MECHANICAL PROPERTIES OF THE CHITIN-CALCIUM-PHOSPHATE "CLAM SHRIMP" CARAPACE (BRANCHIOPODA: SPINICAUDA): IMPLICATIONS FOR TAPHONOMY AND FOSSILIZATION

Timothy I. Astrop, Vasav Sahni, Todd A. Blackledge, and Alyssa Y. Stark

1 Department of Biology and Biochemistry, The University of Bath, Claverton Down, Bath, Somerset, UK
2 Department of Polymer Science, The University of Akron, Akron, OH, USA
3 Department of Biology, Integrated Bioscience Program, The University of Akron, Akron, OH, USA

ABSTRACT

Spinicaudata (colloquially ‘the clam shrimp’) are freshwater branchiopod crustaceans that occur worldwide in lakes and temporary pools. The spinicaudatan carapace is unique among arthropods in that it is subject to only partial molting. During ecdysis (molting), the outer surface of the carapace is not shed, resulting in the retention of the ontogenetic record of an individual through distinct growth-rings representing each molt. When this unusual feature is considered alongside the interesting chemical properties of the carapace, “clam shrimp” present an interesting biological material not seen anywhere else: a multi-laminar calcium-phosphate-chitin composite. In addition, the carapace survives numerous destructive taphonomic processes (including transport, decay, compaction, and desiccation) to become the dominant body component of Spinicaudata preserved in their 380 million year fossil record. Understanding the mechanical properties and chemical composition of this structure may not only aid in a better understanding of the evolutionary history of this group but also facilitate efforts to develop novel materials that retain functional material properties even in harsh aquatic conditions. Therefore, this study aims to provide quantitative information about the composition and mechanics of this unique and interesting biological material and help predict possible biases in the fossilization of different species of Spinicaudata to aid future palaeontological research.

KEY WORDS: arthropod cuticle, Branchiopoda, fracture mechanics, palaeobiology, taphonomy

INTRODUCTION

Arthropoda are without doubt the most diverse, successful, and adaptable animals on earth (Giribet and Edgecombe, 2013). Originating in the Cambrian some 520 million years ago (mya) (Legg et al., 2012), arthropods became essential components of global ecosystems and are now ubiquitous in aquatic, aerial, and terrestrial environments. The quality of the fossil record for Arthropoda ranges from exquisitely preserved amber encased specimens (Barden and Grimaldi, 2013), to isolated partial elements of the exoskeleton. Factors influencing the entrance of arthropodan remains into the fossil record are of key importance to correctly interpreting the evolutionary history of the group. Understanding the biotic and abiotic parameters that allow fossilization is the primary role of the science of taphonomy, and palaeobiologists have long experimented with organismal remains to understand how processes such as decay, transport, predation, and desiccation may ultimately shape the preservational record of life on earth via the process of fossilization (Allison, 1986; Briggs, 1995; Behrensmeyer et al., 2000; Krause et al., 2011).

The cuticle of the arthropods is a key adaptation that provides both structural support and a barrier between an organism’s internal biology and the harsh surrounding environment (Moussian, 2013). This cuticle is comprised of several layers all containing the polysaccharide chitin; a thin outer epicuticle, a mineralized exocuticle, and a less rigid endocuticle (Gupta, 2011); it is the most commonly represented component of arthropods in the fossil record. Chitin is hardened via sclerotization in many arthropods, such as insects, but chitin is also commonly found as a composite material, as in the case with most crustaceans. In many Crustacea, chitin is combined with calcium carbonate into laminae in a unique helical pattern (Bouligand structure, see Cheng et al., 2008) that provides great rigidity for protection.

The ‘clam shrimp,’ Order Spinicaudata, are seemingly inconspicuous crustaceans that inhabit temporary freshwater habitats world-wide and are easily recognized as adults due to their chitinous bivalved carapace, which completely encapsulates them (Fig. 1). This carapace is unique among invertebrates in that molts are partially retained during ecdysis. The outer surface of the carapace is retained after each molt causing distinctive growth lines that record the organism’s ontogeny (Rieder et al., 1984). This results in the carapace forming a ‘layered’ structure, superficially similar to molluscan bivalves, in contrast to the complete discarding of exuviae seen in more familiar arthropods such as crabs, lobsters, and other members of Ecdysozoa.

* Corresponding author; e-mail: tia20@bath.ac.uk

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Additionally, the chemical composition of spinicaudatan carapace itself is unique in that, rather than forming a chitin-calcium complex, the carapace of Spinicaudata is believed to be primarily chitin-phosphate, possibly a product of their freshwater environment that is calcium deficient (Stigall and Hartmann, 2008). Whether there are differences in the mode and level of mineralization among spinicaudatan families is unknown but could lead to differential preservation bias in the spinicaudatan fossil record (Fig. 2).

The strong arthropod cuticle plays a significant role in determining their preservation potential (Edgecombe and Legg, 2013) so that they have an excellent fossil record, extending back to the Devonian (380 mya). The natural ecology confines Spinicaudata to freshwater, making them useful as an indicator of historical freshwater environments. In addition, the accretional carapace growth of spinicaudatan also records information about environmental stresses, fluctuations and seasonality in successive growth ‘rings.’ Several studies explored the possibility of carapace morphology reflecting sexual dimorphism within fossil Spinicaudata populations, in turn allowing the diagnoses of particular sexual systems in geologic time (Astrop et al., 2012; Gallego et al., 2013; Monferran et al., 2013; Stigall et al., 2013). Understanding taphonomic influences of preservation within different lineages of Spinicaudata is of great importance to understanding the reliability of data used for such studies.

The mineralized carapace of Spinicaudata is the main constituent of their excellent fossil record, but the lack of chemical and mechanical characterization of this unique material results in tentative use of this arthropod in palaeobiological studies. We hypothesize that family-level differences in the composition and mechanical properties of the spinicaudatan carapace affect susceptibility to taphonomic processes. In this study, we utilize material testing method to investigate the strength of the carapace of representatives of spinicaudatan families as a proxy for taphonomic susceptibility. Such a preservational bias, should it exist, could have a great numerical effect on the abundance of different taxa and would need to be considered when utilizing the fossil record of this order.

Fig. 1. Taxa investigated in this study. A, Leptestheria compleximanus; B, Eulimnadia feriensis; C, Cyzicus gynecia. Note the accretional growth rings. Scale bar is 2 mm.

Fig. 2. Generic diversity of Spinicaudata over geologic time based on multiple literature sources (primarily Tasch, 1969; Zhang et al., 1976). Note the periodic disappearance of Limnadiidae from the record, the persistence of Cyzicidae, and the recent occurrence of Leptestheriidae. Extinct genera are included for reference.
MATERIALS AND METHODS

Sample Preparation

Three species of Spinicaudata were used for compositional assessment and materials testing, these three species were chosen to represent the three extant families within the spinicaudata, *Cycicus gynecia* (Mattox, 1950), *Leptestheria compleximanus* (Packard, 1877), *Eulimnadia feriensis* Dakin, 1914 (Fig. 1). All populations were reared in the laboratory following the protocol outlined in (Weeks and Zucker, 1999). To control for differences in age, and thus number of molts or layers of cuticle, we only used individuals with the same hatch date. Live samples were refrigerated for sedation and subsequently removed from their carapaces. Values were stored at −10°C for no longer than 3 days while trials where in progress. Samples used for elemental assessment using energy dispersive X-ray spectroscopy (EDAX) were immediately removed from their carapaces and quickly dried via absorption before embedding in epoxy.

Elemental Composition

EDAX was performed using a Princeton Gamma Tech (Princeton, NJ, USA) energy dispersive X-ray spectrometry (EDS) system within an FEI XL-30 environmental scanning electron microscope with a Ge detector. Uncoated samples were embedded in epoxy resin on a glass slide before absorption before embedding in epoxy. Quantitative information was obtained using ZAF Quantification (Standardless) via the EDAX Genesis software package V4.52.

Punch Test

Biological materials testing is generally challenging due to their anisotropic and composite nature and because most biomaterials are viscoelastic (Strait and Vincent, 1998; Aranwela et al., 1999; Edwards et al., 2000). We measured the material properties of individual spinicaudatan shells using a modified punch test or punch-and-die test (Choo et al., 1992; Aranwela et al., 1999; Sanson et al., 2001). In these tests, a punch is lowered at a controlled rate normal to the sample until fracture occurs. To control the region of the sample loaded, punches are small enough to fit through a die, which holds the sample (see Fig. 3 for a schematic of our experimental set-up).

Due to the nature of material failure, the area of fracture can be larger than the die, thus punch tests sometimes inflate measured values, especially for complex biological materials (Lucas et al., 1991). Furthermore, when punching through a material, resistance to penetration involves multiple material properties, such as shear and compressive strength and resistance to crack propagation (Vincent, 1992; Aranwela et al., 1999; Edwards et al., 2000) so that the actual material property measured is not clear. However, the test clearly mimics real physical trauma both from predation and taphonomic process. Moreover, other authors argue that the punch test is a good indicator of both material and structural properties and suggest that the variation in sample fracture can actually enhance our understanding of the material’s performance (Evans and Sanson, 2005; Freeman and Lemen, 2007).

As a result of the inherent challenges of both biological materials testing and punch tests in particular, some suggest this test method should not be adopted (Aranwela et al., 1999; Edwards et al., 2000). Compressive properties of some biomaterials like bone and wood can instead be characterized from milled samples (Vincent, 1992). However, this was not deemed feasible with the small, thin, and fragile spinicaudatan carapaces. Spinicaudata are aquatic and their shells are highly sensitive to desiccation. In preliminary investigations we observed the shells curling and noted qualitatively different material properties after only a few minutes in air. Our use of the punch test addresses these problems as samples can be quickly mounted as whole shells and tested before extensive drying or damage occurs. We also chose the punch test as it investigates material and structural properties (Evans and Sanson, 2005), both of which may be relevant for taphonomic and fossilization processes. Finally, the curved nature of our samples provided additional complexity which seemed best controlled by a punch test (see methodology below).

Several material properties are commonly reported when using a punch test and these are outlined in Table 1 (Edwards et al., 2000; Sanson et al., 2001; Evans and Sanson, 2005). Force and displacement were measured during experimental trials and further used to calculate specific material properties (Table 1). The force-displacement curve gives the maximum force and displacement at break (Fig. 4). The area under the force-displacement curve (grey in Fig. 4) represents the total work to break the specimen. To calculate additional material properties, we normalized force at break to the area of the punch to estimate the compressive “punch strength” of the carapace and then expressed punch strength relative to shell thickness to account for differences among samples as “specific punch strength” (Table 1). How much work is required to punch through a surface depends in part on how much material is under the area of impact. To address this we normalized work to break in two ways (Table 1). Work to punch, expressed as work relative to the area of the probe impacting the shell, while specific work to punch is expressed as work relative to the volume of material directly under the impact site (Table 1) and is analogous to many common measures of toughness. In reality, while our definitions allow clear, repeatable comparison across species, these values probably

Fig. 3. A, Schematic of the experimental set-up showing the spinicaudatan shell suspended across a nylon lock nut. B, Location of the glass rod (punch) as it makes contact with the center of the spinicaudatan shell. A glass rod was attached to a movable cross head grip which was lowered at a speed of 1.00 × 10⁻² mm/s until it punctured the shell to failure. The resulting load on the sample of failure was recorded by the bottom force sensor. To allow for complete puncture of the shell, it was positioned and glued to lie across a nylon lock nut. The lock nut was held on to the bottom force sensor grip by a standard push pin. C, picture of sample set up (carapace on a lock nut and push pin).
Table 1. Elemental composition of six individual Spinicaudatan carapace valves. Two specimens for *L. compleximanus* and *C. gynecia* and one specimen for *E. ferriensis* were used to investigate intra-specific variation.

<table>
<thead>
<tr>
<th>Species</th>
<th>C (%)</th>
<th>O (%)</th>
<th>Na (%)</th>
<th>P (%)</th>
<th>Ca (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leptestheria compleximanus</em></td>
<td>78.87</td>
<td>16.49</td>
<td>0</td>
<td>1.56</td>
<td>3.09</td>
</tr>
<tr>
<td><em>Eulimnadia ferriensis</em></td>
<td>54.30</td>
<td>29.16</td>
<td>3.15</td>
<td>0.23</td>
<td>1.24</td>
</tr>
</tbody>
</table>

underestimate the total amount of material involved in breaking as fractures likely propagated longitudinally from the impact site.

We chose a fixed speed of $1.00 \times 10^{-2}$ mm/s for the probe because high speeds generally result in brittle fracture of the material (Aranwela et al., 1999). Aranwela et al. (1999) argued that punch diameter and hole size of the die should be similar so that shear is enhanced and deformation of the surrounding area is minimized (Aranwela et al., 1999). In contrast, we used a 0.2 mm diameter glass rod for our punch while the shells were mounted on a die with an internal hole diameter of 2.5 mm. We chose a small diameter punch so that it would make full contact with the curved shells and we used the large die hole diameter to minimize the effects of that curvature (Fig. 3b), which was not consistent across species and also not easy to characterize at smaller spatial scales. Moreover, the large die size allows the shape of the shell (i.e., curvature) to influence punch resistance, similar to the way that curvature would influence resistance to forces during taphonomy.

### Sampling Method

Ten valves from the purely selfing-hermaphrodite species *C. gynecia* and *E. ferriensis* were used for materials testing. Each individual was dissected to provide up to two valves, however not all valves were used due to premature damage during dissection or material testing. Therefore, of the ten valves for each species, six different individuals contributed valves to the *C. gynecia* sample group and eight different individuals contributed to the *E. ferriensis* sample group. Fourteen valves were used in the dioecious (male/female) species *L. compleximanus*. Of the fourteen valves from *L. compleximanus*, six valves from three different females and eight valves from five different males were used.

Immediately before testing, sample valves were removed from vials filled with water and blotted dry to remove excess water. Samples that had been removed from water for over five minutes were not used in data analysis due to clear structural changes observed in the valve (i.e., curling) and subsequent inflated material property values. Samples were then mounted on a Nano Bionix tensile tester (Agilent Technology, Amstelveen, The Netherlands) and positioned for the punch test. In order to load the sample to failure at the punch site we suspended the samples on a nylon lock nut (No. 6-32). Ethyl cyanoacrylate glue was brushed lightly around the top rim of the nut and allowed to cure slightly to avoid wicking of glue onto the sample. After curing the sample was placed on the glued edge of the nut and pressed lightly into place along the circumference of the nut. All samples were larger than the nut diameter to ensure consistent attachment of the sample to the testing apparatus and to control for variations in size of the individual. Although there was some size variation within and across species, the standard nut diameter controlled for variations in shell size by limiting the sample testing area to 4.91 mm² for all samples. Prior to removing the sample valve, the base of the nut was glued firmly to the plastic top of a standard push pin mounted in the bottom grip of the Nano Bionix tensile tester (Fig. 3). This grip is attached to a force sensor with a force and displacement resolution of 1-2 μN and 1 μm, respectively. The glass rod was positioned in the top movable crosshead grip normal to the sample (Fig. 3). The rod was then lowered at a fixed speed (0.01 mm/s). As the rod contacted and pressed the sample, displacement occurred and force and displacement was recorded using Testworks 4.0 software (MTS, Eden Prairie, MN, USA). Samples were punctured to failure. In some cases, the force-displacement curve showed a jump in data that we associated with the glass rod sliding down the concave shell because we often could visually observe the glass rod jumping or scraping along the shell. While the rod was positioned above the most concave portion of the shell, it was not possible to confirm ideal placement of the rod by eye alone (ideal would be placement in the center of the shell so that none of the shell curvature is directly experienced by the punch (Fig. 3b)). We determined that an aberrantly fast change in force indicated sliding of the rod down the concave wall of the sample. All samples that had this “jump” in force were removed from analysis. Jumps ranged from about 0.6 mN to about 3.5 mN and generally occurred within 0.025 mm displacement, though in one test the jump occurred over 0.5 mm displacement. By removing jumps we were able to control for the puncture location in test samples as force curves that show a smooth displacement, without jumps, signifies that the rod was in the most concave portion of the shell. For this reason the samples could not be tested in the convex position, where slipping and uncontrolled structural deformations preeminent. Preliminary tests in this orientation showed a smooth force-displacement curve was integrated using a sigmoid function to fit the data and is reported as “toughness.” An example of the fitting function overlaid on the raw data values is shown in Fig. 5. Due to the fragility of the samples and rapid rate of desiccation, we were unable to measure thickness of each sample prior to testing. In order to estimate sample thickness we averaged the measured thickness of ten additional valves from six individuals from *E. ferriensis* and five individuals from *C. gynecia*. The valves were harvested on a similar timescale before being measured, separated and refrigerated in a manner similar to those used for material analysis. Thickness was measured using a flat digital caliper which measured the whole shell. Samples for *L. compleximanus* were more limited and we were unable to measure a standard thickness value for this group, therefore properties normalized by thickness could only be estimated for *L. compleximanus*. See the Results section for a detailed description of the thickness estimation.

### Statistical Analysis

To determine if using two valves from the same individual had any effect on statistical conclusions (lack of independence), we randomly picked one valve per individual (if two were available) from the final data set and performed statistical tests on this subset similar to Evans and Sanson (2005). Force and displacement data were analyzed using Igor Pro software version 6.2.2.2 (WaveMetrics, Lake Oswego, OR, USA). An analysis of variance (ANOVA) and a Tukey HSD post hoc test of all species combinations for each measurement and material property was performed. We also performed a Student’s *t*-test to test for a difference between sexes in *L. compleximanus*. Statistical analyses were performed with JMP version 10 (SAS Institute, Cary, NC, USA). All errors are reported as mean ± 1 SEM.
RESULTS

Energy Dispersive X-ray Spectroscopy (EDAX)

We undertook energy dispersive X-ray spectrographic analyses of three spinicaudatan taxa (Fig. 6). Regions of elemental signal are clearly localized to the thin section of the spinicaudatan carapace (as indicated by color), rather than the resin matrix. These results confirm that the spinicaudatan carapace is mainly composed of a calcium-phosphate complex (Ca, P) that most likely represents mineralization of the chitinous component (C, O) of the cuticle. Trace amounts of zinc, sulfur and other elements also occurred in very low quantities. A weak signal for silicon was also reported but this is likely an artifact of the abrasive used in the preparation of resin-embedded specimens. Only relevant elemental percentages are reported in Table 1. Percentage of C and O, the chitinous component, is relatively similar in all three species. Quantitative differences are clear, however, in the mineralized component of the carapace where C. gynecia has a relatively high calcium and phosphate percentage, and both E. feriensis and L. compleximanus have considerably less strongly mineralized carapaces (lower calcium/phosphate percentage). Interestingly, the lowest calcium/phosphate signal comes from E. feriensis, which has a sodium component (the only one observed) that is equivalent in magnitude to the calcium-phosphate signal in L. compleximanus (about 3-4%).

Materials Testing

Our data include 12 individuals where both sides of their valves were tested and 10 individuals where only a single valve was tested. We found no difference in the outcome of
our results using a randomly selected subset of valves \( n = 22 \), where each individual was only represented once, thus we treated the complete data set \( n = 34 \) as independent in our analyses. The overall ANOVA models which compare all three species, \( C. \) gynecia, \( L. \) compleximanus and \( E. \) feriensis, in each of the measured mechanical properties (Table 2) shows that there is a significant difference between the three groups in all of our measured material properties (Table 3). Specifically, when testing each species pairing we found that \( L. \) compleximanus was statistically lower than both \( C. \) gynecia and \( E. \) feriensis in all measured properties. \( C. \) gynecia and \( E. \) feriensis did not differ significantly in any of the measured properties except when standardized by thickness of the sample, which is not surprising as the punch test is strongly dependent on sample thickness (Choong et al., 1992) (Table 3).

Our results are complicated by our measurement of sample thickness. Thickness measurements were collected from a subset of samples that were not used in testing due to the fragility of the samples and their propensity to desiccate rapidly. This was also done for the whole valve and not for the specific punch site. For both \( C. \) gynecia and \( E. \) feriensis we measured 10 valves each and took the average to represent the thickness of their respective species groups \( (1.81 \pm 0.18 \times 10^{-3} \text{ m} \) for \( C. \) gynecia and \( 0.83 \pm 0.07 \times 10^{-3} \text{ m} \) for \( E. \) feriensis). Although sample thickness was determined in this way, we do not believe it has a significant impact on our results as the differences between the groups were very large and thickness was measured the same way across species. In contrast, we were unable to measure fresh samples from \( L. \) compleximanus, instead measuring an average thickness \( (1.80 \pm 0.19 \times 10^{-5} \text{ m}) \) from only four. When we compare fresh and slightly decayed samples in \( C. \) gynecia and \( E. \) feriensis we found that decay seems to increase thickness. Thus our report of estimated thickness for \( L. \) compleximanus, and subsequently material properties that take into account sample thickness, are likely lower than their true values. Interestingly, we found no difference in any of the measured material properties based on sex in \( L. \) compleximanus \( (p > 0.5 \) for all).

### Discussion

The ultrastructure of the spinicaudatan carapace is a multi-layered cuticle composed of successive layers of the endocuticle and exocuticle retained during the partial molt (Rieder et al., 1984; Martin, 1991; Olempska, 2004). The accreted, multi-laminar ‘macro-structure’ of the spinicaudatan carapace is resilient to taphonomic processes such as decay, desiccation, and compaction compared to the non-mineralized cuticle of the organism within the carapace (Krishnan, 1958). Our analysis of the spinicaudatan carapace clearly shows differences in both chemical composition and specific mechanical properties between the three extant families of Spinicaudata. Our results from elemental analysis show that the proportion of mineralized cuticle is highest in \( C. \) gynecia, containing some 16-21% (approx.) calcium-phosphate, considerably more than the 5% (approx.) seen in representatives of Leptestheriidae (Daday, 1923) (\( L. \) compleximanus), and 2% (approx.) in the representative of Limnadiidae (Burmeister, 1843) (\( E. \) feriensis). Results from the materials testing, however, show that \( C. \) gynecia and \( E. \) feriensis are no different from each other in all mechanical properties.

Table 3. Measured and calculated mechanical properties from the punch test for three species of Spinicaudata. Degrees of freedom (df), \( F \)-ratios and \( p \)-values are reported for an ANOVA which tests for differences in a particular material property across species. Pair-wise comparisons between species are reported below the associated material property. Species with the same letter (i.e., \( A \), \( B \) or \( C \)) do not differ from one another. Error is reported as mean \( \pm 1 \) SEM.

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force at break ( (F_b) )</td>
<td>Recorded during test</td>
</tr>
<tr>
<td>Work to break ( (W_b) )</td>
<td>Area under force-displacement curve</td>
</tr>
<tr>
<td>Punch strength ( (S_p) )</td>
<td>( F_b/A )</td>
</tr>
<tr>
<td>Specific punch strength</td>
<td>( S_p/T )</td>
</tr>
<tr>
<td>Work to punch ( (W_p) )</td>
<td>( W_b/A )</td>
</tr>
<tr>
<td>Specific work to punch</td>
<td>( W_p/T )</td>
</tr>
</tbody>
</table>

Table 2. Measured and calculated mechanical properties for the punch test, adapted from Edwards et al. (2000), Sanson et al. (2001) and Evans and Sanson (2005). \( F \) is the maximum force measured during testing, \( T \) is the thickness of the sample, \( A \) is the area of punch and \( D \) is the displacement of the punch mounted in the moving crosshead of the test machine.
properties except those that take into account the distinct difference in valve thickness. *Leptestheria compleximanus* on the other hand is significantly lower in all measured material properties (Table 3). We conclude that, despite the variation in cuticle chemistry, the degree of mineralization alone does not predict the mechanical properties of the valves.

We hypothesized that more strongly mineralized valves would resist force better and require more work to fracture. This was not entirely the case (Table 3). While the highly mineralized *C. gynecia* reported high values for the material properties measured, the weakly mineralized *E. feriensis* was surprisingly no different from *C. gynecia*. In fact, once the thicker shells of *C. gynecia* were taken into account, the specific punch strength and specific work to punch of *E. feriensis* were both almost twice as high as *C. gynecia*. Thus, it is possible that the low mineralization of *E. feriensis* and their thin carapace actually help to resist puncture in terms of strength and work to punch. For instance the force-displacement curves for *C. gynecia* and *E. feriensis* (Fig. 4) clearly show that the thin-shelled *E. feriensis* failed at a similar force (not normalized by area or thickness) as *C. gynecia* but only after deforming over about a 25% greater distance. Hence, *E. feriensis* are more deformable at high loads over longer distances than the thick, mineralized shells of *C. gynecia*. We believe that *E. feriensis* and *C. gynecia* behave similarly in all material properties except those that normalize by thickness via two different approaches; high mineralization and likely stiffness (steepest slope of the force-displacement curve) versus higher extensibility (longer displacement or ‘stretching’ in colloquial terms) of the sample prior to fracture. The result of these two approaches causes these two species with different material compositions to have similar strength and the carapace requires more work to punch. It is important to remember here that when we take into account valve thickness, which is much lower in *E. feriensis* than *C. gynecia*, *E. feriensis* has significantly higher strength and work required to punch thus valve thickness clearly also has a significant effect on fracture resistance. *Leptestheria compleximanus* on the other hand does not reach high maximum force values seen in *C. gynecia* and *E. feriensis* nor does it reach high displacement values characteristic of *E. feriensis* (Fig. 4) and thus performs lower than both *C. gynecia* and *E. feriensis* in all materials tests. Interestingly this is despite being the intermediately mineralized example of the three and what we expect to be only slightly less thick than *C. gynecia*. The direct comparison between *C. gynecia* and *L. compleximanus*, which has similar thickness (approximated) yet different levels of mineralization suggests that material properties can be directly related to mineralization however, mineralization requirements can be overcome by producing a thinner, less mineralized, and therefore more deformable carapace like *E. feriensis*.

Interestingly, we found a sodium signature in *E. feriensis* in our chemical analysis. To our knowledge sodium has not been reported as a component of the spinicaudatan carapace previously, and intriguingly, this was only found in one of the three species analyzed. The Na-Ca-P total percentage from the elemental analysis of *E. feriensis* was similar to the Ca-P total percentage for *L. compleximanus*, suggesting the possibility of an alternate method for mineralization in Spinicaudata. This species was also more extensible and had similar material properties as the highly mineralized *C. gynecia* when thickness was not taken into account. When we normalized for thickness, these extremely thin shelled species (*E. feriensis*) still had higher specific punch strength and specific work to punch than the thicker shelled *L. compleximanus*. Further investigation using multiple specimens from this species and contemporaneous taxa within Limnadiidae is required to deduce whether or not this is a particular anomaly or a feature of the species or clade. It is interesting to consider, however, an alternative approach to building a strong yet flexible shell in a calcium deficient environment.

The spinicaudatan carapace readily enters the fossil record where conditions allow and seems much more resistant to taphonomic processes than the soft parts of branchiopod crustaceans, as is evidenced from the thousands of instances of carapace preservation (Astrop and Hegna, 2015), and the relatively few (Zhang et al., 1990) instances of fossilized spinicaudatan soft tissue. When considering the fossil record of proposed members of these lineages there are far more fossil taxa with proposed affinities to the extant Cyzidae than there are to the extant Limnadiidae (Novojilov, 1961; Tasch, 1969), despite both families having a similar diversity in the present (Brendonck et al., 2008) (Fig. 2). To avoid potential bias, the materials testing performed in this analysis allows us to take a unique empirical look at how mechanical forces associated with naturally occurring, destructive taphonomic processes (post-mortem transportation, abrasion, predation and scavenging) may affect the entrance of spinicaudatan remains into the fossil record. More specifically, if major differences in material properties existed between spinicaudatan taxa, it would not be unreasonable to predict subsequent differences in the proportional representation of associated taxa in the fossil record.

We found that the material properties of the carapace of *L. compleximanus* to be consistently weaker in all measurements taken. In the fossil record we see that Leptestheriidae are represented less frequently, consistent with their weak carapace when compared to *E. feriensis* and *C. gynecia*. In contrast, the lack of significant differences between *E. feriensis* and *C. gynecia* with respect to resistance to the mechanical forces generated in this experiment insinuates that they are equally as likely to resist physically destructive taphonomic effects despite substantial differences in their carapace composition. By extension, one would not expect mechanical forces associated with destructive taphonomic processes to produce severe numerical bias in the fossil record of either Cyzicidae (Stebbing, 1910), Limnadiidae, or related ancestral groups assuming similar carapace positions in the past. This is not the case however, as we see that presence of Cyzicidae is consistent throughout the fossil record whereas Limnadiidae is patchy, ‘appearing’ and ‘disappearing’ throughout time (Fig. 2). In this case, perhaps the low level of mineralization compared to Cyzicidae contributes more significantly to the very poor fossil record for this family than the equally resistant material properties (when considering overall structure and material components). This would suggest that members of Cyzicidae are
not inherently less resistant to destructive taphonomic effects such as post-mortem transport and abrasion, scavenging, or predation, rather they are perhaps less likely to promote positive effects such as permineralization that would help them become more prevalent in the fossil record (Briggs, 2003; Astrop and Hegna, 2015).

When considering phylogeny and ontogeny, we find that the poorly performing (thin, weak shells) representatives of Leptestheriidae (L. compleximanus) are more closely related to the better performing Cyziciidae (C. gynecia) (thick, strong shells). Although the thinner shelled representative of Limnadiidae (E. feriensis) performs similarly to Cyziciidae, and when normalized for thickness they perform better, interestingly, they are more distantly related to either of the two other groups with which they share either similar thickness, or similar (or better) material properties. This lack of phylogenetic bias in material performance suggests there is another factor driving the mechanical properties of these three species. In terms of ontogeny, the results of this study indicate that the thinner carapace of Limnadiidae (represented by E. feriensis) is less mineralized and is more extensible, conferring a different dimension of material strength without the need for costly investment in mineral deposition. This could be due to ontogenetic differences, in that limnadiids are often characterized by their quick growth to sexual maturity (Brown et al., 2014). Cyziciidae on the other hand molt at more infrequent intervals for longer periods of time and often inhabit less ephemeral water bodies. The longer lived, slower growing cyzicids do not exhibit the ‘rush’ to sexual maturity seen in limnadiids. Further investigation on evolutionary factors driving the material properties of these species, such as environment, behavior and time to sexual maturity are necessary to fully understand why these differences arise.

The less mineralized, extensible limnadiid carapace and the thicker, more mineralized carapace of cyzicids are both likely to resist the physical stresses associated with post-mortem transport of biological remains in aqueous environments, implying that pre-depositional processes might not play a large role in producing taxonomic bias in the fossil record of either group. However, the relative weakness of the leptasteriid carapace could explain their near-absence from the fossil record. Further integrated studies testing different aspects of how this material behaves are required for both a better understanding of the spinicaudatan carapace as a biological structure and how such a structure behaves when subjected to the destructive forces of other taphonomic process than those mentioned here, specifically post-depositional effects such as permineralization and dissolution. Regardless, this study provides a unique approach in understanding the forces involved in organismal remains entering the fossil record and how different parameters of the ‘taphonomic window’ may vary for different taxa as a result of differing biogenic composition. Using modern analogues and heuristic integrative approaches to understand how organic remains of different taxa may enter the fossil record is an extremely useful and an underutilized approach that has the potential to open up areas of palaeobiological and biological material science that were previously unreachable.

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