Structure and Preservation of Chiton Valves: Resolution of a Taphonomic Quandary

Bryce A. Peebles

A thesis submitted in fulfilment for the degree of Doctor of Philosophy at the University of Otago

May 2017
Abstract

Chitons are marine molluscs common worldwide that form their shells out of aragonite and are rare in the sediment and fossil records. The rarity of chitons in these records is not surprising, as dissolution readily removes aragonite from the fossil record. Yet, recent studies have suggested that chitons have an unusual capacity to resist dissolution, which implies that dissolution does not remove chiton material from the sedimentary and fossil records. The purpose of this thesis was to address this apparent contradiction of chiton preservation. The valve structure and the effects of abrasion and dissolution on eight New Zealand chiton species (*Acanthochitona zelandica*, *Notolax violacea*, *Chiton glaucus*, *Onithochiton neglectus*, *Sypharochiton pelliserpentis*, *Sypharochiton sinclairi*, *Ischnochiton maorianus*, and *Leptochiton inquinatus*) were examined. Scanning electron microscopy (SEM), X-ray diffractometry (XRD), and Raman spectroscopy were used to determine the mineralogy, ultrastructure, and organic components of chiton valves. The ability of these chiton valves to resist abrasion and dissolution was directly tested by tumbling isolated valves in a rock tumbler for 96 hours and subjecting another group of isolated valves to two treatments of different pH: 8.10 (ambient) and pH 7.70 (projected pH for the year 2100) for 12 days. XRD and Raman spectra confirmed that valves from all analysed species were made of aragonite. The tegmenum was primarily granular and contained one or two carotenoid pigments. The articulamentum was formed by alternating crossed lamellar and spherulitic structures and a ventral-most acicular sublayer. The number of sublayers varied among species, and *L. inquinatus* displayed a unique crossed lamellar structure in its valves. The valves lost 9-44% of their initial weight after tumbling, which is unusually resistant compared to other molluscs. Abrasion damaged the tegmentum more than the articulamentum. Valves lost 1 to 5% of their initial weight after 12 days in a pH of 7.70 and less than 1% in the ambient pH control. SEM images of valves in the pH treatments revealed that dissolution damaged the articulamentum more than the tegmentum. While these results suggest that chiton valves are resilient, taphonomic forces do not act in isolation. The two different main layers of the valves are vulnerable to different forces; abrasion will remove the tegmentum and expose the articulamentum to rapid dissolution. Chiton valves are estimated to last about 5 years before being rendered unrecognizable. It is likely that chiton valves require a rapid burial
event to be preserved since they can be exposed to taphonomic forces for hundreds of years in temperate intertidal and shelf environments.

**Acknowledgments**

Both the work presented in the thesis and who I am as a person would be lesser if not for the following people. I must acknowledge them as I owe them all gratitude for their support and would not be where I am, or who I am, today without them.

I need to thank my incredible supervisors Abby Smith and Hamish Spencer for their constant academic support, advice, and patience. Thank you to Erin Bowkett for her patience and support throughout the entire journey. Thanks to my parents, Aunt Anitra, and Uncle Jack for their mental and financial support. This journey would have been impossible without these people.

Thanks to Geoffrey Smith and Keith Gordon for their advice and expertise on Raman Spectroscopy; to Doug Mackie for his guidance on pH photospectroscopy methods and the proper use of the tris and amp buffers; to Reuben Pooley and Linda Groenewegen for their help with setting up space and tanks for the OA treatments; Rebecca Zitoun for inspiring me with her previous work on chitons; to Brent Pooley for his advice on resin casting, cross-sectioning, and polishing of chitons material; to Liz Girvan for her support and tutelage on proper use of the SEM, and understanding of the sessions that went into the night; to Gemma Kerr and Damian Walls for guiding me through the process of using the XRD.

I also wish to thank the Departments of Marine Science and Zoology for their support and my dear friends at Abbey College whom I have had the pleasure of meeting and getting to know over the duration of my studies. Thank you to the members of Destreza Pacifica, Humboldt State Fencing, the Golden Gate School of Arms, and Salle Angelo for keeping me physically and mentally agile during the final stages of the thesis.
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Chapter 1 – Introduction

Chitons are common intertidal molluscs found in shallow marine environments worldwide, but are one of the least studied classes in the Mollusca (Fontoura-da-Silva et al. 2013; Sigwart and Sumner-Rooney 2015). They have a large ventral foot, a head adjacent to the foot, and gills positioned near the posterior side of the foot. The head has a centrally positioned mouth and lacks eyes and tentacles. Chitons have three main mineralised body parts: the shell plates (valves), the radula, and some species have girdle spicules (Figure 1.1).

Figure 1.1: Chiton glaucus (centre, right) and Acanthochitona zelandica (left). A. zelandica has girdle spicules while C. glaucus lacks spicules.

Chitons are an exclusively marine group and are sister to the Aplacophora (Chaetodermomorpha and Neomeniomorpha) in the Aculifera clade (Todt et al. 2008; Kocot et al. 2011; Figure 1.2). There are two main orders of Polyplacophora, Chitonida and Lepidopleurida, which are subdivided into 3 suborders, 5 superfamilies, and 21 families (Table 1).
Figure 1.2: Summary of the current consensus of the relationship between major clades of Mollusca (after Kocot et al. 2016).

Table 1: Higher Taxonomy of Polyplacophora down to family with families analysed in this species highlighted in grey (WoRMS Editorial Board, 2017).

<table>
<thead>
<tr>
<th>Class</th>
<th>Order</th>
<th>Suborder</th>
<th>Superfamily</th>
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<tr>
<td>Polyplacophora</td>
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It is estimated that there are currently 930 extant species and 430 fossil species of chitons, most of which are found in the intertidal zone (Puchalski et al. 2008; Schwabe 2008).
The exclusive economic zone (EEZ) of New Zealand has over 8.5% of the world’s chiton population. There are a total of 63 species found in the EEZ of New Zealand; 35 species can be found in the southern half of New Zealand’s South Island, 32 species on the northern coast of North Island, and 30 species between these two zones (Spencer et al. 2016).

Chitons hatch as trochophores and develop both their musculature and shell before settlement and metamorphosis into a juvenile chiton (Creese 1986; Wanninger and Haszprunar 2002). They tend to stay under boulders during the day and graze on algae at night during the juvenile and adult phases of their life cycle. Some species display homing behaviour and return to a specific location on a boulder after foraging (Crozier 1921; Chelazzi et al. 1990; Barbosa et al. 2008).

Most chitons live in boulder crevices in the intertidal zone, some (such as Rhysoplax canaliculate) are also found on the continental shelf. However, the lepidopleuridans are mostly deep-sea dwelling. They are found from 500m to over 6000m depth on sunken wood and in other abyssal habitats (Sirenko 2001; Saito 2006; Sigwart 2009). Chiton population densities can be as large as 228 individuals/m² in surf zones of North Island, New Zealand (Boyle 1970; Gordon et al. 2010). Chitons play a key role in intertidal reefs by promoting coralline algal growth. In some cases, chitons remove macroalgae which allows other microalgae to flourish and provides a food source for other molluscs such as limpets (Deither and Duggins 1984; Littler et al. 1995).

The chiton shell consists of eight overlapping valves, which are primarily composed of aragonite (Baxter and Jones 1981; Carter 1990; Lécuyer et al. 2004; Speiser et al. 2011; Connors et al. 2012; Figure 1.3). These valves are typically numbered i-viii with valve i also called the head valve and valve viii the tail valve; the remaining valves (ii-vii) are termed the intermediate valves. The valves contain two main aragonitic layers and a thin organic layer on the dorsal surface, which slowly wears away over the chiton’s life (the periostracum; Schwabe 2010; Connors et al. 2012). The two mineral layers are the tegumentum, the dorsal layer that contains nerve tissue (aesthete channels; Schwabe 2010), and the articulamentum, which is the ventral layer and makes up the bulk of the valve material (Figure 1.4).
Figure 1.3: *Sypharochiton sinclairi* with the valves numbered (i-viii). Valve i is the head valve, valve viii is the tail valve, and valves ii-vii are the intermediate valves.

Figure 1.4: A sketch of the two main mineral layers, the tegmentum and the articulamentum, of a chiton valve.

The first detailed description of valve microstructures was by Carter (1990), who described the ultrastructure of the valves of *Acanthopleura granulata*. There have been only two other studies since that detail the ultrastructures of chiton valves (Chen 2010; Connors *et al.* 2012). Wilmot *et al.* (1992) mentions a crossed lamellar sublayer of *A. brevispinosa*, but does not give a full description of the rest of the valve sublayers. In addition to this lack of knowledge of chiton valve ultrastructures, only 3% of chiton species have been mineralogically studied so far (Peebles *et al.* 2016). Although the data gathered consistently show that extant chitons use aragonite to form their valves (Clarke and Wheeler 1922; Lowenstam 1962; Kirschvink and Lowenstam 1979; van der Wal 1991; Lécuyer *et al.* 2004; Chen 2010; Connors *et al.* 2012) and extinct chitons show remnants of what was likely
aragonite (Cherns and Wright 2000; Wright et al. 2003; Sanders 2003), mineralogical examination of other chiton species is important since so few species have been surveyed.

Since chitons are commonly found and are ecologically important, it is necessary to understand how they will respond to projected future change in coastal waters, especially changes such as decreasing pH and increasing temperatures. The key to understanding how well chitons can adapt to decreasing pH lies in their shell, as decreasing pH can cause either shell deformation or dissolution (Orr et al. 2005, Doney et al. 2009). Research involving ocean acidification has been increasing over the past decades with interest in how marine calcifiers respond to a reduced pH, but chitons have been ignored. There have only been three studies that mentioned the effects of ocean acidification on chiton skeletal material, and none of them focused on the effects of decreased pH on chiton valve structure (Green 2012; Sigwart and Carey 2014; Sigwart et al. 2015).

The primary purpose of the experiment conducted by Sigwart and Carey (2014) was to determine how the grazing behaviour and radula structure of chitons were affected by lowered pH of 7.5, but the authors noted that the valves showed no evidence of pitting, dissolution, or variance in aesthete size. Sigwart et al. (2015) measured the force needed to fracture valves from Mopalia muscosa, M. lignosa, and Katharina tunicata after exposure to reduced pH; they found no significant difference between those from the control group (pH of 8.0) and those subjected a lowered pH (7.5). Green (2012) also looked at the fracture resistance of two mopaliids (M. muscosa and M. lignosa). M. muscosa valves fractured more easily if they were subjected to pH of 7.495, but valves from M. lignosa showed no difference in strength when subjected to reduced pH. This apparent ability of chiton valves to resist decreasing pH makes them unusual among molluscs (McClintock et al. 2009; Waldbusser et al. 2011; Wolfe et al. 2012; Gazeau et al. 2013) and suggests that their calcification composition and/or ultrastructure are unusual.

The lack of dissolution of chiton valves when exposed to decreased pH is counter-intuitive because aragonite, being metastable, should dissolve as pH decreases (pK$_1$ = 5.88, pK$_2$ = 9.03 at T = 20 ° and S = 35) (Mucci 1983; Millero et al. 2002). The observation that chitons may be able to resist dissolution is anomalous and unexpected, since they are rare in the sediment and fossil records (Cherns 1999; Puchalski et al. 2008; Vendrasco 2008). For example, chitons are not mentioned in the temperate carbonate sediment records of New Zealand (Andrews 1973; Nelson 1978; Probert et al. 1979; Nelson and Hancock 1984; Nelson et al. 1988; Hayton et al. 1995). Bivalves and gastropods make up over 20% of deposited material on the carbonate shelves of New Zealand (Nelson et al. 1988), which
implies either that chiton valves are much more vulnerable to removal than other molluscan shells or that chiton material does not reach the shelf from the rocky shore easily.

Extinct chiton species are generally represented by only a few valves and are rarer than other taxa even when “abundant” (Cherns 1999; Hoare and Farrell 2004; Puchalski et al. 2008; Cherns and Wright 2009). New Zealand also has the most complete molluscan Cenozoic record in the Southern Hemisphere, yet chitons rarely appear in the fossil record (Cherns 1999; Puchalski et al. 2008). The fossil record has a calcite bias, and aragonite is commonly silicified and/or dissolves out of the record (Sanders 2003; Cherns and Wright 2009). If chiton valves resist dissolution, then some other taphonomic force may be responsible for removing them from the sediment and fossil records, assuming they reach the shelf.

The main focus of this thesis is to determine how dissolution and abrasion affect chiton valves as taphonomic processes. Since crystal size and shape determine how the valves break down (Harper 2000; Smith and Nelson 2003), it is necessary to examine the microstructure and the distribution of organic material within chiton valves, especially since descriptions of chiton microstructures are lacking. This thesis examines the taphonomy of eight New Zealand chiton species in six chapters. Chapters 2-5 are manuscripts that are in preparation for submission, have been submitted for publication, or have been published. Chapter 2 details the microstructure of eight chiton species and Chapter 3 is an analysis of the pigments found within the shell and the distribution of proteins within the valves. These two chapters lay the groundwork so the effects of abrasion and dissolution can be understood. The abrasion resistance of chiton valves is examined in Chapter 4, and the response of chiton valves to lowered pH is described in Chapter 5. Chapter 6 serves as a discussion chapter that links the results of the previous four chapters together to describe the cognitive space of this thesis (Figure 1.5).
Figure 1.5: The cognitive structure of the thesis. When chitons in the biosphere die, their skeletal material is subjected to various forces and is either returned to the hydrosphere (dissolution) or to the geosphere (broken down into sediments via abrasion, breakage, or bioerosion), all of which are still exposed to the same taphonomic forces.
Chapter 2 - Valve Microstructure and Phylomineralogy of New Zealand Chitons

This chapter focuses on the physical structure of chiton valves (Figure 2.1). The size and shape of the crystals are key determiners of how calcareous material will break down, yet chiton valve ultrastructures are understudied. Detailing the ultrastructure of chiton valves will provide insight to their taphonomy as well as expand our knowledge of their mineralogy.

![Diagram showing the cognitive space of Chapter 2]

Figure 2.1: The cognitive space that Chapter 2 fills in the thesis. This chapter examines the microstructure of chiton valves which is necessary to understand their taphonomy.

The following chapter was published in the Journal of Structural Biology in 2017. I collected the data, designed the experiments, and wrote the original draft. A.M. Smith and H.G. Spencer supervised the project, helped edit and provided feedback on the drafts. The reviewers’ concerns have been addressed and this paper has been re-formatted to fulfill the requirements of a PhD thesis of the University of Otago. The references have been moved to the References section after Chapter 6 (page 96).
Valve Microstructure and Phylomineralogy of New Zealand Chitons

B.A. Peebles¹*, A.M. Smith¹ and H.G. Spencer²

¹ Department of Marine Science, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
² Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
*Corresponding Author: e-mail: bryce.peebles@gmail.com, phone: +64 21 148 6478

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Abstract

The microstructure and mineralogy of chiton valves has been largely ignored in the literature and only described in 29 species to date. Eight species: Acanthochitona zelandica, Notoplax violacea (Family Acanthochitonidae, Suborder Acanthochitonina, Order Chitonida), Chiton glaucus, Onithochiton neglectus, Sypharochiton pelliserpentis, Sypharochiton sinclairi (Family Chitonidae, Suborder, Chitonina, Order Chitonida), Ischnochiton maorianus (Family Ischnochitonidae, Suborder, Chitonina, Order Chitonida), and Leptochiton inquinatus (Family Leptochitonidae, Suborder Lepidopleurina, Order Lepidopleurida) were collected from the Otago Peninsula, South Island, New Zealand. The valves of these chitons were measured with X-ray diffractometry, Raman spectrometry, and Scanning Electron Micrography (SEM) to determine their mineralogy and microstructure. Both the XRD and Raman data show that the valves consisted solely of aragonite. The observed microstructures of the valves were complex, typically composed of four to seven sublayers, and varied among species. The dorsal layer, the tegmentum, of each species was granular and the ventral layer, the articulamentum, was predominately composed of a spherulitic sublayer, a crossed lamellar sublayer, and an acicular sublayer. The chitonids Sypharochiton pelliserpentis and S. sinclairi had the most complex microstructure layering with three crossed lamellar, two spherulitic sublayers, and a ventral acicular sublayer while the acanthochitonids Acanthochitona zelandica and Notoplax violacea as well as the ischnochitonid Ischnochiton maorianus had the simplest structure with one spherulitic, one crossed lamellar sublayer, and a ventral acicular sublayer. Terminal valves were less complex than intermediate valves and tended to be dominated by the crossed lamellar structure. The leptochitonid Leptochiton inquinatus generated a unique crossed lamellar sublayer different from the other analyzed chitonids. Acanthochitona zelandica is the only analyzed chitonid that utilizes two different crossed
Molluscs use organic-matrix mediation to form specific polymorphs of calcium carbonate, and a range of microstructures, that compose their skeletal material. Molluscs are special among the invertebrates for their complex, layered skeletal structures that incorporate to various degrees organic material (Bøggild 1930). For example, nacre, also known as mother-of-pearl, is composed of highly organised, polygonal uniform plates of aragonite that can form either columns or sheets. Nacreous layers in molluscs have been a focal point of study over the past few decades due to their biomechanical strength (Barthelat et al. 2006; Currey et al. 2001; Jackson et al. 1988; Sun and Tong 2007). Prismatic layers paired with nacreous layers in gastropod shells are well studied (Barthelat et al. 2006; Marin and Luquet 2004). They are composed of column-like crystals, primarily calcite in most shells, and make up the dorsal layer of many bivalve and gastropod shells (Furuhashi et al. 2009; Marin and Luquet 2004). Spherulitic layers also have prism-like crystals, but the prisms fan out from a central point (Marin et al. 2012; Mutvei 1997). If the crystals are needle-like instead of prism-like then the layer is termed an acicular layer (Marin et al. 2012). In crossed-lamellar layers, by contrast, sheet-like crystals form different orders of lamellae (Carter 1990; Wilmot et al. 1992). Usually three orders of lamellae are formed, the lowest (third) order is composed of thin (< 1µm) lamellae that are oriented in the same direction. These lamellae are themselves grouped into layers to form second-order layers. First-order lamellae are similarly formed from the second-order layers. Homogenous layers are layers in which the crystals appear to have no particular structure; course-grained homogenous layers are typically referred to as granular.

Among the molluscs, chitons (Class Polyplacophora) are both atypical and understudied. The shell of a chiton is composed of eight overlapping valves, this feature being unique among the molluscs. The valves are typically numbered i through viii starting with the head valve and can be categorized as terminal valves (i and viii) or intermediate valves (ii through vii). Mineralogical examination of chiton valves has shown the valves to be exclusively aragonite, but only 3% of chiton species have been measured, with several higher taxa ignored (Baxter and Jones 1981; Carter 1990; Connors et al. 2012; Lécuyer et al. 2004; Spieser et al. 2011) (Table 2.1).

<table>
<thead>
<tr>
<th>Order (2)</th>
<th>Suborder (3)</th>
<th>Family (6)</th>
<th>Species (29)</th>
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<tr>
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<td>Acanthochitonon fascicularis</td>
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<td>Lowenstam 1962, Towe and Lowenstam 1967, Kirschvink and Lowenstam 1979; van der Wal 1991; Weaver et al. 2010</td>
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<td>Katharina tunicata</td>
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<td>Lowenstam 1962; van der Wal 1991</td>
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<td>Mopalia muscosa</td>
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<td>Connors et al. 2012</td>
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<td>Chitoninae</td>
<td>Acanthopleura brevispinosa (Acanthopleura spinigera)</td>
<td>Girdle Spicules - Aragonite</td>
<td>Treves 1998; Treves et al. 2003</td>
</tr>
<tr>
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<td>Radula - Apatite, Ferrhydrite, Magnetite, Lepidocrocite</td>
<td>Brooker et al. 2003; Brooker and Shaw 2012</td>
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<td>Lowenstam 1962; Spieser et al. 2011</td>
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<td></td>
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<td>Radula - Lepidocrocite, Magnetite, Geothite, Ferrihydrite, Apatitic Calcium Phosphate, Iron Oxide, Apatite</td>
<td>Lee et al. 1998; Saunders et al. 2009</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Acanthopleura sp.</td>
<td>Radula - Magnetite</td>
<td>Brooker and Shaw 2012;</td>
</tr>
</tbody>
</table>
Each valve is formed separately and has two main aragonitic layers, the tegmentum and the articulamentum. The tegmentum is the dorsal-most layer that incorporates sensory tissue (aesthetes) (Boyle 1969; Schwabe 2010). These aesthetes are housed in channels that expand throughout the dorsal layer of the valve and in some species contain ocular cells (Li et al. 2015; Schwabe 2010). The ocular structures of *Acanthopleura granulata* have large aragonitic crystals that may allow its shell-eyes to form rudimentary images (Li et al. 2015).
The articulamentum is the ventral layer that lacks aesthetes and is composed of aragonite and an organic matrix. These two main layers are subdivided into up to seven aragonitic sublayers and a thin, organic layer on the dorsal surface of the valve, which slowly wears away over the chiton’s life (Connors et al. 2012; Schwabe 2010). Although chitons have received a large amount of attention in the field of biomineralization, most of this effort has been targeted at their radulae; studies of the microstructure of the valves are lacking (see Table 2.1).

Studies of the valves of recent chitons have centred on the structure of shell-eyes (Spieser et al. 2011), the overall organization (Baxter and Jones 1981; Schwabe 2010), the aetheste channels (Vendrasco et al. 2008), and fossil assemblages (Dell’Angelo et al. 2011; Puchalski et al. 2008), but not the microstructure of the valves. The only described valve microstructures in a chiton species to date are from Tonicella marmorea (Mopaliidae; Connors et al., 2012), Acanthopleura granulata (Chitonidae; Carter, 1990) and one layer of an A. brevispinosa valve (Wilmot et al., 1992); these species are all members of the Order Chitonida. Connors et al. (2012) described seven sublayers of an intermediate valve of T. marmorea and Carter (1990) described the microstructure of A. granulata as having three sublayers for the head and tail valves and five sublayers in the intermediate valves. The crossed lamellar sublayer of A. brevispinosa was observed by Wilmot et al. (1992), but no pictures or description was given aside from the width of the first and third order lamellae. Second-order lamellae were not mentioned in the paper so it may be that A. brevispinosa lacks second-order lamellae.

There has been no comparison of microstructures in relation to phylogeny in chitons to date. Recent phylogenetic work on chitons has focused on using genetics to elucidate evolutionary relationships (Okusu et al. 2003) but had not addressed variation in mineralogy (as suggested by Smith et al. 2012). Moreover, although the biogenic minerals chitons produce have been consistently identified as aragonite in the valves and girdle spicules and various forms of iron oxide in the radulae, few species have been surveyed (Connors et al. 2012; Schawbe 2010).
**Material and methods**

Four individuals of each of eight species of chitons were collected (Figure 2.2) from the Otago Peninsula, South Island, New Zealand. Individuals were pried off rocks, except for *Onithochiton neglectus*, which was pulled off beach-cast bull-kelp holdfasts, and all were immediately put into a 75% ethanol solution. Valves were removed from each individual using a scalpel and forceps, then were brushed clean.

A PANalytical X’Pert Pro MPD PW3040/60 X-ray diffractometer (XRD) with a copper core was used to examine the valves of each collected species. Valves selected for X-ray diffraction were cleaned in reverse osmosis (RO) water with ultrasonication for two minutes, ground into a fine powder using a mortar and pestle, and then smeared onto a glass slide. A short scan (26-33° 2θ, speed of 1.2°/min, total running time 5.83 minutes) was performed on each sample. This region was selected since it can easily show the difference between polymorphs of calcium carbonate (Kontoyannis and Vagenas 1999).

All eight valves of three individuals from each analyzed species were cast in an epoxy-resin solution and dried at 60°C (24 valves per species). The resin brick containing the valves was sectioned with a diamond saw, before being polished with a series of sandpapers and grits (240 grit, 600 grit, 1200 grit, 3μm, and 1μm). The polished and exposed valve cross sections were then analysed using a Senterra confocal Raman microscope (Bruker Optics, Ettlingen, Germany). The parameters used for Raman microscopy included: a laser wavelength of 785 nm, 100 mW laser power, a 50 μm pinhole aperture, a 20× objective, a scan time of 5 seconds × 6 co-additions, and a spectral resolution of 9-18 cm⁻¹. These parameters were chosen since they gave the clearest signal while creating a minimal amount of background noise and did not have enough energy to cause the sample to fluoresce or burn. Three line maps which contained 20 spectra each were taken vertically through each of the valves.
Figure 2.2: *Chiton glaucus* (top) with the valves numbered, i is the head plate, viii is the tail plate, and ii-vii are the intermediate valves (a). The other images show a tail, an intermediate, and a head valve from each of the eight collected species: *Leptochiton inquinatus* (b), *Acanthochitona zelandica* (c), *Notoplax violacea* (d), *Ischnochiton maorianus* (e), *Chiton glaucus* (f), *Onithochiton neglectus* (g), *Sypharochiton pelliserpentis* (h), and *S. sinclairi* (i).

A JEOL 6700 FEG scanning electron microscope (SEM) was used to image the microstructure of an intermediate valve and the tail valve of four individuals of each species. Valves were cleaned in reverse osmosis (RO) water with ultrasonication for two minutes, then allowed to air dry before being fractured by hand and mounted onto aluminium stubs then coated with a 10nm layer platinum palladium using an EmiTech Carbon Coater. A micrograph of the total cross section was taken at a suitable magnification, before micrographs of each sublayer were taken at standard magnifications of 200x and 3000x to
investigate in detail the crystal structure.

**Results**

The XRD and Raman data showed that aragonite was the only mineral found in the valves. This result was unsurprising since every other chiton species described to date also uses solely aragonite to form their skeletal material. Figure 2.3 shows a typical XRD spectrum of a chiton valve, the peaks at 26.16 and 27.18 are typical of aragonite and were present in all valve samples. Figure 2.4 shows typical Raman spectra from the articulamentum of each species, which indicated pure aragonite. The lack of other peaks indicates that aragonite is the only mineral present. Figure 2.5 shows a typical Raman spectrum of *Chiton glaucus* beside aragonite and calcite standards. The band at 1085 indicated calcium carbonate, but the band at 702 confirmed that the valve material was made up of specifically aragonite.

![Diagram](image.png)

Figure 2.3: XRD aragonite and calcite standard spectra with a typical spectrum of a valve from *Chiton glaucus*. The peaks at 26.16 and 27.18 are typical of aragonite and were present in all spectra taken from the valves of each analysed species.
Figure 2.4: Typical Raman spectra from the articulamentum of each species. The bands at 702 and 1085 indicate aragonite.

Figure 2.5: Raman spectra of calcite and aragonite standards and a typical spectrum from the articulamentum of a *Chiton glaucus* valve. Standard spectra were obtained from the RRUFF project database (Lafuente *et al.* 2015).

SEM images showed that the microstructure of the valves varied significantly among species but not within species (Figure 2.6). The tegmentum of each species was primarily granular, with no distinct crystal size or orientation. The articulamentum was split into two to six sublayers depending on the species. Figure 2.7 shows the articulamentum of *Sypharochiton pelliserpentis*, which, along with its sister species, *S. sinclairi*, had the most
complex sublayering of the species analysed. The dorsal-most sublayer of the articulamentum was a thin crossed lamellar sublayer that lay immediately below the tegmentum and was only present in the intermediate valves of both *Sypharochiton* species (and in the terminal valves of *Leptochiton inquinatus* and *Onithochiton neglectus*). This sublayer was dorsal to a spherulitic sublayer that varied in depth between species and made up the bulk of the articulamentum on the areas of the valves where the muscles attached. A second crossed lamellar sublayer was ventral to the spherulitic sublayer; the lamellae lay perpendicular to the dorsal sublayer of the shell so that the upper surface of the lamellae were exposed. A homogenous, granular sublayer was located ventral to this second crossed lamellar sublayer. A third crossed-lamellar sublayer was located ventrally to the granular sublayer was thickest in the centre of the valve and tapered to a point towards the edges of the valves where the muscles attached and the initial spherulitic sublayer was thickest. The ventral-most sublayer was a thin (< 0.5 – 3 µm thick) acicular sublayer.
Figure 2.6: Phylogenetic context of the different mineral structures used by each analysed chiton species (Phylogenetic tree based on Okusu et al. 2003).
Figure 2.7: The cross section of a *Sypharochiton pelliserpentis* intermediate valve showing all mineral layers. Layer 1 is the Tegmentum (with obvious aesthete channels) and layers 2-7 form the articulamentum. Layers 2, 4, and 6 have a rod-type crossed lamellar microstructure, layers 3 and 5 have a spherulitic microstructure and layer 7 is the ventral-most acicular sublayer.

Terminal valves typically had a simpler articulamentum than the intermediate valves and were composed of a thin spherulitic sublayer located between the tegmentum and a thick rod-type crossed-lamellar sublayer that made up most of the valve (Figure 2.8). A thin acicular sublayer was the ventral-most sublayer in all species analysed. *Leptochiton inquinatus* and *Onithochiton neglectus* lacked any spherulitic sublayers and had an articulamentum composed solely of the rod-type crossed-lamellar sublayer and the ventral acicular sublayer.
Figure 2.8: Difference in articulamentum complexity between an intermediate plate (left) and a terminal plate (right) of *Chiton glaucus*. Sublayers are indicated by arrows; the tegmentum is labelled “1” and the articulamentum is labelled “2”.

Our results showed that *Acanthochiton zelandica* (Figure 2.9a) and *Notoplax violacea* (Figure 2.9b) (Acanthochitonidae) had few channels penetrating the tegmentum. *Leptochiton inquinatus* (Lepidopleurida) had consistent channel penetration throughout the tegmentum (Figure 2.9c), while *Chiton glaucus, Onithochiton neglectus, Sypharochiton pelliserpentis, S. sinclairi*, (Chitonidae) and *Ischochiton maorianus* (Ischnochitonidae) had a tegmentum that is heavily permeated with aesthete channels (Figure 2.9d).
Figure 2.9: The tegmenta of *Acanthochitona zelandica* (a), *Notoplax violacea* (b), *Leptochiton inquinatus* (c), and *Chiton glaucus* (d). The holes in the mineral are the aesthetes.

The articulamentum of each species analysed showed the most variation in the crossed-lamellar sublayer. *Acanthochitona zelandica*, *Notoplax violacea* (Acanthochitonidae) and *Ichnochiton maorianus* (Ischnochitonidae) had the simplest articulamentum with three sublayers in the articulamentum: a spherulitic sublayer located between a crossed lamellar sublayer and the tegmentum, and the ventral-most acicular sublayer. *Leptochiton inquinatus* (Lepidopleurida) was similar to the acanthochitonids and the ischnochitonid, but also had a second crossed lamellar sublayer present between the tegmentum and the spherulitic sublayer. *Onithochiton neglectus* and *Chiton glaucus* (Chitonidae) lacked the dorsal-most thin crossed lamellar sublayer found in the other chitons (*Sypharochiton pelliserpentis* and *S. sinclairi*) but otherwise had the same valve structure overall.

The analysed New Zealand chitons lacked a second-order lamella structure and had a rod-type crossed lamellar structure in the articulamentum, which has only been described in *Acanthopleura granulata* by Carter (1990). The first-order lamellae of the chitonid and ischnochitonid species, and the acanthochitonid *N. violacea* were rod-shaped and intersect each other orthogonally, instead of at an angle closer to 45° as seen in conchiferan structures and shown in *Acanthopleura granulata* by Carter (1990). *Leptochiton inquinatus* (Lepidopleurida) formed a unique crossed lamellar structure of the analysed chitons: it does not use rods, but instead forms true lamellae like the conchiferan molluscs which intersect at a 45° angle, but it still lacked the second-order lamellae. *A. zelandica* had a crossed lamellar
sublayer that also lacked second order lamellae, but had much flatter lamellae and transitioned to a rod-type crossed lamellar structure in the ventral-most area of the sublayer. *A. zelandica* also formed a crossed lamellar structure that resembles a hybrid of the two different crossed lamellar sublayers of the Leptopleuridan and the Chitonidae. The lamellae were the same size as those from the Chitonidae, but intersected at a 45° angle and had the same shape and organisation as *L. inquinatus*. The terminal valves of *A. zelandica* had a structure of what appeared to be a transition between the rod-type crossed lamellar observed in the other Chitonidae members and the *L. inquinatus* crossed lamellar organization, and the inter-valve crossed lamellar structure was similar to that from the conchiferan molluscs. The size of the lamella also varied significantly among species, the lamella from most Chitonidae were larger than those from the Leptopleuridae and *A. zelandica*. Figure 2.10 shows the different crossed lamellar structures for each analysed chiton species.

![Image](image)

Figure 2.10: Variations of the crossed lamellar substructure of the articulamentum among all eight analyzed chiton species. The crossed lamellar substructure of the *Acanthochiton zelandica* terminal valves (a) and inter-valve microstructure (b) are both shown in the top row.

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Discussion

This study is the first to describe chiton valve microstructure for the intermediate and terminal plates of *Acanthochitona zelandica*, *Notoplax violacea*, *Chiton glaucus*, *Onithochiton neglectus*, *Sypharochiton pelliserpentis*, *S. sinclairi*, *Ischnochiton maorianus*, and *Leptochiton inquinatus*. The XRD and Raman spectroscopy results clearly show that the valves of these species are made of aragonite, in keeping with previous findings (Baxter and Jones 1981; Connors et al. 2012; Lécuyer et al. 2004). This report is the first account of chiton phylomineralogy and shows how the mineralized structures of chitons may vary with phylogeny, which may prove useful for future phylogenetic work or in identification of fossilised chiton valves.

The main source of variation between the chiton taxa examined was due to either the amount of aesthete channel permeation throughout the tegmentum or the crystal structure of the crossed lamellar sublayer (Figure 2.5). The sole lepidopleuridan in our study, *Leptochiton inquinatus*, generated a completely different crossed lamellar structure than the other analysed chitons (Order Chitonida). The crossed lamellar sublayer specific to *L. inquinatus* lacked second-order lamella, similar to some of the other species examined, but each first-order lamella was only one third-order lamella thick instead of the three to ten lamellae shown by the species from the Chitonidae and Ischnochitonidae, as well as the acanthochitonid, *Notoplax violacea*.

The variation of aesthete channels within the Chitonida along with the complexity of the articulamentum allowed for identification down to at least family level. The acanthochitonids and the ischnochitonid showed a simple tegmentum, which differentiated them from the Chitonidae. *Ischnochiton maorianus* had a large amount of aesthete penetration, whereas both *Acanthochitona zealandica* and *Notoplax violacea* had few aesthetes. This difference is interesting since the girdle of the former covers much less of the valves than in the latter two, which may mean that there is no need for the sensory tissue to be present in the valves since they are covered by the girdle; this reasoning may also explain why the Superfamily Cryptoplacoidae have a reduced tegmentum and adult *Cryptochiton stelleri* (Mopaliidae) lacked a tegmentum entirely (Schwabe 2010). *A. zealandica* also varied in crossed lamellar types between the terminal and intermediate valves while *N. violacea* had a consistent, rod-type crossed lamellar sublayer. The chitonids had a complex articulamentum, but *Chiton glaucus* and *Onithochiton neglectus* lacked a thin crossed lamellar sublayer ventral to the tegmentum that the two *Sypharochiton* species displayed.
Although there is enough variation among families to differentiate them, these differences do not appear to reflect phylogeny. The Order Chitonida shows a large amount of variation in all three factors that varied among species (aesthete penetration, articulamentum complexity, and lamellae crystal type). Chitonidans had the most complex (Chitonidae) and the simplest (Acanthochitonidae and Ischnochitonidae) articulamentum structures. The acanthochitonid *A. zelandica* had the fewest aesthetes while the chitonids showed the most. Each analysed chitonidan except for *A. zelandica* had a rod-type crossed lamellar sublayer, but *A. zelandica* showed both rod-type lamellae in the intermediate valves and normal lamellae structures in the terminal valves. The tegmentum of all analysed species had the same overall structure: a granular sublayer with the aesthete channels clearly visible throughout. The terminal valves differed less among species than the intermediate valves. The only variations of crystal structures among species were in *O. neglectus* and *L. inquinatus* which lacked the spherulitic sublayer between the respective crossed lamellar sublayers and the tegmentum and in *A. zelandica* which had a rod-type crossed lamellar sublayer in the intermediate valves but a non-rod-type crossed lamellar sublayer in the terminal valves. These variations do not appear to reflect phylogeny as closely related species seem no more similar to each other than more distantly related species, yet some features, such as the unique crossed lameallar structure of *L. inquinatus*, do appear to be phylogenetically informative. The analysis of families not included in this study would help to clarify if the microstructure of chiton valves reflects phylogeny.

**Conclusions**

The valves of eight New Zealand chitons -- *Acanthochitona zelandica*, *Notoplax violacea* (Acanthochitonidae), *Chiton glaucus*, *Onithochiton neglectus*, *Sypharochiton pelliserpentis*, *Sypharochiton sinclairi* (Chitonidae), *Ischnochiton maorianus* (Ischnochitonidae), and *Leptochiton inquinatus* (Lepidopleurida) -- were imaged with an SEM and analysed using X-ray diffraction and Raman spectroscopy. All valves were composed of aragonite and had varying ultrastructures among families. The tegmentum of Acanthochitonidae shows few aesthete channels while the Chitonidae have the most. The articulamentum of the Chitonidae is the most complex with six sublayers, whereas the Acanthochitonidae and Ischnochitonidae have simpler articulamentums with only three sublayers. Our single lepidopleuridan has a unique crossed lamellar structure in the articulamentum that lacked second order lamellae. Terminal valves of an individual chiton
had simpler ultrastructures than intermediate valves. Although the microstructure of the valves varied among species, these variations do not appear to reflect phylogeny.

**Acknowledgements**

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Chapter 3 – First record of carotenoid pigments and indications of unusual shell structure in chiton valves

This chapter focuses on the organic components of chiton valves (Figure 3.1). The presence of organic tissue determines how mineral will abrade or dissolve (Harper 2000; Smith and Nelson 2003), therefore it is important to determine both the distribution and concentration of proteins in the valves in order to understand their taphonomy.

Figure 3.1: The cognitive space that Chapter 3 fills in the thesis. This chapter examines the organic components of chiton valves which determines how quickly the valves break down.

The following chapter was published by the Journal of Molluscan Studies in 2017 as a research note. I designed the experiments and wrote the original draft of the paper. G.P.S. Smith and I collected the data. A.M. Smith supervised the project and along with K.C. Gordon helped edit the paper and provided feedback. This paper has been re-formatted to fulfill the format requirements of a PhD thesis of the University of Otago. The references have been moved to the reference section following Chapter 6 (page 96).
First record of carotenoid pigments and indications of unusual shell structure in chiton valves

B.A. PEEBLES1*, K.C. GORDON2, A.M. SMITH1 and G.P.S. SMITH2
1 Department of Marine Science, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
2 Department of Chemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
* Correspondence: Bryce A. Peebles, E-mail address: bryce.peebles@gmail.com

Introduction

Mollusc shells are typically made of calcite, aragonite or, in rare cases, vaterite (Weiner et al. 2009; Ehrlich 2010). These minerals are interspersed with protein matrix and sometimes pigments (Comfort 1949a; Hedegaard et al. 2006). Typically, the organic matrix is composed of β-chitin, silk-like proteins rich in alanine and glycine, and aspartic acid-rich proteins (Wiener & Traub 1984; Addadi et al. 2006; Furuhashi et al. 2010). This matrix creates an environment isolated from the surrounding seawater that the mollusc can supersaturate with calcium carbonate. Mineral layers of the shell develop by the nucleation of crystals of calcium carbonate within the protein matrix (Wiener & Traub 1984). The proteins of the organic matrices of bivalve and gastropod shells have been the most intensively studied. It has been shown that these proteins determine the growth of specific crystal polymorphs of calcium carbonate (Marin & Luquet 2004; Furuhashi et al. 2010; Marin et al. 2012; Suzuki & Nagasawa 2013).

Pigments found within the shell are typically melanins and tetrapyrroles, specifically porphyrins and bilins (Comfort 1949a, b, c; Nicholas & Comfort 1949; Gysi et al. 1979; Cai et al. 2011). Comfort (1949b) identified porphyrins by observing their specific fluorescence in over 3000 mollusc species. He also identified porphyrins using talc-adsorption chromatography in four pterioid bivalves, two caenogastropods and two vetigastropods (Comfort 1949a). Nicholas & Comfort (1949) confirmed the presence of uroporphyrin in several mollusc shells using this method. In addition, Comfort (1949c) noted an unusual biliprotein initially suspected to be an indigoid. Cai et al. (2011) demonstrated a blue polyene in the shell of Haliotis discus by using a combination of UV-vis, infrared, NMR and ESI-mass spectroscopy. Using thin-layer chromatography and electrophoresis, Gysi et al. (1979) identified the pigments of Haliotis californiensis as bilipeptides.
Raman spectroscopy can also be used to identify molluscan shell pigments. Raman spectroscopy is a nondestructive analytical technique that uses lasers to identify compounds present within an unknown sample. Raman microscopy combines Raman spectroscopy with optical microscopy, thus allowing Raman spectra to be obtained from precise locations within a sample. Using Raman spectroscopy, coloured pigments in a wide range of gastropods, bivalves and *Nautilus* have been demonstrated to be polynes (Thompson *et al.* 2014), or specifically carotenoid polynes (Barnard & Waal 2006; Hedegaard *et al.* 2006). The characteristics of Raman spectra are determined by the specific chemical bonds present in the analysed material. While the technique cannot conclusively identify the compounds present, identification is attempted by comparison of spectra with known standards.

As in the case of shell proteins, work on shell pigments has focused on bivalves and gastropods. Reports on shell pigments from other classes of Mollusca are few (Matsuno 2001; Barnard & Waal 2006; Hedegaard *et al.* 2006; Karampelas *et al.* 2009; Thompson *et al.* 2014) and entirely lacking for the clade Aculifera (Polyplacophora and Aplacophora; Kocot *et al.* 2011; Kocot 2013). This lack is surprising in the case of chitons, which are common in the intertidal zone worldwide and include some species with a wide range of colour variation (Sigwart 2016).

Chitons have a shell structure that differs from that of other molluscs. The shell of a chiton is composed of eight overlapping aragonitic plates, or valves. Each valve has an outer organic layer (properiostracum) covering the shell and two primary mineral layers, the outer (dorsal) tegmentum and the inner (ventral) articulamentum. The articulamentum forms the bulk of the valve and is a composite prismatic layer (Connors *et al.* 2012). The dorsal tegmentum is also a prismatic layer which, uniquely in molluscan shells, incorporates sensory structures called aesthetes (Schwabe 2010; Connors *et al.* 2012). These are composed of a canal system, with large and small surface pores (megalaesthetes and micraesthetes, respectively; Schwabe 2010). *Acanthopleura granulata* also incorporates eye-like structures into the tegmentum that may have the ability to form rudimentary images and in which the pigment pheomelanin has been identified (Speiser *et al.* 2011; Li *et al.* 2015). In addition, pigments in the tissues of six chiton species from Japan have been shown to include 27 different carotenoids, although the extracellular pigments within the mineralized layers of chiton valves, or in girdle spicules, have not yet been studied (Tsushima *et al.* 1989; Schwabe 2010; Maoka 2011; Speiser *et al.* 2014; Sigwart 2016). Carotenoids were identified using Raman spectroscopy within the valves of eight chitons from New Zealand: *Acanthochitona zelandica*, *Chiton glaucus*, *Ischnochiton maorianus*, *Leptochiton inquinatus*, *Notoplax*.
violacea, Onithochiton neglectus, Sypharochiton pelliserpentis and S. sinclairi.

Methods

Chitons were collected from the Otago Peninsula, South Island, New Zealand. At least three individuals of each of the eight species were collected and killed in 75% ethanol immediately after collection. The valves were dissected post-mortem and the soft tissues removed. The isolated valves were soaked overnight in a 0.5 M bleach solution to remove the properiostracum and any algal contaminants. Surface pigments exposed to the bleach were thus removed, but pigments incorporated in the shell structure remained intact. The valves were then embedded in epoxy resin and warmed at 60 °C for 2 h to harden. The resin block was cut with a diamond saw to section the valves. The exposed valve material was polished with a series of sandpapers (240 grit, 600 grit, then 1200 grit) followed by polishing on a Kent 3 automatic lapping machine with grit sizes of 3μm then 1μm. The polished valve sections were examined using a Senterra confocal Raman microscope (Bruker Optics, Ettlingen, Germany). The parameters used for Raman microscopy were: laser wavelength 785 nm, 100 mW laser power, 50 μm pinhole aperture, 20× objective, scan time 5 s with six co-additions and a spectral resolution of 9-18 cm⁻¹. Three line maps (each containing 20 spectra) were taken vertically through each valve, taking care to avoid the aesthete channels so that signals were strictly from the mineral layers. For each chiton species, line maps were obtained from each of the valves (I–VIII) for one of the three individuals and, from the head (I), fourth (IV) and tail (VIII) valves for the remaining two individuals. The Raman microscope was calibrated daily using a polystyrene standard.

Results

The Raman spectra from complete valves showed prominent Raman bands at 704 cm⁻¹ (aragonite), 1085 cm⁻¹ (calcium carbonate), 1099-1126 cm⁻¹ (C-C bond stretching) and 1498-1513 cm⁻¹ (C=C bond stretching). The pigment signals were of the same relative strength as the aragonite signal at 1085 cm⁻¹. The aragonite signal at 704 cm⁻¹ was the smallest of the prominent peaks (Fig. 1). Aragonite signals were present in all spectra from all valves. The Raman bands at 1099-1126 cm⁻¹ and 1486-1513 cm⁻¹ appeared only in the tegument; these bands are consistent with other Raman studies of molluscan shell pigments that have suggested carotenoids, based on peaks within the ranges 1070-1210 cm⁻¹ and 1450-
1680 cm$^{-1}$ (Britton et al. 1997; Barnard & Waal 2006; Hedegaard et al. 2006) (Figure 3.2, Table 3.1). The occurrence of these putative carotenoids can be further refined by closer examination of the spectra. A green pigment was found in all analysed chiton valves with signals between 1486-1500 cm$^{-1}$ and 1099-1122 cm$^{-1}$. *Onithochiton neglectus*, *S. pelliserpentis*, *S. sinclairi*, and *I. maorianus* (Chitonina) had an additional brown pigment in their tegmenta with signals at 1510-1513 cm$^{-1}$ and 1122-1126 cm$^{-1}$. The sole lepidopleuridan, *L. inquinatus*, the acanthochitonids (*A. zelandica* and *N. violacea*) and *C. glaucus* only contained the green pigment. The pigment signals detected in *L. inquinatus* were generally weaker than those found in the other chitons. Pigment signals detected from each species only varied by at most 8 cm$^{-1}$, except for those found in *O. neglectus*, which were shifted by at least 12 cm$^{-1}$. The Raman spectra of the chitons did not show features at 1400 or 1588 cm$^{-1}$, suggesting that eumelanin was not present at high concentrations within the valves (Huang 2004; Capozzi et al. 2005; Coccato et al. 2015; Perna et al. 2016).

![Figure 3.2: Raman spectra from the tegmentum and articulamentum of *Leptochiton inquinatus*, *Onithochiton neglectus* and *Sypharochiton sinclairi*. Bands at 704 and 1085 cm$^{-1}$ indicate aragonite, peaks at 1099-1117 cm$^{-1}$ and 1486-1499 cm$^{-1}$ indicate C=C and C-C bonds of carotenoids. Raman intensity is measured in arbitrary units (au).](image-url)
Table 3.1: Stretching modes indicating presence of carotenoid pigments in chiton valves. $\nu_1$ can be attributed to C=C, $\nu_2$ can be attributed to C-C.

<table>
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<th>Species</th>
<th>$\nu_1$/cm$^{-1}$</th>
<th>$\nu_2$/cm$^{-1}$</th>
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<td>1501</td>
<td>1118</td>
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<tr>
<td>Chiton glaucus</td>
<td>1506</td>
<td>1122</td>
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<td>Ischochiton maorianus</td>
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<td>1115</td>
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<td>1099, 1122</td>
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<td>Sypharochiton pelliserpentis</td>
<td>1498, 1510</td>
<td>1116, 1122</td>
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<td>Sypharochiton sinclairi</td>
<td>1499, 1510</td>
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</tbody>
</table>

Small Raman bands appeared at 832-835 cm$^{-1}$ (C-N bond stretching), 1270-1291 cm$^{-1}$ (amide III band stretching), 1534-1536 cm$^{-1}$ (amide II band stretching) and 1618-1620 cm$^{-1}$ (C=O bond stretching) and were consistent between and within each examined species. These are indicative of protein, which is most likely part of the protein matrix of the shell. These signals were smaller than both aragonite signals and were found primarily in the outer mineralized layer, the tegmentum (Figure 3.2). Few or no protein signals were found in the articulamentum, which only showed the strong aragonite signals (Figure 3.3).
Figure 3.3: Raman bands from a representative line map across valve of *Sypharochiton sinclairi*. The lack of protein signals at 1110-1600 cm⁻¹ from the tegmentum, and of pigment signals at 1117 and 1499 cm⁻¹ from the articulamentum, were observed in all analysed species. The inset shows location of typical line maps on cross section of a valve; each grey dot is the location of a spectrum taken by the Raman microscope. Raman intensity is measured in arbitrary units (au). Scale bar = 1mm.

**Discussion**

These green and brown carotenoids found in the tegmentum of chitons are the first instances of carotenoids reported in chiton skeletal material. Tsushima *et al.* (1989) have previously identified carotenoids within the tissue of *Liolophura japonica, Placiphorella japonica, P. stimpsoni, Acanthochitona defilippii, A. rebrolineata* and *Cryptochiton stelleri*, but did not analyse the valves or spicules of these species. Pigment signals in *L. inquinatus* (order Lepidopleurida) were weaker than those of the other seven species examined and the
brown pigment was restricted to four members of the order Chitonida. Despite this apparent correlation between pigments and phylogeny, the presence of carotenoids could also be linked with the environment of the chitons. Since carotenoids can only be synthesized by plants (Barnard & Waal 2006), it is likely that the chitons incorporate pigments from their diet of intertidal algae. Dietary differences could explain the results for *O. neglectus*, which was found on kelp holdfasts rather than on rock as with the other sampled chitons. It is unlikely that these pigment signatures reported here are from algae living on or in the chiton valves, since in our data there were no cellular or nonpigment signals from algae (such as adhesion proteins, haemoglobin, chlorophylls and carbohydrates, as previously associated with algae; Parab & Tomar 2012).

Decalcification of chiton valves was attempted using 0.5 M ethylenediaminotetraacetic acid (EDTA) in order to remove the dominant aragonite signals and measure the isolated proteinaceous component of the chiton valves. This decalcification process resulted in the aragonite material dissolving, leaving a firm protein structure. Since the polysaccharides α-chitin and β-chitin have been described as major components of chiton valves, the decalcified valves should theoretically reveal the presence of chitin (Treves *et al.* 2003; Evans & Alvarez 1999; Weaver *et al.* 2010; Connors *et al.* 2012). However, while the decalcified valves showed stronger protein-related peaks in the same location as in the mineralized valves, and no aragonite signal, the spectra did not conclusively indicate the presence of chitin when compared with a chitin standard (Figure 3.4; De Gelder *et al.* 2007; Ehrlich *et al.* 2007). The decalcified valves consistently showed peaks at 534, 930, 955, 1267 and 1621 cm\(^{-1}\), whereas reference spectra from α-chitin showed peaks at 953-955 cm\(^{-1}\), 1267 cm\(^{-1}\) and 1621-1622 cm\(^{-1}\) (Figure 3.4). The similarities indicate only that both the decalcified material and α-chitin have a C-C bond (955 cm\(^{-1}\)) and an amide group (1267 cm\(^{-1}\)). Notably, α-chitin also shows peaks at 1621 cm\(^{-1}\) and 1656 cm\(^{-1}\) (Mikkelsen *et al.* 1997), of which the decalcified valves show only the first, at 1621 cm\(^{-1}\). The decalcified material also appears to be missing the characteristic chitin peaks at 1087, 1109, 1149, 1204, 1324, 1374, 1417 and 1450 cm\(^{-1}\) (Mikkelsen *et al.* 1997). The match between the spectra of the decalcified valves and the α-chitin standard is not sufficiently close to identify chitin with confidence.
Figure 3.4: Raman spectra of a decalcified *Sypharochiton sinclairi* valve and of α-chitin. The major peaks of α-chitin do not appear in the spectrum from *S. sinclairi*. Raman intensity is measured in arbitrary units (au).

**Conclusion**

These are the first data to suggest an unusual composition of chiton shells among molluscs. In other mollusc shells each aragonite crystal is surrounded by an organic matrix of protein and chitin, which is integral to the process by which the shell is formed (Bøggild 1930; Weiner & Traub 1984; Addadi *et al.* 2006). The absence of evidence of α-chitin and β-chitin in the chiton valves is therefore intriguing, especially since chitin has previously been reported in chitons. Treves *et al.* (2003), using IR spectroscopy, found that chitin was abundantly present in the valves of *Acanthopleura villanii* although absent from its girdle spicules. Weaver *et al.* (2010) examined the radula of *Cryptochiton stelleri* using both transmission electron microscopy and Raman spectroscopy and found α-chitin. FTIR spectroscopy has been used to detect α-chitin in the matrix component of valves and girdle spicules of *Tonicella marmorea* and in the radula of *Sypharochiton pelliserpentis* (Evans & Alvarez 1999; Connors *et al.* 2012). These conflicting results should be examined by future studies using both Raman and IR microscopy. The lack of protein signals in the articulamentum is also unexpected, since the organic matrix used for calcification should be present throughout the valve. It is likely that matrix proteins were present in the articulamentum but below detection levels, since there solid material did remain when the valves were decalcified.
Acknowledgements

Thanks to Professor Hamish Spencer and Erin Bowkett for collecting chitons with us for this study. Many thanks to Brent Pooley for his support in sectioning, polishing and resin-embedding of samples. We acknowledge support from the Departments of Marine Science and Chemistry, University of Otago.
Chapter 4 – Abrasion Provides Clues on a Chiton Taphonomic Conundrum

This chapter directly examined how chiton valves resist abrasion (Figure 4.1). The few studies that measure how chiton valves respond to lowered pH suggest that they are resistant to dissolution. However, there is little data on how chitons resist other taphonomic forces, such as abrasion. Therefore, determining how well chiton valves resist abrasion may provide insight into why they are rare in the sediment and fossil records.

Figure 4.1: The cognitive space that Chapter 4 fills in the thesis. This chapter examines how well chiton valves withstand abrasion and provides insight to the taphonomy of chitons.

The following chapter was submitted to Palaeogeography, Palaeoclimatology, Palaeoecology in April 2017. I collected the data and wrote the original draft of the manuscript. A.M. Smith supervised the project and helped edit and improve the draft. As with the other manuscripts, the reviewers’ concerns have been address and the following chapter has been slightly modified to fit the thesis formatting and the references moved to the reference section beginning on page 96.
Abrasion provides clues on a chiton taphonomic conundrum

B.A. Peebles and A.M. Smith

1 Department of Marine Science, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

*Corresponding Author: e-mail: abby.smith@otago.ac.nz, phone: +64 3 479 7470

Abstract

Chitons are common marine invertebrates found worldwide that use aragonite to form their shell and are rare in the sedimentary and fossil records. Their rarity in the fossil record is interesting due to how abundant live chitons are, but may appear unremarkable since metastable aragonite is known to disappear from the fossil record over time. However, previous work suggests that chitons resist dissolution, which appears inconsistent with their rarity in the sediment and fossil records and aragonite composition. This result warrants examination of other taphonomic processes on chiton skeletal material. If dissolution is not removing chiton skeletal material from the fossil record, then other taphonomic processes may play a larger role in removal than previously thought. As many chitons are intertidal, mechanical abrasion of chiton valves is likely to occur; its extent is largely unknown and yet necessary to provide insight to the taphonomy of chiton material. Eight species of chitons were collected from the Otago Peninsula, South Island, New Zealand. A total of 129 valves were isolated, cleaned, weighed, and tumbled in a Lortone 3A rock tumbler for 96 hours. The valves from each species lost an average of between 9-44 % of their weight; damage varied among species. Leptochiton inquinatus (the sole lepidopleuran) lost the most material while the acanthochitonids (Acanthochitona zelandica, and Notoplax violacea) and the chitonids (Chiton glaucus, Onithochiton neglectus, Sypharochiton pelliserpentis, and S. sinclairi) were more resistant to abrasion. The dorsal layer of the valves (the tegmentum), which is penetrated by aesthete tissue and has a granular crystal microstructure, was the most damaged by abrasion in all species. The ventral layer (the articulamentum), which has a highly-organised crystal structure with fewer organic components than the tegmentum, showed the least signs of abrasion. The valves are estimated to last about seven years when in the intertidal zone, which is much less than the hundreds to thousands of years material is exposed.

Keywords: Polyplacophora, Aragonite, Taphonomy
**Introduction**

Chitons are common molluscs worldwide. They are exclusively marine and are characterised by their eight-part shell, composed of overlapping aragonite plates called valves. There are an estimated 930 extant species and 430 fossil species, most of which are found in the intertidal zone (Fontoura-da-Silva *et al.* 2003; Puchalski *et al.* 2008; Schwabe 2008). New Zealand has a large and diverse chiton population, with over 8.5% of the world’s chiton species live in its Exclusive Economic Zone (Gordon *et al.* 2010). Population densities of the very common chiton *Sypharochiton pelliserpentis* may reach 228 individuals/m², but most species are found in New Zealand at population densities of 3-8 individuals/m² (Boyle 1970). Creese 1988 shows other mollusc abundances to be typically lower than those of chitons (see table 1; Creese 1988).

Despite being locally common, chitons rarely appear in the sediment and fossil records (Cherns 1999; Puchalski *et al.* 2008; Cherns and Wright 2009). The vast majority of carbonate shelf deposits are skeletal material, yet literature describing carbonate sediments from New Zealand has no mention of chitons, despite bivalves and gastropods comprising over 20% of the deposit (Ashby 1930; Andrews 1973; Nelson 1978; Probert *et al.* 1979; Nelson and Hancock 1984; Nelson *et al.* 1988; Hayton *et al.* 1995). Fossilised New Zealand chitons are discussed only in some of the literature detailing the fossil records of New Zealand (Beu and Maxwell 1990; Crampton *et al.* 2006).

Although the live-to-dead fidelity of molluscs is high for intertidal, sub-coastal and open shelf habitats (Kidwell and Flessa 1995; Behrensmeyer *et al.* 2000), chitons also rarely appear in the fossil record (Cherns 1999; Vendrasco *et al.* 2012). Extinct chiton species are generally represented by only a few valves and are rarer than other taxa even when reported as abundant (Puchalski *et al.* 2008). The tegmentum and valve shape are used to identify chiton species, so the valve must be in good condition to be recognised and identified. However articulated chiton valves are rare in the fossil record and can sometimes be found as silicified fossils, which occurs when silica precipitates and replaces the calcareous material (Dell’Angelo *et al.* 2003; Sigwart and Sutton 2007). For example, Cherns (1999) reported that chitons from the Silurian of Gotland, Sweden showed an “unusually abundant, diverse, and well-preserved assemblage”, but Paleoloricata valves were 7% as common as bivalves. In a rare exception, Cherns and Wright (2009) described a lagerstätten with Paleoloricata fossils representing 3.5% of silicified molluscs in the Devonian period and dominating in the Ordovician period (18% of molluscs). Hoare and Farrell (2004) also found both Paleoloricata
and Neoloricata from the Devonian period in the Garra Formation in New South Wales. In all, however, chitons accounted for only 3.5% of the silicified molluscs from the Paleozoic to Early Mesozoic in this area (Cherns and Wright 2009). Hoare and Pojeta (2006) collected 55,000 silicified mollusc fossils from the several localities in the USA and only 1.9% (1,083) of them were chitons. Sigwart et al. (2007) collected 458 chiton valves from Denmark, which is was the first reported occurrence of chitons from the region. The only report of chiton fossils that number above 10,000 comes from the San Diego Formation, with more than 15,000 chiton valves from 22 species. The large numbers of intact chiton valves in the San Diego Formation are most likely a result of rapid burial at the relatively shallow depth of 25m (Vendrasco et al. 2012).

The relative absence of chitons in the fossil records is apparent even in New Zealand, which has the most complete Cenozoic molluscan fossil record in the Southern Hemisphere (Crampton et al. 2006). Other molluscs, particularly bivalves and gastropods, are common throughout the record and both cephalopods and opisthobranchs make regular appearances (Stilwell 1993; Stilwell and Grebneff 1996; Beu et al. 2012; Beu, 2012). Beu (2012) and Stilwell (1993) reported on molluscs in the Cenozoic, but listed no chitons. Beu et al. (2012) reviewed data collected in 1865 and did not list any chitons. Beu and Maxwell (1990) listed only 28 species of chitons in the checklist of New Zealand Cenozoic molluscs. Notoplax rubiginosa is the most common chiton in the Plio-Pleistocene. Acanthochitona flebilis, Notoplax sp., and Chiton sp. are representative species of the Pliocene. Cryptoplax sp. was found in sediment washings from the Early/Mid Miocene. Isolated chiton valves were found in the base of the Kauru formation and in faunules from Waitakian and Cenozoic periods but were not identified to species.

Puchalski et al. (2008) argue that the rarity of chitons in the fossil record may be due to sampling bias, since collector curves for Europe and North America are incomplete. A collector curve is a plot of the cumulative number of species compared to the total number of individuals sampled over time. The intention behind this method is to show when adequate sampling is achieved, a collector curve assumes sampling from an area where the biodiversity has not been fully described theoretically results in new species being recorded. The curve should approach a horizontal asymptote when all species have been found in an area; if the curve has not plateaued, it is likely there are additional species in the area that have not been recorded (Weller 1952; Fountaine et al. 2005). While increased sampling may not reveal more species, the curve does not suggest that sampling is complete when the curve reaches an asymptote. It is possible that the rarity of chitons in the fossil record is due to lack of
sampling, given that the main source of disagreement in the live and dead records is lack of sampling over time for current environments. However, lack of sampling does not address chiton fossil rarity in the assemblages from Australasia, Africa, and South America since their collector curves appear to have reached a plateau (Puchalski et al. 2008). In fact, Fleming (1965) shows that the collector curve for New Zealand molluscs began to stabilise around 1945. Therefore, it seems most likely that the sparsity of chitons in the sediment and fossil record is the effect of taphonomic processes removing them from these records or causing them to be unrecognisable.

Taphonomic processes are non-random, since abrasion, breakage, dissolution, and bioerosion act differently on different shapes, sizes, and crystal structures of calcareous material (Harper 2000; Sanders 2003; Smith and Nelson 2003; Cherns and Wright 2009). There is, for example, a bias in the fossil record against aragonite, as it either is removed by dissolution (Sanders 2003) or is replaced by siliceous material which works against dissolution (Cherns and Wright 2000; Wright et al. 2003). Organisms that use calcite to form their skeletal components are more likely to be preserved than those that use aragonite. Since chiton valves are made of aragonite, it seems logical to conclude that dissolution is the cause of removal, especially since silicified chiton fossils are found from the Devonian era (Cherns 1999; Hoare and Farrell 2004). Strangely, however, chiton valves appear to resist dissolution when introduced to environments with a reduced pH of 7.5 (Sigwart and Carey 2014; Sigwart et al. 2015). Chiton valves were exposed to a pH of 7.5 for four weeks at three different temperatures (10, 15, and 20°C) yet the valves remained undamaged (Sigwart and Carey 2014). Valves also showed no reduction in fracture resistance after 10 days of treatment in a pH of 7.5 (Sigwart et al. 2015). However, Sigwart and Carey (2014) were primarily interested in the grazing behaviour under stressful conditions and neither study involved other molluscs or aragonitic shells; while the evidence that chitons may resist dissolution is equivocal it is important enough to consider examining other taphonomic forces since dissolution may not affect chiton valves as much as previously suspected. If chiton valves are indeed able to withstand dissolution, then either biological or physical forces may play a larger role than expected in removing them from the fossil record.

Chiton fossils have been shown to provide information on past salinity and temperature levels (Rojas and Urteaga 2011) so understanding chiton taphonomy can aid in constructing palaeoceanographic conditions. Chitons also appear to have fossilisation potentials that vary among families (Puchalski and Johnson 2009; Sigwart et al. 2014), which adds to their importance as a study organism. The effect of taphonomic forces on chiton
skeletal material, particularly forces other than dissolution, remains understudied. Abrasion in particular affects post-mortem mobile organisms in the intertidal and shelf environments to a higher degree than sessile organisms (Johnson 2006) yet the effects of abrasion have been examined on only three chitonid species: *Acanthochitona crinita* (Family Acanthochitonidae), *Tonicella marmorea* (Family Toncellidae), and *Lepidochitona cinerea* (Family Lepidochitonidae) (Sigwart et al. 2014). This study seeks to expand the current knowledge of taphonomic processes on chiton skeletal material by measuring the effect of abrasion on eight species from four different families (Acanthochitonidae, Chitonidae, Ischnochitonidae, and Leptochitonidae) in two different orders (Chitonida and Lepidopleurida).

**Methods**

Eight species of chitons were collected by hand from the Otago Peninsula on the South Island of New Zealand: *Acanthochitona zelandica, Notoplax violacea* (family Acanthochitonidae, order Chitonida), *Chiton glaucus, Onithochiton neglectus, Sypharochiton pelliserpentis, S. sinclairi* (family Chitonidae, order Chitonida), *Ischnochiton maorianus* (family Ischnochitonidae, order Chitonida), and *Leptochiton inquinatus* (family Leptochitonidae, order Lepidopleurida) (Figure 4.2). Three individuals of each species, except *N. violacea* of which two individuals were collected, then immediately killed and preserved in a 75% ethanol solution.
Figure 4.2: *Chiton glacus* with valves numbered and girdle labelled (a) and the head, tail and an intermediate valve of the eight chiton species analysed in this study (b-i): *A. zelandica* (a), *N. violacea* (b), *C. glaucus* (c), *O. neglectus* (d), *S. pelliserpentis* (f), *S. sinclairi* (g), *I. maorianus* (h), and *L. inquinatus* (i).

Three individuals of each species, except *N. violacea* which had two, were used. Valves were dissected and disarticulated, brushed clean of soft tissue, dried in a 60° oven overnight, then weighed with a precision of 0.1 mg. Any valves that were broken or chipped before collection or during the dissection process were set aside and not used. Terminal valves (head and tail valves) and intermediate valves were tumbled together. A Lortone 3A rock tumbler with a 0.7 l capacity was used to tumble 13-22 valves (129 total) along with 10 ml of sediment collected from North Beach, Otago Peninsula (lat 45.8938 °S, long 170.6509 °E), where the majority of chitons were collected, and tap water was added to fill the tumbling container halfway. The tumbler rotated at a speed of 53 rpm and the valves were tumbled for 96 hours. The sediment and valves travelled about half of the circumference (15 cm) before they fell back down to the bottom of the barrel a distance equal to the diameter (10 cm) on each rotation (Figure 4.3; 25 cm/rotation or 13 m/min). After 96 hours the grains would have travelled about 74 km. Since grains in the intertidal zone can be moved back and
forth for an equivalent distance of 3 km/day (Smith and Nelson 2003), this experiment would have been theoretically the same as 24 days 18 hours in the surf zone. Each species was tumbled individually, with all isolated valves in the rock tumbler. After the valves were tumbled, they were cleaned in an ultrasonic bath of reverse osmosis (RO) water for two minutes, dried in an oven at 60 °C, then re-weighed.

As a control treatment, 9-17 valves (109 total) were dried, weighed, and submerged in water for 96 hours but were not tumbled. Since the process of chemical dehydration with ethanol, dissection, oven drying, and re-submerging into water varies from the natural decay process that chiton valves undergo in the marine environment, this control group was necessary to make sure that these changes did not affect how the valves responded to abrasion. The control group was also necessary to ensure that the use of tap water instead of sea water did not affect the results. The non-tumbled valves were then cleaned, dried, and re-weighed.

Figure 4.3: The path taken by the sediment and valves in the rock tumbler. “A” is the arc of the circle equal to ½ the circumference and “D” is the diameter. The material in the tumbler moves a distance roughly equal to A+D each rotation.

Results

The control treatment showed that the drying, submerging, and cleaning processes caused the valves to lose 0.0-0.3 mg (0.0-6.7 % of their initial weight). This weight loss from the non-tumbling processes was negligible (less than 1 %) for all species except *L. inquinatus*, which weighed so little (0.1-1.7 mg) that these processes alone accounted for an average 6.8 % weight loss (Figure 4.4). *I. maorianus* (Ischnochitonidae) lost the least weight
(0.38-0.41 with a mean of 0.40 %) among the analysed chitons (0.0-0.1 mg with a mean of 0.06 mg). The chitonids (C. glaucus, O. neglectus, S. pelliserpentis, and S. sinclairi) lost 0.0-0.2 mg (0.06-0.23 %) with means of 0.08, 0.06, 0.08, and 0.03 mg (0.14, 0.21, 0.20, 0.07 %) respectively. L. inquinatus (Leptochitonidae) and the acanthochitonids (A. zelandica and N. violacea) lost slightly more than the other species (0.0-0.3 mg with means of 0.09, 0.10, and 0.06 mg respectively; 0.11-6.78% with means of 6.77, 0.13, and 0.85 % respectively).

Chiton valves appeared to resist abrasion fairly well. Most of the samples lost less than 25 % of initial weight after 96 hours in some fairly abrasive conditions and negligible weight in the control treatment (Figure 4.5).

Figure 4.4: Percent weight lost by valves in the control treatment. Boxes represent the average ± the standard deviation and the whiskers indicate the 95 % confidence interval. n is the number of individual valves tumbled for each species.
Figure 4.5: An unabraded valve (left valve in all images) and an abraded valve (right valve in all images) of *Acanthochitona zelandica* (A and B), *Sypharochiton pelliserpentis* (C and D), and *Leptochiton inquinatus* (E and F). The left-hand images (A, C, and E) show the damage done to the tegmentum and the right-hand images (B, D, and F) show the articulamentum of same two shells.

The chiton valves lost an average of 9.2-44.0 % (0.6-16.4 mg) of their weight after 96 hours in the rock tumbler, much more than the 0.0-6.7 % (0.0-0.3 mg) of the control treatment (Figure 4.6; Table 4.1). The acanthochitonids and chitonids were the most resistant groups while the lepidopleuran (*Leptochiton inquinatus*) was the least resistant. The acanthochitonids (*Acanthochitona zelandica* and *Notoplax violacea*) lost 15.2 ± 4.9 % (2.1 ± 1.6 mg) and 14.7 ± 2.7 % (4.9 ± 1.2 mg) respectively. *Chiton glaucus* was the most resistant species overall and lost 9.2 ± 1.8 % (4.9 ± 2.0 mg) of valve weight. *Onithochiton neglectus* lost 13.0 ± 1.1 % (3.5 ± 1.4 mg) and *Sypharochiton pelliserpentis* and its sister species *S. sinclairi* lost 18.8 ± 2.5 % (10.6 ± 7.8 mg) and 20.2 ± 5.4 % (5.1 ± 1.8 mg) respectively. *Ischnochiton maorianus* was the only ischnochitonid studied and lost 21.1 ± 4.6 % (16.4 ± 3.0 mg) of its valve weight. *Leptochiton inquinatus*, the only member of order Lepidopleurida and sole leptochitonid, was the only species to lose over 20% of its weight with an average of 44 ± 21.8 % (0.6 ± 0.1 mg) lost and was the only species in which not all valves were recovered from the rock tumbler. Two intermediate valves of *L. inquinatus* were destroyed in the tumbling process.
Figure 4.6: Valve weight loss after 96 hours in the tumbler. The boxes contain the mean ± one standard deviation and the whiskers extend to the 95\% confidence interval. n is the number of individual valves tumbled for each species.

Table 4.1: Average valve weight loss in the control and experimental treatments.\(^1\)

<table>
<thead>
<tr>
<th>Chiton Species</th>
<th>Control</th>
<th>Experimental</th>
<th></th>
<th></th>
<th></th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthochitona zelandica</td>
<td>0.02 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>2.1</td>
<td>15.2</td>
<td>0.21</td>
<td>16</td>
</tr>
<tr>
<td>Notoplax violacea</td>
<td>0.01 ± 0.002</td>
<td>0.85 ± 0.01</td>
<td>4.9</td>
<td>14.7</td>
<td>0.20</td>
<td>17</td>
</tr>
<tr>
<td>Chiton glaucus</td>
<td>0.05 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>4.9</td>
<td>9.2</td>
<td>0.12</td>
<td>16</td>
</tr>
<tr>
<td>Onithochiton neglectus</td>
<td>0.02 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>3.5</td>
<td>13.0</td>
<td>0.18</td>
<td>15</td>
</tr>
<tr>
<td>Sypharochiton pelliserpentis</td>
<td>0.04 ± 0.03</td>
<td>0.20 ± 0.01</td>
<td>10.6</td>
<td>18.8</td>
<td>0.25</td>
<td>15</td>
</tr>
<tr>
<td>Sypharochiton sinclairi</td>
<td>0.02 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>5.1</td>
<td>20.2</td>
<td>0.27</td>
<td>13</td>
</tr>
<tr>
<td>Ischnochiton maorianus</td>
<td>0.02 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>16.4</td>
<td>31.1</td>
<td>0.28</td>
<td>15</td>
</tr>
<tr>
<td>Leptochiton inquinatus</td>
<td>0.001 ± 0.0004</td>
<td>6.77 ± 0.02</td>
<td>0.6</td>
<td>44.0</td>
<td>0.59</td>
<td>22</td>
</tr>
</tbody>
</table>

\(^1\) Values are reported as the average ± standard deviation. n is the number of individual valves tumbled for each species.

The terminal valves (head and tail valves) lost a different mean of weight than the intermediate valves of all analysed species. The terminal valves of *N. violacea*, *S. pelliserpentis*, and *I. maorianus* lost more weight than the intermediate valves while the opposite was true for the other chiton species (Table 4.2). While there appears to be variation on how the different valves behaved, the bulk of the data is from intermediate valves and the data from each species only includes 2-3 head and 2-3 tail valves. Due to the large difference in sample size, there cannot be any meaningful statistics or conclusions drawn from this, especially since there is no consistent pattern in how the terminal valves responded in comparison to the intermediate valves.
Single factor ANOVA tests with phylogenetic independent contrasts (Felsenstein, 1985) were performed in R (R Core Team, 2017), where contrasts are applied to each node on a phylogenetic tree to exclude any effect of phylogeny, using the ape and phytools packages (Paradis et al., 2004; Revell, 2012) and revealed a significant difference in weight loss between orders, families, genera, and species of chitons except within Acanthochitonidae and between the two *Sypharochiton* species, and between the Chitonidae and *I. maorianus* (Table 4.3).

Table 4.2: Percent weight lost in the experimental treatment by the intermediate, head, and tail valves.

<table>
<thead>
<tr>
<th>Chiton Species</th>
<th>Intermediate (%)</th>
<th>Head (%)</th>
<th>Tail (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acanthochitona zealandica</em></td>
<td>17.9</td>
<td>11.7</td>
<td>17.6</td>
</tr>
<tr>
<td><em>Notoplax violacea</em></td>
<td>14.3</td>
<td>15.4</td>
<td>18.4</td>
</tr>
<tr>
<td><em>Chiton glaucus</em></td>
<td>9.6</td>
<td>7.8</td>
<td>8.8</td>
</tr>
<tr>
<td><em>Onithochiton neglectus</em></td>
<td>14.6</td>
<td>14.1</td>
<td>13.4</td>
</tr>
<tr>
<td><em>Sypharochiton pelliserpentis</em></td>
<td>18.3</td>
<td>14.1</td>
<td>17.9</td>
</tr>
<tr>
<td><em>Sypharochiton sinclairi</em></td>
<td>17.8</td>
<td>20.7</td>
<td>24.0</td>
</tr>
<tr>
<td><em>Ischnochiton maorianus</em></td>
<td>17.4</td>
<td>18.7</td>
<td>19.9</td>
</tr>
<tr>
<td><em>Leptocheiton inquinatus</em></td>
<td>44.0</td>
<td>34.8</td>
<td>36.0</td>
</tr>
</tbody>
</table>

Table 4.3: P-values from the single factor ANOVA results showing significant variation in weight loss.

<table>
<thead>
<tr>
<th>Node</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitonida / Lepidopleurida</td>
<td>0.020</td>
</tr>
<tr>
<td>Acanthochitonina / Chitonina</td>
<td>0.001</td>
</tr>
<tr>
<td>Chitonidae / Ischnochitonida</td>
<td>0.060</td>
</tr>
<tr>
<td>Acanthochitona / Notoplax</td>
<td>0.340</td>
</tr>
<tr>
<td>Chiton / Onithochiton / Sypharochiton</td>
<td>0.030</td>
</tr>
<tr>
<td>S. sinclairi / S. pelliserpentis</td>
<td>0.470</td>
</tr>
</tbody>
</table>

There appears to be a correlation between average valve thickness and weight loss (Figure 4.7). Thicker valves appear to be more resistant to abrasion since they lose a smaller
proportion of their initial weight, however they lose more mg than thinner valves. It is likely that the thicker valves lose more material simply because of their size, but the material lost by the thicker valves is proportionally smaller than that lost by thinner valves.

![Figure 4.7: Weight lost, in both percent of original weight and mg, plotted against valve thickness.](image)

**Discussion**

Chitons appear to be less vulnerable to abrasion than other molluscs. *Mytilus edulis* (Bivalva), *Tritia obsoleta* (reported as *Nassarius obsoletus*), and *Urosalpinx cinerea* (Gastropoda) lost roughly 56 %, 27 %, and 15 % of their weight respectively after tumbling 1700 hours (Driscoll and Weltin 1973). *U. cinerea* and *N. obsoletus* lost a similar range of weight as the majority of the chitons in this study (9-21 %), but *M. edulis* lost more than double this weight and more than *L. inquinatus* (44 %). Rate loss due to abrasion is not linear and changes depending on grain size and shell structure (Driscoll and Weltin 1973; Smith and Nelson 2003). The figures Driscoll and Weltin (1973) provide show an initial rapid loss of weight, typically within the first 100 h, then weight loss begins to stabilise afterwards. The tumbler used in Driscoll and Weltin’s (1973) study was hexagonal and a parameter for distance tumbled is not reliable, so it is difficult to directly compare between studies. However, these molluscs lost a similar range of weight as the chitons in this study but they
became perforated and large holes developed in the shell by the end of the experiment. Perforation did not happen with any of the chiton valves. A similar range of weight loss but with less perforation implies that the chiton valves are more resistant to abrasion. The bivalves *Mercenaria mercenaria*, *Mya arenaria*, and *Aequipecten irradians* were exposed on a beach for 100 hours and lost only 1.5-3.5 % of their weight, which is much less than the chitons lost (Driscoll 1967). However, Driscoll (1967) suggests that 100 hours of exposure to the beach environment does damage to shells equivalent to two hours in a rock tumbler. This suggestion makes sense mathematically; if the reported average movement of 3 km/day in the surf zone is used (Smith and Nelson 2003) then a tumbler would need to move shells 208.3 cm/min for 24 hours in order to move material the same amount (3 km in 24 hours), which is roughly 7.5 rotations/min with a 10 cm diameter barrel. Typical tumbler rotations are over 50 rotations/min, well over the 7.5 needed to closely mimic the surf zone environment. Smith and Nelson (2003) reported that the average loss of temperate carbonate sediments due to abrasion is 12 %/km surf zone transport; the chitons in this study lost two orders of magnitude less than this (0.12-0.59 %/km; see Table 1).

Each species analysed in this study has been found in the New Zealand fossil record (Beu and Maxwell 1990). The relatively common species of chitons in the Cenozoic New Zealand fossil record are those from the genera *Acanthochitona*, *Notoplax*, *Chiton*, and *Cryptoplax*. The chitonids (*Chiton glaucus*, *Onithochiton neglectus*, *Sypharochiton pelliserpentis*, and *Sypharochiton sinclairi*) and acanthochitonids (*Acanthochitona zealandica* and *Notoplax violacea*) tumbled in this experiment also were the most resistant to abrasion and either appear in or are closely related to the chitons that appear most in the fossil record. *Leptochiton inquinatus* and *Ischnochiton maorianus* are found in the record as well, but are rarer than the other chiton species, which is consistent with the results of our abrasion tests since they are more vulnerable to abrasion than the other species tested.

The results of the ANOVA tests indicate that the amount of abrasion a chiton valve can resist may be linked to phylogeny, as has been reported elsewhere (Puchalski and Johnson 2009; Sigwart *et al*. 2014). Puchalski and Johnson (2009) ascertained the preservation potential of chiton species by collecting *Katharina tunicata* and *Mopalia muscosa* valves from the sediment on San Juan Island, Washington, USA and assessing the amount of damage done to the collected valves by examination with a dissecting microscope, then assigning the valves a total taphonomic grade based on: fragmentation, bioerosion, colour, edge modification, and surface erosion. Their results indicated that *M. muscosa* has a higher chance of being preserved than *K. tunicata* since the taphonomic grades of *M.*
muscosa valves were generally lower (meaning the valves were more pristine) than the grades of *K. tunicata* valves. This result implies that different species within Mopaliidae have different potential to fossilise. Sigwart *et al.* (2014) also found a difference in preservation potential between families. The chitons used in their study were sacrificed, placed into sealed containers to decay for 120 days, then tumbled in a rock tumbler for 18 hours. They reported that *Acanthochitona crinita* (Acanthochitonidae) was the most resistant to abrasion, while *Lepidochitona cinerea* (Lepidochitonidae) and *Tonicella marmorea* (Toncillidae) fared less well, with *T. marmorea* suffering the most damage.

The chitons examined by our study also appear to have different capacity to resist abrasion depending on genus. It is interesting that the chitonids are statistically different from each other, yet the acanthochitonids show no statistical difference in material loss. Ischnochitonidae and Leptochitonidae only had one member, so no conclusion can be drawn about how members of those families differ in their fossilisation potential. The data gathered by this study also show the acanthochitonids *Acanthochitona zealandica* and *Notoplax violacea* to be the most resistant to abrasion, similar to the results presented by Sigwart *et al.* (2014). However, the specimens tested by this study did not fracture or lose as much material described by Sigwart *et al.* (2014)’s study. This observed difference in abrasion resistance may be because the chitons examined here underwent a drying and rehydrating process and did not undergo a decay experiment prior to tumbling. In marine sediments, organic tissue decays at a rate should decay rapidly over roughly 70 days, then slow to a rate which can take several years to dissolve (Westrich and Berner 1984). This change in decay rate is because there are two fractions of tissue, the first fraction dissolves at a rate proportional to its mass (Berner 1964; Westrich and Berner 1984) and the second dissolves at a logarithmic rate (Westrich and Berner 1984; Middleburg 1989). It is likely in the experiments performed by Sigwart *et al.* (2014) that the first fraction would have completely been removed while the second fraction of tissue would still be attached to the valves. However, the proportion of these two fractions in chiton valve material is unknown and the valves were subjected to decay for less than a year, so it is unlikely that the second fraction of tissue would be heavily removed. Regardless, decay of organic tissue would increase the rate of deterioration and increase the damage done by abrasion to the valves (Kidwell and Baumiller 1990; Glover and Kidwell 1993). The chitons examined in this study were also tumbled as isolated valves instead of connected to their tissue. This would mean the articulamentum and apophysis were exposed to a larger degree and that there was no connecting tissue for microbes to decay. The articulamentum would be more damaged by abrasion when the valves are isolated than with
the tissue on initially since there is no tissue to act as a barrier. If the tissue remains connected to the shell long enough to decay, the microbial decay process would locally dissolve the shell (Glover and Kidwell 1993; Smith and Nelson 2003). Local dissolution would remove additional material than abrasion alone. The difference in methods would cause the chitons in this study to have less microbial decay and dissolution but more damage due to abrasion than those tested by Sigwart et al. (2014).

_Leptochiton inquinatus_, which was the most vulnerable to abrasion among the examined chitons, has a unique crossed-lamellar microstructure in the articulamentum (Peebles et al. 2017). The architecture of the aesthete channels also varies between lepidopleurans and chitonidans (Sirenko 2006; Peebles et al. 2017). This structure may cause it to be more vulnerable to abrasion than the crystalline structures formed by the other chitons in this study. However, there does not appear to be a correlation between variations in mineralogy and ability to resist abrasion among the chitonidans. All members of Chitonidae have a similar amount of aesthete tissue in their tegmentum and articulamentum structures that closely resemble each other (Peebles et al. 2017). The only variation of microstructures among the Chitonidae is that the Sypharochiton spp. have an additional, thin (< 1 µm) rod-type crossed lamellar sublayer ventral to the tegmentum. Ischnochiton maorianus and the acanthochitonids (A. zelandica and N. violacea) all have a simpler articulamentum of 3 sublayers, but vary in aesthete structure. The chitonidans all showed a high resistance to abrasion, except for the two Sypharochiton spp., which does not match the variation in valve microstructure reported by Peebles et al. (2017). The size of the valves may also play a part as _L. inquinatus_ had the thinnest valves of the collected chitons, and their small size may have led to the lowest observed resistance to abrasion (Table 4.3; Figure 4.6). Sigwart et al. (2015) also noted that valve thickness may play a role in preservation. They reported that _Katherinea tunicata_ valves resisted abrasion to a higher degree than the other examined chitons (Mopalia mucosa and M. lignosa) and argue that this was likely because the valves were thicker.
Table 4.3: Abrasion resistance of analysed chiton valves compared to phylogeny and valve composition, size, and persistence time.

<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acanthochitonina</td>
<td>Acanthochitonidae</td>
<td>Acanthochitona zelandica</td>
<td>high</td>
<td>3.99</td>
<td>Aragonite</td>
<td>Low aesthete penetration, porous tegmentum, 1 Crossed-lamellar and 1 spherulitic sublayer in articulamentum</td>
<td>Green Carotenoid</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Notoplax violacea</td>
<td>high</td>
<td>4.20</td>
<td>Aragonite</td>
<td>Low aesthete penetration, porous tegmentum, 1 Crossed-lamellar and 1 spherulitic sublayer in articulamentum</td>
<td>Green Carotenoid</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chitonida</td>
<td>Chitonidae</td>
<td>Chiton glaucus</td>
<td>high</td>
<td>7.00</td>
<td>Aragonite</td>
<td>High aesthete penetration, porous tegmentum, 2 Crossed-lamellar and 2 spherulitic sublayers in articulamentum</td>
<td>Green Carotenoid</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Onithochiton neglectus</td>
<td>high</td>
<td>4.67</td>
<td>Aragonite</td>
<td>High aesthete penetration, porous tegmentum, 2 Crossed-lamellar and 2 spherulitic sublayers in articulamentum</td>
<td>Green, Brown Carotenoid</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sypharochiton pelliserpentis</td>
<td>moderate</td>
<td>3.36</td>
<td>Aragonite</td>
<td>High aesthete penetration, porous tegmentum, 3 Crossed-lamellar and 2 spherulitic sublayers in articulamentum</td>
<td>Green, Brown Carotenoid</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sypharochiton sinclairi</td>
<td>moderate</td>
<td>3.11</td>
<td>Aragonite</td>
<td>High aesthete penetration, porous tegmentum, 3 Crossed-lamellar and 2 spherulitic sublayers in articulamentum</td>
<td>Green, Brown Carotenoid</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ischnochitonidae</td>
<td>Ischnochiton maorianus</td>
<td>moderate</td>
<td>2.99</td>
<td>Aragonite</td>
<td>High aesthete penetration, porous tegmentum, 1 Crossed-lamellar and 1 spherulitic sublayer in articulamentum</td>
<td>Green, Brown Carotenoid</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Lepidopleurida</td>
<td>Lepidopluerina</td>
<td>Leptochitonidae</td>
<td>Leptochiton inquinatus</td>
<td>low</td>
<td>1.41</td>
<td>Aragonite</td>
<td>Moderate aesthete penetration, porous tegmentum, 2 Crossed-lamellar and 1 spherulitic sublayers in articulamentum</td>
<td>Green Carotenoid</td>
<td>0.19</td>
</tr>
</tbody>
</table>
The ability of Acanthochitonidae to resist taphonomic forces is interesting since Acanthochitonidae and Cryptoplax are more common in the fossil records and have a larger articulamentum than other chiton families, with some Cryptoplax species lacking a tegumentum entirely. Sigwart et al. (2015) also found a species with a reduced tegumentum, Katharina tunicata, was more resistant to fracturing than Mopalia muscoa and M. lignosa; however, their resistance was due to the valves being thicker than the other examined chitons. The ability to resist abrasion due to a reduced tegumentum may not be surprising since the tegumentum is porous and contains an increased amount of organic material due to the aesthetes.

Organic tissue has been demonstrated to be a limiting factor of dissolution on aragonitic material since the amount of microbial decay is related to the amount of organic matrix in the microstructure (Glover and Kidwell 1993; Harper 2000). The presence of organic tissue may explain why the tegument was heavily abraded since it contains tissue from the aesthetes as well as the organic matrix. Porous, organic-rich structures have been shown to be prone to abrasion (Smith and Nelson 2003), and our data agree with this result. The tegument was indeed the most abraded section of the valves after the abrasion treatment (Figure 4.4). The articulamentum was also polished, particularly around the eaves, which also matches the results of previous studies. Since the microstructure of the articulamentum is well-organised and has a low amount of organic tissue it may be that, similar to how dissolution and microbial decay act on shell material, low organic matrix allows an aragonitic shell to better withstand abrasion (Glover and Kidwell 1993; Harper 2000; Smith and Nelson 2003).

The chiton valves examined lost 0.12-0.59% of their weight per km of transport (Table 3). Assuming that the surf zone moves the material on average 3 km/day (Smith and Nelson, 2003), and that the rate of material lost to abrasion is proportional to the remaining mass, then chiton valves can last for 1-7 years, but will lose 90% of their weight within the first two years of exposure in an environment that is solely abrasive (Figure 4.8). This weight loss over time was calculated using:

\[ W_{i+1} = W_i \times r \times (t_{i+1} - t_i) \]

where weights \( W_{i+1} \) and \( W_i \) correspond to the remaining weight of the valves at time \( t_{i+1} \) and \( t_i \), in days and \( r \) is the rate of weight loss due to abrasion seen in the data. For example, C. glaucus lost 9.2% / 74.3 km or 0.12%/km; at 3km/day the rate used for C. glaucus is 0.36%/day, meaning it retains 99.64% of its valve weight each day, so \( r \) for C. glaucus was set at 0.9964 day\(^{-1}\) (Table 4.3). This means that the loss due to abrasion is dependent on the
remaining mass, non-linear, and follows an exponential pattern. However, the rate at which the material decays is likely not constant and dependent on surface area of the valve, grain size, and size of the remaining valve material. Interestingly, similar calculations with other molluscs show that they also last a short (6-7 years) period of time yet they are readily preserved into the fossil record. While tumbling experiments can show relative vulnerability of skeletal materials, using the rate of decay seen in a tumbler may not provide a realistic proxy for the time a shell can remained exposed to taphonomic forces in the marine environment.

Table 4.3: Average valve weight loss after 96 hours / 74.3 km and the rates used in the abrasion damage curve.

<table>
<thead>
<tr>
<th>Chiton Species</th>
<th>% lost after 74.3 km</th>
<th>% lost / km</th>
<th>Rate (/day)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acanthochitona zealandica</em></td>
<td>15.2 ± 4.9</td>
<td>0.21 ± 0.07</td>
<td>0.9937</td>
<td>16</td>
</tr>
<tr>
<td><em>Notoplax violacea</em></td>
<td>14.7 ± 2.7</td>
<td>0.20 ± 0.04</td>
<td>0.9940</td>
<td>17</td>
</tr>
<tr>
<td><em>Chiton glaucus</em></td>
<td>9.2 ± 1.8</td>
<td>0.12 ± 0.02</td>
<td>0.9964</td>
<td>16</td>
</tr>
<tr>
<td><em>Onithochiton neglectus</em></td>
<td>13.0 ± 1.1</td>
<td>0.18 ± 0.01</td>
<td>0.9946</td>
<td>15</td>
</tr>
<tr>
<td><em>Sypharochiton pellisepentis</em></td>
<td>18.8 ± 2.5</td>
<td>0.25 ± 0.03</td>
<td>0.9925</td>
<td>15</td>
</tr>
<tr>
<td><em>Sypharochiton sinclairi</em></td>
<td>20.2 ± 5.4</td>
<td>0.27 ± 0.07</td>
<td>0.9919</td>
<td>13</td>
</tr>
<tr>
<td><em>Ischnochiton maorianus</em></td>
<td>21.1 ± 4.6</td>
<td>0.28 ± 0.06</td>
<td>0.9916</td>
<td>15</td>
</tr>
<tr>
<td><em>Leptochiton inquinatus</em></td>
<td>44 ± 21.8</td>
<td>0.59 ± 0.29</td>
<td>0.9823</td>
<td>22</td>
</tr>
</tbody>
</table>
Figure 4.8: Calculated time chiton valves last under abrasive conditions. All analysed species except *Chiton glaucus* are reduced to less than 1% of their initial weight within 5 years.

While the valves seem to be resistant in comparison to other calcareous materials, they will be subjected to the temperate shelf environment of New Zealand. This environment can leave material exposed for hundreds to thousands of years (Smith and Nelson, 2003). It is unlikely that the valves would stay recognisable after an exposure time magnitudes longer than the time they can resist abrasion alone. Skeletal material needs to be able to resist destructive forces long enough to be buried past the taphonomic active zone and preserved into the fossil record. Preservation into the fossil record is determined by: the amount of material present, the microstructure of the material, the environment that the material is exposed to. An environment that allows deposited material to be buried rapidly or avoid microboring, bioturbation, and storm events is advantageous for preservation. A microstructure with a low organic content theoretically aids resistance to microbial decay, fracturing, and abrasion. This microstructure is seen in the articulamentum of chiton valves and its resistance to abrasion is supported by our data. While the chiton material is estimated to last seven years in a constantly abrasive environment, the majority of that time is wearing down the articulamentum. The tegmentum of the chiton valve is likely be to removed easily by abrasion. This unique shell layer of the chitons is porous and would be full of decaying
organic tissue, which would also increase localised dissolution to a part of the shell that is vulnerable to abrasion. Since the tegmentum is used to help identify chiton species it is possible that chiton fossils are present in the fossil record but in an unrecognisable form, which may explain why they are rarer to find than other aragonitic molluscs after exposure of hundreds to thousands of years.

Conclusions

The effects of abrasion on valves from eight species of chitons from New Zealand were studied in order to better understand chiton taphonomy. Since chitons are rare in the fossil and sediment records but may be able to resist dissolution in low-pH environments, it was necessary to examine the effects of other early sea-floor processes on chiton skeletal material. The amount of organic tissue present in the microstructure of the valves determines how well each of the main layers of the valve break down. The tegmentum, which has a high amount of organic content, is abraded easily. The articulamentum showed little damage from abrasion due to the low organic matrix content of the valves. ANOVA tests show there is a difference in preservation potential among species, which is a result that agrees with previous literature. The sole lepidopleuran was twice as vulnerable as the studied chitonidans, possibly due to its unique crossed-lamellar layer or due to the thinness of the valves it produces but both of these ideas require further testing to determine any correlation. The species that were more resistant to abrasion (Acanthochiton zelandica, Notoplax violacea, Chiton glaucus, and the Sypharochiton spp.) are also relatively more common in the fossil record. All species of chiton tested appear to be more resistant to abrasion than other molluscs since they showed no signs of perforation. The abrasive environment of the intertidal zone that these valves are in could theoretically erode the valves in less than the hundreds to thousands of years which they are exposed to early sea-floor taphonomic processes if they are constantly exposed to abrasive forces for the entirety of their exposure. It is likely that during exposure the tegmentum would be easily removed due to abrasion and may cause the deposited material to be unidentifiable or unrecognisable.

Acknowledgments

The authors would like to acknowledge support from the Department of Marine Science at the University of Otago.
Chapter 5 – Effects of Dissolution on New Zealand Chiton Valves

This chapter is the second that directly determined how chitons resist taphonomic forces. Previous studies have suggested that chitons can resist dissolution, which seems counter-intuitive and inconsistent with their apparent rarity in the sedimentary and fossil records. This study was designed to measure how isolated chiton valves respond to immersion in sea water with lowered pH in order to address this apparent contradiction (Figure 5.1).

Figure 5.1: The cognitive space that Chapter 5 fills in the thesis. This chapter examines how well chiton valves withstand dissolution in order to address the apparent contradiction between their suggested ability to resist reduced pH and their rarity in the sediment and fossil records. This study also provides insight to the taphonomy of chitons.

The following manuscript was submitted to PLoS One in August 2017. I designed the experiment, collected the data, and wrote the original manuscript. A.M. Smith supervised the project and helped edit and improve the paper. The references are included in the reference list beginning on page 96.
Wasting away in the Intertidal: the fate of chiton valves in an acidifying ocean

B.A. Peebles¹ and A.M. Smith¹*

¹ Department of Marine Science, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
*Corresponding Author: e-mail: abby.smith@otago.ac.nz, phone: +64 3 479 7470

Keywords: Aragonite, Ocean Acidification, Dissolution, Taphonomy

Abstract

Chitons have been largely ignored in literature discussing ocean acidification. The few studies which have examined chitons suggested that their valves are resistant to dissolution, but did not directly examine the effects of a reduced pH on chiton valve microstructure. This resistance to dissolution seems odd since the valves are primarily made of aragonite. Chitons are also rarely recorded in the sediment and fossil records, so determining how resistant chiton skeletal material is to dissolution will expand our understanding of how likely they are to survive projected future oceanic conditions and how taphonomic forces affect chiton material. Eight species of chitons were collected from the Otago Peninsula, South Island, New Zealand. The valves were dissected, brushed clean, dried, weighed, then subjected to one of two pH treatments, one at the ambient pH of 8.10 and another at a reduced pH of 7.70. After 12 days in the treatments, the valves were dried and weighed again. The valves lost an average of 0.3-3.3% of their mass in the ambient treatment and lost 0.7-3.5% mass in the experimental treatment. Notoplax violacea, Sypharochiton pelliserpentis, S. sinclairi were the most resistant to dissolution while Acanthochitona zelandica, Chiton glaucus, Onithochiton neglectus, and Ischnochiton maorianus showed a significant difference in mass loss between the two treatments. Leptochiton inquinatus lost the most mass of any of the examined species, but did not show a significant difference between the two treatments. SEM images of the dorsal and ventral surfaces at three locations on each valve were used to assess damage to the crystal structures. The dorsal tegmentum showed no significant difference, but the ventral articulamentum was damaged by the decrease in pH. While chiton skeletal material in general does not appear to resist dissolution any more than other examined molluscs, the resistant tegmentum is exposed to the environment when the chiton is alive, thus conferring considerable resilience to lowered pH.
Introduction

Increasing atmospheric carbon dioxide (CO$_2$) over the last century has caused numerous changes to the marine environment; one of the major changes is a reduction in pH (ocean acidification) (Doney et al. 2009; Keeling et al. 2009). As pH is reduced the marine environment becomes less alkaline, the calcium carbonate saturation state ($\Omega$) and availability of CO$_3^{2-}$ ion are lowered, all of which may make it harder for some calcifying organisms to form shells (Gattuso et al. 1998; Feely et al. 2004). If the shells or skeletal elements that support these organisms structurally and protect against predation are thus thinner or weaker, then the calcifying organisms may be at risk, with consequent changes to marine ecosystems. It is important to be able to predict how environmental changes caused by increasing anthropogenic CO$_2$ will affect future ecological conditions, since the loss of marine calcifiers may result in changes to marine ecosystems and resources (Gutiérrez et al. 2003; FAO 2016). In particular many marine molluscs, which have ecological importance and economic value, produce calcium carbonate shells and thus may respond to ocean acidification.

Marine molluscs compose an estimated 23% of marine species (Gazeau et al. 2013) and are a well-studied group that are important both ecologically and economically (Gutiérrez et al. 2003; Gazeau et al. 2013; FAO 2016). Mollusc shells can function as substrata for attachment by other organisms or create a micro-environment free of predation and environmental stressors for other organisms (Vance 1978; Arnold 1984; Lohse 1993; Buschbaum 2000). Bivalves act as a water filter by consuming plankton, which also increases light levels and promotes algal growth in coastal ecosystems (Asmus and Asmus 1991; Newell and Koch 2004). Grazing activities of gastropods and chitons can promote the growth of primary producers (Borowitzka 1981; Littler et al. 1995). Some molluscs are also farmed for human consumption, such as mussels, or other commercial/industrial products (e.g., paua jewellery, decoration, souvenirs, crushed shell for roading). Global aquaculture production in 2014 (worth US$144.4 billion) produced 16,113,200 tonnes of molluscs, which accounted for 21.8% of farmed seafood (FAO 2016). The most recent report by the Food and Agriculture Organization of the United Nations (FAO 2016) lists ocean acidification and warming as drivers for reduced growth, survival, and shell production. The report also states that more data on how molluscs respond to harsher conditions is needed in order to inform a response.

Bivalves and Gastropods are the most well-studied classes of Mollusca with respect to ocean acidification (Gazeau et al. 2013). Gazeau et al. (2013) summarised the results of 22
experiments on mollucan responses to ocean acidification and reported that 13 out of the 22 studies show mollusc shell production exhibited a neutral response to increased pCO$_2$ and 8 out of 22 show a negative impact on shell growth. While most results show that adult molluscs can survive a decrease in pH, the shells produced were 15-30% thinner and less resistant to fracturing (Bibby et al. 2007; Beniash et al. 2010; Dickinson et al. 2012). Hale et al. (2011) shows that more acidic conditions led to a lower diversity and abundance of molluscs.

Fewer studies have examined larval and juvenile responses. Larval bivalves (Crassostrea gigas and Mytilus galloprovincialis) depend on the aragonite saturation state to form their shell rather than pCO$_2$ (Waldbusser et al. 2015); C. gigas larvae thus had increased difficulty forming their shell when exposed to reduced pH (Waldbusser et al. 2013). Juvenile clams (Ruditapes decussatus) have shown reduced respiration and ingestion rates while juvenile pteropods (Limacina helicina) showed a decrease in shell diameter and an increase in shell degradation and mortality when subjected to reduced pH (Fernández-Reiriz et al. 2011; Lischka et al. 2011). While the literature on molluscan responses to ocean acidification focuses on bivalves and gastropods, little is known about how the other four classes of Mollusca (Cephalopoda, Scaphopoda, Monoplacophora, and Polyplacophora) respond to ocean acidification.

Chitons (Polyplacophora) are a diverse and common group of Mollusca that have yet to have their response to ocean acidification examined. The chiton shell is formed by eight overlapping aragonitic plates, called valves. Aragonite is metastable, and has been shown to dissolve in acidic conditions, both theoretically (Mucci 1983) and practically in coral reefs and molluscs (Orr et al. 2005; McClintock et al. 2009; Gazeau et al. 2013; Busch et al. 2014). Yet, there have been suggestions in the past few years that chitons may have an ability to resist lowered-pH conditions mirroring projected future changes in the ocean (Sigwart and Carey 2014; Sigwart et al. 2015). Sigwart and Carey (2014) noted that valves showed no evidence of pitting in valve surfaces of Leptochiton asellus while testing how grazing responses changed with decreasing pH. Other chitons Katharina tunicata, Mopalia muscosa, and M. lignosa showed no significant difference in fracture strength after exposure to acidic conditions (Sigwart et al. 2015). This apparent capacity of chiton valves to resist dissolution despite being formed of aragonite is counter-intuitive and deserves further study. However, there has been no study to date analysing the effects of lowered pH on chiton skeletal material directly.
The apparent ability of chitons to resist acidic conditions also directs attention to their relative absence from the sediment and fossil records. Chitons are not even mentioned in the literature describing New Zealand carbonate shelf deposits (Andrews 1973; Nelson and Hancock 1984; Nelson et al. 1988; Hayton et al. 1995) and rarely appear in the fossil record (Beu and Maxwell 1990). Yet, the dead-to-live fidelity of molluscs is, in general, high (Kidwell and Flessa 1995) and New Zealand has the most complete Cenozoic molluscan fossil record (Crampton et al. 2006) in the Southern Hemisphere. Aragonite tends to be removed from the fossil record through dissolution (Cherns and Wright 2000; Sanders 2003; Wright et al. 2003), therefore it seems the likely mechanism to remove the aragonitic chiton material from the fossil record. However, if chitons resist dissolution then they should, in theory, be common in the fossil record. Measuring how resistant chiton valves are to dissolution will not only allow more accurate ecological predictions, but it may explain the contradiction between how the valves are expected to dissolve and what has been observed to date. This study examines the how the calcified valves of eight species of New Zealand chitons respond to lowered-pH marine conditions.

**Methods**

A flow-through system was used to direct water from Otago Harbour, South Island, New Zealand into six tanks: three controls and three experimental treatments. The water was filtered and passed through UV light in order to remove microbial contaminants. The control tanks used the ambient pH (average of 8.10-8.00 on total scale) while the experimental tanks had CO₂ input connected to a TUNZE brand pH/CO₂ controller set (pH controller 7070/2, CO₂ valve 7074.110 and power supply unit 5012.101) to regulate the pH to 7.70 ± 0.01. This experimental pH was chosen since the average ocean pH is projected to drop 0.3 units by the end of the century (IPCC 2014).

The pH probe was calibrated daily using tris and amp buffers prepared by Kim Currie (Department of Chemistry, University of Otago) and standardised against standards prepared by the Dickson group (see Dickson 1993). The buffers were allowed to reach room temperature (18°C) before measurements were made. Calibration of the pH probe was done by measuring the potential (mV) read by the probe in each of the calibration solutions and a water sample from each of the treatments. This calibration method was based on those presented by the Carbon Dioxide Information Analysis Center (CDIAC) standard operating procedures (SOP) 6a (Dickson et al. 2007). All buffers and the water samples were allowed
to reach room temperature before measuring the potential of the probe since pH is temperature sensitive. The actual slope of potential (mV) and pH was compared to the theoretical slope of: 0.1984*T where T is the temperature in °K. The pH of the sample was further checked by using the tris and amp buffers and the following calculation:

\[ pH_x = pH_{sample} + \frac{E_s - E_x}{1.9841 \times 10^{-4} \times T} \]

where T is temperature in °K, \( E_s \) is the potential for the tris or amp buffer, \( E_x \) is the potential of the sample, and \( pH_{sample} \) is the calculated pH of the tris or amp buffers.

Spectroscopy was used in tandem with the buffer solutions to make sure the pH probe reported accurate readings. The photospectroscopy methods were taken from (CDIAC SOP 6b; Dickson et al. 2007). Absorbance measurements were taken at 434 nm, 578 nm, and 730 nm from water samples from each tank, then 50 µl of the dye m-cresol purple was added to each water sample and the same absorbance measurements were made for the seawater and dye mixture. This dye can be added in minute concentrations without affecting the precision of the pH measurements required for biological studies. pH was calculated using the following formulae:

\[
\frac{A_1}{A_2} = \frac{(A_{578dye} - A_{578sw}) - (A_{730dye} - A_{730sw})}{(A_{434dye} - A_{434sw}) - (A_{730dye} - A_{730sw})}
\]

and

\[
pH = 8.0056 + \log\left(\frac{A_1}{A_2} - 0.00691 \right)
\]

\[
\left(\frac{2.222 - \left(\frac{A_1}{A_2} \times 0.1331\right)}{2.222 - \left(\frac{A_1}{A_2} \times 0.1331\right)}\right)
\]

(from SOP 6b in Dickson et al. 2007).

This pH was not corrected for the addition of the dye but the correction is typically <<0.01 pH units (CDIAC SOP 6b; Dickson et al. 2007). pH variation in the replicate tanks over the two weeks of the experiment in both control (ranges of ± 0.02, 0.03, and 0.03 pH units) and experimental tanks (ranges of ± 0.05, 0.07, and 0.08 pH units) were within the margin of control recommended by Standard Operating Procedure 22 of the Carbon Dioxide Information Analysis Center (CDIAC) best practices guide (Dickson et al. 2007; Figure 5.2). Control tanks had an average pH of 8.18 ± 0.02, 8.20 ± 0.03, and 8.17 ± 0.03 while the
experimental tanks maintained an average pH of 7.68 ± 0.08, 7.70 ± 0.05, and 7.72 ± 0.07. Although the variation in pH units are within acceptable limits, the difference in \([\text{H}^+]\) between a pH of 7.61 and 7.77 (the largest amount of variation among tanks) is ± 15.5% from the average, which could be a significant variation.

Figure 5.2: Average mean charts (X chart) of the variance of calculated pH using photospectroscopy for each of the experimental and control treatments. The upper and lower warning limits (UWL and LWL) and the upper and lower control limits (UCL and LCL) are defined using calculations from SOP 22 in the CDIAC methods.

Eight species of chitons (Acanthochitona zelandica, Notoplax violacea, Chiton glaucus, Onithochiton neglectus, Sypharochiton pelliserpentis, S. sinclairi, Ischnochiton maorianus, and Leptochiton inquinatus) were collected from the Otago Peninsula, South Island, New Zealand, then killed and stored in a 75% ethanol solution. Valves were dissected, brushed clean, dried in a 60ºC oven for 24 hours and weighed. The valves were then separated into two groups. The first group included the head valve and valves III, V, and VII and the second group contained the remaining four valves (II, IV, VI, and the tail valve). One of these two groups was randomly selected to be introduced to the experimental treatment while the other was used in the control. (Random selection was done by generating a random number between 0-1; if the number was equal to or greater than 0.5, the group containing the
head valve was selected, otherwise the group with the tail valve was used). The chiton material was introduced directly into both tanks. In order to keep track of the valves from the same individual, squares of about 4 cm² were cut from a strip of medical grade gauze and tied around each group of chiton shells (one square of gauze per individual chiton), and labelled (resembling a tea bag). Gauze was used since it allowed water to freely flow around the valves and kept the valves isolated at the same time (Figure 5.3). Valves were left in the tanks for 12 days while the pH was monitored daily. Dry weights of the package of valves were taken again after the two-week period.

Figure 5.3: An example of chiton valves contained in a gauze “teabag”.

Three separate experiments were run at different times. *Leptochiton inquinatus*, *Sypharochiton pelliserpentis*, and *S. sinclairi* were measured first, *Notoplax violacea* and *Ischnochiton maorianus* were in the second set and *Chiton glaucus, Acanthochitona zelandica*, and *Onithochiton neglectus* were in the final set. These experiments were performed in a two-month period when the seawater temperature was within a range of 16 to 18°C.

A JEOL 6700 FEG Scanning Electron Microscope (SEM) was used to take images of an intermediate valve from each replicate in a treatment to access damage to the surface and crystal structure (two valves per individual chiton, one of which was exposed to a lowered pH and the other the ambient pH). The valves were coated with 10 µm of Pt/Pd using a sputter coater. Three images of the lateral area, apophysis, and pleural area were taken at 30x, 500x, and 3000x magnification on both the dorsal and ventral surfaces (Figure 5.4). While the chitons were collected live from the intertidal zone, and therefore may have sustained damage
unrelated to this experiment, any damage incurred prior to this study would be consistent within the same individual used in both treatments, therefore experimental damage could still be compared.

Figure 5.4: SEM photos of an intermediate *Ischnochiton maorianus* valve with sampling locations for the dorsal and ventral surfaces designated by squares.

**Results**

Each species in the control groups lost an average of less than 2% of their original mass except for *Leptochiton inquinatus*, which lost average of 3.3% ± 0.5%. *A. zelandica*, *C. glaucus*, *O. neglectus*, *S. pelliserpentis*, and *S. sinclairi* each lost less than 1% of their initial weight (0.4 ± 0.1%, 0.4 ± 0.1%, 0.5± 0.1%, 0.3 ± 0.1%, and 0.6 ± 0.1% respectively; Table 1). *N. violacea* and *I. maorianus* lost less than 3% of their weight and showed a wider variation among individuals (1.5 ± 0.5% and 1.1 ± 0.1% respectively).
The chiton valves exposed to reduced pH lost less than 5% of their original weight. *Leptochiton inquinatus* and *Onithochiton neglectus* lost the most mass overall in the experimental treatments (3.5 ± 0.5% and 3.5 ± 0.3% respectively; Table 5.1) while *Sypharochiton pelliserpentis* and *S. sinclairi* were the most resistant to reduced pH (0.7 ± 0.1%). The acanthochitonids (*Acanthochitona zelandica* and *Notoplax violacea*) lost 1.7 ± 0.1% and 2.2 ± 0.4% weight respectively. The sole ischnochitonid (*Ischnochiton maorianus*) lost 1.6 ± 0.1% and *Chiton glaucus* (Family Chitonidae) lost 2.1 ± 0.1% weight.

Table 5.1: Average percentage weight loss by chiton valves in 12 days immersion (mean of three replicates). Significance was determined by one-way ANOVA tests, a value of < 0.05 is significant (*), a value of < 0.01 is highly significant (**), and a value of > 0.05 is not significant (-).

<table>
<thead>
<tr>
<th>Species</th>
<th>Control Group</th>
<th>Treatment Group</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valves I, III, V, VII</td>
<td>Valves II, IV, VI, VIII</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ambient pH 8.1</td>
<td>lowered pH 7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td><em>N. violacea</em></td>
<td>1.5</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td><em>A. zelandica</em></td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td><em>C. glaucus</em></td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td><em>O. neglectus</em></td>
<td>0.5</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td><em>S. pelliserpentis</em></td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td><em>S. sinclairi</em></td>
<td>0.6</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td><em>I. maorianus</em></td>
<td>1.1</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td><em>L. inquinatus</em></td>
<td>3.3</td>
<td>1.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

The valves in the experimental treatment lost slightly more weight than the valves in the control (Figure 5.5). *N. violacea*, *S. pelliserpentis*, *S. sinclairi*, *I. maorianus*, and *L. inquinatus* lost a difference of less than 1% between treatments (0.70 ± 0.45%, 0.39 ± 0.04%, 0.10 ± 0.05%, 0.52 ± 0.06%, and 0.19 ± 0.47% respectively). *A. zelandica* and *C. glaucus* lost a difference of 1.30-1.74% between treatments. *O. neglectus* showed the greatest difference in weight loss between treatments (2.97 ± 0.19%). Single factor ANOVA tests revealed that *N. violacea*, *S. sinclairi*, and *L. inquinatus* showed no significant difference.
between treatments (p-values of 0.590, 0.533, and 0.895 respectively). *A. zelandica* and *I. maorianus* had a significant difference in weight loss between treatments (p-values of 0.024 and 0.015 respectively; Table 1). The remaining chitonids (*C. glaucus*, *O. neglectus*, and *S. pelliserpentis*) showed highly significant differences between treatments (p-values of 0.001, 0.005, and 0.006 respectively).

Figure 5.5: Percent weight loss of each chiton species analysed due to dissolution for the experimental group (dark-coloured, species names have a “E” at the end) and the control group (light-coloured, species names have a “C” at the end). The boxes indicate one standard deviation from the average and the whiskers are the 95% confidence interval.

The ventral surface valve images showed little-to-no damage in the control treatment. One replicate of each analysed species (8 valves total) displayed damage on less than 25% of its ventral surface area while the ventral surfaces of all other valves appeared unharmed. The dorsal images of the control treatment showed a damaged tegmentum on all analysed valves. The apophysis remained unharmed on a majority (20 of 24) analysed valves, the other four valves (one from *N. violacea*, *S. pelliserpentis*, *I. maorianus*, and *L. inquinatus*) showed slight damage to the crystal structure.

SEM images of the dorsal surface of the valves in the experimental treatment showed consistent damage on the tegmentum and apophysis. Less than 50% of the dorsal surface area was damaged on 18 of the 24 analysed valves. One valve of *C. glaucus*, *S. sinclairi*, and *L. inquinatus* showed no damage on the tegmentum while one *S. pelliserpentis* and two *S.
*sinclairi* valves sustained no damage on the apophysis. 25-50\% of the ventral surface area of 21 valves from the experimental treatment was eroded. Two *S. sinclairi* valves showed no damage and one *I. maorianus* valve had the ventral-most acicular layer heavily etched and in some parts completely removed, which exposed the adjacent crossed lamellar structure.

The damage on the dorsal surfaces of both treatments was not different between the control and treatment (Figure 5.6). The ventral surfaces were much more damaged in the experimental treatment than the control treatment (Figure 5.7).

*N. violacea, S. pelliserpentis, S. sinclairi, and L. inquinatus* showed little-to-no difference in crystal damage between treatments. The tegmentum showed the same type and proportion of damage on valves, from the same individual, that were in different treatments while the apophysis and ventral layer consistently showed little to no damage to the crystal structure. The other species (*A. zelandica, C. glaucus, O. neglectus, and I. maorianus*) showed increased etching and ultrastructure damage over a larger surface area in the experimental treatment than in the control.

There does not appear to be a clear consistent source of variation in these data. ANOVA tests with phylogenetic independent contrasts (Felsenstein 1985) applied were performed in R with the ape and phytools package (Paradis *et al.* 2004; Revell 2012; R Core Team 2017). The results of the tests showed that number of pigments (p-value of 0.899), aesthete penetration (p-value of 0.851), number of mineral sublayers in the valve (p-value of 0.937), and valve size (p-value of 0.06) did not have a significant effect on weight loss.
Figure 5.6: SEM images of the dorsal valve surfaces of *Chiton glaucus, Leptochiton inquinatus*, and *Notoplax violacea* exposed to the experimental pH (experimental) and the ambient pH (control).
Figure 5.7: SEM images of the ventral valve surfaces of *Chiton glaucus*, *Leptochiton inquinatus*, and *Notoplax violacea* exposed to the experimental pH (experimental) and the ambient pH (control).
Discussion

Chiton valves exposed to reduced pH seawater lost more weight than the controls kept in ambient sea water. This weight-loss appears to have been mainly from the articulamentum, which is displayed in the SEM images of the valves (the apophysis on the dorsal view and the entirety of the ventral view). The articulamentum is concealed when the chiton is alive. Etching and corrosion was far more evident on the surface in the lowered-pH treatment. In contrast, the tegmentum showed no visible difference in damage between treatment and control.

These results match the theory presented by Harper (2000) and Smith and Nelson (2003) that mineral layers with thin, well-organised crystals and minimal organic components are more vulnerable to dissolution than those with a high organic content. The presence of organic tissue, specifically the aesthete channels and the periostracum, may protect the tegmentum when the chiton is alive from acidic conditions (Smith and Nelson 2003). This idea was also put forward by Sigwart et al. (2014) after they subjected live Leptochiton asellus to a lowered pH of 7.5 to assess any changes in grazing behaviour. Although they did not examine valve damage, they report that the valves did not appear to deteriorate and suspected it was due to the intact periostracum.

Sources of Variation

There does not appear to be a strong phylogenetic signal in dissolution rates among species (Table 5.2). O. neglectus and C. glaucus (Chitonidae), along with Acanthochiton zelandica (Acanthochitonidae) were the most vulnerable to dissolution, losing a difference of 1 to 3% of their total mass between treatments respectively. S. pelliserpentis (Chitonidae) and Ischnochiton maorianus (Ischnochitonidae) were able to resist dissolution fairly well, losing only < 1% of their total mass.

L. inquinatus (Lepidopleuridae) was unusual in that it showed no significant difference between control and treatment (p-value of 0.895 with a single factor ANOVA test; Table 1) yet lost the most weight in both treatments among the examined species. The four chitonids showed inconsistent responses to dissolution; two of them (C. glaucus and O. neglectus) were the most susceptible to dissolution, S. pelliserpentis, was highly resistant to dissolution and S. sinclairi showed no significant difference between treatments (p-value of 0.533). While the two Sypharochiton spp. resisted dissolution better than the other chitonids,
there is not a significant difference in mineralogy, pigments, ultrastructures, or size of the valves to indicate why this variation in susceptibility may be. The acanthochitonids also had varying responses to dissolution: *N. violacea* showed no significant difference between the two treatments (p-value of 0.590), but *A. zelandica* was vulnerable to dissolution (p-value of 0.024). Theoretically, the acanthochitonids should have responded poorly to dissolution due to their reduced tegmentum, but that does not appear to be the case. The acanthochitonids are also among the most common chitons found in the New Zealand Cenozoic molluscan fossil record, implying that they may be able to better resist taphonomic forces than other chitons (Beu and Maxwell 1990).

All valves were consistently aragonitic, so mineralogical composition cannot be the source of variation. The ANOVA tests revealed that the number of pigments, sublayers, and aesthete penetration did not correlate with weight loss. *C. glaucus, A. zelandica, N. violacea,* and *L. inquinatus* all have only a green pigment in their valves (unpublished data), but these four chitons did not have consistent responses to reduced pH. *N. violacea* and *L. inquinatus* were resistant to dissolution, but *C. glaucus* and *A. zelandica* showed significant differences in weight loss between treatments. The chitons with green and brown carotenoids also showed no consistent pattern amongst themselves. The difference in treatments was significant for *I. maorianus*, highly significant for *O. neglectus* and *S. pelliserpentis* and insignificant for *S. sinclairi*.

The only two species with few aesthete channels were the Acanthochitonids (*A. zelandica* and *N. violacea*), but *N. violacea* was able to resist dissolution unlike *A. zelandica*. Chitons with the most aesthete tissue (*C. glaucus, O. neglectus, S. pelliserpentis, S. sinlcairi,* and *I. maorianus*) showed a variety of responses to lowered-pH, ranging from not significant (*S. sinclairi*) to highly significant (*C. glaucus, O. neglectus,* and *S. pelliserpentis*).

The chitons with 3-4 sublayers in their valves (*L. inquinatus* and the chitonids) lost more weight than those with 2 or 5 sublayers (*A. zelandica, N. violacea, I. maorianus*), but there is no statistical validation for this relationship. The difference between 4 and 5 sublayers is a thin (< 1 µm) crossed-lamellar sublayer located adjacent to the tegmentum (Peebles *et al.* 2016), it seems unlikely that the addition of a thin layer of the same microstructure already in the valve would result in the valve gaining the ability to resist dissolution.
Table 5.2: Dissolution Resistance of the analysed chiton valves with information on phylogeny and valve composition, size, and persistence time.

<table>
<thead>
<tr>
<th>Phylogeny</th>
<th>Persistance Time (Years)</th>
<th>Composition</th>
<th>Pigments</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitonida</td>
<td></td>
<td></td>
<td>Acanthochitonina</td>
<td>low</td>
</tr>
<tr>
<td>Chitonina</td>
<td></td>
<td></td>
<td>Notoplax violacea</td>
<td>high</td>
</tr>
<tr>
<td></td>
<td>Chitonida</td>
<td></td>
<td></td>
<td>low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Onithochiton neglectus</td>
<td>low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sypharochiton pelliserpentis</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sypharochiton sinclairi</td>
<td>high</td>
</tr>
<tr>
<td></td>
<td>Ischnochitonidae</td>
<td></td>
<td></td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>Lepidopleurida</td>
<td></td>
<td>Lepidopleurina</td>
<td>Leptochitonidae</td>
</tr>
</tbody>
</table>
The factor that correlates best with dissolution rate is morphological: the weight of the initial valves, with larger valves resisting dissolution more than those from smaller individuals or species (Figure 5.8). While the size of the valves statistically did not yield a significant result (p-value of 0.06), it was much closer to correlating with weight loss than the other factors, which all had p-values of >0.80. The species that showed no significant difference between treatments were among the species had the heaviest valves (N. violacea and S. sinclairi). Species with lighter valves generally showed a significant difference between treatments (O. neglectus, C. glaucus, A. zelandica, I. maorianus). The two species that appear to violate this trend are L. inquinatus and S. pelliserpentis. S. pelliserpentis had the heaviest valves, but showed a highly significant difference between treatments. L. inquinatus lost the most weight in both treatments and had the lightest valves among the analysed species, but showed no significant difference between treatments. Crystal structure may well override the size factor for L. inquinatus, but it does not explain the variation among the Sypharochiton spp. Even though both Sypharochiton spp. resist dissolution, only S. sinclairi had no significant difference between treatments.

Figure 5.8: Weight loss plotted against average initial valve weight (g).
Are chitons especially resistant to dissolution?

The ranges of weight lost due to dissolution in our data are within the ranges of previously reported dissolution studies in molluscs (McClintock et al. 2009; Waldbusser et al. 2011; Wolfe et al. 2012; Table 5.3). Waldbusser et al. (2011) reported that *Crassostrea virginica* lost 0.18% of its weight per day, which equates to 2.14% over 12 days. McClintock et al. (2009) determined the dissolution rates of four Antarctic species and reported that the Antarctic molluscs lost 1.755-4.037% of their weight over 63 days. Assuming that the rate of mass loss remained constant, this range of mass loss is also within the range observed by the chitons in this study (0.28-13.32% after 63 days). The mass lost by chitons in a pH of 7.7 is within the same range as that lost by *Argonauta nodosa* in a pH of 7.6-7.4 (Wolfe et al. 2012), but only the most resistant chitons were comparable to the mass lost by *A. nodosa* in a pH of 7.8 (0.70% lost by *A. nodosa* compared to 0.71-3.51% lost by the chiton species). Comparisons to the ability to resist dissolution by other molluscs suggest that chitons are either no more or slightly more vulnerable to acidic conditions than other molluscs.

If chiton valves continued to dissolve at the same rate as was observed in the control, assuming that: the pH and localized saturation state remain constant, dissolution is the only taphonomic force, and the rate of decay also remains constant, then they could last 4-45 years before being reduced to < 1% of their initial weight (Figure 5.8). Dissolution curves are not linear, as the amount of material dissolved at any point in time is dependent on the remaining mass. While *S. pelliserpentis* and *S. sinclairi* are among most resistant chitons in ambient conditions (lasting up to 45 years), *A. zelandica*, *C. glaucus*, and *O. neglectus* have the ability to resist dissolution for over 20 years. *N. violacea* and *I. maorianus* can last for over 10 years, and *L. inquinatus* lasts for an average of 4 years. At the reduced pH of 7.7, in conditions that may be reached by the end of this century, every species except for the two *Sypharochiton* spp. would have been reduced to < 1% of its initial weight over the course of about 4-9 years (Figure 5.9). The *Sypharochiton* spp. would last significantly longer than the other analysed species, just over 21 years.
Table 5.3: Reported percent mass loss from various organisms exposed to lowered pH.

<table>
<thead>
<tr>
<th>Organism</th>
<th>% loss</th>
<th>pH</th>
<th>Time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachiopods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liothyrella uva</td>
<td>0.410</td>
<td>8.2</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Bivalves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laternula elliptica</td>
<td>0.313</td>
<td>8.2</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Aequiluidia eigthasi</td>
<td>0.000</td>
<td>8.2</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Aequiluidia eigthasi</td>
<td>3.705</td>
<td>7.4</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Crassostrea virginica</td>
<td>0.18</td>
<td>7.2</td>
<td>1 Day</td>
<td>Waldbusser et al. 2011</td>
</tr>
<tr>
<td>Crassostrea virginica</td>
<td>0.20</td>
<td>7.4</td>
<td>1 Day</td>
<td>Waldbusser et al. 2011</td>
</tr>
<tr>
<td>Crassostrea virginica</td>
<td>0.14</td>
<td>7.6</td>
<td>1 Day</td>
<td>Waldbusser et al. 2011</td>
</tr>
<tr>
<td>Gastropods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nacella concinna</td>
<td>0.278</td>
<td>8.2</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Nacella concinna (with coralline algae)</td>
<td>1.061</td>
<td>8.2</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Nacella concinna (with coralline algae)</td>
<td>4.037</td>
<td>7.4</td>
<td>63 Days</td>
<td>McClintock et al. 2009</td>
</tr>
<tr>
<td>Cephalopods</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argonauta nodosa</td>
<td>0.00</td>
<td>8.10</td>
<td>14 Days</td>
<td>Wolfe et al. 2012</td>
</tr>
<tr>
<td>Argonauta nodosa</td>
<td>0.70</td>
<td>7.80</td>
<td>14 Days</td>
<td>Wolfe et al. 2012</td>
</tr>
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<td>Argonauta nodosa</td>
<td>1.49</td>
<td>7.60</td>
<td>14 Days</td>
<td>Wolfe et al. 2012</td>
</tr>
<tr>
<td>Argonauta nodosa</td>
<td>2 - 5</td>
<td>7.40</td>
<td>14 Days</td>
<td>Wolfe et al. 2012</td>
</tr>
<tr>
<td>Argonauta nodosa</td>
<td>4 - 6</td>
<td>7.20</td>
<td>14 Days</td>
<td>Wolfe et al. 2012</td>
</tr>
<tr>
<td>Chitons</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Acanthochitona zelandica</td>
<td>1.71</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Notoplax violacea</td>
<td>2.19</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Chiton glaucus</td>
<td>2.12</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Onthochiton neglectus</td>
<td>3.51</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Sypharochiton pelliserpentis</td>
<td>0.72</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Sypharochiton sinclairi</td>
<td>0.71</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Ischnochiton maorianus</td>
<td>1.64</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
<tr>
<td>Leptochiton inquinatus</td>
<td>3.51</td>
<td>7.70</td>
<td>12 Days</td>
<td>This Study</td>
</tr>
</tbody>
</table>
Figure 5.9: Time needed to dissolve chiton valves at a pH of 8.1 and a pH of 7.7.

**Long-term implications**

If the pH is lowered by 0.3 units then the deceased chiton material lasts for significantly less time than in ambient conditions (p-value of 0.022). Low sedimentation rates typical of temperate shelves, however, leave deposited material exposed for hundreds to thousands of years (Smith and Nelson 2003), which is much longer than the 4-45 years that the valves could last under any of the conditions tested. This difference between the exposure time and the amount of time the valves can persist as deposited material implies that dissolution alone is enough to remove chiton valves from the sediment and fossil records over time.
Conclusion

Two pH treatments (8.1 and 7.7) were set up to test how chiton valves resist dissolution. Isolated dead chiton valves appear to resist dissolution to about the same degree as other molluscan shells, losing 0.3-3.3% of their mass after two weeks’ exposure in ambient conditions and 0.7-3.5% in lowered-pH seawater. This weight loss is not surprising since the valves are composed of primarily aragonite, which in theory dissolves easily. SEM images showed that the tegmentum is more resistant than the articulamentum, likely due to the aesthete channels that are incorporated into the mineral. The initial size and weight of the valves seems to be the main factor determining dissolution resistance. Once a valve is deposited into the shelf it could last for up to an estimated 45 years in ambient conditions, however temperate carbonates can be subject to the taphonomically active zone for hundreds to thousands of years. This difference in time may explain why chiton valves are rarely present or in a recognisable form in the sediment and fossil records.

Acknowledgments

The authors would like to acknowledge the Department of Marine Science at the University of Otago and for its support. We would like to thank Doug Mackie and Reuben Pooley for their support and advice.
Chapter 6 – General Discussion

The purpose of this thesis was to examine the apparent contradiction in chiton preservation: although they are abundant and reportedly resistant to dissolution, they are very poorly represented in the sediment and fossil records.

In order to achieve this purpose, I analysed chiton valve structure, composition and pigments, and determined how the taphonomic processes abrasion and dissolution affected chiton valves (Figure 6.1).

The most important findings in these studies, which I will discuss in more detail below were:

- Valve microstructures vary among species and include a rod-type crossed-lamellar microstructure unique to chitons.
- Chiton valves contain carotenoid pigments in the tegmentum, which reflects their diet.

Figure 6.1: Taphonomic processes acting on chiton skeletal material.
• α-chitin appears to be absent in chiton valves, which is a result at odds with other studies.
• Chiton valves resist abrasion fairly well and can last seven years in constantly abrasive conditions with a constant rate of decay and no other acting taphonomic forces before breaking down to an unrecognisable form.
• Chitons resist dissolution about as well as other molluscs.
• The tegmentum in chitons is the most damaged by abrasion, but resists dissolution.
• The articulamentum, in contrast, is vulnerable to dissolution but resistant to abrasion.
• Abrasion appears to remove the valves from the intertidal environment quicker than dissolution (7 years as opposed to 45 years).

Figure 6.2: The red boxes indicate the questions answered in thesis chapters. Chapters 2 and 3 looked at the chitons in the biosphere and determined what microstructures and organic components chiton valves contain, chapter 4 determined the effects of abrasion on chiton valves and chapter 5 examined the effects of dissolution on the valves.
The physical structure of chiton valves

The XRD and Raman spectra obtained in chapters 2 and 3 clearly showed that both the tegmentum and articulamentum in all the species considered were composed of aragonite. This result is completely consistent with the literature (Lowenstam 1962; Baxter and Jones 1981; Speiser et al. 2011; Connors et al. 2012). It is tempting to generalise that all chiton valves are formed of only the one biomineral, but there are whole families that have yet to have their mineralogy determined. Only five out of 21 chiton families have been examined so far (Acanthochitonidae, Mopaliidae, Chitonidae, Ischnochitonidae, and Leptochitonidae; Clarke and Wheeler 1922; Kirschvink and Lowenstam 1979; Baxter and Jones 1981; Speiser et al. 2011; Connors et al. 2012; This thesis). There are reports that either assume that the valve is aragonite, but do not verify or list only “Chiton sp.” as aragonite (as with Cornish and Kendell 1888), which makes species level comparison difficult or impossible. Since 16 of 21 families have yet to be mineralogically surveyed, caution should be exercised before generalising that all chitons precipitate solely aragonite in their valves.

Pigments within chiton tissues are known to be carotenoids (Tsushima et al. 1989; Schwabe 2010; Maoka 2011; Speiser et al. 2014). However, studies on valve pigments are lacking, which is peculiar since some chitons can display a variety of colours in their shells and colour is important to species identification (Sigwart 2016). This thesis provides the first study that identified chiton shell pigments. The tegmentum in all species analysed so far has a granular microstructure, contains one or two carotenoid pigments, and produces a weak protein signal in the Raman spectra. The articulamentum has a more complex microstructure with 3-6 sublayers, but does not contain any pigments, and does not produce a detectable protein signal (see Chapter 2, Figure 2.5 and Chapter 3, Figure 3.3). The carotenoid pigments are likely to be a result of their diet, since molluscs cannot synthesise carotenoids (Barnard and Waal 2006). O. neglectus showed different Raman bands from the other analysed chitons, and thus had different pigments, perhaps related to its different diet because of its distribution on kelp holdfasts and not on rocks with a thin algal film.

Many other molluscs have a simpler shell structure than chitons; the main difference between chiton valves and shells of other molluscs is the presence of aesthete channels (Table 6.1). Chitons are the only molluscs to have aesthetes (nerve tissue) running through their shell. These nerve channels penetrate the entirety of the tegmentum, which makes up roughly
30% of the valve. The presence of the aesthetes makes the calcified structure porous and full of organic tissue, which is also unusual among the molluscs. Other molluscan shells tend to be non-porous and mostly calcareous with organic matrix and periostracum (protective dorsal organic layer) as non-mineral components.

Table 6.1: Shell mineralogy, organic content, and pigments of major molluscan groups. Grey boxes show areas where data is lacking.

<table>
<thead>
<tr>
<th>Molluscan Group</th>
<th>Mineralogy</th>
<th>Organic Content</th>
<th>Shell Pigments</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaphopoda</td>
<td>Aragonite</td>
<td></td>
<td></td>
<td>Marin 2012</td>
</tr>
<tr>
<td>Solengastres</td>
<td>Aragonite, Calcite</td>
<td></td>
<td></td>
<td>Marin 2012</td>
</tr>
<tr>
<td>Caudofovaeta</td>
<td>Aragonite, Calcite</td>
<td></td>
<td></td>
<td>Marin 2012</td>
</tr>
<tr>
<td>Polyplacophora</td>
<td>Aragonite</td>
<td>Asp/Gly-rich Proteins, α-chitin (radula)</td>
<td>Carotenoids, Melanin</td>
<td>Treves et al. 2003; Addadi et al. 2006; Speiser et al. 2011; Connors et al. 2012; Marin 2012; Li et al. 2015; This Study</td>
</tr>
</tbody>
</table>
Generally, molluscs form 2-5 calcareous layers in their shell and display a large variety of crystal structures, the most common of which are: nacre, crossed lamellar, homogenous, spherulitic, acicular, and prismatic layers (Carter 1990; Wilmot et al. 1992; Barthelat et al. 2006; Marin and Luquet 2004; Furuhashi et al. 2009; Marin et al. 2012; Figure 6.3). Chitons only use four of these crystal types: granular (also called course homogenous), crossed lamellar, spherulitic, and acicular. They typically have more mineral layers in their shell (4-7) than other molluscs. The organisation of two main aragonite mineral layers (the tegmentum and articulamentum) that chitons have is similar to some gastropods which have a paired prismatic and nacre layer; but the ventral layer in chitons is far more complex. The tegmentum is granular and the articulamentum alternates between crossed lamellar and spherulitic sublayers until the ventral-most acicular sublayer, which tends to be very thin (< 1 µm).

Chitons also have a rod-type crossed lamellar layer first described by Carter (1990) (Chapter 2, Figure 2.9), and in the case of Leptochiton inquinatus, a unique crossed lamellar layer I have yet to see documented in any other skeletal carbonate (Figure 6.4). The rod-type crossed lamellar layer appears to be unique to chitons, as the only descriptions of it in the literature are describing members of Chitonida. Carter (1990) first describes this layer as “rod-type concentric crossed lamellar” when describing ultrastructures of Acanthopleura granulata valves. The rod-type structure is next documented by Chen (2010) when describing the valves of Liolophura japonica. The rod-type lamellae are clearly visible in Figure 1k of Chen (2010), although there is no comment on the shape of the lamellae and the structure is termed “crossed lamellar”. A rod-type structure appears again in Figure 7k of Connors et al. (2012), but is labelled “simple crossed lamellar”. The structure appears to have rod-type crystals instead of the lamellae typically seen in conciferan molluscs. Although it is hard to tell since the best view of the lamellae (Connors et al. 2012; Figure 7f) is when the shell was polished and etched, which may have changed the shape of the crystals. Neither Chen (2010) or Connors et al. (2012) use the terminology “rod-type”, but the images they share clearly show rod-type lamellae that deviate from the flat lamellae of other molluscan shells. The chitons I examined all had the rod-type crossed lamellar layer in their articulamentum as well.
Figure 6.3: Common mineral structures forming shell-layers in the phylum Mollusca. a) Stacked tablets layer from the nacre of abalone *Haliotis iris*, b) prismatic layer from the oyster *Ostrea chilensis*, c) crossed-lamellar layer from the chiton *Acanthochitona zelandica*, d) granular layer from the clam *Austrovenus stutchburyi*, and e) spherulitic layer from the chiton *Onithochiton neglectus*. Scale = 1 µm (a-d), 10 µm (e).
Figure 6.4: The unique crossed lamellar layer in the articulamentum of *Leptochiton inquinatus*.

There were only two chitons that deviated from solely forming rod-type lamellae in their crossed lamellar layers. *Acanthochitona zelandica* also had a typical crossed lamellar layer on the eaves of its intermediate valves in addition to a rod-type crossed lamellar layer in its articulamentum cross section. *Leptochiton inquinatus* is the only analysed chiton species, to date, that did not have a rod-type crossed lamellar layer. Instead, it had flattened lamellae similar to other molluscs, but each first order lamellae was only one crystal thick, instead of multiple as typical in other molluscs and chitons (Figure 6.4).

The few chiton species that have had their ultrastructures detailed but were not examined in this study also show the same complexity and mineral types (Carter 1990; Wilmot *et al.* 1992; Chen 2010; Connors *et al.* 2012). In total, however, only 11 chiton species have had their ultrastructures fully described; *Acanthopleura brevispinosa* has been shown to have a crossed lamellar layer (Wilmot *et al.* 1992), but the other layers of its valves are not detailed. It is important to note that generalisations about chiton valve mineralogy and mineral layering really should not be made from only 11 species.

Typically, crossed lamellar layers are anisotropic (that is, the amount of stress the structure can handle varies with the direction it is coming from) (Currey and Kohn 1976; Liang *et al.* 2008) and have a flexural strength of 70 MPa perpendicular to the long axis of the first lamellae and 200 MPa parallel to the lamellae (Currey and Kohn 1976). However, the angle at which the rod-type cross lamellae of chitons cross is close
to 90°, which would help mitigate the anisotropic properties of the crossed lamellar layer since each first order lamella layer is perpendicular to the next. The crossed lamellar structure would thus better resist crushing and fracturing damage that could be implemented by predators. This angle of intersection is on the low end for typical molluscs (90-130°; Wilmot et al. 1992).

The alternating mineral ultrastructures (rod-type crossed lamellar to spherulitic) of the articulamentum help to protect the chiton by making it more difficult for predators to break the shell (Wang et al. 2009). Physical stress can be mitigated by a multi-layered structure because stress can be transferred to a layer that can dissipate the stress rather than having one structure to absorb and deflect all incoming forces. In addition, chitons articulate their valves when they move or defensively curl; having more than one structure type may aid in flexibility. A shell of only a crossed lamellar structure would be stiff and would grant protection (Neves and Mano 2005), but likely would sacrifice movement for it; having multiple layers may provide a better balance between mobility and defence. Since the articulamentum is partially exposed when the chiton defensively curls (Connors et al. 2012), it makes sense that it is the most protective layer. The tegmentum is most likely on the surface for sensory input from the aesthetes, leaving the articulamentum for defence; which results in the differing ultrastructures between the two layers.

Thus, the special structures and mineral layers of chiton shells reflect their lifestyle and environment, where the pigments therein reflect diet.

**Effects of Abrasion and Dissolution**

Taphonomic work on calcareous structures demonstrated that mineralogy, crystal shape and size, and the presence of organic tissue determine how a shell breaks down (Harper 2000; Sanders 2003; Smith and Nelson 2003). Aragonite is metastable and dissolves more easily than calcite (Mucci 1983; Sanders 2003). In addition, if aragonitic and high-Mg calcite shells are in a taphonomically-active zone, they could be further selected against due to increased acidity due to organic decay and re-oxidation (Sanders 2003; Cherns et al. 2011). Aragonitic material can be preserved by silicification (Cherns and Wright 2000; Wright et al. 2003) or by rapid burial (Cherns et al. 2011). However, in low energy environments such as the temperate shelves of New
Zealand, it is likely they will dissolve before burial and not be recorded in the fossil record (Davies et al. 1989).

The organic matrices of different microstructures also have different preservation potentials (Clark II 1999). Nacreous organic matrices of *Nautilus* and *M. californianus* preserved well over time, staying mostly intact in a Cretaceous sample but showing decay in a Pennsylvanian sample. Crossed lamellar matrices of *M. mercenaria* and *Chione* sp. do not preserve as well of nacreous material, most of the matrix was lost in a Pleistocene sample. Prismatic matrices of *M. mercenaria* and *M. edulis* do not preserve as well due to the large sheaths that form between crystals, which encourages microbial activity (Clark II 1999; Glover and Kidwell 1993). Exposed organic shell material can provide a substrate for microbes, which increases microboring and degradation of the shell. When the organic components deteriorate, the crystal structure of the shell breaks apart. The individual crystals are then exposed and prone to dissolution. Once the organic components are completely removed, the mineralogy dictates how the shell will break down.

In chitons, theoretically, the tegmentum should abrade easily due to its porous structure and the presence of the aesthete channels, but be able to initially resist dissolution until the organic components begin to decay. The articulamentum, due to its low amount of organic tissue, multiple sublayers, and organised crystal structure should be vulnerable to dissolution but able to resist abrasion and fracturing. While the results of the abrasion and dissolution studies showed that chitons were able to resist these forces fairly well compared to other molluscs, taphonomic processes do not act in isolation. The two main mineral layers oppose each other in their weaknesses and resistances, and both structures will be vulnerable to dissolution once the organic material of the tegmentum has decayed. Therefore, an environment that subjects the valves to both abrasion and dissolution will leave each mineral layer of a chiton valve vulnerable to removal.

The granular structure and organic-rich components of the tegmentum make it vulnerable to abrasion. The aesthetes will allow the tegmentum to resist dissolution initially, but also lower the preservation potential of the tegmentum in non-sterile environments since they will act as a microbial substrate (Kidwell and Jablonski 1983). Once the aesthetes and periostracum decay, the porous structure of the tegmentum will be vulnerable to dissolution due to its large surface area. The tegmentum is far more likely to be removed from the sediment and fossil records than the articulamentum due
to its initial vulnerability to abrasion, then its susceptibility to organic decay and dissolution.

The articulamentum should behave more like a typical molluscan shell, due to its organic-poor structure and high crystal surface area. The surface mineral layers will be vulnerable to dissolution as the non-sterile environments of the shelf and intertidal zone start to degrade the organic matrix (Sanders 2003). Once the organic material is completely removed, its surface area will be less than the tegmentum, allowing it to have a larger chance to be recorded in the sediment or fossil records.

Previous taphonomic work on chitons focused more on assessing bias in the fossil record (Puchalski et al. 2008) or observing damage isolated valves had endured in the intertidal zone (Puchalski and Johnson 2009). There is only one other study describing how abrasion affects chiton valves (Sigwart et al. 2014) and two demonstrating that dissolution has no significant effect on chiton valve fracture strength (Sigwart et al. 2015). The results of the abrasion and dissolution experiments presented in this thesis confirmed that the tegmentum was more vulnerable to abrasion but resisted dissolution, and vice versa for the articulamentum.

It has been theorised that chiton valves resist dissolution well (Sigwart and Carey 2014; Sigwart et al. 2015) and the results presented in this thesis support that idea. The SEM images of the tegmenta of all analysed species showed no significant difference between the two dissolution treatments (ambient and lowered pH). The valves tested by Sigwart and Carey (2014) also showed no pitting or evidence of dissolution. However, the tegmentum was the only part of the valve exposed to the experimental conditions by Sigwart and Carey (2014). Since the tegmentum is the most dissolution-resistant layer of the valve, it is not surprising that no evidence of dissolution was observed. The level of organic tissue present in the tegmentum is unique among molluscs and its presence is likely the cause of this apparent quality to resist dissolution while the chiton is alive or still has organic material present after death. However, once the organic material decays, the channels that previously contained aesthetes will be exposed. The experiments described in Chapter 5 used UV light to remove microbial influence to measure dissolution directly, which allowed the organic tissue to remain on the valves. The absence of microbes and presence of the organic tissue throughout the experiment, and those conducted previously (Sigwart and Carey 2014, Sigwart et al. 2015) may have allowed the valves to resist dissolution for the duration for the experiments.
Isolated chiton valves also showed no significant difference in fracture strength between ambient and lowered pH treatments (Sigwart et al. 2015). This result is interesting, since the articulamentum is exposed to reduced pH, unlike the conditions tested by Sigwart and Carey (2014) where only the tegmentum was exposed. Fracture strength is dependent on the thickness of the shell, therefore it should only be affected if exposure to a lowered pH reduces shell thickness or destroys the crystal structure. The chiton valves analysed in this thesis lost less than 5% of their weight over 12 days in a reduced pH of 7.70. The material tested by Sigwart et al. (2015) was subjected to lowered pH of 7.50 for 10 days; it is possible that there was not enough weight loss to affect valve strength.

Chiton valves will be reduced to less than 1% of their original weight in 3-5 years by abrasion. Dissolution, on the other hand, reduces valves to less than 1% of their mass after 4-46 years in ambient conditions and after 4-21 years in a reduced pH of 7.70. If both forces act on chiton valves, the valves will last for less than 5 years (Figure 6.5). However, the shelf environment off of New Zealand leaves deposited material exposed for hundreds or thousands of years. If valves cannot last under this combination of taphonomic forces for more than five years, it is unlikely they will remain after 20-200 times that amount of time. Vulnerability to the combination of forces may explain their rarity in the sediment and fossil records, as it is highly unlikely a valve will resist taphonomic forces and remain recognisable for fossilisation.
Figure 6.5: Percent mass loss of chiton valve material exposed to dissolution and abrasion over 5 years. The top graph assumes an ambient pH of 8.10 and the bottom assumes a reduced pH of 7.70.

The Sedimentary and Fossil Record of Chitons

Interestingly, chitons are absent from all accounts of the carbonate sediment record of New Zealand (Andrews 1973; Nelson 1978; Probert et al. 1979; Nelson and Hancock 1984; Nelson et al. 1988; Hayton et al. 1995). New Zealand’s EEZ contains 63 species of chitons which represent 8.5% of the world’s chiton population (Gordon et al. 2010; Spencer et al. 2016). The majority of chiton species that are found in New Zealand have population densities of 3-8 individuals/m², but S. pelliserpentis can reach 228 individuals/m² off the coast of the North Island (Boyle 1970). Since chitons in New Zealand are abundant and diverse, it is odd that they do not appear in the carbonate sediment record.
The only mentions of chitons in sedimentary records elsewhere are reported in studies that look at magnetite in sediments (Kirschvink and Chang 1984; Kirschvink and Lowenstam 1979) and the few valves found in the sedimentary records of Silicy, Italy (Dell’Angelo and Bonfitto 2005; Dell’Angelo et al. 2007a,b, 2012, 2013, 2014), Romania (Dell’Angelo and Bonfitto 2005), and the western coast of Washington state, U.S.A (Dell’Angelo et al. 2011). However, there is no solid conclusion that chitons produced the magnetite present in the sediments, and the characteristics of the grains imply they originate from magnetobacteria (Kirschvink and Chang 1984; Chang and Kirschvink 1989; Aïssaoui and Kirschvink 1991). The rarity of chitons in the sedimentary record seems odd since the sheer number of chitons should supply ample material to the shelf. Although calcareous material is not typically transported from the intertidal zone to the shelf regardless of tidal current strength (Fürsich and Flessa 1987; Meldahl and Flessa 1990), Meldahl and Flessa (1990) showed that dead Mytilus edulis shells can be found in high abundance within 300 m of the breakwater and were found up to 3 km away from their original location. This result would imply that material from the intertidal zone can reach the shelf or become a relict of lowered sea level. This transport from the intertidal zone to the shelf is likely to include at least some chitons due to their large abundances. Even in the intertidal zone, chiton material can only be exposed on the surface or contained in a taphonomically-active zone for a total of 5 years; which is unlikely since material can remain on the surface of the intertidal zone for an average of 400 years (Flessa et al. 1993). The destruction of chiton valves is not immediate, so there should be a small window of time where chiton material can be identified in the sediment record; especially with a large and constant supply of freshly dead chitons. However, this window of time does not appear to exist. Either the material is abraded so heavily as it is transported to the shelf from the intertidal zone that the material is unrecognizable, or the material does not reach the shelf at all and stays in the intertidal zone where it is removed relatively quickly. Regardless, chiton material does not appear in the sediment records of New Zealand, which implies that it is indeed vulnerable to taphonomic forces.

I have shown that chiton material can only last about 5 years when subjected to destructive forces of dissolution and abrasion, assuming they move the reported average of 3 km/day in the surf zone (Smith and Nelson 2003). If chiton material is removed at this rate, it may seem odd that chitons fossilise at all. Special circumstances will be
required: there are multiple lines of evidence that rapid burial is essential to preserve chiton valves.

Rapid burial appears to account for most recorded chitons in the sediment or fossil records. The San Diego Formation is evidence of rapid burial as it is the most plentiful site of chiton fossils found to date (Vendrasco et al. 2012) and preserved its specimens by a rapid burial event. The damage done to preserved chiton valves from Sicilian deposits (Dell’Angelo et al. 2007a,b, 2012, 2013, 2014), strata in the western U.S.A. (Dell’Angelo et al. 2011), Romania (Dell’Angelo and Bonfitto 2005) and from New Zealand (Beu and Maxwell 1990) show damage due to weathering and abrasion, or are damaged in a similar manner to the valves subjected to reduced pH in this thesis. Valves collected by Puchalski and Johnson (2009) also appear to have similar damage to valves tumbled in this study; the articulamentum appears smooth and shiny and the tegmentum is partially eroded. Since the damage appears to be specific from abrasion and not due to weathering, it is unlikely that the chiton valves remained on the surface for long (Behrensmeyer 1978). Further evidence for rapid burial is the lack of any taphonomic feedback on chiton valves. If chiton valves were exposed for a long period of time, then they should have served as a substrate for other organisms, particularly algae or bryozoans (Kidwell and Jablonski 1983). The absence of chitons in the sedimentary record also implies that if a chiton valve is not buried or bioturbated under the sediment, it will quickly be destroyed before preservation.

**Phylogenetic Patterns**

**Phylomineralogy**

The acanthochitonids (*A. zelandica* and *N. violacea*) studied here shared a similar valve structure. Both species had a reduced tegmentum with few aesthete channels and a green carotenoid pigment. Their articulamentum had three sublayers, a dorsal-most spherulitic sublayer, a rod-type crossed lamellar sublayer, and a ventral acicular sublayer. The ischnochitonid, *I. maorianus*, also had three sublayers that made up the articulamentum. However, *I. maorianus* had more aesthete tissue and an additional brown carotenoid in the tegmentum. The chitonids *C. glaucus* and *O. neglectus* had similar valve structures; both species had five sublayers in their articulamentum. These layers started with a dorsal-most spherulitic sublayer then
alternated between rod-type crossed lamellar and spherulitic structures until the final ventral-most acicular sublayer. *S. pelliserpentis* and *S. sinclaire* (also chitonids) had almost the same ultrastructure as the other chitonids, but had a thin (< 1 µm), additional crossed lamellar sublayer as the dorsal-most sublayer of the articulamentum. *C. glaucus* had a green carotenoid in the tegmentum while *O. neglectus, S. pelliserpentis, and S. sinclaire* had both green and brown pigments. *L. inquinatus* had four sublayers, which alternated between crossed lamellar and spherulitic with a ventral-most acicular sublayer. The crossed lamellar sublayer of *L. inquinatus* was unique among the analysed molluscs.

*L. inquinatus* was the most vulnerable to taphonomic forces; it lost the most mass in both the abrasion and dissolution experiments even though there was no statistically significant difference between the two pH treatments (Table 6.2; Figure 6.6). *A. zelandica, C. glaucus, and O. neglectus* all were able to resist abrasion but were vulnerable to dissolution. *S. pelliserpentis, S. sinclaire, and I. maorianus* resisted both dissolution and abrasion. *N. violacea* lost the least amount of mass in both experiments and was the most resistant species tested.
Figure 6.6: The taphonomy of the chiton species analysed in this study. Phylogenetic tree based on Okusu et al. 2003.
Table 6.2: Mass loss for abrasion and dissolution with the relative resistance labelled. Abrasion resistances were labelled “high” if the valves lost less than 0.25% mass/km, “moderate” for a value between 0.25-0.50%, and “low” for values over 0.50%. Dissolution resistances were labelled “high” if there was no significant difference between treatments, “moderate” if the valves lost less than 1% mass, and “low” for a loss of over 1%.

<table>
<thead>
<tr>
<th>Chiton Species</th>
<th>Abrasion % lost / km</th>
<th>Relative Resistance</th>
<th>pH = 8.1 Mean Difference</th>
<th>pH = 7.7 Mean Difference</th>
<th>Surface Relative Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthochiton zealandica</td>
<td>0.21 ± 0.07</td>
<td>HIGH</td>
<td>0.41 ± 0.05</td>
<td>1.71 ± 0.18</td>
<td>1.30 ± 0.13</td>
</tr>
<tr>
<td>Notoplax violacea</td>
<td>0.20 ± 0.04</td>
<td>HIGH</td>
<td>1.49 ± 0.48</td>
<td>2.19 ± 0.43</td>
<td>0.70 ± 0.45</td>
</tr>
<tr>
<td>Chiton glaucus</td>
<td>0.12 ± 0.02</td>
<td>HIGH</td>
<td>0.38 ± 0.04</td>
<td>2.12 ± 0.08</td>
<td>1.74 ± 0.06</td>
</tr>
<tr>
<td>Onithochiton neglectus</td>
<td>0.18 ± 0.01</td>
<td>HIGH</td>
<td>0.54 ± 0.04</td>
<td>3.51 ± 0.27</td>
<td>2.97 ± 0.19</td>
</tr>
<tr>
<td>Sypharochiton pelliseptensis</td>
<td>0.25 ± 0.03</td>
<td>MODERATE</td>
<td>0.33 ± 0.02</td>
<td>0.72 ± 0.05</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>Sypharochiton Sinclairi</td>
<td>0.27 ± 0.07</td>
<td>MODERATE</td>
<td>0.61 ± 0.06</td>
<td>0.71 ± 0.04</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>Ischnochiton maorianus</td>
<td>0.28 ± 0.06</td>
<td>MODERATE</td>
<td>1.12 ± 0.04</td>
<td>1.64 ± 0.07</td>
<td>0.52 ± 0.06</td>
</tr>
<tr>
<td>Leptochiton inquinatus</td>
<td>0.59 ± 0.29</td>
<td>LOW</td>
<td>3.51 ± 0.47</td>
<td>3.32 ± 0.47</td>
<td>0.19 ± 0.47</td>
</tr>
</tbody>
</table>

The ability to resist abrasion or dissolution does not appear to have any strong phylogenetic trends. Puchalski and Johnson (2009) and Sigwart et al. (2015) both point out that different chiton species have different preservation potentials; this variation does not appear to be consistent with phylogenetic position. The best indicators of abrasion resistance were the size of the valves and the structure of the tegmentum. *Leptochiton inquinatus* performed poorly in both studies in terms of weight loss, but its initial weight was much smaller than the other species. The loss of 0.1-0.3 mg of mass would already skew the results since that could be over 6% of the valves weight already, whereas that amount removed from a different species would be negligible (see Chapter 4). *A. zealandica* and *N. violacea* (members of the suborder Acanthochitonina) were able to resist abrasion well due to their reduced tegmentum, which is a phenotypic result instead of genetic. The majority of their valves were articulamentum, which due to its highly organised and low organic structure resists abrasion. The members of the suborder
Chitonina (C. glaucus, O. neglectus, S. pelliserpentis, and S. sinclairi, and I. maorianus) did not seem to have any consistent responses among themselves, although they showed a moderate resistance to removal overall instead of being completely susceptible (L. inquinatus) or resilient (A. zelandica and N. violacea).

Sigwart et al. (2014) collected Acanthochitona crinita, Lepidochitona cinerea, and Tonicella marmorea, sacrificed them, allowed them to decay in sealed containers for 120 days, then tumbled them for 18 hours. The decay experiments done prior to tumbling would have exposed the valves to acidic conditions before tumbling, and tumbling was done with organic tissue still attached to the valves. Although the methodology used in the previous study differs from this thesis, both experiments show that acanthochitonids resist abrasion. Sigwart et al. (2014) concluded that A. crinita was the most resistant to abrasion and this thesis showed that A. zelandica and N. violacea were among the more resistant species studied. However, the Acanthochitonidae was the only family examined in both studies, therefore a comparison between the other families cannot be made.

Acanthochitona, Notoplax, Chiton, Sypharochiton, and Cryptoplax are the common fossil genera in the New Zealand Cenozoic record (Beu and Maxwell 1990). These genera are also the ones that were able to resist abrasion better than the other analysed species. The ability of Acanthochitona, Notoplax, and Cryptoplax to record well is due to their resistance to abrasion thanks to their reduced tegmentum. Since the tegmentum is the more vulnerable layer to abrasion, the appearance of genera with reduced tegmenta implies abrasion is a more important factor than dissolution for removing material from the New Zealand fossil record. This ability is less of a phylogenetic one and more due to their phenotype, since it is the structure of their shell that determines how well they will record. The common appearance of Chiton spp. in the fossil record is most likely due to their large abundance rather than the ability to resist taphonomic forces. It is important to note that Beu and Maxwell (1990) listed Sypharochiton pelliserpentis in the genus Chiton, which means it was also relatively common in the Cenozoic fossil record. The common appearance of S. pelliserpentis makes sense as it is also one of the most common found in the intertidal zone of New Zealand.

The shell structure and taphonomy of additional chiton species – especially lepidopleurans – need to be analysed in order to really delineate any phylogenetic
influences. Only 11 species have had their valve ultrastructures described to date, eight of which were done in this thesis. In addition, less than 5% of chiton species have been mineralogically examined. The phylomineralogical study performed in chapter 2 was the first time microstructures of acanthochitonids, an ischnochitonid, and a leptochitonid were described. The deep-sea chitons may have different ultrastructures than chitons with developed tegmentums found on the rocky shore, as seen with the unique crossed lamellar layer organisation of *L. inquinatus*.

**Organic Tissue Presence**

The proteins and polysaccharides that make up the organic matrices of chiton valves are still under investigation, and the specific proteins used have yet to be identified. The apparent absence of α-chitin described in chapter 3 was unexpected and is at odds with the published literature. It is important to point out that while α-chitin is expected to be within chiton valves, its expectation was based on observations of molluscs in general and only three other chiton species analysed (Table 6.3). Treves *et al.* (2003) reported a lack of α-chitin in *Acanthopleura villantii* girdle spicules, which seemed unlikely when compared to other molluscan studies. However, no study to date has been able to properly refute this result. Furuhashi *et al.* (2009) came close, but used a different species (*A. japonica*) as an argument that chitin should be within the spicules of *A. villantii*. It seems reasonable to argue that unless the same species is tested and chitin found within the spicules we cannot ignore the work done by Treves *et al.* (2003). The assumption that members of the same genus have the same organic components is difficult to make with only three species surveyed. In fact, due to the findings of this thesis, the majority of analysed chiton species to date have been shown to lack α-chitin in their valves. I cannot comment if this result is a trend for chitons in general or not, but the Raman data are very consistent and convincing. At the very least, this result should serve as a warning not to generalise based on small sample sizes.
Table 6.3: Current available data on the organic components of chiton valves and how chiton species respond to taphonomic forces. Grey boxes indicate a lack of data for a listed species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Organic Valve Components</th>
<th>Taphonomic Resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthochitona zelandica</td>
<td>None (valves)</td>
<td>Green Carotenoid</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Acanthochiona crinita</td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Notoplax violacea</td>
<td>None (valves)</td>
<td>Green Carotenoid</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Katharina tunicata</td>
<td>None (valves)</td>
<td>Green Carotenoid</td>
<td>High</td>
</tr>
<tr>
<td>Mopalia muscosa</td>
<td></td>
<td></td>
<td>Sigwart et al. 2015</td>
</tr>
<tr>
<td>Mopalia lignosa</td>
<td></td>
<td></td>
<td>Sigwart et al. 2015</td>
</tr>
<tr>
<td></td>
<td>Found (spicules)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepidochiton cinerea</td>
<td>Low</td>
<td></td>
<td>Sigwart et al. 2014</td>
</tr>
<tr>
<td>Acanthopleura villanitii</td>
<td>Found (valves)</td>
<td></td>
<td>Treves et al. 2003</td>
</tr>
<tr>
<td></td>
<td>None (spicules)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthopleura japonica</td>
<td>Found (spicules)</td>
<td></td>
<td>Furuhashi et al. 2009</td>
</tr>
<tr>
<td></td>
<td>Found (valves)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiton glaucus</td>
<td>None (valves)</td>
<td>Green Carotenoid</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Onithochiton neglectus</td>
<td>None (valves)</td>
<td>Green and Brown Carotenoid</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Sypharochiton pelliserpentis</td>
<td>None (valves)</td>
<td>Green and Brown Carotenoid</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Sypharochiton Sinclairi</td>
<td>None (valves)</td>
<td>Green and Brown Carotenoid</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Ischnochiton maorianus</td>
<td>None (valves)</td>
<td>Green and Brown Carotenoid</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Leptochiton asellus</td>
<td></td>
<td></td>
<td>Sigwart and Carey 2014</td>
</tr>
<tr>
<td>Leptochiton inquinatus</td>
<td>None (valves)</td>
<td>Green Carotenoid</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
</tbody>
</table>

An analysis of shell pigments from deep-sea chitons, chitons with a large variation of shell colours (e.g. *Tonicella lineata*), or chitons with a reduced or absent tegmentum would also be interesting and valuable. Examining deep-sea chitons could also test the hypothesis that chiton shell pigments are obtained from their diet, since deep-sea chitons and intertidal chitons have different diets. The varieties of shell colours could be analysed to determine if there are multiple carotenoids in the algal diet that cause different colour patterns, or if a pigment changes structure when it bonds to the shell resulting in the
observed colour variety. If a chiton species were to be spawned and raised in the laboratory and fed a specific diet with known pigments, the shell pigments could then be analysed to see if the dietary pigments were incorporated into the shell.

**Future Research**

A study that identifies the proteins of the organic matrix and uses additional spectroscopy methods to specifically determine the presence of chitin in the same species analysed in this thesis and/or *A. villantii* would help clarify if α-chitin is indeed a component of chiton valves. Work that analyses the organic shell components in general of chiton species other than *Acanthochitona zelandica, Notoplax violacea* (*Acanthochitonidae*), *Tonicella marmorea* (*Tonicellidae*), *Acanthopleura villantii, A. japonica, Chiton glaucus, Onithochiton neglectus, Sypharochiton pelliserpentis, S. sinclairi* (*Chitonidae*), *Ischnochiton maorianus* (*Ischnochitonidae*), and *Leptochiton inquinatus* (*Leptochitonidae*) would also be valuable, since these are the only chiton species to date that have had their valve proteins examined.

The studies performed by this thesis detail how chiton valves break down due to taphonomic forces, but still need to be expanded to include more species in order to draw any true phylogenetic conclusions. Any phylogenetic pattern cannot be accurately concluded from the few species studied (see Table 6.2). Work that describes the microstructures of currently unexamined chiton species is needed to understand how these microstructures vary among species, especially for the lepidopleuridans as *Leptochiton inquinatus* is very different from other chitons, having a unique crossed lamellar layer and five sublayers; it is the only lepidopleuridan with its ultrastructure detailed to date. If variation of microstructures among chiton species is well understood, then the taphonomy of the analysed species can be studied more in-depth. Determining how additional chiton species respond to reduced pH will also increase the accuracy of future ecological predictions.

It would be valuable to repeat the abrasion experiments from chapter 4 with the same species, but leave the girdle and connective muscle tissue attached to the valves to determine how the presence of organic tissue affects abrasion. In theory, the organic tissue
will protect the mineral from abrasion, but will also decay which may decrease the pH of the water surrounding the mineral. The tegmentum is exposed in both cases so there may not be a significant difference between treatments that vary only in organic tissue presence since the tegmentum is the most vulnerable layer of the shell. However, the dissolution of shell material due to decay may affect abrasion resistance. The presence of organic tissue may also affect the results from the ocean acidification experiments. Although the girdle and any connecting muscle tissue would help separate the valves from the lowered pH, the decay may cause additional dissolution. If the decay of organic tissue has an effect on the valves, it should be measured and understood in order to accurately understand the taphonomy of chitons.

Conclusions

Chiton valves studied so far are composed of aragonite with complex sublayering. The tegmentum has a granular microstructure, permeated by the aesthete channels, and contains carotenoid pigments and proteins. The articulamentum contains sublayers alternating between spherulitic and crossed-lamellar microstructures and shows no pigment or protein signals. There is no obvious phylogenetic component to variance in valve structures, though *L. inquinatus* had a unique crossed lamellar layer in its articulamentum and displayed weaker protein signals than the other chitons. In theory, the tegmentum should be vulnerable to abrasion but resist dissolution, while the articulamentum is vulnerable to dissolution, but resistant to abrasion.

Abrasion is important in chiton taphonomy, removing an average of 9.2–44% of the valve mass after 96 hours. The acanthochitonids (*A. zelandica* and *N. violacea*) and the chitonids (*C. glaucus* and *O. neglectus*) were the most resistant to abrasion, while the lepidopleuridan (*L. inquinatus*) lost the most mass of the analysed species. The tegmentum was the most abraded layer, while the articulamentum was able to resist abrasion. Valve dissolution in lowered-pH water was less significant than abrasion, and did not appear to have a phylogenetic component. Chiton valves probably can last for 1-6 years in ambient conditions. They are, however exposed to taphonomic forces on the temperate New
Zealand shelf for hundreds or thousands of years, so that very few are found in sedimentary deposits or preserved in the fossil record.

The lack of taphonomic feedback and weathering on preserved chiton valves implies that rapid burial is needed to produce a chiton fossil. In fact, burial must occur more quickly than is necessary to preserve other molluscs, as bivalves and gastropods are still found in both records. Chiton species that either have a reduced tegmentum (such as *Acanthochitona* sp., *Notoplax* sp. and *Cryptoplax* sp.) or are incredibly abundant (*Chiton glaucus*, *Sypharochiton* sp.) have the best chance of being preserved. A reduced tegmentum, which is more vulnerable to removal than the articulamentum, maximizes the chance of enduring taphonomic forces and staying identifiable until burial.

The apparent conundrum of a relatively resistant mollusc structure that is almost never found in the sedimentary or fossil records is thus resolved. The apparent resistance of chiton valves is observed when the valves are exposed to isolated taphonomic forces in controlled conditions. The tegmentum allows the valves to resist dissolution, assuming there is no microbial interference, and the articulamentum allows them to resist abrasion. While these results do suggest the valves are relatively resistant, the results are an artefact of isolating taphonomic forces in the laboratory. When the valves are exposed to a combination of abrasion and dissolution, it is a different story. Abrasion damages the tegmentum, which is the layer protecting the valve from dissolution. The removal of the tegmentum exposes the now-vulnerable articulamentum to rapid dissolution. The removal of the tegmentum could be sped up if microbial activity is allowed to decay the aesthetes and periostracum. Taphonomic forces remove the valves from the sedimentary and fossil records by working in tandem, regardless if the valves remain in the intertidal zone or are transported to the shelf environment.
References


