. Here we use the TT thermometer on two Jurassic shells, nearly 200 Ma, where much of the shell has undergone diagenesis, thus making it impossible for any chemical analysis method to estimate the formation T. Our T estimates from TT in these Jurassic shells are realistic.

Why is this important?

In our world of changing climate it is particularly valuable to reconstruct the climates of the past and observe their biological effects on marine ecosystems. Nacre TT has the potential to be a popular proxy because it is easily accessible: after the validation work included here, which took 4 years of hard work and many collaborators, now *anybody with an SEM can measure TT, and thus reconstruct T in deep time*. This paper is the first describing the nacre TT thermometer, it may thus be widely used and cited.

Since nacre is so beautiful, we are happy to provide an attractive image showing nacre's luster and structural colors for the graphical abstract.

We look forward to your thoughts on this exciting manuscript, and remain at your disposal for any changes.

Sincerely,

Pupa Gilbert and Andrew H. Knoll

Nacre tablet thickness records formation temperature in recent and fossil shells

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Stasio

Abstract

Nacre, the iridescent outer lining of pearls and inner lining of many mollusk shells, is composed of periodic, parallel, organic sheets alternating with aragonite (CaCO_3) tablet layers. Nacre tablet thickness (TT) is comparable to the wavelength of visible light, thus generating nacre's iridescence and remarkable resistance to fracture. Despite extensive studies on how nacre forms, the mechanisms controlling TT remain unknown, even though they determine the most conspicuous of nacre's characteristics, visible even to the naked eye.

Thermodynamics predicts that temperature (T) will affect both physical and chemical components of biomineralized skeletons. The *chemical* composition of biominerals is well-established to record environmental parameters, and has therefore been extensively used in paleoclimate studies. The *physical* structure, however, has been hypothesized but never directly demonstrated to depend on the environment. Here we observe that the physical TT in nacre from recent and fossil shallow-water shells of the bivalves *Pinna* and *Atrina* correlates with T as measured by the carbonate clumped isotope thermometer. Based on the observed TT vs. T correlation, we anticipate that TT will be used as a paleothermometer, useful to measure T in shallow-water paleo-environments. The increase of TT with T is consistent with greater aragonite growth rate at higher T, and with greater metabolic rate at higher T. Thus, it reveals a complex, T-dependent biophysical mechanism for nacre formation.

Keywords

Paleo-climate, Proxy, Biomineral, mollusk, PEEM, PIC-mapping.

1. Introduction

Calcareous skeletons record animal diversity in marine environments through Phanerozoic time (<541 Ma) (Alroy et al., 2008; Sepkoski Jr, 1981), and geochemical proxies document climatic variation through the same interval (Zachos et al., 2001). The integration of these paleobiological and paleoclimatic records, however, can be challenging because the skeletal fossils that document animal evolution (Jablonski and Shubin, 2015) originate mostly from shallow continental shelf and platform environments. In contrast, the most widely used proxies of marine paleoclimate originate from deep sea sediments, especially for the record of the last 100 million years when modern ecosystems were shaped (Kim et al., 2008; Zachos et al., 2001). Here we report a promising new paleotemperature proxy stored in the nacre of bivalve mollusks in the family Pinnidae, which have lived in shallow marine environments for more than 400 million years. We evaluate this proxy by comparing the physical structure of nacre (Cartwright and Checa, 2007; England et al., 2007) through geologic time with a geochemical proxy for temperature: carbonate clumped isotope thermometry (Eiler, 2007).

We focus on pinnid bivalves for several reasons. Previous work indicated that bivalves hold promise as temperature proxies (Olson et al., 2012). More specifically, members of the Pinnidae, or pen shells, including the genera *Pinna* and *Atrina* (Lemer et al., 2014), are fast-growing sessile suspension-feeders, widespread in tropical to temperate, coastal, shallow shelf environments, both today and in the past (Aucoin and Himmelman, 2011; Grave, 1911; Richardson et al., 2004; Schultz and Huber, 2013; Turner and Rosewater, 1958). The pinnid fossil record goes back to the Silurian Period (~444 Ma) (Enzhi et al., 1986), and both *Pinna* and *Atrina* have been reported from Mississippian rocks

(359-323 Ma)([Wheelton, 1905](#)) ([Figure S1](#)). Their shell morphology is unique and easily identified, even from fragments. Like many other marine bivalves, pen shells combine an outer layer of prismatic calcite (CaCO_3) with an inner nacre layer. Uniquely in Pinnidae, prismatic calcite and nacre aragonite layers easily separate as the shells dry, providing two distinct carbonate minerals to analyze using carbonate clumped isotope thermometry. When present in the fossil record, shells tend to be well-preserved although the prismatic and nacre layers rarely remain attached.

In this study we quantify the physical parameters of nacre ultrastructure, including nacre tablet thickness (TT), and compare them to independent estimates of temperature based on carbonate clumped isotope analysis for both recent and ancient shells of the sister taxa *Pinna* and *Atrina*. Three recent shell samples are included, from a range of latitudes and seasonal temperatures (Florida, USA; Mallorca, Spain; Bocas del Toro, Panama). The carbonate clumped isotope temperatures, when compared to nearby buoy sea surface temperatures, are in good agreement, increasing our confidence in clumped isotope paleotemperature estimates from fossil shells. Nine fossil specimens were analyzed, drawn from different geologic times, places, and temperatures.

2. Materials and Methods

Detailed samples and methods are described in the online Supporting Information. Briefly, we obtained fossil shells from various museums, and recent shells from marine biology laboratories. The shells were embedded, cut, embedded again, polished to expose cross-sections and TT, and coated as described previously ([De Stasio et al., 2003](#); [Gilbert, 2014](#)). Images were acquired using the PhotoElectron Emission Microscope (PEEM) at the

Advanced Light Source. All images were acquired at 534 eV photon energy, the oxygen K-edge π^* peak (DeVol et al., 2014), with linear polarization. Stacks of 19 images were acquired at different linear polarization angles, from horizontal to vertical in 5° intervals, and used to produce PIC-maps (DeVol et al., 2014; Gilbert et al., 2011; Metzler et al., 2007; Metzler et al., 2008), using Igor Pro Carbon® and software developed for this purpose (GG-Macros, 2016).

PIC-maps were then analyzed in Adobe Photoshop® to measure nacre TT. Temperatures were measured using the clumped isotope thermometer (Eiler, 2007), then TT and T were plotted on the same graph using KaleidaGraph 4.5®.

3. Results and Discussion

3.1. Tests for Diagenetic Alteration

If nacre is to provide a reliable indication of ancient shallow water temperatures, we must first demonstrate that it can be preserved intact over long time intervals. Figure 1 shows results from PhotoElectron Emission spectromicroscopy (PEEM) for both Polarization-dependent Imaging Contrast (PIC)-mapping (DeVol et al., 2014; Gilbert et al., 2011; Metzler et al., 2007; Metzler et al., 2008) and x-ray absorption spectra at the oxygen K-edge (DeVol et al., 2014), for Recent, Cretaceous, and Jurassic shells. The Recent and Cretaceous specimens are essentially identical, documenting mineralogical and crystallographic preservation on nearly 70 million year timescales, consistent with previous results on the organic and mineral components (Weiner et al., 1976). The Jurassic specimen shows clear signs of diagenesis, with only few tablets preserved. For the modern and Cretaceous shells, x-ray absorption spectra (Figure 1D) extracted from microscopic

areas of aragonite and calcite are identical to those from their geologic and synthetic counterparts, exhibiting 6 and 4 peaks, respectively (DeVol et al., 2014). X-ray microdiffraction of both Cretaceous shells confirms that nacre and prismatic layers are still aragonite and calcite, respectively, as shown in Figure S2. EPMA measurements confirm excellent preservation of one of the Cretaceous shells (Figure S3, Table S3).

Thus, both crystallographic and chemical analyses confirm excellent preservation of Recent-Cretaceous shells and lack of diagenetic alteration of crystal structure in these and most other areas of the fossil shells. Diagenetic change was observed sporadically in the PIC-maps of Eocene and Cretaceous specimens, predominantly near cracks where water presumably penetrated, dissolved and re-precipitated aragonite crystals with greater-than-normal angle spreads in c-axis orientation; this is shown in Figure S4. Major but still incomplete diagenesis was observed in the Jurassic specimens as shown in Figures 1C and 2F, where calcite was re-precipitated.

3.2. Nacre Tablet Thickness in Recent and Ancient Shells

Figure 2 shows PIC-maps of shells from each of the five geologic epochs in this study: Recent, Miocene, Eocene, Late Cretaceous, and Early Jurassic. In PIC-maps, color (hue and brightness) illustrates crystal orientation in each 20nm pixel (DeVol et al., 2014). Thus, Figure 2 shows that each aragonite tablet behaves as a single crystal and is similar, though not identical, in orientation to other tablets (within $\pm 15^\circ$). This is consistent with previous observations in modern nacre (England et al., 2007; Olson et al., 2013). Tablet layers are interspersed with 30nm-thick organic sheets (Levi-Kalisman et al., 2001), visible as black lines in Figure 2A,B but not always preserved in fossils (Figure 2C,D). The

orientation-color contrast, therefore, enables identification of individual tablets even where organics are absent, e.g., in the blue tablets below the word “Eocene” in Figure 2C. This enables the observation and measurement of TT in samples of variable preservation and age.

We analyzed three recent (Figures S5-S7) and nine fossil pen shells (Figures S8-S11) with a continuous series of PIC-maps (complete data set in Figure S12), in the same location in the shell, that is, the thickest nacre region magnified in Figure S6 and indicated by red arrows in Figures S5, S7, S10. Average TT was measured by dividing the vertical field of view of each image by the number of tablet layers present, counted digitally, as shown in Figure S13 and described in detail in the SM Methods. In Figure S14 we present the results of all TTs in each shell cross-section, which shows the variability of TT through time as nacre is deposited.

3.3. Clumped Isotope Temperature Estimates

To quantitatively explore the relationship between temperature and nacre biomineralization, we utilized carbonate clumped isotope thermometry to compare independent estimates of paleotemperature with nacre TT for all shells, except for the diagenetically altered Jurassic specimens (Eiler, 2007). This thermodynamically-controlled, temperature-dependent geochemical method measures the preferential association of multiple heavy isotopes within a single carbonate ion (Δ_{47}). Unlike the $\delta^{18}\text{O}$ paleothermometer, the carbonate clumped isotope thermometer is independent of the isotopic composition of seawater.

First, we validated the clumped isotope thermometer by comparing its results in three recent shells collected from locations with known water temperature. As shown in [Figures S15-S17](#), the agreement is good. Given the likelihood that the transect near the umbo in two of these shells was formed over less than a year ([Aucoin and Himmelman, 2011](#); [Butler and Brewster, 1979](#); [Richardson et al., 1999](#)) deviations from the mean annual temperature are not surprising. Then, we completed carbonate clumped isotope analyses on Miocene, Eocene and Cretaceous specimens ([Table S2](#)). The Jurassic specimens were not analyzed, as extensive diagenesis made bulk analysis meaningless. Reconstructed paleotemperatures for the fossil shells are consistent with T estimates from other proxies ([Rosales et al., 2004](#); [Zachos et al., 2001](#)).

3.4. Nacre Tablet Thickness Quantitatively Reflects Temperature

[Figure 3](#) shows the observed strong correlation of TT with formation T. In each of the pen shells, Recent-Cretaceous, mean T from clumped isotopes was measured along with average TT in each PIC-map across the entire thickness of nacre. All measurements and all error bars, from each specimen were used (630 PIC-maps total) to examine the relationship between TT and T. The most probable linear fit between TT and T, and its confidence level (CL) intervals were determined using one randomly selected data point per shell, randomly positioned within its error bars, then linear-fitting the 9 points, and repeating 100,000 times ([Figure S20](#)). (Neglecting all error bars and averaging all data, as is commonly done but less accurate, gives $R = 0.98$ ($R^2 = 0.96$), as shown in [Figure S21](#).) The observation of such a strong correlation between mean TT and mean T, despite fluctuations in each shell (some strong, as in Px1, discussed below) ([Figure S14](#)), indicates

that nacre somehow preserves a physical record of carbonate formation temperature. As noted, each shell displays a range of TT values depending on the location within the shell where the measurement was taken, and may reflect the contribution of other underlying physical factors, but the average value of the TT distribution clearly shows a shift to higher value with temperature (Figs. 3 and S21). For each shell, we have therefore taken TT measurements in complete transects to account for the intrinsic fluctuations in TT.

This empirical observation may be due to several factors, including metabolic rate, which increases with T (Gillooly et al., 2001). Greater metabolic rate increases all biological activities, including synthesis and transport of nacre precursor phases (DeVol et al., 2015), polysaccharides, and proteins to forming nacre. In addition, a simple experiment in the absence of any organics confirms that the kinetics of crystal growth may also affect TT. We measured abiotic aragonite growth rates at different temperatures over a standard time range. The results confirm a linear, positive correlation of particle size with T, with a correlation coefficient $R = 0.97$ ($R^2 = 0.94$) (Figure S18). Importantly, the correlation is positive in both Figures 3 and S18; thus, crystal growth rate and metabolic rate may both contribute to TT, and both are sensitive to T in the same direction: they increase with T.

Within a shell, we sometimes see large fluctuations in the local TT as a function of position. For instance, using the TT vs. T equation to measure temperature vs. position for shell Px1-1 in Figure 3 and Figure S14, the minimum and maximum T obtained are unrealistically low and high, respectively, at 2°C and 50°C. These fluctuations may be due to factors other than T that affect TT: possibly salinity, pH, or nutrients. The extreme end-member TTs, however, are averaged out in the all-inclusive analysis, which is precisely what is observed in the average linear fit of Figure 3 or the linear fit of the averages in

Figure S21. This suggests that despite the influences of other factors, temperature has an overarching role in determining nacre tablet thickness. A similar behavior is observed in foraminifera and T estimates from $\delta^{18}\text{O}$, which exhibit out of range values locally, that are averaged out across the entire shell (Kozdon et al., 2009; Rollion-Bard et al., 2008).

Finally, we used the TT vs. T relationship observed here to estimate the T of formation for Jurassic *Pinna* shells, measuring TT in the few well-preserved tablets, shown as horizontal black lines in **Figure 3**. Two transects from the Pfo1 shell provide very similar most-probable temperatures, $T=23^{\circ}\text{C}$ and 21°C respectively, whereas for the Ps8 shell we obtained $T=14^{\circ}\text{C}$. 50% confidence levels (CL) are: $21\text{-}27^{\circ}\text{C}$ and $19\text{-}24^{\circ}\text{C}$ for Pfo1, $12\text{-}16^{\circ}\text{C}$ for Ps8; 90% CL are: $18\text{-}34^{\circ}\text{C}$ and $16\text{-}31^{\circ}\text{C}$ for Pfo1, $8\text{-}20^{\circ}\text{C}$ for Ps8. All three temperatures are consistent with expected T values for the Early Jurassic (Rosales et al., 2004). From these estimates, the shell transects (away from the umbo) are likely to have formed in a Jurassic summer and winter, respectively. Notably, diagenesis had made these Jurassic shells inaccessible to most chemical proxies, but left them within reach for the nacre TT thermometer.

4. Conclusions

The physical structure of biominerals has seldomly been related to environmental parameters. Olson et al. hypothesized that nacre ultrastructure (Olson et al., 2012) may depend on environmental temperature at the time of biomineral deposition. Other authors confirmed that other physical parameters in bivalves (Milano et al., 2015; Schöne et al., 2002) and in foraminifera (Be, 1968; Frerichs et al., 1972) correlate with temperature. Here we demonstrated that pen shells record and preserve a quantitative indication of

average temperature in the thickness of nacre tablets formed during shell growth. TT was measured in PIC-maps here, however, SEM images can provide quick and easy access to the required TT data. All paleoenvironmental proxies have their strengths and weaknesses, but temperature estimates from nacre TT are more forgiving than many, simply requiring good preservation of a few aragonite tablets even when other regions of the shell experienced dissolution, recrystallization, or replacement by calcite. The strength of the new TT proxy, thus, complements the strengths and compensates for the weaknesses of existing geochemical methods, especially in shallow marine environments that preserve most of the invertebrate fossil record. Continuing research on other fossil taxa will enable us to know to what extent the relationship shown in [Figure 3](#) is taxon-specific, and may disentangle the effects of metabolism and crystal growth rate on nacre TT. Finally, the observed T-dependence reveals that the formation of nacre's TT, iridescence, and fracture toughness ([Barthelat et al., 2016](#)) is exquisitely bio-physical in origin, combining diverse T-dependent parameters, such as bio-metabolic rate and physical crystal growth rate, that can be harnessed for biomimetic nacre-like materials synthesis ([Finnemore et al., 2012](#); [Tang et al., 2003](#); [Wegst et al., 2015](#)).

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Figure 1. PIC-maps of three *Pinna* shells in cross-section. One recent (A: Pn1) and one from the Late Cretaceous (B: Ps6) show the excellent preservation of prismatic calcite layer at the bottom (c1-4) and aragonite nacre (a1-4) at the top, with dark organic sheets between prisms (o) and between prisms and nacre. (C: Ps8) A poorly preserved Jurassic *Pinna* shell: dissolved and re-crystallized regions show mis-oriented calcite (c5-6) interspersed with intact aragonite nacre (a5-6), and holes filled with epoxy (e). Colors identify different c-axis crystal orientations (color legend at bottom right). (D) Oxygen spectra extracted from $\sim 2\mu\text{m}$ squares correspondingly labeled and colored regions in all PIC-maps. Aragonite and calcite have 6 and 4 peaks, respectively (vertical lines). Peak amplitudes differ for differently oriented crystals, generating the colors in PIC-maps.

Figure 2. Nacre PIC-maps from the five geologic epochs in this study. Colors identify different c-axis crystal orientations (see color legend) and differentiate individual nacre tablets, even in the presence of surface scratches (A) or missing fragments of nacre tablets (black in C,D,E,F). Despite the disruption in structure in E, the crystal orientations are intact with a nearly vertical c-axis (cyan and neighboring colors, thus $\pm 15^\circ$). Even in F some nacre tablets show no diagenesis, even in immediate contact with holes (black), or diagenetic, recrystallized calcite (purple).

Figure 3. Nacre tablet thickness (TT) of recent and fossil shells is correlated with formation temperature (T). All TTs in each shell cross-section are measured microscopically from each of 630 PIC-maps, as shown in **Figures S12-S14**. T was independently measured with carbonate clumped isotope analysis from a shell fragment adjacent to the PIC-mapped area. The blue “line” is the most probable estimate fit, $TT (\mu m) = 0.2830 + 0.0144 T (^{\circ}C)$, obtained from 100,000 linear fits, each including all errors. The darker and lighter blue shaded areas indicate the 50% and 90% confidence level (CL) intervals, respectively. The minimum and maximum error bars are shown in magenta on top left for clarity. These are St Dev for TT and St Error of the Mean for T.