THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Decay of the sea anemone Metridium (Actiniaria): Implications for the preservation of softbodied diploblast-grade animals

OPEN ACCESS

Research Article
DECAY OF THE SEA ANEMONE *METRIDIUM* (ACTINIARIA): IMPLICATIONS FOR THE PRESERVATION OF CNIDARIAN POLYPS AND OTHER SOFT-BODIED DIPLOBLAST-GRADE ANIMALS

SEAN MCMAHON, LIDYA G. TARHAN, and DEREK E.G. BRIGGS
Department of Geology and Geophysics, Yale University, 210 Whitney Avenue, New Haven, Connecticut 06511, USA
email: sean.mcmahon@yale.edu

ABSTRACT: Fossils preserving the anatomy of soft tissues provide rare but essential evidence for the reconstruction of metazoan evolutionary history. Decay is inherent to the fossilization process and features may be distorted, displaced, or missing even in exceptionally preserved fossils, and non-anatomical artifacts may be introduced. Here we describe the results of experimental decay of the epibenthic actinian (sea anemone) *Metridium senile* and document serial changes in its morphology. Decay proceeded rapidly and followed a consistent, reproducible trajectory, which we divide into six stages; in the final stage, no anatomically illuminating information remained. The column, one of the most salient anatomical features of actinians, contracted near the time of death and changed shape dramatically thereafter. The tentacles decayed from their distal ends. Fibrous bundles representing the interior musculature were among the morphological features most resistant to degradation, and taphonomically induced transverse bands were also long lasting. These experimental results provide a semi-quantitative and predictive framework which can be applied to the interpretation of putative polypoid cnidarian organisms. Furthermore, this decay series shows that diploblast- and triploblast-grade fossils are unlikely to be confused and provides the means to evaluate the taxonomic validity of the long-standing assumption that many enigmatic soft-bodied Ediacaran and lower Cambrian taxa are of actinian grade.

INTRODUCTION

Although scarce relative to fossils of biomineralized taxa, fossils preserving soft tissues yield unique ecological and evolutionary insights. However, they are not facsimiles of living or freshly killed organisms but snapshots of the process of decay, in which tissues and organs are distorted, displaced, or lost, and non-anatomical taphonomic artifacts are introduced, impeding the recovery of fundamental biological information. The goal of experimental taphonomy is to reconstruct how and to what extent taphonomic processes have filtered anatomical information, in order to facilitate the reconstruction, based on fossil specimens, of original morphology. The serial decay of modern analogue organisms under controlled environmental conditions and comparison of experimental results to the morphology of fossil taxa provide powerful tools for elucidating this taphonomic 'gap'. In recent decades, experimental work has been successful in demonstrating how decay obscures anatomical information and thus taxonomic affinity, and illuminating the nature of historically problematic forms (e.g., Sansom et al. 2010; Raff and Raff 2014; review by Briggs and McMahon 2016). So far, this experimental program has largely focused on triploblastic animals (particularly worms and arthropods). Patterns and processes of decay in the diploblasts—animals such as cnidarians and ctenophores, which possess only two primary tissue layers—are less well resolved. However, an array of fossils, particularly those from Ediacaran–Cambrian strata, has been attributed to cnidarians, including actinians (Actiniaria: the sea anemones).

Interpretation of Ediacaran–Cambrian soft-bodied fossil forms as 'diploblast-grade', and particularly as actinian, frequently is premised on the presence of a limited number of non-diagnostic characters—e.g., radial symmetry or a columnar body plan—coupled with a lack of bilaterian synapomorphies (Conway Morris 1990). Such an assignment has, in some cases, verged on a 'waste-bin'-style solution to classification: macroscopic size, some level of morphological differentiation, and a lack of obvious macrophytic characters suggest a metazoan affinity but a lack of diagnostic bilaterian characters prohibits assignment to Bilateria.

The difficulty of interpreting putatively cnidarian fossils is compounded by a poor understanding of how the morphology of diploblast-grade taxa is altered by taphonomic processes. Previously published experimental taphonomic studies of cnidarians (Norris 1989; Bruton 1991) have been limited to analysis of the impressions made by hydrozoan and scyphozoan medusae and pennatulids in wet sand (Briggs 1995). There is a pressing need for decay experiments on diploblastic organisms to develop a predictive framework for the decay and fossilization of cnidarians, as well as to ground-truth and refine the taxonomic validity of fossil forms long presumed to have diploblastic affinities. New diploblast-focused experimental taphonomy is critical to provide a resource for the interpretation of fossils, particularly enigmatic taxa from intervals of Earth history contemporaneous with the appearance and radiation of metazoans. Here we report the results of an experimental investigation of the serial decay of the epibenthic actinian *Metridium senile*, which provide a prediction for which actinian synapomorphies are likely to be retained during fossilization, and in what sequence these features are lost or taphonomic artifacts introduced during decay. Our results provide a sequence of decay stages, which represent a semi-quantitative predictive framework that can be applied to the fossil record of putative diploblast-grade soft-bodied organisms. This sequence can also be used for the assessment of taphonomic fidelity and likely loss of anatomical characters. More broadly, actualistic decay data provide a means to evaluate the validity of the long-standing assumption that many enigmatic soft-bodied Ediacaran and lower Cambrian taxa are of actinian grade.

MATERIALS AND METHODS

Metridium senile is a large, plumose, epibenthic anemone and a widespread colonizer of hard substrates in northern marine waters (Fig. 1). The body wall is smooth, brown, translucent, and finely patterned with transverse and longitudinal lineations, which express the internal muscle fields. When the column contracts, the body wall folds and buckles. The pedal disc, which anchors the anemone to its substrate, is slightly broader than the column, and has an irregular margin. The oral end of the body is delineated by a sharp fossa around the column, defining a collar, over which the remainder of the body wall hangs. The plume of tentacles, which is whitish and translucent with longitudinal lineations, terminates in a convoluted fringe covered in hundreds of fine-pointed tips. The mouth/anus at the center of the oral disc is about one-third the width of the column, and pinches out at the sides into (typically) two grooves (siphonoglyphs). The wall of the pharynx is patterned with longitudinal ribs that terminate as slight radiating protuberances around the mouth, giving the latter a "puckered" appearance. Below the pharynx lies the broad coelenteron (gastric cavity). Around the coelenteron are the thread-like, nematocyst-bearing acontia. The gastrovascular cavity is divided radially by septum-like mesenteries, which extend longitudinally between the pedal disc and the oral disc.

A number of attributes of *Metridium senile* recommend it as a model organism for the taphonomic study of cnidarian polyps. First, individuals are large and morphologically well differentiated, with clearly defined features that make it possible to track the sequence of decay: pedal and oral discs, latitudinal and transverse lineations on the body wall, and structurally complex tentacles. Thus, the progressive transformation and/or loss of several

2

discrete features can be readily tracked. Second, these anemones contract immediately in response to tactile stimulation and relax again shortly afterwards. This behavior persists until very near death, and enables the time of death to be determined. Third, this species is widely distributed around the world and its biology has been well studied over many decades. Thus specimens for experimental analysis and detailed anatomical information are readily available.

Eight living specimens of *Metridium senile*, each about 10 cm in height (when fully extended) were obtained from the Marine Biological Laboratory, Woods Hole, MA, USA. Each anemone was transferred to an individual glass bowl (Pyrex, 950 ml) filled with artificial seawater which was made according to the recipe of Berges et al. (2001) and sterilized in an autoclave. The anemones were prodded with a glass rod and the occurrence or non-occurrence of the contraction reflex was used to constrain the time of death to within approximately 15 hours. Prodding usually also stimulated the expulsion of the thread-like, nematocyst-bearing acontia, which were also ejected spontaneously by some specimens when close to death. All specimens died within three days of transfer to the experimental vessels, probably from starvation rather than asphyxiation, since the vessels were not lidded and *M. senile* is highly tolerant of oxygen depletion (Wahl 1984). We allowed the anemones to die passively, rather than killing them with chemical poisoning, freezing, or high temperatures, as such methods might have destroyed tissues or modified their volatility and thus compromised our taphonomic data. Rather than introducing an exogenous inoculum, the carcasses were allowed to decay under the influence of the microbial communities already associated with them. After death, the vessels were lidded and sealed with parafilm. The vessels were placed in an incubator set to 25 °C (approximating room temperature) in order to minimize temperature variation resulting from either sampling or diurnal fluctuation. As the object was to establish a predictive baseline for serial decay, rather than to investigate the role of particular environmental conditions upon sample taphonomy, we did not bury the anemones in sediment. Further, burial would have made it impossible to track the sequence of decay in individual specimens (as sampling would then have required exhumation and disruption of the specimens, effectively terminating the experiment). The specimens were photographed daily over the following seven days; by the end of this interval, all morphological details had been lost. In the later stages of decay, visibility of the specimens was commonly obscured by turbidity due to microbial proliferation in the seawater medium and the development of biofilms at the water-air interface. Water and biofilms were therefore siphoned into secondary vessels with a plastic serological pipette during each data collection session and poured back in as soon as the carcass had been photographed. These fluid transfers were conducted as gently as possible to avoid disturbing the tissues.

RESULTS

The decaying anemones rapidly changed in color, luster, texture, and three-dimensional shape. Concurrent changes in these parameters facilitated the identification of six discrete stages of decay (from Stage 1: carcass pristine, to Stage 6: tissues thoroughly degraded), defined as follows (Fig. 2):

Stage 1 (Fig. 2A)—The body of the fresh carcass was smooth, glossy, slightly translucent and richly hued, with both transverse and longitudinal lineations clearly visible. The tentacles remained fully extended and the mouth/anus was visible. For specimens in which the body was fully extended, the pedal disc was observed to be broader than the column.

Stage 2 (Fig. 2B)—This stage was defined by the darkening of the body wall, which also became less lustrous and less translucent, and dimmed to an orange-brown color. The tentacles became more opaque and began to soften in definition and lose their fine tips so that they appeared less sharply pointed. These were the first visible postmortem changes. Even

individual anemones that were extended prior to death began to contract shortly thereafter, resulting in a more bulbous shape, such that the width of the column was greater than the pedal disc. The oral disc also narrowed, and the tentacles were partially retracted. Acontia were ejected from some specimens (apparently passively) at this time.

Stage 3 (Fig. 2C)—This stage was defined by a dramatic change in the exterior appearance of the column, which developed a reticulate and pustulose surface texture, and darkened to a dull gray-brown. Longitudinal and fine transverse lineations (corresponding to underlying muscle fields) were lost. As the column contracted, deep transverse creases (more widely spaced than the fine transverse lineations) developed in the ectoderm. The pedal disc widened and its margin became lobate; the oral disc narrowed dramatically and its collar began to lose definition. The tentacles continued to lose definition, becoming more truncated and losing the appearance of tapering; they assumed the appearance of fibrous tufts. By this stage, all individuals that were originally oriented vertically (i.e., attached by their pedal disc to the base of the glass vessel) were lying horizontally on the bottom of the vessel.

Stage 4 (Fig. 2D)—During this stage, the wall of the column became spongy and began to slough away. In four of the eight specimens, surfaces exposed by this sloughing thinned and extruded into blisters under the outward pressure of trapped gases (visible bubbles). In most specimens, the pedal disc ruptured, and white, fibrous, gelatinous internal matter (e.g., gastrodermis and mesoglea) was extruded through the opening. This rupture likely resulted from the microbial gas production that generated the blistering, and was perhaps enhanced by increased coelenteric fluid pressure associated with contraction of the oral disc and column. Deterioration of the tentacles continued; they were truncated near the base, and in four of the eight specimens the body wall was observed to have peeled away from the fossa.

Stage 5 (Fig. 2E)—At this stage the column lost structural integrity, almost entirely collapsed, and spread outward. The tentacles were no longer discernible. The remains of the body wall became sparse and diffuse, exposing a pink and white mass of degraded coelenteral or muscle material. In some specimens, the outermost brown layer of the wall disappeared, exposing an interior, white layer (Fig. 2E) still retaining the creases introduced during Stage 3, which constituted the only well-defined structures remaining. Wispy, fibrous bundles of internal tissue emerged from ruptures in the column walls.

Stage 6 (Fig. 2F)—At this final stage, the gross structure was poorly defined; the anemone was a cloudy, disorganized and diffuse mass of tissues, spread out across the bottom of the vessel. Some density stratification occurred: pinkish flakes sank to the bottom while diffuse, wispy remains of muscle fibers floated at the top of the seawater medium.

All eight specimens decayed at approximately the same rate. With the exception of two specimens on Day 2, every specimen remained within +/- 1 stage of the average reached by the eight specimens on any given day (Fig. 3). Stage 6 was achieved by each specimen by 4–6 days after death (Fig. 3).

DISCUSSION

The Effect of Decay on Morphology

The physical conditions in our experiments were not intended to mimic the environmental conditions under which an anemone might be fossilized. The objective was not to test the role played by particular environmental factors (e.g., rapid burial or authigenic mineralization, which may impede or delay decay) in the preservation of actinian-grade fossils, but to track the sequence of decay of salient morphological features, in order to reconstruct the *relative* longevity of particular anatomical characters and differentiate primary (anatomical) from secondary (taphonomically introduced) features. Therefore, the absolute time associated with each decay stage in the experiments should not be interpreted as a literal predictor for duration of decay. It is the patterns observed across the eight specimens that facilitate a

number of predictions concerning the preservation of cnidarian soft tissues, particularly those of polypoid cnidarians. These experiments indicate that anemones are unlikely to be preserved in the fully extended life-state because the column contracts and rapidly loses volume upon or immediately preceding death. Although tentacles typically remain partially extended after death, they decay from the distal end, becoming increasingly squat and truncated in appearance; thus, any comparison of tentacle shape between fossil specimens is as likely to reveal taphonomic as taxonomic differences. Since the flexible column can swell, pinch and deform rapidly and unpredictably as the coelenteric fluid is redistributed, both during life and over the course of postmortem decay, a high degree of morphological variability is expected between specimens of the same taxon, without reflecting taxon-level anatomical differences. Textures observed on the ectoderm of the column wall are likely to be taphonomic artifacts rather than primary ornamentation. The pustulose and reticulate texture observed on the column wall of *Metridium* is a postmortem feature which appeared early in the experiments (Stage 2) and may be evident in fossil cnidarians (cf. the rough surface of the soft-bodied Chengjiang fossil *Xianguangia sinica* [Hou et al. 2005]; see below for further comments on this taxon). Although fossil anemones are likely to preserve strong transverse ridges along the column wall, these may, like those in *Metridium*, relate to contraction, furrowing, and degradation of columnar muscle fibers (decay Stage 3). These features could be confused with genuine anatomical structures such as the ectodermal expression of transverse musculature which, in *Metridium*, consisted of notably finer-scale, relatively short-lived features (Fig. 2C). Even if individuals were fossilized prior to reaching this decay stage, finer lineations and fibrous textures are more likely to represent musculature or its expression on the ectoderm than true ornamentation (Fig. 2E).

Ediacaran and Cambrian Organisms Interpreted as Cnidarians

There has been a tendency to consider many of the discoidal, frondose, 'sac-like', or other enigmatic Ediacaran taxa as having cnidarian affinities (Conway Morris 1990). Putative polypoid cnidarians of Ediacaran age have been described from the Doushantuo and Lantian Formations of China, the Ediacara Member of Australia, the lower Fermeuse Formation of Newfoundland, and from assemblages of tubular fossils worldwide.

The Lantian Biota of South China, which occurs within Member II of the Lantian Formation—lithostratigraphically and chemostratigraphically correlated to Member II of the Doushantuo Formation (632.5 Ma; Condon et al. 2005; Yuan et al. 2011)—contains a rich assemblage of exceptionally preserved carbonaceous compressions of macroalgae, including fan-shaped, conical, and filamentous forms (Yuan et al. 2011). The Lantian assemblage also includes a number of forms that appear unlikely to be macroalgae, including fossils of the "Type C" morphotype (Yuan et al. 2011). Type C fossils are characterized by a conical to pyramidal and fusiform body about 20 mm long, marked by longitudinal lineations (which have been interpreted as either ornamentation, retractor muscles, or mesentery-like structures), with a crown-like cluster of filaments at its apex. These features, together with the association of these fossils with taphonomically similar and presumably benthic, holdfast-bearing forms, has prompted the comparison of Type C fossils to cnidarian polyps (Yuan et al. 2011). However, if the pronounced postmortem contraction observed in *Metridium senile* is typical of anemones and also occurs under conditions of rapid burial, then the extended, elongate posture of Type C fossils does not support an actinian affinity.

Tubular fossils are a common and globally distributed component of upper Ediacaran fossil assemblages. They occur in a range of lithofacies and variety of taphonomic modes, from silicification to glauconization, phosphatization, preservation in carbonate, as carbonaceous compressions, and Ediacara-style preservation as sandstone casts and molds, and some have been interpreted to be of diploblast grade or even polypoid cnidarians (e.g., Droser and Gehling 2008; Liu et al. 2008; Cai et al. 2010, 2015; Cortijo et al. 2010; Penny et

al. 2014; Tarhan et al. 2014, 2016; Smith et al. 2016). Although exceptional preservation of Ediacaran tubular fossils is not uncommon, associated labile soft tissues (e.g., respiratory or feeding structures, or musculature) are not preserved, making assessment of their affinity challenging. Tubular taxa are typically macroscopic (> 1 mm in length), multicellular, and morphologically differentiated, and are therefore very probably eukaryotic. Such tubes comprised a significant portion of late Ediacaran biodiversity and are likely a polyphyletic group encompassing an assortment of organisms with organic tubes as well as the earliest macroscopic, animal-grade biomineralizing taxa such as *Sinotubulites* and *Cloudina*—taxa which have also been interpreted to have polypoid cnidarian affinities (e.g., Cortijo et al. 2010; Cai et al. 2015).

The Doushantuo Formation contains a diversity of phosphatized tubular fossils within the Weng'an and Miaohe Biotas, diverse macroalgae preserved as carbonaceous compressions or via phosphatization or silicification, and putative metazoan embryos preserved in phosphate. Among the phosphatized tubular microfossils of the Weng'an Biota, several taxa (e.g., *Ramitubus increscens, R. decrescens, Quadratitubus orbigoniatus, Crassitubus costatus,* and *Sinocyclocyclicus guizhouensis*) have been interpreted as stem-group or even crown-group cnidarians (Liu et al. 2008) although not without controversy (Cunningham et al. 2015). The cylindrical or tetragonal tubular morphology of *Ramitubus*, coupled with the presence of cross-walls, dichotomous branching and a large terminal chamber, have prompted its interpretation as a stem-anthozoan or stem-tabulate coral (Liu et al. 2008). *Protoconites minor* and *Calyptrina striata* from the Miaohe Biota, which are characterized by, respectively, cylindrical and conical organic tubes, have been interpreted as scyphozoans but their lack of diagnostic crown-group cnidarian characters has hindered confident taxonomic placement (Xiao et al. 2002). None of the tubular forms of the Doushantuo Formation retains evidence of internal soft tissues (e.g., polypoid structures).

Corumbella werneri, a tubular body fossil known from Ediacaran strata in Brazil and Paraguay, occurs as carbonaceous compressions and is characterized by a differentiated, bipartite morphology, consisting of an elongate tetragonal, flexible tube with transverse annulations and longitudinal ornamentation, terminating in an apical structure interpreted as a holdfast (Babcock et al. 2005). Scanning electron microscopy indicates that *Corumbella* possessed an organic carapace of polygonal carbonaceous plates, analogous to the ultrastructure of conulariids as well as certain early Paleozoic scyphozoan taxa (Warren et al. 2012). However, only the organic skeleton is preserved. Non-biomineralized tissues may vary significantly in their susceptibility to decay (e.g., cuticle versus muscle: Briggs and Kear 1993). The tissues within *Corumbella* and other tubular fossils were presumably soft and more labile than the kerogenous carapace and therefore, like the internal tissues of *Metridium*, decayed more rapidly leaving only the more recalcitrant tubes.

The late Ediacaran Ediacara Member of the Rawnsley Quartzite of Australia (taxonomically the richest Ediacara Biota fossil assemblage; Droser and Gehling 2015) includes a number of soft-bodied taxa, characterized by Ediacara-style preservation as sandstone casts and molds, which have been interpreted as poylpoid cnidarians. *Inaria karli* (Gehling 1988), which also occurs in the White Sea assemblage of northern Russia (Grazhdankin 2000), possesses a lobate disc in conjunction with a flattened axial tube. The lobes are separated by pronounced radiating grooves, and covered in fainter radiating lineations, features that have been interpreted as, respectively, the mesenteries and pharynx-hosted gastrodermal and longitudinal muscles of an epibenthic actinian (Gehling 1988). However, this interpretation has been challenged by Grazhdankin (2000) who interpreted the linear radiating features not as primary anatomical features but as nested creases resulting from the collapse of the organism under current-induced tensile stress (albeit still ordered to some extent by the underlying fibrous structure of the outer wall). Grazhdankin (2000)

therefore adopted a neutral stance on the position of *Inaria* within the eumetazoans. The transverse creases that appeared in the body wall of experimentally decayed *Metridium* in Stage 3, and were retained in Stage 4 (and to some extent Stage 5), are clearly the result of longitudinal contraction and passive collapse, and are similar in size and shape to the radiating lineations of *Inaria karli*. The difference in orientation between the transverse creases of *Metridium* and the radial ridges of *Inaria karli* may reflect the current- and sediment-mediated stress regime experienced by specimens of this taxon during decay and burial, rather than the original anatomy of the once-living organism, particularly as the internal structure of the wall of *Metridium* includes both transverse and radial/longitudinal elements (Fig. 2A). These observations support Grazhdankin's (2000) interpretation of the ridges in *Inaria* as the result of deformation of the outer wall, but they clearly do not negate the possibility of an actinian affinity for *Inaria*.

Eoandromeda octobrachiata, which is known from both the Ediacara Member (in Ediacara-style preservation) and the Doushantuo Miaohe Biota (as carbonaceous compressions), is characterized by an octoradial, spiral morphology (Zhu et al. 2008). This symmetry, and the presence of transverse bands along each arm, have led to the interpretation of *Eoandromeda* as a benthic diploblast-grade organism with potential cnidarian or ctenophore affinities (Zhu et al. 2008; Tang et al. 2011). Its spiral morphology and lack of bilateral symmetry, however, have thwarted confident placement in the crown group of either of these phyla. *Funisia dorothea*—one of several tubular taxa described from the Ediacara Member—is distinct in its arrangement of serial iterations attached to a holdfast and its occurrence in dense assemblages of up to > 1000 similarly sized individuals/m^2 (Droser and Gehling 2008). The density of these aggregations, coupled with the rarity of branching, indicates that the affinities of *Funisia* are more likely to lie with stem-group poriferans or stem-group cnidarians than with macroalgae (Droser and Gehling 2008).

Haootia quadriformis, which is preserved in positive and negative epirelief in the upper Ediacaran lower Fermeuse Formation of Newfoundland, Canada, and displays an approximately radiosymmetrical morphology, has also been described as a polypoid cnidarian (Liu et al. 2014). The finer closely spaced features observed on the surface of *Haootia quadriformis* do not recall the taphonomic creases observed in experiments on *Metridium* in either spacing or orientation, supporting their interpretation as musculature (Liu et al. 2014); they also compare well with muscle structures co-occurring with but clearly distinguishable from taphonomic creases in some fossil medusae (e.g., Leich 1995).

The Cambrian fossil record includes a number of soft-bodied forms which have been interpreted to have polypoid cnidarian, and in some cases actinian, affinities. The lowermost Cambrian Kuanchuanpu Formation of China yields *Eolympia pediculata*, a phosphatized microfossil (< 1 mm long) characterized by a cylindrical and radially symmetrical body associated with a stalk-like element (Han et al. 2010). Micro-tomographic analysis suggests that *Eolympia* possessed 18 paired mesenteries and 18 hollow tentacles. The presence of these features, in conjunction with the stalk-like element (interpreted as a pedicle), suggests that it may have been a solitary polyp within stem-group Hexacorallia, and thus both the oldest known anthozoan and the only known coral with fossilized polyps (Han et al. 2010; Van Iten et al. 2014). Other microscopic phosphatized putative polyps in the Kuanchuanpu include one probable scyphozoan and several possible (albeit controversial) cubozoans (Han et al. 2010; Van Iten et al. 2014).

The lower Cambrian Chengjiang Biota (Yu'anshan Shale Member, Qiongzhusi Formation) contains a rich fossil assemblage remarkable for its exceptional preservation of non-mineralized soft tissues. Among the Chengjiang forms is *Archisaccophyllia kunmingensis*, a compressed cylindrical fossil ~ 20 mm long with longitudinal ridges, associated with two discs and up to 12 tentaculate elements (Hou et al. 2005).

7

Archisaccophyllia has been interpreted as an actinian bearing intact oral and pedal discs, mesenteries, and a crown of tentacles (Hou et al. 2005). The longitudinal structures in the outer wall of the column of *Archisaccophyllia kunmingensis* are sharply defined, regular, equally spaced ridges that represent the primary feature of the fossil—Hou et al. (2005) suggested that these reflect the intersection of the mesenteries with the body wall. The clear preservation of this wall is in sharp contrast to the poor preservation of the tentaculate elements, counter to the pattern observed in the experiments reported here, where the tentacles and column deteriorated in tandem. These observations imply that the ectoderm of *Archisaccophyllia* was more robust than that of *Metridium*, and may cast doubt on its interpretation as an actinian. The good preservation of a circular pedal disc-like feature in *Archiscaccophyllia* also contrasts with the experimental finding that the pedal disc of *Metridium* ruptured relatively early; however, this result may partly reflect the fact that *Metridium* tended to fall over so that the disc was no longer attached to the base of the vessel. In the decay of the anemone *Actinia equine*, the pedal disc has been found to remain attached and to outlast the wall and tentacles (Adler 2013).

Other Chengjiang taxa characterized by a cylindrical morphology and tentaculate elements have been mooted as actinians, notably *Xianguangia sinica*, which has 16 branching feather-like "tentacles" or "arms" (Chen and Erdtmann 1991; Lei et al. 2014). The texture of the column in *Xianguangia* is irregular but the branching "tentacles" may reflect rapid preservation and/or inhibition of decay. The consistent straightness and rigidity of this organism are difficult to reconcile with an actinian affinity (Hou et al. 2005), however, not only because anemones are relatively flexible and even plastic in life (although some anemones are less flexible than *Metridium*, e.g., *Anthopleura xanthogrammica*), but also, as our experiments showed, because they deform after death. Thus other interpretations of *Xianguangia*—as a frondose taxon analogous to Ediacara forms (Han et al. 2010), a non-actinian anthozoan-like animal (Lei et al. 2014), or a biomineralized organism of unknown affinity (Hou et al. 2005)—may be more plausible.

Mackenzia costalis from the middle Cambrian Burgess Shale is a sac-like fossil bearing longitudinal ridges, which has also been suggested to have actinian affinities (Conway Morris 1990). Alternatively, *Mackenzia* has been interpreted as a problematic form without sufficient diagnostic characters to justify its placement in any living animal group (Hou et al. 2005). Certainly it does not resemble any stage in the decay of *Metridium senile*, which may suggest a non-actinian affinity.

Implications for the Differentiation of Putative Diploblastic and Triploblastic Taxa

Loss of characters due to decay can result in misinterpretation of the phylogenetic placement of a fossil (Donoghue and Purnell 2009; Sansom 2010; Briggs and McMahon 2016). The phylogenetic position of living cnidarian groups is difficult to determine based on polyp morphology, even without the effects of decay. In our *Metridium* decay experiments, all morphological features that would clearly identify the specimen as a sea anemone were lost by decay Stage 4 (of 6), while muscle tissue was retained. In contrast, previous decay experiments suggest that structures diagnostic of triploblast-grade animals tend to outlast the muscles; it is therefore unlikely that, under similar environmental conditions, a decayed triploblast-grade animal could be mistaken for a diploblast-grade animal. In the polychaete *Nereis*, for example, the gut, cuticle, bristles and jaws remain present after the muscles have deteriorated beyond recognition (Briggs and Kear 1993). In shrimps, the exoskeleton outlasts the muscles (Briggs and Kear 1994). Similarly, the grasping spines in chaetognaths (Casenove et al. 2011); the arms, eyes, and ink sac in coleoid cephalopods (Kear et al. 1995), and the gill bars in enteropneusts (Nanglu et al. 2015) each survive longer than the associated musculature. Together with these results, our decay experiments on *Metridium senile* imply that diploblast-grade organisms are, at nearly all stages of decay, unlikely to be mistaken for

triploblast-grade organisms and vice versa. Therefore, the lack of distinctive triploblast-grade synapomorphies in enigmatic soft-bodied Ediacaran and lower Cambrian taxa may reflect a paucity of true crown-group bilaterian organisms in these assemblages.

CONCLUSIONS

Experimental results documenting the serial decay of *Metridium senile* represent a first step in the actualistic investigation of the taphonomy of cnidarian polyps. We find that (1) the actinian body is rapidly deformed during decay such that the relative proportions of the oral disc, the pedal disc, and the column are modified in an unpredictable fashion; (2) tentacles may be retained until relatively late in the decay series, albeit in a truncated, anatomically unrepresentative form; and (3) the original pattern of lineations on the ectoderm wall is rapidly replaced by an increasingly pustulose and spongy texture during decay, while strong transverse creases also develop through folding, contraction, and collapse. Such taphonomic features might easily be mistaken for anatomical structures. These changes and others can be used to distinguish six distinct decay stages, providing a rigorous semi-quantitative metric that can be applied to fossils of putatively diploblastic soft-bodied organisms. Many such fossils have been identified in Ediacaran and lower Cambrian successions, and resolving their affinities is critical to the reconstruction of the base of the eumetazoan tree.

REFERENCES

ADLER, L.B., 2013. The taphonomy of soft-bodied cnidarians: Unpublished doctoral dissertation, University College Dublin, 296 p.

BABCOCK, L.E., GRUNOW, A.M., SADOWSKI, G.R., AND LESLIE, S.A., 2005, *Corumbella*, an Ediacaran-grade organism from the late Neoproterozoic of Brazil: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 220, p. 7–18, doi: 10.1016/j.palaeo.2003.01.001.

BERGES, J.A., FRANKLIN, D.J., AND HARRISON, P.J., 2001, Evolution of an artificial seawater medium: improvements in enriched seawater, artificial water over the last two decades: Journal of Phycology, v. 37, p. 1138–1145. doi: 10.1046/j.1529-8817.2001.01052.x.

BRIGGS, D.E.G., 1995, Experimental taphonomy: PALAIOS, v. 10, p. 539–550.

BRIGGS, D.E.G. AND KEAR, A.J., 1993, Decay and preservation of polychaetes: taphonomic thresholds in soft-bodied organisms: Paleobiology, v. 19, p. 107–135.

BRIGGS, D.E.G. AND KEAR, A.J., 1994 Decay and mineralization of shrimps: PALAIOS, v. 9, p. 431–456.

BRIGGS, D.E.G. AND McMAHON, S., 2016, The role of experiments in investigating the taphonomy of exceptional preservation: Palaeontology, v. 59, p. 1–11. doi: 10.1111/pala.12219.

BRUTON, D.L., 1991, Beach and laboratory experiments with the jellyfish *Aurelia* and remarks on some fossil 'medusoid' traces, *in* A. Simonetta and S. Conway Morris (eds.), The Early Evolution of Metazoa and the Significance of Problematic Taxa: Cambridge University Press, Cambridge, p. 125–129.

CAI, Y., HUA, H., XIAO, S., SCHIFFBAUER, J.D., AND LI, P., 2010, Biostratinomy of the late Ediacaran pyritized Gaojiashan Lagerstätte from southern Shaanxi, South China: importance of event deposits: PALAIOS, v. 25, p. 487–506, doi: 10.2110/palo.2009.p09-133r.

CAI, Y., XIAO, S., HUA, H., AND YUAN, X., 2015, New material of the biomineralizing tubular fossil *Sinotubulites* from the late Ediacaran Dengying Formation, South China: Precambrian Research, v. 261, p. 12–24, doi: http://dx.doi.org/10.1016/j.precamres.2015.02.002.

CASENOVE, D., OJI, T., AND GOTO, T., 2011, Experimental taphonomy of benthic chaetognaths: implications for the decay process of Paleozoic chaetognath fossils: Paleontological Research, v. 15, p. 146–153.

CHEN, J.Y. AND ERDTMANN, B.D., 1991, Lower Cambrian fossil Lagerstätte from Chengjiang, Yunnan, China: insights for reconstructing early metazoan life, *in* A. Simonetta and S. Conway Morris (eds.), The Early Evolution of Metazoa and the Significance of Problematic Taxa: Cambridge University Press, Cambridge, p. 57–76.

CONDON, D., ZHU, M., BOWRING, S., WANG, W., YANG, A., AND JIN, Y., 2005, U-Pb ages from the Neoproterozoic Doushantuo Formation, China: Science, v. 308, p. 95–98, doi: 10.1126/science.1107765.

CONWAY MORRIS, S., 1990, Late Precambrian and Cambrian soft-bodied faunas: Annual Review of Earth and Planetary Sciences, v. 18, p. 101.

CORTIJO, I., MUS, M.M., JENSEN, S., AND PALACIOS, T., 2010, A new species of *Cloudina* from the terminal Ediacaran of Spain: Precambrian Research, v. 176, p. 1–10, doi: http://dx.doi.org/10.1016/j.precamres.2009.10.010.

CUNNINGHAM, J.A., VARGAS, K., PENGJU, L., BELIVANOVA, V., MARONE, F., MARTÍNEZ-PÉREZ, C., GUIZAR-SICAIROS, M., HOLLER, M., BENGTSON, S., AND DONOGHUE, P.C., 2015, Critical appraisal of tubular putative eumetazoans from the Ediacaran Weng'an Doushantuo biota: Proceedings of the Royal Society B, v. 282, 20151169, doi: 10.1371/journal.pone.0096998.

DONOGHUE, P.C.J. AND PURNELL, M.A., 2009, Distinguishing heat from light in debate over controversial fossils: BioEssays, v. 31, p. 178–189. doi: 10.1002/bies.200800128.

DROSER, M.L. AND GEHLING, J.G., 2008, Synchronous aggregate growth in an abundant new Ediacaran tubular organism: Science, v. 319, p. 1660–1662, doi: 10.1126/science.1152595.

DROSER, M.L. AND GEHLING, J.G., 2015, The advent of animals: the view from the Ediacaran: Proceedings of the National Academy of Sciences, v. 112, p. 4865–4870, doi: 10.1073/pnas.1403669112.

GEHLING, J.G., 1988, A cnidarian of actinian-grade from the Ediacaran Pound Subgroup, South Australia: Alcheringa, v. 12, p. 299–314, doi: http://dx.doi.org/10.1080/03115518808619129.

GRAZHDANKIN, D., 2000, The Ediacaran genus *Inaria*: a taphonomic/morphodynamic analysis: Neues Jahrbuch fur Geologie und Palaontologie-Abhandlungen, v. 216, p. 1–34.

HAN, J., KUBOTA, S., UCHIDA, H.O., STANLEY, Jr., G.D., YAO, X., SHU, D., LI, Y., AND YASUI, K., 2010, Tiny sea anemone from the lower Cambrian of China: PLoS One, v. 5, p.e13276.

HOU, X.G., STANLEY, G., ZHAO, J., AND MA, X.Y., 2005, Cambrian anemones with preserved soft tissue from the Chengjiang biota, China: Lethaia, v. 38, p. 193–203, doi: 10.1080/00241160510013295.

KEAR, A.J., BRIGGS, D.E.G., AND DONOVAN, D.T., 1995, Decay and fossilisation of non-mineralised tissue in coleoid cephalopods: Palaeontology, v. 38, p. 105–131.

LEI, Q.P., HAN, J., OU, Q., AND WAN, X.Q., 2014, Sedentary habits of anthozoa-like animals in the Chengjiang Lagerstätte: adaptive strategies for Phanerozoic-style soft substrates: Gondwana Research, v. 25, p. 966–974. doi: 10.1016/j.gr.2013.01.007.

LEICH, H., 1995, Fossile Quallen aus den Solnhofener Plattenkalken: Archaeopteryx, v. 13, p. 75–84.

LIU, A.G., MATTHEWS, J.J., MENON, L.R., MCILROY, D., AND BRASIER, M.D., 2014, *Haootia quadriformis* n. gen., n. sp., interpreted as a muscular cnidarian impression from the late Ediacaran period (approx. 560 Ma): Proceedings of the Royal Society B, v. 281, 20141202.

LIU, P., XIAO, S., YIN, C., ZHOU, C., GAO, L., AND TANG, F., 2008, Systematic description and phylogenetic affinity of tubular microfossils from the Ediacaran Doushantuo Formation at Weng'an, South China: Palaeontology, v. 51, p. 339–366, doi: 10.1111/j.1475-4983.2008.00762.x.

NANGLU, K., CARON, J.-B., AND CAMERON, C.B., 2015, Using experimental decay of modern forms to reconstruct the early evolution and morphology of fossil enteropneusts: Paleobiology, v. 41, p. 460–478.

NORRIS, R.D., 1989, Cnidarian taphonomy and affinities of the Ediacara biota: Lethaia, v. 22, p. 381–393, doi: 10. 1111/j.1502-3931.1989.tb01439.x.

PENNY, A.M., WOOD, R., CURTIS, A., BOWYER, F., TOSTEVIN, R., AND HOFFMAN, K.H., 2014, Ediacaran metazoan reefs from the Nama Group, Namibia: Science, v. 344, p. 1504–1506, doi: 10.1126/science.1253393.

RAFF, R.A. AND RAFF, E.C., 2014, The role of biology in the fossilization of embryos and other soft-bodied organisms: microbial films and Lagerstätten, *in* M. LaFlamme, J.D. Schiffbauer, and S.A.F. Darroch (eds.), Reading and Writing of the Fossil Record: Preservational Pathways to Exceptional Fossilization: Paleontological Society Papers, v. 20, p. 83–100.

SANSOM, R.S., GABBOTT, S.E., AND PURNELL, M.A., 2010, Nonrandom decay of chordate characters causes bias in fossil interpretation: Nature, v. 463, p. 797–800, doi: 10.1038/nature08745.

SMITH, E.F., NELSON, L.L., STRANGE, M.A., EYSTER, A.E., ROWLAND, S.M., SCHRAG, D.P., AND MACDONALD, F.A., 2016, The end of the Ediacaran: two new exceptionally preserved body fossil assemblages from Mount Dunfee, Nevada, USA: Geology, v. 44, p. 911–914, doi:10.1130/G38157.1.

TANG, F., BENGTSON, S., WANG, Y., WANG, X.L., AND YIN, C.Y., 2011, *Eoandromeda* and the origin of Ctenophora: Evolution and Development, v. 13, p. 408–414, doi: 10.1111/j.1525-142X.2011.00499.x.

TARHAN, L.G., HUGHES, N.C., MYROW, P.M., BHARGAVA, O.N., AHLUWALIA, A.D., AND KUDRYAVTSEV, A.B., 2014, Precambrian–Cambrian boundary interval occurrence and form of the enigmatic tubular body fossil *Shaanxilithes ningqiangensis* from the Lesser Himalaya of India: Palaeontology, v. 57, p. 283—298, doi: 10.1111/pala.12066.

TARHAN, L.G., V.S. HOOD, A., DROSER, M.L., GEHLING, J.G., AND BRIGGS, D.E., 2016, Exceptional preservation of soft-bodied Ediacara Biota promoted by silica-rich oceans: Geology, v. 44, p. 951–954, doi: 10.1130/G38542.1.

VAN ITEN, H., MARQUES, A.C., LEME, J.D.M., PACHECO, M.L., AND SIMÕES, M.G., 2014, Origin and early diversification of the phylum Cnidaria Verrill: major developments in the analysis of the taxon's Proterozoic–Cambrian history: Palaeontology, v. 57, 677–690, doi: 10.1111/pala.12116.

WAHL, M., 1984, The fluffy sea anemone *Metridium senile* in periodically oxygen depleted surroundings: Marine Biology, v. 81, p. 81–86, doi: 10.1007/BF00397629.

WARREN, L.V., PACHECO, M.L.A.F., FAIRCHILD, T.R., SIMÕES, M.G., RICCOMINI, C., BOGGIANI, P.C., AND CÁCERES, A.A., 2012, The dawn of animal skeletogenesis:

ultrastructural analysis of the Ediacaran metazoan *Corumbella werneri*: Geology, v. 40, p. 691–694, doi: 10.1130/G33005.1.

XIAO, S., YUAN, X., STEINER, M., AND KNOLL, A.H., 2002, Macroscopic carbonaceous compressions in a terminal Proterozoic shale: a systematic reassessment of the Miaohe biota, South China: Journal of Paleontology, v. 76, p. 347–376, doi: http://dx.doi.org/10.1666/0022-3360(2002)076<0347:MCCIAT>2.0.CO;2.

YUAN, X., CHEN, Z., XIAO, S., ZHOU, C., AND HUA, H., 2011, An early Ediacaran assemblage of macroscopic and morphologically differentiated eukaryotes: Nature, v. 470, p. 390–393, doi:10.1038/nature09810.

ZHU, M., GEHLING, J.G., XIAO, S., ZHAO, Y., AND DROSER, M.L., 2008, Eight-armed Ediacara fossil preserved in contrasting taphonomic windows from China and Australia: Geology, v. 36, p. 867–870, doi: 10.1130/G25203A.1.
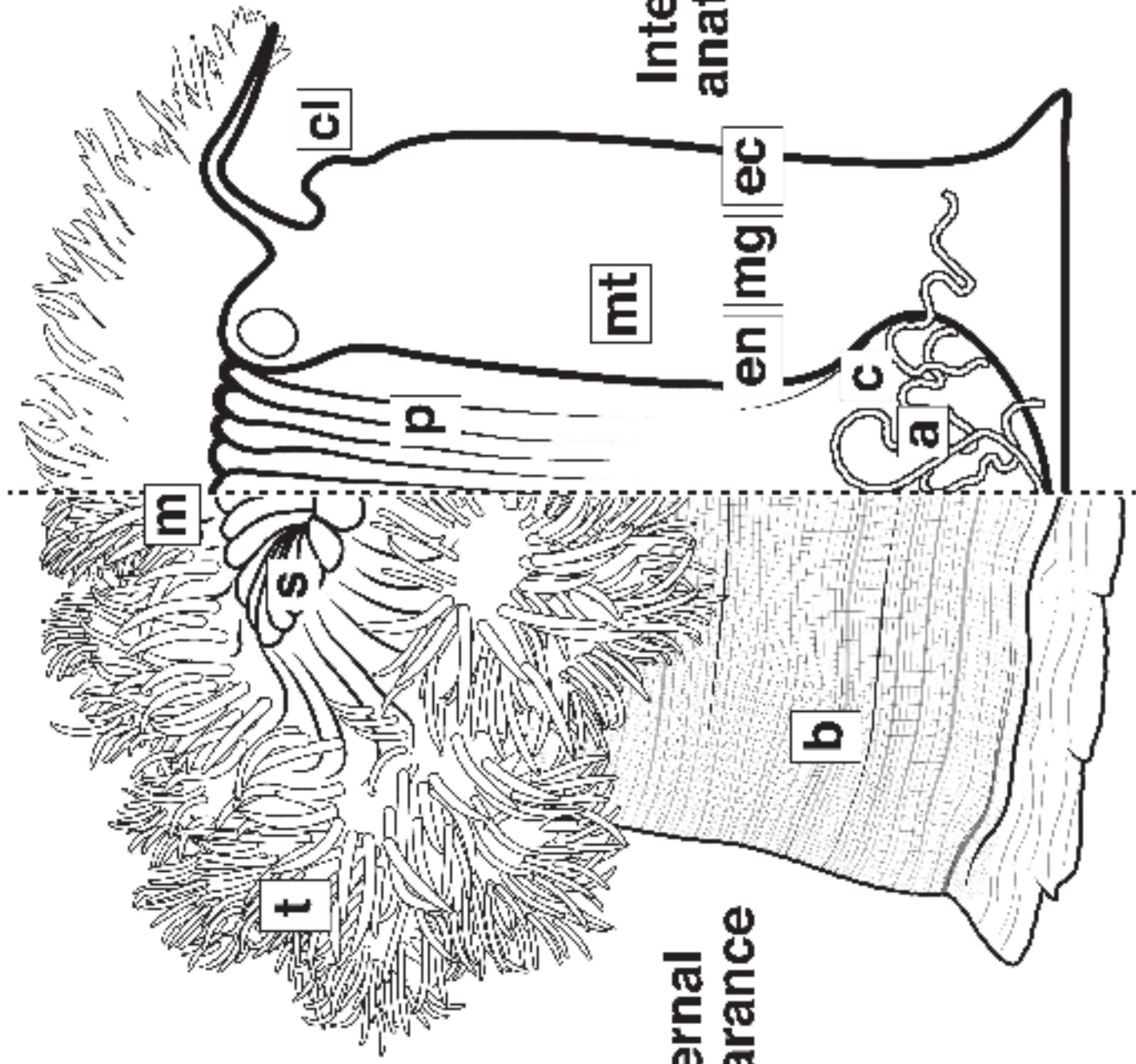
FIGURE CAPTIONS

FIG. 1.—External appearance and internal anatomy of the sea anemone *Metridium senile*. Abbreviations: a = acontia; b = body wall showing lineations; c = coelenteron (digestive cavity); cl = collar; ec = ectoderm; en = endoderm; m = mouth/anus; mg = mesoglea; mt = mesentery; p = pharynx (throat); s = siphonoglyphs; t = tentacles. Scale bar = ~ 3 cm.

FIG. 2.—Six consecutive stages in the decay of the sea anemone *Metridium senile*. Photographs represent stages in the decay of *Metridium* and have been edited for contrast. Interpretative drawings indicate key features visible in the photographs. Insets illustrate the appearance of the outer wall and the tentacles. All scale bars = 2 cm. **A**) Decay Stage 1. The column is smooth and glossy and the tentacles are finely pointed. **B**) Decay Stage 2. Slightly turbid water indicates microbial population growth and incipient biofilm development. Surface lineations are slightly closer together than in Stage 1 because of contraction. The tentacles are reduced in sharpness. **C**) Decay Stage 3. The column has darkened and become pustulose and deeply creased (inset, arrowed). Tentacle decay (distally to proximally) has notably progressed beyond that of Decay Stage 2. **D**) Decay Stage 4. The outermost brown layer of the wall has become spongy and begun to flake away. A spheroidal blister has developed in the underlying white layer (arrowed). The tentacles are truncated at the base or entirely absent. **E**) Decay Stage 5. The anemone has collapsed into the horizontal plane. The loss of the brown ectoderm has exposed a creased white interior layer. **F**) Decay Stage 6. The diffuse remains of this anemone have settled; some density stratification is apparent, with loose, disaggregated bundles of fibers floating near the surface (arrowed).

FIG. 3.—Results of *Metridium senile* decay experiments, demonstrating progression through the six taphonomic stages depicted in Figure 2. Results are aligned to the time of death for each specimen (Day 0). Above: Decay progression of each anemone (gray trend lines) and of the average (black trend line). Below: Daily decay stages for each specimen (with assemblage averages, rounded to one decimal place). Specimens are labeled A–H. Decay stages combining features of two stages were awarded a score halfway between them.

Oral disc

Internal anatomy

Pedal disc

External appearance

cl

mt

en | mg | ec

p

c

a

b

m

s

t

Decay stage vs Day 0–Day 6

Legend: Each individual; Average

Anemone specimen	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	1	2.5	4	4	5	5	6
B	1	3	3	4	5	6	6
C	1	2	3	4	5	5	6
D	1	2	3	3.5	4	5	6
E	1	2	3	3	4	5	6
F	1	2	2	5	6	6	6
G	1	3	5	5	6	6	6
H	1	2	3.5	4	4	6	6
Average	1	2.3	3.3	4.1	4.9	5.5	6