Elongated thoracic spines as potential predatory deterrents in olenelline trilobites from the lower Cambrian of Nevada

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Abstract

Repaired injuries offer the opportunity to study predation in the fossil record and test hypotheses concerning predation intensity, anti-predatory characters, and predator selectivity. Many studies have used the late Palaeozoic and Mesozoic records but quantitative explorations of early Palaeozoic predation, especially during the Cambrian, are lacking. Trilobites, a group of biomineralised arthropods with a high early Palaeozoic disparity and diversity are an ideal study system for exploring repaired injury frequencies. The Ruin Wash *Lagerstätte* (Nevada, USA; Stage 4, Cambrian Series 2) provides a bulk sample of closely related but morphologically distinct taxa. The five most abundant, *Olenellus chiefensis, O. fowleri, O. gilberti, O. terminatus,* and *Nephrolenellus geniculatus* differ in the spinosity of the cephalon, axial lobe of the thorax, and pleural lobes of the thorax. A total of 26 repaired injuries on 330 articulated trilobite cephalothoraces reveal

that *O. terminatus* has the highest incidence of repaired injuries (repair frequency = 0.18) and *N. geniculatus* has the lowest (repair frequency = 0.043). The role of enlarged spines on the third thoracic segment, which are most elongate in *N. geniculatus,* are suggested as predation deterrents. Predator selection for prey of a larger size may have played a minor role, though we did not find statistical support for this. Three cephala with repaired injuries were noted in a sample of 685 specimens: a much lower repair frequency compared to thoraces. This indicates that cephalic attacks were more often fatal when compared to thoracic attacks. Our study provides the first quantitative support for species specific predation on Cambrian trilobites.

1. Introduction

The dramatic increase in diversity and disparity during the Cambrian period (the Cambrian Explosion) was likely a result of numerous biological, ecological and environmental factors (Smith and Harper 2013; Zhang et al. 2014). Among these, predation was likely an important evolutionary shaper of events and morphologies (e.g. Bengtson 2002; Knoll 2003; Wood and Zhuravlev 2012; Erwin and Valentine 2013; Sperling et al. 2013; Zhang et al. 2014; Bicknell and Paterson 2018). Predation has been the subject of numerous large scale studies through geological time (e.g. Kowalewski et al. 1998; Huntley and Kowalewski 2007; Klompmaker et al. 2017) and has been touted as a major component of the Cambrian Explosion and the development of biomineralised exoskeletons and shells (Vermeij 1989, 2013; Bengston 2002; Babcock 2003; Knoll 2003). However, few studies on Cambrian predation intensity or selection have been conducted (but see Pratt 1998) and

quantitative evidence for a Cambrian 'arms race' or escalation event is currently lacking (Leighton 2011; Bicknell and Paterson 2018).

Predation is an important agent of selection when predators and prey encounter each other often, and when predators fail to kill prey (Vermeij 2002). Repaired injuries (on bone, exoskeletons, or shells) are useful indicators of predation, as these fossils are common across depositional environments and present throughout the Phanerozoic (Kowalewski 2002). Furthermore, a repaired injury shows that the attack occurred during the animal's life, unlike unrepaired broken sclerites or shells that can also reflect post-mortem damage (e.g scavengers or taphonomy), complicating quantitative predation studies (Pates et al. 2017). Predation events that result in damage only to the soft-parts, or no damage at all, would not be measured by repaired injuries on hard exoskeletons, and so the predation pressure considered here pertains only to predation damage to a hard exoskeleton.

Repair frequency (a measure of the proportion of injured animals in a sample) is a useful proxy for predation intensity in the fossil record (e.g. Vermeij et al. 1981; Dietl et al. 2010; Harper 2016; Harper and Peck 2016), as supported by studies of modern gastropods (Molinaro et al. 2014; Stafford et al. 2015). Repair frequency is affected by the frequency of attacks (predation intensity), the proportion of failed attacks (predator efficiency) (Alexander 1981), and, as shown by a theoretical analysis of age-structured populations, the frequency of other sources of injury and death (Schoener 1979). These models show that repair frequency for animals damaged or killed only by predation is a result of only predator efficiency (Schoener 1979; Budd and Mann 2018). Schoener (1979) presented three competing hypotheses to explain differences in repaired specimens between samples: i) increased predator efficiency; ii) increased predation intensity, coupled with other causes of

non-fatal injuries; and iii) decreased predation intensity coupled with a significant source of alternative mortality. We explore these three hypotheses to assess a record of differing repair frequencies in five Cambrian trilobite taxa. Our interpretation is complemented by data relating to the relative preservation frequency of near-articulated cephalothoraces (specimens where the length of the cephalothorax could be confidently measured) and isolated cephala.

2. Background

2.1 Trilobites as a study system

Repair frequency in brachiopods and molluscs is affected by prey morphology (e.g. Alexander 1986; Dietl et al. 2000; Alexander and Dietl 2001; Dietl 2003a, b; Dietl and Hendricks 2006; Harper et al. 2009) and defensive adaptations like spiny shells and reduced aperture sizes (Vermeij 1977, 1987; Kelley 1989; Vendrasco et al. 2011; Klompmaker and Kelley 2015). Such records have been extensively studied as shell drilling and crushing spans the Phanerozoic. Conversely, trilobites have been less explored, despite their large disparity and high diversity through the early Palaeozoic (Foote 1991; Adrain et al. 1998; Webster 2007a).

The high preservation potential of Cambrian trilobites, coupled with their diversity and global distribution, makes this group ideal for providing quantitative data and evidence of early Palaeozoic predation. Repaired injuries on Cambrian trilobite exoskeletons are well known (see Owen 1985; Babcock 1993, 2003; Vinn 2018; Bicknell and Paterson 2018 for reviews) and trilobite rich gut contents (Conway Morris 1977; Bruton 1981; Zhu et al. 2004; Vannier 2012; Zacaï et al. 2016; Jago et al. 2016) and coprolites (Sprinkle 1973; Conway Morris and Robison 1988; Nedin 1999; Babcock 2003, Skinner 2005; Vannier and Chen 2005; English and Babcock 2010; Daley et al. 2013; Kimmig and Strotz 2017) confirm that trilobites

were prey items. Additionally, recent biomechanical analyses that built on functional morphological studies have confirmed that at least some Cambrian predators, such as *Sidneyia inexpectans* of the middle Cambrian Burgess Shale, were mechanically capable of injuring trilobites (Bicknell et al 2018a, b). Trilobites likely engaged in anti-predatory behaviors, such as burrowing, hiding, and enrolment (Fortey 1986; Esteve et al. 2011, 2013; Fatka and Budil 2014), and had select exoskeletal characters with anti-predatory roles: elongate spines and thick biomineralised exoskeletons (Fortey and Owens 1999; Clarkson and Ahlberg 2002). However, the anti-predatory effectiveness of these features has not yet been explored.

Studies of repaired injuries in trilobites are complicated by ecdysis, which can cause injuries, death, and discarded moults bearing similarities to carcasses. Different groups of trilobites utilized a vast array of moulting modes, that varied inter- and intra-generically (Daley and Drage 2016). The two genera considered in this study, *Olenellus* and *Nephrolenellus,* used the same two major moulting modes: the Marginal Suture Mode and Salterian Mode (Hennigsmoen 1975; Webster 2015; Drage in review; Drage et al. 2018). Moulting events likely regenerated injured areas, removing evidence for injuries from the fossil record (Pates et al. 2017; Bicknell et al. 2018c). Also problematic moulting is another potential cause for injuries and death (e.g. Owen 1985). Ultimately, the repair frequencies of trilobites should not be compared to non-trilobite groups without considering these factors. To assess potential trilobitic anti-predatory features, a bulk sample (either direct or indirect, *sensu* Kowalewski 2002) containing multiple species with different anti-predatory features are needed from a single site. A bulk sample (the collection of every specimen in a deposit) precludes collection bias for species, animal sizes, and preferential collection of injured or uninjured specimens. Additionally, the trilobites must be autochthonous (as

different sources could have different environments, predators and injury sources), and not

be size sorted (size can affect predator preference and therefore repair frequency, see Paine

1976 or Harper et al. 2009). Finally, the collection should be large enough (relative to injury

frequency) to allow for statistical comparisons between species.

Extensive work at the Ruin Wash *Lagerstätte*, Nevada, USA (Cambrian Series 2, Stage

4; Fig. 1C) has documented the ontogeny, systematics, and taphonomy of the trilobite fauna

(e.g. Palmer 1998; Webster and Hughes 1999; Webster 2007b, 2015; Webster et al. 2008).

Here, predator selection is explored by comparing the relative frequency of injuries in the

five most common Ruin Wash *Lagerstätte* trilobite taxa. Disparity in prothorax spinosity is

used to assess the role of different spines as predatory deterrents and subsequent

differences in repair frequencies between these species.

2.2 Ruin Wash

The Ruin Wash *Lagerstätte* (Fig. 1A, B), the top 43 cm of the Combined Metals

Member, Pioche Formation (Nevada, USA), is uppermost Dyeran in age (Cambrian Series 2,

Stage 4; Fig. 1C). A field study in 1999 (Webster et al. 2008) collected every specimen,

providing an indirect bulk sample (*sensu* Kowalewski 2002) for this study. Bottom currents

likely removed the smallest (<2 mm length) specimens but no other evidence for size-

sorting or faunal transport is present. Trilobites therefore represent an autochthonous

assemblage deposited in a low energy environment, buried by tempestite events (Webster

et al. 2008). Slight variations in the pre-burial exposure time and current intensity in certain

horizons resulted in some beds containing more articulated specimens than others

(Webster et al. 2008).

The five most common species, *Olenellus chiefensis, O. fowleri, O. gilberti, O. terminatus,* and *Nephrolenellus geniculatus* can be recognized by morphological differences in the cephalon and prothorax. The opisthothorax is well known in *N. geniculatus* as it is often preserved, but is rarely preserved in *Olenellus* species and unknown for *O. chiefensis* and *O. terminatus* (Fig. 2; Palmer 1998). All five taxa bear genal spines, but the length of these spines relative to the overall cephalic and thoracic lengths varies: *Nephrolenellus geniculatus* and *O. fowleri* have the shortest genal spines, and *O. chiefensis* and *O. gilberti* have the longest. All five taxa bear an enlarged third thoracic segment (T3), with associated elongated pleural spines on the prothorax. T3 is hyperpleural (*sensu* Palmer 1998) in *N. geniculatus*, as it distorts both the thoracic segment immediately anterior (T2) and posterior (T4). The *Olenellus* species are macropleural (*sensu* Palmer 1998), as only T4 is distorted in the genus. *Olenellus* species are varyingly macropleural: weakly (amplipleural *sensu* Palmer 1998) in *O. fowleri*, moderately in *O. terminatus*, and more strongly in *O. chiefensis* and *O. gilberti* (Palmer 1998). The pleural spine on T3 extends significantly beyond the posterior prothorax in *N. geniculatus* (dolichospinous *sensu* Palmer 1998). The T3 pleural spines are larger than adjacent spines, but do not extend beyond the prothorax in the four *Olenellus* species (macrospinous *sensu* Palmer 1998). *Olenellus fowleri* has proportionally longer pleural spines along the entire prothorax, with *O. chiefensis* and *O. gilberti* the taxa with the next most spinous prothoraces. *Nephrolenellus geniculatus* and *O. terminatus* have the proportionally shortest pleural spines on the prothorax. All *Olenellus* species bear an axial spine on T15, which is unknown in *N. geniculatus*.

Individual taxa can therefore be considered the most or least spinous from different perspectives. When only genal spines are considered, *Olenellus chiefensis* and *O.* gilberti are the most spinous. When only T15 is considered, the *Olenellus* taxa are more spinous than *N.*

geniculatus. When T3 spines and all other thoracic spines are considered together, *O. fowleri* is the most spinous. When T3 is considered most important and the other thoracic spines have secondary importance, *N. geniculatus* would be considered the most spinous and *O. terminatus* the least spinous. We consider each of these in turn, in relation to the injury frequencies discussed below.

3. Methods

3.1 Institutional abbreviations.

CMCP, CMNH, Cincinnati Museum Center, Cincinnati, Ohio, USA; DMNH, Denver Museum of Natural History, Denver, Colorado, USA; FMNH, Field Museum of Natural History, Chicago, Illinois, USA; ICS, Institute for Cambrian Studies, University of Chicago, Chicago, Illinois, USA; MCZ, Museum of Comparative Zoology, Harvard, Boston, Massachusetts, USA; UCR, University California Riverside, Riverside, California, USA; YPM, Yale Peabody Museum, New Haven, Connecticut, USA.

3.2 Data collection

The bulk sample of Ruin Wash *Lagerstätte* trilobites from a 1999 field study is accessioned across a large number of institutes (see *Institutional abbreviations* above); with the ICS collection also holding latex peels of specimens accessioned at the DMNH. Ruin Wash trilobites at these institutions were examined (latex peels at the ICS were used in place of DMNH specimens). Additional Ruin Wash specimens from earlier, non-exhaustive, excavations and donations from collectors are present in the ICS. These were also included in the study to increase sample size, introducing a minor sampling bias to the indirect bulk

sample. Evidence for repaired injuries on trilobites, as defined and discussed in Bicknell and

Paterson (2018) and Bicknell et al. (2018c), was noted, including single spine injuries (SSIs).

The length from the anteriormost point of the cephalon to the posteriormost point of the

axial lobe of the prothorax was measured for all injured trilobites, and those with at least an

articulated or nearly articulated cephalothorax (referred to as cephalothoraces from this

point, even if they also had the opisthothorax or additional body parts preserved) and well

preserved spines (i.e. where an injury could be identified if present). Measurements were

performed using digital calipers. It is worth noting that telescoping of thoracic segments and

slight cephalic disarticulations in some specimens will have introduced a small degree of

error. Cephalic measurements (e.g. just cephalon width, or sagittal length) would have not

introduced such errors, however cephalothoracic length was gathered as the cephalothorax

as a whole was assessed for damage.

Injured specimens were photographed with a Canon EOS 500 DSLR camera, and

injury location (side, body part, location on that body part) was noted. All isolated cephala

in the CMCP, CMNH, DMNH, ICS, YPM and UCR collections were counted and examined for

injuries, to provide an additional injury frequency dataset.

3.3 Data analysis

3.3.1. Frequency of preservation.

Isolated cephala can result from the disarticulation of complete skeletons after

moulting, predation, or scavenging, and the disruption of carcasses by bottom currents.

Specimens including at least a cephalothorax reflect either Marginal Suture moulting (MSM)

mode (Drage in review), or carcasses. If all taxa were attacked and killed without predator

selection, and the proportions moulting through the MSM mode were not significantly

different, the percentage of each taxon in an assemblage would be the same for both isolated cephala and cephalothoraces. The frequency of isolated cephala and cephalothoraces for taxa from the Webster et al. (2008) field study were compared with our dataset using a Chi-squared test (α=0.05). The test was performed using the 'MASS' package in RStudio (Venables and Ripley, 2002). A higher proportion of isolated cephala from a taxon compared to cephalothoraces thereof could reflect i) higher predation, scavenging, or disarticulation frequency relative to other taxa, or ii) lower frequency of MSM mode and non-predatory death (not resulting in exoskeletal disarticulation). The following hypothesis was tested:

H_0: cephalothoraces and cephala are preserved in the same relative frequency between taxa. There is therefore no predatory selection or difference in moulting mode frequency.

H_1: cephalothoraces and cephala are not preserved in the same relative frequency between taxa. Predatory selection, moulting behavior, and/or propensity to disarticulate prior to burial is significantly different between taxa.

3.3.2 Frequency of repair.

 Repair frequencies have been calculated in two ways. The first, dividing the total number of injuries by total number of animals (F-value in Pates et al. 2017) gives an inflated percentage value of injured animals (Dietl et al. 2000). The second, dividing total number of injured animals by total number of animals (R-value in Pates et al. 2017), gives an underestimate for the percentage value of injured animals (Alexander and Dietl 2003). Here, no articulated cephalothorax had multiple injuries: F- and R-values are therefore the

same. One example of an *O. gilberti* from Ruin Wash with two injuries (one on T4 and T5, the second on T8) is known in the literature (Palmer 1998, fig. 9.2).

A Bayesian inference method was used to assess the effect of sample size on calculated repair frequencies (following Pates et al. 2017). This method produces a distribution of possible outcomes, therefore accounting for uncertainty induced by low sample sizes by producing a broader distribution. In contrast, a higher sample size will produce a more constrained posterior distribution. Here, an uninformative prior was used, as we had no knowledge of injury abundance before sampling. The total number of specimens and number of injured specimens were input to produce the posterior distribution, from which the 5th and 95th percentile values were taken as confidence limits. This approach is preferred to a Chi-squared test for two reasons: i) Chi-squared tests perform poorly with low expected values and ii) a Bayesian-inference value allows the expected spread of values to be examined for individual taxa. The 5th and 95th percentile Bayesian Inference repair frequencies were calculated for each taxon. The 5th percentile value gives a lower bound where 95% of the inferred distribution of possible repair frequencies is expected to have a higher repair frequency, and the 95th percentile value gives an upper bound where 95% of the inferred distribution is expected to have a lower repair frequency.

3.3.3 Size selection.

The distributions of the cephalothoracic lengths of two groups (injured and not injured trilobites) were compared using a Mann-Whitney U test using the 'stats' package in R Studio (R Core Team 2017). This was done for the bulk sample (all trilobites) and for a

subset (*Olenellus* specimens), to assess possible relationship between the cephalothoracic length of trilobites and injury likelihood (i.e. size selection by predators).

3.3.4 Injury location.

Preferential injury location (stereotypy) for either pleural lobe of trilobite specimens was tested using two-tailed binomial tests calculated using the 'stats' package in RStudio (R Core Team 2017). The hypothesis tested was:

H_0: Injuries are equally likely on both trilobite pleural lobes.

H_1: Injuries are more likely on one pleural lobe or the other of the trilobite. This would provide evidence for lateral asymmetry of injuries (Babcock and Robison 1989; Babcock 1993, 2003).

A second test for injury stereotypy explored the role T3 pleural spines in active defense, and the suggestion that elongated spines were more commonly injured during problematic moulting. The T3 segment bears larger pleural spines than other prothoracic segments in all five taxa considered. Tips of the T3 spines were not always visible due to sediment cover, and an injury was only counted if an injury could be confirmed (and differentiated from sediment cover). The hypothesis tested was:

H_0: Enlarged spines on the third thoracic segment are equally likely to be injured as other spines.

H_1: Enlarged spines are more or less likely to be injured than other spines. This provides evidence for use of these spines in defense or evidence for problematic moulting preferentially causing injuries to the longest spines. If used for active defense, these spines would be broken, but the animal would survive and spines subsequently repair. If instead

they were used as deterrents, or had no anti-predatory function, these spines would not be more likely broken than other spines, as the predator would not attack the animal.

The axial spine on T15 is only known in *Olenellus*, and due to its orientation outside the plane of the rest of the body, we were not confident in identifying repaired injuries in this structure and distinguishing them from variation in taphonomic compression. No quantitative data was collected for this spine, and so its likelihood of injury during defense or accidental injury was not analyzed. Furthermore, genal spine injuries were not analyzed statistically due to the apparent rarity of cephalic injuries in general (see results below).

4. Results

4.1 Frequency of preservation.

The five taxa were not recovered in equal proportions, and the relative frequency of taxa preserved was not consistent between cephala and cephalothoraces. *Olenellus gilberti* was the most common taxon recovered, with *O. chiefensis* and *Nephrolenellus geniculatus* the next most abundant respectively (Table 1). The Chi-squared test indicates that the taxon-frequency of cephala from Webster et al. (2008) (Table 1, N cephala Webster et al. 2008) differs significantly from the taxon-frequency of cephalothoraces in our results (Table 1, N cephalothoraces this study) (χ^2= 12.35, p-value = 0.015). The Webster et al. (2008) cephala sample also differs from the cephalothorax frequency in the same study (Table 1, N cephalothoraces Webster et al. 2008) (χ^2 = 10.54, p-value = 0.032). The percentage of animals belonging to each taxon in the two cephalothoraces samples are not significantly different when treated with a Chi-squared test (χ^2= 5.97, p-value = 0.20); however, the result is only tentative as the field sample had a small sample size (n=17) (Webster et al. 2008).

More *Nephrolenellus geniculatus* cephalothoraces were preserved relative to the frequencies of isolated cephala reported by Webster et al. (2008). *Olenellus terminatus* cephalothoraces are preserved in a lower proportion compared to other taxa than isolated cephala. For the other three *Olenellus* taxa, the percentage of each taxon represented as isolated cephala is broadly similar to the percentage of each taxon in the cephalothoraces sample (Table 1, columns 1 and 2).

4.2 Frequency of thoracic repair.

Twenty-three injuries were found across 298 complete specimens of *Olenellus* and *Nephrolenellus*. An additional three injuries were noted on 32 *Olenellus* specimens that could not be identified to species level (Table 2; all the specimen and injury data is available to download through the Open Science Framework - Pates & Bicknell 2018). All *Olenellus* species record higher repair frequencies than *Nephrolenellus geniculatus,* with highest repair frequency calculated for *O. terminatus* (Table 2).

Repair frequencies for all *Olenellus* species were below the 95% Bayesian-inferred value for *Nephrolenellus geniculatus* (0.13) with the exception of *O. terminatus* (0.18). Bayesian-inference values for *N. geniculatus* equaled the measured repair frequencies of *Olenellus gilberti* at 70% (0.74), *O. chiefensis* at 77% (0.83) and *O. fowleri* at 94% (0.12). The *N. geniculatus* repair frequency (0.043) is similar to the 5% Bayesian-inferred value for *O. chiefensis* (0.044)*, O. fowleri* (0.047)*,* and *O. gilberti* (0.048), and below the 5% Bayesian-inferred value for *Olenellus* as a genus (0.062) and *O. terminatus* (0.072). *Olenellus terminatus* has the highest repair frequency (0.18), with the 5% Bayesian-inferred value lower than the recorded repair frequencies for all other *Olenellus* species (Table 2).

4.3 Description of injuries.

Thoracic injuries are limited to shortened pleural spines, on T3 and other segments. Eleven of 26 injuries are visible as two adjacent short spines, representing a single injury that damaged adjacent thoracic segments (e.g. Figs 3A, B; 4A, B), with the remaining 15 injuries limited to SSIs (e.g. Figs. 3C, D; 4C, D). No thoracic specimens showed signs of cicatrisation, suggesting a recent injury. Injured spines are at various stages of regeneration, but nonetheless are i) shorter than undamaged spines on the opposite side and ii) shorter than expected length relative to adjacent spines. In some cases they are much shorter (e.g. Fig. 3C) or show signs of distortion (e.g. Fig. 4A). Injured spines are distinguished from missing and broken spines (which have jagged or sharp edges) as the spine edge is smooth, indicating that some regeneration has occurred.

Three injured cephala, one *Olenellus fowleri* and two *O. terminatus*, at different stages of repair were identified (Fig. 5). The smallest specimen, *O. fowleri* (Fig. 5A), displays a cicatrised injury with a raised margin immediately interior to the 'U'-shaped indentation, and a missing genal spine. The second smallest specimen (Fig. 5B) has an uncicatrised 'V'-shaped injury to the right cephalon that is approximately as wide as it is deep. The presence of genal spines cannot be confirmed due to sediment cover. The largest specimen (Fig. 5C) shows damage and repair on both sides of the cephalon. Both genal spines are missing and the lateral borders immediately interior to these indents are slightly distorted.

4.4 Size selection.

Specimens of the largest and smallest trilobites were *Olenellus gilberti,* likely reflecting the large number of specimens (n=163, Table 1). On average (median and mean)

Nephrolenellus geniculatus were the smallest, *O. fowleri* the largest, and *O. terminatus* second largest (Table 3; Fig. 6A).

The median and mean length of injured specimens (23.90 mm and 25.72 mm) is higher than specimens without injuries (20.50 mm and 22.69 mm), both when all trilobites are treated as a bulk sample (Fig. 6B) and when considering just *Olenellus* taxa (Table 3, Fig. 6C). However, these size distributions of injured and not injured populations are not resolved as statistically different when interrogated with a Mann-Whitney U test (bulk sample: W = 2803.5, p-value = 0.06913; *Olenellus* only: W = 2813.5, p-value = 0.2114).

4.5 Injury location.

Fourteen of 26 thoracic injuries were on the right side of the body, 12 on the left side, and none present on the axial lobe. Three injuries are present on T3, with the remaining 23 on other prothoracic segments. The two-tailed binomial tests failed to reject either null hypothesis: injuries were equally likely to occur on either thoracic side (p-value = 0.845) and T3 was injured as frequently as other thoracic segments (p-value = 0.4743). Injured thoraces (repair frequency = 0.079, n=26 out of 330) are more common than injured cephala (repair frequency = 0.004, n= 3 out of 685).

5. Discussion

5.1 Differences in thoracic repair frequency

The three hypotheses that might explain the lower repair frequency of *Nephrolenellus geniculatus* relative to *Olenellus terminates* are considered (Schoener 1979): i) increased predator efficiency; ii) higher predation intensity with a significant alternate

cause of non-fatal injury; or iii) a lower predation intensity with a significant alternate cause of mortality. The third cause appears most likely, as it is supported by both injury frequency data (Table 2) and cephalothorax preservation data (Table 1). Fewer potentially injury-inducing encounters for *N. geniculatus* relative to *O. terminatus,* coupled with an alternate explanation for cephalothoraces in the fossil record (e.g., death during ecdysis) would result in more *N. geniculatus* cephalothoraces and a higher injury frequency for *O. terminatus*. The three other *Olenellus* species have repair frequencies higher than *N. geniculatus* and lower than *O. terminatus*, and also preserve more cephalothoraces than *O. terminatus* but fewer than *N. geniculatus*.

Hypotheses i) and ii) inadequately explain the differential preservation frequency of cephalothoraces and isolated cephala. For i) an increased success of predatory attacks to *Nephrolenellus geniculatus* relative to *Olenellus terminatus* predicts that *N. geniculatus* cephalothoraces would be rarer than *O. terminatus*. This assumes that successful predation produces fragmentary or disarticulated specimens. A major difference in the relative frequency of moulting modes employed by Ruin Wash trilobites would also be needed to explain why the *N. geniculatus* cephalothoraces are more abundant than expected and *O. terminatus* are lower than expected. The more frequent preservation of *Nephrolenellus geniculatus* opisthothoraces compared to *O. terminatus* suggests that the *N. geniculatus* thorax was less prone to disarticulation after death or moulting. This may contribute to the higher-than-expected percentage of cephalothoraces for *N. geniculatus* relative to *O. terminatus*. However, this difference does not account for the lower-than-expected percentage of cephalothoraces for *O. terminatus* compared to all other *Olenellus* species (Table 1). Similarly for hypothesis ii) a higher predation pressure on *N. geniculatus* coupled with an alternative injury source and significant differences in the MSM modes between the

five species are required to produce the relative proportions of articulated specimens for *N. geniculatus* and *O. terminatus* (Table 1). However, assuming there is no difference in the predator efficiency, a higher predation pressure on *N. geniculatus* compared to *O. terminatus* would result in fewer articulated *N. geniculatus* than expected, with more *O. terminatus* (as successful predation would remove these from the record). This is the opposite of what is observed at Ruin Wash. In addition, exuviae with attached cephalon an thorax may also have been disturbed – a study using models of Mesozoic oysters showed that predators in the modern ocean (specifically crabs) attack suitable-looking prey even when no soft parts or nutrition are available (LaBarbera 1981). Such behavior at Ruin Wash (the action of a different, now long extinct predator) would have removed additional cephalothoraces of taxa experiencing the highest predation pressure. For hypothesis ii) to hold, an additional further mechanism is needed to explain the lower percentage of articulated *O. terminatus* compared to other *Olenellus* species, as under this hypothesis *O. terminatus* experienced the lowest predation pressure. Interspecific differences in the use of the Salterian Moulting mode could provide this.

In summary, hypothesis iii) is considered the most likely explanation for the differences in repair frequency and preservation of articulated material reported here, as it requires no additional explanation beyond predation pressure. Hypotheses i) and ii) cannot be completely ruled out, as we do not know the relative frequencies of the moulting mode used by these taxa. However, these differences in moulting mode would have to align perfectly to exactly counteract the signal from higher predation efficiency or pressure on *Nephrolenellus geniculatus*.

5.2 Moulting – a non-predatory cause of injury frequency differences?

Spine length could potentially influence another, non-predatory, cause of injury: problematic moulting. Longer spines may be damaged more often than shorter specimens during moulting (Babcock 1993). However, in this study the T3 injury frequency (the macro- or dolichospinous segment) is the same as for other pleural spines. Additionally, the incidence of injuries is highest in *Olenellus terminatus*, and lowest in *Nephrolenellus geniculatus,* which have similarly sized prothoracic segments, except for T3. This suggests that either self-inflicted moulting injuries are not the major cause of shortened spines in, or that spine length does not influence the likelihood of moulting injuries for these animals.

A second consideration is whether differences in the relative frequency of the moulting mode (MSM or Salterian), with no differences in predation efficiency or intensity, could produce the observed differences in the frequency of predator-induced injuries. Modelling approaches using data from modern crabs have shown that the expected size-distribution of corpses and exuviae in fossil assemblages are very similar (Hartnoll and Bryant 1990). This is because corpses and exuviae together comprise the total number of individuals to reach each instar (Hartnoll and Bryant 1990). Assuming that the presence or absence of an injury did not affect moulting mode used by the a given trilobite, the proportion of exuviae preserving a cephalothorax (through the MSM mode) and injured carcasses should be the same. The repair frequency of a species (which is calculated using both carcasses and exuviae) is therefore unaffected by differences in relative frequency of moulting mode, assuming that the presence of an injury does not affects the moulting mode used by a given trilobite.

5.3 Spines as a predation deterrent?

The variation in predation intensity across taxa requires consideration. Differences in spinosity between the taxa provides a possible explanation; however, there are a number of different spine morphologies and locations on these taxa, and we discuss each in turn. As the predation intensity on *Olenellus* was likely higher than on *Nephrolenellus*, there is no evidence that the axial T15 spine acted as a predatory deterrent for attacks to pleural lobes (a T15 spine is present on all *Olenellus* taxa but absent in *Nephrolenellus*). This does not preclude this axial spine from having performed a defensive or deterrent function for attacks to other body parts, such as the axial lobe. Similarly, although genal spines would have overlapped and perhaps offered some defense for the anterior prothorax, the relative length of these spines does not appear to have a relation to the inferred predation intensity. Thus they were unlikely an effective deterrent for attacks on the prothoracic pleural lobes. Genal spines may have played helped defense or deter attacks to the anteriormost segments of the prothorax, or the cephalon. A larger sample size of injuries is required to test these hypotheses (only three injured cephala were identified in this study).

When considering the thorax as a whole, *Olenellus fowleri* is arguably the most well defended taxon, with longer pleural spines on all segments except T3 relative to the other taxa assessed here. Conversely, *O. terminatus* and *Nephrolenellus geniculatus* have the shortest pleural spines. Although *O. terminatus* has the highest incidence of repair, the apparently well-defended *O. fowleri* has the second highest, and *N. geniculatus* the lowest. There is therefore no clear relationship between prothoracic pleurae spinosity and predation intensity, when all thoracic spines are considered together.

There is some support for the elongate T3 pleural spine acting as a deterrent. This spine is longest (dolichospinous) in *Nephrolenellus geniculatus*, the species that experienced the lowest predation intensity. As this spine extends to the posterior of the prothorax in all

species considered, a dolichospinous T3 may have offered no more protection than a macrospinous T3. Thus any damage differences would be expected on the opisthothorax, as this area is protected by the dolichospinous condition but not the macrospinous. However the injury frequency on T3 compared to other thoracic segments does not provide evidence supporting a role for these spines in active defense as the frequency of T3 spine injuries is statistically indistinguishable from injuries on other thoracic pleural spines. Instead, we suggest that T3 spines likely acted primarily as a deterrent, dissuading predators from attacking altogether (and thus resulting in a lower predation intensity). Thus a trilobite with a longer spine may have been a less appealing target for a predator than one with shorter spines, resulting in *N. geniculatus* experiencing the lowest predation pressure with its doliichospinous T3, compared to the macrospinous *Olenellus*. As *Olenellus fowleri* has the relatively shortest T3 spines, it is possible that the remaining thoracic pleural spines provided less substantial deterrent. This would explain why *O. terminatus* has the highest repair frequency with *O. fowleri* second highest. However, as the confidence intervals for *O. terminatus* and *O. fowleri* overlap substantially (Table 2), perhaps no such secondary deterrent explanation is required, and a larger sample size would reveal a higher repair frequency in *O. fowleri* than *O. terminatus*.

5.4 Other explanations

Size is not considered a major factor affecting predation pressure, as the lengths of injured and non-injured trilobite cephalothoraces are not statistically distinguished, however size may have played a minor role. Injured trilobites are on average larger than non-injured trilobites (Fig. 6). Additionally, *Olenellus fowleri* and *O. terminatus,* the taxa with the highest repair incidences and consequently the highest inferred predation pressure,

have the largest mean and median sizes (Table 3). This may suggest that larger trilobites (regardless of the species) experienced higher predation pressure than smaller trilobites, perhaps as they offer a more substantial 'reward' for successful predators. The overall smaller size of *Nephrolenellus geniculatus* may therefore partly explain the lower predation pressure experienced by this species.

Other potential explanations for the lower predation pressure on *Nephrolenellus geniculatus* include behavioral adaptations. Predation pressure, as measured here, reflects failed fatal attacks on trilobites which caused some damage. Interactions with predators are more complex than just attacking, as predators must observe, catch, then kill prey. If *N. geniculatus* was more effective at hiding or escaping from predators, the number of injury-causing events would be lower, regardless of the number of interactions, producing the observed signal. Unfortunately, we do not have knowledge of the relative hiding, escaping, and surviving capabilities of these taxa, and so we are limited to considering morphological features and injury-causing events.

5.5 Location-selectivity of repair

Thoracic injuries are far more common than cephalic examples in this study. Five cephalic injuries are present in the literature (Resser and Howell 1938; Alpert and Moore 1975; Cowie and McNamara 1978; Babcock 1993, 2007; Skinner 2004; Bicknell and Paterson 2018; Bicknell et al. 2018c). Two abnormal *Olenellus gilberti* prothoraces in Ruin Wash trilobites were reported by Palmer (1998, figs 9.2, 9.4), and thoracic injuries are known in other *Olenellus* taxa (Rudkin 1979; Whittington 1989), the closely related nevadiid trilobites (*sensu* Lieberman 1998) *Buenellus higginsi* (Babcock and Peel 2007), and *Nevadia weeksi* (Whittington 1989). No axial injuries are known to any Cambrian trilobite (Owen 1985;

Babcock 1993; Bicknell and Paterson 2018), supported by the data in this study. Higher repair frequency of injuries to pleural lobes compared to axial lobes and cephala adds support to previous suggestions that trilobites are more likely to survive damage to pleural lobes than other exoskeletal parts. This is because vital nervous, alimentary and circulatory organ systems were likely housed in the axial lobe and cephalon (Babcock 1993), and likely exacerbated by the difficulties in identifying biologically (rather than taphonomically) damaged axial spines.

No evidence for thoracic injury stereotypy was found. This contrasts with data in Babcock and Robison (1989) and Babcock (1993), where a right-side injury stereotypy for Cambrian trilobites was demonstrated. The absence of stereotypy in this study mirrors the findings of Pates et al. (2017), for injuries to *Eccaparadoxides pradoanus* from the Murero Formation. Evidence for injury lateralization on Cambrian trilobites presented by Babcock may potentially reflect lumping data from multiple taxa, across different temporal and geographical regions, masking taxon-specific palaeoecological signals. Further work exploring location-specific and taxon-level sampling of injuries on Cambrian trilobites is needed. A useful avenue would be the targeted analysis of taxa in the bulk sample of Babcock and Robison (1989) and Babcock (1993), to determine which individual species were responsible for the right-sided signal.

6. Conclusions

This is the first study to show evidence for predator selectivity on trilobites from the same site and suggests that phenotypic differences resulted in differences in predation intensity. We identified injuries on thoraces and cephala of five trilobite taxa from the Ruin Wash *Lagerstätte: Olenellus chiefensis, O. fowleri, O. gilberti, O. terminatus,* and

Nephrolenellus geniculatus. Thoracic injuries are far more common than cephalic injuries. The highest injury repair frequency of the five taxa was found for *O. terminatus* and the lowest for *N. geniculatus*. Differences in repair frequencies are likely a result of predation intensity, not predator (in)efficiency. This is suggested as the taxon with the highest percentage of articulated remains experienced the lowest repair frequency. If instead predation on this taxon was the most efficient, *N. geniculatus* would be expected to be the taxon with the lowest percentage with articulated remains.

We propose that elongate spines on T3 spines on these taxa acted as predator-deterrent features for this group. Of the proposed measures of spinosity, the elongate spines on T3 offer the best case for a morphological feature affecting predation intensity. These spines were not used in active defense, and instead it is suggested that T3 pleural spines on olenelline taxa were predation deterrents in the Ruin Wash *Lagerstätte*. The role of genal or axial T15 spines as predation deterrents or in defense of the axial lobe or cephalon could not be tested as too few injuries were recorded on these parts. Size may have played an additional minor role in predator selection as injured trilobites were on average larger than trilobites without injuries, but no statistical support for this was found.

7. Acknowledgments

8. References

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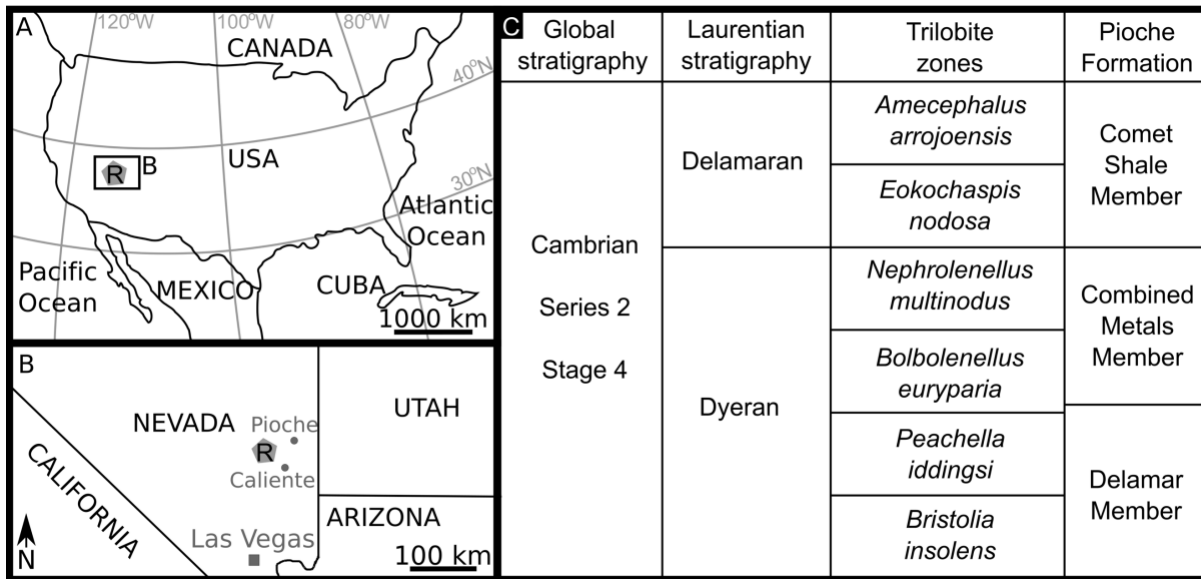R Core Team. (2014). R: A language and environment for statistical computing.

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Competing interests

There are no competing interests to report

| Global stratigraphy	Laurentian stratigraphy	Trilobite zones	Pioche Formation
Cambrian Series 2 Stage 4	Delamaran	*Amecephalus arrojoensis*	Comet Shale Member
		Eokochaspis nodosa	
	Dyeran	*Nephrolenellus multinodus*	Combined Metals Member
		Bolbolenellus euryparia	
		Peachella iddingsi	Delamar Member
		Bristolia insolens	

Fig. 1. A. Map showing geographic location of the Ruin Wash *Lagerstätte* (R in grey pentagon). **B.** Inset from A. **C.** Global and local stratigraphy with trilobite zones of Ruin Wash and neighbouring units. The Ruin Wash *Lagerstätte* is found at the top 43 cm of the Combined Metals Member, and is in the *Nephrolenellus multinodus* biozone. A small-scale map showing the detailed position of localities can be found in Webster et al. (2008, fig. 1).
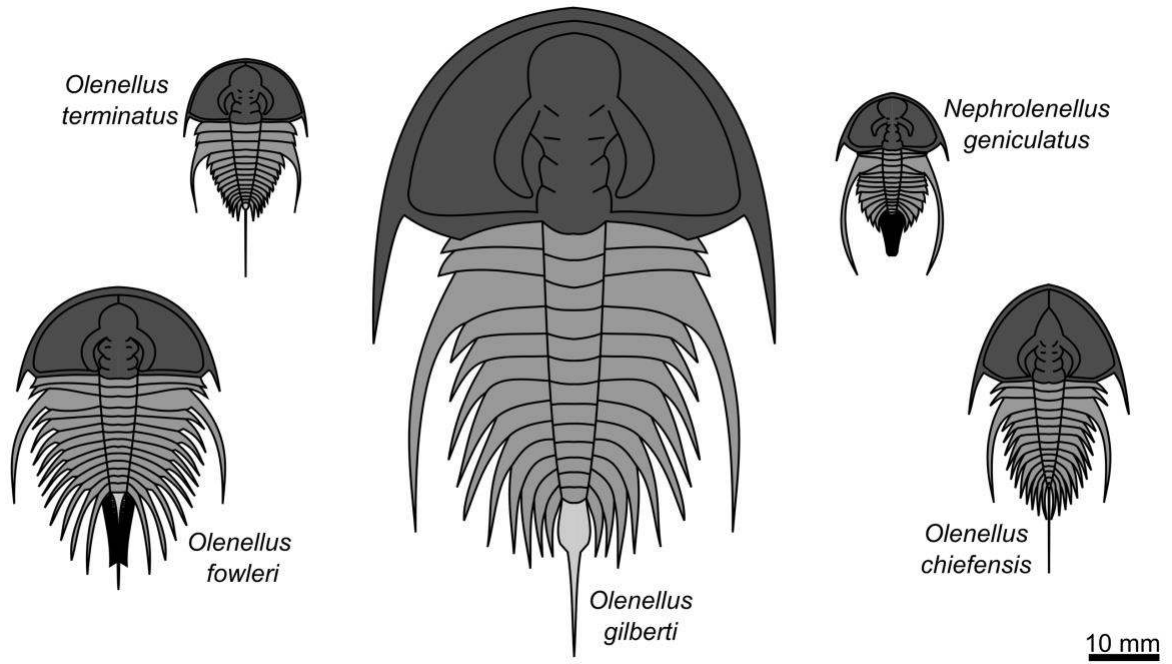
Fig. 2. The five most abundant taxa at Ruin Wash *Lagerstätte*, shown at their maximum measured sizes measured in this study. Cephalon in dark grey, prothorax in medium grey, spine on 15th thoracic segment in light grey. Opisthothorax visible as black shading in *Olenellus fowleri* and *Nephrolenellus geniculatus*.
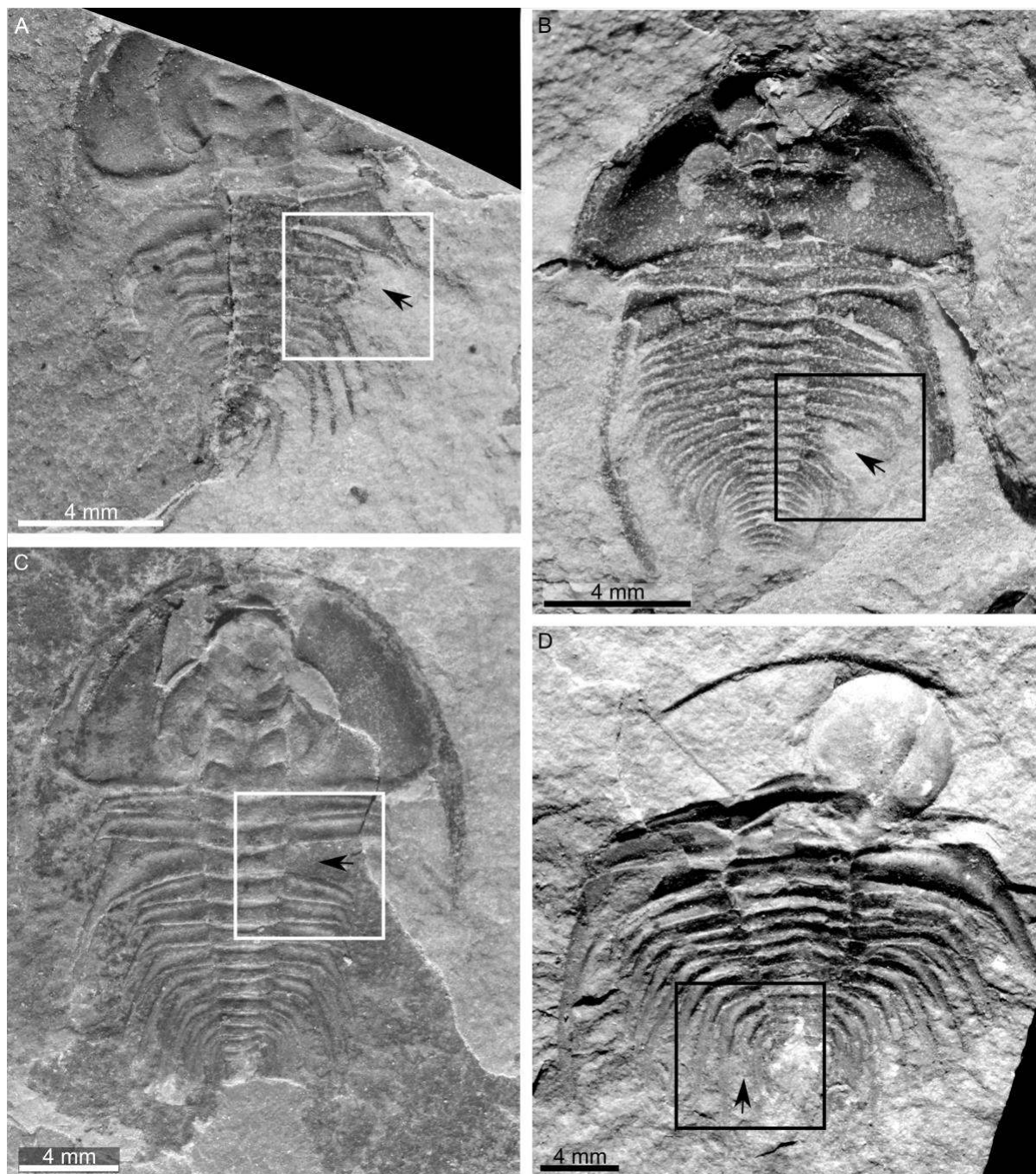
Fig. 3. Examples of repaired injuries on Ruin Wash trilobite cephalothoraces. **A, B.** Injuries to two adjacent thoracic segments. **C. D.** Single spine injuries. **A.** MCZ 188159, *Olenellus gilberti*. **B.** MCZ 188351, *Nephrolenellus geniculatus*. **C.** MCZ 188149, *Olenellus gilberti*. **D.** MCZ 188467, *Olenellus fowleri*. Injuries marked by arrows, boxes show areas inset figured in Fig. 4. Image credit: Museum of Comparative Zoology, Harvard University.
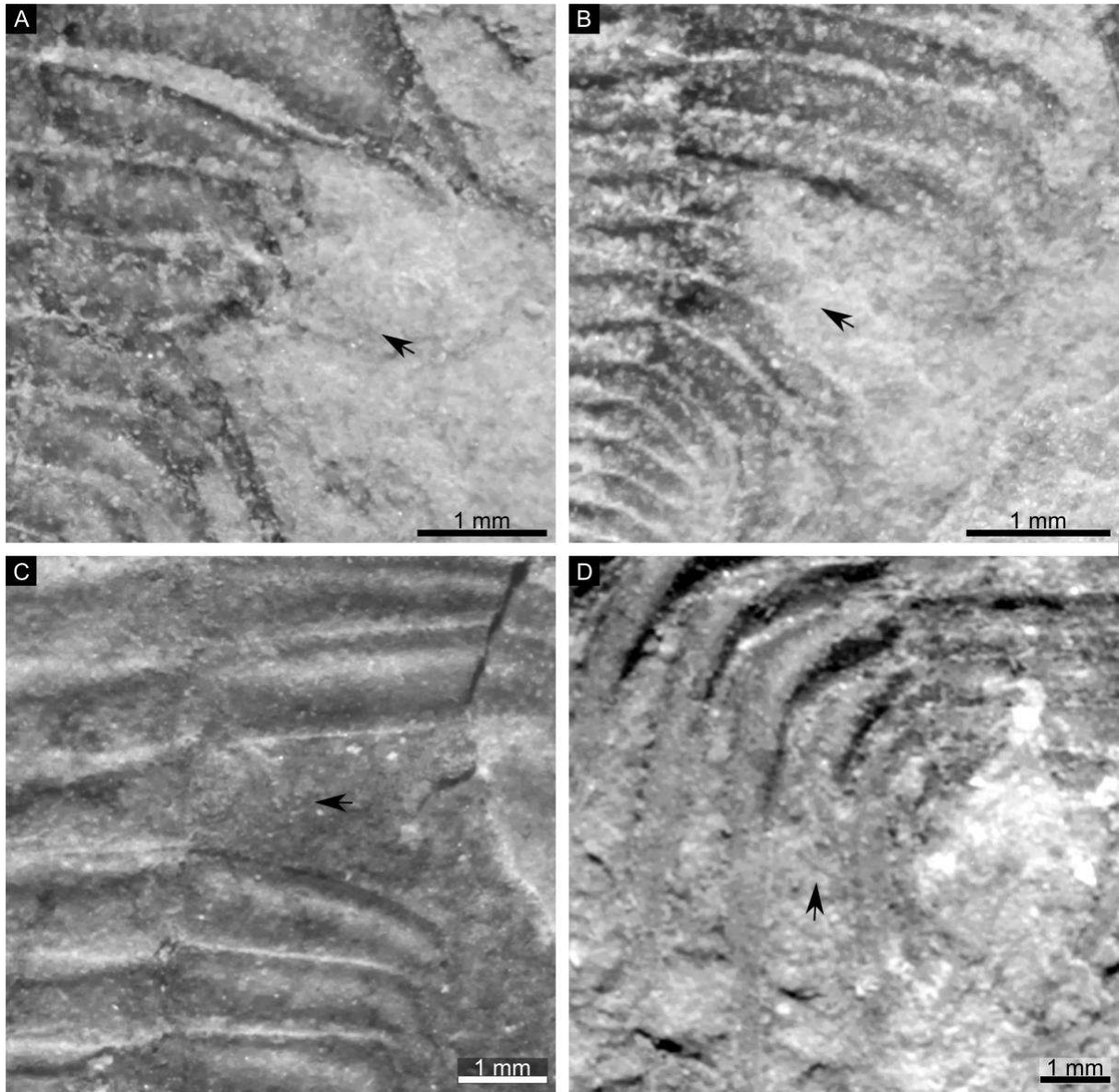
Fig. 4. Enlarged areas showing repaired injuries from Figure 3. **A.** MCZ 188159, *Olenellus gilberti*. **B.** MCZ 188351, *Nephrolenellus geniculatus*. **C.** MCZ 188149, *Olenellus gilberti*. **D.** MCZ 188467, *Olenellus fowleri*. Injuries marked by arrows. Image credit: Museum of Comparative Zoology, Harvard University.

Fig. 5. Examples of repaired injuries on Ruin Wash isolated *Olenellus* cephala. **A.** *Olenellus fowleri* cephalon with cicatrized injury on the right hand side. FMNH PE 58503. **B.** *Olenellus terminatus* cephalon with indent to the right hand side. FMNH PE 58505. **C.** *Olenellus terminatus* with bilateral injury and missing genal spines. FMNH PE 58504. Injuries marked by arrows.
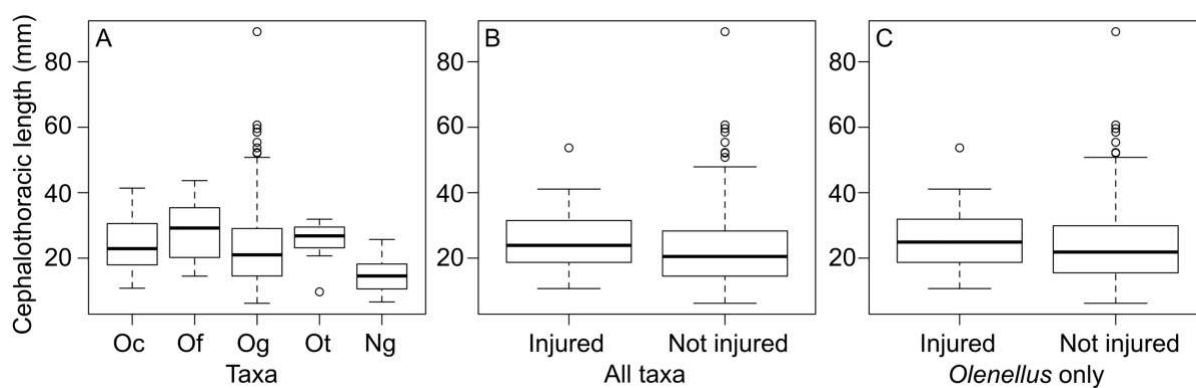
Fig. 6. Boxplots showing relative lengths of trilobite cephalothoraces from Ruin Wash *Lagerstätte* included in this study. **A.** Each of the five taxa studied, Oc = *Olenellus chiefensis*, Of = *Olenellus fowleri*, Og = *Olenellus gilberti*, Ot = *Olenellus terminatus*, Ng = *Nephrolenellus geniculatus*. **B.** Lengths of all injured trilobites and all not injured trilobites. **C.** Lengths of all injured *Olenellus* trilobites and all not injured *Olenellus* trilobites.

Table 1. Relative proportions of each taxa in this study compared to field sample

	N cephalothoraces (this study)	N cephala (Webster et al. 2008)	N cephalothoraces (Webster et al. 2008)
Olenellus chiefensis	60 (20%)	47 (24%)	1 (6%)
Olenellus fowleri	17 (6%)	16 (6%)	1 (6%)
Olenellus gilberti	163 (55%)	131 (52%)	9 (53%)
Olenellus terminatus	11 (3%)	27 (11%)	0 (0%)
Nephrolenellus geniculatus	47 (16%)	28 (11%)	6 (35%)

Number of specimens used in this study, and reported from a field study by Webster et al. (2008). Raw values given for each of the five taxa considered in this study, with the percentage of each group made up by that taxon given in brackets. Cephalothoraces refers to a specimen where at least the cephalothorax is present. Cephala refers to isolated cephala.

Table 2. Repair frequencies for individual taxa

Taxon	N	Inj.	F, R	5%	95%
Olenellus chiefensis	60	5	0.083	0.044	0.12
Olenellus fowleri	17	2	0.12	0.047	0.31
Olenellus gilberti	163	12	0.074	0.048	0.12
Olenellus terminatus	11	2	0.18	0.072	0.44
Olenellus sp.	32	3	0.094	0.042	0.22
Olenellus (total)	283	24	0.088	0.062	0.12
Nephrolenellus geniculatus	47	2	0.043	0.017	0.13

Number of specimens (N), number of injured specimens (Inj.), repair frequency (F, R), and 5th and 95th percentile Bayesian Inference values for each taxon considered in this study.

Table 3. Length distributions

Taxon	Smallest	1st quartile	Median	Mean	3rd quartile	Largest
Olenellus chiefensis	10.80	18.02	22.90	24.78	30.48	41.40
Olenellus fowleri	14.50	20.20	29.20	27.92	35.40	43.70
Olenellus gilberti	6.20	14.55	21.00	23.92	29.05	89.20
Olenellus terminatus	9.70	23.15	26.80	25.40	29.50	31.90
Nephrolenellus geniculatus	6.60	10.60	14.55	14.61	18.20	25.70
Injured (all taxa)	10.70	18.75	23.90	25.72	31.43	53.70
Not injured (all taxa)	6.20	14.50	20.50	22.69	28.30	89.20
Injured (*Olenellus* only)	10.70	18.70	24.90	26.62	31.90	53.70
Not injured (*Olenellus* only)	6.20	15.53	21.85	24.26	29.90	89.20

Length distributions for taxa considered in this study. All lengths in mm.