A 3,000-yr high-resolution reconstruction of forest disturbances in the Cascade Range, Oregon, USA

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Summary

Current outbreaks of *Dendroctonus* bark beetles across the Pacific Northwest are widely regarded as unprecedented with the increased scale and severity of such outbreaks attributed to anthropogenic warming over the 19th and 20th Centuries. Despite such severe outbreaks observed in recent years, very little is known of historic occurrence of these forest pests. Much of the historic reconstructions of forest disturbance in this region are focussed on the Coastal Mountain Range and Willamette Valley, Oregon, and southwestern Washington. This thesis aimed to address important knowledge gaps associated with forest disturbances by producing a high-resolution fire history reconstruction at three sites within the crest of the Cascade Range, Oregon, along with the development of sedaDNA-methodologies for the direct detection of several key forest pests, including the mountain pine beetle (*Dendroctonus ponderosae*), the Douglas-fir beetle (*Dendroctonus pseudotsugae*), and the genus of budworm *Choristoneura*. The fossil charcoal records identified a period of heighted fire activity pre-700 yr BP, attributed to the Medieval Climate Anomaly, followed by a period of suppressed fire activity between 700 yr BP and present, associated with the effects of the Little Ice Age. DNA-based methods revealed the presence of *D. pseudotsugae* and *Choristoneura occidentalis* within lake sediments during periods of known outbreaks as inferred by aerial detection survey data. These results increase our understanding of fire dynamics within the Pacific Northwest and further emphasise the potential role of DNA-based methods in the field of palaeoecology.

Declarations and statements

Declaration

This work has not previously been accepted in substance for any other degree and is not being concurrently submitted in candidature for any degree.

Signed: [redacted]

Date: 14th January 2022

Statement 1

This thesis is a result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in the footnote(s). Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

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The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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List of abbreviations

AB – Alberta, Canada
AD – Anno Domini
aDNA – Ancient DNA
ADS – Aerial detection survey
BLAST – Basic Local Alignment Search Tool
bp – Base pairs
BP – Before present (1950 AD)
Cal. yr. BP – Calendar years before present
CHAR – Charcoal accumulation rate
eDNA – Environmental DNA
FC3 – Forlorn Camp Lake 3
GIS – Geographic information systems
HRM – High resolution melting
LIA – Little Ice Age
LML – Little Monon Lake, Oregon
LOD – Limits of detection
MCA – Medieval Climate Anomaly
MSH – Mount Saint Helens
MT – Montana, USA
NCBI – National Centre for Biotechnology Information
OR – Oregon, USA
PCA – Principal components analysis
PCR – Polymerase chain reaction
PYR – Pyramid Lake, Oregon
qPCR – Quantitative polymerase chain reaction
sedaDNA – Sedimentary DNA
Spp. – Species
SUM – Summit Lake, Oregon
USDA FS – United States Department of Agriculture Forest Service
WA – Washington, USA
WY – Wyoming, USA

CHAPTER ONE

GENERAL INTRODUCTION

1. Research context

The detection of historic insect and disease outbreaks within palaeoecological records was recently identified as one of the top fifty priority research areas within the field of Quaternary science (Seddon et al., 2014), further emphasising the demand for widely applicable, standardised methodologies, capable of the direct detection of historic outbreak dynamics highlighted in the current literature (Morris and Brunelle, 2012; Morris et al., 2010; Brunelle et al., 2008; Raffa et al., 2008). The demand for a deeper understanding of historic outbreak occurrence has been heightened by the increased scale, severity, and synchronicity of current outbreaks of *Dendroctonus* spp. bark beetles (Coleoptera, Scolytinae) within coniferous forest ecosystems of North America, Europe, and Asia, which are widely regarded as unprecedented (Brunelle et al., 2008; Raffa et al., 2008; Bentz et al., 2010; Morris et al., 2015; Mitton and Ferrenberg, 2012). As relatively little is known of historic outbreak dynamics (Morris et al., 2015), our understanding of the spatial and temporal dynamics of past forest disturbances and of the naturally occurring resistance and resilience of these forest ecosystems remains limited (Morris et al., 2017; Hessburg et al., 2019).

Past dynamics of some key disturbance agents, such as fire, are relatively well understood (Long et al., 1998; Flannagan et al., 2001; Long et al., 2007; Brunelle et al., 2008; Bergeron et al., 2010) and methodically assessed within the palaeoecological record, e.g., the presence and morphology of charcoal particles which can indicate occurrence and severity of historic fire events. However, unlike wildfires, unequivocal evidence of insect pest outbreaks is rarely preserved (Jenkins et al., 2008; Hebertson and Jenkins, 2008; Morris et al., 2013), therefore, we can typically only measure the inferred indirect effects these agents inflict on forest communities. In addition, the reconstruction of insect outbreaks has been largely neglected due to the absence of an effective detection tool (Girona et al., 2018). Consequently, most of our understanding is derived from proxy evidence of recent known, large-scale, highly destructive events, such as the 1940's spruce beetle (*Dendroctonus rufipennis*) outbreak in Colorado, USA which killed over 90% of Engelmann spruce (*Picea engelmanii*) in White River National Forest (Eisenhart and Veblen, 2000). This bias towards primarily large-scale events results in a skewed understanding of forest pathogen occurrence and impact (Tittiger and Blomquist, 2016).

The knowledge gaps associated with historic forest pathogen occurrence are compounded by a lack of standardised detection methods, within which there are no commonly accepted quantitative thresholds used to identify periods of outbreak, and a bias towards calibrating such methods on large-scale outbreaks. The ability of these methods to directly detect smaller, more localised outbreaks which still impact the overall forest ecology or create ideal conditions for subsequent outbreaks is unknown. Therefore, I argue the development of more sensitive, direct detection of disturbance agents over longer timescales is required to better understand historic outbreak dynamics. The study sites chosen for this thesis are all located within the Cascade Range of the Pacific Northwest of North America, a region whereby disturbance, notably by fire and insects, is very much a natural part of the ecosystem, and where over the last few decades, evidence suggests that the scale and severity of these disturbance events are increasing. The aims of this thesis are to explore current methods of detecting and reconstructing historic disturbance events, assess the degree to which they are capable of detecting all levels of disturbance by a wide range of agents, and explore the potential role of DNA-based methodologies in the field of palaeoecology.

2. Forest disturbance

This thesis aims to focus primarily on one aspect of forest ecology, disturbance. Defined as a discrete event in time that disrupt ecosystems, changes resources, substrate availability, or the physical environment, disturbances play vital roles in determining forest composition, structure, and patterns of succession (Minckley and Long, 2016; Masek et al., 2008; White and Pickett, 1985). The frequency, severity, and scale of disturbance events through time are referred to as a disturbance regime (Suffling and Perera, 2008; Turner, 2010; White and Pickett, 1985) and an understanding of ‘natural’ disturbance regimes is integral to our understanding of how natural forest ecosystems function, how they change through time, and the impact humans inflict on these regimes (Suffling and Perera, 2008). Therefore, the accurate reconstruction of such disturbances forms one of the fundamental aspects of ecology and palaeoecology.

Disturbance events can be seen as facilitators of both destruction and renewal for example, the selective clearing of older, weakened trees by bark beetles facilitates regeneration of new growth (Burton, 2008), and are integral to maximise biological diversity and promote successional patterns (Burton, 2008; Resh et al., 1988; Brookes, 2019). The organisms or processes that cause disturbance, referred to as disturbance agents, can be broadly split in to selective- and non-selective agents. Selective agents, such as forest insects, often induce mortality in single and/or small group of species, as they have evolved to exploit a narrow group of species or tissue types (Raffa et al., 2015). Perhaps some of the most studied examples of widespread, selective forest disturbance during the Holocene, include the Elm decline which led to an almost synchronous decline in *Ulmus* spp. across much of Britain and continental Europe around 5.5 ka BP (Parker *et al.*, 2002) and the mid-Holocene hemlock decline observed as an almost instantaneous decline of *Tsuga canadensis* across much of eastern North America around 5,000 yr BP (Bennett and Fuller, 2002: Fuller 1998). These rapid, species-specific declines have recently been attributed to a highly successful symbiotic relationship of fungi, transported via a bark beetle vector to viable hosts (Santini and Faccoli, 2015). In contrast, non-selective agents, such as fire, often result in widespread mortality of multiple or all species. Each of these agents will, therefore, differ in their observed impacts on stand structure, composition, and age (Burton, 2008) and influence the magnitude and duration of the event as well as the timeframe of recovery (Amiro et al., 2010). Regardless of specificity, each type of forest disturbances typically results in some level of stand modification, and it is the ability to detect these effects, within a wide range of archives, through time, that form the basis of historic reconstructions. These distinguishing features facilitate the detection and reconstruction of historic events within palaeoenvironmental samples.

3. The Pacific Northwest

3.1. Geography, geology, and climate

The Pacific Northwest of North America comprises British Columbia, Oregon, Washington, and Idaho, and covers nearly 20 degrees of latitude, extending from the Gulf of Alaska to northern California. This region is characterised by high topographic variability arising from the north-south trending mountain ranges including the Cascade Range, Coast Range, Olympic Mountains, and the British Columbia Mountain Range, and several lowland areas, including the Willamette Valley and the Puget Trough (Franklin and Dyrness, 1973). Altitudes range from sea-level to up to the highest point of 4,440m at the summit of Mt. Rainier, Washington. This high level of topographic variability within a relatively small geographical area results in highly variable geological, climatic, and ecological zones. The Pacific Northwest contains many lakes and bogs making it highly suitable for palaeoecological research (Whitlock, 1991). Consequently, there have been a number of reconstructions of historic disturbance events throughout this region, however, these are limited to the Coast Range and the Willamette Valley, leading to major knowledge gaps among the Cascade Range. The sites chosen for this study are all based within the northern Cascade Range of Oregon and the southern Cascade

Range of Washington, with the aim of extending our knowledge of disturbance into these understudied regions.

The geology of the Cascade Range consists of two major Cenozoic Age units: the underlying, older, more abundant section consists of largely deformed flows and pyroclastic rocks of Eocene origin, and the younger, volcanic rocks, consisting of andesitic and basaltic flows and breccia, which range in age from Pliocene to present (Peck et al., 1964). This combination of geologies has resulted in a high level of topographic variation within the Cascade Range with areas ranging from high plateaus to river cut valleys and slopes. High levels of volcanicity are also a predominant feature of the Cascade Range, with many highly documented eruptions over the last 40,000 years, including the eruption of Mt Mazama 7,700 yr BP and the eruption of Mt St Helens, in May 1980 AD, both of which are commonly used as time markers within palaeoenvironmental samples (Minckley and Long, 2016; Long et al., 2014; Whitlock et al., 2008; Brunelle and Whitlock, 2003**;** Millspaugh et al., 2000; Zdanowicz et al., 1999; Sea and Whitlock, 1995; Cwynar, 1987; Barnosky, 1985; Lichti-Federovich, 1970). On the timescale of palaeoenvironmental reconstructions, volcanism acts as both a driver of environmental change (landscape modification) and as an agent of disturbance (ash deposits and lahar flows) in this region (Figure 1.1).

The climate of the Pacific Northwest has undergone many changes since the last glacial maximum (LGM) (Sea and Whitlock, 1995). These dramatic shifts between glacial and interglacial environments over the last 20,000 years have greatly influenced the distribution and composition of plant, soil, and geological communities (Franklin, 1988; Whitlock, 1992). The present climate is driven by western maritime air masses and their interaction with the north-south trending mountains of the Cascade Mountain Range. Complex interactions between large-scale (latitude, longitude, and altitude) and small-scale (slope, aspect, hydrology) environmental factors produce many diverse climatic niches. The predominant climate for the Cascade Range is characterised by warm dry summers and cool wet winters (Peck et al., 1964). Precipitation patterns exhibit strong east-west, seasonal, and altitudinal gradients. The western side of the Cascades receives up to 3,800mm annual precipitation while a strong rain-shadow effect results in less than 500mm on the eastern side (Sproles et al., 2013). There is also a high seasonal effect, with up to 80% of precipitation falling between November and March. At higher elevations, over half of this precipitation falls as snow with annual snowpacks accumulating up to 5,000mm deep in some areas (Serreze et al., 1999). The average winter temperatures at high elevation sites average ~0°C, making these sites particularly vulnerable to climate change as small temperature shifts can greatly alter snow and ice dynamics. Spring snowmelt forms a principal component of the hydrological regime in these areas. Several small glaciers are present on the highest peaks (Peck et al., 1964). Recent warming has resulted in less overall snowfall, decreased snow cover, and earlier snow melt (Mote, 2006; Sproles et al., 2013). In the McKenzie River basin, central Oregon, current spring runoff occurs an average of 17-days earlier than in the mid-20th Century (Sproles et al., 2013). Earlier spring run-off and warmer summer temperatures lead to an increase in the number of warm and dry days, which create drought-like conditions conducive to both fire and insect outbreaks (Westerling et al., 2006).

3.2. Ecology

Over one-third of Oregon and Washington is covered by forests (Brookes et al., 1996), making this region one of the most densely vegetated areas of North America (Franklin and Dyrness, 1973). The complex climatic niches produced by localised variability in geology, soils, elevation, aspect, slope, are reflected in vegetation patterns across this region. Dense forests dominated by some of the largest and longest-lived coniferous species, result in some of the highest productivity and greatest biomass anywhere in the world (Franklin, 1988). The current climate of mild, wet winters and warm, dry summers greatly favours the growth of evergreen and needle-leaved vegetation as these species capitalise on extended growing seasons while minimising transpiration during dry summer months (Waring and Franklin, 1979). These prolonged periods of warm and dry weather which facilitate extended growing seasons also provide optimal conditions for wildfire occurrence. Consequently, fire is and always has been an important catalyst for environmental change in this region, with the historic impacts of fire and fire suppression greatly influencing current forest composition. When describing such ecosystems, it is common to highlight several aspects of fire behaviour, notably, fire return time (frequency and time between fires), fire types and intensity (crown fires vs. ground fires and fire severity), and size/extent of fires (Heinselman, 1981). These are collectively referred to as a fire regime and can differ greatly by region/subregion (which will become evident during the charcoal analysis section of this thesis).

Large expanses of productive temperate forest ecosystems make the Cascade Range highly suitable for timber harvest. Consequently, industrial levels of timber harvest have been one of the primary land use types in this area for at least the last 100 years (Grant and Wolff, 1991).

Activities associated with logging (e.g. felling, road construction, slash disposal) have greatly altered stand structure, age, and composition, with wider impacts on soil erosion and hydrological dynamics (Beschta, 1978). These activities, more commonly found at lower elevations, would also impact characteristic signatures associated with other proxy methodologies typically used to reconstruct environmental change, such as pollen/macrofossil data. Therefore, to allow these types of analyses to be conducted on my samples in the future, higher elevation sites were favoured for this study to reduce the potential for significant levels of human influence on reconstructive signatures.

With the exception of several niche areas whereby large stands of single species are found for example, the Olallie Scenic Area, in the central Cascade Range, which is dominated by dense *Pinus contorta* stands, three major vegetation 'zones' or 'series' exist in the sampling region, named after the dominant climax species with many localised variations in understory species (See Table 1.1. for a summary of dominant species within these zones.).

Zone	***Tsuga heterophylla***	***Abies amabilis***	***Tsuga mertensiana***
Altitude	< 1,000m a.s.l.	1,000 – 1,500m a.s.l.	> 1,500m a.s.l.
Main species	*Tsuga heterophylla* *Pseudotsuga menziesii* *Thuja plicata* *Abies grandis* *Pinus monticola*	*Abies amabilis* *Tsuga heterophylla* *Abies procera* *Pseudotsugae* *Thuja plicata* *Pinus monticola*	*Tsuga mertensiana* *Abies amabilis* *Abies lasiocarpa* *Picea engelmanii* *Pinus contorta* *Pinus monticola* *Pseudotsuga menziesii*

Table 1.1. The main vegetation zones of the Cascade Mountain Range, Oregon. Adapted from Sea and Whitlock, 1995 and Franklin and Dyrness, 1988. Also see Figure 1.1. for the distribution and extent of the above zones.

The *Tsuga heterophylla* (western hemlock) Zone (Franklin and Dyrness, 1988) is found in the low-to-mid elevations of the westside Cascades (<1,000m a.s.l.). *Tsuga heterophylla,* a highly shade-tolerant species is the dominant or co-dominant climax-species, alongside *Pseudotsuga menziesii* or *Thuja plicata*, in this ecological zone. *Tsuga heterophylla* grows in dense stands, and their thin bark, shallow root systems, and highly flammable bark increase their susceptibility to fire. Despite such high vulnerability to fire, the fire regime in this zone ranges between 150 and 400 years, owing to the cool, wet climates that dominate these altitudes (Arno and Davis, 1980; Parminter, 1983.

Above the *Tsuga heterophylla* zone, the *Abies amabilis* (pacific silver fir) Zone (Franklin and Dyrness, 1988) extends into the middle elevations of this region (1,000 – 1,500m a.s.l.). The climax species of *Abies amabilis* is often found alongside smaller proportions of *Pseudotsuga menziesii, Tsuga mertensiana, Abies procera,* and *Thuja plicata*. *Abies amabilis* is not a fire tolerant species (Agee, 1991) due to its thin bark, shallow root systems, and highly flammable foliage (Parminter, 1998). Here, the fire regime ranges from 200 to 500 years and are primarily controlled by levels of precipitation. These altitudes are characterised by high humidity and high levels of precipitation; however, prolonged drought-like conditions can lead to an increased occurrence of fire. The *Tsuga mertensiana* (mountain hemlock) Zone (Franklin and Dyrness, 1988) occupies the higher elevations, greater than 1,500m a.s.l., and represents the highest forested zone in Oregon and Washington. *Tsuga mertensiana* often occurs as a dominant or co-dominant, with *Abies amabilis, Abies lasiocarpa,* or *Chamaecyparis nootkatensis*. Despite having thicker bark than *Tsuga heterophylla,* dense stands and low-hanging, highly flammable foliage make *Tsuga mertensiana* stands highly susceptible to fire. While fire occurrence in this zone is relatively low, often occurring every 600 to 800 years, fires that do occur are predominantly stand replacing events. Above the timber line of the *Tsuga mertensiana* Zone, there exists areas of meadow or parkland (Franklin and Dyrness, 1988). This area is characterised by open boggy marshes, meadows, and moors, which form in poorly drained depressions. The dominant species include *Eriophorum polystachion, Equisetum palustre, Viola palustris, Sphagnum squarrosum, Sphagnum magellanicum, Scirpus cespitosus,* and *Trientalis arctica.*

In addition to the ecological 'climax species' classifications described by Franklin and Dyrness, (1988), Thorsen et al., (2003), developed the Ecoregions of Oregon, which denotes areas of general similarity in vegetation but also includes similarity in geology, physiography, climate, soil, land use, and hydrology (See Figure 1.1). A comprehensive understanding of the ecological zones and the species and features therein are vital in understanding the dynamics of forest disturbances i.e., how these forests respond to and recover from disturbance events. For example, mixed conifer forests will behave very differently to fire and insect outbreaks than single species stands, or how steep slopes can facilitate the rapid spread of fire and insect outbreaks. Each region consists of a two-character code made up of a number and a letter (i.e.

4a). The Cascade Range, denoted by the number 4, is divided into 6 Ecoregions, a, b, c, d, e, and f, all of which are characterised as having a moist, temperate climate that supports dense, extensive, highly productive coniferous ecosystems, with highly variable topography.

Sites chosen for this study lie within subdivisions 4b – The Western Cascades Montane Highlands and 4c – The Cascade Crest Montane Forest. Region 4b is similar to the *Tsuga heterophylla* zone outlined above, whereby abundant precipitation supports dense forests of *Pseudotsuga menziesii, Tsuga heterophylla, Abies amabilis,* and *Abies procera*. The topography in this zone is characterised by steeply sloped mountains dissected by river and stream valleys. Region 4c, which is akin to the *Abies amabilis* and *Tsuga mertensiana* zone outlined above, sits at slightly higher elevation than 4b, and is dominated by *Tsuga mertensiana* and *Abies amabilis*. The topography here consists of more open, undulating plateaus intersected by volcanic peaks, in comparison to the steep, stream cut slopes observed in region 4b. The combination of differing vegetation and topography will result in varying taphonomic and source area considerations for both fire and insect remains at sites found across different Ecoregions. For example, fossil charcoal is typically transported aerially or via surface hydrological processes. The topography of an area greatly influences the dynamics of these processes, with steeper areas effectively producing barriers for charcoal transportation while more open environments lead to greater potential source areas as charcoal can be blown in or washed in from a wider area (Scott and Damblon, 2010).

3.3. Disturbance and tree defences

In regions such as the Pacific Northwest which are characterised by disturbance, repeated historic exposure often results in physiological adaptations in host species which allow the ecosystem to persist through and recover from disturbance events (See Table 1.2) (Burton et al., 2008). These adaptations vary from fire ‘loving’ behaviour to preventative measures. For example, *Pinus contorta* exhibits serotinous cones, encased in a thick resin which require intense heat associated with wildfires to release their seeds (Teste et al., 2011; Hellum and Wang, 1985). The additional advantage of this fire strategy is that seeds germinate in the fresh ash beds of the preceding fire(s). Therefore, the general fire ecology of areas dominated by *Pinus contorta* are typically infrequent, high severity, stand replacement fires. In contrast, *Pseudotsuga menziesii* has evolved thick, cork-like bark which protects the delicate cambium during fire events and results in much longer intervals between destructive events (Kohnle et al., 2012). Knowledge of how individual species interact with fire is critical to our understanding of how varying forest compositions react to and recover from fire events, which form a key component of assessing fire dynamics.

Figure 1.1. The Ecoregions of The Pacific Northwest (Thorsen et al., 2013). The Cascade Range consists of 6 ecoregions 4a – Western Cascades Lowlands and Valleys, 4b – Western Cascades Montane Highlands, 4c – Cascade Crest Montane Forest, 4d – Cascades Subalpine/Alpine, 4e – High Southern Cascades High Montane Forest, 4f – Low Southern Cascades Mixed Conifer Forest.

With regards to tree defences against insect attacks, the primary defence mechanism of Pinaceae (including *Pinus, Abies, Picea, Tsuga, Larix,* and *Cedrus*), is the production of oleoresin (Ferrenberg et al., 2014; Himejima et al., 1992; Smith et al., 1963). Resin exudation provides physical defences against attack, essentially reducing mobility or trapping insects, but also a chemical defence as resins are commonly laden with monoterpenes and diterpene acids, both of which have been shown to reduce bark beetle vigour (Raffa *et al.*, 2005). Coniferous trees exhibit formidable defences against insect attack (Franceschi *et al.*, 2005), with some species displaying defences strong enough to overcome epidemic-level outbreaks (Strom et al., 2002). These resin defence capabilities appear to be heritable genetic traits (Ferrenberg et al., 2014) with resin duct characteristics (flow rate, chemistry and duct number, density, and size) highly associated with host resistance to attack (Moreira et al., 2012; Gaylord et al., 2013). Outbreak mitigation strategies are often based on the assumption that slow-growing trees are 'weaker' trees compared to fast growing trees, and are therefore, at greater risk of being killed by insects. However, plant defence theory (Kant et al., 2015) suggests that defence mechanisms require high energy investment, thus, fast growing trees must be compromising the allocation of resources to developing defences (Heil and Baldwin, 2002).

Climate change is also drastically affecting tree defence capabilities in two principal ways (Huang et al., 2020; Raffa et al., 2015; Bentz et al., 2010). Firstly, drought-like conditions induced by a greater occurrence of warm and dry weather patterns, drastically limit the moisture available to assign to resin production of these species, greatly reducing their defence capabilities against insect attacks (Raffa et al., 2015; Negron et al., 2009). Secondly, warmer temperatures effectively extend the climatically accessible range of bark beetle outbreaks to higher elevation sites in the Cascade Range, whereas historic temperatures were too low to facilitate the breeding and development of bark beetle eggs and larvae (Morris et al., 2015). As such the trees in these habitats naturally have supressed and or underdeveloped resin defences, leaving these individuals potentially disproportionately vulnerable to attack (Morris et al., 2015; Morris et al., 2012).

Tree species	Fire tolerance	Fire ’strategy’	Mortal fire ‘type’
Lodgepole pine (*Pinus contorta*)	Low	Late-opening (serotinous) cones keep the seedbank protected until the end of the fire season. Cones then open and release seeds into fresh ash bed.	Scorching of cambium layer or crown
Douglas-fir (*Pseudotsuga menziesii)*	High	Thick, cork-like bark that insulates the cambium layer. This species is slow growing, effective fire adaptation occurs after ~40 years growth.	Crown fires
Western hemlock (*Tsuga heterophylla*)	Very Low	Little to no fire adaptation. Thin bark (even on mature individuals), thin shallow roots, and highly flammable foliage. Seed dispersal distance is very high (>3,500 ft)	Root char, crown fire, or core burn
Western larch (*Larix occidentalis*)	Very high	Thick bark, high open crown, and deep roots. Annual regrowth of needle result in high water content	Survives all but the most severe fires. Widely regarded as the most fire-tolerant tree species in western North America.

Table. 1.2. Key tree species of the Pacific Northwest and their typical level of fire tolerance and major fire adaptation strategies (Adapted from Agee, 1993; Hood et al., 2018)

4. Disturbance agents of the Pacific Northwest

Evidence derived from archival records (Marlon et al., 2012), tree-ring records (Lauvaux et al., 2016; Merschel et al., 2018), and the analysis of particulate charcoal matter derived from lake sediments (Long et al., 1998; Hoffman et al., 2016) suggests that the conifer-dominated forests of the Pacific Northwest were historically strongly influenced by various natural disturbances such as fire, insect outbreaks, and volcanic activity. Palaeoecological reconstructions in this region show that fire has likely had both the greatest severity and occurrence, with the natural fire regime providing the primary control on patterns of community structure, composition, succession, and diversity (Taylor and Skinner, 1998). Current observations of the Pacific Northwest show a landscape overwhelmed with large areas of burnt vegetation or stands which exhibit the characteristic discolouration of the canopy associated with insect attacks. This dramatic shift in the dynamics of all disturbance agents is widely attributed to high levels of fire suppression since European settlement in the mid 17th Century, although the levels of suppression have drastically increased in the recent Century (Minckley and Long, 2016; Voelker et al., 2019; Columbaroli and Gavin, 2010; Walsh et al., 2008; Weisberg and Swanson, 2003; Skinner et al., 2002; Long et al., 1998; Teensma, 1988). The next section discusses the notable agents responsible for both current and historic disturbance events, their role within this ecosystem, and the current methods of detecting historic occurrence of these agents.

4.1. Forest insects

Under normal conditions, bark beetles perform highly valuable roles within forest ecosystems (Müller *et al.*, 2008), notably, thinning of weakened trees, promoting ecological succession, initiating decomposition processes which contribute to nutrient cycling, and provide nesting opportunities for other organisms, and, perhaps more directly, provide a food source for birds and other vertebrates (Harvey *et al.*, 2013; Raffa et al., 2016). Natural constraints, including the cool and wet winters typical of the Cascade Range, usually prevent most species from undergoing wide-scale population eruptions (Raffa *et al.*, 2008). Forest insect pests can be broadly split into two distinct groups based on the types of tissue they exploit. These groups consist of bark beetles, which as their name suggests, spend the majority of their life history beneath the bark of host trees, and defoliators, which reproduce in needle, cone, or seed tissue. These insects compete with humans for resources and their life cycle often leads to tree mortality and are, therefore, both considered pests. The next section explains the physiology, life history, and behaviour of both groups and explores how they have caused widespread tree mortality across much of the Pacific Northwest in recent years.

4.2. Bark beetles and the genus Dendroctonus

The order Coleoptera is the most species-rich order of insects, containing 400,000 described species (Hammond, 1992) across 257 genera. Of these, a third are categorised as bark beetles (Six, 2020) as they spend the majority of their life cycle in the trees' phloem, the nutrient rich tissue underlying the bark (Raffa et al., 2015). One genus, *Dendroctonus*, contains only 19 described species (Six and Bracewell, 2015) yet, is disproportionately responsible for high levels of tree mortality, making them one of the most destructive disturbance agents of coniferous ecosystems in the world (Six and Bracewell, 2015). This genus contains a wide range of life history strategies, from highly aggressive tree-killers to facultative colonisers of weak and weakened trees, to saprophagous exploiters of dead trees (Paine et al., 1997; Klepzig and Six, 2004; Rene, 2016). Bark beetle attacks induce significant physiological effects on their host species and the surrounding forest communities and environments. These key physiological effects include the disruption of nutrient and water transport between the roots and canopy during bark beetle egg gallery construction and the introduction of pathogenic fungi during entry to the new host tree, both leading to rapid, synchronous, species-specific mortality (Raffa et al., 2015; Fuller et al., 1998). Crucially, these physiological stresses result in a characteristic discolouring of canopy foliage (from healthy green to red, orange, purple, or grey), allowing the remote monitoring of the proportion of trees within a stand that are affected or have succumbed to an attack.

The term bark beetles refer solely to species who establish galleries in the phloem of woody plants (Lindgren and Raffa, 2013) and must be distinguished from the closely related ambrosia beetles (Coleoptera, Curculionidae) which depend entirely on cultivated fungi and similar species that feed on other plant tissues, such as cone beetles (Conophthorus Hopkins) (Lindgren and Raffa, 2013). All *Dendroctonus* species are morphologically similar (Wood, 1982). Adult beetles have cylindrical bodies with steep convex declivity, enlarged heads to accommodate large mandibles, and short retractable legs and antennae (Hulcr et al., 2015; Six and Bracewell, 2015), features that are perfectly adapted to tunnelling. Colours range from reddish brown, through brown, to black. While body shapes are very similar between *Dendroctonus* species, body size fluctuates greatly. The smallest species, the southern pine beetle (*Dendroctonus frontalis*) is three times smaller than the largest species, the red turpentine beetle (*Dendroctonus valens*), averaging 2.8mm and 7.3mm, respectively (Wood, 1982) and is likely correlated to the climate in which they are found, i.e., warmer regions facilitate growth of larger individuals. The genus has undergone several reclassifications since they were first described in 1836 (Erichson, 1836), however, recent molecular analysis of mtDNA COI sequences (Reeve et al., 2012) reveal 6 clades (See Figure 1.2) (Six and Bracewell, 2015). The growing number of DNA-based assessments of *Dendroctonus* spp. has generated a sufficient library of DNA sequences to expand the possibilities of further molecular methodologies to address other aspects of *Dendroctonus* dynamics. To date, there have been no attempts to use molecular methods (PCR, qPCR, or metabarcoding) to directly detect *Dendroctonus* within environmental samples in either a monitoring or reconstructive capacity.

While bark beetles often adapt to exploit localised environmental conditions, leading to slight variations in reproductive behaviours, feeding strategies, and interactions with other organisms (Schebeck et al., 2017), most species share general life attributes, each stage of which affords an opportunity for detection within palaeoenvironmental archives (Six and Bracewell, 2015). Bark beetles develop through four stages – egg, larva, pupa, and adult (Gibson et al., 2009). Adult beetles emerge from their brood galleries in late spring/early summer to find a mate and a new host (Raffa et al., 2015). This dispersal is typically limited to several hundred metres as the pheromone-mediated ‘mass-attack’ strategy is a key component in overwhelming new host tree defences mechanisms (Six and Bracewell, 2015; Reeve, 1997; Salom and McLean, 1989).

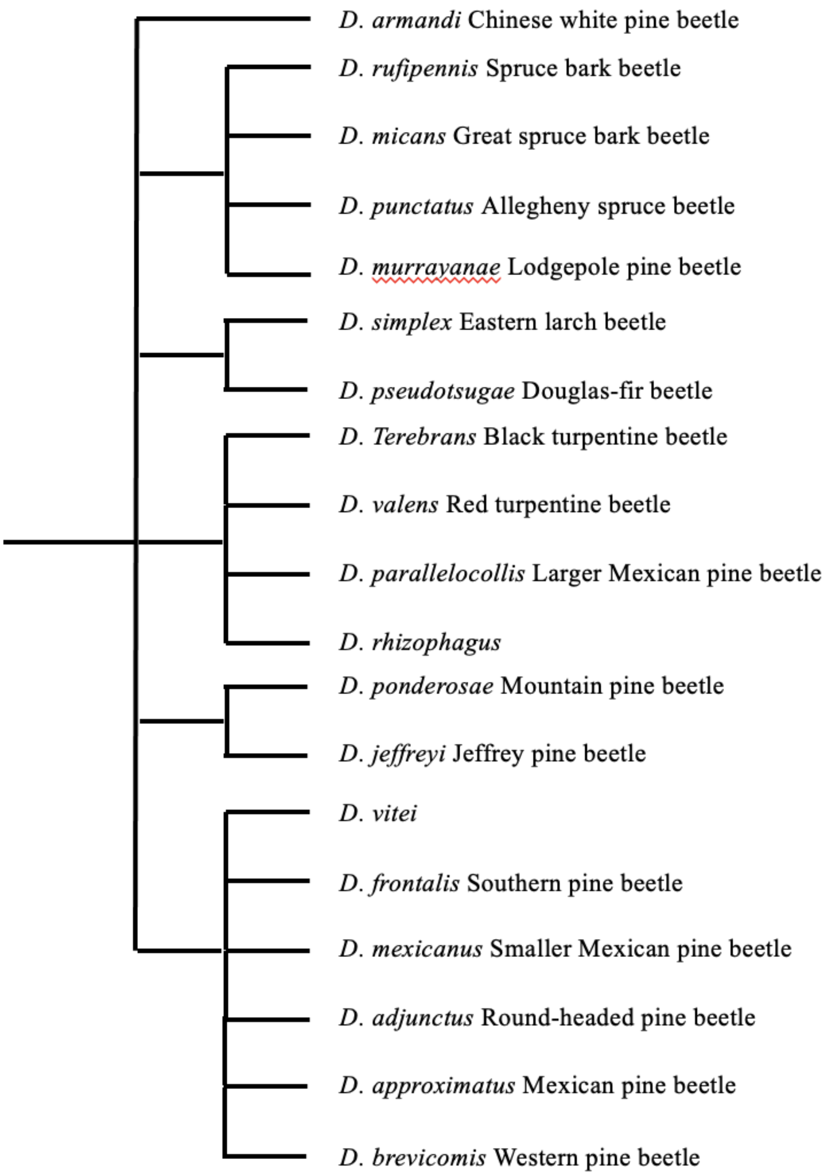


Figure 1.2. Phylogenetics of the genus Dendroctonus, adapted from Six and Bracewell, 2015.

Bark beetles face numerous challenges at all aspects of host selection, from highly toxic resin defences of the host, ensuring the host can support nutritional requirements of sustaining a brood, intense competition for less well-defended trees, vulnerability to predation *en route* to the new host, and time constraints due to the relatively short lifespan of the adult beetles (Raffa et al., 2016). Only a small proportion of bark beetles attack live trees (Lindgren and Raffa, 2013) and, of these, an even smaller proportion risk entering healthy live trees (Raffa et al., 2016). In addition, the host must be the right species, suitable age, adequate size, and be within a relatively narrow set of physiological conditions. Consequently, finding a poorly suited host (i.e. healthy, high resin capacity) inevitably always ends in death of the beetle (Six and Bracewell, 2015).

Once a new host has been selected, adult beetles burrow through the bark tissue and enter the phloem. Reproductive strategies broadly fall into two categories: monogamous species (e.g. *Dendroctonus* spp.), whereby the female initiates the attack on the new host and is joined by a single male, and, polygamous species (e.g. *Ips* spp.), whereby, the male initiates the attack on the new host and is joined by, and mates with, several females (Raffa et al., 2015). The difference in reproductive strategies significantly alters the brood size, which ultimately controls the outbreak dynamics of the next generation.

Gallery morphology and oviposition patterns differ between these groups and can be used to taxonomically identify most species (Wood, 1982). Monogamous species construct a single nuptial chamber constructed by the male, while polygamous species exhibit multi-chamber galleries constructed by each of the females. Eggs are deposited singly along these galleries and typically hatch between 10 and 14 days (Gibson et al., 2009). The construction of egg galleries within the fragile phloem greatly disrupts nutrient and water transport, which severely affects growth dynamics and/or results in the death of host trees (Brunelle et al., 2008). The disrupted growth dynamics or death of the host tree facilitates the most widely implemented methods of reconstructing insect outbreaks through the identification of divergence from ‘expected’ growth patterns in tree rings as the host tree is affected by the attack, or the loss in pollen abundance as a result of the death of the host tree(s).

Once hatched, larvae feed on phloem tissue and, occasionally, symbiotic fungi (Six and Bracewell, 2015; Raffa et al., 2015). Larvae develop in 3-5 stages, with the final instar constructing a small chamber in which to pupate (Six and Bracewell, 2015). Metamorphosis is completed in less than 10 days (Raffa et al., 2015) before the cycle begins again as adults flee the host tree to find a new host. The variation in body size discussed above is also a key control on the generational time and also the development rate (Six and Bracewell, 2015). Larger species, such as the mountain pine beetle (*Dendroctonus ponderosae*) are univoltine or semivoltine (one generation per year or one generation every two years), while smaller species such as the western pine beetle (*Dendroctonus brevicomis*) can be bivoltine or multivoltine (two, or more, generations per year) (Six and Bracewell, 2015).

Various disturbance agents, such as fire, have been widely studied in regards to their relationships to climatic factors (Brunelle et al,. 2008; Westerling et al., 2006), however, the effects of climate change on the morphology, survival rates, and spatial distribution of insects is a rapidly increasing area of research. Temperature has the greatest influence on the timing, development, survival, and dynamics of the bark beetle life cycle (Gibson et al., 2009) with the number of generations per year the key control on growth and population dynamics (Schebeck et al., 2017). Cold-induced mortality is a key factor driving *Dendroctonus* population dynamics (Regniere and Bentz, 2007). All *Dendroctonus* species are capable of overwintering in any life stage; certain species have a preference for specific life stages. For example, *Dendroctonus ponderosae* favours overwintering in the larval stage, while *Dendroctonus pseudotsugae* typically overwinter as adults (Wood, 1963). While the physiology of overwintering is not well understood (Lester and Irwin, 2012), it is thought that winter survival plays a critical role in range limitation and expansion, outbreak frequency, and severity (Logan and Powell, 2001; Campbell et al., 2007; and Aukema et al., 2008). Warmer winter temperature across much of the PNW has resulted in a greater proportion of bark beetle species successfully overwintering, leading to exponential growth patterns (Morris et al., 2015).

The morphology of individual beetles, their development rates, and their reproductive rates affect the amount of biomass available while dispersal strategies determine the period/duration of time by which insects are freely available in the environment to become incorporated into depositional environments. Therefore, understanding the dynamics of the bark beetle life history is critical in identifying the likelihood and mechanisms responsible for their remains, and therefore, their DNA being freely available in the environment to become deposited. The earliest example of *Dendroctonus* spp. presence in north-western America is attributed to a taxonomically identified macrofossil retrieved from a sedimentary core at Baker Lake, Montana, dating to 8,331 BP (Brunelle et al., 2008). However, between this early Holocene example and the current epidemic, very little information is known on historic outbreak occurrence.

4.3. Defoliators

In addition to bark beetles, defoliators are another highly destructive group of biological disturbance agents in the Pacific Northwest (Stehr, 1967; Carolin and Coulter, 1959). Of the defoliators, the most destructive species belong to the *Choristoneura* genus of budworms. Unlike bark beetles, who spend the majority of their life cycle beneath the bark of host trees, *Choristoneura* develop within external tissues, such as needles, seeds, or cones (Royama, 1984). While *Choristoneura occidentalis, C. lambertiana*, and *C. rosaceana* are native to the Pacific Northwest (Carolin and Coulter, 1958), an increase in conditions favourable for outbreaks (warm and dry), has led to a shift in equilibrium, resulting in dramatic levels of mortality in *Pseudotsuga menziesii* and true firs across Oregon and Washington, suggesting their dynamics are changing (Swetnam, 1995; Franklin et al., 1995; Powell, 1994; Fellin, 1982).

Balsam woolly adelgid (*Adelgies picea*) is also a significant pest among the true fir *(Abies* spp.*)* forests of North America (Matthews, 1990). While not considered a pest in Europe, a lack of natural enemies has led to vast areas of true fir (mostly, *Abies lasiocarpa*) mortality across much of North America, since its' accidental introduction in the early 20th century (Matthews, 1990). Several widespread outbreaks of balsam woolly adelgid have occurred in this region over the last 100 years (Mitchell and Buffam, 2001; Smith, 1967; Tunnock and Rudinsky, 1959).

4.4. Fire

Fire is not a recent phenomenon. Fire is, and always has been, an integral process within all North American forest ecosystems (Agee, 1993). Evidence of fire can be found as early as the Carboniferous Period, aptly named for its' extensive coal deposits that contain a significant amount of fusain, a form of fossilised charcoal. Evidence suggests that fire has been the primary driver of changes to community structure, composition, succession, and diversity in the Pacific Northwest across many temporal and spatial scales (Merschel et al., 2018; Lauvaux et al., 2016; Long et al., 1998; Whitlock et al., 2010; Taylor and Skinner, 1998; Franklin, 1988). While the interactions between vegetation, fire, and climate are well understood on short timeframes, i.e., recent individual events (Westerling et al., 2006), comparatively little is known about historic interactions (Minckley and Long, 2016; Long et al., 2007). Dendrochronology-based assessments of fire are limited by the lifespan of the tree species; therefore, the examination of fossil charcoal remains preserved in depositional environments extend our knowledge of wildfire occurrence over longer timescales (Long et al., 2007). Several other major disturbance agents, including insect and disease activity and volcanic eruptions have undoubtedly played a key role in shaping vegetation change throughout the Holocene in the Pacific Northwest (Long et al., 2007; Agee, 1993), however, fire has historically had the greatest influence. A combination of low precipitation and high levels of thunderstorm development mean that the primary ignition source for fires in the PNW is lightning strikes (Agee, 1993). Recently, however, the number of fires attributed to anthropogenic ignition has exceeded those by natural sources, for example, in 2018, 65% of fires in Oregon were attributed as ‘human-caused’ while 35% were ‘lightning-caused’ (Bureau of Land Management, 2018).

Fire regimes in the Pacific Northwest are broadly categorised into three types: low severity, whereby only small percentage of trees are killed, moderate severity, where some are killed and a similar percentage survive, and high severity, whereby most, if not all, trees are killed (Wetzel and Fonda, 2000; Agee, 1993). Areas with short fire intervals, often have fire regimes where low to mid severity fires are dominant, as there is less time for fuel loads to accumulate. In contrast, longer periods of time between fires allow fuel loads to build and the resulting fires are typically high severity, stand replacement fires (Wetzel and Fonda, 2000).

5. Current methods of reconstructing historic outbreaks

Our baseline knowledge of historic bark beetle dynamics comes from traditional palaeoecological-based methods such as dendroecology (Berg et al., 2006; Alfaro et al., 2009; Smith et al., 2012), palynology (Morris et al., 2010; Morris and Brunelle, 2012), and the identification of subfossil remains (Brunelle et al., 2008; Morris et al., 2015), which provide information on long-term population dynamics, while GIS/remote sensing methods give insights into current outbreaks or the repercussions of those in the recent past (Meddens and Hicke, 2014). Very few of these reconstructions pre-date the 20th Century (Brunelle et al., 2008; Morris et al., 2015) and each have associated limitations. Fossil pollen can provide long records of ecological disturbance, however, fluctuations in pollen abundance largely only ‘infer’ impacts, while dendroecological assessments require trees to survive and subsequently recover, which is a rare occurrence during particularly severe outbreaks. These studies do, however, highlight an important principle, that when insect outbreaks reach epidemic levels, they can be ‘directly’ (their remains) or ‘indirectly’ (their effects) detectable within environmental samples (Brunelle et al., 2008). Yet, the reconstruction of insect outbreaks, including bark beetles and defoliators, has been largely neglected due to the absence of effective direct detection tools (Girona et al., 2018). This section aims to provide a review of the most widely applied methodologies of reconstructing insect outbreaks within the current literature and highlight how a lack of standardised methods and an absence of quantitative thresholds indicative of periods of infestation have contributed to our relatively poor understanding of insect outbreaks beyond recorded history.

5.1. Dendroecology

Bark beetles spend most of their life history within host trees (Raffa et al., 2008). As such, measuring the effects beetles induce on attacked trees and the surrounding vegetation forms a significant proportion of bark beetle research. The tree ring sampling methods (see Young et al., 2010 for further information) associated with dendroecological assessments are relatively standardised (Davis and Loader, 2020; Alfaro et al., 2010; McCarroll and Loader, 2004). It is, therefore, the interpretation of the tree ring measurements where some level of disparity within these methodologies arises.

To identify historic disturbance events, tree ring data are typically deconstructed into a background component and a peak component. The background component usually consists of a running mean or a statistical analysis to identify the expected levels of tree growth, while the peak component is calculated using an index to assess the rate of growth in relation to expected levels. These indices identify periods whereby tree growth diverges from expected i.e. values >1 indicate a period whereby a tree is growing more than expected, while values <1 indicated periods where a tree is growing less than expected. These are termed accelerated or restricted growth, respectively. This type of analysis constitutes the most widely implemented measurement of outbreak events as measurements serve as a proxy for canopy disturbance (Reid and Robb, 1999; Alfaro et al., 2003). Periods of accelerated growth, termed release, occur as disturbance creates openings within which surviving host trees, non-attacked individuals of the host species, non-host canopy species, or understory species can thrive (Morris et al., 2010). As surviving hosts are rare, particularly during severe outbreaks, most assessments focus on heightened growth rates in non-host species or non-attacked individuals (Alfaro et al., 2003; Campbell et al., 2007; Zhang and Alfaro, 2002).

Table 1.3. highlights several examples of published dendroecological reconstructions of bark beetle outbreaks. While each set of growth rate parameters successfully observed known outbreaks in the 20th Century or identified several previously unknown outbreaks, I think that the parameters chosen show high levels of variation which may have led to key ecological data being lost. Alfaro et al., 2004, chose to dismiss release periods less than 5 years in duration, suggesting severe outbreaks would likely cause opening of the canopy for at least 5 years. In this instance, it is possible that low-level, short duration, or more isolated/scattered outbreaks or outbreaks within mixed species stands may remain undetected, reemphasising the bias towards large-scale outbreaks. During a reconstruction of mountain pine beetle (*Dendroctonus*

ponderosae) outbreaks in lodgepole pine across British Columbia and Alberta, Canada, a series of 121 host and non-host stand chronologies was constructed (Alfaro et al., 2010). Growth release rate was defined as a period in which 30% of trees within a stand exhibited a 25% increase in growth in mean ring width for 3 consecutive years. Outbreaks in this region were known to occur between 1977 and 1983 (from aerial detection survey data) and in the late 1930's (from historical records) and were reflected in relative growth rates

Agent	**Host**	**Location**	**Growth rate def.**	**Reconstructed outbreaks**	**Ref.**
Mountain pine beetle (*D. ponderosae*)	*P. contorta*	Chilcotin Plateau, BC, Canada	50% increase in relation to mean ring width of previous 5 years	1890's AD 1940's AD 1980's AD	*Alfaro et al., 2003*
Mountain pine beetle (*D. ponderosae*)	*P. contorta*	White River Plateau, CO, USA	30% of trees within a stand exhibit a 25% increase in growth in mean ring width for 3 consecutive years	1930's AD 1977-1983 AD	*Alfaro et al., 2010*
Spruce beetle (*D. rufipennis*)	*P. engelmannii*	BC and Alberta, Canada	200% increase in mean ring width between consecutive groups of 5 years	1716-1750 AD 1827-1845 AD 1860-1870 AD 1940-1960 AD	*Eisenhart and Veblen, 2000*
Spruce beetle (*D. rufipennis*)	*P. engelmannii*	White River Plateau, CO, USA	250% increase in the mean ring width when consecutive groups of 5 years or more	1716 AD 1827 AD 1949 AD	Veblen et al., 1994

Table 1.3. Several key dendroecological reconstructions of bark beetle outbreaks in North America, emphasising the disparity in agreed definitions for growth release.

No other periods of outbreaks were identified within the time series. A reconstruction of *Picea engelmanii* (Engelmann spruce), outbreaks in Colorado, USA termed growth release as a 200% increase in mean ring-width between consecutive groups of 5 years (Eisenhart and Veblen 2000). Using these parameters, several outbreak occurrences were identified, in 1716–1750 AD, 1827–1845 AD, 1860–1870 AD, and 1940–1960 AD. In a reconstruction of spruce beetle outbreaks in Engelmann spruce, White River Plateau, Colorado, growth release was defined as a 250% increase in the mean ring width when consecutive groups of 5 years or more are compared against the mean ring width for the entire series (Veblen et al., 1994). Standing dead trees were also sampled and successfully cross-dated, extending the length of the chronology to 500 yr BP. Evidence of outbreaks was found in 1716 AD, 1827 AD, and 1949 AD. While expected outbreak periods were identified, these examples emphasise a lack of standardised thresholds for several parameters associated with growth release. The amount of growth, the proportion of trees affected, and the period over which these effects must be observed to be deemed a period of disturbance all vary between these studies. Variations in localised conditions, community structure, the subject of the study (host or non-host species), make standardised methods difficult, and subsequently fair comparison problematic.

Tree-ring records are highly suitable for reconstructing disturbance events, as they offer annual resolution and therefore the possibility of identifying single events over multiple centuries, beyond recorded history (Morris et al., 2017). Trees of the family Pinaceae, which form the primary host of bark beetles, are relatively long-lived species, although there is a high level of variation among several key bark beetle host taxa (See Table 1.4). Douglas-fir (*Pseudotsuga menziesii*), the primary host of the Douglas-fir beetle (*Dendroctonus pseudotsugae*), are not only long-lived species, typically average ~1,200 years, but are also resistant to low- to moderate-level fires due to their thick bark. Long-term survival of the tree is critical for the longevity of the record. In contrast, the mountain pine beetle (*Dendroctonus ponderosae*) favour lodgepole pine (*Pinus contorta*), a much shorter-lived, typically around 200 years, fire-dependent species (Lotan and Brown, 1985). Communities comprising fire-dependent species that exhibit stand-replacement fire behaviour, such as lodgepole pine, impact the ability to reconstruct disturbance events as there are frequently no surviving trees available to produce records. These examples show how variation in event duration, host, stand composition, agent, expected fire regime, complicate uniform analysis, highlighting the difficulty in assigning standardised threshold for release for all stands.

Tree species	Life expectancy (years)	Typical Height (metres)	Shade Tolerance
Abies amabilis (Pacific silver fir)	400	45-55	Very tolerant
Abies lasiocarpa (Subalpine fir)	250	25-35	Tolerant
Picea engelmanii (Engelmann spruce)	500	45-55	Tolerant
Picea sitchensis (Sitka spruce)	800	70-75	Tolerant
Pinus contorta (Lodgepole pine)	250	25-35	Intolerant
Pinus monticola (Western white pine)	400	60	Intermediate
Pseudotsuga menziesii (Douglas-fir)	750	70-80	Intolerant
Thuja plicata (Western red cedar)	1000	60	Tolerant
Tsuga heterophylla (Western hemlock)	400	50-65	Very tolerant
Tsuga mertensiana (Mountain hemlock)	400	25-35	Tolerant

Table 1.4. Life expectancy, height, and shade tolerance for several key tree species in the Pacific Northwest. Shade tolerance is an important factor in determining how species respond to post-disturbance canopy opening and closing. Adapted from Franklin and Dyrness, 1988.

One method of increasing the temporal resolution of dendroecological methods, involves matching diagnostic features, such as a unique ring-width pattern or a fire scar, shared between dead standing trees and live trees (Fritts, 1976), extending the chronologies far beyond recorded history. One notable example of successful cross-dating (Perkins and Swetnam, 1996) facilitated the reconstruction of mountain pine beetle in whitebark pine (*Pinus albicaulis*) in central Idaho over the last 1,000 years. A series of narrow ring structures (the diagnostic feature within the series which allowed matching of tree rings) in the late 19th Century enabled successful cross-dating of records of multiple age. To identify outbreak periods, each ring width was divided by the mean width for the entire series (the expected growth) to identify periods of enhanced or restricted growth. Three outbreak periods were identified, one well documented period in the 1920's and two previously unknown occurrences, in 1730 and 1887. This example is, to our knowledge, the longest dendroecological reconstruction of bark beetle outbreaks.

Areas in North America where bark beetle outbreaks occur are typically also impacted by a variety of disturbance agents (fire, snow, avalanche, flooding) and climatic influences. While indirect methods infer periods of outbreak, we cannot always discount the effects of these other agents, or the combination of several agents, on forest structure, therefore, growth dynamics.

5.2. Palynology

Unlike fire, which is a largely non-selective agent, forest pathogens often adapt to exploit a single host species (Marajo et al., 2007). Measurements of single species decline, inferred through fossil pollen abundance, allow the identification of shifts in vegetation, and therefore, possible periods of pathogenic outbreaks through time. One major advantage of palynology is that under favourable conditions, pollen seemingly preserves indefinitely, often providing the longest records of environmental change (Bennett and Willis, 2002) with some of the greatest forest disturbances identified through these methodologies. Notable examples include the mid-Holocene elm decline, during which levels of elm (*Ulmus* spp.) pollen decreased by 73% in less than ten years across much of northwest Europe (Peglar, 1992; Parker et al., 2002) and the mid-Holocene hemlock decline observed as an almost instantaneous decline of *Tsuga canadensis* across much of eastern North America around 5,000 yr BP (Bennett and Fuller, 2002: Fuller 1998). These have both since been attributed to pathogenic outbreaks likely carried by bark beetle vectors (Parker et al., 2002). More recently, the introduction of balsam woolly adelgid (*Adelgies picea)* to eastern North America in the early 20th Century, has led to a rapid and widespread decline in *Tsuga canadensis* which is reflected in pollen records across this region (Allison et al., 1986; Calcote, 2003).

While not all events result in such dramatic fluctuations as those outlined above, quantification of these vegetational shifts, and the associated pollen fluctuations, during periods of known outbreaks, allow the identification of these characteristic signatures through time, extending our knowledge of historic occurrences (Parducci et al., 2017). These fluctuations can be assessed through qualitative (relative abundances of pollen) or quantitative (set thresholds for periods of infestation) measurements, both of which are employed in the current literature.

A calibration of a known 1986 spruce beetle outbreak in Wasatch Plateau, Utah, (Morris et al., 2010), against pollen signatures retrieved from lake sediments, aimed to qualitatively identify periods of outbreak using a sharp decline in pollen of host *Picea engelmanii*, coinciding with an increasing relative abundance non-host fir and understory species, as a parameter for identifying periods of infestation. While the 1986 outbreak was identified within the pollen sequence, no other dramatic fluctuation was observed within the 750-year sedimentary sequence (Morris et al 2010). It is possible that purely qualitative parameters, e.g. sharp declines in host species, may fail to identify lower level outbreaks as not all outbreaks result in such observable dramatic shifts in pollen abundances.

Pollen-based methods often calculate ratios between key taxa to identify vegetational shifts indicative of disturbance. A high-resolution pollen analysis of the 1939-1951 spruce beetle outbreak at Antler Pond, CO, which killed >90% of *Picea engelmanii,* by volume, in the area was used to calibrate a *Picea/Abies* ratio during severe outbreaks (Anderson et al., 2010). A pre-outbreak ratio of 1.36 was reduced to 0.45 for the 40 years (1955-1996) following the outbreak. A ratio of 0.45 used to identify periods of outbreak pre-1939, although no other periods were identified. Morris and Brunelle, (2012), scaled up the basin-level approaches of Morris et al., (2010) and Anderson et al., (2010), discussed above, to determine whether palynological responses are robust at landscape-level assessments. In addition to Wasatch Plateau discussed in Morris et al., (2010), Morris and Brunelle, (2012), assessed a further six sites across Utah. Combinations of percentage data, influx data, and pollen ratios were assessed to provide the closest association with changes in vegetation during known period of outbreak during the 20th Century (a decline in spruce coinciding with an increase in fir and understory species). Within this study, the ratio of spruce and fir to total non-arboreal pollen (NAP) most closely reflected these changes, yet no quantitative threshold (i.e. no specific ratio) was determined to identify historic outbreaks.

There are several factors that complicate the direct relationship between pollen abundances/percentages and vegetation biomass (Birks and Berglund, 2018), the first of which is the ability, or perhaps more importantly, the inability, to distinguish pollen grains to the desired taxonomic level. Palynology has historically suffered with the inability to identify taxa to the lowest possible level. As such, there are several groups of taxa, particularly within coniferous species, that have largely indistinguishable pollen grains (Birks and Birks, 2000), some of which are only differentiated through the presence/absence of very subtle features which may or may not be preserved (Moore et al., 1991). This is the case for several *Pinus* spp., the principal host of bark beetles. As such, they are often grouped into two pollen types: diploxylon-type in western North America e.g. lodgepole pine (*P. contorta*), Ponderosae pine (*P. ponderosae*), and Jeffrey pine (*P. jeffreyi*) and haploxylon-type e.g. whitebark pine (*P. albicaulis*), western white pine (*P. monticola*), and sugar pine (*P. lambertiana*) (Brunelle et al., 2008). While these species mostly occupy distinct ecological zones, there are communities where multiple species are found, and consequently, reconstructing historic outbreaks in these areas based on palynological assessments alone are particularly problematic. For instance, if an outbreak occurs in a single host, the pollen abundance from this single species declines, yet the surrounding vegetation, including a palynologically indistinguishable non-host species, increases as a result of the newly available canopy space. The overall net pollen production for that pollen type may therefore remain consistent through the period of infestation and the outbreak remains undetected. A mountain pine beetle outbreak reconstruction in the northern Rocky Mountains (Brunelle et al., 2008), measured the ratio of host lodgepole pine pollen (diploxylon-type) to non-host whitebark pine pollen (haploxylon-type) to identify periods of infestation. During the analysis, the authors concluded that the possibility of Ponderosae pine contributing to diploxylon-type, and western white pine and limber pine (*Pinus flexilis*) contributing to haploxylon-type pollen, cannot be completely dismissed as they both occupy the northern Rocky Mountains.

The amount of pollen produced varies between species (Birks and Birks, 2000), with anemophilous (wind-dispersed) species generally producing significantly more pollen than entomophilous (insect-dispersed) species. As such, pollen deposited with an environment can be over- or under- representative of the contemporary vegetation. It is therefore vital to consider the source of pollen (local signal vs. regional signal). The literature shows signals vary greatly by catchment characteristics and vegetation composition i.e., dense forests comprise more vegetation typical represent local taxa, while open tundra, with very little vegetation, depicts more regional, wind-blown signals. Bark beetle outbreaks occur in dense coniferous ecosystems, so it is possible that the signals of low-level outbreaks, a decline in a local population, is masked by the regional signal. In addition, many bark beetle hosts, including *Tsuga*, *Abies,* and *Picea* are fast growing species. As such, they can recover from reduced pollen production (associated with death of a population or supressed pollen production during periods of attack) to full production in less than 20 years (Cruz and Alexander, 2010). If sampling resolutions are too coarse, they can essentially ‘miss’ periods of supressed pollen production associated with the disturbance event, with the pollen record showing consistent abundances of these taxa across the sampling intervals.

5.3. Taxonomic identification of preserved remains

Assessments of tree ring growth rates and pollen assemblages are indirect measurements of beetle events, i.e., they observe the effects of the agent on the surrounding vegetation.

Taxonomic identification of sufficiently preserved bark beetle remains within depositional environments is, to date, the only reconstructive method of analysis aimed at the direct detection of this disturbance agent. Adult dispersal typically occurs in June, and is particularly brief, with beetles leaving the inner bark for only several days within their 1- to 2-year life cycle (Raffa et al., 2008; Alfaro et al., 2010) and typically travelling less than 300m from their original host (Seidl and Rammer, 2017). Unfavourable conditions during this short window, such as heavy rainfall, can lead to interrupted flight patterns, and the subsequent incorporation of beetle remains within depositional environments (Brunelle et al., 2008). For example, the 1949 spruce beetle epidemic on the White River Plateau, Colorado, coincided with the wettest June on record (NCDC, 2010), resulting in an accumulation of adult beetle carcasses six inches deep, six feet wide, and more than a mile along the eastern shore of Trappers Lake (Frye et al., 1977; Morris et al., 2010).

It seems however, that the conditions required for large masses of beetle remains to be incorporated, preserved, and recovered to a level which is taxonomically identifiable, are rare. There are only two notable examples (Brunelle et al., 2008) of successful recovery of taxonomically identifiable remains (head and elytra) of adult bark beetles from lake sediments that provide information on historic occurrence - remains at Baker Lake, Montana, corresponding to the highly cited 1920/30's outbreak and three early Holocene examples, dating to 8331, 8410, and 8529 cal yr BP, along with two further early Holocene examples recovered from Hoodoo Lake, Idaho, dating to 7954 and 8163 cal yr BP (Brunelle et al., 2008). These early Holocene examples significantly pre-date any other known example from any type of reconstructive archive and provide the earliest examples of physical bark beetle presence in North America. A later assessment (Morris et al., 2013) found that within 30 sedimentary sequences from British Columbia, Montana, Idaho, Wyoming, Utah, and Colorado, no diagnostic remains for primary bark beetles were recovered, which seems to confirm the rare nature of these conditions. The major limitation of this method is that even when remains are found, they can often be too poorly preserved for taxonomic identification. Taxonomic analysis of the remains, consisting of beetle elytra and head capsules, were deemed 'most likely' to be associated with adult mountain pine beetle. This raises two points that are worth elaborating on. Firstly, bark beetles are morphologically very similar and, therefore, require a high level of taxonomic skill to distinguish, and secondly, diagnostic features are not always preserved. This can be seen in Morris et al., 2010, whereby chitinous remains that temporally correspond to very recent outbreaks were recovered, however, preservation levels were too poor to allow taxonomic identification.

While the examples of taxonomically identifiable remains are rare, they highlight a fundamental principle, that bark beetle remains can become incorporated into depositional environments. I, therefore, hypothesise that DNA-based methods could be used to identify such remains with a higher level of sensitivity than taxonomic identification alone.

6. The potential role of DNA-based methodologies in palaeoecology

6.1. Introduction to DNA-based methodologies

Since the revolutionary work on the retrieval, extraction, and analysis of ancient DNA (hereafter aDNA) from ancient samples in the 1980's (Pääbo, 1989; Pääbo, 1985), the field of DNA-based methodologies to address key environmental and ecological questions has expanded exponentially (Crump 2021; Edwards, 2020; Parducci et al., 2019; Taberlet et al., 2018; Parducci et al., 2017; Pansu et al., 2015; Pedersen et al., 2015; Thomsen and Willerslev, 2015; Bohmann et al., 2014; Giguet-Covex et al., 2014; Rees et al., 2014; Pedersen et al., 2013; Epp et al., 2012; Jorgensen et al., 2012; Yoccoz et al., 2012; Ficetola et al., 2008; Bennett and Parducci, 2006; Coolen and Overmann, 1998). This type of DNA is characteristically found in low abundance and is subject to degradation through time and consequently, initially requires amplification to produce a sufficient quantity and quality of DNA molecules to be detected and analysed (Nichols et al., 2019). The breakthrough technique which facilitated such analysis was the polymerase chain reaction (PCR), an enzymatic reaction which exponential increases the number of copies of DNA molecules (Kelly et al., 2019; Nichols et al., 2019; Mullis and Faloona, 1987).

Of the various types of PCR, quantitative PCR (qPCR) is highly advantageous for such applications due to its cost effectiveness, high-sensitivity, high sequence specificity, and little to no post-amplification processing (Tsuji et al., 2019; Cao and Shockey, 2012). qPCR differs from traditional PCR in that it incorporates a fluorescent dye into the reaction which binds to double-stranded DNA, allowing the measurement of fluorescence within the reaction which can be used as a proxy for DNA abundance or level of gene expression (Robinson et al., 2018). In addition to the quantitative aspect of this technique, qPCR also allows the incorporation of post amplification high-resolution melt curve analysis (HRM), a technique used to differentiate species due to the variation in their melting temperatures (Tm), associated with changes in fragment length and sequence composition between species (Ririe et al., 1997). For this study, a SYBR Green qPCR assay with HRM was favoured as the various assays were designed to produce species-specific melting temperatures and it was hypothesised that the scale/severity of the insect outbreak could be estimated by the quantitative aspect of qPCR. In addition, my research group has previously validated the use of SYBR Green verses other polymerases and it has been shown to produce less amplification artifacts and produce more accurate levels of expression (see Robinson et al., 2018; Cao and Shockey, 2012).

6.2. sedaDNA

One branch of DNA-based methodologies involves the extraction of DNA from such sediments, referred to as sedaDNA. Lake sediments are a well-established source of long-term biological and environmental information (Brunelle et al., 2008; Arnaud et al., 2016) and have been widely used to provide proxy evidence of past biological communities and environmental change (Birks and Birks, 2008 Parducci et al., 2017) as evidence of this change is preserved for thousands of years. Additionally, lakes are found in almost all environments, making these methodologies widely applicable. Sediments typically contain varying levels of organic matter (Meyers and Teranes, 2001; von Wachenfeldt et al., 2008), sourced from within lake (autochthonous) biological processes, as well as material in-washed (allochthonous) from the surrounding catchment and the atmosphere (Giguet-Covex et al., 2019).

These ubiquitous DNA molecules are released from a wide range of sources and can persist in the environment for periods of several hours to several millions of years, depending on depositional conditions (van der Valk et al., 2021; Taberlet et al., 2018; Thomsen and Willerslev, 2015; Bohmann et al., 2014; Taberlet et al., 2012; Willerslev et al., 2007). The analysis of DNA from sediments is still a relatively new and exciting tool within this field (Edwards, 2020; Nichols et al., 2019) with the majority of research focussed on the identification of taxa and the reconstruction of plant communities (Sjögren et al., 2017; Alsos et al., 2016; Willerslev et al., 2014; Boessenkool et al., 2014; Jørgensen et al., 2012; Bennett and Parducci, 2006). As the previous literature review has highlighted, macrofossil remains of forest pests, notably bark beetles, have been successfully recovered from lake sediments (Morris et al., 2015; Brunelle et al., 2008) and are currently our oldest direct record for historic presence of these species. The preservation of beetle remains highlights the potential for the successful recovery of DNA from decomposed and/or indistinguishable remains from sedimentary archives, aiding in our understanding of historic outbreak occurrence.

While the combination of the wide range of depositional environments on a wide range of timescales makes DNA-based methodologies highly suitable for addressing palaeoenvironmental questions, these techniques have remained largely underutilised in historic detection or reconstructive capacities. To date, there have been no published investigations using DNA-based methodologies to detect forest pathogen outbreaks, current or historic. Of the few DNA-based studies of bark beetles that exist, most concern phylogenetics, the relatedness within and between species and populations (Stauffer et al., 2001; Schrey et al., 2005; Maroja et al., 2007; Peters et al., 2014; Godefroid et al., 2019). Analysis of mtDNA COI sequences and allele frequency for 9 microsatellite loci across the present-day population of spruce beetle has been used to assess timing of divergence and possible introduction, the results of which suggest these pathogens have been impacting North American forest ecosystems far longer than previously thought (Maroja et al., 2007). Two haplotypes were identified between Newfoundland and Alaska where white spruce is a primary host, and a third haplotype in the Rocky Mountains, where Engelmann spruce is favoured. The two northern lineages were 3-4% divergent from each other and from the Rocky Mountain population. Together with pollen records, used to confirm the historic availability of suitable hosts, it is highly possible that spruce beetles have been present in North America for the last 1.7 million years (Maroja et al., 2007).

7. Thesis structure and aims

Whether an insect outbreak occurs in preferred hosts or occasional hosts, in weakened trees or healthy trees, is curtailed by tree defences or causes widespread mortality, some level of stand modification occurs, and it is the ability to detect these effects, within a wide range of archives, through time, that form the basis of historic reconstructions. The vast majority of the current bark beetle ecological literature states that current outbreaks of forest insects are unprecedented in both scale and severity, and while modern outbreaks suggest this to be true, without adequate reconstructions, this cannot be confirmed. What has become apparent during this review is that a lack of standardisation in the quantification of these stand modifications associated with bark beetle outbreaks, a combination of highly variable outbreak dynamics, and no standardised method of assessing them is partly responsible for our failing to fully understand the true occurrence and dynamics of past events at all levels.

The rapid growth of molecular techniques and the occurrence of beetle remains within lake sediments highlight why DNA-based methodologies have been widely ‘called for’ by several high-profile bark beetle research groups (Morris and Brunelle, 2012; Morris et al., 2010; Brunelle et al., 2008; Raffa et al., 2008) to deepen our understanding of historic occurrence. It must be emphasised that due to the relative infancy of the use of DNA-based methods to address environmental change questions, these techniques are not without associated limitations and there are still some key fundamental ideas which need a deeper understanding. This thesis aims to extend our knowledge of several of these limitations, including the assessment of the effects cellular degradation (fragmentation and deamination) has on the recovery of DNA from historic samples, the factors associated with DNA taphonomy and catchment area, and how reliably DNA-based methods detect historic outbreak dynamics.

The underlying aim of this thesis is to deepen our understanding of historic disturbance in the previously unstudied crest area of the Cascade Mountain Range in the Pacific Northwest and assess the potential role of DNA-based methodologies in palaeoenvironmental reconstrucitions. To achieve these aims, this thesis has the following objectives:

To review the current methods of assessing historic disturbance in this region, outlining the associated strengths and weaknesses of each method, to better understand why we have such a limited knowledge of past disturbance occurrence in this region.

To assess the role of tephrochronology in providing key time markers associated with historic disturbance events and develop the reference database for such types of analyses.

To produce a continuous high-resolution fire reconstruction at three ecologically important, previously understudied sites within the Crest area of Cascade Range, Oregon, USA using a novel method of statistically analysing charcoal accumulation data.

To assess the degree to which DNA-based methodologies have the potential to better our understanding of historic outbreak dynamics, through the development of species-specific qPCR protocols for the direct detection of *D. ponderosae*, *D. pseudotsugae* and a genus-specific qPCR protocol for *Choristoneura* spp. within modern and palaeoenvironmental samples.

To identify the key areas within DNA-based methodologies which require further assessment and use the results generated within this thesis to attempt to develop these ideas.

CHAPTER TWO

METHODS

1. Site selection

1.1. Current and recent evidence of forest disturbance

Much of the current literature concerning the reconstruction of forest disturbance in this region is focussed on the Coast Range and the Willamette Valley areas of Oregon (Walsh et al., 2010a; Walsh et al., 2010b; Long and Whitlock, 2002; Long et al., 1998). Therefore, the first set of criteria for site selection was to focus on sites within the higher elevation, Cascade Range, with the aim of addressing important data gaps and extending our understanding of historic disturbance regimes into these previously understudied regions. The effects of recent, large-scale, highly destructive disturbance events are evident across much of the Cascade Range with nearly all forested areas exhibiting signs of recently burnt vegetation and/or the characteristic discolouring of the forest canopy associated with insect and disease. For example, Fig 2.1. shows Little Monon Lake, Jefferson County, Oregon, on the day of sediment collection in July 2016. In the foreground, standing burnt trees can be seen in the immediate vicinity of the lake. This area of burnt vegetation extends 2 km east and 3.5 km south and are the remnants of a major fire in the Olallie catchment in 2001. The mountainous peak observable in the background of the photograph is Olallie Butte, a shield volcano which sits 2,200m a.s.l. At the time of sampling, major signs of discolouration were evident along the entire western slope, attributed to from a *D. ponderosae* outbreak in *Pinus contorta,* which has been ongoing since 1995. The second set of criteria was the choice of sites, such as Little Monon Lake, whereby there was evidence of recent disturbance in the immediate vicinity of the lake as it was hypothesised that the ongoing/recent disturbance could be used as a positive control to check the reliability of any signals observed in the sedimentary archives i.e., evidence of recent fire at the site should be reflected in the charcoal record.

In addition to current observations and published reconstructions, the Pacific Northwest has a relatively unique, highly detailed record of historic disturbances which cover the last 75 years (1946 to 2021). Since 1946, a co-operative comprised of the USDA Forest Service, Forest Health Protection (FHP), Oregon Department of Forestry (ODF), Forest Health Management Section (FHMS), and the Washington Department of Natural Resources (WDNR), have been performing annual aerial surveys of forest disturbances across all forested federal, state, and private land within the Pacific Northwest. The surveys are conducted by entomologists aboard light aircraft and follow a gridded system to allow comparison between the data from multiple years. The dataset contains information on the responsible agent (which include insects, disease, fire, wind-throw, snow, drought, mammals), the host tree species, the area affected, and the severity of the outbreak. The duration of the event(s) and their movement through time can be inferred by analysing data over multiple years. Publicly available as Geographic Information Systems (GIS) datasets, these surveys have served as a highly valuable resource of disturbance trends within this area and are regularly used in land management plans and forest service reports. It seems, however, that this comprehensive database has been largely underutilised for research purposes. This dataset firstly helped with site selection as it identified sites recently affected by disturbance, but also allowed a deeper understanding of the dynamics associated with recent outbreaks, their extent, duration, and the responsible agent, which would provide invaluable in the interpretation and discussion of results generated in this thesis.

Figure 2.1. A view of Little Monon Lake, Olallie, Oregon, showing areas of burnt trees associated with the 2001 fire in the foreground, with characteristic canopy discolouring associated with insect outbreaks on the western slopes of Olallie Butte in the background.

To construct insect outbreak maps, individual annual polygon shapefiles of ADS data were combined in ArcMap (ESRI, 2021) to produce yearly records of disturbance from 1947 to 2016 across the Mount Hood National Forest and Willamette National Forest, Oregon, and the Gifford Pinchot National Forest, Washington. The resulting analysis presented a series of maps consisting of forested areas, topography, and hydrological features, overlain with outbreak data, including the responsible agent(s), the year of outbreak, with the extent of the outbreak marked with a translucent red polygon. The use of these polygons to demarcate area affected by outbreak facilitated the identification of outbreak extent and also areas repeatedly associated with outbreaks, as the accumulation of these translucent layers over multiple years result in darker shaded regions on the output files (See Final Site Selection below for final analyses). To identify potential geographical features (such as catchments, valleys, slope features etc.) that may influence dispersal/spreading behaviour of disturbance agents, taphonomic processes, and potential source areas for charcoal and DNA, Hydrological Unit Code 10 (HUC 10) boundaries which represent watershed level units were used in combination with 10-metre contour lines. Watersheds with the United States are categorised into a standardised hierarchical drainage system, known as Hydrological Units, with each representing varying levels of hydrologic features i.e. Hydrological Unit Code 2 represent regional level drainage, while Hydrological Unit 12 represents sub watershed drainage (Johnson, J. *pers. comm.*). HUC 10, or watershed-level boundaries, were used in this study as they likely best represented the source areas of palaeoenvironmental data, while 10-metre contour lines highlights subtle features within the immediate lake vicinity that may influence taphonomic processes. GIS analysis identified that the primary agents of tree mortality in this region since 1946 have been fire, bark beetles (Curculionidae: Scolytinae), and tree defoliation attributed to the genus of budworm, *Choristoneura* (Coleman et al., 2018). As such, the final sites chosen for full analyses were located within watersheds which have been inundated with either fire, bark beetles, and/or budworms, with suitable catchments that would likely lead to in-washing of material, including charcoal, pollen, and DNA.

1.2. Final site selection and core descriptions

GIS analysis of ADS data and consultation with the US Forest Service identified fifteen small, shallow lakes within the Cascade Range with no major inlets or outlets which exhibit high levels and/or distinct periods of disturbance associated with *Dendroctonus ponderosae*, *Dendroctonus pseudotsugae*, *Choristoneura* spp., and/or fire in the immediate vicinity, either from current observations or ADS data (See Table 2.2. for a summary of outbreak data obtained from GIS analysis of ADS data). All sites avoided any major human influences (notably, timber harvest) to reduce the potential influence these activities may have on the interpretation of results, while being accessible on foot and with topographic characteristics likely to lead to the inclusion of palaeoenvironmental material becoming incorporated into lake sediments. From these fifteen sites, a total of thirty-eight individual lake sediment cores and two peat cores were retrieved from the Mt Hood National Forest and Willamette National Forest, Oregon, and the Gifford Pinchot National Forest, Washington, across two field seasons in 2016 and 2017 (See Table 2.1 for the full list of cores obtained). The target time-period for this study was the mid-to late-Holocene. As such, only single-drives were collected at each site as it was thought that this depth of sediment would provide sufficient coverage for this time-period.

Of the forty total cores, six sites were chosen due to their proximity to recent insect, disease, and/or fire events, ecological interest, and core length. These included Little Monon Lake (44°47'50"N, 121°47'17"W), Pyramid Lake (45° 8' 48" N, 121° 55' 35" W), Summit Lake (45°01′53″N, 121°47′17″W), Scorpion Lake East (44°49'54.9"N 121°59'16.2"W), Little Shadow Camp Lake (43°41'55.0"N 122°01'55.8"W), and Forlorn Camp Lake (45°57'19.7"N 121°45'31.8"W). Radiocarbon dating of middle and basal levels revealed poor sedimentation rates at both Little Shadow Camp Lake and Scorpion Lake East and, therefore, both sites were omitted from final analyses.

The cores obtained from Little Monon Lake, Pyramid Lake, Summit Lake, and Forlorn Camp Lake 3 consisted of homogenous light brown (5YR 3/6) lake sediments with very little variation except for the visible tephra bands observed at Forlorn Camp Lake 3. No macrofossils were observed during subsampling of sediment cores.

Site	Cores obtained
Beargrass Lake	BGL_B1
	BGL_D2
Little Shadow Camp Lake	LSCL_B1
	LSCL_L2
Gold Lake Bog	GLB_L1
	GLB_L2
	GLB_L3
Forlorn Camp Lake 3*	FC3_B1
	FC3_D2
Hidden Big Lake	HBL_B1
	HBL_B2
	HBL_D3
Horseshoe Lake	HSH_B1
	HSH_D2
Rock Island Lake	RIL_B1
	RIL_B2
	RIL_D3
	RIL_D4
Barefoot Lake	BFL_B1
	BFL_D2
Little Monon Lake*	LML_B1
	LML_D2
	LML_L3
	LML_L4
Little Three Creeks Lake	LTC_B1
	LTC_B2
	LTC_D3
	LTC_B4
Hideaway East Pond	HEP_L1
	HEP_L2
	HEP_D3
Pyramid Lake*	PYR_L1
	PYR_B2
	PYR_L3
	PYR_D4
Scorpion Lake East	SCO_B1
Summit Lake*	SUM_B1
	SUM_D2
Little Fish Lake	LFL_D1
	LFL_B2

Table 2.1. All cores obtained during the 2016 and 2017 fieldwork season. Code B refers to cores obtained with a Bolivia-style corer. Code L refers to cores obtained with a Livingstone-style corer. Code D refers to cores obtained with a Bolivia corer with the addition of a full decontamination of the PVC barrel prior to core collection. *Sites used in this thesis

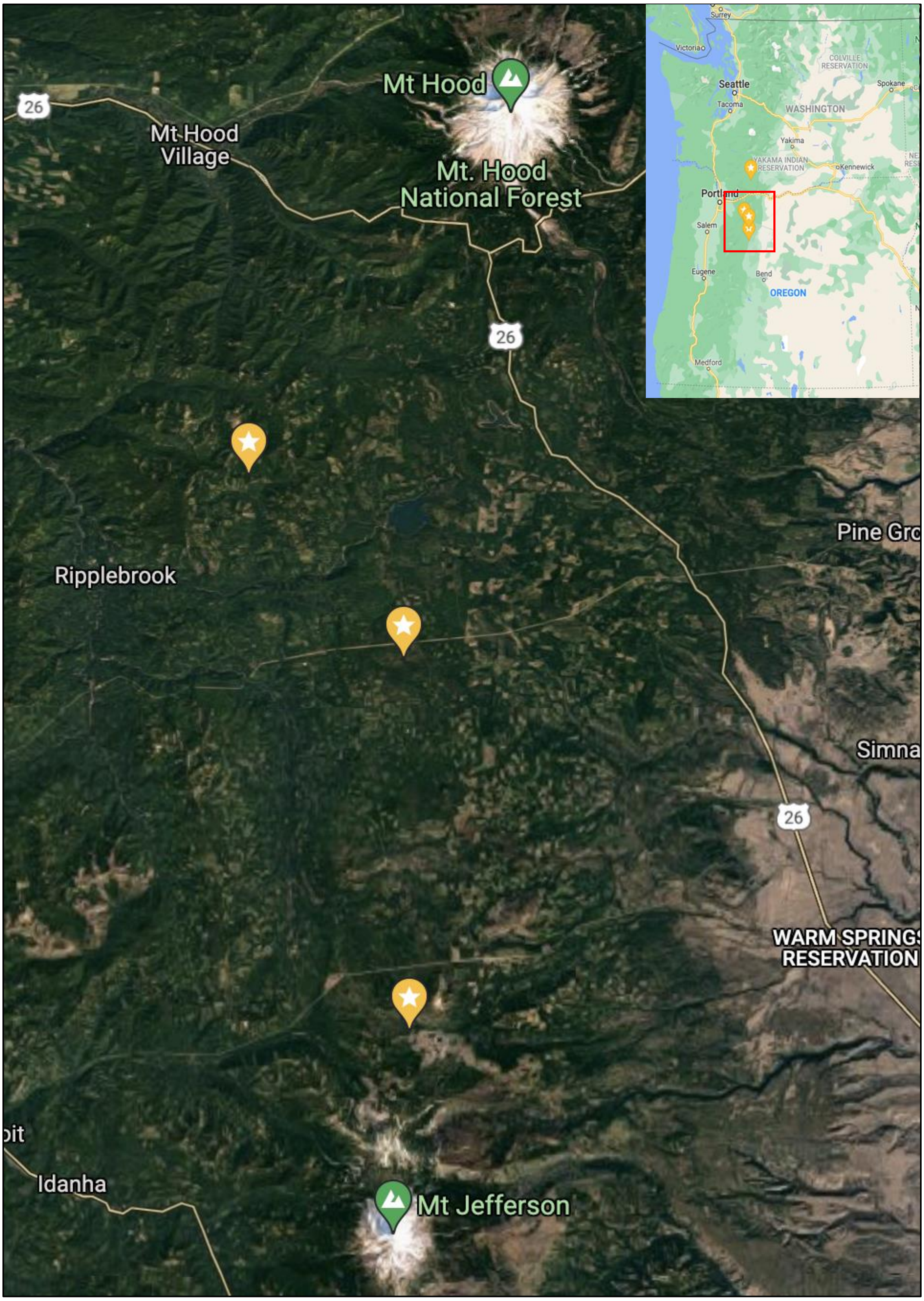


Figure 2.2. Site locations of (from north to south) Pyramid Lake, Summit Lake, and Little Monon Lake, Oregon.

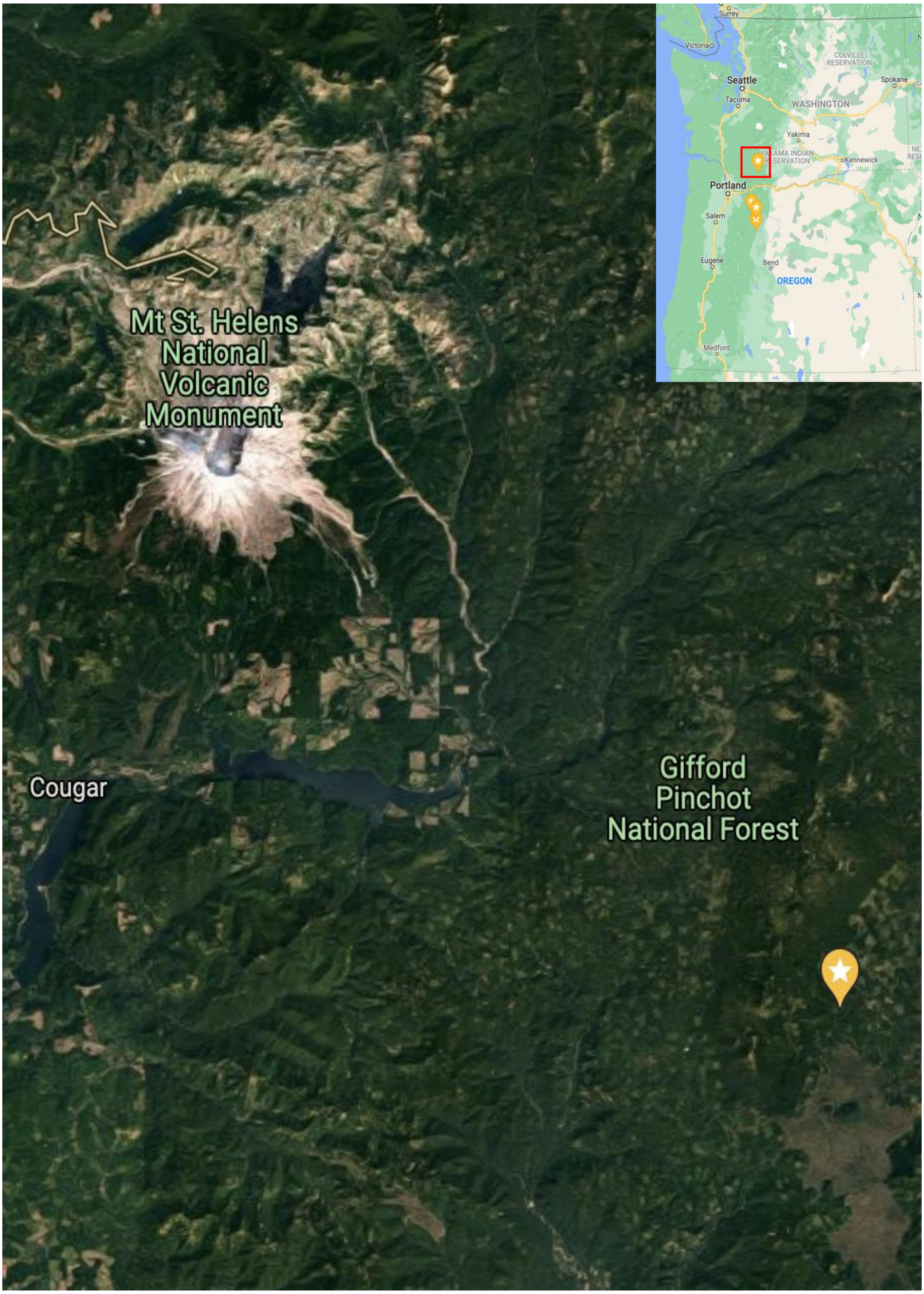


Figure 2.3. Site location of Forlorn Camp Lake, Gifford Pinchot National Forest, Washington.

Site	Agent	Outbreak year(s)
Little Monon Lake		
	D. ponderosae (Mountain pine beetle)	1950, 1980, 1986, 1989, 1991, 2000, 2001, 2003, 2004, 2005, 2006, 2007
	D. pseudotsugae (Douglas-fir beetle)	1987, 1999
	Adelgies picea (Balsam woolly adelgid)	1957, 1958, 1964, 1966, 2008, 2010, 2011, 2012, 2015
	Scolytus ventralis (Fir engraver)	1997, 2003, 2004, 2007
	Pseudohylesinus sericeus (Silver fir beetle)	2004
Pyramid Lake		
	D. ponderosae (Mountain pine beetle)	2006, 2007, 2008
	D. pseudotsugae (Douglas-fir beetle)	1992
	Adelgies picea (Balsam woolly adelgid)	2011
	Scolytus ventralis (Fir engraver)	2004, 2016
	Choristoneura occidentalis (Western spruce budworm)	1986, 1987, 1988, 1989, 1991, 1992
	Pseudohylesinus sericeus (Silver fir beetle)	2012
Summit Lake		
	D. ponderosae (Mountain pine beetle)	1962, 1962, 1963, 1965, 1971, 1972, 2006, 2007, 2008, 2009, 2010, 2011, 2012
	D. pseudotsugae (Douglas-fir beetle)	1954, 1995
	Adelgies picea (Balsam woolly adelgid)	1968, 1970, 1973, 2003, 2011
	Scolytus ventralis (Fir engraver)	1971, 1981, 1995, 2008
	Choristoneura occidentalis (Western spruce budworm)	1987, 1992, 1993

Table 2.2. A summary of outbreak data at Little Monon Lake, Pyramid Lake, and Summit Lake, obtained from GIS analysis of ADS data. As a consequence of the frequent, widespread outbreaks of *D. ponderosae, D. pseudotsugae,* and *Choristoneura occidentalis,* these species were chosen to be the targets of qPCR protocols designed within this thesis.

1.3. Little Monon Lake

Little Monon Lake (44°47'50"N, 121°47'17"W, 1,511m elevation) is situated within Jefferson County, Mt Hood National Forest, Oregon. It is a shallow, 150m wide, 5-acre lake situated within Ecoregion 4c - Cascade Crest Montane Forest (Thorson et al., 2003) with the dominant catchment vegetation consisting of *Pinus contorta* (lodgepole pine), *Tsuga* *mertensiana* (mountain hemlock), and *Callitropsis nootkatensis* (Alaska yellow cedar), all of which are either fire-dependent or not well adapted to fire (Minckley and Long, 2016). Beyond the immediate vicinity of Little Monon Lake, the extra-local vegetation is characterised by widespread single species stands of *Pinus contorta* forests to the east and south of the site, which are relatively uncommon in the Cascade Range and will likely impact the behaviour and response to disturbance regimes. At the time of sampling, Little Monon Lake was surrounded by open areas and standing dead trees to the south and east, the remnants of a widespread fire in 2001, and there was evidence of an ongoing *D. ponderosae* outbreak in *Pinus contorta* on the western slope of Olallie Butte. Little Monon Lake is bordered to the south by Monon Lake (65 acres) and to the north by Olallie Lake (240 acres). A Holocene fire reconstruction exists for a core obtained from Breitenbush Lake, 4km south of Little Monon Lake (Minckley and Long, 2016; Gavin et al., 2006) which would serve as a good comparison of fire regimes in this region. ADS data from Little Monon Lake shows this watershed has been inundated with disturbance since the origins of the survey, in 1947. Frequent and widespread outbreaks of *D. ponderosae*, *D. pseudotsugae*, *Scolytus ventralis*, *Adelgies picea*, and *Pseudohylesinus sericeus* can be observed for the duration of the survey period (See Figure 2.2. and Table 2.3. for more information).

Figure 2.4. Little Monon Lake, Jefferson County, Oregon.

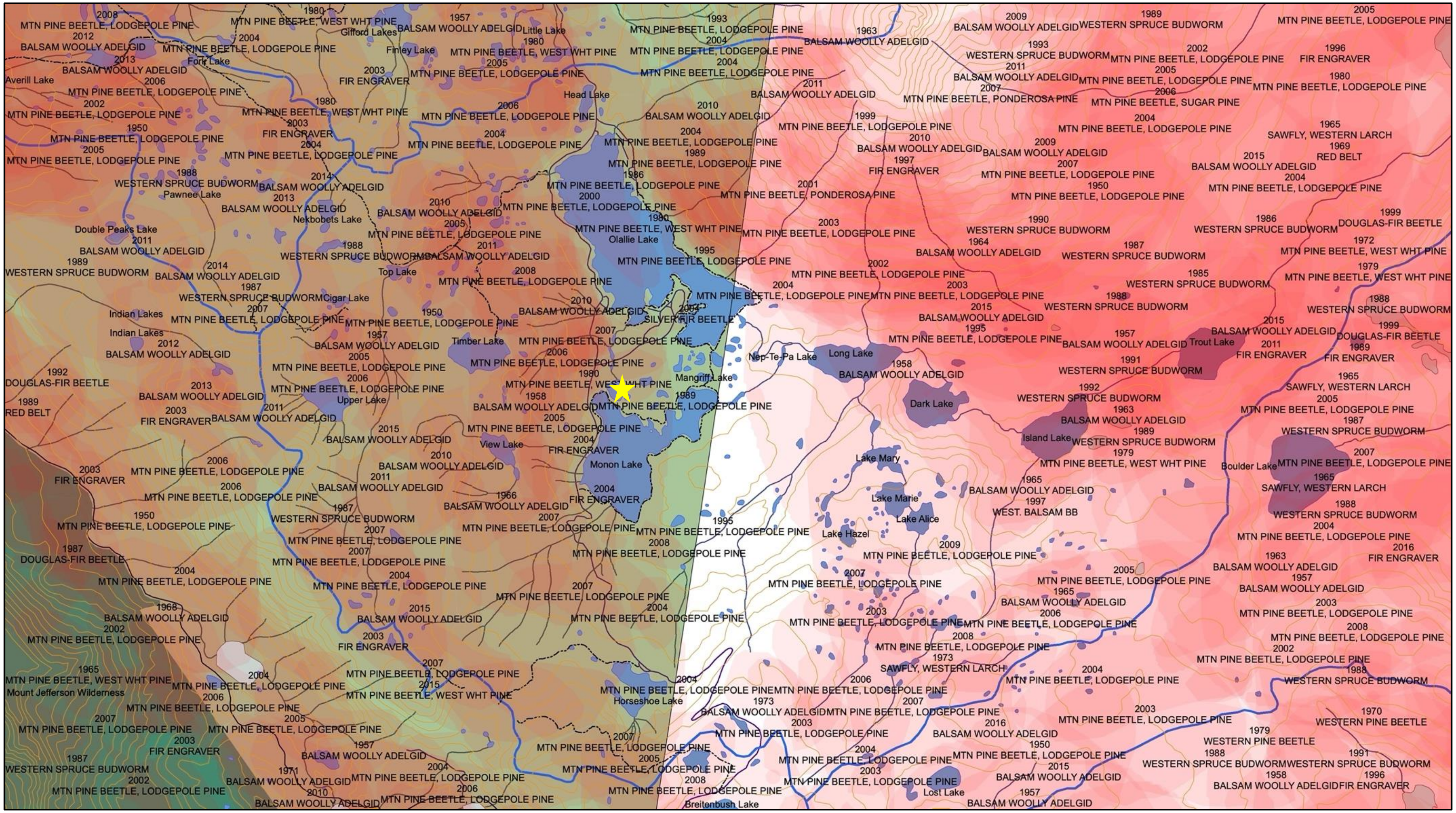


Figure 2.5. GIS analysis of ADS data at Little Monon Lake, Olallie, Oregon. This watershed has observed highly frequent and widespread outbreaks *D. ponderosae*, *D. pseudotsuga*, *Scolytus ventralis*, *Adelgies picea*, and *Pseudohylesinus sericeus* since the origins of the ADS survey in 1947.

1.4. Pyramid Lake

Pyramid Lake (45° 8' 48" N, 121° 55' 35" W, 1,213m elevation) is a 283m wide, 5-acre high elevation site situated within the Roaring River Wilderness Area, Mt Hood National Forest, Oregon. site is situated within ecoregion 4b – Western Cascades Montane Highlands (Thorson et al., 2003). Dominant watershed vegetation consists of *Tsuga mertensiana* (mountain hemlock), *Tsuga heterophylla* (western hemlock), *Abies procera* (noble fir), *Pseudotsuga menziesii* (Douglas-fir), *Abies amabilis* (Pacific silver fir), *Pinus monticola* (western white pine), with *Alnus rubra* (red alder), *Salix* spp. (willow), *Pteridium* spp. (bracken), and *Rhododendron macrophyllum* (Pacific rhododendron) forming the main understory species. The lake sits within a niche of a small west to east trending slope. Recent timber harvest is evident to the south. GIS analysis of the ADS insect and disease database shows Pyramid Lake has experienced several severe insect outbreaks in the last 70 years, including a series of *D. ponderosae* events in the 2006, 2007, and 2008, a *D. pseudotsugae* outbreak in 1992, and a period of widespread *Choristoneura occidentalis* (western spruce budworm) outbreaks throughout the 1980's and 1990's.

Figure 2.6. Pyramid Lake, Mt Hood National Forest, Oregon.

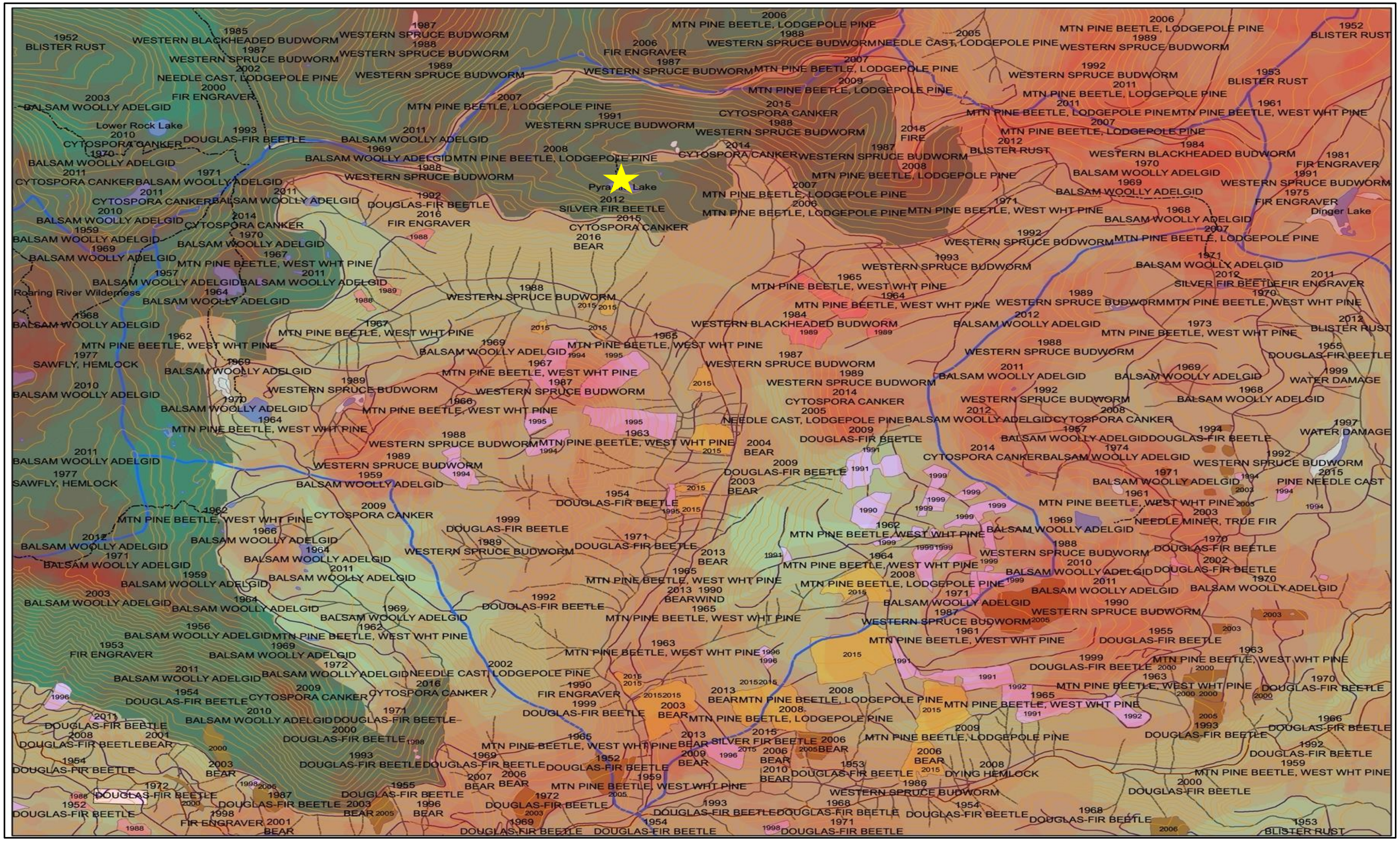


Figure 2.7. GIS analysis of ADS data at Pyramid Lake, Mt Hood National Forest, Oregon. Similar to Little Monon Lake, this watershed has observed highly frequent and widespread outbreaks *D. ponderosae*, *D. pseudotsuga*, *Scolytus ventralis*, *Adelgies picea*, and *Pseudohylesinus sericeus* since the origins of the ADS survey in 1947. Pyramid Lake has also experienced highly severe *Choristoneura occidentalis* outbreaks in the immediate vicinity of the lake.

1.5. Summit Lake

Summit Lake (45°01′53″N, 121°47′17″W, 1,275m elevation) is a 10-acre, 320m long lake situated ~10km south of Timothy Lake, Mt Hood National Forest, Oregon. The site is located within Ecoregion 4c – Cascade Crest Montane Forest (Thorson et al., 2003) with dominant stand species consisting of *Pinus contorta* (lodgepole pine), *Tsuga mertensiana* (mountain hemlock), *Cupressus nootkatensis* (Alaska yellow cedar). The catchment within which Summit Lake sits is the largest and most open of the three sites characteristic of the undulating plateaus found throughout Ecoregion 4c, rather than the river-cut valleys found throughout Ecoregion 4b. Therefore, Summit Lake potentially has the largest catchment out of the three sites chosen for full analysis, producing signals more associated with regional change than local change. At the time of sampling, a large area of standing burnt trees were evident across the north shore of Summit Lake, however, the timing of this burn is unknown.

ADS data shows that Summit Lake has been subject to outbreaks of *D. ponderosae* in nearly every decade since the beginning of the ADS survey, along with *Choristoneura occidentalis* outbreaks in 1989, 1992, and 1993. There have been two outbreaks of *D. pseudotsugae*, the first in 1954 and a second in 1995. There are also outbreaks from other notable agents including *Adelgies picea* (Balsam woolly adelgid), *Scolytus ventralis* (fir engraver), however, these species were not chosen to be target species for DNA-based methodologies.

Figure 2.8 – Summit Lake, Oregon (obtained from google image search. Credit: Skeels, J)

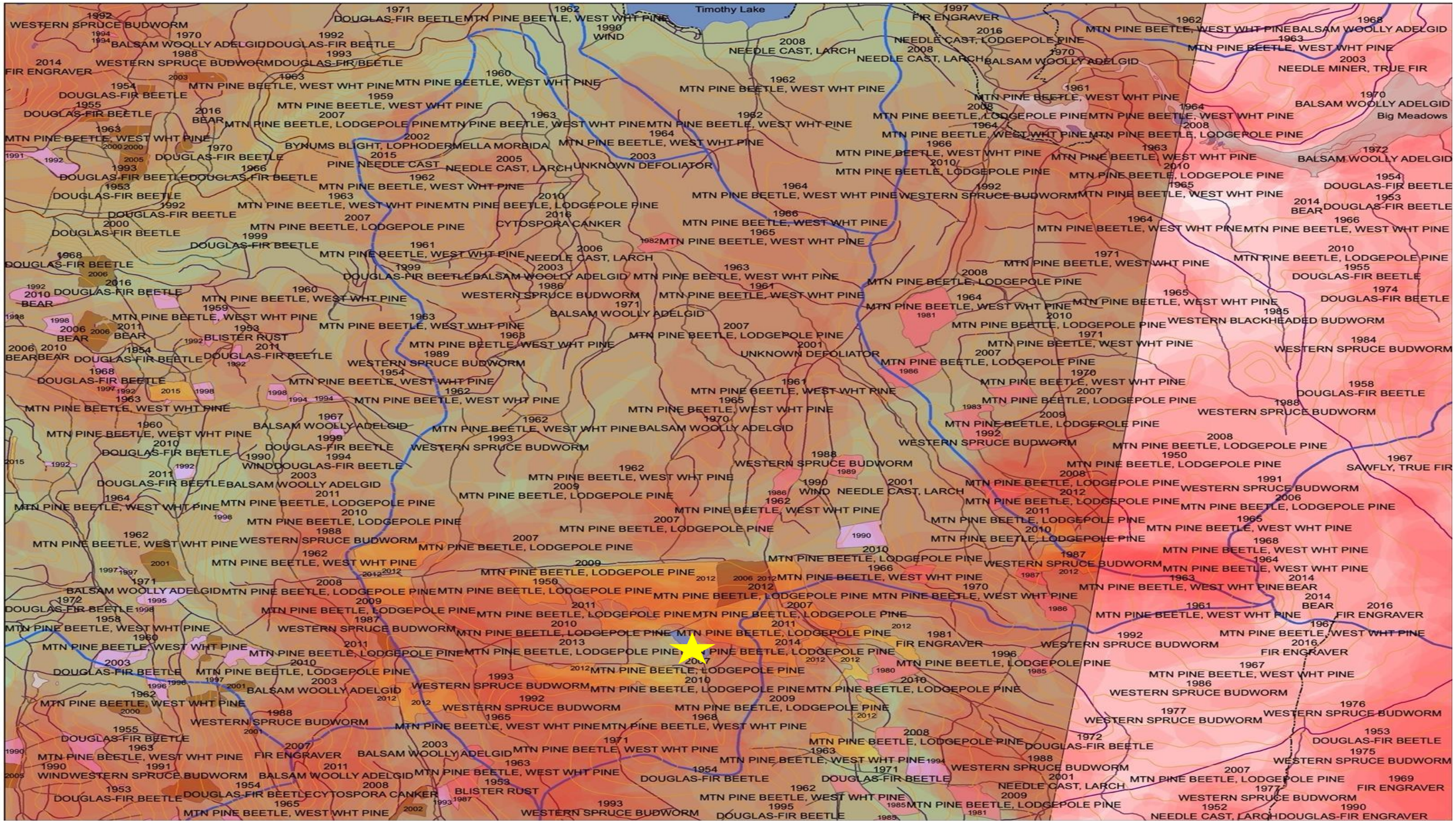


Figure 2.9. GIS analysis of ADS data at Pyramid Lake, Mt Hood National Forest, Oregon. Similar to Little Monon Lake, this watershed has observed highly frequent and widespread outbreaks *D. ponderosae, D. pseudotsuga, Scolytus ventralis, Adelgies picea*, and *Pseudohylesinus sericeus* since the origins of the ADS survey in 1947. Pyramid Lake has also experienced highly severe *Choristoneura occidentalis* outbreaks in the immediate vicinity of the lake.

1.6. Forlorn Camp Lake 3

Forlorn Camp Lake 3 (45°57'19.7"N 121°45'31.8"W, 1132m a.s.l.) is a 200m wide lake situated within the Gifford Pinchot National Forest, Washington. The site sits within Ecoregion 4b – Western Cascades Montane Highlands (Thorsen et al., 2003). The dominant overstorey vegetation at this site consists of *Tsuga mertensiana* (mountain hemlock), *Tsuga heterophylla* (western hemlock), *Pinus contorta* (lodgepole pine), *Pseudotsuga menziesii* (Douglas-fir), and *Abies amabilis* (Pacific silver fir), with *Vaccinium membranaceae* (black huckleberry), *Cornus canadensis* (dogwood), and *Rosa gymnocarpa* (dwarf rose) forming the main understory species.

Figure 2.10. Forlorn Camp Lake 3, Gifford Pinchot National Forest, Washington.

Forlorn Camp Lake 3 lies 42km southeast of the summit of Mount St Helens and upon extrusion of core, three distinct tephra bands were observed. The uppermost band, termed FC3 B1, is located ~5cm from the surface and consists of dark grey, dense pumice. As there were no observable tephra bands above this layer it was assumed that it must derive from the most recent eruption of Mt St Helens, in 1980 AD. This would provide a critical dating control within the period covered by the ADS dataset and therefore this site was chosen for tephrogeochemical analyses. Should these observable tephra layers be associated with Mt St Helens, then analysis of cryptotephra would provide an additional dating control within this study. Two deeper tephra bands, termed FC3 B2 and FC3 B3 consisted of two yellowish grey layers of ash which occur in quick succession 23cm deep within the core.

Figure 2.11. - Core FC3_B1 obtained from Forlorn Camp Lake 3, Gifford Pinchot National Forest, Washington. Extraction revealed three distinct ash bands, the upper layer around 5cm from the surface, and two further layers between 23 and 25cm deep.

2. Sediment collection

Three lake sediment cores obtained from Little Monon Lake, Summit Lake, Pyramid Lake, Mt Hood National Forest, Oregon, were selected for charcoal and sedaDNA analyses. Single Bolivia cores were obtained from each site as it was believed that these would cover the relatively short time-period associated with this study. At all sites, the shallow bedrock and/or thick tephra layers prevented coring beyond the first Bolivia section. Cores measured 230 mm, 600 mm, 670 mm respectively. In addition, a fourth lake sediment core retrieved from Forlorn Camp Lake, Gifford Pinchot National Forest, Washington, measuring 360mm, was selected for tephrogeochemical analysis due to its' proximity to Mt St Helens and several observable tephra layers. All sediment cores were obtained using a Bolivia corer, a modified Livingstone-type rod piston corer (Livingstone, 1955). The replacement of the steel coring barrel with a single-use, decontaminated PVC pipe (Wright, 1967; Wright and Myrbo, 2008), is more suited to unconsolidated/watery sediments, limits the need to extrude the sample in the field and, critically for samples intended for DNA methodologies, allows the decontamination of all sampling equipment prior to collection (Morris et al., 2015). A traditional Livingstone corer was used to retrieve two peat cores from Gold Lake Bog, Oregon, however, these were not used for final analyses as there was no decontamination protocol implemented prior to sampling with this corer.

All sediment cores were obtained from the deepest part of the lake using a stabilised inflatable dingy. Once retrieved, sediments (which remained in their PCV pipes) were capped with floral foam to protect the sediment-water interface and prevent mixing of sediments and sealed with plastic caps and duct tape. All cores were refrigerated while on fieldwork and frozen at -20^{o}C on return to Swansea University until sub-sampling, to limit DNA degradation. For subsampling, cores were sawn into 10 cm sections and left to thaw horizontally overnight to prohibit sediment mixing while thawing. During thawing, no fracturing or loss of sediment coherence occurred. High-resolution, contiguous 2mm sampling strategy was chosen to provide a comprehensive reconstruction of disturbance regimes and to address concerns in the literature that coarse sampling methodologies lead to a bias towards only detecting large-scale, destructive events (Long et al., 1998). Samples were taken every 2mm and isolated into the required volumes for charcoal, DNA, and radiocarbon dating, with the remaining sediment moved into falcon tubes and refrigerated. For DNA analyses, three replicates for each depth (each weighing 0.25g) were placed directly into the first step lysis tube of the QIAGEN PowerLyzer PowerSoil Kit (QIAGEN, UK). All subsampling equipment was decontaminated with a bleach, water, ethanol wash in between samples. DNA extractions were performed immediately after subsampling to limit the potential of DNA degradation.

3. AMS radiocarbon dating

A total of forty-three 1 cm^3 bulk-sediment samples were taken across four sites to be AMS dated by myself at iThemba LABS, University of the Witwatersrand, Johannesburg, South Africa, via the 6MV Tandem AMS system (Mbele et al., 2017). The six provisional cores were sampled for dates at the surface, middle, and base, in addition to several further samples taken near the surface (See Table 2.3 or Supplementary Material for full AMS analyses) to provide robust chronologies with an emphasis on the last few hundred years for which there is ADS data and published reconstructions of forest disturbance with which to compare results. No macrofossils were obtained for dating.

At iThemba LABS, samples were subjected to a standard Acid-Base-Acid (A-B-A) pre-treatment (De Vries and Barendsen, 1952; Brock et al., 2010). The sediments were treated with hot 1% HCl (70°C for 45 minutes), washed with deionised water, treated with 0.4% NaOH (20°C for 30 minutes), washed with deionised water until pH was neutral, before a final reacidification of the sediment in 1% HCl (20°C for 10 minutes) and washed with distilled water. Samples were weighed and transferred into a combustion tube along with 80mg each of copper oxide and silver. Combustion tubes were connected to a vacuum and sealed using a blowtorch. Sealed samples were then placed in a furnace at 500°C for 6 hours to generate CO_2 and then graphitized. Samples were analysed using standard 6MV Tandem AMS system protocol (Mbele et al., 2017).

Raw radiocarbon dates were converted (two sigma) to calendar ages (cal. yr. BP) in OxCal (Bronk Ramsey, 2009) using the Bomb13NH1 calibration curve (Hua et al., 2013) which is prepended with IntCal13 (Reimer et al., 2020) (See Table 2.3). Before present (BP) refers to 1950 AD. Age depth models were constructed using Bayesian statistics in the R package "rBacon" (Blaauw et al., 2021) (See Figures 2.12, 2.13, and 2.13). Several iterations of age depths models were calculated using varying sampling slices and interpolation methods. Each iteration used the same the Bomb13NH1 calibration curve (Hua et al., 2013) which is prepended with IntCal13 for any pre-1950 dates (Reimer et al., 2020). Final age depth models were selected on goodness of fit and the determination of realistic transitions in sedimentation rates, using linear interpolation, with a 95% confidence interval.

Each age-depth model output contains four plots. The main plot shows the distribution of individual radiocarbon dates in blue, with the age-depth model outlined in grey. The red curve shows the mean age value, and the dashed grey lines show 95% confidence intervals. The upper left plot shows the goodness of fit of all Markov Chain Monte Carlo (MCMC) iterations and is used to identify the robustness of the age-depth model. The upper middle plot depicts average sedimentation rate. The upper right plot indicates variation in accumulation rates. Basal ages at each site were 2494-2307 cal. yr. BP at Little Monon Lake, 5441-5221 cal. yr. BP at Pyramid Lake, and 3595-3439 cal. yr. BP at Summit Lake. Average sedimentation rates at each site were 10.45 yr mm^{-1} at Little Monon Lake, 9.57 yr mm^{-1} at Pyramid Lake, and 5.71 yr mm^{-1} at

Summit Lake. This temporal resolution is comparable to published sedimentation rates from the lower elevation Coastal Range and Willamette Valley due to the fine-resolution sampling intervals chosen for this study. Upon the discovery of slow sedimentation rate, Pb210 dating was considered, however, no financial budget was available to complete this additional analysis due to the abundance of radiocarbon dates already obtained. Due to such high sampling resolution we instead chose to reconstruct the last 2-3,000 years of disturbance at each site, as inferred by the age-depth model, as the underlying theme of this thesis is to move from long-term, coarse sampling methodologies, and aim to produce high-resolution, continual records, capable of detecting all levels of disturbance.

Site	Depth (mm)	Age	Age error	Age BP	Age AD/BC
Summit Lake					
	4	-80	66	260--7	1700-1957 AD
	8	0	60	149-20	1804-1936 AD
	32	930	79	959-684	861-1176 AD
	40	230	34	317-142	1761-1804 AD
	54	300	66	503-267	1652 AD
	60	300	60	498-273	1651 AD
	300^{+}	2110	36	2152-1991	230-90 BC
	600^{*}	3290	49	3595-3439	1439-1244 BC
Little Monon Lake					
	2	120	30	151-9	1800-1898 AD
	8	70	30	140-32	1810-1920 AD
	14	150	31	283-0	1717-1789 AD
	20	360	40	496-314	1510-1669 AD
	26	670	38	676-555	1204-1355 AD
	32	900	45	917-727	960-1139 AD
	38	910	33	910-735	974-1105 AD
	44	1030	33	977-902	854-985 AD
	50	1170	34	1176-974	712-847 AD
	58	1220	48	1278-1055	634-825 AD
	115^{+}	1610	46	1586-1382	248-431 AD
	230^{*}	2350	42	2494-2307	485-318 BC
Pyramid Lake					
	2	-500	31	-53	2004 AD
	8	40	31	256-33	1867-1918 AD
	16	90	32	266-22	1806-1930 AD
	24	460	34	543-473	1422-1557 AD
	36	590	36	649-532	1288-1431 AD
	40	590	42	651-529	1276-1433 AD
	340^{+}	2160	35	2307-2004	281-142 BC
	670^{*}	5330	55	5441-5221	3492-3272 BC
Forlorn Camp Lake					
	Above B1	1080	81	1154-900	774-1050 AD
	Below B1	660	39	676-554	1274-1396 AD
	Above B2	2510	53	2746-2436	796 -486 BC
	Below B3	4380	61	5665-5452	3715-3502 BC

Table 2.3. Summary of AMS dates at sites used in this study. Each site contained a surface, middle, and basal date. Forlorn Camp Lake core FC3_B1 was used for tephrogeochemical analyses. Above B1 refers to a bulk sample taken above the grey ash layer found 5cm from the core surface. Below B1 refers to a bulk sample taken below this layer. Above B2 refers to a bulk sample taken from the younger of the two lower ash bands found at 25 and 28cm depth. Below B3 refers to a bulk sample taken below the older of two ash layers found between 25 and 28cm deep. $^{+}$Middle date, *Basal date

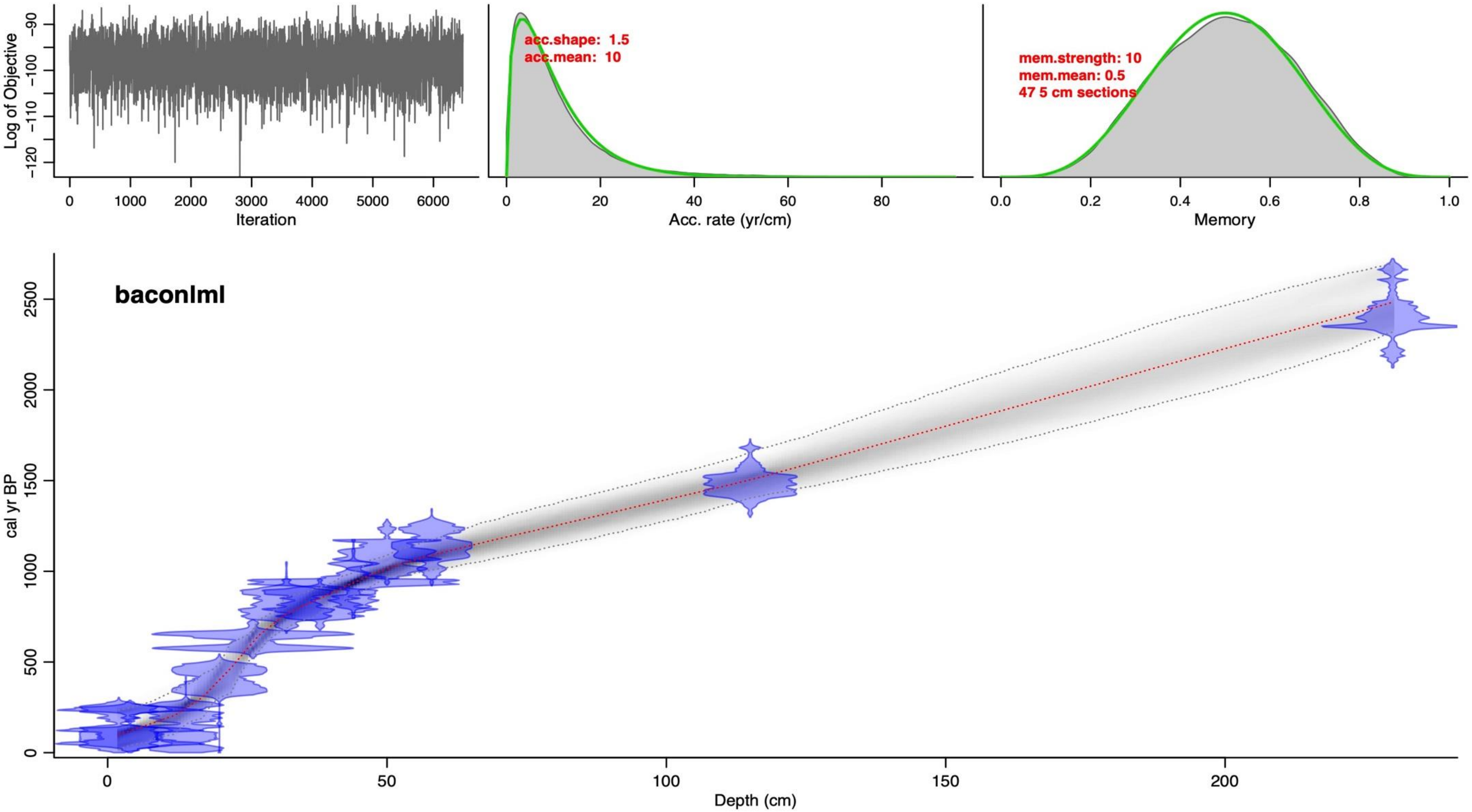


Figure 2.12. Age-depth model from Little Monon Lake, constructed using the R package "rBacon" (Blaauw et al., 2021).

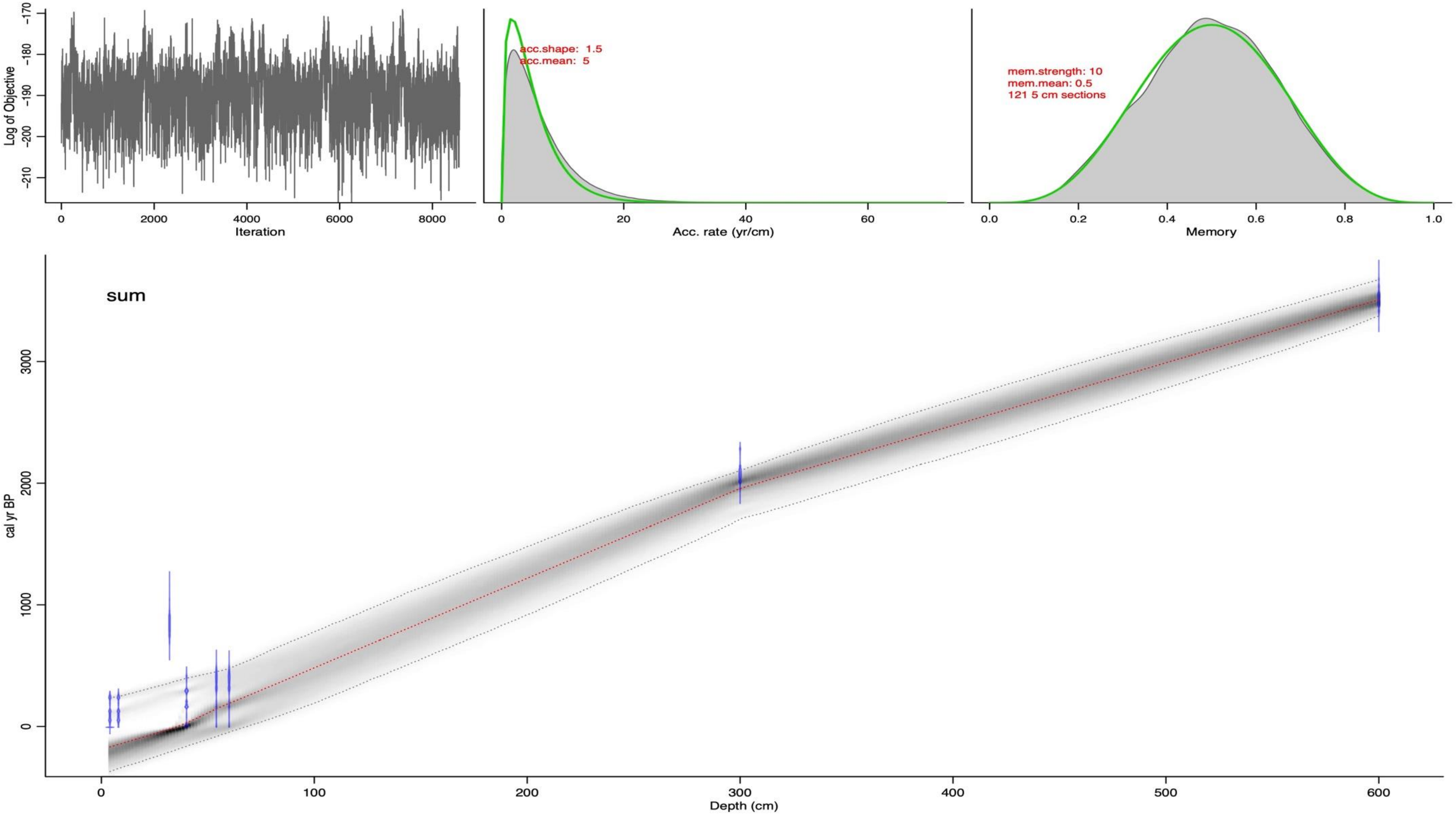


Figure 2.13. Age-depth model from Summit Lake, constructed using the R package "rBacon" (Blaauw et al., 2021)

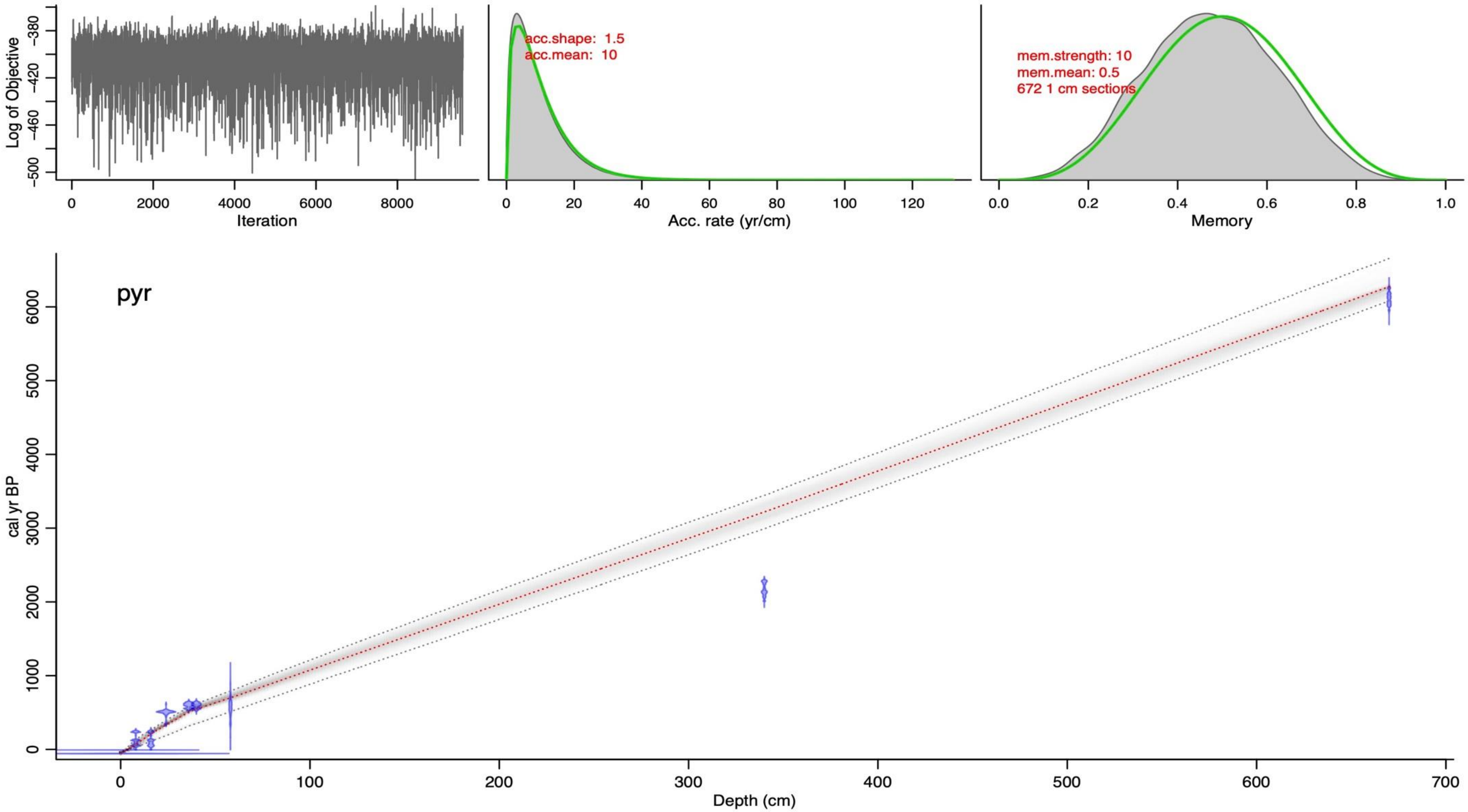


Figure 2.14. Age-depth model from Pyramid Lake constructed using the R package "rBacon" (Blaauw et al., 2021)

4. Tephrochronology

The Cascade Volcanic Arc is a prominent feature within the Pacific Northwest, ranging from northern California to British Columbia. Several catastrophic eruptions have occurred during the Holocene including the 7,700 BP eruption of Mt Mazama and the May 1980 eruption of Mt St Helens, both of which provide critical time markers within published palaeoenvironmental reconstructions (Minckley and Long, 2016; Long et al., 2014; Whitlock et al., 2008; Brunelle and Whitlock, 2003**;** Millspaugh et al., 2000; Zdanowicz et al., 1999; Sea and Whitlock, 1995; Cwynar, 1987; Barnosky, 1985; Lichti-Federovich, 1970. Consequently, much research has been conducted on the geochemical composition of these ash layers, for example, Mount St Helens has over 100 chemically described tephra layers from the past 40,000 years (Mullineaux, 1986). Geochemical correlation of such tephra layers to reference databases facilitate not only the dating of sedimentary records but to transfer ages across multiple sites/sequences by linking geochemically unique tephra layers (Fiebel, 1999; Lowe, 2011). Upon retrieval of core FC3 B1 from Forlorn Camp Lake, Gifford Pinchot National Forest, Washington, three separate and distinct layers of volcanic ash were observed. Sediment cores obtained from sites near Forlorn Camp (Chamber's Lake, Washington and Elk Pass, Washington (Zobel and Antos, 1991) report similar ash bands which correlate to 1980's eruption of Mt St Helens. Successful matching of the tephra found within FC3 B1 to reference data for Mt St Helens would provide a key time marker for the reconstruction of recent forest disturbances.

At Forlorn Camp Lake, 1 cm^3 tephra samples were taken from three distinct bands, each measuring between 3 and 5cm, observable within the stratigraphy. In addition, separate 1 cm^3 bulk sediment samples were taken above and below the visible tephra layers to provide radiocarbon dating constraints for all events. Samples were freeze-dried, weighed to 0.5g, and then ashed at 550ºC for 4 hours to remove any organic contamination (Turney, 1998; Jones et al., 2018). The ashed samples were then soaked overnight in 10% HCl to disaggregate the sediment and remove any carbonates. Samples were sieved through 80 and 25µm mesh. The 80-25µm fraction was density separated following the heavy liquid separation technique (Turney, 1998; Blockley et al., 2005). The 2.3 to 2.5g cm^{-3} fraction were mounted onto microscope slides and encased in epoxy resin (Stevenson et al., 2012; Lakeman et al., 2008; Davies et al., 2005). A polarised light microscope was used to confirm the presence of tephra shards. Where present, samples were polished using progressively finer grades of silica carbide paper to expose a cross section of the shards for geochemical analysis (Jones et al., 2018). Major and minor element composition of the tephra shards were measured at the Tephra Analytical Unit (TAE) at the University of Edinburgh using a wavelength dispersive spectrometer electron microprobe (WDS EMPA) following standard running protocol (Hayward, 2012). A second set of analyses on FC3 were conducted at The University of Alberta, Canada, as the first set of analyses produced inconclusive results. Raw tephra results were normalised to 100%, a method commonly employed within tephra research to eliminate the variability of post deposition hydration and to make signatures comparable between published assemblages (Jones et al., 2018; Lowe et al., 2017; Eastwood et al., 2009; Smith and Westgate, 1968).

5. Macrofossil charcoal

5.1. Sampling

In the laboratory, 0.5cm^3 samples were taken at contiguous 2 mm intervals at Little Monon Lake, Pyramid Lake, and Summit Lake, except for the uppermost 30 mm at Little Monon Lake, in which 0.25 cm^3 was used due to a lack of sediment. Several samples across three sites (27 samples from 433 depths) yielded insufficient sediment and were omitted from final analyses. Samples were taken using a calibrated syringe. Samples were soaked in 10% sodium hexametaphosphate for 7 days to deflocculate the sediment, prior to washing with a shower head tap attachment through a nest of sedimentology sieves at 500, 250, and 125 microns with each size class reserved to provide three sets of analyses for each depth (Schlacter and Horn, 2010; Froyd, 2006; Whitlock and Larsen, 2002; Patterson et al., 1987). A small sable paint brush was used to gently break up any remaining sediment. Prepared samples were washed onto a gridded petri dish and tallied under a 40x stereomicroscope to produce total counts for the following size classes - 125-250µm, 250-500µm, and >500µm. Identification of charcoal fragments were based on morphology, reflectance, and the nature of the fragment when crushed (Froyd, 2006; Whitlock and Larsen, 2002).

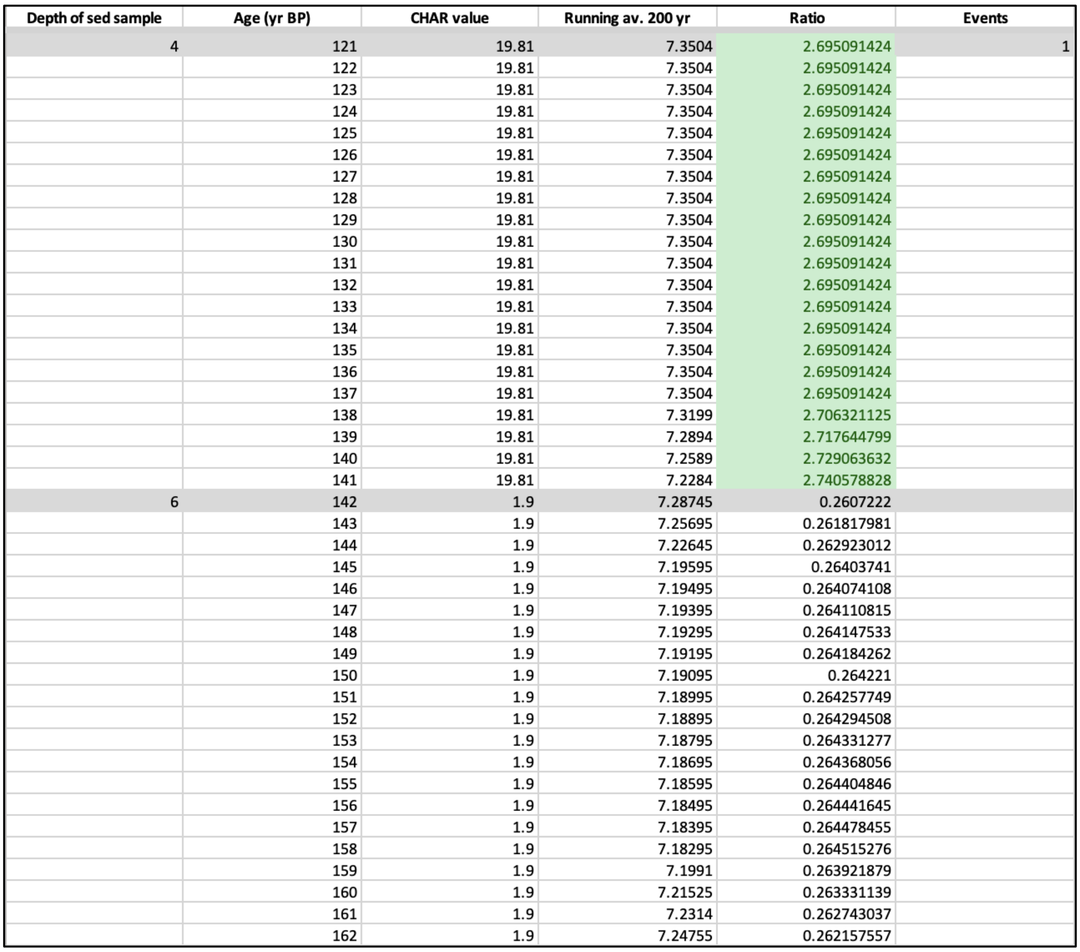
5.2. Creation of charcoal accumulation rates (CHAR)

Raw charcoal counts do not account for variation in sedimentation rates; therefore, each size class was combined with an age-depth model (constructed using the R package “rBacon” (Blaauw et al., 2021)) to produce charcoal accumulation rates (CHAR) using the R package “paleofire” (Blarquez et al., 2014). CHAR was calculated for each size class and presented in particles mm^{-1} yr^{-1}. Sample depths whereby a lack of sediment resulted in the absence of charcoal data were interpolated by the R package "paleofire" (Blarquez et al., 2014) to produce contiguous records. The input volume within the CHAR construction model was amended to account for a smaller sample volume throughout the top 30mm at Little Monon Lake, whereby a lack of sediment resulted in sampling volumes of 0.25cm^3.

5.3. The development of 'fire event' indices

It has been highlighted in the literature that the comparison of fire episodes between studies is problematic due to the variation in the resolution of different reconstructive methods i.e., tree rings provide annual resolution, whereby analysis of sediments typically analyse periods of time, dictated by the sampling methodology. The use of a contiguous sampling methodology and robust dating controls throughout facilitated the development of a novel method of identifying peak components within the charcoal record, associated with fire events. A spreadsheet was constructed with each row accounting for an individual year. Using age-depth models constructed in the R packages "OxCal" (Bronk Ramsay, 2009) and "rBacon" (Blaauw et al., 2021), each sampling depth was placed onto the corresponding age along with the CHAR value for that sample depth. CHAR values were then repeated to the next sampling point, for example, the 4mm sample at Little Monon Lake, which dates to 121 yr BP, has a CHAR value of 19.81 particles mm^{-1} yr^{-1}. The value of 19.81 was ascribed to every year until we reach the next sampling point, at 6mm which dates to 142 yr BP and, whereby the CHAR value was 1.9 particles mm^{-1} yr^{-1} (See Figure 2.15). This method of analyses generated a charcoal history with a simulated annual resolution, akin to those constructed by dendroecological methods (See Figure 2.15). The aim of this approach was to test whether this method could identify peaks in charcoal data as well as calculate return intervals comparable with those calculated from annual tree ring datasets from nearby sites (Minckley and Long, 2016) using data already used for fire reconstruction (CHAR values and an age depth model).

In order to identify fire events, we must first determine what is classed as a fire event. The most widely implemented method of identifying fire events is to determine periods of time within the charcoal records whereby the fire activity at any depth exceeds that of the background fire behaviour. The creation of event indices within the CHAR series involved a three-step decomposition of the dataset (Higuera et al., 2010). Firstly, to construct the background component, or *backgroundCHAR*, three variations of running mean were constructed for the series using moving windows of 100, 150, and 200 years i.e. for the 100-yr window, an average for the values 50 years above and 50 years below a given value, excluding that given value were used. CHAR values at each depth were then divided by this background component to create a ratio, known as *peakCHAR* (Hawthorne and Mitchell., 2016; Finsinger, 2014; Leys et al., 2013; Higuera et al., 2005; Whitlock and Larsen, 2002; Long et al., 1998).



Depth of sed sample	Age (yr BP)	CHAR value	Running av. 200 yr	Ratio	Events
4	121	19.81	7.3504	2.695091424	1
	122	19.81	7.3504	2.695091424	
	123	19.81	7.3504	2.695091424	
	124	19.81	7.3504	2.695091424	
	125	19.81	7.3504	2.695091424	
	126	19.81	7.3504	2.695091424	
	127	19.81	7.3504	2.695091424	
	128	19.81	7.3504	2.695091424	
	129	19.81	7.3504	2.695091424	
	130	19.81	7.3504	2.695091424	
	131	19.81	7.3504	2.695091424	
	132	19.81	7.3504	2.695091424	
	133	19.81	7.3504	2.695091424	
	134	19.81	7.3504	2.695091424	
	135	19.81	7.3504	2.695091424	
	136	19.81	7.3504	2.695091424	
	137	19.81	7.3504	2.695091424	
	138	19.81	7.3199	2.706321125	
	139	19.81	7.2894	2.717644799	
	140	19.81	7.2589	2.729063632	
	141	19.81	7.2284	2.740578828	
6	142	1.9	7.28745	0.2607222	
	143	1.9	7.25695	0.261817981	
	144	1.9	7.22645	0.262923012	
	145	1.9	7.19595	0.26403741	
	146	1.9	7.19495	0.264074108	
	147	1.9	7.19395	0.264110815	
	148	1.9	7.19295	0.264147533	
	149	1.9	7.19195	0.264184262	
	150	1.9	7.19095	0.264221	
	151	1.9	7.18995	0.264257749	
	152	1.9	7.18895	0.264294508	
	153	1.9	7.18795	0.264331277	
	154	1.9	7.18695	0.264368056	
	155	1.9	7.18595	0.264404846	
	156	1.9	7.18495	0.264441645	
	157	1.9	7.18395	0.264478455	
	158	1.9	7.18295	0.264515276	
	159	1.9	7.1991	0.263921879	
	160	1.9	7.21525	0.263331139	
	161	1.9	7.2314	0.262743037	
	162	1.9	7.24755	0.262157557	

Figure 2.15. Sample output of the novel method of identifying event indices within CHAR data. Samples were placed onto their corresponding ages along with their associated CHAR values. These values were then extrapolated to the sample below to provide simulation annual resolution of CHAR data. Running means were then constructed from these continual records to produce the background component. Individual CHAR values were then divided by the background component to produce a ratio. Ratios greater than or equal to 1 were classed as fire events.

There is no agreed upon threshold for indices which represent fire events. In a simplistic manner, indices greater than or equal to 1 indicate periods whereby the signal at a given depth is greater than or equal to the background component and one could argue that this alone could be considered a fire event. Methods outlined in Long et al., 1998, ascribed a range of values to determine which threshold best suited the data with regards to the 'real life' behaviour and expected fire patterns of the vegetation type found at a given site. Therefore, an additional test, using a range of values, 1, 1.05, 1.12, and 1.2, as thresholds for fire events, were compared, using the 100-yr window as the source of backgroundCHAR. Throughout all CHAR analysis, periods whereby a lack of sediment led to zero charcoal values, a linear interpolation was used to fill in missing data and prevent any division by zero within the dataset.

5.4. Fire events and return fire intervals

Due to the variation in age and sedimentation rate among sites, the CHAR records for Little Monon Lake, Pyramid Lake, and Summit Lake were split into 100-year periods before present (i.e., 0-99, 100-199 yr BP, with BP fixed at 1950 AD) as identified using age depth models. This additional set of analysis allowed the comparison of the total fire events between sites. Accurate mean return intervals are typically limited to dendroecological methodologies with annual resolution. To test whether continuous, high-resolution records are capable of producing return fire intervals comparative to those derived from tree ring data, mean fire return intervals were calculated by measuring the periodicity of fire events as identified by the time between events identified by *peakCHAR* and then temporally compared to events identified within published reconstructions using age-depth models.

6. DNA

6.1. DNA extractions and qPCR primer design

Taxonomically confirmed positive controls are vital in all aspects of DNA research, used for sequencing, primer design, and for validating successful amplification within PCR protocols. Ten taxonomically identified individuals of mountain pine beetle (*Dendroctonus ponderosae*), Douglas-fir beetle (*Dendroctonus pseudotsugae*), and western spruce budworm (*Choristoneura occidentalis*) were obtained by Beth Willhite, Regional Entomologist for USDA FS Region 6, Oregon. DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, UK) following manufacturer's instructions. Tissues samples were preserved in molecular grade ethanol upon collection to reduce enzymatic degradation until time of DNA extraction (Srinivasan et al., 2002). Several published examples of successfully recovered macrofossil beetle remains from lake sediments (Brunelle et al., 2008; Morris et al., 2015) suggest the most commonly preserved body parts consist of head and elytra, likely due to a high content of chitin, a robust biopolymer, which forms the basis of the insect exoskeleton. Chitinous remains are, therefore, becoming increasing used as a source of ancient DNA (aDNA) (Campos and Gilbert, 2011; Stankiewicz et al., 1997; Miller et al., 1993). The extraction of DNA from modern specimens for the use of positive PCR controls and to test the efficacy of DNA protocols would also facilitate the analysis of DNA yields from different tissue types, as these would be the source material of any fossil DNA.

The morphology of the target species chosen for DNA analyses within this study are highly different. The bark beetles, *D. ponderosae* and *D. pseudotsugae*, are characterised by hard exoskeletons, while the budworms of *Choristoneura* spp., are formed primarily of soft body parts with only a few chitinous components. To test how various types of remains and digestion times impact the potential for DNA release, which ultimately determines the likelihood of recovering target species DNA within environmental samples, a series of whole beetles, head and elytra, and leg tissue samples from *D. ponderosae* and *D. pseudotsugae* and whole pupae of *C. occidentalis* were extracted using a QIAGEN Blood and Tissue Kit to use as positive controls for PCR reactions. Samples were extracted after 4 hours digestion and 20 hours digestion in proteinase K to assess how longer incubation periods affected DNA yields from chitinous remains. DNA concentrations were measured using a Qubit dsDNA HS Assay Kit with a Qubit 3.0 Fluorometer (Invitrogen, Inc.). High resolution melting (HRM) analysis from the above extractions were used to provide reference melting temperature (Tm) of target species (Tm ±0.1^{o}C) to confirm any successful amplifications within sedaDNA samples.

Modern tissue samples of *D. ponderosae*, *D. pseudotsugae* and *C. occidentalis* were sequenced at IBERS (Aberystwyth University). The primer pairs used were Pat and Jerry and Rust and Marty (See Table 2.4 for primer pairs used in sequencing) which amplify ~650bp fragments of the COI mtDNA gene (Simon et al., 1994; Sperling et al., 1994) and the Cole01F and Cole01R (Taberlet et al., 2018) primer set which amplify a ~115bp fragment of the 16s mtDNA. These various primer sets were used to help identify regions whereby *D. ponderosae* and *D. pseudotsugae* differ to aid in the design of species-specific protocols. PCR conditions for all sequencing reactions were as follows: 95^{o}C for 5:00, followed by 30 cycles at 95^{o}C for 00:10 and 54.5^{o}C for 00:30, with a final elongation stage at 72^{o}C for 5:00. All amplified PCR products were checked for expected amplicon size using 2% gel electrophoresis. Sequences were aligned with BioEdit v. 5.0.9 using Clustal W.

6.2. Dendroctonus *protocol*

Sanger sequencing revealed no regions within the 16s mtDNA obtained using Cole01 primer sets (Taberlet et al., 2018) with sufficient difference to design species-specific primers. Primer sets DpondF and DpondR and DpseudoF and DpseudoR (see Table 2.4) were designed to amplify 84 bp fragments of the cytochrome c oxidase subunit I (hereafter COI) mtDNA gene of *D. ponderosae* and *D. pseudotsugae*, respectively. All primer sets were designed using Oligo Architect™ (Sigma-Aldrich Co.) and checked for cross-amplification using NCBI Primer-BLAST (Ye et al., 2012). While the product of DpondF and DpondR has a similarity of 98% with *D. simplex* (Eastern larch beetle), and the product of DpseudoF and DpseudoR has a similarity of 98% with *D. simplex* (Eastern larch beetle) and 90.48% similarity to *D. rhizophagus* (Mexican pine beetle), the geographical distribution of *D. ponderosae* and *D. pseudotsugae* in relation to the other amplified species do not overlap, therefore, the potential for cross-contamination is extremely unlikely.

6.3. Choristoneura *spp, protocol*

Choristoneura spp. primers were designed using sequences from modern *Choristoneura occidentalis* (Western spruce budworm) pupae obtained through Sanger sequencing using the Cole01F and Cole01R primer set (Taberlet et al., 2018) which amplifies a ~115 bp fragment of the 16s mtDNA gene. ChoristF and ChoristR are a genus-specific primer set designed to amplify an 82 bp fragment of the 16s mtDNA gene in all 49 described *Choristoneura* species. This primer set was designed at genus-level as the distribution of *Choristoneura* species in the Pacific Northwest is largely unknown, yet all are defoliators and considered pests. Therefore, information about the presence of any species within this genus would be insightful.

Primer set	Target species	Target gene	Sequence (5’ to 3’)	Product length
DpondF	*D. ponderosae*	COI	GCA GTT CCA ACC GGA ATT AAA A	84 bp
DpondR	“		TCA AAG GGA GGA GGG AGT TAA T	
DpseudoF	*D. pseudotsugae*	COI	GCA GTT CCT ACC GGA ATT AAA A	84 bp
DpseudoR	“		CCA AAG AGA AGA AGG AGT TAG A	
ChoristF	*Choristoneura*	16s	TGT GAT GAG CTC AAA CTA CAA ACC	82 bp
ChoristR	“		AAA GAA ACT TTC GGA TGT TTA GGA	
Jerry[a]	Univ.	COI	CAA CAT TTA TTT TGA TTT TTT GG	632 bp
Pat[a]	“		TCC AAT GCA CTA ATC TGC CAT ATT A	
Rust[a]	“	COI	ACT GTG AAT ATA TGA TGG GCT CA	650 bp
Marty[a]	“		TAC AAT TTA TCG CCT AAA CTT CAG CC	
Cole01F[b]	Coleoptera	16s	TGC WAA GGT AGC ATA ATM ATT AG	115 bp
Cole01R[b]	“		TCT ATA GGG TCT TCT CGT C	
matKF[c]	Plants	matK	TCG GCA ATG TTA TTT CC	97 bp
matKR[c]	“		CCT CAG AAA ATA ACC TGG	

Table 2.4. Primer sets for mountain pine beetle (*Dendroctonus ponderosae)*, Douglas-fir beetle (*D. pseudotsugae)* and *Choristoneura* spp. matK, and those used in NGS. [a]Simon et al., 1994, [b]Taberlet et al., 2018, [c]Kim et al., 2018.

6.4. qPCR optimisation

All primers sets were designed to amplify short fragments to accommodate for DNA degradation in older samples, allowing the same qPCR protocol to be applied in both modern biodiversity assessments and for historical reconstructions. Primers were assessed using positive control tissue from the initial sequencing reactions. Temperature gradient tests for all primer sets were performed between 55-65°C to provide optimum annealing temperatures and increase specificity, as higher annealing temperatures reduce the risk of non-specific amplification. Limits of detection (LOD) were identified using a ten-fold dilution series with tissue DNA from each species. Dilutions ranged from 1:1 to 1:1,000,000, extended to 1:10,000,000 in the case of DpondF and DpondR assay. All qPCR runs were carried out in 10µl reactions with 1µl of template DNA. For all PCRs and qPCRs, three PCR replicates were used throughout unless otherwise stated. The amplification master mix contained 5µl SYBR Green, 1µl of each primer and 2µl of molecular grade H_2O. Optimised qPCR protocols are as follows:

DpondF and DpondR (Target: *D. ponderosae*)*:* 95°C for 5:00, followed by 45 cycles at 95°C for 00:10 and 61.5°C for 00:30. High resolution melt (HRM) curve analysis ran from 60 to 90°C in 0.1°C increments.

DpseudoF and DpseudoR (Target: *D. pseudotsugae*)*:* 95°C for 5:00, followed by 45 cycles at 95°C for 00:10 and 61.5°C for 00:30. High resolution melt (HRM) curve analysis ran from 60 to 90°C in 0.1°C increments.

ChoristF and ChoristR (Target: *Choristoneura* spp.)*:* 95°C for 5:00, followed by 45 cycles at 95°C for 00:10 and 61.5°C for 00:30. High resolution melt (HRM) curve analysis ran from 60 to 90°C in 0.1°C increments.

6.5. sedaDNA extractions

All sediments were subsampled at 2mm resolution under sterile conditions consistent with established sedaDNA extraction methodologies (Pedersen et al., 2015; Parducci et al., 2017; Taberlet et al., 2018). At each depth, three replicates, weighing ~0.25g, were extracted within a UV treated hood using a Qiagen Powerlyzer PowerSoil Kit (Qiagen, UK) according to manufacturer's instructions, except for a final elution volume of 60 μl used to increase DNA yield. DNA concentrations from sedaDNA extractions were quantified using a Qubit dsDNA HS Assay Kit with a Qubit 3.0 Fluorometer (Invitrogen, Inc.). All qPCRs were run in triplicate, unless otherwise stated, and a positive result was classed as at least one positive from three replicates with a melt rate above 200 -d(RFU)/dt. Positive controls for each species were included in each PCR run along with extraction blanks and PCR blanks. Any samples containing potential amplifications using the qPCR protocols were re-run as conventional PCRs using the same protocols and sent for Sanger sequencing to confirm species.

6.6. Confirmation of sedaDNA

As the historic presence of these *D. ponderosae, D. pseudotsugae* and *C. occidentalis* is largely unknown within this region, beyond the time-period associated with the ADS survey, these species could not be used to check the presence and preservation of useable DNA within the sedimentary cores. Palynological reconstructions (Long et al., 1998; Long et al., 2002; Briles et al., 2005; Walsh et al., 2010; Minckley and Long, 2016) from northwest Oregon show this region has been continuously vegetated for the timescale of this study. Therefore, a maturase K assay (Kim et al., 2018), which amplify a 97 bp fragment of the plant plastidial gene, was used to assess the levels of DNA preservation within the sediment i.e., if vegetation was not detected, this would suggest issues with DNA preservation. *Pinus lambertiana* was used as a positive control within the matK assay with DNA extracted from a positively identified seed cone obtained from Little Monon Lake using a QIAGEN DNeasy Plant Mini Kit (QIAGEN, UK) following manufacturer's instructions. qPCR was conducted in 10µl reactions containing 0.25µl each of forward and reverse primer, 5µl SYBR Green and 4.5µl sedaDNA. The qPCR conditions consisted of an initial denaturation phase of 5 mins at 95°C, followed by 40 cycles of 10 seconds at 95°C and 30 seconds at 55°C. High resolution melt curve analysis ran between 60 and 90°C in 0.1°C increments. In addition to the matK assay, a second control of DNA preservation was used. As Coleoptera are commonly preserved in lake sediments (Elias, 2010; Smol et al., 2001; Coope, 1977), the general Cole01 primer pair (Taberlet et al., 2018) used in sequencing were also used to confirm the presence of Coleopteran DNA within sedaDNA samples.

6.7. Testing for potential PCR inhibition

Humic acids are known to be very potent PCR inhibitors which could present false negatives within sedaDNA results (Sidstedt et al., 2015). To assess the level of PCR inhibition within sedaDNA samples, varying concentrations of sedaDNA from the uppermost samples at Little Monon Lake, Pyramid Lake, and Summit Lake were added to various volumes of molecular grade H_2O, and 1µl of *D. ponderosae*. Final volumes measured 5.5µl. All samples were amplified using the Dpond protocol. The hypothesis for this experiment was that samples with higher concentrations of sedaDNA would likely introduce a greater abundance of PCR-inhibiting humic acids into the reaction, potentially delaying or preventing the amplification of pure *D. ponderosae* DNA i.e., samples with no difference in Ct value between no sedaDNA and 4.5 µl sedaDNA would suggest no PCR inhibition.

CHAPTER THREE

RESULTS

1. DNA

1.1. Tissue extractions of target species

1.1.1. Dendroctonus

To test how target species morphology and the source tissue type affects DNA recovery, the DNA extractions from *D. ponderosae* and *D. pseudotsugae* were performed on various combinations of body parts. During this test, DNA extracted from intact whole adult specimens of *D. ponderosae* and *D. pseudotsugae* generated the highest overall DNA yields, while extractions of leg tissue only consistently had the lowest yields (See Table 3.1.). In addition, these combinations of body parts were subjected to varying periods of digestion in proteinase K, ranging from four hours to twenty hours, to assess the degree to which organisms characterised by high levels of chitin may require additional levels of digestion to release DNA. Tissues subjected to a twenty-hour digestion in proteinase K did not always lead to higher DNA yields over four-hour incubation times. While longer digestion periods resulted in almost double the DNA yield in whole beetles in both *D. ponderosae* and *D. pseudotsugae*, increasing from 48.0 to 100.2 ng/µl and 51.6 to 91.5 ng/µl, respectively, longer digestion periods led to lower yields in combined head and elytra and leg tissues in both species. While whole beetles yielded higher quantities of DNA, combined head and elytra tissues are typically the body parts recovered from palaeoenvironmental samples (Morris et al., 2015; Brunelle et al. 2008), and since sufficient levels of DNA were obtained from these tissue types, these extractions were used at all stages of primer design, protocol optimisation, sequencing, and as PCR and qPCR positive controls.

1.1.2. Choristoneura *spp.*

As only taxonomically confirmed larvae of *Choristoneura occidentalis* was obtained from colleagues in the USDA Forest Service, a 'body part' test was not conducted, instead whole larvae were used for DNA extractions, sequencing, and as PCR and qPCR controls. Interestingly, despite a short digestion time of 4 hours, DNA yields were higher than those obtained from whole adult specimens in both beetle species (based on 10 individuals per 'body part' type) highlighting the variation in DNA recovery from 'hard' bodied species versus those comprised of mostly soft tissues. See Discussion for further interpretation of how these results may influence the recovery and detectability of DNA between these two different groups of forests insects.

Species	Body part	Digestion period (hrs)	DNA yield (ng/µl)	σ
D. ponderosae				
	Whole	4	48.0	2.13
	Head and elytra	4	46.2	1.96
	Leg	4	6.1	0.88
	Whole	20	100.2	4.79
	Head and elytra	20	35.5	2.70
	Leg	20	4.2	0.48
D. pseudotsugae				
	Whole	4	51.6	3.67
	Head and elytra	4	19.5	1.08
	Leg	4	0.4	0.10
	Whole	20	91.5	3.19
	Head and elytra	20	3.6	0.56
	Leg	20	0.3	0.09
C. occidentalis				
	Whole	4	110.1	7.76

Table 3.1. DNA yields from whole beetles, head and elytra tissue, and leg tissue samples for *D. ponderosae* and *D. pseudotsugae* and for *Choristoneura occidentalis* pupae. 10 individuals were used in each scenario.

1.2. Quantification of sedaDNA extractions and background DNA checks

Quantification of DNA yields using Qubit 3.0 assays indicated DNA was present in nearly all sedaDNA samples and exhibit declining levels of total DNA concentration through time, typical of expected levels of DNA degradation with age (See Figures 3.1 to 3.3). Sufficient levels of DNA preservation were confirmed as the matK (Kim et al., 2018) assay evidenced vegetation at all depths at all sites, while general Coleoptera primers (Cole01 protocol, outlined in Taberlet et al., 2018), also indicated the presence of Coleopteran DNA within the sedimentary cores.

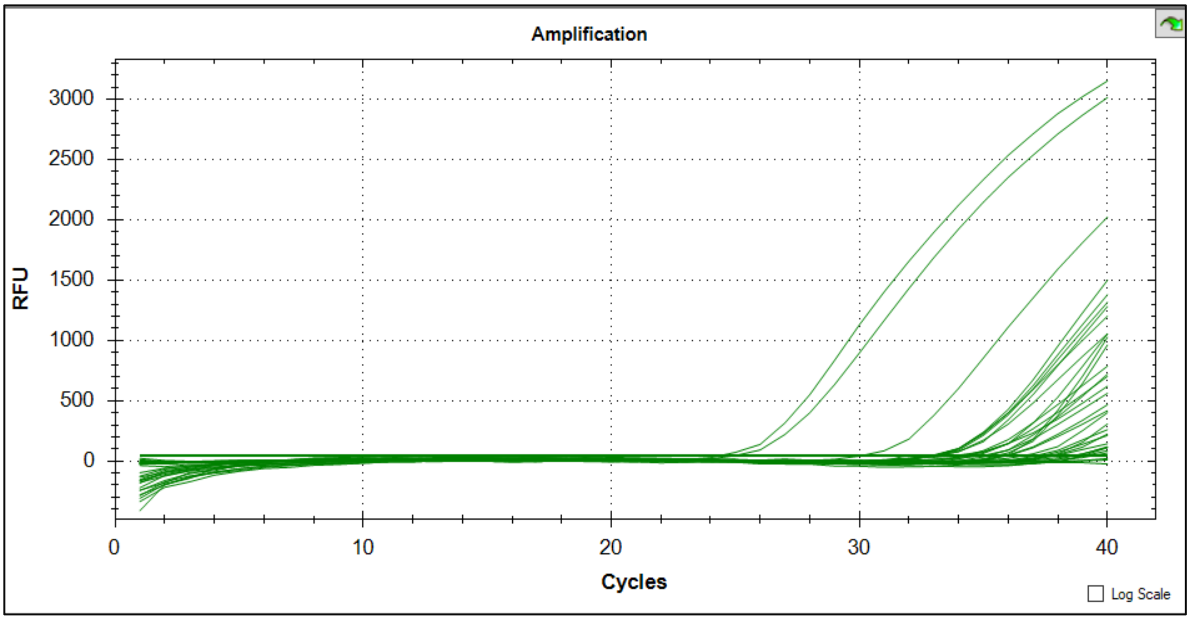
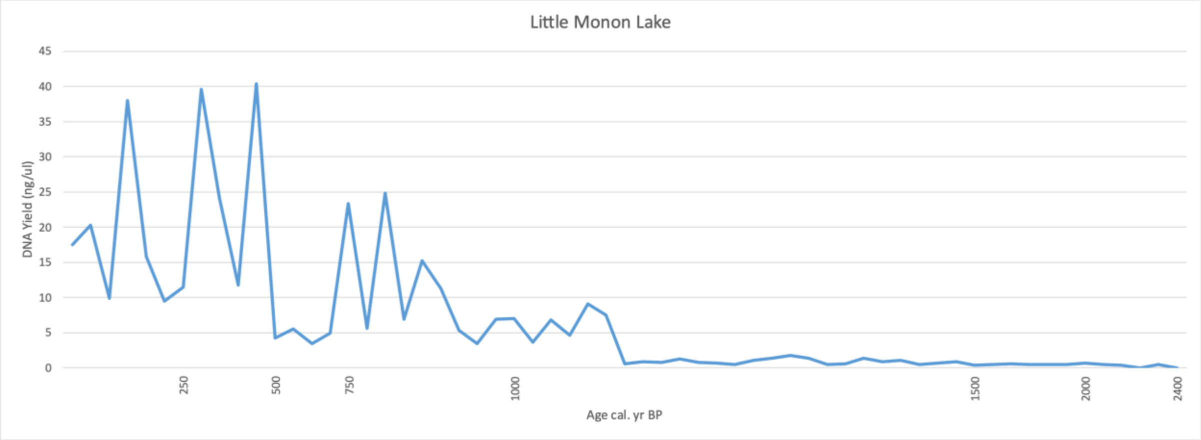


Figure 3.1. Output of MatK qPCR assay indicating presence of DNA throughout the sediment cores obtained at Little Monon Lake, Pyramid Lake, and Summit Lake.

1.3. Primer specificity, efficiency, and limits of detection

Primer sets DpondF and DpondR and DpseudoF and DpseudoR are designed to be species-specific and were tested in vitro against positive controls for *D. ponderosae* and *D. pseudotsugae* obtained from the Mt Hood National Forest, Oregon, U.S.A. These individuals were obtained from locations nearby to the sites chosen within this thesis to ensure any sequences recovered from sedaDNA would be comparable to modern specimens from local populations. Dpond, Dpseudo, and Chorist qPCR protocols were run with tissue of both target and non-target species to highlight specificity (Table 3.2). Primer pairs Dpond and Dpseudo demonstrated high levels of species-specificity amplifying only the target species and not the closely related non-target species. ChoristF and ChoristR were designed to be genus specific and were validated with *C. occidentalis* as a positive control and *D. ponderosae* as a negative control. Amplicons were Sanger sequenced (Men et al., 2008) and the resulting sequences were checked against published reference material for target species using NCBI-BLAST. All amplified sequences matched expected corresponding reference sequences within the NCBI database.



Figures 3.2. sedaDNA yields from Little Monon Lake with corresponding age inferred from age-depth model

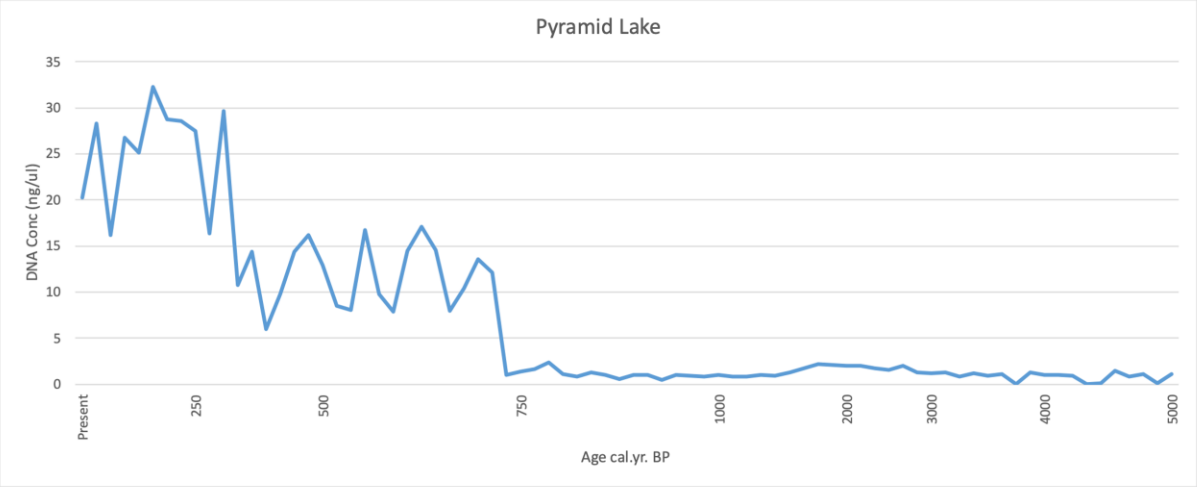
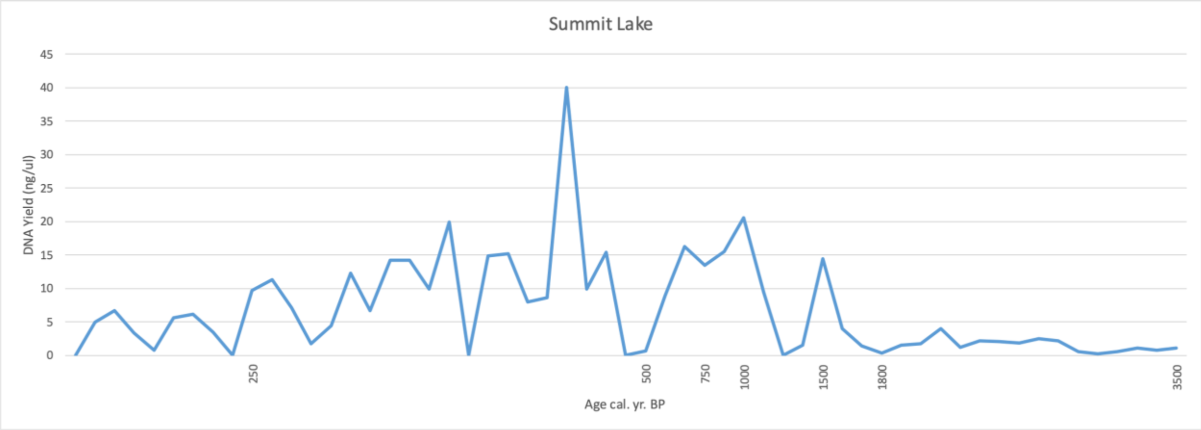


Figure 3.3. sedaDNA yields from Pyramid Lake with corresponding age inferred from age-depth model



Figures 3.4. sedaDNA yields from Summit Lake with corresponding age inferred from age-depth model

Primer set	**Samples**	**Ct value**
DpondF and DpondR		
	D. ponderosae #1	17.3
	D. ponderosae #2	16.2
	D. ponderosae #3	16.4
	D. pseudotsugae #1	No amp.
	D. pseudotsugae #2	No amp.
	D. pseudotsugae #3	No amp.
DpseudoF and DpseudoR		
	D. ponderosae #1	No amp.
	D. ponderosae #2	No amp.
	D. ponderosae #3	No amp.
	D. pseudotsugae #1	17.9
	D. pseudotsugae #2	17.7
	D. pseudotsugae #3	17.7
ChoristF and ChoristR		
	C. occidentalis #1	16.7
	C. occidentalis #2	16.6
	C. occidentalis #3	16.6
	D. ponderosae #1	No amp.
	D. ponderosae #2	No amp.
	D. ponderosae #3	No amp.

Table 3.2. qPCR results summary showing the species-specificity of designed primer sets Dpond, Dpseudo, and Chorist.

Temperature gradient tests were run between 55 and 65°C revealed primer efficiencies were optimal at both 60 °C and 61.5 °C, and so annealing temperatures for all primer sets were optimised at 61.5°C to provide increased specificity. High resolution melting (HRM) analysis of *D. ponderosae*, *D. pseudotsugae*, and *C. occidentalis* tissue samples using the newly designed primer sets yielded consistent melting temperatures of 71.7°C, 72.8°C, and 70.7°C, respectively. These melting temperatures were used to indicate presence of target species in any positive sedaDNA samples. A ten-fold dilution series of positive controls of *D. ponderosae*, *D. pseudotsugae*, and *C. occidentalis* DNA obtained from taxonomically verified modern samples show that primer efficiencies for each primer set were 104.0%, 89.3%, and 91.1%, respectively, with limits of detection of 0.00000462 ng/µl, 0.000195 ng/µl, and 0.00011 ng/µl, respectively.

1.4. sedaDNA results

1.4.1. Pyramid Lake

1.4.1.1. Dpseudo protocol

Two positive sets of amplifications of *D. pseudotsugae* were identified using the Dpseudo protocol (target: *D. pseudotsugae*) at Pyramid Lake. The first at 2mm (in 2/3 replicates, Tm: 72.6°C) and the second at 22mm (in 1/3 replicates, Tm: 72.0°C). The Tm of these amplifications correspond to the Tm of *D. pseudotsugae* (Figure 3.4). Age depth models indicate these depths correspond to 1955 - 2004 AD and 543 - 473 cal. yr. BP, respectively. The uppermost sample, 2mm, shows a high level of amplification, with positives occurring in 2 out of 3 replicates. This sample coincides with the period associated with ADS data, during which there was a known outbreak of *D. pseudotsugae* in 1992 within the immediate vicinity of Pyramid Lake.

1.4.1.2. Dpond protocol

Six positive sets of amplifications were identified using the Dpond protocol (target: *D. ponderosae*) at Pyramid Lake. These amplifications occurred at 64mm (in 2/6 replicates, Tm: 74.0°C), 66mm (in 1/6 replicates, Tm: 74.1°C), 70mm (in 1/6 replicates, Tm: 74.1°C), 72mm (in 1/6 replicates, Tm: 73.5°C), 74mm (in 3/6 replicates, Tm: 73.9°C), 80mm (in 2/6 replicates, Tm: 73.9°C) (Figure 3.5). Age depth models indicate these depths correspond to a period between 752 cal. yr. BP (64mm) and 896 cal. yr. BP (80mm). The Tm of the replicates was consistent in all cases, but different from that from modern reference material from the target species.

1.4.1.3. Chorist protocol

No positive amplifications of *Choristoneura* spp. occurred at any depth at Pyramid Lake using the Chorist protocol. All positive controls amplified in all qPCR reactions and there were no amplifications in the negative controls.

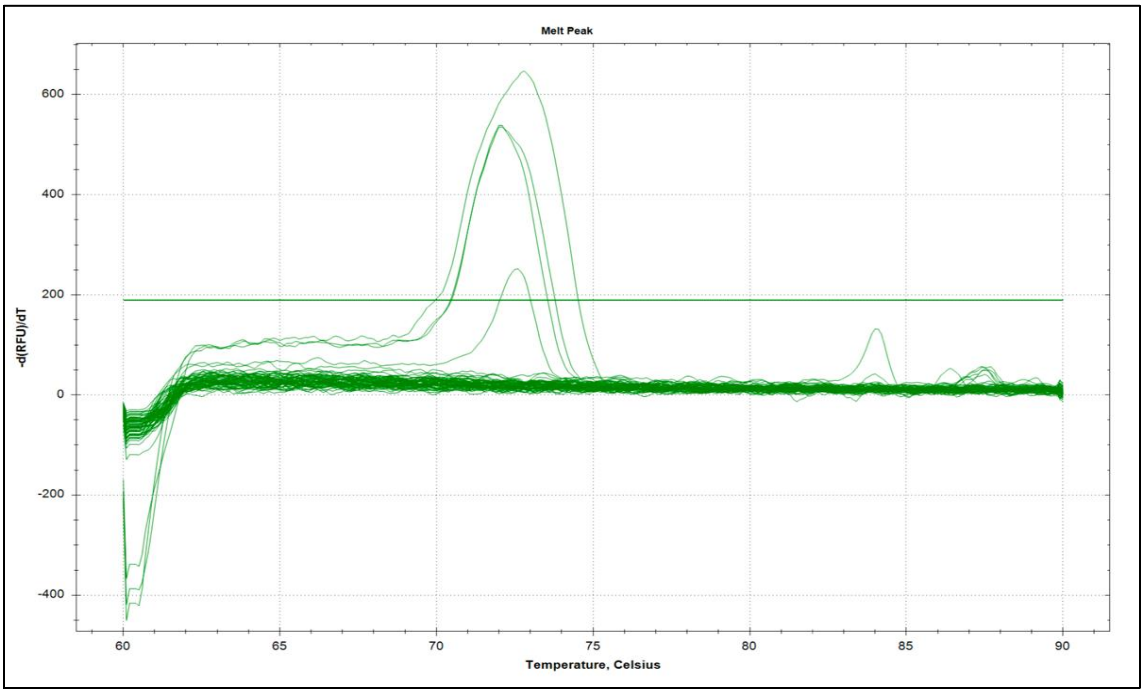


Figure 3.5. HRM analyses of Dpseudo protocol at Pyramid Lake indicating three positive amplifications matching Tm of the target species (*D. pseudotsugae*)

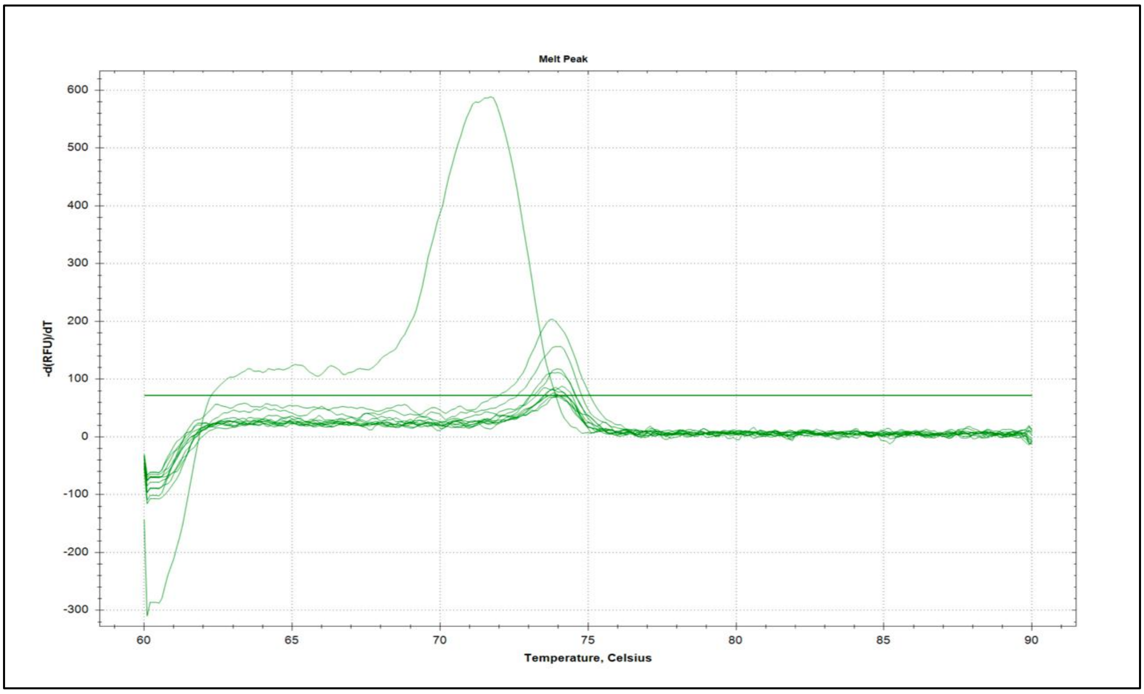


Figure 3.6. HRM analyses of Dpond protocol at Pyramid Lake indicating the multiple amplifications of an unknown target

1.4.2 Little Monon Lake

1.4.2.1. All protocols

The use of the Dpond, Dpseudo, and Chorist qPCR protocols resulted in no positive amplifications of *D. ponderosae, D. pseudotsugae* or *C. occidentalis* at any sample depth at Little Monon Lake. All qPCR positive controls amplified successfully and there was no evidence of contamination in any negative controls. The surface sample predates the period covered by the ADS survey dataset, as such, there is no information on which, if any, species were expected at Little Monon Lake.

1.4.3. Summit Lake

1.4.3.1. Chorist protocol

One positive amplification from three replicates was identified using the Chorist qPCR protocol in the uppermost (2mm) sample at Summit Lake. While no radiocarbon date exists for the uppermost sample, the sample directly below dates to 1810-1925 AD, which suggests the uppermost sample could originate from the early-to-mid 20th Century. High resolution melting (HRM) analyses indicated that the Tm of the positive amplification (72.4°C) was different from the positive control of *Choristoneura occidentalis*, however, several other *Choristoneura* species are known to inhabit the Mt Hood National Forest, including sugar pine tortrix (*C. lambertiana*) and the Obliquebanded leafroller (*C. rosaceana*) (Figure 3.4). Samples which showed potential amplifications using the qPCR protocols were re-run as conventional PCR with the Chorist protocol. This PCR produced four positive amplifications that matched the expected product length of the *Choristoneura* spp. The resulting sequences were not clear enough to distinguish the species, therefore, the presence of the target species cannot be confirmed. Several repetitions to reproduce the positive amplification were attempted, however, no further positive amplifications occurred.

1.4.3.2. Dpond and Dpseudo protocols

Neither *D. ponderosae* or *D.* pseudotsugae were detected at any depth using the Dpond and Dpseudo protocols.

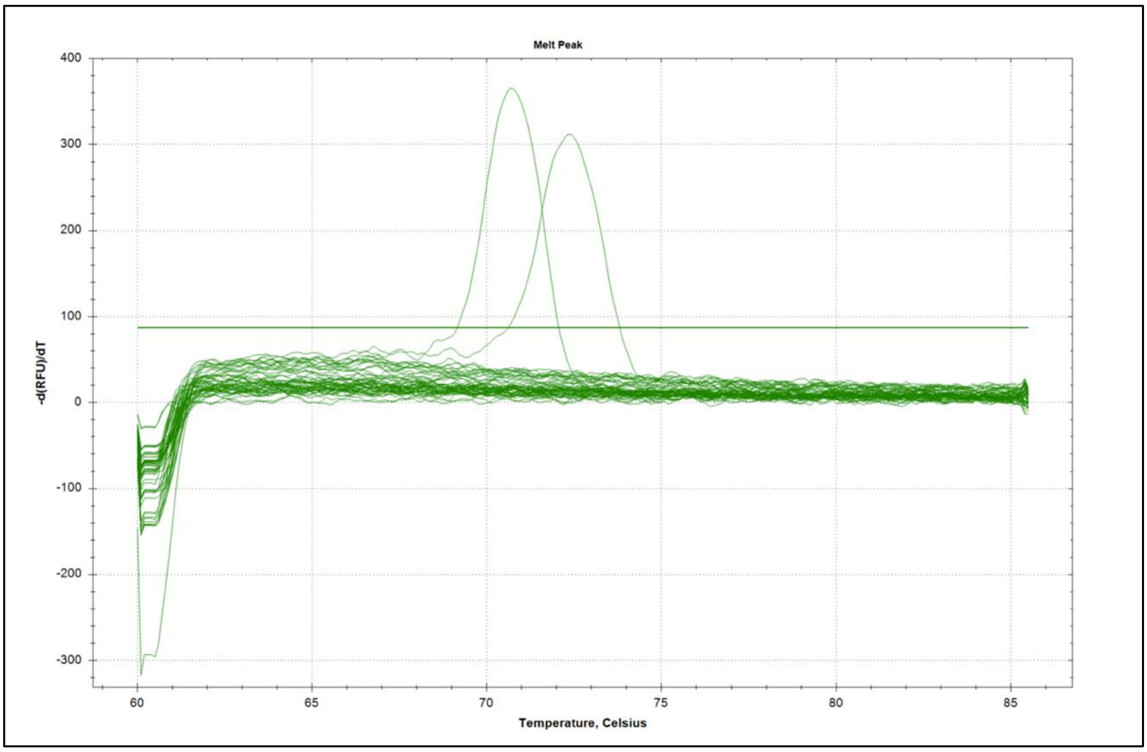


Figure 3.7. HRM analyses of Dpseudo protocol at Summit Lake indicating the amplification of an unknown target

1.5. PCR inhibition trials

To assess the degree to which PCR inhibition associated with humic acid content may have contributed to the lack of positive amplifications outlined above, a dilution series containing varying levels of sedaDNA with 1µl of *D. ponderosae* tissue DNA were run with the Dpond qPCR protocol using the uppermost sample at each site (Table 3.3). The hypothesis was that samples containing higher concentrations of sedaDNA would introduce more potential PCR inhibitors into the reaction and delay the amplification of pure *D. ponderosae* DNA.

At Pyramid Lake, the sample containing no sedaDNA + 1µl DNA (Sample 6) amplified three cycles earlier than the sample containing 3.5µl sedaDNA + 1µl DNA (Sample 2). This suggests the potential for significant levels of PCR inhibition at the surface sample at Pyramid Lake. At Summit Lake, the sample containing no sedaDNA + 1µl DNA (Sample 6) amplified at the same cycle as the sample containing 3.5µl sedaDNA + 1µl DNA (Sample 2) indicating there is no PCR inhibition within the surface sediment at Summit Lake. At Little Monon Lake, the sample containing no sedaDNA + 1µl DNA (Sample 6) amplified 1.3 cycles earlier than the sample containing 3.5µl sedaDNA + 1µl DNA (Sample 2). This suggests there could be moderate levels of PCR inhibition at Little Monon Lake.

Site	Sample #	sedaDNA:DNA Mixture	Ct value
Pyramid Lake	1	4.5µl sedaDNA - no DNA	0
	2	3.5µl sedaDNA - 1µl DNA	19.4
	3	2.5µl sedaDNA - 1µl DNA + 1µl H20	19.0
	4	1.5µl sedaDNA - 1µl DNA + 2µl H20	19.5
	5	0.5µl sedaDNA - 1µl DNA + 3µl H20	17.4
	6	no sedaDNA – 1µl DNA + 3.5 H20	16.5
Summit Lake	1	4.5µl sedaDNA - no DNA	0
	2	3.5µl sedaDNA - 1µl DNA	15.0
	3	2.5µl sedaDNA - 1µl DNA + 1µl H20	14.9
	4	1.5µl sedaDNA - 1µl DNA + 2µl H20	15.2
	5	0.5µl sedaDNA - 1µl DNA + 3µl H20	15.1
	6	no sedaDNA – 1µl DNA + 3.5 H20	15.0
Little Monon Lake	1	4.5µl sedaDNA - no DNA	0
	2	3.5µl sedaDNA - 1µl DNA	19.6
	3	2.5µl sedaDNA - 1µl DNA + 1µl H20	18.8
	4	1.5µl sedaDNA - 1µl DNA + 2µl H20	18.8
	5	0.5µl sedaDNA - 1µl DNA + 3µl H20	18.5
	6	no sedaDNA – 1µl DNA + 3.5 H20	18.3

Table 3.3. Ct values for varying concentrations of sedaDNA:DNA to test potential PCR inhibition

2. Charcoal

2.1. Summary statistics of raw charcoal counts

The maximum number of charcoal particles found within any particular depth at Little Monon Lake were 5, 35, and 181 particles in the > 500 µm, 250 – 500 µm, and 125 – 250 µm size classes, respectively. The 5 particles found within the >500 µm size class, at 114mm depth (1,497 cal. yr. BP), were the highest number of particles found within this size class at any site. All samples within the 125 – 250 µm size class contained charcoal particles. Little Monon Lake contained the highest mean number of particles per 2mm sample across all sites in both the 250 – 500 µm and >500 µm size classes, with an average of 9.06 and 0.31 particles per depth, respectively. The maximum number of particles found within a particular depth at Pyramid Lake were 2, 17, and 133 particles in the > 500 µm, 250 – 500 µm, and 125 – 250 µm size classes, respectively. All samples within the 125 – 250 µm size class contained charcoal particles. Pyramid Lake contained the lowest mean number of particles across all size classes, with an average of 0.12, 3.98, and 45.07 particles within the >500 µm, 250 – 500 µm, and 125 – 250 µm size classes, respectively. The maximum number of particles found within a particular depth at Summit Lake were 2, 22, and 182 particles in the > 500 µm, 250 – 500 µm, and 125 – 250 µm size classes, respectively All samples within the 125 – 250 µm size class contained charcoal particles. Summit Lake contained the highest mean number of particles per 2mm sample in the 125 – 250 µm size class at all sites with an average of 87.56 particles.

		>500 µm	**250-500 µm**	**125-250 µm**
Little Monon Lake				
	Min	0	0	5
	Max	5	35	181
	Mean	0.31	9.06	76.31
	Var	0.46	39.50	1264.16
Pyramid Lake				
	Min	0	0	9
	Max	2	17	133
	Mean	0.12	3.98	45.07
	Var	0.13	10.78	675.28
Summit Lake				
	Min	0	0	12
	Max	2	22	182
	Mean	0.21	7.47	87.56
	Var	0.21	21.89	1908.33

Table 3.4. Summary statistics of raw charcoal count data

2.2. Distribution of charcoal particles

The violin plots outlined in Figure 3.7 combine a typical box plot with a kernel density plot to show the frequency of sample depths which contain a given number of particles. Patterns of distribution within the 125 – 250 µm size classes are very different between the three sites. Little Monon Lake is normally distributed with the main population of the data grouped around the median particle count of 77 particles. The same size class at Pyramid Lake is unimodally distributed, however, the main population is skewed towards the lower end of the particle counts, with most sample depths containing between 20 and 30 particles. The data for Summit Lake shows a multimodal distribution with two main populations. The first population contains a group of samples each with around 25 particles with a second, much larger, population focused around 100 particles. The distribution of the 250 – 500 µm size class is similar for all three sites with the main populations skewed towards a lower number of charcoal particles per depth. The distribution of the >500 µm size class is also similar across all three sites, as most of the sampling depths contained no charcoal particles within this size class. Little Monon Lake shows the greatest variation as a single sample within this site contains 5 charcoal particles, which is the highest count within this size class at any site.

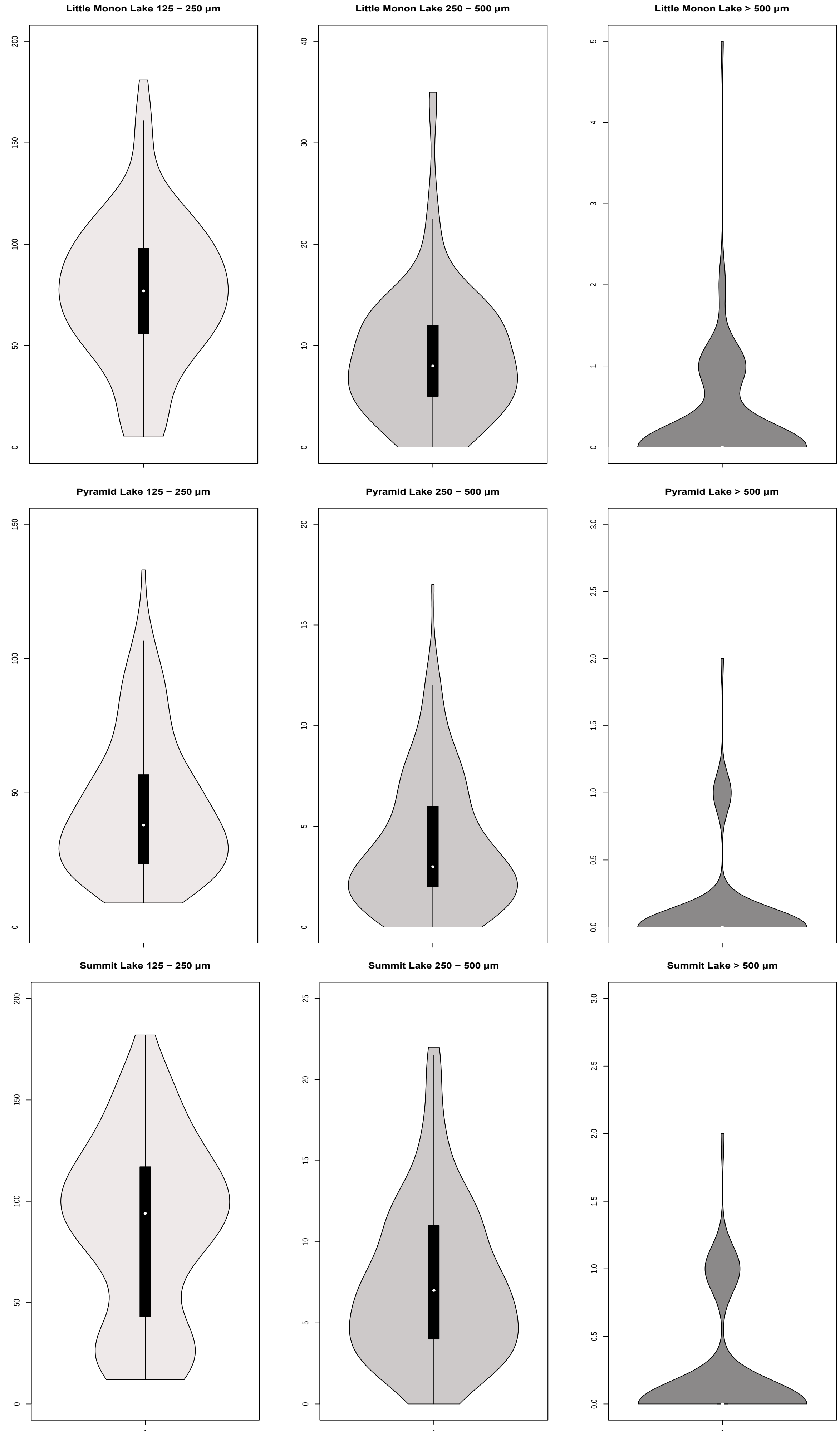


Figure 3.8. – Violin plots of raw charcoal counts at Little Monon Lake, Pyramid Lake, and Summit Lake.

2.3. Charcoal accumulation rates (CHAR)

2.3.1. Little Monon Lake

2.3.1.1. 250-125µm

A total of 8,013 charcoal particles were observed in this size class across all depths. The record for Little Monon Lake begins with moderate level CHAR values around 25 particles mm^{-1} yr^{-1}. These decline to 10.12 by 2,400 yr BP. This decline is followed by a steady increase in CHAR values to 36 particles mm^{-1} yr^{-1}. By 2,200 yr BP. The peak is followed by a sharp decline in CHAR values to 11 particles mm^{-1} yr^{-1} by 1,900 yr BP. The next 400-year period, between 1,900 yr BP and 1,500 yr BP, sees three distinct CHAR peaks followed by rapid declines in CHAR values. The first peak in this sequence occurs ~1,850 yr BP with a value of 35 particles mm^{-1} yr^{-1}, the second, occurs 100 years later, in 1,750 yr BP whereby values peak at 40 particles mm^{-1} yr^{-1}. This period is followed by a period of low CHAR, falling to 10 particles mm^{-1} yr^{-1} between 1,700 yr BP and 1,550 yr BP. Around 1,500 yr BP, CHAR dramatically increases to the highest value for the series with CHAR around 48 particles mm^{-1} yr^{-1}. The period 1,400 yr BP to 1,100 yr BP is characterised by a steady increase to 30 particles mm^{-1} yr^{-1} followed by a gentle decrease in CHAR to ~5 particles mm^{-1} yr^{-1} by 750 yr BP. The period 750 yr BP to 250 yr BP is characterised by very low CHAR values, less than 5 particles mm^{-1} yr^{-1}. The most recent 250 years exhibit a rapid increase in CHAR values which range from 2 particles mm^{-1} yr^{-1} at 200 yr BP to 20 particles mm^{-1} yr^{-1} in the uppermost sample.

2.3.1.2. 500-250µm

A total of 951 particles were observed in this size class with an average of 8.29 particles (σ 6.55) observed per sample depth. The first 1,000 years (2,500 yr BP to 1,500 yr BP) of CHAR values in this size class are highly fluctuating ranging between 0.5 and 5.5 particles mm^{-1} yr^{-1}. The distinct peak observed in the >500µm size class ~1,500 yr BP is also observed in the 500-250µm size class. This peak, which has a top value of 10 particles mm^{-1} yr^{-1} is the highest peak throughout the 500-250µm size class CHAR record. This peak is followed by around 800 years of similar fire activity to the first 1,000 years, fluctuating between 0.5 and 4 particles mm^{-1} yr^{-1}. There is a noticeable decline in CHAR values around 750 yr BP whereby values fall to less than 0.3 particles mm^{-1} yr^{-1}. The most recent 200 yr BP sees an increase in CHAR values to around 0.8 particles mm^{-1} yr^{-1}.

2.3.1.3. >500µm

The >500µm size class expectedly contains far few numbers of charcoal particles than the two smaller size classes. A total of 33 particles were observed within this size class with an average of 0.29 (σ 0.66) per sample depth. CHAR values are higher at the beginning of the record with near consistent values ranging from 0 to 0.2 particle mm^{-1} yr^{-1} for the period of 2,400 BP to 2,050 BP. During this period, there is a distinct peak in CHAR ~2,200 yr BP whereby values rise to 0.5 particles mm^{-1} yr^{-1} from a background value of 0.2 particles mm^{-1} yr^{-1}. This interval is followed by a period of no charcoal particles between 2,000 yr BP and 1,700 yr BP. The second peak occurs at 1,500 yr BP where CHAR values rapidly increase to 1 particle mm^{-1} yr^{-1}, which is the largest CHAR value observed in this size class at Little Monon Lake. A third peak occurs around 1,350 BP where CHAR values rise to 0.6 particles mm^{-1} yr^{-1}. Three small peaks in CHAR are observed ~1,000 yr BP with values ~0.2. CHAR values within the last 1,000 years are much reduced compared to earlier in the record. Two periods of fire activity are observed around 500 and 250 yr BP with CHAR values ~0.1 particles mm^{-1} yr^{-1}.

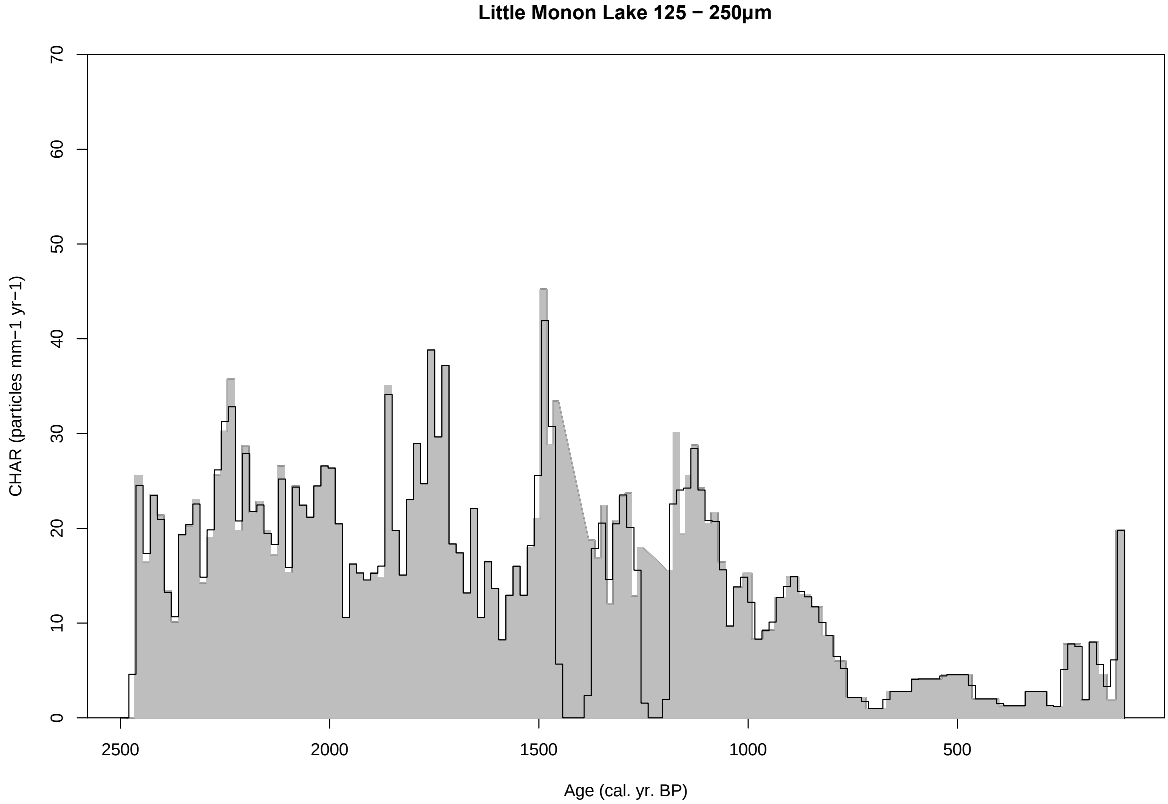


Figure 3.9 Little Monon Lake - CHAR 125 – 250 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

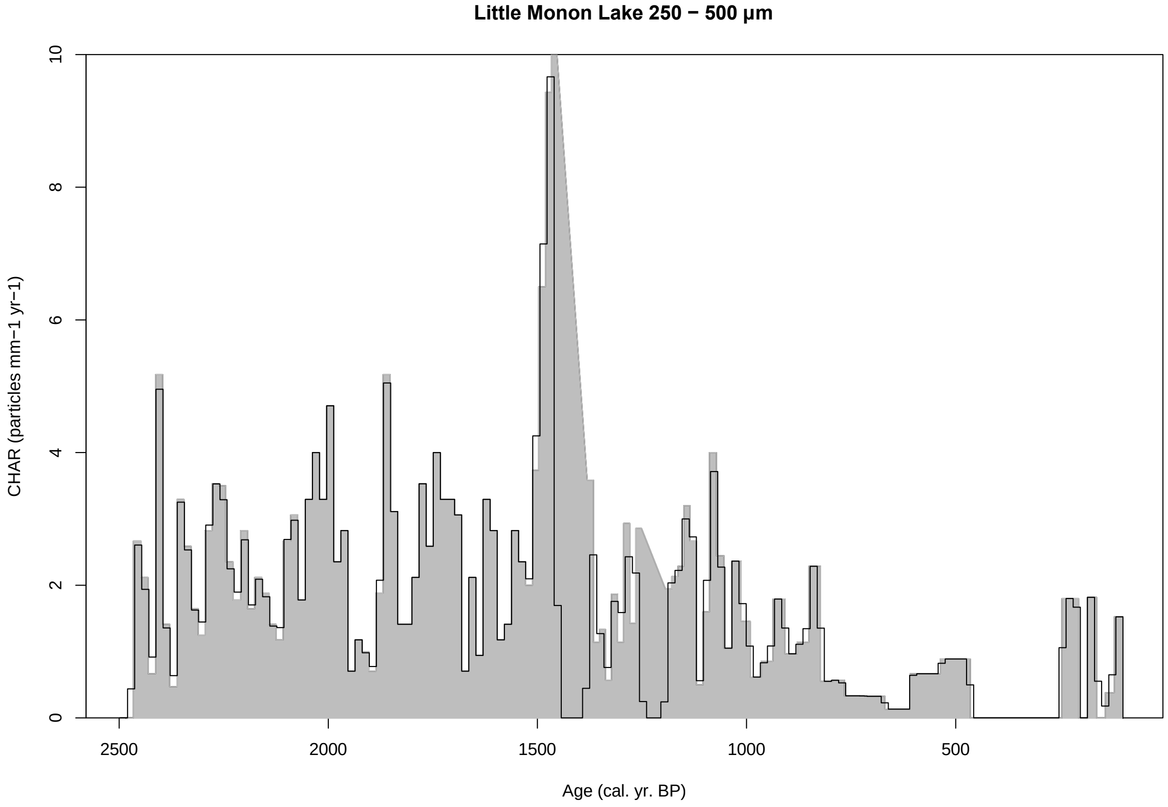


Figure 3.10. Little Monon Lake – CHAR 250 – 500 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

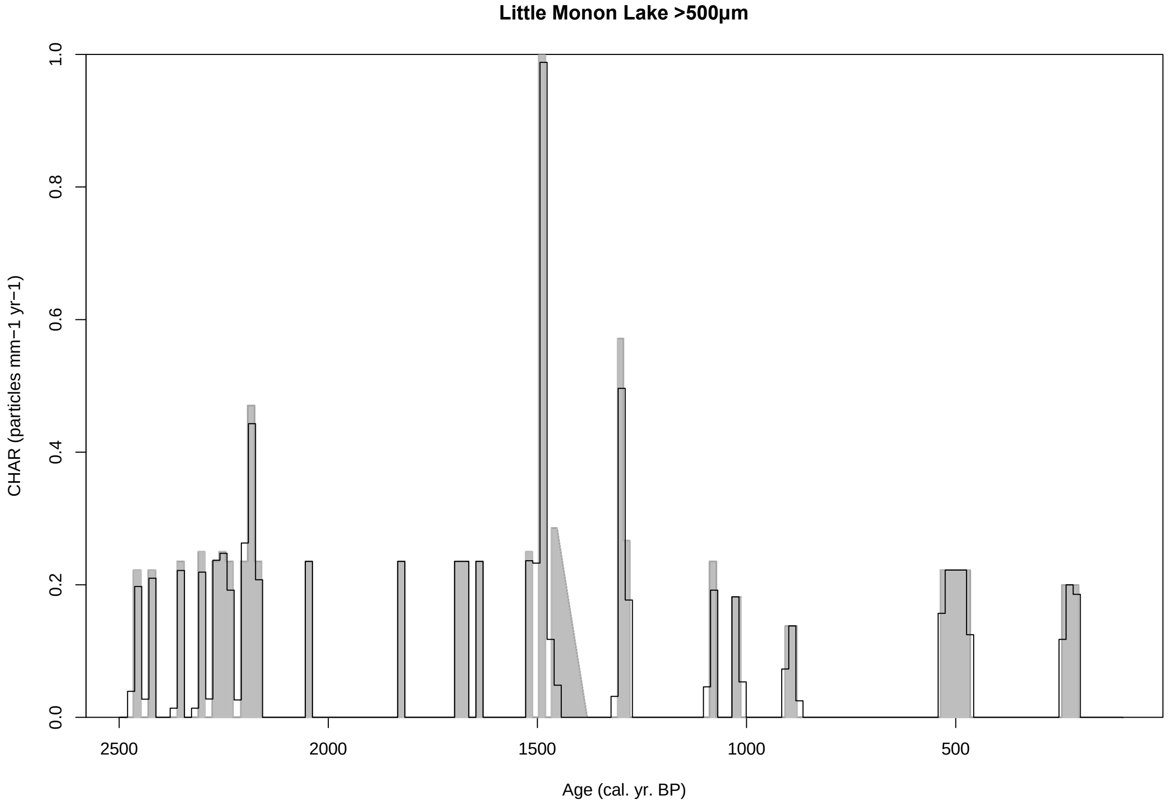


Figure 3.11. Little Monon Lake CHAR >500 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

2.323. Pyramid Lake

2.3.2.1. >250-125µm

CHAR values for this site are considerably lower than the records for Little Monon Lake and Summit Lake. Background CHAR levels are fairly uniform, ranging between 5-8 particles mm^{-1} yr^{-1} throughout. There are several episodic peaks observed throughout the record, but perhaps not as abrupt as the other sites. The largest peak occurs ~1,650 yr BP where CHAR values rise dramatically to 32 particles mm^{-1} yr^{-1}. This peak is followed by a period of around 300 years whereby CHAR values steadily deline to a low of 4 particles mm^{-1} yr^{-1}. By 1,200 yr BP, CHAR starts to rise again, culminating in a peak of 25 particles mm^{-1} yr^{-1} around 750 yr BP. An abrupt decline in CHAR values is observed after this period whereby CHAR value decrease from 20 to less that 10 particles mm^{-1} yr^{-1} where they remain until the present-day sample.

2.3.2.2. >500-250µm

The pattern of CHAR for this size class is characterised by relatively low background CHAR with values ~1 particles mm^{-1} yr^{-1}, throughout. Upon these fairly stable background levels exist a series of evenly spaced peaks of equals magnitude which range between 2 and 3 particles mm^{-1} yr^{-1}. The largest peak occurs at 800 yr BP whereby values rise above 4 particles mm^{-1} yr^{-1}. Interestingly, this peak is not as distinct in the other size classes for Pyramid Lake. Between 800 yr BP and present, there is a distinct reduction in CHAR values with very low background levels and several low amplitude peaks of 1-1.5 particles mm^{-1} yr^{-1}.

2.3.2.3. >500 µm

Two distinct peaks occur towards the beginning of the record. The first, at 3,000 yr BP, where CHAR rises to 0.5 particles mm^{-1} yr^{-1} and the second, around 2,300 yr BP, with values of 0.45 particles mm^{-1} yr^{-1}. The remainder of the record consists of 10 evenly spaced, small peaks with values around 0.2 particles mm^{-1} yr^{-1}. Between 750 yr BP and present, only one small peak is evident, occurring at ~350 yr BP with a value of 0.15 particles mm^{-1} yr^{-1}.

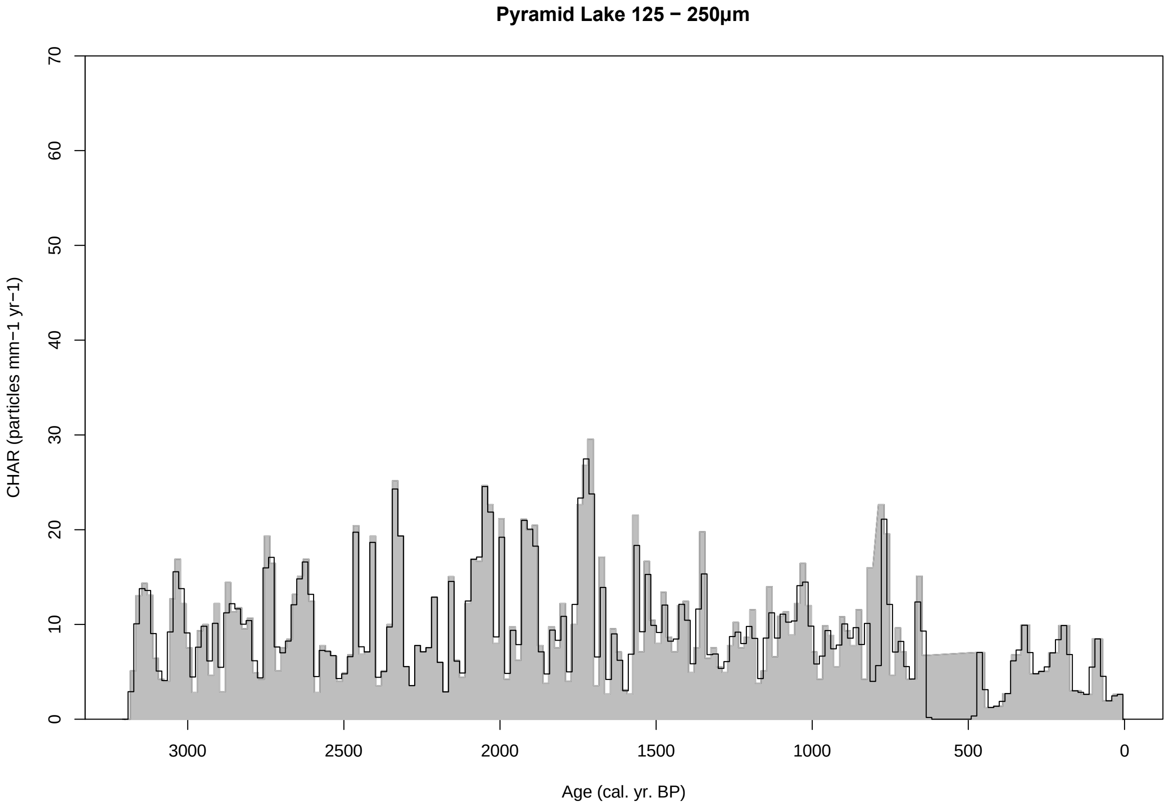


Figure 3.12. Pyramid Lake - CHAR 125-250 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

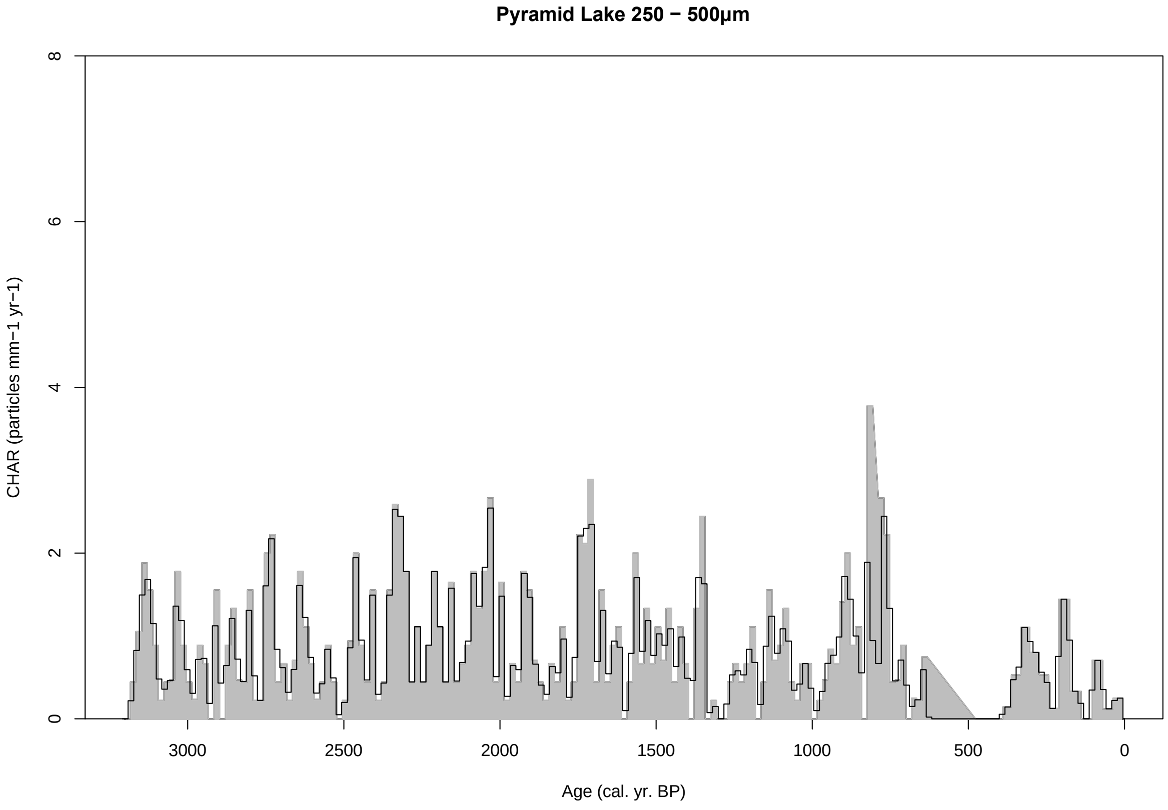


Figure 3.13. Pyramid Lake - CHAR 250 - 500 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

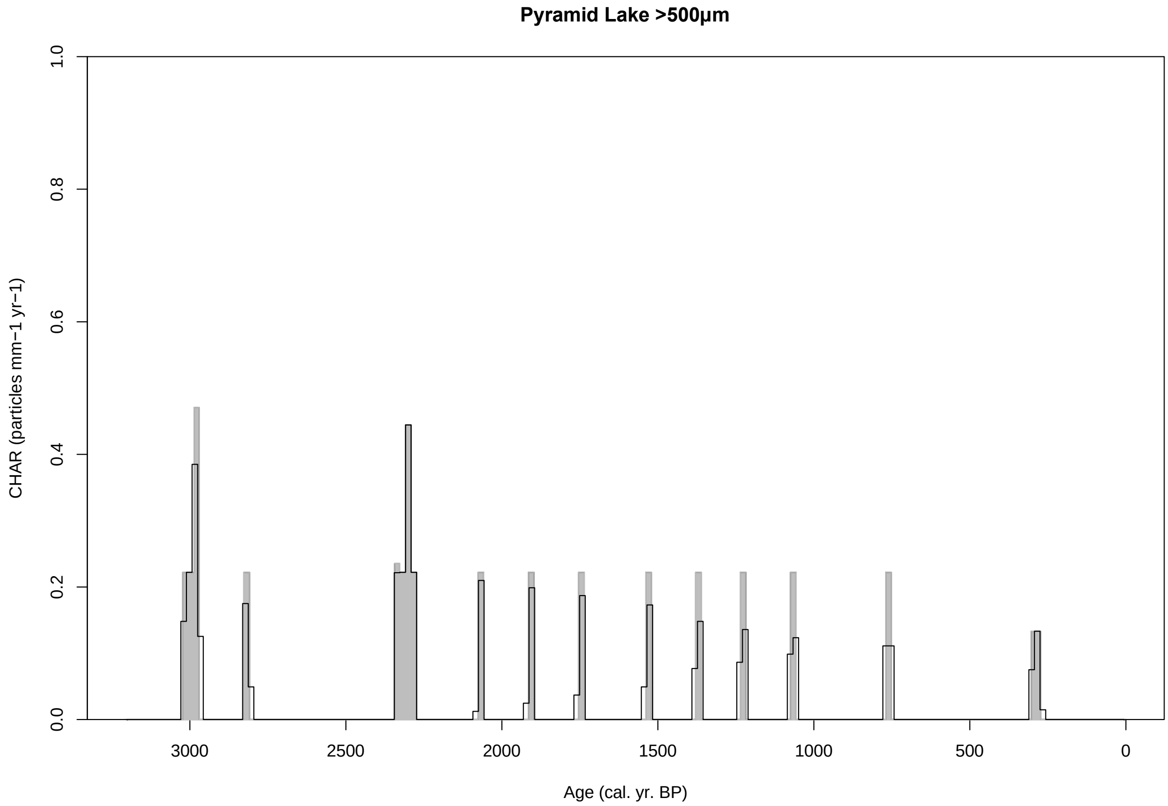


Figure 3.14. – Pyramid Lake - CHAR > 500 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

2.3.3. Summit Lake

2.3.3.1. >250-125µm

CHAR values for Summit Lake are the highest between all sites, characterised by very high background signals around 25 particles mm^{-1} yr^{-1}. Between 1,800 to 1,350 yr BP there is an apparent upward trend in CHAR values. During this period, background signals steady rise from 10 to 40 particles mm^{-1} yr^{-1}, with several peaks reaching 50 to 55 particles mm^{-1} yr^{-1}. Between 1,350 and 1,100 yr BP, background signals are dramatically reduced to a stable level of 30 particles mm^{-1} yr^{-1} throughout this brief period. While background CHAR remains relatively low, several notable peaks are observed within this period, which range 45-55 particles mm^{-1} yr^{-1}. The period 1,100 to 900 yr BP contains the highest level of CHAR at Summit Lake. Background levels of 35 particles mm^{-1} yr^{-1} and peaks ranging from 55 to 68 particles mm^{-1} yr^{-1} represent the highest CHAR levels found at any site. Between 900 and 650 yr BP, there is an overall declining trend in CHAR following the peak values observed in the previous period. There is one distinct peak within this downward trending period, occurring at 850 yr BP where CHAR values rise dramatically to 60 particles mm^{-1} yr^{-1}. At 650 yr BP, there is an obvious and abrupt change in the fire regime. A dramatic decline in CHAR from 40 to 5 particles mm^{-1} yr^{-1} occurs within a few decades. From this period to the present day, CHAR remains low, never exceeding 15 particles mm^{-1} yr^{-1} and takes the form of a more background component over high variation.

2.3.3.2. >500-250µm

The record for this size class is much more fluctuating than the smaller size class. Background signals are low, ranging from 1 to 2 particles mm^{-1} yr^{-1}, however, this record is characterised by very large and distinct peaks in CHAR. The upward trend between 1,800 and 1,300 yr BP is also observable in this record, with numerous peaks between 6 and 7.8 particles mm^{-1} yr^{-1} occurring throughout this period. From 1,300 yr BP onwards, there is an overall decline in CHAR values. Despite the overall decline, there are still numerous dramatic peaks within the record, although the amplitude decreases moving towards the present day. One other significant peak occurs during this period, at 1,000 yr BP with a value of 7 particles mm^{-1} yr^{-1}. Unlike the abrupt decline observed at 650 yr BP in the smallest size class, CHAR values here trend downward more smoothly, with less of an instantaneous change and more like a smooth transition. The values for the last 650 years are less stable than those observed in the smallest size class. Although background levels are low, less than 1 particles mm^{-1} yr^{-1}, there are numerous peaks within the period comparable with earlier in the record. Two peaks occur at 450 and 200 with values over 3 particles mm^{-1} yr^{-1}, which are more akin with pre 650 yr BP levels. The last 200 years see a downward trend towards 1 particles mm^{-1} yr^{-1}.

2.3.3.3. >500 µm

The >500µm record for Summit Lake is interesting as it does not follow the patterns observed in the two smaller size classes. Higher peaks are observed in the more recent samples, than earlier in the record. There is a clustering of CHAR peaks between 1,800 yr BP and 1,200 yr BP, mostly of equal amplitude of around 0.4 particles mm^{-1} yr^{-1}. There is one peak of 0.7 particles mm^{-1} yr^{-1} around 1,300 yr BP which greatly exceeds the values surrounding it. A similar peak is observed in the 500-250µm record. This peak is followed by a period of very little fire activity with no CHAR between 1,250 to 1,000 yr BP. Between 1,000 and 950 yr BP there is a cluster of three CHAR peaks with values ~0.4 particles mm^{-1} yr^{-1}. These peaks are followed by another hiatus in CHAR. Two peaks occur at 700 yr BP measuring 0.4 particles mm^{-1} yr^{-1}. The last 500 years contain several peaks in CHAR, including the highest peak observed throughout the record. Three peaks of 0.4 particles mm^{-1} yr^{-1} are separated by one large peak around 400 yr BP where CHAR values rise dramatically to 0.8 particles mm^{-1} yr^{-1}, which is the highest value for the series.

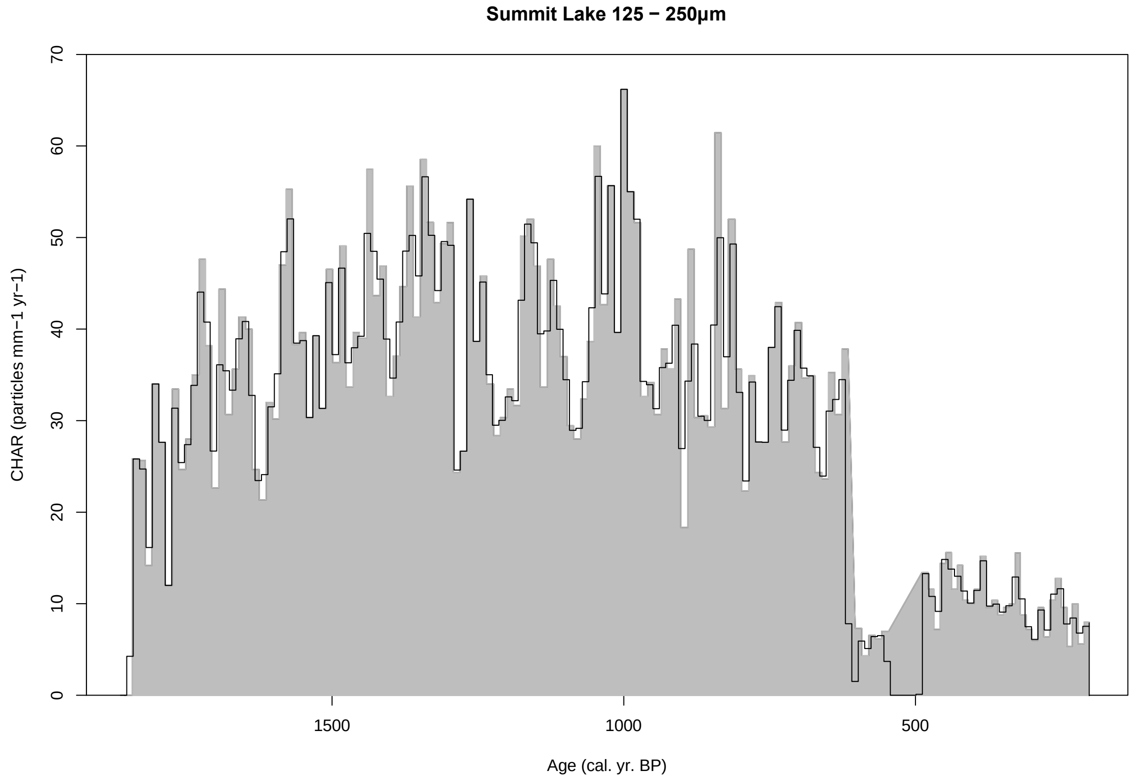


Figure 3.15. Summit Lake - CHAR 125-250 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

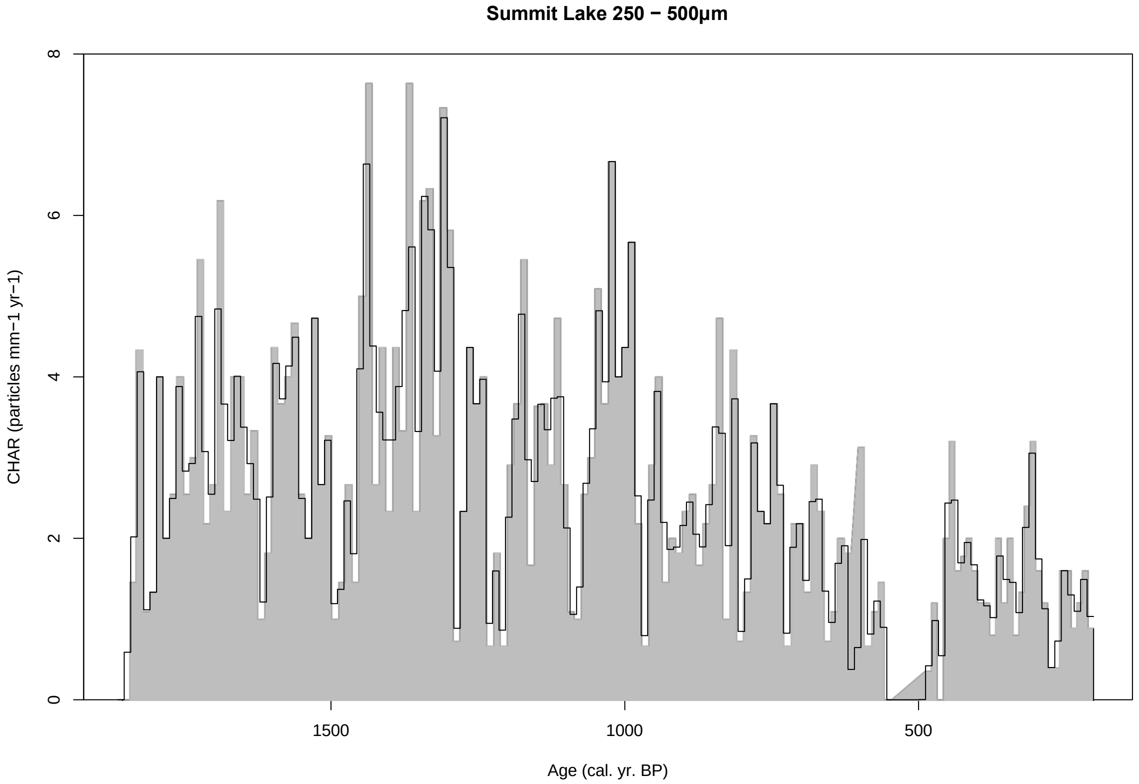


Figure 3.16. - Summit Lake - CHAR 250 - 500 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

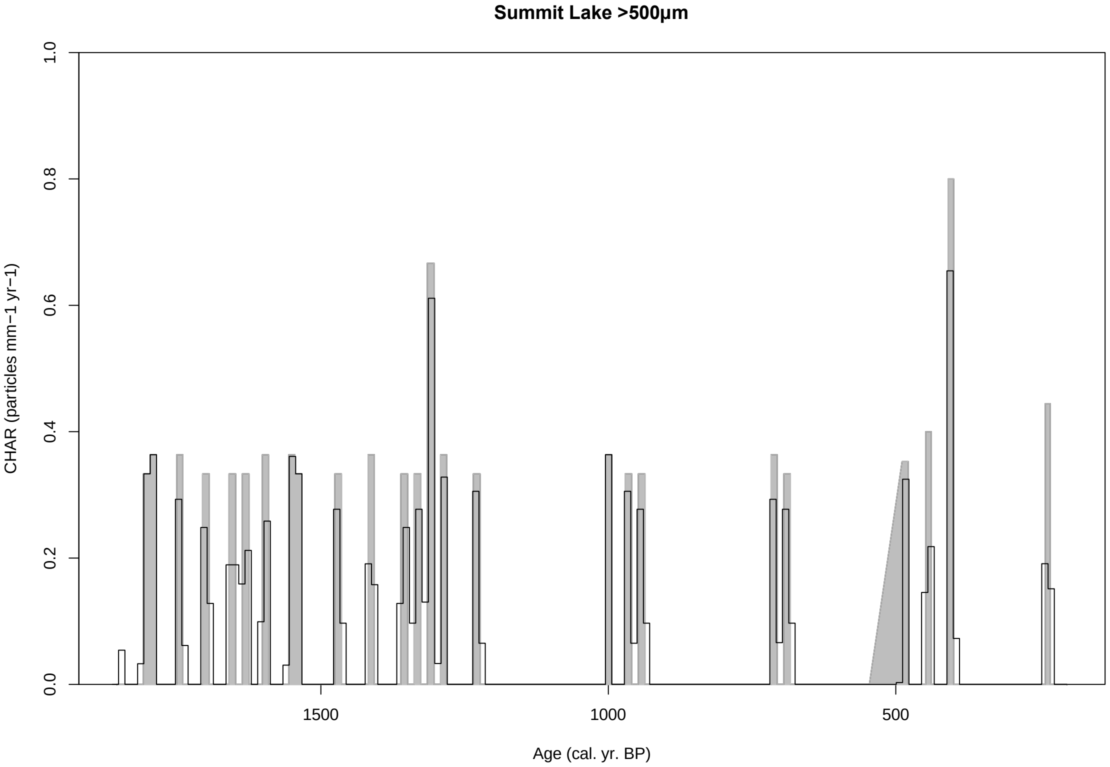


Figure 3.17. – Summit Lake - CHAR >500 µm size class. Original accumulation rates are represented by the shaded area while the histogram represents interpolated accumulation rates at equal time intervals.

2.4. Fire 'event' indices

Several running mean intervals (200-yr, 150-yr, and 100-yr) were used to assess how various methods of calculating *backgroundCHAR* in the 125 – 250 µm size class affected the number of fire events identified using the event indices (Table 3.5). Little Monon Lake showed the greatest disparity in number of fire events identified between the differing lengths of running mean. The 100-yr window resulted in the identification of 65 fire events throughout the series, compared to 58 and 53 events identified using the 150-yr and 200-yr windows, respectively. Therefore, 7-12 more fire events were identified using the shorter running mean. The results for Summit Lake show much less variation in the number of fire events identified using the varying length of running mean, with 72 events identified using the 100-yr window, 71 events identified using the 150-yr window, and 70 events identified using the 200-yr window. At Pyramid Lake, 69 fire events were identified using both the 200-yr and 150-yr windows, however, a further 10 events, were identified using the shorter 100-yr window. This analysis was done manually using conditional formatting in Microsoft Excel, highlighting the sample depths at which the event thresholds were met.

Figures 3.17 to 3.25 show the varying running means for each site, as calculated by the 100-yr, 150-yr, and 100-yr windows, plotted with the values for CHAR. These results clearly demonstrate how the 100-yr window is more variable and reflective of the *peakCHAR* component. While detecting the highest number of events does not necessarily result in the highest quality reconstruction, a 100-yr running mean was used as it was relative to the 500-yr running means implemented when using 1cm sampling resolutions, cited in the literature (Minckley and Long, 2016; Walsh et al., 2008; Long et al., 2007; Long et al., 1998).

There was a high level of variation between the number of events identified using values of 1, 1.05, 1.12, and 1.2, as thresholds for fire occurrence. Table 3.6 shows the number of events identified by each threshold along with the percentage of events identified by the 1.05, 1.12, and 1.2 threshold, in comparison to a threshold of 1. At Little Monon Lake, 65 events were identified using an event index of 1, compared to just 25 events identified using a threshold of 1.2. This is a 62% reduction in the number of events between these set of thresholds, which is the greatest difference across all three sites. Summit Lake shows similar results, with 72 events identified using a threshold of 1, with only 33 events identified using a threshold of 1.2, which accounts for a 55% reduction in the total number of events identified. At Pyramid Lake, there was much less variation in the number of events detected by the range of indices, however, there was still a 32% reduction in the number of events identified using a threshold of 1.2, in comparison to the 79 events identified using a threshold of 1.

	Little Monon Lake	**Pyramid Lake**	**Summit Lake**
100-yr window	65	79	72
150-yr window	58	69	71
200-yr window	53	69	70

Table 3.5. Total number of fire events identified using 100, 150, and 200-yr running windows.

Index	**Little Monon Lake**	**Pyramid Lake**	**Summit Lake**
1	65	79	72
1.05	50	72	65
1.12	35	58	47
1.2	25	54	33
% of Index = 1			
1	100	100	100
1.05	76.92	91.14	90.28
1.12	53.85	73.42	65.28
1.2	38.46	68.35	45.83

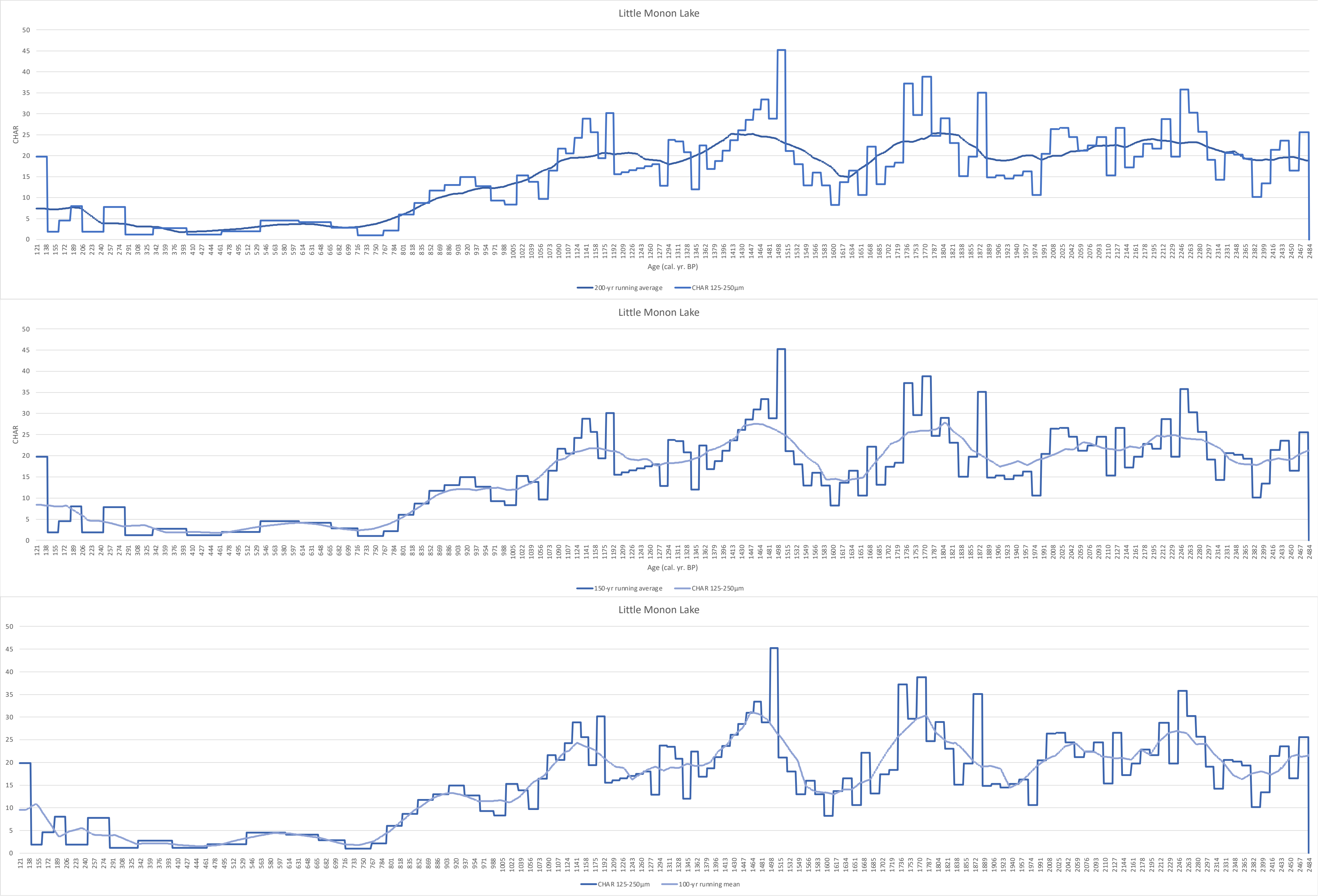
Table 3.6. The number of fire events identified using 1, 1.05, 1.12, and 1.2 as a threshold for fire events (as used in Long et al., 1998) and the percentage of events identified by the 1.05, 1.12, and 1.2 threshold, in relation to a threshold of 1.

2.5. Fire return intervals

Fire return intervals were calculated using a 100-yr window and an event threshold of 1.0. At Little Monon Lake, the average fire return interval (FRI) for the entire series was 36.64 years (σ 36.31 years), however, there is a high level of variation in the FRI behaviour at Little Monon Lake. FRI ranges from 22.8 years between 1,000 to 1,499 yr BP to 83.2 years in the most recent 500-yr period. The Pyramid Lake record show a similar level of fluctuation. The average FRI for the entire series is 41.34 years (σ 29.17 years), however, this ranges from 28.4 years in the period between 3,000 and 3,499 yr BP, to 91.8 years in the most recent 500-year period. These dramatic increases in FRI are indicative of the fire suppression patterns observed in both CHAR records described above. The record at Summit Lake is much more consistent. The average FRI for the series was 22.45 years (σ 15.52 years) with a high of 20.2 in the most recent 500-yr period and a low of 27.8 years in the period 1,000 to 1,499 yr BP.

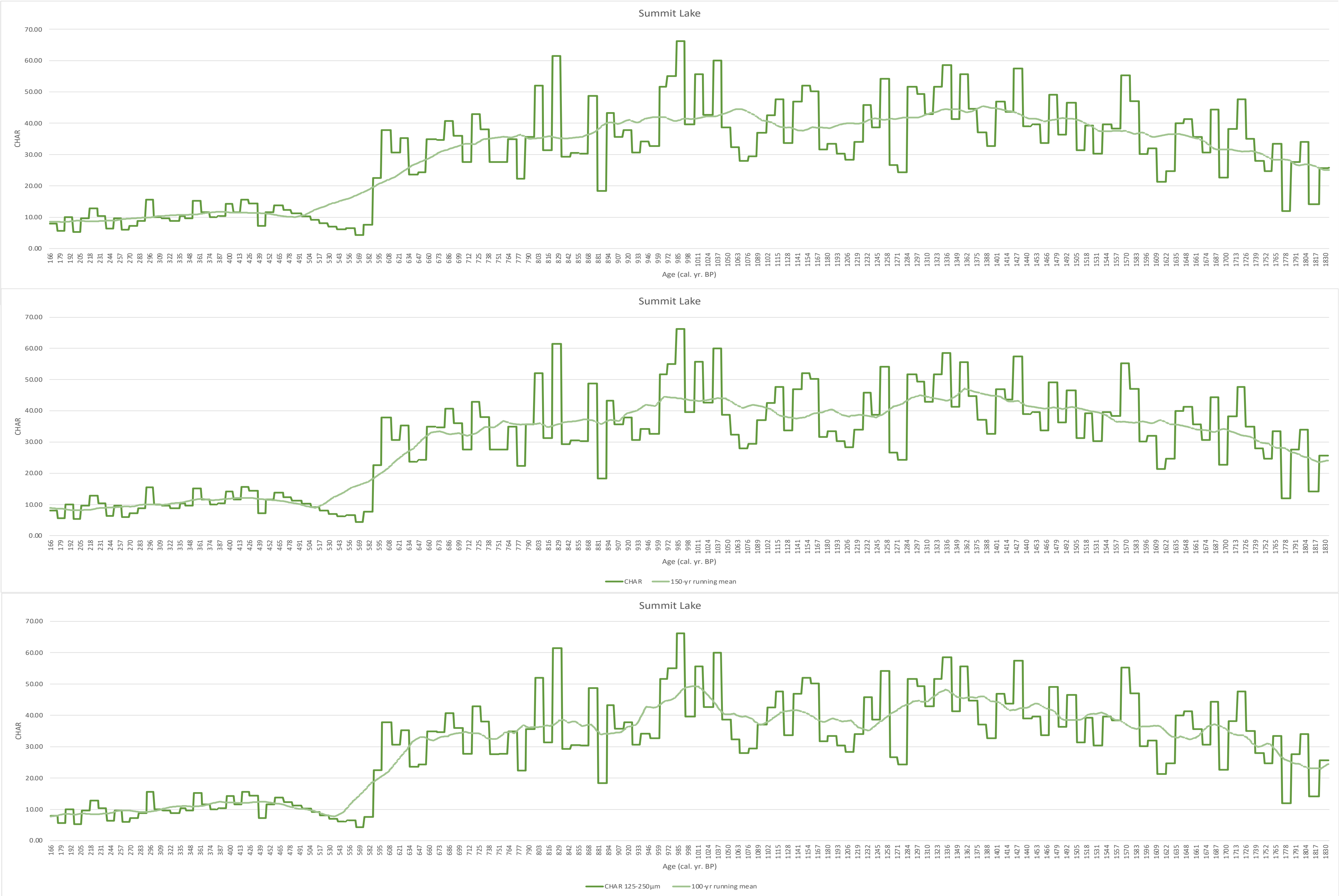
	Little Monon Lake	**Pyramid Lake**	**Summit Lake**
Mean fire return interval	36.6	41.3	22.4
0-499 yr BP	83.2	91.8	20.2
500-999 yr BP	68.0	32.2	20.7
1000-1499 yr BP	22.8	37.0	27.8
1500-1999 yr BP	36.8	38.5	20.8
2000-2499 yr BP	28.8	45.5	
2500-2999 yr BP		32.1	
3000-3499 yr BP		28.4	

Table 3.7. Mean fire return interval for the entire series at Little Monon Lake, Pyramid Lake, and Summit Lake with the mean fire return intervals for 500-yr periods before present. These were calculated using a 100-yr running mean and an event threshold of 1.0.



Figures 3.18, 3.19, and 3.20. 200-yr running mean (top), 150-yr running mean (middle), and 100-yr running mean (bottom) at Little Monon Lake, Oregon.

Figures 3.21, 3.22, and 3.23. 200-yr running mean (top), 150-yr running mean (middle), and 100-yr running mean (bottom) at Pyramid Lake, Oregon.



Figures 3.24, 3.25, and 3.26. 200-yr running mean (top), 150-yr running mean (middle), and 100-yr running mean (bottom) at Summit Lake, Oregon.

2.6. Fire events per 100-yr period

The following results are based on an event threshold of 1.0 and a running mean of 100 years.

2.6.1. Little Monon Lake

At Little Monon Lake the most common number of fire events per century is 3, with 7 100-yr periods containing 3 events. The record shows a highly fluctuating number of events with a clustering of higher fire activity within the middle of the Little Monon Lake record, between 2,099 yr BP and 999 yr BP. With the exception of 1,500 to 1,599 yr BP, every 100-yr period contains at least 2 fire events. The 100-yr period with the highest number of fire events occurs between 1,400-1,499 yr BP with a total of 7 events. One period, 1,100 to 1,199 yr BP contains 6 events. Two periods, 2,000-2,099 yr BP and 1,300-1,399 yr BP contain 5 fire events. The number of fire events per 100-yr period dramatically decreases between 800-yr BP and present, whereby mostly 0 or 1 events are observed, with the exception of 100-199 yr BP, whereby two events are identified.

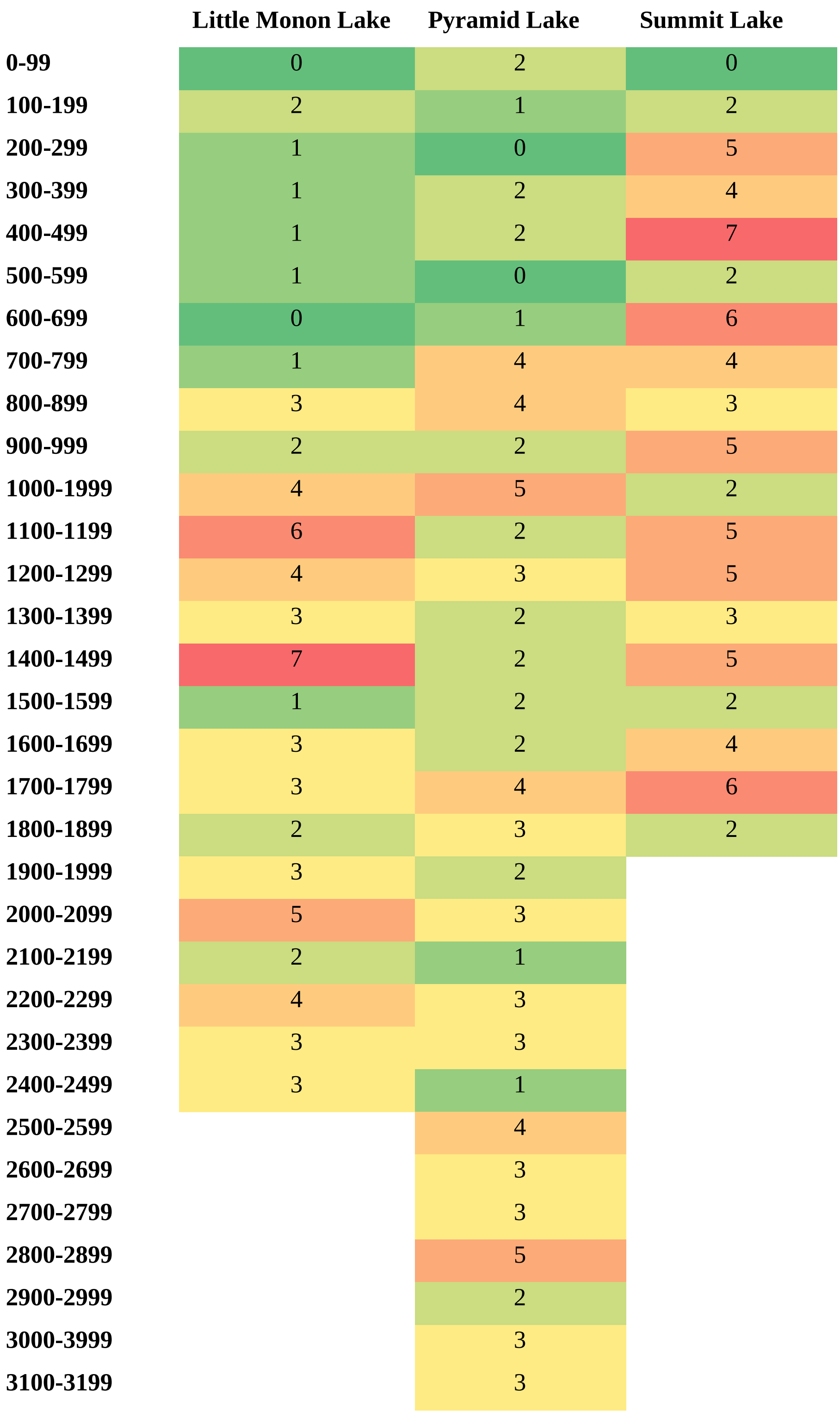
2.6.2. Pyramid Lake

The record for Pyramid Lake is seemingly less variable than those observed at Little Monon Lake or Summit Lake with less dramatic fluctuation in the number of events. The most common number of fire events per century at Pyramid Lake is 2 events, which can be found within 11, 100-yr periods. No 100-yr periods contain 6 or 7 fire events as observed in both the Little Monon Lake and Summit Lake records. Two periods, 2,800-2,899 yr BP and 1,000 to 1,099 yr BP, contain the most fire events, each with 5 events observed within their respective periods. A change in fire regime is observable from the 700-799 yr BP to present as the number of events per 100-yr period is noticeable reduced to between 2, 1, or 0 events.

2.6.3. Summit Lake

The record for Summit Lake clearly highlights that fire activity is greatest and most variable at this site. Of the 19 100-yr periods within the record, 14 periods contain at least 3 fire events. One period, 400 – 499 yr BP contains 7 fire events, the most observed at Summit Lake. Two periods 1700-1799 yr BP and 600-699 yr BP contain a total of 6 events. Only the most recent 100-yr period, 0-99 yr BP, contained no events, which is attributed to a lack of sample during this time period.

Interestingly, none of the 100-yr periods contain the same number of fire events across all three sites.



	Little Monon Lake	Pyramid Lake	Summit Lake
0-99	0	2	0
100-199	2	1	2
200-299	1	0	5
300-399	1	2	4
400-499	1	2	7
500-599	1	0	2
600-699	0	1	6
700-799	1	4	4
800-899	3	4	3
900-999	2	2	5
1000-1999	4	5	2
1100-1199	6	2	5
1200-1299	4	3	5
1300-1399	3	2	3
1400-1499	7	2	5
1500-1599	1	2	2
1600-1699	3	2	4
1700-1799	3	4	6
1800-1899	2	3	2
1900-1999	3	2	
2000-2099	5	3	
2100-2199	2	1	
2200-2299	4	3	
2300-2399	3	3	
2400-2499	3	1	
2500-2599		4	
2600-2699		3	
2700-2799		3	
2800-2899		5	
2900-2999		2	
3000-3999		3	
3100-3199		3	

Figure 3.27. The number of events per 100-yr period at Little Monon Lake, Pyramid Lake, and Summit Lake as identified by the 100-yr running window.

3. Tephra

All geochemical results presented, along with reference database material, are normalised to 100% to eliminate the variability of post deposition hydration and to make signatures comparable between publications (Jones et al., 2018; Lowe et al., 2017; Eastwood et al., 2009; Smith and Westgate, 1968).

Site	Depth	Age	Age error	Age BP	Age AD/BC
Forlorn Camp Lake 3					
	Above B1	1080	81	1154-900	774-1050 AD
	Below B1	660	39	676-554	1274-1396 AD
	Above B2	2510	53	2746-2436	796 -486 BC
	Below B3	4380	61	5665-5452	3715-3502 BC

Table 3.8. Radiocarbon dates and calibrated ages for samples associated with tephra analyses at Forlorn Camp Lake, Washington.

3.1. FC3 B1

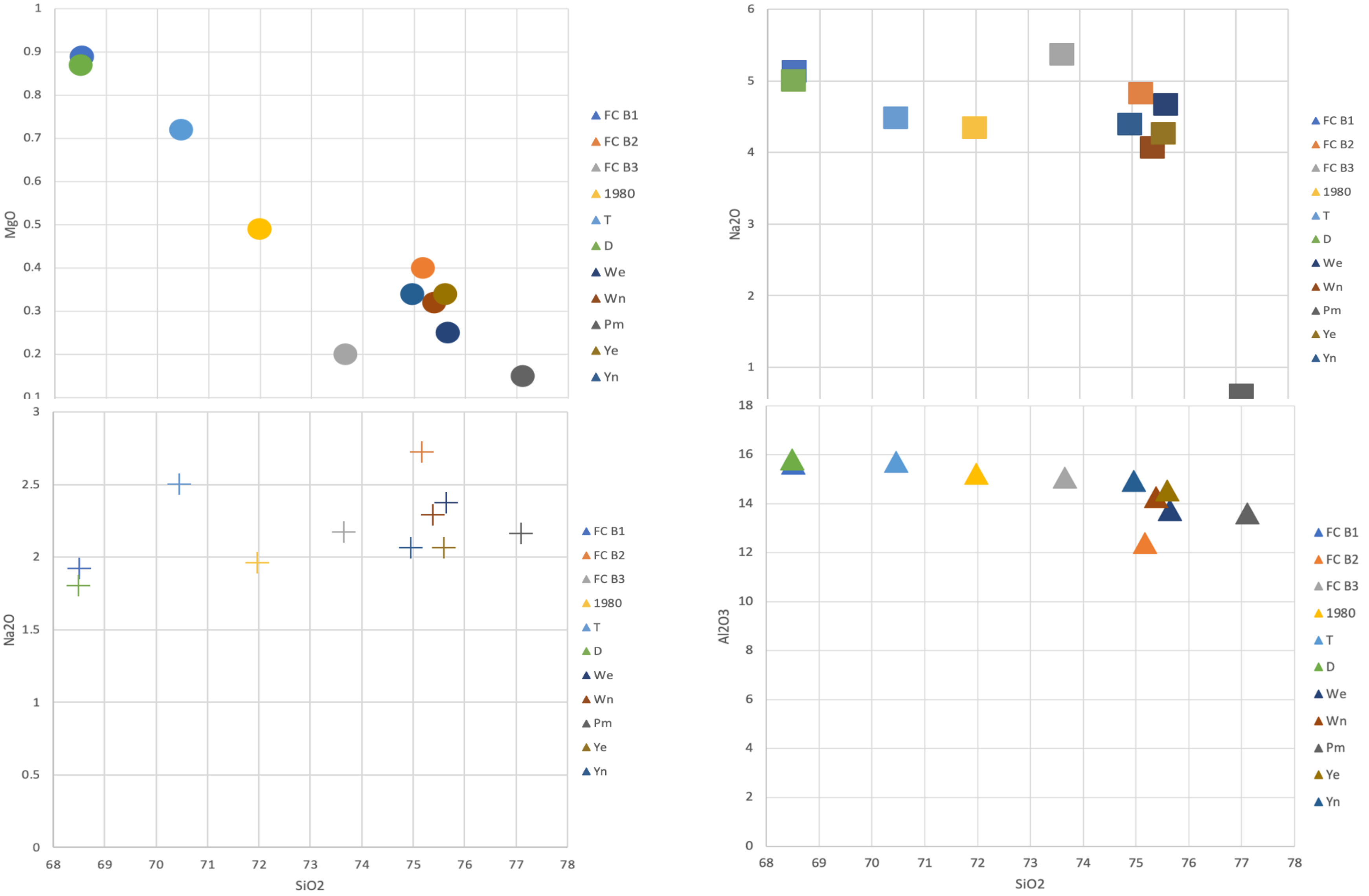
There are several factors which suggest with high probability that tephra layer FC3 B1 obtained from Forlorn Camp Lake 3 is derived from Mt St Helens Tephra Layer D. Firstly, reference material of Tephra Layer D obtained from the source at Mt St Helens has previously been radiocarbon dated from a charcoal macrofossil contained within the deposit to 1150 BP (Mullineaux, 1986). A radiocarbon date obtained from a bulk sample directly below FC3 B1 gives an age range of 1154-900 BP which temporally associates these layers. Secondly, Figures 3.27, 3.28, 3.29, and 3.30 below show PCA of major element geochemical signatures of FC3 B1, FC3 B2, and FC3 B3, against reference material from major eruptions over the last 4,000 years at Mt St Helens obtained from Mullineaux, 1981. The four plots which combine SiO_2 with Al_2O_3, SiO_2 with MgO, SiO_2 with Na_2O, and SiO_2 with K_2O, show major grouping of geochemical signatures of layer FC3 B1 and Tephra Layer D as a distinct population with no overlap to any other set of reference chemistry. This correlation is further confirmed by assessing one of the more abundant elements, SiO_2. Tephra layer FC3 B1 contains unusually low levels of silica, with an average value of 68.52%. Reference material for Mt St Helens Tephra Layer D also exhibits this unusually low level of silica with an average abundance of 68.5%. These uncommon values further evidence the correlation of these layers as the abundance of silica within these layers is significantly different to the higher levels, between 70-75%, found throughout the history of Mt St Helens. Therefore, a combination of the visual similarities among these layers, their correlation in age and, strong correlation in geochemical signatures is sufficient to correlate FC3 B1 with Mount St Helens Layer D.

Tephra Set	SiO2	TiO2	Al2O3	FeO	MnO	MgO	CaO	Na2O	K2O
FC B1	**68.52**	**0.56**	**15.62**	**2.98**	**0.06**	**0.89**	**4.21**	**5.13**	**1.92**
σ	9.96	0.77	5.29	4.03	0.08	1.80	3.85	1.33	1.12
FC B2	**75.17**	**0.48**	**12.38**	**2.15**	**0.05**	**0.40**	**1.71**	**4.82**	**2.72**
σ	6.47	0.32	2.82	1.82	0.04	0.37	1.24	1.13	0.87
FC B3	**73.66**	**0.15**	**15.06**	**0.72**	**0.02**	**0.20**	**2.60**	**5.36**	**2.17**
σ	6.16	0.07	4.02	0.38	0.02	0.19	2.09	1.52	1.01
1980	71.98	0.44	15.22	2.62	0.06	0.49	2.78	4.34	1.96
T	70.46	0.42	15.71	2.90	0.05	0.72	2.82	4.48	2.50
D	68.50	0.42	15.80	2.00	0.05	0.87	3.10	5.00	1.80
We	75.65	0.26	13.74	1.53	0.06	0.25	1.37	4.67	2.37
Wn	75.39	0.19	14.26	1.73	0.02	0.32	1.63	4.06	2.29
Pm	77.11	0.24	13.59	0.93	0.03	0.15	1.01	0.61	2.16
Ye	75.60	0.14	14.54	1.25	0.03	0.34	1.68	4.26	2.06
Yn	74.96	0.15	14.93	1.24	0.04	0.34	1.79	4.39	2.06

Table 3.9. – Summary of geochemical data from Forlorn Camp Lake samples with reference material from all Mount St Helens eruptions from the last 4,000 years (Mullineaux, 1996).

3.2. FC B2 and FC B3

Layers FC3 B2 and FC3 B3 both appear to have several clusters of differing geochemistry, suggesting they are each composed of numerous populations or may be contaminated. As such, significant correlation of these layers against reference material from Mt St Helens could not be achieved. Radiocarbon dates obtained from bulk sediments above and below these tephra layers result in an age range of 2,436 to 5665 cal. yr. BP, which suggests that these layers should derive from Mt St Helens Tephra Set Y or Mt St Helens Tephra Set P. However, analysis of raw geochemical data and PCA failed to provide a strong correlation between these layers. Since Layers FC3 B2 and FC3 B3 are within such close proximity to each other within core FC3, there is a possibility that FC3 B3 could have contaminated FC3 B2, or vice versa, either through reworking in the core or sediment deposition into the lake at the time from material on the landscape. Layers FC3 B2 and FC3 B3, therefore, remain uncorrelated.



Figures. 3.28. (top left), 3.29. (top right), 3.30. (bottom left), and 3.31 (bottom right). Scatter plots depicting major element geochemistry of FC B1, FC B2, and FC B3 against reference material for major eruptions of Mt St Helens over the last 4,000 years show clear correlation between Tephra Set D and FC B1.

CHAPTER FOUR

DISCUSSION

1. Dating controls

1.1. Original rationale for this study

Most of the study sites chosen within this study are located along the crest of the Cascade Range, an area which is disproportionately understudied with much of the published literature concerning the reconstruction of historic disturbance in western North America focussed on the western and eastern slopes of the Cascade Range, Oregon and the Rocky Mountains, Colorado (Long et al., 2016; Morris and Brunelle, 2012; Morris et al. 2010; Anderson et al., 2010; Colpron-Tremblay and Lavoie, 2010; Long et al., 1998). With an abundance of suitable lake sites, these higher-elevation sites would afford the opportunity to address important data gaps within this region and expand our knowledge of historic fire and insect occurrence across the western North America as a whole, which was a key factor in site selection.

The original objective for this study was to calibrate known forest disturbance events in the Cascade Range over the last 70 years, obtained from USDA Forest Service Aerial Detection Survey (ADS) data, with pollen signals obtained from near-annual/sub-decadal sampling of lake sediment cores (as per, Morris et al., 2012; Anderson et al., 2010 outlined in the General Introduction). This is important to highlight as it formed the key rationale for site selection, sampling strategy, etc. The hypothesis was that insect-induced tree mortality during periods of outbreaks would result in quantifiable changes in pollen abundances and produce ‘signatures’ associated with such events. These characteristic signatures could then be identified through time to provide valuable insights of historic insect occurrence. These records of historic outbreak occurrence would also facilitate exploratory analysis into the use of DNA-based methodologies in detecting forest insects during periods of outbreak, using the palynological reconstruction as a proxy for identified events. As timber harvest has been a major landscape use in the Cascade Range for the period of the ADS dataset and could interfere with the event calibration (for example, a decline in tree pollen associated with logging activity mistaken for a period of beetle-induced tree mortality), high elevation sites, often adjacent to wilderness areas, were favoured to limit the potential of human influence on forest disturbances signals, providing information on an ecosystem functioning as close to ‘natural’ as possible.

Robust chronologies, particularly within the 70-year calibration stage were critical in identifying insect-induced modifications, which would ultimately determine the accuracy of the historic reconstructions. An abundance of radiocarbon dates focussed near the surface of the three final sites chosen for full analyses revealed one significant and unanticipated disadvantage of high elevation sites in the Cascade Range; slower sedimentation rates than those observed at lower elevation sites such as the Coast Range and at sites in the Willamette Valley. Sedimentation rates within published palaeoenvironmental reconstructions range from ~5 years cm-^{1} in the lower-elevation Coast Range (Long and Whitlock, 2002) to ~34 yr cm^{-1} in the lowland Willamette Valley (Minckley and Long, 2016). Radiocarbon dates and age-depth models outlined in this thesis revealed sedimentation rates at the higher elevation sites of the Cascade Range were between 50- and 360-years cm^{-1} (based on an average of 10-72 years 2mm^{-1} observed at Little Monon Lake, Pyramid Lake, and Summit Lake).

As a consequence of poor sedimentation rates, the number of sampling points with dates correlating to ADS data were insufficient to facilitate the original objective of producing a robust calibration of disturbance signatures over the last 70 years. Therefore, a contiguous high-resolution, 2mm sampling methodology was chosen to achieve continual records of environmental change at time intervals which still reflect the timescales of the processes occurring in these ecosystems and with those in the published literature.

1.2. Radiocarbon dating and tephrochronology

The proximity of Little Monon Lake, Pyramid and Summit Lake to several major volcanoes within the High Cascade Range, highlighted that any identified tephra layers could serve as an additional dating tool, providing distinct chronological markers within the timeframe of the ADS dataset, most notably, the May 1980 eruption of Mt St Helens. Any recovered tephra layers could also be used to cross-correlate events between cores (Lowe et al., 2011). Well-documented tephra layers, such as the eruption of Mt Mazama, central Oregon, in 7,630 yr BP, are readily used to provide key time markers of environmental change across North America (Minckley and Long, 2016; Long et al., 2014; Whitlock et al., 2008; Brunelle and Whitlock, 2003**;** Millspaugh et al., 2000; Zdanowicz et al., 1999; Sea and Whitlock, 1995; Cwynar, 1987; Barnosky, 1985; Lichti-Federovich, 1970). The distinct bands of tephra found within core FC3, recovered from Forlorn Camp Lake, Washington, provided an opportunity to test the applicability of these methods. A thick band of ash found near the surface of core FC3 was believed to be associated with the 1980 eruption of Mt St Helens, as there were no other tephra bands observed above this layer, suggesting that it could only have been associated with the most recent eruption. In addition, soil samples from two nearby sites contained 53mm ash (from Chamber’s Pass (22km from MSH)) and 44mm ash (from Elk Pass (44km from MSH)) which correlated to the May 1980 eruption of Mount St Helens (Zobel and Antos, 1991).

Tephra Set	Eruptive Stage	Age of onset (yr BP)
1980		1980 AD
T	*Goat Rocks*	1800 AD
X		1500 AD
W	*Kalama*	1480 AD
FC3 B1		1154-900
D	*Sugar Bowl*	1,200
B	*Castle Creek*	2,500 – 1,600
FC3 B2		2746 - 2436
P	*Pine Creek*	3,000 - 2,500
Y	*Smith Creek*	4,000
FC3 B3		5665 - 5452
J		10,500
S	*Swift Creek*	13,000
K		19,000
M	*Cougar*	20,500
C	*Ape Canyon*	50,000 – 40,000

Table. 4.1. The temporal context of tephra layers obtained from Forlorn Camp Lake (highlighted in grey) in relation to late-Pleistocene and Holocene eruptions at Mt St Helens. Tephra layer FC3 B1 was successfully geochemically matched to reference material from Tephra Set D owing to their unusually low levels of silica and close temporal association. FC3 B2 and FC3 B3 remain uncorrelated.

Analysis of major elemental chemistry and radiocarbon dating of the tephra layers recovered from Forlorn Camp Lake revealed that the sediments were much older than first thought. The uppermost tephra layer expected to match the 1980 eruption was instead successfully matched to reference material from Mt St Helens Tephra Layer D, confirmed by their close temporal association, and correlation in geochemical signatures, notably, their unusually low levels of SiO_2 ~ 68%, compared to the silica abundance at adjacent levels, where values are between 70 and 75%. Layers FC3 B2 and FC3 B3 remain uncorrelated to any reference material for any eruption of Mt St Helens. The radiocarbon date obtained from above layer FC3 B2 places this layer between Mt St Helens Tephra Sets B and P (See Table 4.1), however, geochemical analysis of this layer failed to identify the origin. Interestingly, the radiocarbon date obtained from below FC3 B3 places this layer, between Mt St Helens Tephra Sets Y and J, in a period of dormancy at Mt St Helens. Tephra layer FC3 B2, which dates to between 2,746 – 2,436 yr BP, falls within a period whereby both Mt Rainier and Mt Adams, Washington, have documented eruptions (Sisson and Vallance, 2008; Donoghue et al., 2007). Both volcanoes are within close proximity to Forlorn Camp Lake; however, no correlation could be made with the geochemistry available (Sisson and Vallance, 2008). The date associated with Layer FC3 B3 (5,665 to 5,452 yr BP) coincides with the Osceola collapse event (Vallance and Scott, 1997) whereby a great lahar which flowed northeast of the summit to cover an area around 212 square-miles across the Puget Trough. Forlorn Camp Lake sits to the southwest of Mt Rainier and despite the temporal correlation of these events, it is unlikely that they are related. It cannot be discounted that tephra layers FC3 B2 and FC3 B3 may not derive from Mt St Helens, but instead from other volcanoes within the Cascade Volcanic Chain.

During analyses it became apparent that there is a distinct lack of reference material for North American tephras, which hindered the successful matching of tephra layers FC3 B2 and FC3 B3. Reference databases of tephra geochemistry are typically generated from deposits located at the source, whereas the taphonomic processes associated with the transport, deposition, and incorporation of tephra into sediment results in less clear signals i.e., mixing of closely erupted events or mineral contamination (Stevenson et al., 2013; Naeser et al., 1981). ‘Tephra Base’ (http://www.tephrabase.org/), is a European-collaborative database of names, ages, and geochemical signatures of tephra layers found in Iceland, northwest- and northern-Europe, Russia, and central Mexico (Newton et al., 2007), facilitating the speedy and efficient matching of sediment-derived tephra layers within a comprehensive database of reference material. Since a ‘Tephra Base’ style database does not yet exist for North American tephra and there are only several published collections of Mt St Helens geochemistry (Mullineaux et al., 1975, Mullineaux, 1986; Mullineaux, 1996).

While the tephra layers from Forlorn Camp Lake failed to provide distinct time periods within the original scope of the project, analyses have generated a set of radiocarbon dated geochemical signatures which could add to the reference database of tephra from Mount St Helens. It appears a high level of variation within the chemistry of ash recovered from both layers coupled with a lack of reference material were crucial limiting factors in the successful correlation with reference material. Therefore, it is hoped that the tephra geochemistry of the samples within the core obtained from Forlorn Camp Lake, Washington, along with any further layers identified in future analyses of other cores obtained during this thesis, will be used in the future to help calibrate a comprehensive database for North America, ensuring locally sourced reference material significantly matches with ‘real world’ samples which have been subjected to environmental processes and potential contamination.

2. DNA

The development of qPCR protocols for the direct detection of *D. ponderosae, D. pseudotsugae,* and *Choristoneura* spp. outlined in this thesis show promise for the potential role of DNA-based methodologies in palaeoenvironmental research. The sites chosen for this thesis have been inundated with outbreaks of a wide range of forest insects at least for the last 70 years, yet the number of positive amplifications of target species were much lower than expected. This section aims to discuss the results obtained through the novel use of qPCR to detect historic occurrence of these species, highlight the potential factors which may have contributed to the results observed, and summarise the lessons learnt from this thesis to outline areas within sedaDNA research which still require further development.

2.1. sedaDNA

While radiocarbon dating and age-depth models revealed that an insufficient number of samples covered the periods associated with the ADS data set to provide robust calibrations of individual outbreak extent and location with sedimentary evidence of pathogen presence, several samples near the surface of Pyramid Lake and Summit Lake do indeed correlate to the time periods associated with the ADS dataset (Table 4.2). The surface sample at Pyramid Lake dates to 1955 – 2004 AD which nearly spans the entire period associated with the ADS dataset. In addition, the 8mm sample dates to 1867 – 1918 AD suggesting that the 4mm and 6mm samples could derive from the 20th Century and potentially cover a short period of the ADS dataset. At Summit Lake, the uppermost sample failed to be dated due to a lab error in the preparation process. With the sample below the uppermost sample, at 4mm, dating to 1800 to 1957 AD, age depth models suggest there is also potential for parts of this sample and the uppermost sample covering periods of time associated with the ADS dataset. This highlights the great advantage of having robustly dated sediments which produce high-quality age depth models, particularly in time periods by which there is valuable proxy data, as these often provide useful additional information. The surface sample at Little Monon Lake pre-dates the ADS dataset and therefore there is no direct comparison of results at this site to any reference material. Analysis of ADS did, however, facilitate a direct comparison of known periods of outbreaks with potential DNA positives obtained from sedimentary records, which crucially, facilitated the interpretation of the results over this period.

Site	Depth	RC Age	Age error	Age BP	Cal. Age AD/BC
Summit Lake					
	4mm	-80	66	260—7	1800-1957 AD
	8mm	0	60	149-20	1804-1936 AD
Pyramid Lake					
	2mm	-500	31	-53--5	2004 AD
	8mm	40	31	256-33	1867-1918 AD
	16mm	90	32	266-22	1806-1930 AD

Table 4.2. Radiocarbon dates from Summit Lake and Pyramid Lake which overlap with the period associated with the ADS survey data.

Site	Agent	Outbreak years	Corresponding sample
Pyramid Lake			
	D. ponderosae	2006, 2007, 2008	None
	D. pseudotsugae	1992	2mm, 4mm*, 6mm*
	C. occidentalis	1987, 1988, 1991	2mm, 4mm*, 6mm*
Summit Lake	*D. ponderosae*	1950, 1962, 1963, 1965, 1968, 1975, 2007, 2008, 2009, 2010, 2011, 2012.	2mm
	D. pseudotsugae	1954, 1995	2mm
	C. occidentalis	1986, 1987, 1988, 1992, 1993	2mm
	Adelgies picea	1970, 1973, 2003, 2011	2mm

Table 4.3 shows the outbreak occurrence at Pyramid Lake and Summit Lake alongside samples with corresponding ages. *Depths not confirmed by radiocarbon dating to coincide with ADS data, but indicated by age-depth models.

2.1.1.. Pyramid Lake

2.1.1.1946 – 2017 AD

The date of the surface sample, 0-2mm, at Pyramid Lake ranges from 1955 to 2004 AD, which means that Pyramid Lake is the only site at which sample ages can be confidently associated with the period of ADS survey data, and, therefore, the only site at which we can directly compare the results from DNA analyses with known outbreaks. During this period there were several outbreaks of *D. ponderosae* to the northwest of the site, several *Choristoneura occidentalis* outbreaks to the west, north, and east of the site, and a *D. pseudotsugae* outbreak to the southwest of the site (See Table 4.3 and Figure 4.1). Within this uppermost sample, the Dpseudo protocol (target: *D. pseudotsugae*) produced successful amplifications in two out of three replicates with an amplicon melting temperature corresponding to that of *D. pseudotsugae* tissue.

The topography of the Pyramid Lake catchment and the geographical location of the 1992 outbreak of *D. pseudotsugae* in relation to Pyramid Lake is critical in the recovery and detection of *D. pseudotsugae* DNA. Within the sub catchment depicted in Figures 4.1 to 4.3., it is evident that Pyramid Lake sits on a small, plateau-like shelf, with the southern slope feeding into Pyramid Lake and the west and northern areas draining away from Pyramid Lake. The outbreak occurred to the south of Pyramid Lake (shown in red in Figure 4.1) and lies along the leeward side of a ridge, situated 165m above Pyramid Lake, with the natural run-off regime likely to wash material from this crest into Pyramid Lake. This is evidence by signs of erosion along the downward 'outbreak' slope which has been identified as a key indicator of potential sources of in-washed material, including DNA (Giguet-Covex et al., 2014). Alsos et al., 2018, found that the correlation between catchment relief and total DNA ($r^2 = 0.42$) were higher than the correlation between total DNA and local vegetation ($r^2 = 0.34$), suggesting that particularly in small catchments, such as Pyramid Lake, processes associated with slope, run off or snowmelt are critical in sediment delivery (Parducci et al., 2019). High levels of precipitation, in the forms of snow, snowmelt, and rain, are typical in this region of the Cascade Range, which increases the likelihood that taphonomic processes would result in material, including DNA, from the outbreak ending up incorporated into the sediment at this site. Outbreaks of species 'downward' of Pyramid Lake would therefore likely have to fly into the lake in order to become incorporated into the sediment.

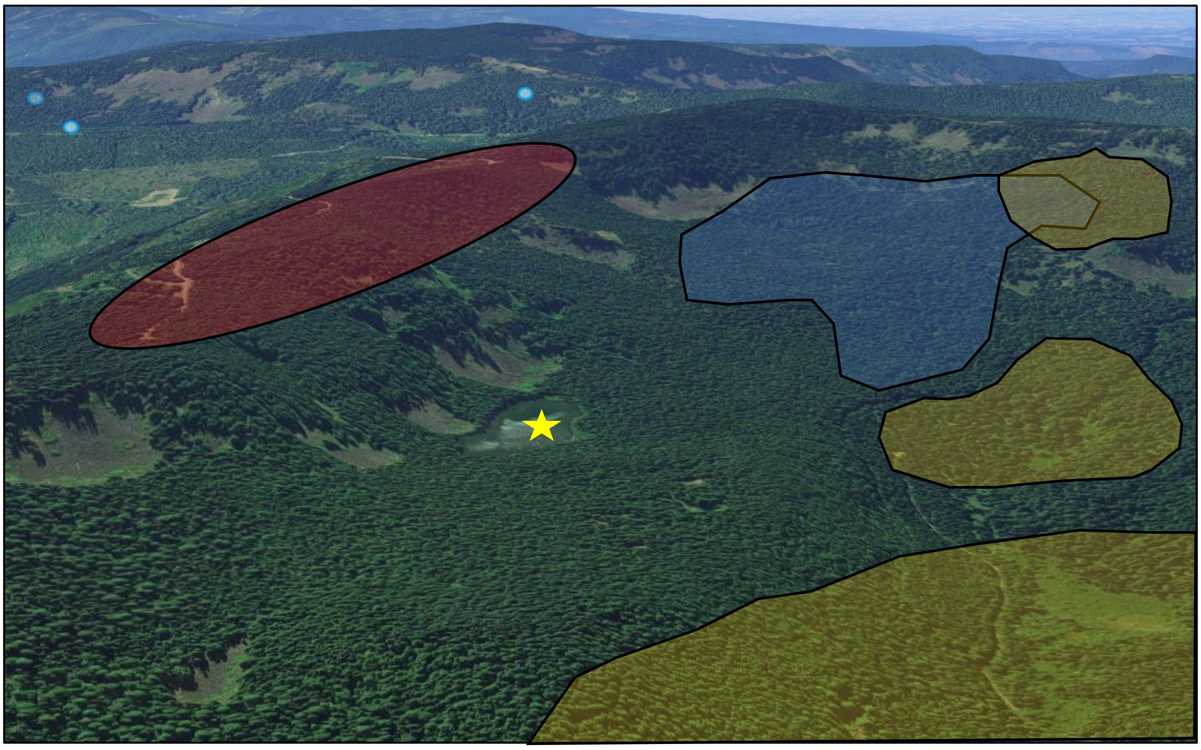


Figure 4.1. The location of Pyramid Lake (starred) and known outbreaks of *D. ponderosae*, *D. pseudotsugae* and *Choristoneura occidentalis* obtained from ADS data. The outbreak of *D. pseudotsugae* of 1992 is shown in red while the *D. ponderosae* outbreaks of 2006, 2007, and 2008 are shown in blue. The outbreaks of *Choristoneura occidentalis* in 1987, 1988,1989, and 1992 are shown in yellow.

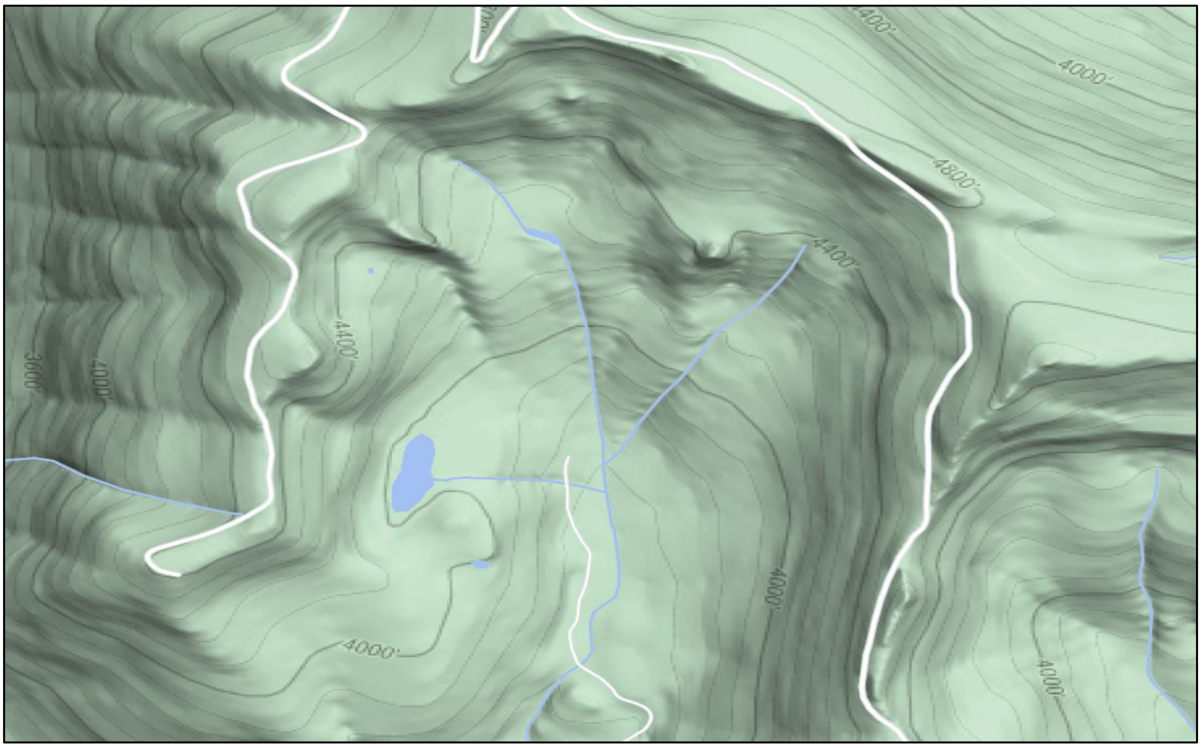


Figure 4.2. A topographic map of Pyramid Lake, showing how catchment dynamics may explain the results found during sedaDNA analysis.

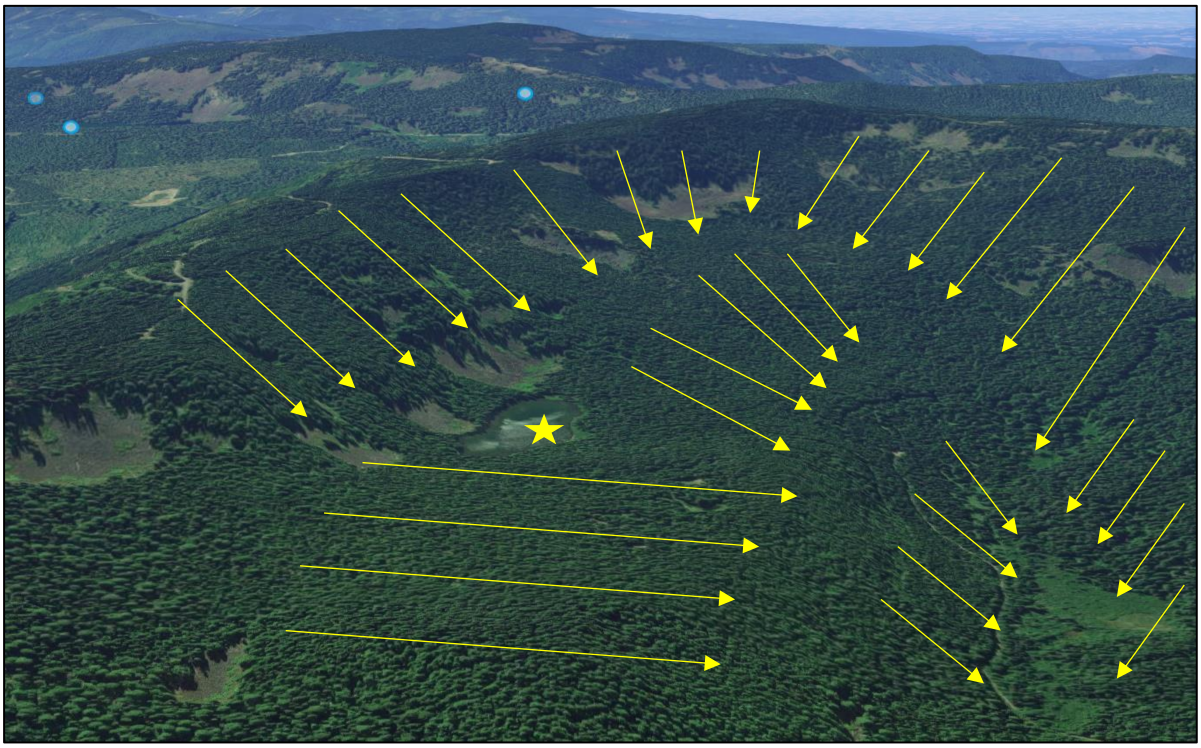


Figure 4.3. A schematic of the Pyramid Lake catchment, showing drainage patterns which have been shown to influence the source areas of material, including DNA.

A lack of *Choristoneura* species qPCR amplifications at Pyramid Lake, despite several outbreak occurrences of *Choristoneura occidentalis* during this time evidence the strong influence of taphonomic processes, outlined above, in delivering DNA to the site. The location of the outbreaks, between 900m and 1,200m to the north and at lower elevation than Pyramid Lake, suggests material from these outbreaks is likely to wash away from Pyramid Lake instead of becoming incorporated into the sediment. From an outbreak in this location, it seems the only mechanisms for remains of *Choristoneura* spp. to become incorporated into Pyramid Lake would have been for individuals to fly into the lake itself. Adult *Choristoneura* have shown to readily fly 20km, with a record dispersal distance documented at 450km (Sturtevant et al., 2013; Greenbank et al., 1980). Effective dispersal is a key component in the mass attack dynamics of budworm species linking spatially disjunct populations (Régnière, et al. 2019; Anderson et al., 2011). These flight behaviours show that the *Choristoneura* have the capacity to reach Pyramid Lake, however, no target DNA was recovered. One hypothesis is that the majority of the newly hatched populations moved further down into the watershed to find suitable hosts there, with only several individuals dispersing in the direction of Pyramid Lake. This highlights the importance of considering the outbreak scenario as a whole and understanding the various factors that may contribute to the recovery of target species DNA i.e. the number of individuals that are flying in the right direction to encounter a lake, how many are incorporated into the lake, how many avoid being eaten, and then how many become deposited into the lake sediment. When outlining all these factors, it is perhaps more understandable how only several positive amplifications were obtained in this study. In future studies, a comparison between several lakes within a watershed, each with differing topographic constraints could help to further develop the understanding of the variables outlined above.

The age range of the 0-2mm surface sample (1955 to 2004 AD) predates the known outbreaks of *D. ponderosae* in 2006, 2007, and 2008 (shown in Figure 4.1 and Table 4.3). This suggests the most recent sediment was missed during sediment collection, likely forming a part of the unconsolidated surface/sediment interface. A lack of positive amplification of *D. ponderosae* within the uppermost sample potentially addresses the concerns of leaching of DNA to lower depths within sedimentary archives (Haile et al., 2007; Sjögren et al., 2017). The results here highlight that a radiocarbon dated sample obtained 2-4 years prior to a series of highly severe outbreaks in the immediate vicinity shows no evidence of DNA moving downwards within the core and mixing with older sediments. These results concur with those found in Sjogren et al.,

(2017) whereby abrupt/diagnostic signals of events do no not appear in sediments prior to the event(s), although, it cannot be discounted that the small sample volume may have just failed to detect *D. ponderosae* at this depth.

2.1.1.2. Pre-1946 AD

In addition to the positive amplifications within the period covered by ADS data, there were two positive amplifications which pre-date the ADS survey. Firstly, there was a positive amplification (in one of three replicates) of *D. pseudotsugae* within the 22mm sample using the Dpseudo assay (target: *D. pseudotsugae*) with a Tm matching *D. pseudotsugae* tissue. Age depth models suggest this sample dates to between 543 and 473 yr BP. While this outbreak clearly occurred before the onset of ADS data, resulting in no information about the spatial occurrence of this outbreak, the catchment dynamics which likely led to the positive amplification in the uppermost sample suggests that this outbreak may have occurred across the same crest, to the south of Pyramid Lake, which would result in material washed into Pyramid Lake. This outbreak coincides with the end of a moderate-level fire period as shown by fossil charcoal data, suggesting this period may have been warmer and drier than surrounding periods leading to a suppression of tree defences, making them susceptible to disturbance by both insects and fire. Secondly, several successful amplifications using the Dpond protocol occurred in a period between 752 cal. yr. BP (sample depth 64mm) to 896 cal. yr. BP (sample depth 80mm) at Pyramid Lake. The melting temperature of the amplicons were ~1.6-2^{o}C higher than that from *D. ponderosae* reference material suggestive of some level of DNA degradation (see *2.2.4. DNA degradation*, for further discussion). NCBI-BLAST data suggests that the Dpond qPCR protocol only amplifies *D. ponderosae* and *D. simplex*, which suggests that the amplicon could potentially be associated with a unsequenced *D. ponderosae* population or an unspecified amplification of a non-target species. The period between 752 and 896 BP whereby positive amplifications using the Dpond qPCR protocol were identified coincides with an intense fire period, observed within the fossil charcoal record at Pyramid Lake. This period contains the highest CHAR values found throughout the 250-500 µm size class as well as appearing as a distinct peak in both the 125-250µm and >500 µm records. The correlation of timing of these events coincides with the end of the Medieval Climate Anomaly (MCA) (Walsh et al., 2008), which is characterised by a period of prolonged warm and dry conditions which could have led to heightened drought stress in host trees leading to a period of insect outbreaks with a subsequent period of heightened fire activity in the resulting stands of dead trees. Another scenario in this instance could be that fires disturbed surface sediments and lead to a heightened period of erosion of all terrestrial matter, including charcoal (see Colombaroli and Gavin, 2010; Whitlock and Larsen, 2002)

2.1.2.. Summit Lake

Due to a lab error while dating, the age range of the uppermost sample (0-2mm) at Summit Lake is unknown. However, the date obtained from the 2-4mm sample ranges from 1810-1925 AD, meaning the surface sample may derive from the mid- to late-20th Century and, therefore, cross-over with the ADS dataset. During the period covered by the ADS dataset, there were frequent and widespread outbreaks of *D. ponderosae*, *D. pseudotsugae*, *C. occidentalis*, and *Adelgies picea* in the immediate vicinity of Summit Lake (See Table 4.3 for more info). A positive amplification using the Chorist (target: *Choristoneura* spp.) qPCR protocol was identified in the uppermost sample, in one of three replicates. The melting temperature of the amplicon was 1.6°C higher than positive control tissue from *C. occidentalis.* Conventional PCR produced four positive amplifications which matched the expected product length of *Choristoneura* spp. Sanger sequencing produced a mixture of DNAs which along with the varying Tm found in the *D. ponderosae* positives at Pyramid Lake, suggests some level of DNA degradation at these sites. ADS data shows an outbreak of *C. occidentalis* in 1993, along the southern shoreline of Summit Lake, which could account for the potential positive as the topography indicates that material from this outbreak may become incorporated into the sediment at Summit Lake. Several other *Choristoneura* species are known to inhabit the Summit Lake watershed, including the Oblique-banded leafroller (*C. rosaceana*) and the sugar pine tortrix (*C. lambertiana*), however, a lack of successful sequencing failed to facilitate the matching of sequences to reference material for these other potential species. As described in the Methods Chapter, the catchment for Summit Lake is the widest, and most open of the three final sites chosen, characterised by undulating plateaus typical of Ecoregion 4c (Thorsen et al., 2013). Unlike Pyramid Lake where the highly undulating landscape and distinct topographic features provides a better understanding of potential source areas for material, a wide, open catchment affords a much greater disparity in the potential sources of material. A lack of barriers, such as steep river cut valleys which may interrupt flight patterns, potentially increase the source area of insects able to fly into this area. Equally, a larger catchment increases the hydrological influence, providing much more opportunity for material to be in-washed into Summit Lake. Despite this, only one set of amplifications occurred at Summit Lake. Perhaps the openness of the catchment decreased the density of biomass of the target species, allowing dispersal in any direction, in contrast to the dynamics of Pyramid Lake whereby topography essentially controls dispersal direction.

Fig. 4.4 The location of Summit Lake in relation to the extent of the known outbreak of *Choristoneura occidentalis* in 1993.

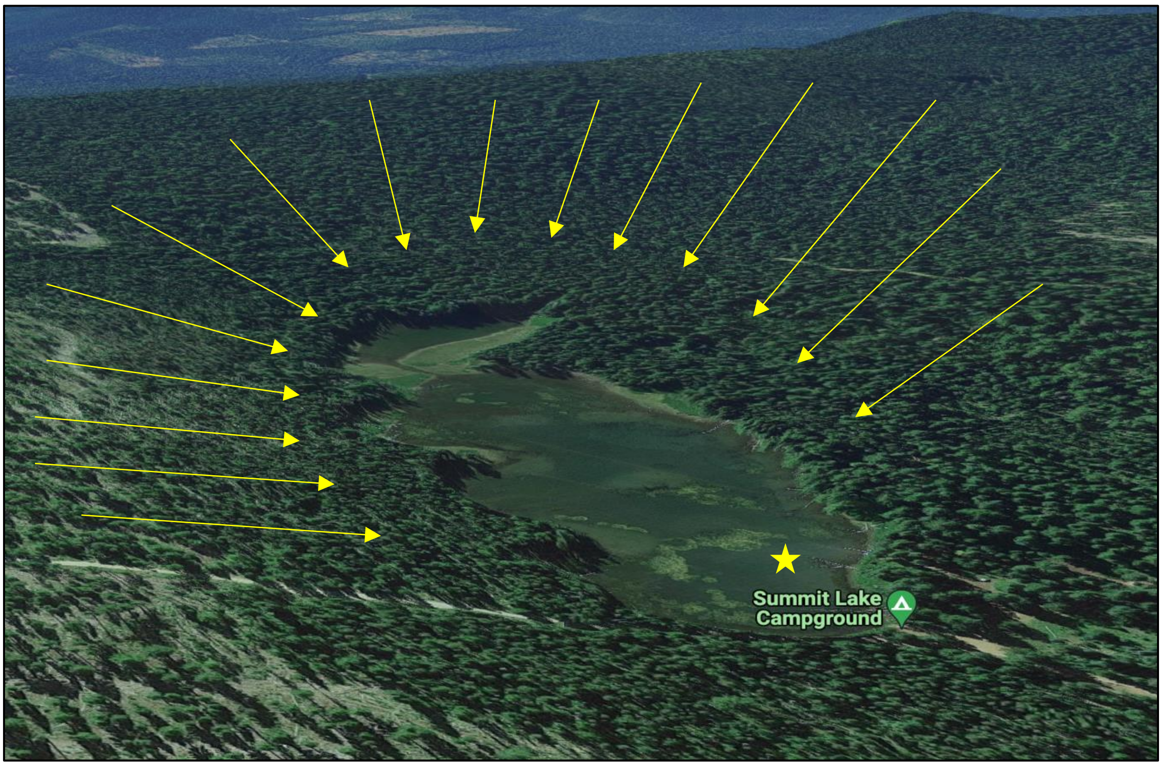


Fig. 4.5 A schematic of the Summit Lake drainage showing in-wash patterns which may explain the results from sedaDNA analyses.

2.1.3. Little Monon Lake

Current observations of Little Monon Lake show an area highly impacted by disturbance in the recent past. The devastating effects of the highly severe fire, which occurred in 2001, can be seen across much of the south of Little Monon Lake, with evidence of ongoing forest insect outbreaks across the slopes of Olallie Butte. The fossil charcoal record shows high fire activity at Little Monon Lake, suggesting fire has been a major disturbance agent in this catchment over recent centuries, further evidence by the fire record at Breitenbush Lake, in the adjacent catchment (Minckley and Long, 2016).

Despite such high insect activity observed at the site and within the ADS dataset, no positive amplifications occurred at any depth using the Dpond, Dpseudo, and Chorist qPCR assays. The age range of the surface sample at Little Monon Lake (1800 to 1898 AD) pre-dates the time period covered by the ADS survey dataset, therefore, there was no opportunity to compare expected forest insect outbreaks identified using the ADS dataset with any results from sedaDNA. The topography of the Olallie catchment (See Figure 4.7) suggests that had the most recent sediments been recovered, DNA from these outbreaks may likely have in-washed into Little Monon Lake from all directions. One hypothesis as to why no insect DNA was recovered could be linked to such high fire activity. The charcoal record for Little Monon Lake indicates high levels of fire between 2,500 yr BP and 600 yr BP, therefore, there is potential that frequent fires may have hindered the establishment of insect populations due a lack of suitable hosts. A deeper discussion on the interplay between fire and insects can be found in the Charcoal Discussion section below. The lack of results found at Little Monon Lake failed to extend our knowledge of past insect occurrence beyond the period associated with the ADS survey data.

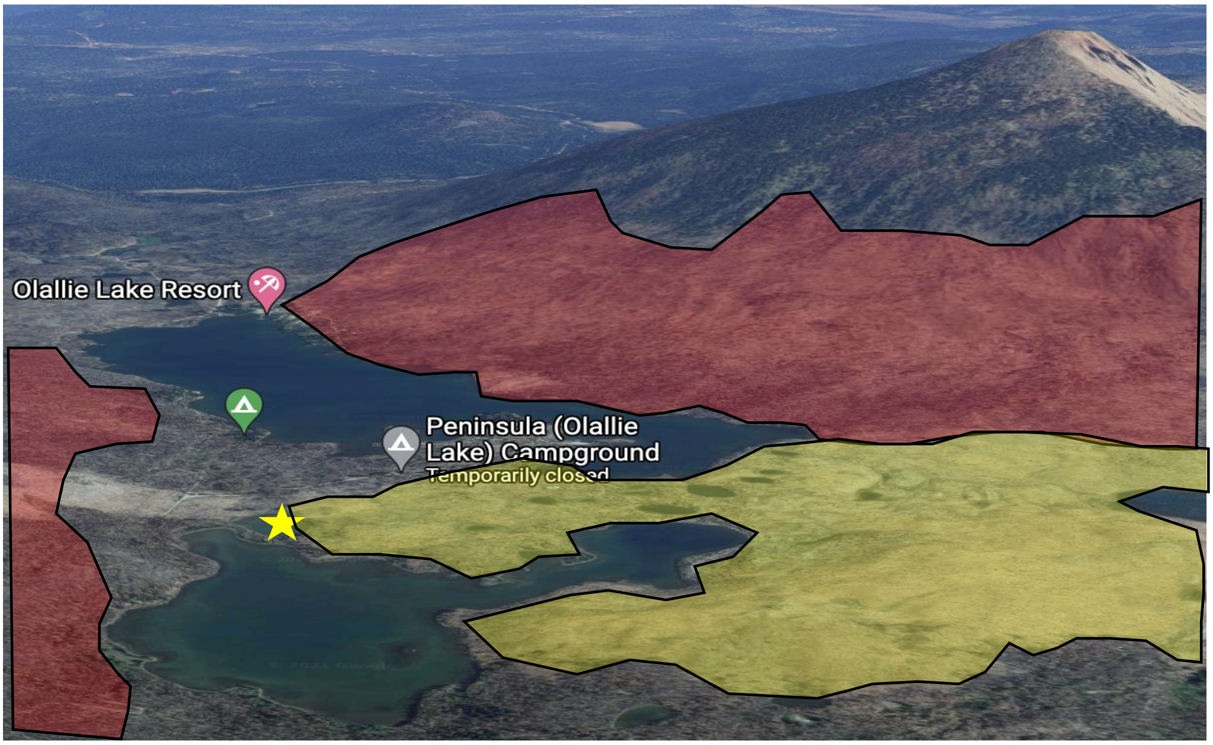


Figure 4.6. The location of Little Monon Lake in relation to *D. ponderosae* outbreaks (shown in red) obtained from ADS data, and the effects of the most recent fire at within the Olallie catchment (shown in yellow).

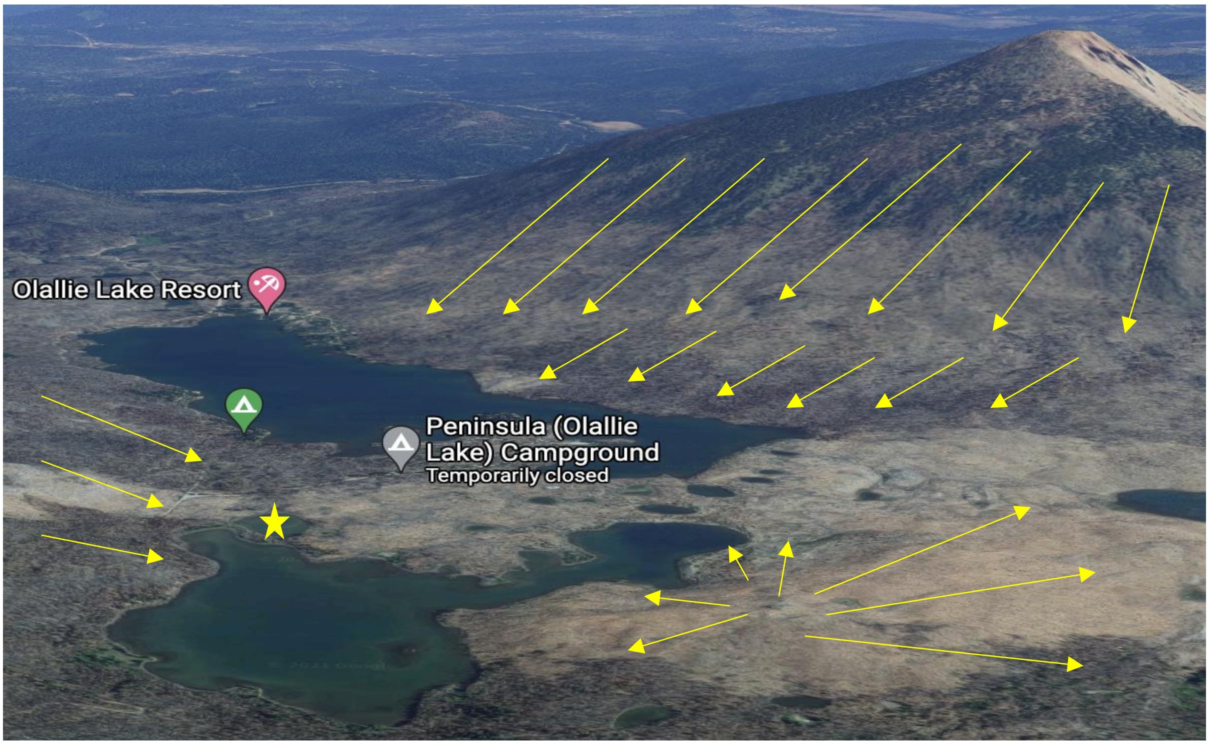


Figure 4.7. A schematic of the Little Monon Lake catchment, identifying potential sources of in-washed material.

In addition to the ADS dataset which spans the period of 1946 to 2017 (the year of sampling), the General Introduction contained a discussion of the current methods of detecting historic insect outbreak occurrence based on tree ring data, palynology, and taxonomically identified remains on longer timescales, beyond that of the ADS dataset. In each study, the authors calibrated signals within their respective proxy to identify characteristic signatures during known periods of outbreak. These signatures were then used to identify any period within the record which contained the same behaviour that suggest periods of outbreak. This facilitated a discussion on any correlation between the two set of positive amplifications observed at Pyramid Lake, obtained between 543 and 473 yr BP and 752 and 896 yr BP, of which there is no ADS data with which to compare results. A meaningful calibration requires a sufficient number of samples to be robust and useable. It was thought that basing a calibration on only two periods of outbreak failed to produce adequate calibration.

The earliest evidence of insect outbreaks in western North America as inferred by dendroecological methods was a *D. rufipennis* (spruce beetle) outbreak in 1716-1750 AD across British Columbia and Alberta, Canada (Eisenhart and Veblen, 2000). Therefore, evidence obtained from tree ring data does not cover a long enough timeframe with which to compare the results observed at Pyramid Lake. The records utilising lake sediments to conduct palynological analyses extend the length of insect reconstructions to the time periods associated with my sites. Reconstructions based on pollen analysis only detected insect outbreaks within the 20th Century (Morris and Brunelle, 2012; Anderson et al., 2010; Morris et al., 2010) with no further outbreaks detected in older samples. Evidence of historic insect outbreaks as inferred by taxonomically identified remains (Brunelle et al., 2008) have provided the longest reconstructions of outbreak occurrence, which span the entire Holocene. Evidence of bark beetle remains, most likely attributed to *D. ponderosae*, were recovered from 8331, 8410, and 8529 cal yr BP, along with two further early Holocene examples recovered from Hoodoo Lake, Idaho, dating to 7954 and 8163 cal yr BP (Brunelle et al., 2008). Therefore, these provide no additional information on the historic outbreak patterns observed at Pyramid Lake. The sedaDNA results presented here are most analogous with those of Brunelle et al., (2008), whereby insect remains were identified only from the early Holocene and in the 20th Century, with no evidence recovered in between these time intervals. It seems highly unlikely, given the abundance of suitable hosts and periods of warmer and drier climate, such as the MCA, that bark beetles have only been impacting the forests of western North America in the early Holocene and in the most recent few hundred years. Therefore, one highly important aspect of forest pest dynamics, which requires further understanding, is the behaviour of these species in between periods of outbreaks i.e., are species continuously present at low levels or do they only survive in areas where environmental conditions are suitable for supporting such high-level outbreaks? I believe the more likely scenario in this instance is that varying levels of outbreaks are almost continual and that current proxies are just not capable of detecting all levels of outbreak. As DNA-based methodologies are directly detecting the agent, not the effects of the agent, these are in theory the most sensitive method of identifying presence of beetles which could correspond to low-level populations as well as epidemics.

2.2. Factors affecting DNA recovery

Forest Service ADS data shows that Pyramid Lake, Monon Lake, and Summit Lake are situated in catchments which have been inundated with outbreaks of a wide range of forest pathogens at least for the last 75 years, yet an underwhelming recovery of target species DNA was observed at all sites. Palynological reconstructions from the region show consistent levels of suitable host species to support outbreaks large/severe enough to cause significant modification to the surrounding forest ecosystem, for at least 13,000 years (Minckley and Long, 2016). This section aims to address one of the fundamental questions within this thesis and within wider environmental DNA research: what are the controlling factors that ultimately affect the likelihood that DNA from target species is released into the environment, becomes incorporated into depositional environments, and is subsequently preserved, avoiding levels of degradation which prevent amplification and sequencing?

2.2.1. Species morphology

Most studies concerning the recovery of DNA from sediments have focussed on vertebrates and plants (Kisand et al., 2018; Kistler, 2012; Schlumbaum and Tensen, 2008; Willerslev and Cooper, 2005; Parducci and Petit, 2004; Willerslev et al. 2004; Pääbo, 1993), while the of recovery of DNA from insects has been disproportionately understudied, limited to museum specimens dating to only several hundred years old (Watts et al., 2006; Hartley et al., 2006; Harper et al., 2006; Goldstein and Desalle, 2003) or amber-encased specimens of millions of years old (Cano et al., 1992; Desalle et al. 1992). Therefore, the mechanisms of recovery of DNA from specimens between these two extremes, on the timescales concerned with most palaeoenvironmental reconstructions, requires further development (Thomsen et al., 2009).

Here, *D. ponderosae, D. pseudotsugae,* and *C. occidentalis* represented species with diverse morphological characteristics which posed different challenges for the preservation, and therefore, the potential for recovery of DNA. The two groups of insects targeted for DNA analyses within this thesis, bark beetles and budworms, have highly variable morphology and life history behaviours. Bark beetles have hard exoskeletons formed of chitin and spend the majority of their life history beneath the bark of host trees (Henriques et al., 2020), while *Choristoneura* are primarily formed of soft tissue with several chitinous components and spend the majority of their lives on the external tissue types of host trees (Volney and Fleming, 2007). As such, the characteristics of DNA released between these two groups are likely very different. The two primary sources of invertebrate DNA release into the environment are from moulting of exoskeletons at various life stages (Chequer et al., 2019; Deiner and Altermatt, 2014) or the post-mortem incorporation of individuals and/or their remains into depositional environments. The release of DNA into the environment, or shedding, is controlled by physical properties of the target species, such as body-type, and/or their behaviour, such as flying (Andruszkiewicz Allen et al., 2021). As most of the literature concerning mechanisms for DNA shedding to the environment has focussed on fish or amphibians (Brantschen et al., 2021; Kirtane et al., 2021; Ratcliffe et al., 2021; Trimbos et al., 2021; Jo et al., 2019; Hänfling et al., 2016) and there has been very little research into the shedding behaviour of insects (Trimbos et al., 2021), this work represents an important insight into the DNA shedding of insects into sediment.

In general, the fossil record is biased towards harder organisms, or harder parts of organisms, such as skeletal remains, as soft tissues typically decay before they are fossilised (Anderson et al., 2018). This likely has implications on the preservation of different tissue types within palaeoenvironmental samples. In the DNA extractions, whole specimens of *D. ponderosae, D. pseudotsugae,* and *C. occidentalis* consistently yielded higher levels of DNA than head and elytra and leg tissues. Within these extractions, those of whole *Choristoneura* spp. yielded levels of DNA double those obtained from both whole adult *Dendroctonus* species, which highlights how the soft body type of *Choristoneura* could result in greater shedding of DNA into the environment and, therefore, increase their potential for detection. The chitinous exoskeleton of bark beetles likely ‘encases’ the soft tissue more likely to leach DNA within this exoskeleton which reduces the amount of DNA leached into the environment. This highlights how, should insect remains become incorporated into sedimentary deposits, the morphology of the target organism likely plays a key role in the probability of detection via

DNA-based methodologies. This theory is further evidenced by leg tissues having the lowest yields in the body part extraction experiment i.e., small, chitinous structures with very little soft tissue within. Thomsen et al., 2009, highlighted that insect groups other than beetles may yield higher levels of DNA as their chitinous exoskeleton is less thick and resilient. Of the very few examples of bark beetle remains successfully recovered from palaeoenvironmental samples, the most commonly preserved body parts consist of head and elytra tissues (Brunelle et al., 2008). Here, despite their 'hard' chitinous nature, these tissue types yielded moderate levels of DNA (46.2 ng/µl *D. ponderosae* and 19.5 ng/µl in *D. pseudotsugae*) during the body part extraction experiment, suggesting that if they are deposited, then they should provide levels of DNA sufficient to be detected by DNA-methodologies.

2.2.2. Biomass

Environmental DNA or sedimentary DNA is typically comprised of a complex mixture of DNA from a wide range of organisms (Taberlet et al., 2018), therefore, we must also consider how the total biomass from multiple individuals or populations of the target organisms coupled with the abundance of signals from non-target species may impact on DNA methodologies. With the exception of two notable examples presented by Brunelle et al., 2008, the majority of beetle remains successfully recovered from palaeoenvironmental samples were from large-scale, widespread outbreaks of the 20th Century, whereby masses of beetles are known to have accumulated (Morris et al., 2015; Eisenhart and Veblen, 2000). This suggests high concentrations of beetles becoming incorporated into depositional environments is rare. Another component of DNA deposition concerns the differences in the life histories of target pests (Barnes and Turner, 2016). Bark beetles spend the majority of their life beneath the bark of host trees, only leaving for a brief flight to a new host. As such, the period of time by which beetles are freely in the environment and can become incorporated into lake sediments is disproportionately limited.

Bark beetles can only survive for several day to a few weeks outside a host tree due to energy depletion and/or predation (Buyers and Löfqvist, 1989; Pope et al., 1980). As such large masses of bark beetles outside of the safety of the host tree is likely a rare occurrence. This may explain why examples of intact adult bark beetle accumulations within lake sediments are rare (Sturtevent, et al 2013; Morris et al., 2015; Schmid and Frye, 1977). In comparison, budworms spend their life history feeding on external plant tissues (needles, cones, and seeds). As such, they are more exposed to the environment and therefore the chance of them becoming incorporated into lake sediment is likely higher than bark beetles (Girona et al., 2018).

In addition to the biomass of the target species we must consider the biomass of the non-target species which are also contributing the DNA signatures. The forests of the Pacific Northwest are some of the most productive of any temperature ecosystem and palynological reconstructions from this region show constant and persistent dense coniferous forest ecosystems since the Last Glacial Maximum, with overmature, dense stands becoming common as a result of fire suppression throughout the 19th and 20th Centuries (Minckley and Long, 2016; Fettig et al., 2007). Therefore, we must consider the levels of terrestrial and lacustrine vegetation biomass contributing to lake sediment deposits, which ultimately determine the levels of total DNA (Barnes and Turner, 2016). The matK assay (Kim et al., 2018) demonstrated that vegetation DNA could be recovered from all sites. However, as levels of eDNA have been shown to correlate with biomass (Barnes and Turner, 2016; Bohmann et al., 2014) the low levels of target DNA detected here could be due to the dense vegetation found in the immediate vicinity of all sites overwhelming target species DNA. This has been shown to occur in other studies of plant-sourced DNA (Pedersen et al., 2013), whereby a high abundance of dominant taxa overwhelmed the presence of rarer taxa, despite their presence confirmed with macrofossil analysis.

2.2.3. Taphonomy and the DNA 'catchment'

Studies aiming to identify the DNA 'catchment' suggest the source area for DNA input is directly correlated with the hydrological catchment, and sites whereby processes such as overland flow, in wash, and run off occur, produce DNA records more closely affiliated with pollen/macrofossil data (Edwards, 2020; Alsos et al., 2018). Sites with minimal in/out wash of allochthonous material incorporated into the sediments via these taphonomic processes tend to overrepresent species from within lake sources (Parducci et al., 2019). The understanding of the taphonomic processes associated with pollen and macrofossil analyses have become increasingly sophisticated over the last 50 years as the mechanisms of production, dispersal, representation, and preservation have been increasingly considered (Wilmshurst and McGlone, 2005; Bennett and Willis, 2002; Campbell, 1999; Sugita, 1993; Pennington, 1979). Evaluations of how the size, geography, and catchment characteristic of a lake site affect DNA recovery has not been explored experimentally (Parducci et al., 2019). To attempt to quantify the DNA catchment area of plant material, Alsos et al., 2018, used metabarcoding of the upper 2cm of lake sediments at 11 sites across Norway and compared the results to plant surveys conducted in the vicinity of the lake. Plants found less than 2m from the lake edge were detected 73% of time, which declined to just 12% of plants identified at 50m from the lake edge. While this study concerns plants and not more mobile species such as forest insects, it tends to suggest that DNA signals are more akin to macrofossil remains, producing more localised representations. This poor understanding of DNA taphonomy has attracted great criticism (Birks and Birks, 2016). It is expected that the development of sedaDNA methods will help this field undergo similar studies on the underlying principles of taphonomy, adding to the authenticity and validity of future results. The greater number of potential positive amplifications from Pyramid Lake than at Summit Lake further highlights the role that topographic features within the catchment may play in determining the source area for DNA.

2.2.4. False negatives

One drawback of sedaDNA-based methodologies is the occurrence of both true and false negatives (Burian et al., 2021). The results from the sedaDNA analysis highlight the potential for the occurrence of both of these types of results. A well-designed assay with high primer efficiency and low limits of detection, such as those generated within this thesis, imply that if no DNA from target species is detected, we can have a good degree of confidence that the target is not present, although (as for any sampling methods) the uncertainty of negative results in higher.

The date attributed to the 8mm sample at Pyramid Lake ranges from 1867-1918 AD, suggesting the 4mm and 6mm samples are more recent and could, therefore, coincide with ADS data. However, there were no positive qPCR amplifications for any target species within the 4mm and 6mm samples, despite a great number of *D. ponderosae* outbreaks documented within this time period. ADS data shows that Summit Lake has been completely decimated by *D. ponderosae* outbreaks for the entirety of the survey period. Outbreaks occur in nearly every decade of the record and in close proximity to the lake, however, no *D. ponderosae* was detected using the Dpond protocol. The surface sample at Little Monon Lake dates to 1800-1898 AD and, therefore, predates the beginning of the ADS dataset. In addition, no positives were identified at any depth using the Dpond, Dpsuedo, or Chorist qPCR protocols at Little Monon Lake. As such, our knowledge of historic pest outbreak occurrence beyond the timeframe of the ADS survey remains limited. Fossil pollen records from the nearby Breitenbush Lake (Minckley and Long, 2016) show this area has been continually forested by suitable host species which could support beetle populations. Fossil charcoal records reveal Little Monon Lake has been subjected to near constant fire throughout its' history. However, as there is no ADS data for this time period, it is difficult to assess whether this negative is true or false.

To date, the greatest levels of DNA recovery has been from frozen or near frozen, anaerobic, pH neutral, and/or dry sediment (Edwards, 2020; Parducci et al., 2019). As a result of high elevation, there are great seasonal temperature fluctuations at the three sites examined in this study, ranging from below freezing during winter months to above 40°C during the summer months. While these conditions are not necessarily experienced on the lakebed, with most literature suggesting that the sediment surface is always exposed to the coldest waters (Parducci et al., 2019; Sobek et al., 2009), it can't be discounted that seasonal fluctuations in temperature of the surface sediments may have been detrimental to DNA preservation (Edwards, 2020). Therefore, our qPCR protocols were intentionally designed to amplify short fragments, of ~85 bp in length, which proved long enough to be species-specific but short enough to allow for DNA degradation with time or from poor preservation conditions (Saito, 2021; Wei et al., 2018).

One further potential reason for a lack of additional positive amplifications within my sites concerns the introduction of several potent PCR inhibitors commonly found within lake sediments (Schrader et al., 2012). Humic acids, salts, and proteins have all been shown to delay PCR and qPCR amplification resulting in erroneously low estimates of DNA abundance and/or false-negative results (Schrader et al., 2012; Lloyd et al., 2010; Yeates et al., 1997; Kreader, 1996). During as assessment of the potential introduction of PCR inhibitors at my sites, Summit Lake was the only site whereby samples containing no eDNA amplified in the same cycles as those containing eDNA. Samples containing eDNA at both Pyramid Lake and Little Monon Lake amplified later. This suggests a certain degree of inhibition at Pyramid Lake and Little Monon Lake, although likely not sufficient levels of inhibition to completely avoid amplification. In fact, while Pyramid Lake contained the 'highest' level of PCR inhibition according to the dilution test, however, two sets of positive amplifications were recovered from this site. No positive amplifications occurred at Little Monon Lake which had moderate levels of PCR inhibition. There was, therefore, no correlation between sites that had positive amplifications within sedaDNA and low levels of PCR inhibitors. A crucial step within designing PCR/qPCR assays from sedaDNA should check for potential PCR inhibition before increasing the concentration of template eDNA within the PCR/qPCR reaction, as one would think they are increasing the abundance of target species, but they could also be increasing the abundance of PCR inhibitors. (Schrader et al., 2012)

2.2.5. DNA degradation

Perhaps the most important discussion that arises from the results observed in the DNA section of this thesis concerns the effects of cellular degradation on the recovery of DNA derived from environmental samples (including eDNA, sedaDNA, aDNA) as this is still a key component of this field that requires further understanding. Several potential positive amplifications of *D. ponderosae* were obtained from Pyramid Lake. These products incurred a consistent Tm but one that was ~1.6-2^{o}C higher than reference tissue for *D. ponderosae*. In addition, Sanger sequencing failed to produce sequences of sufficient quality to confirm the identify of these positive amplifications. Initially, it was concluded that these positive amplifications must belong to an unsequenced, closely related species of *D. ponderosae*, however, I believe these results can be explained by exploring the characteristics of DNA degradation associated with historic samples. Upon death of an organism, the processes associated with cellular repair no longer occur. With regards to the retrieval of DNA from historic or ancient sample, this degradation manifests itself in three primary ways: sequence fragmentation or shortening, the occurrence of damaged regions which block polymerase replication, and/or nucleotide modifications which lead to the misreading of DNA by the polymerase (Dabney et al., 2013). The greater the period between death and retrieval of DNA or unfavourable depositional conditions both increase the likelihood of such degradation and drastically reduces the chances of recovering DNA of sufficient quality to analyse (Pääbo, 1989). Consequently, these factors become a particular concern within studies concerning historic samples. In the context of the results observed at Pyramid Lake, sequence modification could have led to nucleotide changes within the product, resulting in a slightly different melting temperature to that of modern reference material. In addition, deamination has been shown to produce high levels of baseline noise in various sequencing platforms (Chen et al., 2014; Hofreiter et al., 2001) explaining the inability to generate sufficient sequences to fully confirm that these positive amplifications are associated with the target organism.

These uncertainties highlight one of the major limitations associated with environmental DNA and further research needs to be done in order to fully understand the effects degradation has on the ability to recover and study DNA from historic samples. Despite this, I believe the results outlined in this thesis demonstrate the rapid advancement of our understanding of such limitations and the final section of the discussion aims to present the potential role of DNA-based methodologies in addressing palaeoecological questions.

2.3. The potential role of DNA-based methodologies in palaeoecology

The earliest studies utilising sedaDNA are less than 25 years old (Coolen and Overmann, 1998), with most of the major developments concerning these methods occurring only in the last 10 years (Crump, 2021; Edwards, 2020; Pedersen et al., 2013). This study has presented just one method of addressing sedaDNA-based questions, a SYBR Green qPCR assay, which is highly suited to assessing the presence of single species or small groups of known targets such as the *Dendroctonus* spp. bark beetle presence across the Pacific Northwest. This technique has been shown to have higher sensitivity in detecting rare taxa than other DNA-based methodologies (Harper et al., 2018) and was chosen as the most appropriate method of addressing the research questions within this study. It must be highlighted that qPCR is just one option within the array of DNA-based methodologies and that other methods may be favoured, dependent on the objectives and targets of each individual research study. Methods involving high-throughput sequencing (HTS) such as DNA metabarcoding can be used in conjunction with sedaDNA to provide information on whole communities (Hatzenbuhler et al., 2020; Singer et al., 2020; Harper et al., 2018; Deiner et al., 2017). Future studies of identifying historic disturbance by biological agents maybe benefit from the use of these HTS techniques to provide further information on how populations of taxa change through time and how these changes coincide with changes inferred from other proxy datasets.

The final section aims to summarise the lessons learned from within this thesis and the wider literature to outline the key advantages of DNA-based methods, which further emphasise their applicability and potential role within palaeoenvironmental studies, with an emphasis on sedaDNA.

Direct detection of the agent(s)

This thesis has demonstrated that most existing methods of detecting historic insect pathogen outbreaks rely on the indirect detection of the agent, observing the effects they inflict on the environment, rather than the agent themselves. As such, these signals need to be of a great enough magnitude to significantly alter the forest communities and produce an ‘outbreak’ signature within the chosen proxy. This has led to an overall bias within the literature towards the detection of highly severe, large-scale outbreaks. The greatest advantage of DNA-methods is that they involve the direct detection of the agent or agents i.e., if DNA from the target species is recovered, we can confirm the presence of that species, in that location, at that point in time. As we are directly detecting the agent, it is possible that they can be detected at lower abundances than those observed indirectly, as we are not reliant on the production of outbreak signatures in other proxy datasets. Methods of direct detection have been developed for a wide range of species and sources (Le Blanc et al., 2020; Harper et al., 2018; Rees et al., 2014; Xavier et al., 2010; Ficetola et al., 2008; Whiteman et al., 2002;) and are becoming a readily used technique within palaeoenvironmental science. This thesis has generated several robust, highly specific qPCR protocols for the direct detection of *Dendroctonus ponderosae, Dendroctonus pseudotsugae,* and *Choristoneura* spp. within the Pacific Northwest region, with promising results, which we believe will serve as a highly valuable tool in assessing their current and historic abundance and deepening our understanding of forest pest dynamics.

Flexibility in assay design

Protocols can be designed to target single species (as per the Dpond and Dpseudo protocols), multiple species, genera (as per the Chorist protocol), or even families, with primer design being the controlling factor on the specificity of amplicons. Primers within multiplex assays are designed to amplify the same region in multiple species whereby there is variation in the amplicon (Wood et al., 2020). High-resolution melting (HRM) can be used, in conjunction with positive controls for target species, and sequencing, to confirm presence within the sample (Minett et al., 2021; Robinson et al., 2019; Robinson et al., 2018). Study sites whereby species presence and abundance are largely unknown, such as *Choristoneura* spp. across Oregon and Washington, may opt for a genus-specific primer assay which can detect all species within those genera.

High sensitivity

Limits of detection (LOD) refer to the lowest concentration of template that can be detected (Klymus et al., 2020) and are used as a measure of the sensitivity of assays. This is particularly useful when remains are too poorly preserved for taxonomic identification. As well as limits of detection, high sensitivity can also refer to the identification of samples to the lowest taxonomic group, which has proven particularly advantageous when reconstructing floras. Due to morphological limitations, pollen assemblages are often restricted to genus or family level

(Birks and Birks, 2016) with very few taxa routinely identified to species and sub-species level. Much work (Alsos et al., 2018a; Alsos et al., 2018b; Edwards et al., 2018; Willerslev et al., 2014; Bennett and Parducci, 2006 and) has been conducted on increasing the resolution of several key taxa (Poaceae, Cyperaceae, *Pinus, Alnus, Betula*) to provide more detailed palaeoenvironmental reconstructions through the identification of more taxa to species level, either via increased abundance of reference sequences or more thorough filtering processes. These advancements facilitate more comprehensive assessments as the different species within a genus may require varying conditions which help to build a picture of the environmental conditions.

DNA as an additional 'proxy'

The methods associated with DNA recovery (i.e., coring, subsampling, sampling strategies) are near identical to existing palaeoecological methods that analyse charcoal, pollen, macrofossils, tephra, etc. Samples obtained for DNA analysis are the same, for example, as bulk sediment samples taken for radiocarbon dating (Bennett and Parducci, 2006), the only difference is that sterile conditions when coring and subsampling must be maintained to limit contamination. The protocols outlined in this thesis obtained material for radiocarbon dating, charcoal, DNA, and tephra, utilising the same methods of coring and subsampling. Additional proxies such as pollen and macrofossils could also have been sampled to provide additional ecological information. When correct sedaDNA sampling methods are followed, samples intended for DNA-based methodologies are of no greater risk of contamination that any other type of analyses (Edwards, 2020). Studies which utilise molecular ecology and palaeoecology techniques have shown clear benefits in reconstructing environmental change (Parducci et al., 2019). DNA, therefore, can be considered as an additional proxy source of information within existing multiproxy approaches (Dzombier et al., 2021; Polling et al., 2021; Parducci et al., 2020; Parducci et al., 2015).

Detection in modern and palaeoenvironmental samples

This thesis was concerned with the reconstruction of historic insect outbreaks. However, the qPCR assays designed for *D. ponderosae, D. pseudotsugae,* and *Choristoneura* spp. could readily be used in a biomonitoring capacity in modern samples. For example, downstream sampling to detect outbreaks at higher elevations, which has successfully been achieved in riverine environments (Muha et al., 2019; Wacker et al., 2019; Pont et al., 2018; Deiner and

Altermatt, 2014) or the metabarcoding of surface samples across a wide spatial area. This drastically reduces the cost of traditional surveys and facilities the measurements of species abundances in areas too dangerous to sample by traditional means (Crookes et al., 2020). The designing of primer sets to amplify short fragments accommodates degradation in older samples, allowing the same protocols to be used in both modern and palaeoenvironmental samples (Opel et al., 2006). DNA-based protocols are readily use in modern samples, to provide low effort, non-destructive methods of assessing biodiversity, and in a reconstructive capacity, to assess late-Pleistocene and Holocene environmental change. However, to our knowledge, there are no current examples of a qPCR protocol specifically designed to assess the abundance of a species in both a modern and reconstructive capacity. Studies show that DNA-based methodologies can span the timeframes concerned with most palaeoenvironmental reconstruction, with DNA successfully retrieved from terrestrial sediments aged 400 ka BP (Willerslev et al., 2003; Lydolph et al., 2005) and from frozen environments aged 800 ka BP (Willerslev et al., 2007). These methodologies are seemingly limited by degradation of DNA which varies by source and environmental conditions post deposition.

The ability to detect all life stages of the target organism

Some species are typically only distinguishable as adults, and even then, species-specific distinction is not always possible as adults, even in modern specimens (Pochon et al., 2013). Therefore, methodologies which can detect and identify specimens at larval or instar stages provide results with higher resolution than those identified taxonomically (Andruszkiewicz Allan et al., 2021; Deiner et al., 2017; Parducci et al., 2017).

The ability to detect samples too poorly preserved for taxonomic ID

DNA-studies do not rely on levels of preservation required to taxonomically identify morphological characteristics. Instead, if DNA is present, it can, in theory, be detected (Deiner, 2017). Degradation can be attributed to a wide range of factors, including age, environmental conditions, or other biological processes. Morris et al., 2015, highlighted that one possible reason for the lack of Holocene bark beetle remains could be that carcasses are eaten by fish before they become incorporated into sedimentary archives. The use of DNA-based methodologies implies that even if a beetle has been eaten by fish, or a tree containing beetle remains falls into lake, its DNA could still become incorporated into lake sediment, and detected. Several reviews have also highlighted a measurable decline in taxonomic expertise and identification skills across many fields (Oliveira et al., 2011; Boonham et al., 2008;

Hopkins and Freckleton, 2002). While this is a growing concern within paleoenvironmental science, and one that needs addressing, the advantages of DNA-based methodologies is that they do not require any prior knowledge of the target species (Deiner et al., 2017).

Standardisation of protocol

All aspects of DNA-based methodologies, including extraction protocols, primer sets, PCR conditions, number of replicates, could all in theory be standardised (Loeza-Quintana et al., 2020), whether through metabarcoding, single, or multiplex assays, the creation of named/standardised protocols for species or groups of species. This would also address a major disadvantage of palaeoecological science outlined in the General Introduction, that a lack of standardised protocols and thresholds for disturbance, fail to facilitate the fair comparison of results across studies and/or sites. While a drive for standardisation of DNA methodologies is currently ongoing (Loeza-Quintana et al., 2020; Harper et al., 2018), it seems that the abundance of variables associated with each research study (target species or group of species, sample type, budget) may limit the potential for standardisation. Other paleoenvironmental methods, such as pollen analysis, have become standardised in their preparation methods (see Moore, 1991) and it is hoped that as the field develops, we can work towards some level of standardisation as seen in other areas of the field.

2.4. DNA conclusion

DNA-based methodologies have revolutionised the field of Quaternary science through the retrieval of target species DNA from a wide range of depositional environments, overcoming the need for sufficient levels of preservation and taxonomic skills required for species identification from macrofossils. The protocols designed within this thesis have both high-specificity and low limits of detection, yet an underwhelming number of positive amplifications were obtained. Only two sets of potential positive amplifications occurred prior to the period covered by the ADS dataset whereby the Dpseudo protocol identified several positive of *D. pseudotsugae* at Pyramid Lake between 543 and 473 yr BP and the Dpond assay identified several positives of an unsequenced species at Pyramid Lake ranging in age between 752 and 896 cal yr BP. Despite an overwhelming consensus in the literature that current outbreaks of *Dendroctonus* bark beetles are unprecedented and that the habitable range suitable to supporting such large insect populations is increasing due to climate change (Morris et al., 2015), it seems highly unlikely, given the abundance of suitable host trees across all of the sites chosen within this thesis, that insect outbreaks have only been impacting these sites for the last Century and for a brief period between 752 - 896 and 543 - 473 cal yr BP. It is therefore more likely that some aspect of the site selection, sampling methodology, or DNA protocols, led to a failure to detect insect outbreaks throughout the time periods associated with this study, emphases by the much more closed catchment of Pyramid Lake experiencing higher levels of DNA recovery.

It has been highlighted in the literature (Dickie et al., 2018; Klymus et al., 2020) that the fields of eDNA, aDNA, and sedaDNA research are still in their infancy, therefore, the reporting and discussion of negative results such as these are integral in bettering our understanding of the mechanisms and behaviour of sedaDNA signals. Despite an overall lack of positive amplifications, it must be highlighted that there have been several key lessons learned with regards to the catchment dynamics and source areas of DNA, notably the identification of key factors which influenced the detection of *D. pseudotsugae* within the surface sample at Pyramid Lake while a nearby outbreak of *C. occidentalis* was not detected. In addition, the newly designed qPCR assays for *D. ponderosae, D. pseudotsugae* and *C. occidentalis* all show great potential in the detection of their respective targets' DNA with a wide range of environmental samples.

As discussed in the beginning of this chapter, the original aim of this study was to produce a pollen-based calibration of known insect disturbance events obtained from ADS survey data. To limit the potential for human activity influencing the interpretation of this calibration, higher elevation sites were favoured, which seemingly led to much lower sedimentation rates expected and to those found at lower elevation sites. Building upon the deeper understanding of sedaDNA behaviour obtained within this thesis, future studies, at lower elevation sites, with higher sedimentation rates may provide better sampling resolutions and time intervals with which to further develop these methodologies.

3. Charcoal

3.1. BackgroundCHAR *and* peakCHAR

Minckley and Long et al., 2016 and Tweiten et al., 2009, have both highlighted that direct comparison of fire episodes between studies is problematic due to the differences in temporal resolution of each record type, namely, the annual resolution of tree-ring data versus sedimentary methods which analyse periods of time dictated by the subsampling strategy. One novel set of analyses performed on the raw charcoal data was a decomposition of the data into a simulated annual resolution using age-depth models to ascribe CHAR values between known points of data. This facilitated the application of varying sizes of continuous running means to identify levels of *backgroundCHAR* and the calculation of mean fire return intervals comparable with those obtained from annual dendroecological methods. This resulted in a high-resolution analysis strategy which could be widely implemented using the data already typically used for fire reconstructions i.e., raw charcoal counts, CHAR, and age-depth models.

Methods used to calculate *background CHAR* typically implement running means with window sizes of between 500 to 1000 years based on sampling intervals of 1cm (Minckley and Long, 2016; Minckley et al., 2007; Millspaugh et al., 2000; Long et al., 1998; Cleveland, 1979). When windows are too small, *backgroundCHAR* tends to track *peakCHAR* too closely which can lead to a lack of distinct peaks and an erroneous estimate of the number of total fire events. This highlights a balance that must be considered between only detecting high severity events and producing records which class the entire series as a fire event due to the similarity in both *backgroundCHAR* and *peakCHAR* (Long et al., 1998). The above parameters are based on a sampling resolution of 1cm, therefore, due to the high-resolution 2mm sampling methodology used throughout this thesis, a range of 200-yr, 150-yr, and 100-yr running means were tested to provide comparable versions of the parameters outlined above.

Fire events were identified whereby the ratio of *peakCHAR* to *backgroundCHAR* exceeded a certain value, deemed the ‘fire event’ threshold, which is a measure of when the peak component exceeds the background component within the dataset. There is no agreed upon threshold for the identification of fire events within the literature. Millspaugh and Whitlock (1995), developed a calibration of an event threshold based on the parameters that would identify periods of known fire obtained from dendroecological records, however, no tree ring data was not available for this study and could not be used to calibrate the event threshold in my samples. Instead, it was the analysis of Long et al. (1998) which aided the selection of the event threshold parameter for this study. An analysis of Little Lake, Coast Range, Oregon, (210m a.s.l. in the *Tsuga heterophylla* Zone), tested various events thresholds (1, 1.05, 1.12, and 1.2), with *backgroundCHAR* calculated using a 600-yr running window (Long et al., 1998). These various events thresholds were applied to a 9,000-year record of CHAR at Little Lake, and detected 18, 14, 8, and 5 peaks, respectively. Despite identifying the greatest number of events, both the 1 and 1.05 indices were dismissed, as they produced mean fire return intervals of 110 years, or less, which they deemed unlikely considering the typical large, infrequent stand-replacing fire behaviour of the moist temperate *Tsuga heterophylla* forests of the Coast Range and several published fire reconstructions in a similar vegetation zone (Agee, 1993; Agee, 1990). In contrast, the threshold of 1.2 identified only 5 events which included a 1,700 ‘fire-free’ period, which they also considered unlikely for this vegetation type. Consequently, a threshold of 1.12 was chosen as these parameters reflected the expected fire regimes based on current composition and behaviour of these temperate forest ecosystems. A criticism of this method could be how appropriate it is to base the parameters for reconstruction on the most recent behaviour or current forest composition, but palynological reconstructions of this area (such as Long et al., 1998), show very little change in specues composition and abundance which justifies the choice of such parameters.

To assess how these varying event indices affected the number of events identified within my data, the same event thresholds of 1, 1.05, 1.12, and 1.2, used in Long et al., (1998), were applied to the CHAR records for Little Monon Lake, Pyramid Lake, and Summit Lake, using the 100-yr running mean as a source of *backgroundCHAR*. There was a great disparity in the results using the range of 1 to 1.2 as events thresholds. There was a 62% reduction in events identified at Little Monon Lake (65 events where index = 1 versus 25 events where index = 1.2), a 32% reduction in events identified at Pyramid Lake (79 events where index = 1 versus 54 events where index = 1.2), and a 55% reduction in events identified at Summit Lake (72 events where index = 1 versus 33 events where index = 1.2). While detecting the ‘highest number’ of events does not necessarily reflect the highest quality reconstruction, an event threshold of 1 was chosen for this study for several reasons. Firstly, the abundance of fire reconstructions from this region show that fire is a common disturbance process within the Cascade Range and fire intervals are common in this area (Walsh et al., 2010; Long et al., 2007; Briles et al., 2005; Weisberg et al., 2003; Long et al., 1998; Teensma, 1988). Second, the vegetation types of the sites chosen within this thesis, such as the nearly pure stand of *P. contorta* at Little Monon Lake, experience both episodic high severity stand replacement fires, as well as frequent, small-scale fires, and too high an event threshold would fail to detect the latter of these fire types, skewing our understanding of total fire events. Thirdly, the mean fire return intervals produced using a threshold of 1 were comparable to both published reconstructions at nearby sites and in reconstructions of similar vegetation types, which was a key component in the decision making of Long et al., 1998. For example, the mean fire return interval for Little Monon Lake, the site dominated by nearly pure *P. contorta* stands, were calculated as 36.6 years, which compares to a range of 22 to 67 years across similar sites in

Montana, USA, Wyoming, USA, and Alberta, Canada (See Table 4.4). And finally, it seemed counterintuitive to produce such a high sampling resolution and fine running mean and then choose a high event threshold which would only detect large events as this would contradict one of the key themes within this thesis, which is to readdress the imbalance within the published literature of a bias towards the detection of large-scale, high severity events. Instead, we chose a methodology that would result in the more accurate detection of fire events at all levels within the fossil charcoal assemblage, including small, less severe, or more localised events.

3.2. Fire return intervals

Accurate methods of identifying fire return intervals are integral to understanding fire regimes, stand composition, structure, and population demographics (Ford et al., 2010; Cwynar, 1987). Mixed-aged stands occur as a result of low to medium severity fires as only groups of trees within the stand burn, while single-aged stands occur from stand replacement fires, whereby the entire stand burns (Morrison and Swanson, 1990). As stands age they become more flammable, due to increased density, over maturation, or insect attacks that leave large areas of standing dead trees (Lotan et al., 1988), therefore, longer fire intervals fires tend to result in high severity fires when they do burn. The novel use of the simulated continuous record to produce return fire intervals resulted in fire return intervals comparable to those obtained using traditional dendroecological method with annual resolution (See Table 4.4). *Pinus contorta* (lodgepole pine) is relatively abundant across eastern regions of the Pacific Northwest (Lotan et al., 1985) with its' presence attributed to the repeated interruption of successional patterns by fire, leaving pure stands of fire-dependent *Pinus contorta* (Perry and Lotan, 1979; Brown, 1975). Fire dynamics within *Pinus contorta* forests are described as 'all or nothing', occurring as either smouldering, creeping burns across the forest floor, or rapidly moving, intense, stand replacement crown fires (Lotan et al., 1995). A rapidly growing, fire-tolerant species, *P. contorta*, usually capitalises on these intense fire events to become dominant species. Little Monon Lake, and the surrounding Olallie catchment, is an example of an area characterised by pure *P. contorta* stands. Published fire reconstructions from pure *P. contorta* stands in the Rocky Mountains (Tande, 1979; Arno, 1976; Gabriel, 1976; Loop and Gruell, 1973, Day, 1972) calculated mean fire return intervals from tree ring data which range between 22 and 67 years. The mean return interval for Little Monon Lake, as calculated by the simulated annual resolution method using a running mean of 100-yrs and an event threshold of 1.0, ranged from 22.8 years in the period 1,499 – 1,000 yr BP to 83.2 years in the most recent 500-year period, with an average for the entire record of 36.6 years (See Table 3.6 in Results and Table 4.4 below), making these results comparable with published reconstructions. The mean fire return interval for Pyramid Lake and Summit Lake were less similar to published reconstructions of similar ecological zones (Minckley and Long, 2016; Long et al., 1998; Morrison and Swanson, 1990). The records for Pyramid Lake and Summit Lake suggest a mean fire return interval of 41.3 and 22.4 years, respectively, compared to a range of 95 to 280 years found in other mixed conifer sites in the Coastal Range and Cascade Range. Pyramid Lake, Summit Lake, Cook-Quentin Study Area, Deer Study Area (Morrison and Swanson, 1990), Breitenbush Lake (Minckley and Long, 2016), and Little Lake are all mixed conifer stands in the Cascade Range and Coastal Range of Oregon (Long et al., 1998). As these are mixed conifer stands, fire behaviour of each area will vary by the abundance of each conifer species and their respective fire tolerance and behaviour, leading to a greater variation in return intervals that in pure stands, whereby fire behaviour of single species can be more predictable. This suggests it may be less straightforward to determine measurements of mean fire return intervals in mixed conifer stands than in stands dominated by single species.

	FRI	**Dominant vegetation**	**Reference**
Little Monon Lake, OR	36.6 years	*P. contorta*	This study
Pyramid Lake, OR	41.3 years	Mixed conifer	This study
Summit Lake, OR	22.4 years	Mixed conifer	This study
Bitterroot Valley, MT	22 years	*P. contorta*	Arno, 1976
Jasper National Park, AB	27 years	*P. contorta*	Tande, 1979
Bob Marshall Wilderness, MT	40 years	*P. contorta*	Gabriel, 1976
Jackson Hole, WY	50 years	*P. contorta*	Loop and Gruell, 1973
Rocky Mountain Foothills, AB	67 years	*P. contorta*	Day, 1972
Cook-Quentin Study Area, OR	95 years	Mixed conifer	Morrison and Swanson, 1990
Deer Study Area, OR	149 years	Mixed conifer	“
Breitenbush Lake, OR	280 years	Mixed conifer	Minckley and Long, 2016
Little Lake, OR	230	Mixed conifer	Long et al., 1998
South Santiam, OR	50	Mixed conifer	Stewart et al., 1984

Table 4.4 Comparative summary of return fire intervals across western North America, by vegetation type, against Little Monon Lake, Pyramid Lake, and Summit Lake.

3.3. Fire history across the Pacific Northwest

There is an overall lack of published fire regimes for the Cascade Range, making thorough discussion of the patterns of fire behaviour observed at Little Monon Lake, Pyramid Lake, and Summit Lake, in relation to other sites across this region difficult. However, within the fire reconstructions which are available, there are some strong correlations with the fire regimes of my sites.

The only existing published fire reconstruction in the central Cascade Range, Oregon, is derived from Breitenbush Lake and consists of a multiproxy reconstruction of vegetation and fire derived from pollen and charcoal data obtained from lake sediments (Minckley and Long, 2016). Breitenbush Lake sits 3.2 km southeast of Little Monon Lake, within the adjacent watershed and is located 1,678m a.s.l. within the *Abies amabilis* Zone, with major canopy species including *Abies amabilis, Abies procera, Tsuga mertensiana,* with smaller abundances of *Picea engelmanii* and *Pinus contorta*. This vegetation composition differs significantly from Little Monon Lake, which is dominated by nearly pure stands of *Pinus contorta*. The core obtained from Breitenbush Lake spans the last 13,400 years BP therefore we can compare the charcoal record over the last 2,500 years whereby it crosses over with the length of the Little Monon Lake record. During this period, CHAR indices from Breitenbush indicate a period of heightened fire activity around 2,000 yr BP, with CHAR values twice that of the average for the entire series (0.79 particles cm^{-1} yr^{-1} at 2,000 yr BP compared to an average of 0.39 particles cm^{-1} yr^{-1} for the entire series). This pattern is reflected in the Little Monon Lake record, with 5 events during this period and *backgroundCHAR* (Figure 3.24 in Results) values around 27 particles mm^{-1} yr^{-1}, which is particularly high for Little Monon Lake. This compares to 2 events in the 2,000-2,100 yr BP period and 3 events in the 1,999-1,900 yr BP period observed at Little Monon Lake. Worona and Whitlock (1995), found at Little Lake, Oregon, an increase in *Pseudotsuga menziesii* and *Abies* spp. at 1,950 yr BP, which also suggest a drier period in the Coastal Range, which is atypical for this region and, therefore, suggests climate was a principal driver of a change in fire regime at this time as it seems to be simultaneous across much of Oregon.

There is an abrupt and dramatic decline in fire activity ~700 yr BP observable across all three CHAR records in this study (Little Monon Lake, Pyramid Lake, and Summit Lake), however, levels of CHAR remain high for Breitenbush between 2,000 yr BP and present. Pollen analysis of Breitenbush Lake indicated that the median canopy:understory pollen ratios remained fairly constant through this time, suggesting that fire episodes resulted in little to no change to forest structure (Minckley and Long, 2016). Interestingly, the major shifts in pollen assemblages at Breitenbush Lake coincide with periods of very little to no fire activity. *Tsuga* spp., *Abies* spp., and *Picea* spp., which are all found at Breitenbush Lake, are thin barked species mortally susceptible to mid- to -high-severity fires (Agee, 1993). There are also quick to grow and recover from such events, with pollen production returning to full capacity in less than 20 years (Cruz and Alexander, 2010) therefore, pollen records do not always show the changes these types of fires inflict on forest community structure. It is therefore possible that the pollen records at Breitenbush Lake were either overwhelmed by more regional pollen signatures which effectively ‘hid’ the taxa lost to fire, or that the sampling resolution failed to identify declining abundances of burnt species, instead, capturing time periods whereby the pollen production of affected species had recovered.

The only other fire reconstruction within the Cascade Range was produced at Battle Ground Lake, southwestern Washington (Walsh et al., 2008). The site is situated further north within the Cascade Range and much lower elevation than the sites within this study. Although this site is further north than the sites studied within this thesis, the vegetation type is typical of the *Tsuga heterophylla* Zone, dominated by *Pseudotsuga menziesii*, *Thuja*-type, *Tsuga heterophylla*, *Abies*, and *Alnus rubra*. While, the vegetation types are not analogous to my sites, a reconstruction of Holocene fire, vegetation, and climate (Walsh et al., 2008) revealed a period of heightened fire activity between, 1,500 yr BP and 700 yr BP which was the highest observed within the last 4,000 years at Battle Ground Lake. This period of high fire activity between 1,500 and 700-yr BP is observable in both the Summit Lake and Little Monon Lake records. At Little Monon Lake, a large peak in CHAR around 1,450 – 1,500 yr BP is observed in all size classes. At this time, CHAR values in the >500 µm and 250-500 µm size classes are 2x the values found throughout the rest of the series, with values of 1 and 10 particles mm^{-1} yr^{-1}, respectively, indicative if a highly severe localised fire at Little Monon Lake. There is also a high fire activity towards the end of this period at Pyramid Lake, however, overall fire activity is far less than that observed at Little Monon Lake and Summit Lake. This time-period of heightened fire activity coincides with the Medieval Climate Anomaly (MCA) whereby temperatures were comparable, or even higher than those observed in the 20th Century (Mann, 2002). Evidence derived from tree ring records (Cook et al. 2004; Graumlich, 1993) and lake sediments (Brunelle and Whitlock, 2003; Mohr et al., 2000) suggest the MCA in North America was characterised by increased aridity and warmer temperatures, which would effectively extend the fire season and also increase the weather conditions suitable for fire (Walsh et al., 2008).

Following the period of heightened fire activity at Battle Ground Lake there was a rapid decline in fire activity, with only three major fire episodes occurring over the last 700 years, in 1350 AD, 1390 AD, and 1902 AD. This pattern is observable within the CHAR records for Little Monon Lake, Pyramid Lake, and Summit Lake, whereby all sites experience a rapid decline in CHAR values ~700 yr BP. During this period, fire return intervals at Little Monon Lake increase from 22.8 years to 83.2 years and at Pyramid Lake FRI increases from 37.0 to 91.8 years. Walsh et al., (2008) attributed the decline in fire activity at Battle Ground Lake to the effects of the Little Ice Age. The LIA would have had the opposite effect of the MCA, with cooler, wetter conditions leading to much shorter annual fire seasons and a suppression of conditions suitable for fire.

The reconstruction to Battle Ground Lake also consisted of a palynological reconstruction of vegetation. Interestingly, during the periods of both heightened fire activity between 1,500 and 700yr BP and drastically reduced fire activity in last 700 years to present, there was very little change in overall vegetation. Abundances of dominant species inferred through pollen analysis, show *Pseudotsuga*, *Thuja plicata*, and Poaceae, are found at the same levels throughout the MCA. This minimal change in vegetation was also observed during significant shifts in fire regime at Breitenbush Lake (Minckley and Long, 2016). Therefore, the short-term changes in climate must have been the predominant influence on fire regime, rather than longer term climate induced changes in vegetation, forest composition, or stand structure (Walsh et al., 2008).

In addition to reconstructions based on fossil charcoal remains, there are also several key fire reconstructions in the Cascade Range which utilise dendroecological methods. Morrison and Swanson, 1990, produced an 800-year (1150 to 1985 AD) reconstruction of fire history at two sites within the Willamette Valley using fire scars in tree ring records and clear-cut stumps as evidence of past fire events. The Cook-Quentin study and The Deer study area are both categorised as old-growth Douglas-fir stands, situated within the central western Cascade Range where the Cook Creek, Quentin Creek, and Blue River intersect, 6km northeast of the H J Andrews Experimental Forest. Reconstructed fire regimes between the two sites exhibit high variability, reflective of high topographic variation. The Cook-Quentin site is steeper and more dissected, akin to the topography of Pyramid Lake, and experienced low to medium severity fires with an average return interval of 95 years. The Deer Study site, although within the adjacent watershed, has more gentle topography, akin to the topography of Little Monon Lake and Summit Lake, and receives more rainfall than Cook-Quentin and consequently has a slightly longer fire rotation period of 149 years, with the majority of these fires being stand replacement events. The advantage of dendroecological methods is that they provide annual resolution, allowing assessments of the duration of events. In addition, when multiple trees within a stand are analyses, it can provide information on spatial extent of the fire event. Interestingly, these sites both show a decline in fire activity ~800 – 700 yr BP. In the records for both the Cook-Quentin study area and Deer study area, Morrison and Swanson, (1990), identified a high-severity fire event ~800 – 750 yr BP, which lasted for 50 years. Following this period, there was a 250-year gap in fire activity, which is the longest fire free period found within the records at both sites. This distinct change in fire regime coincides with the beginning of the LIA which led to a dramatic change in fire behaviour across the Pacific Northwest, as inferred by the fossil charcoal records outlined above and then data obtained from my sites.

The comparison of fire regimes across the Cascade Range, Coastal Range, and Willamette Valley have revealed that despite high variability in both vegetation zones and catchment dynamics, they broadly show a similar fire behaviour i.e., higher levels of fire pre-700 yr BP, lower levels of fire between 700 yr BP and 200 yr BP, and then a steady increase in fire to the present. These results highlight that climate must be the primary driver of fire dynamics in this region, rather than vegetation.

3.4. Factors affecting charcoal production and deposition

The two principal factors which ultimately affect the amount of charcoal recovered from lake sediments, are firstly, how much charcoal is initially produced, and, secondly, the taphonomic processes that deliver charcoal to the lake and result in its' deposition (Higuera et al., 2010). An exploration of these factors highlights key results obtained from the fossil charcoal records at Little Monon Lake, Pyramid Lake, and Summit Lake.

The evidence obtained from Little Monon Lake Pyramid Lake, and Summit Lake, suggests the fire regimes of the central Cascades are highly variable. The number of fire events and mean return intervals identified from my data, vary considerably, as do the reconstructed fire regimes of this region discussed above. It seems on regional scales, climate has the greatest effect on fire and vegetation, while topography and the spatial distribution of fuels and vegetation having the greatest impact on local scale (Merschel et al., 2018; Kellogg et al., 2008). For example, in the Cascade Range, characterised by high topographic variation and river-cut valleys, steep topographic features may either act as a barrier for the spread of fire or accelerants of fire spreading (Merschel et al., 2018; Taylor and Skinner, 2003). This means that widespread locations within more homogenous catchments, such as the Summit Lake catchment, may vary less than sites in proximity within highly variable catchments. For example, the fire return intervals of Deer Study Area and Cook-Quentin Study Area, vary by a factor of 2.4x, despite being 9km apart. An RFI of 96 years at Deer Study area versus 241 years at Cook-Quentin Study Area despite being of similar vegetation. This is likely attributed to the high variation in topography, elevation, climatic niches, and vegetation composition, found across the Cascade Range, which ultimately influence the abundance of fuel, 'fire weather', and how easily fires can spread (Rothermel, 1983).

Firstly, the amount of charcoal produced is directly correlated to the type of vegetation burned, the area burned, and the intensity of fire. The General Introduction contained a brief discussion regarding the fire behaviours of the major tree species associated with the forest communities within the Pacific Northwest (Tables 1.2 and 1.4). A coevolution of these species alongside fire has resulted in the development of fire behaviours and fire tolerances, the dynamics of which vary greatly. Species with very low fire tolerance, such as *Tsuga heterophylla* (western hemlock), have thin bark, shallow roots, and highly flammable foliage, therefore, when stands of *T. heterophylla* burn more individuals are likely to succumb to the fire event producing a greater abundance of charcoal. In contrast, highly fire tolerant species, such as *Larix occidentalis* (western larch) survive all but the most severe fires, due to their thick bark, open crown, and high canopy. The dominant vegetation at the sites chosen for this thesis consist of either fire-dependent species (such as near pure stands of *P. contorta* at Little Monon Lake) or species with low fire tolerance (such as *T. heterophylla* which form major components of the mixed conifer stands at Summit Lake and Pyramid Lake). Consequently, these species are both prone to burning, which explains the high levels of fossil charcoal recovered from all sites.

The controlling factor on the area burned, in addition to weather patterns, can be thought of as an extension of how the fire behaviour of individual species scales up to their relative abundance within a stand i.e., what proportion of a stand has high fire tolerance, versus low fire tolerance, are stands composed of single species of multiple species. Fire intensity is primarily a factor of climate, with cool wet conditions, such as those observed throughout the LIA result in an accumulation of fuels, resulting in high intensity fires when areas do eventually burn. The intensity of a fire greatly influences the dispersal of charcoal particles as this has been shown to directly correlated with the size of the plume and the abundance of material carried aloft (Whitlock and Larsen 2002). The Olallie catchment, within which Little Monon Lake sits, is characterised by densely-packed near pure stands of fire-dependent *P. contorta*, which at the time of sampling, the entire southern side of the catchment was burned, attributed to a high severity fire in 2001. This combination of a densely packed stand comprised of a single fire dependent species which has undergone several centuries of fire suppression illustrates the three controlling factors which result in large amounts of charcoal production outlined above. The distinctive peak in CHAR ~1,500 yr BP found within the Little Monon Lake fossil charcoal record could therefore be attributed to a similar scenario.

The second critical component to charcoal deposition concerns taphonomy. Taphonomy, or the processes involved between charcoal production and deposition, is not a concern within dendroecological-based reconstructions as the occurrence of fire is directly recorded by the fire scars within affected trees (Long et al., 1998; Morrison and Swanson, 1990), however, these considerations are integral to interpreting fossil charcoal records. There have been attempts to understand the dynamics associated with deposition of fossil charcoal by assessing the patterns of deposition from modern fires within lake sediments, in an effort to better understand how to interpret results from palaeoenvironmental reconstructions. In general, charcoal dispersal distance increases with wind speed and injection height and decreases with particle size and density suggesting that peaks in macroscopic charcoal occur when there is fire in the immediate watershed as larger, heavier particles travel shorter distances, and microscopic charcoal represent more regional signals (Gardner and Whitlock, 2001; Clark, 1988; Patterson et al., 1987). (Peters and Higuera, 2007). The catchment schematics which depict major topographic features (Figures 4.1 to 4.7) outlined in the DNA Discussion aimed to identify potential source areas for palaeoenvironmental material at Little Monon Lake, Pyramid Lake, and Summit Lake. These drainage characteristics which provided a strong correlation to the results observed within sedaDNA analyses, are also important factors in the mechanisms for charcoal transport. Little Monon Lake and Summit Lake have the most comparable fire histories of the sites chosen of this study. Firstly, the abundance of charcoal at these sites are considerably higher than the abundance of charcoal found at Pyramid Lake. The catchment dynamics of Little Monon Lake and Summit Lake highlight how the combination of a site dominated by species with low fire tolerances and a large source areas resulted in the highest abundance of charcoal particles recovered from this site as well as the higher number of events identified, relative to length of the record. Pyramid Lake contained the highest number of positive sedaDNA amplifications yet had the lowest abundance of charcoal with less fluctuation in fire activity than the records for Little Monon Lake and Summit Lake. The variation in results for these differing proxies suggest that ideal site selection may vary by proxy, depending on the nature of the material required for analysis (Edwards, 2020).

3.5. Human influence on fire

Human influence has always played a key role in fire dynamics (DeWilde and Chapin, 2006), with the effects ranging from fire promotion (e.g., clearing of land for grazing or the construction of settlements), to fire suppression (e.g., active controlling of burned areas) (Haugo et al., 2019; Pyne, 2017). Therefore, whenever charcoal records show abrupt shifts in fire behaviour, such as those observed at Little Monon Lake and Summit Lake ~600 – 700 yr BP, the effects of humans in promoting such changes must be assessed. Recent human influence has undoubtedly affected the role of fire within the Cascade Range, however, relatively little is known prior to European settlement in the 17th Century and the role indigenous populations had on fire activity (Haugo et al., 2019; Morrison and Swanson, 1990). Early human settlement sites are believed to have been concentrated at lowe elevation, along the Columbia River, allowing easy access to resources, including salmon, and to provide water for crops (Martindale, 2015; Hajda, 1994; Pettigrew, 1990; Boyd and Hajda, 1987). Indigenous populations are also known to have seasonally used middle to higher elevation sites across the Cascade Range (Teensma, 1987; Boyd, 1986; Minor and Pecor, 1977). There is also evidence of high elevation occupation as early as ~4,500 yr BP, although this is believed to be focussed in the Washington Cascade Range (Mierendorf, 1999). Some indigenous fire-related practises are well documented, including the annual burning of grassland within the Willamette Valley (Burke, 1979) or the use of fire to promote the growth of food within the Columbia River Basin (Boyd, 1990). However, the relatively low population sizes of these indigenous populations suggest that the scale and extent of these fires are likely considered too small to register within fossil charcoal records or to have significantly altered fire regimes (Walsh et al., 2008). The results observed in this thesis can be explained by shifts in climate, as fire behaviour closely reflecting either warm and dry or cool and wet conditions across sites of dissimilar vegetation, such as between Battle Ground Lake in Washington and Little Monon Lake and Summit Lake in Oregon, are concurrent. Full Holocene reconstructions of fire (Minckley and Long, 2016;

Walsh et al., 2008; Long et al., 2007; Long et al., 1998) show the greatest period of fire activity occurred during the early Holocene, at periods of least human influence. Therefore, while we cannot discount the effect indigenous populations had on fire regimes, it appears that they were not the primary driver of altered fire regimes reconstructed in this study.

3.6. Charcoal conclusion

The evidence discussed above suggests the fire regimes of the central Cascades are highly variable. The number of fire events and mean return intervals identified from my data, vary considerably, as do the reconstructed fire regimes of this region discussed above. It seems on regional scales, climate has the greatest effect on fire and vegetation, while topography and the spatial distribution of fuels and vegetation having the greatest impact on local scale (Merschel et al., 2018; Kellogg et al., 2008). For example, in the Cascade Range, characterised by high topographic variation and river-cut valleys, steep topographic features may either act as a barrier for the spread of fire or accelerants of fire spreading (Merschel et al., 2018; Taylor and Skinner, 2003). This means that widespread locations within more homogenous catchments, such as the Summit Lake catchment, may vary less than sites in proximity within highly variable catchments.

Much of the published reconstructions of historic fire occurrence in the Pacific Northwest are based in the Coastal Range and the Willamette Valley, Oregon. The majority of these reconstructions are obtained from only microscopic charcoal particles, which provide information of more regional fire signals, macroscopic charcoal particles sampled at wide time intervals, or tree ring data, which limits our understanding of historic fire occurrence beyond the lifespan of the host tree (Weinman and Mathewes, 1987, Cwynar, 1987, Dunwiddie, 1986; Tsukada et al., 1981). The fire reconstructions produced within this thesis are foccused within the understudied crest area of the Cascade Range, Oregon, and consist of continual sampling over three key size classes, >500 µm, 250 to 500 µm, and 125 – 250 µm, which, in combination with both traditional and novel methods of analyses have resulted in highly detailed robust records of historic fire occurrence over local and regional scales with sampling resolutions that detect a greater range of events magnitudes than previous reconstructions in this region. These reconstructions have address important data gaps within this region and will provide highly useful information for future studies of fire history in the Pacific Northwest.

Recent observations suggest fire activity has significantly increased since the 1930's, attributed to long period of fire suppression which allows fuel build up, increasingly warm and dry conditions, and considerable human alteration of the ecosystem (Reilly et al., 2017; Hulse et al., 2016; Westerling and Bryant, 2006; Gedalof et al., 2005; Morris et al., 1934). The present-day fire regime of the Cascade Range is now categorised by medium to high severity fires (Agee, 1993).

4. Conclusion

Earlier literature (Rudinsky, 1979; Wickman et al., 1973; Childs and Shea, 1967) stated that although insect outbreaks are observed within the Cascade Range, they are more of a threat to individual trees than entire stands. These statements, made just fifty years ago, emphasise the rate at which insect outbreaks have superseded fire to become the most destructive disturbance agent in the Pacific Northwest.

The overwhelming evidence is that the frequency, severity, and scale of all types of forest disturbances are increasing (Kulakowski et al., 2016). Fire and insect outbreaks are both greatly influenced by climate and projected future warming of 1 to 3.5^{o}C and a reduction in precipitation patterns by 2050 are likely to increase the occurrence and severity of extreme weather events, and therefore, create ideal conditions for more intense and widespread disturbance events (Fettig et al., 2013; Hicke et al., 2012; Bentz et al., 2010). Our understanding of how forest ecosystem processes will develop, either through changes in composition or a continued resilience, as climate and fire regimes continue to change is unclear (Minckley and Long, 2016; Thomsen and Willerslev, 2015; MacDonald et al., 2008). This thesis has highlighted that fire and insect outbreaks have been the two primary disturbance agents in the Pacific Northwest and both have shaped the forest landscapes across this region for thousands of years. How specific vegetation types behave during and recover from single agent events is relatively well understood, however, much less is known about the interactions between multiple disturbance agents (Turner et al., 2010). The dramatic increase in beetle-induced tree mortality over the past few decades has raised questions on how vast areas of standing dead trees and altered fuels loads will impact fire dynamics (Raffa et al., 2008; Meddens et al., 2012, Hicke et al., 2012; Harvey et al., 2014). It is thought that bark beetle outbreaks and fire severity may inextricably linked with fire severity greatly affected by pre-fire outbreaks (Harvey et al., 2014; Bond et al., 2009; Jenkins et al., 2008; Berg and Anderson, 2006). Mountain pine beetle outbreaks have been shown to affect the seed release of serotinous species such as lodgepole pine (Teste et at al., 2011). Gradual tree death associated with insect outbreak(s) fail to provide the high temperature 'fire' cues associated with serotinous cone opening and resulting seed release (Hellum and Wang, 1985) which ultimately impacts post-disturbance regeneration patterns (Harvey et al., 2014).

Several review papers (Gugerli et al., 2005; Parducci, 2004) have described the retrieval of DNA from modern and palaeoenvironmental samples as ‘no longer a dream’. As such, the methods associated with DNA-based analyses of such samples are likely to only become more developed, improved, and routinely used across much of Quaternary science. A new wave of research has looked at calibrating environmental changes i.e., contemporary vegetation or pollen assemblages, with DNA signals, however, the calibration of insect outbreaks with the within these records is still an important data gap. This thesis has produced a high-specificity qPCR protocol capable of the direct detection of several notable forest pathogens with high sensitivity, high primer efficiencies, and low limits of detection, which expands on the field of palaeoenvironmental science and will prove a key asset in the monitoring and reconstruction of outbreak occurrence for these key forest pests. The results outlined in this thesis have furthered the discussion of the dynamics associated with sedaDNA and will contribute to the wider understanding of the processes associated with sedaDNA research, notable catchment dynamics and source areas for DNA.

The collaboration of molecular ecologists and palaeoecologists has demonstrated clear benefits when reconstructing past environments (Parducci et al., 2019). As highlighted in Edwards (2020), much of the recent literature concerning sedimentary DNA has suffered from a lack of communication between palaeoecologists and molecular biologists. This thesis demonstrates how inextricably linked these fields are and highlights how successful collaboration of these disciplines will drastically improve the potential for the successful integration of molecular biology into palaeoecological research in the future.I hope that accurate detection methods, including those utilising DNA-based methodologies, will play a key role with ecology and palaeoecology in the years to come.

SUPPLEMENTARY MATERIAL

Sample	Lab #	δ13C	Age	Age error
Summit Lake 4mm	1668	-25.6	-80	66
Summit Lake 8mm	1665	-25.5	0	60
Summit Lake 32mm	1669	-25.4	930	79
Summit Lake 40mm	1670	-25.5	230	34
Summit Lake 54mm	1666	-25.6	300	66
Summit Lake 60mm	1667	-25.8	300	60
Summit Middle	1603	-25.0	2110	36
Summit Basal	1602	-25.6	3290	49
Little Monon Lake 2mm	1641	-21.1	120	30
Little Monon Lake 8mm	1642	-21.1	70	30
Little Monon Lake 14mm	1640	-20.9	150	31
Little Monon Lake 20mm	1636	-21.4	360	40
Little Monon Lake 26mm	1638	-21.7	670	38
Little Monon Lake 32mm	1643	-22.0	900	45
Little Monon Lake 38mm	1647	-21.4	910	33
Little Monon Lake 44mm	1639	-21.2	1030	33
Little Monon Lake 50mm	1644	-21.6	1170	34
Little Monon Lake 58mm	1637	-22.1	1220	48
Little Monon Lake Middle	1633	-23.5	1610	46
Little Monon Lake Basal	1634	-21.7	2350	42
Pyramid Lake 2mm	1601	-23.9	-500	31
Pyramid Lake 8mm	1600	-24.5	40	31
Pyramid Lake 16mm	1599	-24.3	90	32
Pyramid Lake 24mm	1598	-24.7	460	34
Pyramid Lake 36mm	1606	-25.0	590	36
Pyramid Lake 40mm	1607	-24.7	590	42
Pyramid Lake Middle	1610	-25.3	2160	35
Pyramid Lake Basal	1593	-26.5	5330	55
Little Shadow Camp Lake Middle	1649	-26.4	3800	39
Little Shadow Camp Lake Basal	1632	-26.5	2990	34
Scorpion Lake Middle	1609	-27.0	8000	94
Scorpion Lake Middle	1660	-26.3	3980	67
Tephra Layers				
Above B1	1714	-26.3	1080	81

Below B1	1604	-24.9	660	39
Above B2	1631	-26.8	2510	53
Below B3	1663	-24.9	4830	61

Table S1. Raw Radiocarbon dates obtained from iThemba LABS, Gauteng, South Africa

Depth (mm)	>500µm	500-250µm	250-125µm	Total
2				
4	0	2	21	23
6	0	1	16	17
8	0	6	72	78
10	0	0	23	23
12	0	3	27	30
14	0	13	89	102
16	0	1	56	57
18	0	4	38	42
20	1	6	36	43
22	0	8	72	80
24	0	4	51	55
26	0	1	19	20
28	0	0	10	10
30	0	0	9	9
32	0	0	36	36
34				
36				
38				
40				
42				
44				
46				
48				
50	0	3	27	30
52	0	1	68	69
54	0	1	17	18
56	0	0	18	18
58	0	4	32	36
60	0	2	41	43
62	0	2	21	23
64	1	10	88	99
66	0	12	102	114
68				
70	0	17	72	89
72	0	0	19	19
74	0	5	52	57
76	0	4	35	39
78	0	9	42	51
80	0	6	46	52
82	0	3	25	28
84	0	4	42	46
86	0	2	42	44

88	0	1	19	20
90	0	0	32	32
92	0	3	54	57
94	0	3	74	77
96	0	1	55	56
98	1	2	40	43
100	0	6	51	57
102	0	4	49	53
104	0	3	28	31
106	0	7	63	70
108	0	2	23	25
110	0	0	17	17
112	0	5	52	57
114	0	3	39	42
116	1	2	34	37
118	0	3	46	49
120	0	2	35	37
122	0	0	21	21
124	0	0	25	25
126	0	1	34	35
128	0	0	29	29
130	0	11	89	100
132	1	6	34	41
134	0	0	21	21
136	0	3	56	59
138	0	5	54	59
140	0	2	32	34
142	0	6	39	45
144	0	3	57	60
146	0	5	36	41
148	0	3	47	50
150	1	6	75	82
152	0	3	32	35
154	0	9	97	106
156	0	2	12	14
158	0	0	14	14
160	0	5	32	37
162	0	4	43	47
164	0	2	12	14
166	0	7	77	84
168	0	2	16	18
170	0	13	133	146
172	0	9	114	123
174	1	10	102	113
176	0	2	45	47
178	0	1	18	19
180	0	5	55	60
182	0	2	34	36
184	0	3	44	47
186	0	1	17	18
188	0	2	35	37
190	0	3	87	90
192	1	7	90	98
194	0	8	95	103

196	0	2	28	30
198	0	3	44	47
200	0	1	19	20
202	0	7	90	97
204	0	2	36	38
206	0	12	102	114
208	0	8	111	119
210	1	6	75	82
212	0	8	76	84
214	0	4	55	59
216	0	3	20	23
218	0	2	28	30
220	0	7	64	71
222	0	2	13	15
224	0	5	27	32
226	0	8	58	66
228	0	4	34	38
230	0	2	32	34
232	0	5	35	40
234	1	2	16	19
236	2	8	25	35
238	1	11	87	99
240	1	11	107	119
242	0	7	45	52
244	0	2	23	25
246	0	1	16	17
248	0	7	87	94
250	0	2	32	34
252	0	4	31	35
254	0	9	92	101
256	0	4	29	33
258	0	1	22	23
260	0	0	18	18
262	0	2	30	32
264	0	4	32	36
266	0	2	35	37
268	0	1	12	13
270	0	3	56	59
272	0	5	76	81
274	0	8	68	76
276	0	3	56	59
278	0	1	38	39
280	0	3	34	37
282	0	2	23	25
284	0	10	74	84
286	0	9	87	96
288	0	1	19	20
290	0	1	22	23
292	0	7	48	55
294	1	2	43	46
296	0	2	50	52
298	0	6	51	57
300	0	4	65	69
302	0	0	13	13

304	0	7	55	62
306	0	0	21	21
308	0	3	45	48
310	0	4	42	46
312	2	1	12	15
314	1	2	34	37
316	1	4	55	60
318	0	8	76	84
320	0	2	54	56
322	0	2	18	20
324	0	1	19	20
326	0	4	29	33
328	0	7	59	66
330	0	8	61	69
332	0	5	62	67
334	0	2	23	25
336	0	1	19	20

Table S2. Raw charcoal counts for Pyramid Lake

Depth (mm)	>500µm	500-250µm	250-125µm	Total
2	0	4	52	56
4	0	1	5	6
6	0	0	12	12
8	0	5	22	27
10	0	0	5	5
12	1	9	39	49
14	0	0	6	6
16	0	0	18	18
18	0	0	10	10
20	0	0	16	16
22	2	8	41	51
24	0	6	37	43
26	0	1	21	22
28	0	2	6	8
30	0	2	13	15
32	0	4	42	46
34	0	4	63	67
36	0	16	82	98
38	0	8	91	99
40	1	7	108	116
42	0	13	92	105
44	0	6	65	71
46	0	4	54	58
48	0	8	84	92
50	1	13	76	90
52	0	5	46	51
54	0	11	74	85
56	1	17	92	110
58	0	6	77	83
60	0	2	97	99
62	0	10	108	118
64	0	12	96	108
66	0	8	68	76

68	0	8	113	121
70	0	7	56	63
72				0
74				0
76				0
78				0
80	0	10	63	73
82	0	5	45	50
84	1	11	89	101
86	2	4	82	88
88	0	7	78	85
90	0	2	42	44
92				0
94	0	5	84	89
96	0	4	59	63
98	0	13	68	81
100				0
102				0
104				0
106				0
108				0
110	1	35	117	153
112	0	33	101	134
114	5	26	181	212
116	0	14	79	93
118	1	8	72	81
120	0	10	55	65
122	0	12	68	80
124	0	6	55	61
126	0	5	35	40
128	0	12	58	70
130	0	14	70	84
132	1	4	45	50
134	0	9	94	103
136	1	3	56	60
138	1	13	74	88
140	0	14	78	92
142	0	14	158	172
144	0	17	126	143
146	0	11	165	176
148	0	15	105	120
150	0	9	123	132
152	0	6	98	104
154	1	6	64	71
156	0	14	89	103
158	0	22	149	171
160	0	8	63	71
162	0	3	65	68
164	0	4	58	62
166	0	5	65	70
168	0	3	69	72
170	0	12	45	57
172	0	10	87	97
174	0	20	112	132

176	0	14	113	127
178	0	17	104	121
180	1	14	90	105
182	0	8	101	109
184	0	13	104	117
186	0	12	69	81
188	0	5	113	118
190	0	6	73	79
192	0	8	84	92
194	1	9	97	107
196	2	7	92	101
198	1	12	122	135
200	0	8	89	97
202	1	10	152	163
204	1	14	121	136
206	1	15	109	125
208	0	12	81	93
210	1	5	57	63
212	0	7	98	105
214	0	11	86	97
216	1	14	82	97
218	0	2	43	45
220	0	6	57	63
222	0	22	91	113
224	1	3	106	110
226	0	9	70	79
228	1	12	115	128
230	1	11	118	130

Table S3. Raw charcoal counts for Little Monon Lake

Depth (mm)	>500µm	500-250µm	250-125µm	Total
2				0
4	0	2	18	20
6	0	4	14	18
8	0	3	25	28
10	1	2	12	15
12	0	4	24	28
14	0	4	32	36
16	0	1	26	27
18	0	1	16	17
20	0	3	24	27
22	0	4	15	19
24	0	8	18	26
26	0	6	22	28
28	0	3	35	38
30	0	2	25	27
32	0	5	24	29
34	0	3	22	25
36	0	5	26	31
38	0	2	24	26
40	0	3	38	41
42	0	3	29	32
44	2	4	25	31

46	0	5	26	31
48	0	4	32	36
50	0	4	29	33
52	1	8	39	48
54	0	5	36	41
56	0	0	18	18
58	0	3	29	32
60	1	1	38	40
62				0
64				0
66				0
68				0
70				0
72	0	0	21	21
74	0	4	17	21
76	0	3	18	21
78	0	2	13	15
80	0	9	21	30
82				0
84	0	5	104	109
86	0	6	92	98
88	0	3	97	100
90	0	2	65	67
92	0	7	73	80
94	0	8	96	104
96	1	4	104	109
98	0	6	112	118
100	1	6	99	106
102	0	2	83	85
104	0	7	118	125
106	0	11	114	125
108	0	6	76	82
110	0	7	83	90
112	0	9	96	105
114	0	4	67	71
116	0	2	98	100
118	0	13	156	169
120	0	3	94	97
122	0	13	169	182
124	0	8	88	96
126	0	6	84	90
128	0	5	91	96
130	0	7	134	141
132	0	7	55	62
134	0	5	119	124
136	0	6	107	113
138	0	4	104	108
140	1	12	92	105
142	0	8	94	102
144	1	2	98	101
146	0	6	142	148
148	0	17	165	182
150	1	12	182	195
152	0	11	109	120

154	0	20	167	187
156	0	11	128	139
158	0	14	165	179
160	0	9	116	125
162	0	7	89	96
164	0	3	84	87
166	0	3	81	84
168	0	8	111	119
170	0	13	117	130
172	0	8	131	139
174	0	11	101	112
176	0	10	129	139
178	0	5	156	161
180	0	15	138	153
182	0	11	95	106
184	0	8	92	100
186	0	2	91	93
188	0	5	78	83
190	1	2	102	105
192	0	11	126	137
194	0	11	116	127
196	0	12	149	161
198	0	7	80	87
200	1	2	67	70
202	0	16	142	158
204	2	22	148	172
206	0	9	118	127
208	1	19	155	175
210	0	17	161	178
212	1	7	124	132
214	0	21	153	174
216	0	10	134	144
218	0	12	102	114
220	0	7	98	105
222	1	12	129	142
224	0	8	131	139
226	0	21	158	179
228	0	15	117	132
230	0	4	109	113
232	1	8	101	110
234	0	4	135	139
236	0	3	109	112
238	0	9	128	137
240	0	8	94	102
242	0	13	108	121
244	1	6	91	98
246	1	7	109	117
248	0	14	115	129
250	0	11	152	163
252	0	11	141	152
254	1	12	83	96
256	0	5	88	93
258	0	3	64	67
260	1	10	74	85

262	0	7	110	117
264	1	12	124	137
266	0	11	98	109
268	0	7	92	99
270	0	17	122	139
272	1	8	68	77
274	0	6	105	111
276	0	15	131	146
278	0	9	105	114
280	1	7	77	85
282	0	12	74	86
284	0	7	92	99
286	0	6	36	42
288	1	11	76	88
290	1	4	102	107
292	0	3	39	42
294	0	13	77	90
296	0	4	71	75
298	1	10	63	74
300	2	7	88	97

Table S4. Raw charcoal counts for Summit Lake

```
#Charcoal Acc. rates using the "pretreatment" function in "paleofire"

install.packages("paleofire")
library("paleofire")

#Little Monon Lake - all particle sizes
charlmlall <- read.csv("charlmlall.csv")
LCall_=charlmlall[,6]
LPall_=charlmlall[,1:5]
View(LCall_)
View(LPall_)
charlmlall=pretreatment(params=LPall_,serie=LCall_,Int=TRUE)
View(charlmlall)
plot(charlmlall, xlab = "Age (cal. yr. BP)", ylab = "CHAR (particles mm-1 yr-1)", ylim = c(0,75))
charlmlall[["acc"]]

#125-250um ("small" particle range)
charlmlsmall <- read.csv("charlmlsmall.csv")
LCsmall_=charlmlsmall[,6]
LPsmall_=charlmlsmall[,1:5]
View(LCsmall_)
View(LPsmall_)
charlmlsmall=pretreatment(params=LPsmall_,serie=LCsmall_,Int=TRUE)
View(charlmlsmall)
plot(charlmlsmall, main = "Little Monon Lake 125 - 250µm", xlab = "Age (cal. yr. BP)", ylab = "CHAR (particles mm-1 yr-1)",ylim = c(0,70))
charlmlsmall[["acc"]]

#The following uses 0.25cc as vol. for the top 30mm to reflect sampling
#125-250um ("small" particle range)
charlmlsmall05 <- read.csv("charlmlsmall05.csv")
LCsmall05_=charlmlsmall05[,6]
LPsmall05_=charlmlsmall05[,1:5]
View(LCsmall05_)
View(LPsmall05_)
```

```
charlmlsmall05=pretreatment(params=LPsmall05_,serie=LCsmall05_,Int=TRUE)
View(charlmlsmall05)
plot(charlmlsmall05, xlab = "Age (cal. yr. BP)", ylab = "CHAR (particles mm-1 yr-1)",ylim = c(0,70))
charlmlsmall05[["acc"]]


#250um-500um ("medium" particle range)
charlmlmed <- read.csv("charlmlmed.csv")
LCmed_=charlmlmed[,6]
LPmed_=charlmlmed[,1:5]
View(LCmed_)
View(LPmed_)
charlmlmed=pretreatment(params=LPmed_,serie=LCmed_,Int=TRUE)
View(charlmlmed)
plot(charlmlmed, main = "Little Monon Lake 250 - 500 µm", xlab = "Age (cal. yr. BP)", ylim = c(0,10), ylab = "CHAR (particles mm-1 yr-1)")
charlmlmed[["acc"]]

#>500um ("large" particle range)
charlmllarge <- read.csv("charlmllarge.csv")
LClarge_=charlmllarge[,6]
LPlarge_=charlmllarge[,1:5]
View(LClarge_)
View(LPlarge_)
charlmllarge=pretreatment(params=LPlarge_,serie=LClarge_,Int=TRUE)
View(charlmllarge)
plot(charlmllarge, main = "Little Monon Lake >500µm", xlab = "Age (cal. yr. BP)", ylim = c(0,1), ylab = "CHAR (particles mm-1 yr-1)")
charlmllarge[["acc"]]

#Standardised values - samples 2-30mm use 0.25cm3 - values doubled

#125-250um ("small" particle range - "standardised" values for the top 30mm doubled - as 0.25cm3 was used instead of 0.5cm3)
charlmlsmallstand <- read.csv("charlmlsmallstand.csv")
Csmallstand_=charlmlsmallstand[,6]
Psmallstand_=charlmlsmallstand[,1:5]
View(Csmallstand_)
View(Psmallstand_)
charlmlsmallstand=pretreatment(params=Psmallstand_,serie=Csmallstand_,Int=TRUE)
View(charlmlsmallstand)
plot(charlmlsmallstand, xlab = "Age BP", ylab = "CHAR", main = "Little Monon Lake 125 - 250 µm Standardised")
charlmlsmallstand[["acc"]]

#250 - 500um ("med" particle range - "standardised" values for the top 30mm doubled - as 0.25cm3 was used instead of 0.5cm3)
charlmlmedstand <- read.csv("charlmlmedstand.csv")
Cmedstand_=charlmlmedstand[,6]
Pmedstand_=charlmlmedstand[,1:5]
View(Cmedstand_)
View(Pmedstand_)
charlmlmedstand=pretreatment(params=Pmedstand_,serie=Cmedstand_,Int=TRUE)
View(charlmlmedstand)
plot(charlmlmedstand, xlab = "Age BP", ylab = "CHAR", main = "Little Monon Lake 250  to 500 µm Standardised")
charlmlmedstand[["acc"]]

#>500um ("large" particle range - "standardised" values for the top 30mm doubled - as 0.25cm3 was used instead of 0.5cm3)
```

```
charlmllargestand <- read.csv("charlmllargestand.csv")
Clargestand_=charlmllargestand[,6]
Plargestand_=charlmllargestand[,1:5]
View(Clargestand_)
View(Plargestand_)
charlmllargestand=pretreatment(params=Plargestand_,serie=Clargestand_,Int=TRUE)
View(charlmllargestand)
plot(charlmllargestand, xlab = "Age BP", ylab = "CHAR", main = "Little Monon Lake >500 µm Standardised")
charlmllargestand[["acc"]]

#Pyramid Lake - all particle sizes

charpyrall <- read.csv("charpyrall.csv")
PCall_=charpyrall[,6]
PPall_=charpyrall[,1:5]
View(PCall_)
View(PPall_)
charpyrall=pretreatment(params=PPall_,serie=PCall_,Int=TRUE)
View(charpyrall)
plot(charpyrall, xlab = "Age (cal. yr. BP)", ylab = "CHAR (particles mm-1 yr-1)", ylim = c(0,75))
charpyrall[["acc"]]

#125-250um ("small" particle range)
charpyrsmall <- read.csv("charpyrsmall.csv")
PCsmall_=charpyrsmall[,6]
PPsmall_=charpyrsmall[,1:5]
View(PCsmall_)
View(PPsmall_)
charpyrsmall=pretreatment(params=PPsmall_,serie=PCsmall_,Int=TRUE)
View(charpyrsmall)
plot(charpyrsmall, main = "Pyramid Lake 125 - 250µm", xlab = "Age (cal. yr. BP)", ylab = "CHAR (particles mm-1 yr-1)", ylim = c(0,70))
charpyrsmall[["acc"]]


#250um-500um ("medium" particle range)
charpyrmed <- read.csv("charpyrmed.csv")
PCmed_=charpyrmed[,6]
PPmed_=charpyrmed[,1:5]
View(PCmed_)
View(PPmed_)
charpyrmed=pretreatment(params=PPmed_,serie=PCmed_,Int=TRUE)
View(charpyrmed)
plot(charpyrmed, main = "Pyramid Lake 250 - 500µm", xlab = "Age (cal. yr. BP)", ylim = c(0,8), ylab = "CHAR (particles mm-1 yr-1)")
charpyrmed[["acc"]]

#>500um ("large" particle range)
charpyrlarge <- read.csv("charpyrlarge.csv")
PClarge_=charpyrlarge[,6]
PPlarge_=charpyrlarge[,1:5]
View(PClarge_)
View(PPlarge_)
charpyrlarge=pretreatment(params=PPlarge_,serie=PClarge_,Int=TRUE)
View(charpyrlarge)
plot(charpyrlarge, main = "Pyramid Lake >500µm", xlab = "Age (cal. yr. BP)", ylim = c(0,1), ylab = "CHAR (particles mm-1 yr-1)")
charpyrlarge[["acc"]]
```

```
#Summit Lake - all particle sizes
charsumall <- read.csv("charsumall.csv")
SCall_=charsumall[,6]
SPall_=charsumall[,1:5]
View(SCall_)
View(SPall_)
charsumall=pretreatment(params=SPall_,serie=SCall_,Int=TRUE)
View(charsumall)
plot(charsumall, xlab = "Age (cal. yr. BP)", ylim = c(0,75), ylab = "CHAR (particles mm-1 yr-1)")
charsumall[["acc"]]

#125-250um ("small" particle range)
charsumsmall <- read.csv("charsumsmall.csv")
SCsmall_=charsumsmall[,6]
SPsmall_=charsumsmall[,1:5]
View(SCsmall_)
View(SPsmall_)
charsumsmall=pretreatment(params=SPsmall_,serie=SCsmall_,Int=TRUE)
View(charsumsmall)
plot(charsumsmall, main = "Summit Lake 125 - 250µm", xlab = "Age (cal. yr. BP)", ylim = c(0,70), ylab = "CHAR (particles mm-1 yr-1)")
charsumsmall[["acc"]]

#250um-500um ("medium" particle range)
charsummed <- read.csv("charsummed.csv")
SCmed_=charsummed[,6]
SPmed_=charsummed[,1:5]
View(SCmed_)
View(SPmed_)
charsummed=pretreatment(params=SPmed_,serie=SCmed_,Int=TRUE)
View(charsummed)
plot(charsummed, main = "Summit Lake 250 - 500µm", xlab = "Age (cal. yr. BP)", ylab = "CHAR (particles mm-1 yr-1)", ylim = c(0,8))
charsummed[["acc"]]

#>500um ("large" particle range)
charsumlarge <- read.csv("charsumlarge.csv")
SClarge_=charsumlarge[,6]
SPlarge_=charsumlarge[,1:5]
View(SClarge_)
View(SPlarge_)
charsumlarge=pretreatment(params=SPlarge_,serie=SClarge_,Int=TRUE)
View(charsumlarge)
plot(charsumlarge, main = "Summit Lake >500µm", xlab = "Age (cal. yr. BP)", ylim = c(0,1), ylab = "CHAR (particles mm-1 yr-1)")
charsumlarge[["acc"]]

#Exporting acc data as a csv.

#Little Monon Lake
charclmllargedf <- as.data.frame(charlmllarge$acc)
View(charclmllargedf)
write.csv(charclmllargedf, "charlmllargeacc.csv")

charlmlmedacc <- as.data.frame(charlmlmed$acc)
View(charlmlmedacc)
write.csv(charlmlmedacc, "charlmlmedacc.csv")

charlmlsmallacc <- as.data.frame(charlmlsmall$acc)
View(charlmlsmallacc)
```

```
write.csv(charlmlsmallacc, "charlmlsmallacc.csv")

#Pyramid Lake
charpyrlargeacc <- as.data.frame(charpyrlarge$acc)
View(charpyrlargeacc)
write.csv(charclmllargedf, "charpyrlargeacc.csv")

charpyrmedacc <- as.data.frame(charpyrmed$acc)
View(charpyrmedacc)
write.csv(charpyrmedacc, "charpyrmedacc.csv")

charpyrsmallacc <- as.data.frame(charpyrsmall$acc)
View(charpyrsmallacc)
write.csv(charpyrsmallacc, "charpyrsmallacc.csv")

#Summit Lake
charsumlargeacc <- as.data.frame(charsumlarge$acc)
View(charsumlargeacc)
write.csv(charsumlargeacc, "charsumlargeacc.csv")

charpyrmedacc <- as.data.frame(charpyrmed$acc)
View(charpyrmedacc)
write.csv(charpyrmedacc, "charpyrmedacc.csv")

charsumsmallacc <- as.data.frame(charsumsmall$acc)
View(charsumsmallacc)
write.csv(charsumsmallacc, "charsumsmallacc.csv")

#Student's t-test
t.test(charpyrsmallacc, charsumsmallacc)
t.test(charpyrsmallacc, charlmlsmallacc)
t.test(charsumsmallacc, charlmlsmallacc)

#Various plots of acc. data

boxplot(charlmlsmallacc, main = "Little Monon Lake CHAR")

#vioplot
install.packages("vioplot")
library(vioplot)

#Std y axis

#All particles - distribution
par(mfrow=c(1,3))
vioplot(LCsmall_, main = "Little Monon Lake 125 - 250 µm", col ="snow2", ylim = c(0,200))
vioplot(LCmed_, main = "Little Monon Lake 250 - 500 µm", col ="snow3", ylim = c(0,40))
vioplot(LClarge_, main = "Little Monon Lake > 500 µm", col ="snow4", ylim = c(0,5))

par(mfrow=c(1,3))
vioplot(PCsmall_, main = "Pyramid Lake 125 - 250 µm", col ="snow2", ylim = c(0,150))
vioplot(PCmed_, main = "Pyramid Lake 250 - 500 µm", col ="snow3", ylim = c(0,20))
vioplot(PClarge_, main = "Pyramid Lake > 500 µm", col ="snow4", ylim = c(0,3))

par(mfrow=c(1,3))
vioplot(SCsmall_, main = "Summit Lake 125 - 250 µm", col ="snow2", ylim = c(0,200))
vioplot(SCmed_, main = "Summit Lake 250 - 500 µm", col ="snow3", ylim = c(0,25))
vioplot(SClarge_, main = "Summit Lake > 500 µm", col ="snow4", ylim = c(0,3))

vioplot(LCall_, main = "Little Monon Lake", col ="snow2", ylim = c(0,220))
```

```
vioplot(PCall_, main = "Pyramid Lake", col ="snow3", ylim = c(0,220))
vioplot(SCall_, main = "Summit Lake", col ="snow4", ylim = c(0,220))

par(mfrow=c(1,3))
vioplot(LCsmall_, main = "Little Monon Lake 125 - 250 µm", col ="snow2", ylim = c(0,200))
vioplot(PCsmall_, main = "Pyramid Lake 125 - 250 µm", col ="snow3", ylim = c(0,200))
vioplot(SCsmall_, main = "Summit Lake 125 - 250 µm", col ="snow4", ylim = c(0,200))

plot(LCsmall_, main = "Little Monon Lake 125 - 250 µm", col ="snow2", ylim = c(0,200))
plot(PCsmall_, main = "Pyramid Lake 125 - 250 µm", col ="snow3", ylim = c(0,200))
plot(SCsmall_, main = "Summit Lake 125 - 250 µm", col ="snow4", ylim = c(0,200))

vioplot(LCmed_, main = "Little Monon Lake 250 - 500 µm", col ="snow2", ylim = c(0,40))
vioplot(PCmed_, main = "Pyramid Lake 250 - 500 µm", col ="snow3", ylim = c(0,40))
vioplot(SCmed_, main = "Summit Lake 250 - 500 µm", col ="snow4", ylim = c(0,40))

vioplot(LClarge_, main = "Little Monon Lake >500 µm", col ="snow2", ylim = c(0,5))
vioplot(PClarge_, main = "Pyramid Lake >500 µm", col ="snow3", ylim = c(0,5))
vioplot(SClarge_, main = "Summit Lake >500 µm", col ="snow4", ylim = c(0,5))


#CHAR - distribution
par(mfrow=c(1,3))
vioplot(charlmlallacc, main = "Little Monon Lake", col ="snow2", ylim = c(0,220))
vioplot(charpyrallacc, main = "Pyramid Lake", col ="snow3", ylim = c(0,220))
vioplot(charsumallacc, main = "Summit Lake", col ="snow4", ylim = c(0,220))

par(mfrow=c(1,3))
vioplot(LCsmall_, main = "Little Monon Lake 125 - 250 µm", col ="snow2", ylim = c(0,200))
vioplot(PCsmall_, main = "Pyramid Lake 125 - 250 µm", col ="snow3", ylim = c(0,200))
vioplot(SCsmall_, main = "Summit Lake 125 - 250 µm", col ="snow4", ylim = c(0,200))

vioplot(LCmed_, main = "Little Monon Lake 250 - 500 µm", col ="snow2", ylim = c(0,40))
vioplot(PCmed_, main = "Pyramid Lake 250 - 500 µm", col ="snow3", ylim = c(0,40))
vioplot(SCmed_, main = "Summit Lake 250 - 500 µm", col ="snow4", ylim = c(0,40))

vioplot(LClarge_, main = "Little Monon Lake >500 µm", col ="snow2", ylim = c(0,5))
vioplot(PClarge_, main = "Pyramid Lake >500 µm", col ="snow3", ylim = c(0,5))
vioplot(SClarge_, main = "Summit Lake >500 µm", col ="snow4", ylim = c(0,5))

#Scaled to 70 on y axis
par(mfrow=c(1,3))
vioplot(charlmlall, main = "Little Monon Lake All", col ="snow2", ylim = c(0,70))
vioplot(charpyrall, main = "Pyramid Lake All", col ="snow3", ylim = c(0,70))
vioplot(charsumall, main = "Summit Lake All", col ="snow4", ylim = c(0,70))
```

Figure S1. R Script for the package 'Paleofire' used to generate CHAR and corresponding figures

```
#Age depth models using rBacon

setwd("~/Desktop/agedepthmodels")
install.packages('rbacon')
#Do these separately as you need to type Yes after installation
library(rbacon)

#Summit Lake, Oregon, USA
sum <- read.csv("Bacon_runs/sum/sum.csv")
View(sum)
Bacon("sum", thick = 1, postbomb = 2, prob = 0.95, d.min = 0, d.max = 600, acc.mean = 50)
```

```
#Post-run analyses
#Viewing individual dates for any given depth - e.g. 300mm (mid core)
Bacon.hist(300)

#Viewing all possible dates assigned to a particular depth - e.g. 300mm (mid core)
ages.d300 = Bacon.Age.d(300)
View(ages.d300)

#Pyramid Lake, Oregon, USA
pyr <- read.csv("Bacon_runs/pyr/pyr.csv")
View(pyr)
Bacon("pyr", thick = 5, postbomb = 2, prob = 0.95, d.min = 0, d.max = 670, acc.mean = 50)

#Little Monon Lake, Oregon, USA
lml <- read.csv("Bacon_runs/lml/Bacon_runs/lml.csv")
View(lml)
Bacon("lml", thick = 1, postbomb = 2, prob = 0.95, d.min = 0, d.max = 230, acc.mean = 50)
```

Figure S2. R Script for the package ‘rBacob’ used to generate age depth models

	Running av. 100yr	**Ratio**
121	9.5446	2.075519142
122	9.5446	2.075519142
123	9.5446	2.075519142
124	9.5446	2.075519142
125	9.5446	2.075519142
126	9.5446	2.075519142
127	9.5446	2.075519142
128	9.5446	2.075519142
129	9.5446	2.075519142
130	9.5446	2.075519142

131	9.5713	2.069729295
132	9.598	2.063971661
133	9.6247	2.058245971
134	9.6514	2.052551961
135	9.7124	2.03966064
136	9.7734	2.026930239
137	9.8344	2.014357765
138	9.8954	2.001940295
139	9.9564	1.989674983
140	10.0174	1.977559047
141	10.0784	1.965589776
142	10.3185	0.184135291
143	10.3795	0.183053134
144	10.4405	0.181983621
145	10.5015	0.180926534
146	10.5625	0.179881657
147	10.6235	0.178848779
148	10.6845	0.177827694
149	10.7455	0.176818203
150	10.8065	0.175820108
151	10.8675	0.174833218
152	10.7494	0.176754051
153	10.6313	0.17871756
154	10.5132	0.180725184
155	10.3951	0.182778424
156	10.216	0.185982772
157	10.0369	0.189301478
158	9.8578	0.192740774
159	9.6787	0.196307355
160	9.4996	0.200008421
161	9.3205	0.203851725
162	9.1414	0.207845625
163	8.9356	0.511437396
164	8.7565	0.521898019
165	8.5774	0.532795486
166	8.3983	0.544157746
167	8.2192	0.556015184
168	8.0401	0.568400891
169	7.861	0.581350973
170	7.6819	0.594904906
171	7.5028	0.609105934
172	7.3237	0.624001529
173	7.1446	0.639643927
174	6.9655	0.656090733
175	6.7864	0.673405635
176	6.6073	0.691659225
177	6.4282	0.710929965
178	6.2491	0.731305308
179	6.07	0.752883031
180	5.8909	0.775772802
181	5.7118	0.800098043
182	5.5327	0.825998156
183	5.3536	0.853631201
184	5.1745	0.883177119
185	4.9611	1.612545605
186	4.782	1.672940192
187	4.6029	1.738034717

188	4.4238	1.808400018
189	4.2447	1.884703277
190	4.0656	1.96772924
191	3.8865	2.058407307
192	3.7074	2.157846469
193	3.7074	2.157846469
194	3.7074	2.157846469
195	3.7074	2.157846469
196	3.7664	2.12404418
197	3.8254	2.091284572
198	3.8844	2.059520132
199	3.9434	2.028706193
200	4.0024	1.99880072
201	4.0614	1.969764121
202	4.1204	1.941559072
203	4.1794	1.914150357
204	4.2384	1.887504719
205	4.2974	1.861590729
206	4.4174	0.430117264
207	4.4764	0.424448217
208	4.5354	0.418926666
209	4.5944	0.413546927
210	4.6534	0.408303606
211	4.7124	0.40319158
212	4.7714	0.398205977
213	4.8304	0.393342166
214	4.8627	0.39072943
215	4.895	0.388151175
216	4.9273	0.385606722
217	4.9596	0.383095411
218	4.9919	0.380616599
219	5.0242	0.378169659
220	5.0565	0.37575398
221	5.0888	0.373368967
222	5.1211	0.37101404
223	5.1534	0.368688633
224	5.1857	0.366392194
225	5.218	0.364124186
226	5.2503	0.361884083
227	5.2826	0.359671374
228	5.3149	0.357485559
229	5.3472	0.355326152
230	5.3795	0.353192676
231	5.4118	0.351084667
232	5.4441	0.349001672
233	5.4764	0.346943247
234	5.5087	0.344908962
235	5.541	0.342898394
236	5.473	0.347158779
237	5.405	0.351526364
238	5.337	0.356005246
239	5.269	0.360599734
240	5.201	0.365314363
241	5.133	0.370153906
242	5.065	0.375123396
243	4.997	0.380228137
244	4.929	0.385473727

245	4.861	0.390866077
246	4.734	1.64765526
247	4.666	1.671667381
248	4.598	1.696389735
249	4.53	1.721854305
250	4.462	1.748095025
251	4.394	1.775147929
252	4.326	1.803051318
253	4.258	1.831845937
254	4.19	1.861575179
255	4.122	1.892285298
256	4.054	1.924025654
257	4.047	1.927353595
258	4.04	1.930693069
259	4.033	1.934044136
260	4.026	1.937406855
261	4.019	1.940781289
262	4.012	1.944167498
263	4.005	1.947565543
264	3.998	1.950975488
265	3.991	1.954397394
266	3.984	1.957831325
267	3.977	1.961277345
268	3.97	1.964735516
269	3.963	1.968205905
270	3.956	1.971688574
271	3.949	1.975183591
272	3.942	1.97869102
273	3.935	1.982210928
274	3.928	1.985743381
275	3.921	1.989288447
276	3.914	1.992846193
277	3.907	1.996416688
278	3.9	2
279	3.893	2.003596198
280	3.886	2.007205353
281	3.879	2.010827533
282	3.872	2.01446281
283	3.865	2.018111255
284	3.858	2.021772939
285	3.851	2.025447936
286	3.91	0.306905371
287	3.903	0.307455803
288	3.9117	0.306771992
289	3.9204	0.306091215
290	3.9291	0.305413453
291	3.9378	0.304738687
292	3.9465	0.304066895
293	3.9552	0.303398058
294	3.9639	0.302732158
295	3.9726	0.302069174
296	3.9813	0.301409087
297	3.931	0.305265836
298	3.8807	0.309222563
299	3.8304	0.313283208
300	3.7801	0.317451919
301	3.7298	0.321733069

302	3.6795	0.326131268
303	3.6292	0.330651383
304	3.5789	0.335298555
305	3.5286	0.340078218
306	3.4783	0.344996119
307	3.428	0.350058343
308	3.3777	0.355271338
309	3.3274	0.360641943
310	3.2771	0.366177413
311	3.2268	0.371885459
312	3.1765	0.37777428
313	3.1262	0.383852601
314	3.0759	0.390129718
315	3.0256	0.396615547
316	2.9753	0.403320674
317	2.925	0.41025641
318	2.8747	0.417434863
319	2.8244	0.424868999
320	2.7741	0.432572726
321	2.7238	0.440560981
322	2.6735	0.448849822
323	2.6232	0.457456542
324	2.5729	0.466399782
325	2.5226	0.475699675
326	2.4723	0.485377988
327	2.422	0.495458299
328	2.3717	0.505966185
329	2.3214	0.516929439
330	2.2711	0.528378319
331	2.2208	0.540345821
332	2.1705	0.552868003
333	2.1202	0.565984341
334	2.0699	0.579738152
335	2.0196	0.594177065
336	1.9693	0.609353577
337	1.985	0.604534005
338	1.985	1.395465995
339	2.0007	1.38451542
340	2.0164	1.37373537
341	2.0321	1.363121894
342	2.0478	1.352671159
343	2.0635	1.342379452
344	2.0792	1.33224317
345	2.0949	1.322258819
346	2.1106	1.312423008
347	2.1263	1.302732446
348	2.142	1.29318394
349	2.1577	1.283774389
350	2.1734	1.274500782
351	2.1734	1.274500782
352	2.1734	1.274500782
353	2.1734	1.274500782
354	2.1734	1.274500782
355	2.1734	1.274500782
356	2.1734	1.274500782
357	2.1734	1.274500782
358	2.1734	1.274500782

359	2.1734	1.274500782
360	2.1734	1.274500782
361	2.1734	1.274500782
362	2.1734	1.274500782
363	2.1734	1.274500782
364	2.1734	1.274500782
365	2.1734	1.274500782
366	2.1734	1.274500782
367	2.1734	1.274500782
368	2.1734	1.274500782
369	2.1734	1.274500782
370	2.1734	1.274500782
371	2.1734	1.274500782
372	2.1734	1.274500782
373	2.1734	1.274500782
374	2.1734	1.274500782
375	2.1734	1.274500782
376	2.1734	1.274500782
377	2.1734	1.274500782
378	2.1734	1.274500782
379	2.1734	1.274500782
380	2.1734	1.274500782
381	2.1734	1.274500782
382	2.1734	1.274500782
383	2.1734	1.274500782
384	2.1734	1.274500782
385	2.1734	1.274500782
386	2.1734	1.274500782
387	2.1734	1.274500782
388	2.1734	1.274500782
389	2.1577	1.283774389
390	2.142	1.29318394
391	2.1263	1.302732446
392	2.1106	1.312423008
393	2.0949	1.322258819
394	2.0792	1.33224317
395	2.0635	1.342379452
396	2.0478	1.352671159
397	2.0321	1.363121894
398	2.0164	1.37373537
399	2.0007	1.38451542
400	1.985	1.395465995
401	1.985	0.604534005
402	1.9693	0.609353577
403	1.9536	0.614250614
404	1.9379	0.619226998
405	1.9222	0.624284674
406	1.9065	0.629425649
407	1.8908	0.634651999
408	1.8751	0.639965868
409	1.8594	0.645369474
410	1.8437	0.650865108
411	1.828	0.656455142
412	1.8123	0.662142029
413	1.7966	0.667928309
414	1.7809	0.67381661
415	1.7732	0.676742612

416	1.7655	0.679694138
417	1.7578	0.682671521
418	1.7501	0.685675104
419	1.7424	0.688705234
420	1.7347	0.691762264
421	1.727	0.694846555
422	1.7193	0.697958471
423	1.7116	0.701098387
424	1.7039	0.704266682
425	1.6962	0.707463742
426	1.6885	0.710689962
427	1.6808	0.71394574
428	1.6731	0.717231486
429	1.6654	0.720547616
430	1.6577	0.723894553
431	1.65	0.727272727
432	1.6423	0.730682579
433	1.6346	0.734124556
434	1.6269	0.737599115
435	1.6192	0.741106719
436	1.6115	0.744647844
437	1.6038	0.74822297
438	1.5961	0.751832592
439	1.5884	0.75547721
440	1.5807	0.759157335
441	1.573	0.76287349
442	1.5653	0.766626206
443	1.5576	0.770416025
444	1.5499	0.7742435
445	1.5422	0.778109195
446	1.5345	0.782013685
447	1.5268	0.785957558
448	1.5191	0.789941413
449	1.5114	0.793965859
450	1.5037	0.798031522
451	1.496	0.802139037
452	1.504	0.79787234
453	1.512	0.793650794
454	1.52	0.789473684
455	1.528	0.785340314
456	1.536	0.78125
457	1.544	0.777202073
458	1.552	0.773195876
459	1.56	0.769230769
460	1.568	0.765306122
461	1.576	0.76142132
462	1.584	0.757575758
463	1.592	0.753768844
464	1.6	0.75
465	1.6	1.25
466	1.608	1.243781095
467	1.616	1.237623762
468	1.624	1.231527094
469	1.632	1.225490196
470	1.64	1.219512195
471	1.648	1.213592233
472	1.656	1.207729469

473	1.664	1.201923077
474	1.672	1.196172249
475	1.68	1.19047619
476	1.688	1.184834123
477	1.696	1.179245283
478	1.704	1.17370892
479	1.712	1.168224299
480	1.72	1.162790698
481	1.728	1.157407407
482	1.736	1.152073733
483	1.744	1.146788991
484	1.752	1.141552511
485	1.76	1.136363636
486	1.768	1.131221719
487	1.8016	1.110124334
488	1.8352	1.089799477
489	1.8688	1.070205479
490	1.9024	1.051303616
491	1.936	1.033057851
492	1.9696	1.015434606
493	2.0032	0.998402556
494	2.0368	0.981932443
495	2.0704	0.965996909
496	2.104	0.950570342
497	2.1376	0.935628743
498	2.1712	0.921149595
499	2.2048	0.907111756
500	2.2384	0.893495354
501	2.272	0.88028169
502	2.3056	0.867453158
503	2.3392	0.85499316
504	2.3728	0.842886042
505	2.4064	0.831117021
506	2.44	0.819672131
507	2.4736	0.808538163
508	2.5072	0.797702616
509	2.5408	0.787153652
510	2.5744	0.77688005
511	2.608	0.766871166
512	2.6416	0.757116899
513	2.6752	0.747607656
514	2.7088	0.738334318
515	2.7424	0.729288215
516	2.768	0.722543353
517	2.7936	0.715922108
518	2.8192	0.709421112
519	2.8448	0.70303712
520	2.8704	0.696767001
521	2.896	0.690607735
522	2.9216	0.684556407
523	2.9472	0.678610206
524	2.9728	0.672766416
525	2.9984	0.667022412
526	3.024	0.661375661
527	3.0496	0.655823715
528	3.0752	0.650364204
529	3.1008	0.64499484

530	3.1264	0.639713408
531	3.152	0.634517766
532	3.1776	0.629405841
533	3.2032	0.624375624
534	3.2288	0.619425173
535	3.2544	0.614552606
536	3.28	0.609756098
537	3.28	1.390243902
538	3.3056	1.379477251
539	3.3312	1.368876081
540	3.3568	1.358436606
541	3.3824	1.348155156
542	3.408	1.338028169
543	3.4336	1.32805219
544	3.4592	1.318223867
545	3.4848	1.308539945
546	3.5104	1.298997265
547	3.536	1.28959276
548	3.5616	1.28032345
549	3.5872	1.271186441
550	3.6128	1.262178919
551	3.6384	1.253298153
552	3.664	1.244541485
553	3.6896	1.235906331
554	3.7152	1.227390181
555	3.7408	1.21899059
556	3.7664	1.210705183
557	3.792	1.202531646
558	3.8176	1.194467728
559	3.8387	1.187902154
560	3.8598	1.181408363
561	3.8809	1.174985184
562	3.902	1.168631471
563	3.9231	1.162346104
564	3.9442	1.156127985
565	3.9653	1.149976042
566	3.9864	1.143889223
567	4.0075	1.1378665
568	4.0286	1.131906866
569	4.0497	1.126009334
570	4.0708	1.120172939
571	4.0919	1.114396735
572	4.113	1.108679796
573	4.1341	1.103021214
574	4.1552	1.0974201
575	4.1763	1.091875584
576	4.1974	1.086386811
577	4.2185	1.080952945
578	4.2396	1.075573167
579	4.2607	1.070246673
580	4.2818	1.064972675
581	4.3029	1.059750401
582	4.324	1.054579093
583	4.3451	1.04945801
584	4.3662	1.044386423
585	4.3873	1.039363618
586	4.4084	1.034388894

587	4.4295	1.029461565
588	4.425	1.030508475
589	4.4205	1.031557516
590	4.416	1.032608696
591	4.4115	1.03366202
592	4.407	1.034717495
593	4.4025	1.035775128
594	4.398	1.036834925
595	4.3935	1.037896893
596	4.389	1.038961039
597	4.3845	1.040027369
598	4.38	1.04109589
599	4.3755	1.04216661
600	4.371	1.043239533
601	4.3665	1.044314668
602	4.362	1.045392022
603	4.3575	1.046471601
604	4.353	1.047553411
605	4.3485	1.048637461
606	4.344	1.049723757
607	4.3395	1.050812306
608	4.335	1.051903114
609	4.335	0.948096886
610	4.3305	0.949082092
611	4.326	0.950069348
612	4.3215	0.95105866
613	4.317	0.952050035
614	4.3125	0.953043478
615	4.308	0.954038997
616	4.3035	0.955036598
617	4.299	0.956036288
618	4.2945	0.957038072
619	4.2769	0.960976408
620	4.2593	0.964947292
621	4.2417	0.968951128
622	4.2241	0.972988329
623	4.2065	0.977059313
624	4.1889	0.981164506
625	4.1713	0.985304342
626	4.1537	0.989479259
627	4.1361	0.993689708
628	4.1185	0.997936142
629	4.1009	1.002219025
630	4.0833	1.006538829
631	4.0657	1.010896033
632	4.0481	1.015291124
633	4.0305	1.0197246
634	4.0129	1.024196965
635	3.9953	1.028708733
636	3.9777	1.033260427
637	3.9601	1.037852579
638	3.9425	1.042485732
639	3.9249	1.047160437
640	3.9073	1.051877255
641	3.8897	1.056636759
642	3.8721	1.061439529
643	3.8545	1.066286159

644	3.8369	1.071177252
645	3.8193	1.076113424
646	3.8017	1.081095299
647	3.7841	1.086123517
648	3.7665	1.091198726
649	3.7489	1.096321588
650	3.7313	1.101492777
651	3.7137	1.106712982
652	3.6961	1.111982901
653	3.6785	1.117303249
654	3.6609	1.122674752
655	3.6433	1.128098153
656	3.6257	1.133574206
657	3.6081	1.139103683
658	3.5905	1.144687369
659	3.5729	1.150326066
660	3.5598	1.154559245
661	3.5467	1.158823695
662	3.5336	1.163119765
663	3.5205	1.167447806
664	3.5074	1.171808177
665	3.4943	1.176201242
666	3.4812	1.18062737
667	3.4681	1.185086935
668	3.4368	1.195879888
669	3.4186	0.819048733
670	3.3873	0.82661707
671	3.356	0.834326579
672	3.3247	0.842181249
673	3.2934	0.850185219
674	3.2621	0.858342785
675	3.2308	0.866658413
676	3.1995	0.87513674
677	3.1682	0.883782589
678	3.1369	0.892600975
679	3.1056	0.901597115
680	3.0743	0.910776437
681	3.043	0.920144594
682	3.0117	0.929707474
683	2.9804	0.939471212
684	2.9491	0.949442203
685	2.9178	0.959627116
686	2.8865	0.970032912
687	2.8552	0.980666853
688	2.8239	0.991536527
689	2.7926	1.00264986
690	2.7613	1.014015138
691	2.73	1.025641026
692	2.6987	1.037536592
693	2.6674	1.049711329
694	2.6361	1.062175183
695	2.6048	1.074938575
696	2.5735	1.088012434
697	2.5422	1.101408229
698	2.5109	1.115137998
699	2.4796	1.129214389
700	2.4483	1.143650696

701	2.417	1.158460902
702	2.3857	1.173659723
703	2.3544	1.189262657
704	2.3231	1.20528604
705	2.2918	1.221747098
706	2.2605	1.238664012
707	2.2292	1.256055984
708	2.1979	1.27394331
709	2.1666	1.292347457
710	2.1353	1.311291153
711	2.104	1.330798479
712	2.0727	1.350894968
713	2.0414	1.37160772
714	2.0101	1.392965524
715	1.9788	1.414998989
716	1.95936	1.429038053
717	1.93992	1.443358489
718	1.93868	0.505498587
719	1.91924	0.510618787
720	1.9129	0.512311151
721	1.90656	0.51401477
722	1.90022	0.515729758
723	1.89388	0.517456227
724	1.88754	0.519194295
725	1.8812	0.520944078
726	1.87486	0.522705695
727	1.86852	0.524479267
728	1.86218	0.526264915
729	1.85584	0.528062764
730	1.8495	0.529872939
731	1.84316	0.531695566
732	1.83682	0.533530776
733	1.83048	0.535378698
734	1.82414	0.537239466
735	1.8178	0.539113214
736	1.81146	0.541000077
737	1.80512	0.542900195
738	1.79878	0.544813707
739	1.79244	0.546740756
740	1.7861	0.548681485
741	1.77976	0.550636041
742	1.77342	0.552604572
743	1.76708	0.554587229
744	1.79908	0.544722858
745	1.83108	0.535203268
746	1.86308	0.526010692
747	1.89508	0.517128564
748	1.92708	0.50854142
749	1.95908	0.500234804
750	1.99108	0.492195191
751	2.02308	0.48440991
752	2.05508	0.476867081
753	2.08708	0.469555551
754	2.11908	0.462464843
755	2.15108	0.455585101
756	2.18308	0.448907049
757	2.21508	0.442421944

758	2.24708	0.436121544
759	2.27908	0.429998069
760	2.31108	0.42404417
761	2.34308	0.418252898
762	2.37508	0.41261768
763	2.40708	0.407132293
764	2.43908	0.401790839
765	2.47108	0.396587727
766	2.49122	0.869453521
767	2.52322	0.858426931
768	2.55522	0.847676521
769	2.60542	0.831343891
770	2.65562	0.815628742
771	2.70582	0.800496707
772	2.75602	0.785915922
773	2.83312	0.764528153
774	2.91022	0.744273629
775	2.98732	0.725064606
776	3.06442	0.706822172
777	3.14152	0.689475159
778	3.21862	0.672959219
779	3.29572	0.657216026
780	3.37282	0.642192587
781	3.44992	0.627840646
782	3.52702	0.614116166
783	3.60412	0.60097888
784	3.68122	0.588391892
785	3.75832	0.576321335
786	3.83542	0.564736065
787	3.91252	0.553607394
788	3.98962	0.542908848
789	4.06672	0.532615966
790	4.14382	0.522706102
791	4.22092	0.513158269
792	4.29802	0.503952983
793	4.37512	0.495072135
794	4.41388	1.359348238
795	4.49098	1.336011294
796	4.56808	1.313462111
797	4.64518	1.291661464
798	4.72228	1.270572689
799	4.79938	1.250161479
800	4.87648	1.230395695
801	4.98378	1.203905469
802	5.09108	1.178531864
803	5.19838	1.154205733
804	5.30568	1.130863527
805	5.41298	1.108446734
806	5.52028	1.086901389
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808	5.73488	1.046229389
809	5.84218	1.027013889
810	5.94948	1.008491498
811	6.05678	0.990625382
812	6.16408	0.973381267
813	6.27138	0.956727228
814	6.37868	0.940633485

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816	6.59328	0.910017472
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818	6.78416	0.884413103
819	6.8796	0.872143729
820	6.97504	0.860210121
821	7.07048	0.848598681
822	7.16592	0.837296537
823	7.23446	1.20119539
824	7.3299	1.185555055
825	7.42534	1.17031678
826	7.52078	1.155465258
827	7.61622	1.140985948
828	7.71166	1.126865033
829	7.82	1.111253197
830	7.92834	1.096068029
831	8.03668	1.081292275
832	8.14502	1.066909596
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835	8.47004	1.025969181
836	8.57838	1.013011781
837	8.68672	1.000377588
838	8.79506	0.988054658
839	8.9034	0.976031628
840	9.01174	0.964297683
841	9.12008	0.952842519
842	9.22842	0.941656318
843	9.33676	0.930729718
844	9.4451	0.920053785
845	9.5151	0.913285199
846	9.5851	0.906615476
847	9.6551	0.900042465
848	9.7251	0.893564076
849	9.7951	0.887178283
850	9.8651	0.880883113
851	9.9049	1.182243132
852	9.9749	1.173946606
853	10.0449	1.165765712
854	10.1149	1.157698049
855	10.1849	1.149741284
856	10.2549	1.141893144
857	10.3249	1.13415142
858	10.4139	1.124458656
859	10.5029	1.114930162
860	10.5919	1.105561797
861	10.6809	1.096349559
862	10.7699	1.087289576
863	10.8589	1.078378105
864	10.9479	1.069611524
865	11.0369	1.060986328
866	11.1259	1.052499124
867	11.2149	1.044146626
868	11.3039	1.035925654
869	11.3929	1.027833124
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873	11.7489	0.996689052
874	11.811	0.99144865
875	11.8731	0.986263065
876	11.9352	0.981131443
877	11.9973	0.976052945
878	12.0594	0.971026751
879	12.1086	1.073617099
880	12.1707	1.068139055
881	12.2328	1.062716631
882	12.2949	1.057348982
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884	12.4191	1.046774726
885	12.4812	1.041566516
886	12.5433	1.036409876
887	12.5833	1.03311532
888	12.6233	1.029841642
889	12.6633	1.026588646
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891	12.7433	1.020143919
892	12.7833	1.016951804
893	12.8233	1.013779604
894	12.8633	1.010627133
895	12.9033	1.007494207
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897	12.9833	1.001286268
898	13.0233	0.998210899
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900	13.1033	0.99211649
901	13.1433	0.989097107
902	13.1531	0.988360158
903	13.1629	0.987624308
904	13.1727	0.986889552
905	13.1825	0.986155888
906	13.1923	0.985423315
907	13.2021	0.984691829
908	13.1929	1.129395357
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910	13.2125	1.127719962
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913	13.2419	1.12521617
914	13.2517	1.124384041
915	13.2275	1.126441126
916	13.2033	1.128505752
917	13.1791	1.130577961
918	13.1549	1.132657793
919	13.1307	1.134745292
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924	13.0097	1.145299277
925	12.9855	1.147433676
926	12.9613	1.149576046
927	12.9371	1.15172643
928	12.9129	1.153884875

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931	12.8145	1.162745328
932	12.7774	1.166121433
933	12.7403	1.169517201
934	12.7032	1.172932804
935	12.6661	1.176368416
936	12.629	1.179824214
937	12.614	1.006025052
938	12.5769	1.008992677
939	12.5398	1.011977862
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941	12.4558	1.018802486
942	12.4089	1.022653096
943	12.362	1.026532923
944	12.3151	1.030442303
945	12.2682	1.034381572
946	12.2213	1.038351076
947	12.1744	1.042351163
948	12.1275	1.046382189
949	12.0806	1.050444514
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951	11.9868	1.058664531
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953	11.893	1.06701421
954	11.8461	1.071238636
955	11.7992	1.075496644
956	11.7523	1.079788637
957	11.7054	1.084115024
958	11.6585	1.088476219
959	11.5926	1.094663837
960	11.5267	1.100922207
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962	11.3949	1.113656109
963	11.3986	1.113294615
964	11.4023	1.112933356
965	11.44	0.812062937
966	11.4437	0.811800379
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1463	31.06523447	0.997094001
1464	31.03953463	0.997919566
1465	31.0138348	0.998746499
1466	30.98813496	0.999574803
1467	30.93788472	1.08055222
1468	30.91218488	1.081450571
1469	30.88648505	1.082350418
1470	30.86078521	1.083251763
1471	30.83508537	1.084154611
1472	30.80938554	1.085058965
1473	30.7836857	1.08596483
1474	30.75798587	1.086872208
1475	30.70783611	1.088647206
1476	30.65768636	1.090428012
1477	30.60753661	1.092214654
1478	30.52668685	1.095107378
1479	30.4458371	1.098015466
1480	30.36498735	1.100939039
1481	30.32983759	0.951538231
1482	30.24898784	0.95408151
1483	30.16813809	0.956638422
1484	30.08728833	0.959209075
1485	30.00643858	0.961793581
1486	29.92558883	0.964392052
1487	29.84473907	0.967004601
1488	29.76388932	0.969631344
1489	29.65858965	0.973073917
1490	29.55328998	0.976541022
1491	29.4479903	0.980032923
1492	29.34269063	0.983549885
1493	29.23739096	0.987092181
1494	29.13209129	0.990660084
1495	28.97619162	0.9959901
1496	28.82029195	1.001377781
1497	28.50049228	1.587691874
1498	28.34459261	1.596424427

1499	28.18869293	1.605253571
1500	28.03279326	1.614180919
1501	27.87689359	1.623208119
1502	27.72099392	1.632336854
1503	27.56509425	1.641568848
1504	27.38474466	1.65237984
1505	27.20439507	1.663334174
1506	27.02404548	1.674434719
1507	26.84369589	1.685684422
1508	26.6633463	1.69708631
1509	26.48299671	1.708643493
1510	26.30264712	1.720359163
1511	26.12229754	1.732236605
1512	26.21434795	0.80375831
1513	26.06459836	0.808376163
1514	25.91484877	0.813047384
1515	25.76509918	0.817772905
1516	25.61534959	0.822553677
1517	25.4656	0.827390676
1518	25.2913	0.833092803
1519	25.117	0.838874069
1520	24.9427	0.844736135
1521	24.7684	0.850680706
1522	24.5941	0.856709536
1523	24.4198	0.862824429
1524	24.2455	0.869027242
1525	24.0712	0.875319884
1526	23.8969	0.881704321
1527	23.7226	0.888182577
1528	23.579	0.763391153
1529	23.3741	0.770083126
1530	23.1692	0.776893462
1531	22.9643	0.783825329
1532	22.8051	0.789297131
1533	22.6459	0.794845866
1534	22.4867	0.800473169
1535	22.3275	0.806180719
1536	22.1683	0.811970246
1537	22.0091	0.817843528
1538	21.8499	0.823802397
1539	21.6907	0.829848737
1540	21.5315	0.835984488
1541	21.3723	0.842211648
1542	21.2131	0.848532275
1543	21.0539	0.854948489
1544	20.8947	0.861462476
1545	20.7861	0.622531403
1546	20.5799	0.628768847
1547	20.3737	0.635132548
1548	20.0036	0.646883561
1549	19.6335	0.659077597
1550	19.2634	0.671740191
1551	18.8933	0.684898879
1552	18.5232	0.698583398
1553	18.1531	0.712825909
1554	17.783	0.72766125
1555	17.4129	0.743127222

1556	17.0428	0.75926491
1557	16.6727	0.776119045
1558	16.3026	0.793738422
1559	15.9325	0.812176369
1560	15.5624	0.831491287
1561	15.1923	0.851747267
1562	14.7916	1.081695016
1563	14.7174	1.087148545
1564	14.6432	1.092657343
1565	14.569	1.098222253
1566	14.4948	1.103844137
1567	14.4206	1.109523876
1568	14.3464	1.115262365
1569	14.2722	1.121060523
1570	14.198	1.126919284
1571	14.1238	1.132839604
1572	14.0496	1.138822458
1573	13.9754	1.144868841
1574	13.9012	1.150979772
1575	13.827	1.157156288
1576	13.7528	1.163399453
1577	13.6786	1.16971035
1578	13.6044	1.176090089
1579	13.5915	0.952065629
1580	13.5762	0.953138581
1581	13.5609	0.954213953
1582	13.5456	0.955291755
1583	13.5303	0.956371995
1584	13.515	0.95745468
1585	13.4997	0.958539819
1586	13.4844	0.959627421
1587	13.4691	0.960717494
1588	13.4538	0.961810046
1589	13.4385	0.962905086
1590	13.4232	0.964002622
1591	13.4079	0.965102663
1592	13.3926	0.966205218
1593	13.3773	0.967310294
1594	13.362	0.968417902
1595	13.3467	0.969528048
1596	13.429	0.613597438
1597	13.4055	0.614673082
1598	13.382	0.615752503
1599	13.3585	0.616835723
1600	13.335	0.61792276
1601	13.3115	0.619013635
1602	13.288	0.620108368
1603	13.2645	0.621206981
1604	13.241	0.622309493
1605	13.2175	0.623415926
1606	13.194	0.6245263
1607	13.1705	0.625640636
1608	13.147	0.626758956
1609	13.1235	0.627881282
1610	13.1	0.629007634
1611	13.0765	0.630138034
1612	13.053	0.631272504

1613	12.9448	1.054477474
1614	13.006	1.049515608
1615	13.0672	1.04460022
1616	13.1284	1.03973066
1617	13.1896	1.03490629
1618	13.2508	1.030126483
1619	13.312	1.025390625
1620	13.3732	1.020698113
1621	13.4344	1.016048353
1622	13.4956	1.011440766
1623	13.5568	1.006874779
1624	13.618	1.002349831
1625	13.6792	0.997865372
1626	13.7404	0.993420861
1627	13.8016	0.989015766
1628	13.8628	0.984649566
1629	13.924	0.980321747
1630	13.9876	1.177471475
1631	13.99	1.177269478
1632	13.9924	1.177067551
1633	13.9948	1.176865693
1634	13.9972	1.176663904
1635	13.9996	1.176462185
1636	14.002	1.176260534
1637	14.0044	1.176058953
1638	14.0068	1.175857441
1639	14.0092	1.175655997
1640	14.0116	1.175454623
1641	14.014	1.175253318
1642	14.0164	1.175052082
1643	14.0188	1.174850914
1644	14.0212	1.174649816
1645	14.0236	1.174448786
1646	14.026	1.174247825
1647	14.1342	0.749246508
1648	14.2259	0.744416873
1649	14.3176	0.739649103
1650	14.4093	0.734942017
1651	14.501	0.730294462
1652	14.5927	0.725705318
1653	14.6844	0.72117349
1654	14.7761	0.716697911
1655	14.8678	0.712277539
1656	14.9595	0.707911361
1657	15.0512	0.703598384
1658	15.1429	0.699337643
1659	15.2346	0.695128195
1660	15.3263	0.690969118
1661	15.418	0.686859515
1662	15.5097	0.682798507
1663	15.6014	0.678785237
1664	15.5237	1.424918029
1665	15.5707	1.420616928
1666	15.6177	1.416341715
1667	15.6647	1.412092156
1668	15.7117	1.407868022
1669	15.7587	1.403669084

1670	15.8057	1.399495119
1671	15.8527	1.395345903
1672	15.8997	1.391221218
1673	15.9467	1.387120846
1674	15.9937	1.383044574
1675	16.0407	1.378992189
1676	16.0877	1.374963481
1677	16.1347	1.370958245
1678	16.1817	1.366976276
1679	16.2287	1.36301737
1680	16.2757	1.35908133
1681	16.3839	0.804448269
1682	16.591	0.794406606
1683	16.7981	0.784612545
1684	17.0052	0.775057041
1685	17.2123	0.765731483
1686	17.4194	0.756627668
1687	17.6265	0.747737781
1688	17.8336	0.739054369
1689	18.0407	0.730570322
1690	18.2478	0.72227885
1691	18.4549	0.714173472
1692	18.662	0.706247991
1693	18.8691	0.698496484
1694	19.0762	0.690913285
1695	19.2833	0.683492971
1696	19.4904	0.676230349
1697	19.6975	0.669120447
1698	19.9211	0.873947724
1699	20.1117	0.86566526
1700	20.3023	0.857538308
1701	20.4929	0.849562531
1702	20.6835	0.841733749
1703	20.8741	0.834047935
1704	21.0647	0.826501208
1705	21.2553	0.819089827
1706	21.4459	0.811810183
1707	21.6365	0.804658794
1708	21.8271	0.797632301
1709	22.0177	0.79072746
1710	22.2083	0.783941139
1711	22.3989	0.777270312
1712	22.5895	0.770712056
1713	22.7801	0.764263546
1714	22.9707	0.757922049
1715	23.0366	0.79655852
1716	23.2036	0.790825562
1717	23.3706	0.785174536
1718	23.5376	0.779603698
1719	23.7046	0.774111354
1720	23.8716	0.768695856
1721	24.0386	0.763355603
1722	24.2056	0.758089037
1723	24.3726	0.752894644
1724	24.5396	0.74777095
1725	24.7066	0.742716521
1726	24.8736	0.737729963

1727	25.0406	0.732809917
1728	25.2076	0.727955061
1729	25.3746	0.723164109
1730	25.5416	0.718435807
1731	25.7086	0.713768933
1732	25.7767	1.442387893
1733	25.892	1.435964777
1734	26.0073	1.429598613
1735	26.1226	1.423288647
1736	26.2379	1.417034138
1737	26.3532	1.410834358
1738	26.4685	1.404688592
1739	26.5838	1.398596137
1740	26.6991	1.392556303
1741	26.8144	1.386568411
1742	26.9297	1.380631793
1743	27.045	1.374745794
1744	27.1603	1.368909769
1745	27.2756	1.363123084
1746	27.3909	1.357385117
1747	27.5062	1.351695254
1748	27.6215	1.346052894
1749	27.7698	1.067706645
1750	27.8851	1.063291866
1751	28.0004	1.058913444
1752	28.1157	1.054570934
1753	28.231	1.050263894
1754	28.3463	1.045991893
1755	28.4616	1.041754504
1756	28.5769	1.037551309
1757	28.6922	1.033381895
1758	28.8075	1.029245856
1759	28.9228	1.025142794
1760	29.0381	1.021072315
1761	29.1534	1.017034034
1762	29.2687	1.013027569
1763	29.384	1.009052546
1764	29.4993	1.005108596
1765	29.6146	1.001195356
1766	29.6288	1.310211686
1767	29.6759	1.308132188
1768	29.723	1.306059281
1769	29.7701	1.303992933
1770	29.8172	1.301933112
1771	29.8643	1.29987979
1772	29.9114	1.297832933
1773	29.9585	1.295792513
1774	30.0056	1.293758498
1775	30.0527	1.291730859
1776	30.0998	1.289709566
1777	30.1469	1.287694589
1778	30.194	1.285685898
1779	30.2411	1.283683464
1780	30.2882	1.281687258
1781	30.3353	1.27969725
1782	30.3824	1.277713413
1783	30.3823	0.813302482

1784	30.1611	0.819267202
1785	29.9399	0.825320058
1786	29.7187	0.831463018
1787	29.4975	0.83769811
1788	29.2763	0.844027421
1789	29.0551	0.850453105
1790	28.8339	0.856977377
1791	28.6127	0.863602526
1792	28.3915	0.870330909
1793	28.1703	0.877164957
1794	27.9491	0.884107181
1795	27.7279	0.891160167
1796	27.5067	0.89832659
1797	27.2855	0.905609206
1798	27.0643	0.913010867
1799	26.8431	0.920534514
1800	26.6549	1.085729078
1801	26.509	1.091704704
1802	26.4103	1.095784599
1803	26.3116	1.099895103
1804	26.2129	1.104036562
1805	26.1142	1.108209327
1806	26.0155	1.112413753
1807	25.9168	1.116650204
1808	25.8181	1.120919045
1809	25.7194	1.125220651
1810	25.6207	1.129555399
1811	25.522	1.133923674
1812	25.4233	1.138325866
1813	25.3246	1.142762373
1814	25.2259	1.147233597
1815	25.1272	1.151739947
1816	25.0285	1.156281839
1817	24.8969	0.92621973
1818	24.7065	0.933357618
1819	24.6689	0.934780229
1820	24.6313	0.936207184
1821	24.5937	0.937638501
1822	24.5561	0.939074202
1823	24.5185	0.940514306
1824	24.4809	0.941958833
1825	24.4433	0.943407805
1826	24.4057	0.944861241
1827	24.3681	0.946319163
1828	24.3305	0.947781591
1829	24.2929	0.949248546
1830	24.2553	0.950720049
1831	24.2177	0.952196121
1832	24.1801	0.953676784
1833	24.1425	0.955162059
1834	24.326	0.619090685
1835	24.4295	0.616467795
1836	24.3306	0.618973638
1837	24.2317	0.621499936
1838	24.1328	0.62404694
1839	24.0339	0.626614906
1840	23.935	0.629204094

1841	23.8361	0.631814768
1842	23.7372	0.634447197
1843	23.6383	0.637101653
1844	23.5394	0.639778414
1845	23.4405	0.642477763
1846	23.3416	0.645199986
1847	23.2427	0.647945376
1848	23.1438	0.65071423
1849	23.0449	0.65350685
1850	22.946	0.656323542
1851	22.8048	0.660387287
1852	22.6164	0.874586583
1853	22.4799	0.879897153
1854	22.3434	0.885272608
1855	22.2069	0.890714147
1856	22.0704	0.896222996
1857	21.9339	0.901800409
1858	21.7974	0.907447677
1859	21.6609	0.91316612
1860	21.5244	0.918957091
1861	21.3879	0.924821979
1862	21.2514	0.930762209
1863	21.1149	0.936779241
1864	20.9784	0.942874576
1865	20.8419	0.949049751
1866	20.7054	0.955306345
1867	20.5689	0.96164598
1868	20.4912	0.965292418
1869	20.2528	1.73111866
1870	20.1672	1.738466421
1871	20.0816	1.745876823
1872	19.996	1.75335067
1873	19.9104	1.760888782
1874	19.8248	1.76849199
1875	19.7392	1.776161141
1876	19.6536	1.783897098
1877	19.568	1.791700736
1878	19.4824	1.799572948
1879	19.3968	1.807514642
1880	19.3112	1.815526741
1881	19.2256	1.823610186
1882	19.14	1.831765935
1883	19.0544	1.839994962
1884	18.9688	1.848298258
1885	18.9632	1.848844077
1886	19.1679	0.77316764
1887	19.1702	0.773074877
1888	19.1725	0.772982136
1889	19.1748	0.772889417
1890	19.1771	0.772796721
1891	19.1794	0.772704047
1892	19.1817	0.772611395
1893	19.184	0.772518766
1894	19.1863	0.772426158
1895	19.1886	0.772333573
1896	19.1909	0.77224101
1897	19.1932	0.772148469

1898	19.1955	0.772055951
1899	19.1978	0.771963454
1900	19.2001	0.77187098
1901	19.2024	0.771778528
1902	19.2047	0.771686098
1903	19.1646	0.797825157
1904	19.1292	0.799301591
1905	19.0938	0.8007835
1906	19.0584	0.802270915
1907	19.023	0.803763865
1908	18.9876	0.805262382
1909	18.9522	0.806766497
1910	18.9168	0.808276241
1911	18.8814	0.809791647
1912	18.846	0.811312745
1913	18.8106	0.812839569
1914	18.7752	0.81437215
1915	18.7398	0.815910522
1916	18.7044	0.817454717
1917	18.669	0.819004767
1918	18.6336	0.820560708
1919	18.6061	0.779314311
1920	18.3614	0.789700132
1921	18.1167	0.800366513
1922	17.872	0.811324978
1923	17.6273	0.822587691
1924	17.3826	0.834167501
1925	17.1379	0.846077991
1926	16.8932	0.858333531
1927	16.6485	0.870949335
1928	16.4038	0.883941526
1929	16.1591	0.897327203
1930	15.9144	0.911124516
1931	15.6697	0.925352751
1932	15.425	0.940032415
1933	15.1803	0.955185339
1934	14.9356	0.970834784
1935	14.6909	0.987005561
1936	14.4383	1.058988939
1937	14.4948	1.054861054
1938	14.5513	1.050765224
1939	14.6078	1.046701078
1940	14.6643	1.042668249
1941	14.7208	1.038666377
1942	14.7773	1.034695107
1943	14.8338	1.030754089
1944	14.8903	1.026842978
1945	14.9468	1.022961437
1946	15.0033	1.019109129
1947	15.0598	1.015285728
1948	15.1163	1.011490907
1949	15.1728	1.007724349
1950	15.2293	1.003985738
1951	15.2858	1.000274765
1952	15.3423	0.996591124
1953	15.3893	1.055278668
1954	15.4999	1.047748695

1955	15.6105	1.040325422
1956	15.7211	1.033006596
1957	15.8317	1.025790029
1958	15.9423	1.018673592
1959	16.0529	1.011655215
1960	16.1635	1.004732886
1961	16.2741	0.997904646
1962	16.3847	0.99116859
1963	16.4953	0.984522864
1964	16.6059	0.977965663
1965	16.7165	0.971495229
1966	16.8271	0.965109853
1967	16.9377	0.958807866
1968	17.0483	0.952587648
1969	17.1589	0.946447616
1970	17.3339	0.610941565
1971	17.4548	0.606709902
1972	17.5757	0.602536457
1973	17.6966	0.598420035
1974	17.8175	0.594359478
1975	17.9384	0.590353655
1976	18.0593	0.586401466
1977	18.1802	0.582501843
1978	18.3011	0.578653742
1979	18.422	0.57485615
1980	18.5429	0.571108079
1981	18.6638	0.567408566
1982	18.7847	0.563756674
1983	18.9056	0.56015149
1984	19.0265	0.556592122
1985	19.1474	0.553077702
1986	19.2683	0.549607386
1987	19.2825	1.061584338
1988	19.3743	1.056554301
1989	19.4661	1.051571707
1990	19.5579	1.046635886
1991	19.6497	1.041746184
1992	19.7415	1.036901958
1993	19.8333	1.032102575
1994	19.9251	1.027347416
1995	20.0169	1.022635873
1996	20.1087	1.017967347
1997	20.2005	1.013341254
1998	20.2923	1.008757016
1999	20.3841	1.004214069
2000	20.4759	0.999711856
2001	20.5677	0.995249833
2002	20.6595	0.990827464
2003	20.7513	0.986444223
2004	20.7748	1.268363594
2005	20.8242	1.265354732
2006	20.8736	1.26236011
2007	20.923	1.25937963
2008	20.9724	1.256413191
2009	21.0218	1.253460693
2010	21.0712	1.25052204
2011	21.1206	1.247597133

2012	21.17	1.244685876
2013	21.2194	1.241788175
2014	21.2688	1.238903934
2015	21.3182	1.236033061
2016	21.3676	1.233175462
2017	21.417	1.230331045
2018	21.4664	1.22749972
2019	21.5158	1.224681397
2020	21.5652	1.221875985
2021	21.6687	1.227115609
2022	21.7746	1.221147576
2023	21.8931	1.214537914
2024	22.0116	1.207999418
2025	22.1301	1.201530947
2026	22.2486	1.195131379
2027	22.3671	1.188799621
2028	22.4856	1.1825346
2029	22.6041	1.176335267
2030	22.7226	1.170200593
2031	22.8411	1.164129573
2032	22.9596	1.158121222
2033	23.0781	1.152174572
2034	23.1966	1.14628868
2035	23.3151	1.140462619
2036	23.4336	1.13469548
2037	23.5521	1.128986375
2038	23.593	1.037172043
2039	23.6127	1.036306733
2040	23.6527	1.034554195
2041	23.6927	1.032807574
2042	23.7327	1.03106684
2043	23.7727	1.029331965
2044	23.8127	1.027602918
2045	23.8527	1.02587967
2046	23.8927	1.024162192
2047	23.9327	1.022450455
2048	23.9727	1.02074443
2049	24.0127	1.019044089
2050	24.0527	1.017349404
2051	24.0927	1.015660345
2052	24.1327	1.013976886
2053	24.1727	1.012298998
2054	24.2127	1.010626655
2055	24.2268	0.874238447
2056	24.208	0.874917383
2057	24.1892	0.875597374
2058	24.079	0.879604635
2059	23.9688	0.883648743
2060	23.8586	0.88773021
2061	23.7484	0.891849556
2062	23.6382	0.89600731
2063	23.528	0.900204012
2064	23.4178	0.904440212
2065	23.3076	0.90871647
2066	23.1974	0.913033357
2067	23.0872	0.917391455
2068	22.977	0.921791357

2069	22.8668	0.926233666
2070	22.7566	0.930719
2071	22.6464	0.935247986
2072	22.5338	0.939921363
2073	22.4086	1.001401248
2074	22.296	1.006458558
2075	22.296	1.006458558
2076	22.296	1.006458558
2077	22.296	1.006458558
2078	22.296	1.006458558
2079	22.296	1.006458558
2080	22.296	1.006458558
2081	22.296	1.006458558
2082	22.296	1.006458558
2083	22.296	1.006458558
2084	22.296	1.006458558
2085	22.296	1.006458558
2086	22.296	1.006458558
2087	22.296	1.006458558
2088	22.296	1.006458558
2089	22.3172	1.005502482
2090	22.3181	1.096419498
2091	22.3393	1.095378996
2092	22.2664	1.098965257
2093	22.1935	1.102575078
2094	22.1206	1.106208692
2095	22.0477	1.109866335
2096	21.9748	1.113548246
2097	21.9019	1.117254667
2098	21.829	1.120985845
2099	21.7561	1.124742026
2100	21.6832	1.128523465
2101	21.6103	1.132330417
2102	21.5374	1.136163139
2103	21.4645	1.140021897
2104	21.3916	1.143906954
2105	21.3187	1.147818582
2106	21.2787	1.149976267
2107	21.2387	1.15214208
2108	21.2901	0.720052982
2109	21.2759	0.720533561
2110	21.2617	0.721014782
2111	21.2475	0.721496647
2112	21.2333	0.721979155
2113	21.2191	0.72246231
2114	21.2049	0.722946112
2115	21.1907	0.723430562
2116	21.1765	0.723915661
2117	21.1623	0.724401412
2118	21.1481	0.724887815
2119	21.1339	0.725374872
2120	21.1197	0.725862583
2121	21.1055	0.726350951
2122	21.0913	0.726839977
2123	21.0771	0.727329661
2124	21.0503	0.728255654
2125	20.9109	1.271585632

2126	20.9147	1.271354597
2127	20.9185	1.271123647
2128	20.9223	1.270892779
2129	20.9261	1.270661996
2130	20.9299	1.270431297
2131	20.9337	1.270200681
2132	20.9375	1.269970149
2133	20.9413	1.269739701
2134	20.9451	1.269509336
2135	20.9489	1.269279055
2136	20.9527	1.269048858
2137	20.9565	1.268818744
2138	20.9603	1.268588713
2139	20.9641	1.268358766
2140	20.9679	1.268128902
2141	20.9514	1.2691276
2142	21.029	0.816967046
2143	21.0008	0.818064074
2144	20.9726	0.819164052
2145	20.9444	0.820266993
2146	20.9162	0.821372907
2147	20.888	0.822481808
2148	20.8598	0.823593707
2149	20.8316	0.824708616
2150	20.8034	0.825826548
2151	20.7752	0.826947514
2152	20.747	0.828071528
2153	20.7188	0.829198602
2154	20.6906	0.830328748
2155	20.6624	0.831461979
2156	20.6342	0.832598308
2157	20.606	0.833737746
2158	20.5778	0.834880308
2159	20.6152	0.958516046
2160	20.749	0.952335052
2161	20.8828	0.946233264
2162	21.0166	0.940209168
2163	21.1504	0.934261291
2164	21.2842	0.928388194
2165	21.418	0.922588477
2166	21.5518	0.916860773
2167	21.6856	0.911203748
2168	21.8194	0.905616103
2169	21.9532	0.900096569
2170	22.087	0.894643908
2171	22.2208	0.889256912
2172	22.3546	0.883934403
2173	22.4884	0.878675228
2174	22.6222	0.873478265
2175	22.756	0.868342415
2176	22.7466	1.003226856
2177	22.7678	1.002292712
2178	22.6997	1.00529963
2179	22.6316	1.008324643
2180	22.5635	1.011367917
2181	22.4954	1.014429617
2182	22.4273	1.01750991

2183	22.3592	1.020608966
2184	22.2911	1.023726958
2185	22.223	1.02686406
2186	22.1549	1.030020447
2187	22.0868	1.033196298
2188	22.0187	1.036391794
2189	21.9506	1.039607118
2190	21.8825	1.042842454
2191	21.8144	1.04609799
2192	21.7463	1.049373916
2193	21.784	0.993848696
2194	21.81	0.992663916
2195	21.9958	0.984278817
2196	22.1816	0.976034191
2197	22.3674	0.967926536
2198	22.5532	0.959952468
2199	22.739	0.952108712
2200	22.9248	0.944392099
2201	23.1106	0.936799564
2202	23.2964	0.929328137
2203	23.4822	0.921974943
2204	23.668	0.914737198
2205	23.8538	0.907612204
2206	24.0396	0.900597348
2207	24.2254	0.893690094
2208	24.4112	0.886887986
2209	24.597	0.880188641
2210	24.6864	1.162988528
2211	24.7913	1.158067548
2212	24.8962	1.153188037
2213	25.0011	1.148349473
2214	25.106	1.143551342
2215	25.2109	1.138793141
2216	25.3158	1.134074373
2217	25.4207	1.129394549
2218	25.5256	1.124753189
2219	25.6305	1.120149822
2220	25.7354	1.115583982
2221	25.8403	1.111055212
2222	25.9452	1.106563064
2223	26.0501	1.102107094
2224	26.155	1.097686867
2225	26.2599	1.093301955
2226	26.3648	1.088951936
2227	26.4391	1.085891729
2228	26.5567	0.744821457
2229	26.585	0.744028588
2230	26.6133	0.743237404
2231	26.6416	0.742447901
2232	26.6699	0.741660074
2233	26.6982	0.740873917
2234	26.7265	0.740089424
2235	26.7548	0.739306592
2236	26.7831	0.738525413
2237	26.8114	0.737745884
2238	26.8397	0.736967999
2239	26.868	0.736191752

2240	26.8963	0.735417139
2241	26.9246	0.734644154
2242	26.9529	0.733872793
2243	26.9812	0.73310305
2244	27.0212	0.732017823
2245	26.8355	1.332563209
2246	26.8096	1.333850561
2247	26.7837	1.335140403
2248	26.7578	1.336432741
2249	26.7319	1.337727584
2250	26.706	1.339024938
2251	26.6801	1.340324811
2252	26.6542	1.341627211
2253	26.6283	1.342932144
2254	26.6024	1.344239617
2255	26.5765	1.34554964
2256	26.5506	1.346862218
2257	26.5247	1.348177359
2258	26.4988	1.349495071
2259	26.4729	1.350815362
2260	26.447	1.352138239
2261	26.3575	1.147680926
2262	26.2129	1.154011956
2263	26.0683	1.160413222
2264	25.9237	1.1668859
2265	25.7791	1.17343119
2266	25.6345	1.180050323
2267	25.4899	1.186744554
2268	25.3453	1.193515168
2269	25.2007	1.200363482
2270	25.0561	1.207290839
2271	24.9115	1.214298617
2272	24.7669	1.221388224
2273	24.6223	1.228561101
2274	24.4777	1.235818725
2275	24.3331	1.243162606
2276	24.1885	1.250594291
2277	24.0439	1.258115364
2278	24.0088	1.068358269
2279	24.017	1.067993505
2280	24.0252	1.06762899
2281	24.0334	1.067264723
2282	24.0416	1.066900705
2283	24.0498	1.066536936
2284	24.058	1.066173414
2285	24.0662	1.06581014
2286	24.0744	1.065447114
2287	24.0826	1.065084335
2288	24.0908	1.064721803
2289	24.099	1.064359517
2290	24.1072	1.063997478
2291	24.1154	1.063635685
2292	24.1236	1.063274138
2293	24.1318	1.062912837
2294	24.14	1.062551781
2295	24.2105	0.787261725
2296	24.0553	0.792340981

2297	23.9001	0.797486203
2298	23.7449	0.802698685
2299	23.5897	0.807979754
2300	23.4345	0.813330773
2301	23.2793	0.818753141
2302	23.1241	0.824248295
2303	22.9689	0.82981771
2304	22.8137	0.835462902
2305	22.6585	0.841185427
2306	22.5033	0.846986886
2307	22.3481	0.852868924
2308	22.1929	0.85883323
2309	22.0377	0.864881544
2310	21.8825	0.871015652
2311	21.7754	0.654408185
2312	21.6658	0.657718616
2313	21.5562	0.66106271
2314	21.4466	0.664440984
2315	21.337	0.667853963
2316	21.2274	0.671302185
2317	21.1178	0.674786199
2318	21.0082	0.678306566
2319	20.8986	0.681863857
2320	20.789	0.685458656
2321	20.6794	0.68909156
2322	20.5698	0.692763177
2323	20.4602	0.69647413
2324	20.3506	0.700225055
2325	20.241	0.7040166
2326	20.1314	0.707849429
2327	20.0218	0.711724221
2328	19.8487	1.037851345
2329	19.6934	1.046035728
2330	19.5381	1.054350218
2331	19.3828	1.062797945
2332	19.2275	1.071382135
2333	19.0722	1.080106123
2334	18.9169	1.088973352
2335	18.7616	1.097987378
2336	18.6063	1.107151879
2337	18.451	1.116470652
2338	18.2957	1.125947627
2339	18.1404	1.135586867
2340	17.9851	1.145392575
2341	17.8298	1.155369101
2342	17.6745	1.165520948
2343	17.5192	1.175852779
2344	17.3639	1.186369422
2345	17.2122	1.17591011
2346	17.1557	1.179782813
2347	17.0992	1.183681108
2348	17.0427	1.18760525
2349	16.9862	1.191555498
2350	16.9297	1.195532112
2351	16.8732	1.199535358
2352	16.8167	1.203565503
2353	16.7602	1.207622821

2354	16.7037	1.211707586
2355	16.6472	1.215820078
2356	16.5907	1.21996058
2357	16.5342	1.22412938
2358	16.4777	1.228326769
2359	16.4212	1.232553041
2360	16.3647	1.236808496
2361	16.3082	1.241093438
2362	16.3093	1.182760756
2363	16.3809	1.177590975
2364	16.4525	1.172466191
2365	16.5241	1.167385818
2366	16.5957	1.162349283
2367	16.6673	1.15735602
2368	16.7389	1.152405475
2369	16.8105	1.1474971
2370	16.8821	1.14263036
2371	16.9537	1.137804727
2372	17.0253	1.133019682
2373	17.0969	1.128274716
2374	17.1685	1.123569328
2375	17.2401	1.118903023
2376	17.3117	1.114275317
2377	17.3833	1.109685733
2378	17.4549	1.105133802
2379	17.5547	0.576483791
2380	17.5628	0.576217915
2381	17.5924	0.575248403
2382	17.622	0.574282147
2383	17.6516	0.573319133
2384	17.6812	0.572359342
2385	17.7108	0.57140276
2386	17.7404	0.57044937
2387	17.77	0.569499156
2388	17.7996	0.568552102
2389	17.8292	0.567608193
2390	17.8588	0.566667413
2391	17.8884	0.565729747
2392	17.918	0.564795178
2393	17.9476	0.563863692
2394	17.9772	0.562935274
2395	18.0068	0.562009907
2396	18.0071	0.744706255
2397	18.0403	0.743335754
2398	18.0026	0.744892404
2399	17.9649	0.746455588
2400	17.9272	0.748025347
2401	17.8895	0.749601722
2402	17.8518	0.751184754
2403	17.8141	0.752774488
2404	17.7764	0.754370964
2405	17.7387	0.755974226
2406	17.701	0.757584317
2407	17.6633	0.759201282
2408	17.6256	0.760825163
2409	17.5879	0.762456007
2410	17.5502	0.764093856

2411	17.5125	0.765738758
2412	17.4748	0.767390757
2413	17.3666	1.23282623
2414	17.3384	1.234831357
2415	17.3102	1.236843017
2416	17.3729	1.232379165
2417	17.4356	1.227947418
2418	17.4983	1.22354743
2419	17.561	1.219178862
2420	17.6237	1.214841378
2421	17.6864	1.210534648
2422	17.7491	1.206258345
2423	17.8118	1.202012149
2424	17.8745	1.197795743
2425	17.9372	1.193608813
2426	17.9999	1.189451053
2427	18.0626	1.185322157
2428	18.1253	1.181221828
2429	18.188	1.177149769
2430	18.3424	1.167240928
2431	18.4753	1.275216099
2432	18.6297	1.26464731
2433	18.7841	1.254252267
2434	18.6829	1.261046197
2435	18.8373	1.250710027
2436	18.9917	1.240541921
2437	19.1461	1.230537812
2438	19.3005	1.220693764
2439	19.4549	1.211005968
2440	19.6093	1.201470731
2441	19.7637	1.192084478
2442	19.9181	1.182843745
2443	20.0725	1.173745174
2444	20.2269	1.164785508
2445	20.3813	1.155961592
2446	20.5357	1.147270363
2447	20.6572	1.140522433
2448	20.8496	0.789943212
2449	20.9711	0.785366528
2450	21.0926	0.78084257
2451	21.2141	0.776370433
2452	21.3356	0.77194923
2453	21.3662	0.770843669
2454	21.3968	0.76974127
2455	21.4274	0.768642019
2456	21.458	0.767545904
2457	21.4886	0.76645291
2458	21.5192	0.765363025
2459	21.5498	0.764276235
2460	21.5804	0.763192527
2461	21.611	0.762111887
2462	21.6416	0.761034304
2463	21.6722	0.759959764
2464	21.6228	0.761695988
2465	21.5734	0.763440162
2466	21.4331	1.192547975
2467	21.3837	1.195302964

2468	21.3343	1.198070712
2469	21.2849	1.200851308
2470	21.2355	1.20364484
2471	21.257	1.202427436
2472	21.2785	1.201212491
2473	21.3	1.2
2474	21.3215	1.198789954
2475	21.343	1.197582345
2476	21.3645	1.196377168
2477	21.386	1.195174413
2478	21.4075	1.193974075
2479	21.429	1.192776144
2480	21.4505	1.191580616
2481	21.472	1.190387481
2482	21.472	1.190387481
2483	21.472	1.190387481
2484	21.7276	0

Table S.5. Calculation of event indices from CHAR data at Little Monon Lake using the 100-yr running mean

5	2.36	1.110255803
6	2.36	1.110255803
7	2.36	1.110255803
8	2.36	1.110255803
9	2.36	1.110255803
10	2.36	1.110255803
11	2.36	1.110255803
12	2.36	1.110255803
13	2.36	1.110255803
14	2.36	1.110255803
15	2.36	1.110255803
16	2.36	1.110255803
17	2.36	1.110255803
18	2.36	1.110255803
19	2.36	1.110255803
20	2.43	1.080411421
21	2.49	1.05212951
22	2.56	1.025290499
23	2.63	0.999786712
24	2.69	0.975520928
25	2.75	0.954781546
26	2.81	0.934905637
27	2.87	0.915840375
28	2.92	0.897537158
29	2.98	0.879951192
30	3.04	0.863041127
31	3.10	0.846768731
32	3.16	0.831098602
33	3.22	0.815997911
34	3.28	0.801436174

35	3.33	0.787385042
36	3.39	0.773818122
37	3.46	0.560796858
38	3.52	0.551474248
39	3.57	0.542456525
40	3.63	0.533728974
41	3.69	0.525277809
42	3.75	0.517090108
43	3.81	0.509153739
44	3.87	0.501457304
45	3.93	0.493990085
46	3.98	0.486741992
47	4.04	0.479703519
48	4.10	0.472865703
49	4.16	0.466220083
50	4.22	0.459758668
51	4.28	0.453473905
52	4.33	0.447358646
53	4.39	0.441406126
54	4.39	0.441402106
55	4.39	0.441398087
56	4.39	0.441394068
57	4.39	0.441390049
58	4.39	0.44138603
59	4.39	0.441382011
60	4.39	0.441377992
61	4.39	0.441373973
62	4.39	0.441369954
63	4.39	0.441365935
64	4.39	0.441361917
65	4.39	0.441357898
66	4.39	0.44135388
67	4.39	0.441349861
68	4.39	0.441345843
69	4.39	0.441341825
70	4.33	1.956956205
71	4.33	1.95693812
72	4.33	1.956920034
73	4.33	1.956901949
74	4.33	1.956883865
75	4.33	1.95686578
76	4.33	1.956847696
77	4.33	1.956829613
78	4.33	1.956811529
79	4.33	1.956793446
80	4.33	1.956775364
81	4.33	1.956757281
82	4.33	1.956739199
83	4.33	1.956721118
84	4.33	1.956703036
85	4.33	1.956684955
86	4.33	1.956666875
87	4.33	1.956648794
88	4.34	1.95353493
89	4.35	1.948766088
90	4.36	1.944020473
91	4.37	1.939297915

92	4.38	1.934598245
93	4.39	1.929921299
94	4.40	1.925266912
95	4.41	1.920634921
96	4.42	1.916025164
97	4.43	1.911437482
98	4.44	1.906871717
99	4.45	1.902327712
100	4.46	1.897805313
101	4.47	1.893304364
102	4.48	1.888824714
103	4.49	1.884366212
104	4.56	0.57604242
105	4.57	0.574706362
106	4.59	0.573376487
107	4.60	0.572052753
108	4.61	0.570735117
109	4.62	0.569423537
110	4.63	0.568117971
111	4.64	0.566818379
112	4.65	0.565524718
113	4.66	0.564236949
114	4.67	0.562955032
115	4.68	0.561678926
116	4.69	0.560408593
117	4.70	0.559143993
118	4.71	0.557885087
119	4.72	0.556631837
120	4.73	0.555384206
121	4.68	0.561876998
122	4.62	0.568523396
123	4.57	0.575328916
124	4.51	0.58229934
125	4.53	0.580476216
126	4.54	0.578664471
127	4.56	0.576864001
128	4.57	0.575074701
129	4.59	0.573296466
130	4.60	0.571529194
131	4.61	0.569772784
132	4.63	0.568027137
133	4.64	0.566292154
134	4.66	0.564567737
135	4.67	0.562853791
136	4.69	0.561150219
137	4.70	0.559456929
138	4.71	0.557773827
139	4.72	0.635075203
140	4.74	0.633174547
141	4.75	0.631285234
142	4.77	0.629407161
143	4.78	0.627540231
144	4.79	0.625684342
145	4.81	0.623839399
146	4.82	0.622005304
147	4.84	0.620181961
148	4.85	0.618369278

149	4.87	0.61656716
150	4.88	0.614775515
151	4.89	0.612994252
152	4.91	0.611223282
153	4.92	0.609462515
154	4.94	0.607711864
155	5.01	0.598905201
156	5.08	0.590350137
157	5.15	0.58203604
158	5.23	0.573952871
159	5.30	0.566091141
160	5.37	0.558441873
161	5.42	0.553934783
162	5.46	0.549499864
163	5.50	0.545135393
164	5.55	0.540839708
165	5.59	0.536611193
166	5.63	0.532448286
167	5.68	0.528349471
168	5.72	0.524313281
169	5.77	0.520338289
170	5.81	0.516423116
171	5.85	0.51256642
172	5.90	0.508766902
173	5.94	0.505023298
174	5.98	0.501334385
175	5.96	1.6593778
176	6.00	1.647294409
177	6.05	1.635385725
178	6.09	1.623647986
179	6.13	1.612077539
180	6.18	1.600670831
181	6.22	1.589424412
182	6.26	1.578334927
183	6.31	1.567399113
184	6.35	1.556613798
185	6.40	1.545975897
186	6.44	1.535482408
187	6.48	1.52513041
188	6.53	1.514917061
189	6.57	1.504839593
190	6.61	1.495734253
191	6.65	1.486738437
192	6.69	1.477850182
193	6.71	1.473300891
194	6.73	1.468779523
195	6.75	1.46428582
196	6.77	1.45981953
197	6.79	1.455380404
198	6.81	1.450968193
199	6.84	1.446582653
200	6.86	1.442223545
201	6.88	1.437890628
202	6.90	1.433583669
203	6.92	1.429302434
204	6.94	1.425046694
205	6.96	1.420816222

206	6.98	1.416610793
207	7.00	1.412430186
208	7.02	1.408274181
209	7.04	1.404142561
210	7.06	1.400035114
211	7.11	0.984221523
212	7.13	0.981370779
213	7.15	0.978536501
214	7.17	0.975718547
215	7.19	0.972916777
216	7.22	0.970131051
217	7.24	0.967361232
218	7.26	0.964607184
219	7.28	0.961868774
220	7.30	0.959145867
221	7.32	0.956438333
222	7.34	0.953746042
223	7.36	0.951412712
224	7.38	0.949090771
225	7.39	0.946780136
226	7.34	0.953340778
227	7.29	0.959992978
228	7.24	0.966738666
229	7.19	0.973579825
230	7.14	0.980518498
231	7.09	0.987556785
232	7.04	0.994696845
233	6.99	1.001940903
234	6.94	1.009291247
235	6.88	1.016750234
236	6.83	1.02432029
237	6.78	1.032003916
238	6.73	1.039803685
239	6.68	1.047722252
240	6.63	1.055762351
241	6.58	1.063926802
242	6.53	1.072218512
243	6.50	0.779746898
244	6.45	0.785901553
245	6.40	0.79215414
246	6.34	0.798507016
247	6.29	0.804962612
248	6.24	0.811523441
249	6.19	0.818192096
250	6.14	0.824971258
251	6.09	0.831863697
252	6.04	0.838872275
253	6.04	0.838812549
254	6.04	0.838752831
255	6.04	0.838693122
256	6.04	0.838633422
257	6.04	0.838573729
258	6.04	0.838514046
259	6.04	0.838454371
260	6.04	0.838394704
261	6.04	0.838335046
262	6.07	0.8342885

263	6.10	0.83028083
264	6.13	0.82631148
265	6.16	0.822379902
266	6.19	0.818485559
267	6.22	0.814627926
268	6.25	0.810806485
269	6.28	0.80702073
270	6.31	0.803270163
271	6.34	0.799554295
272	6.37	0.795872648
273	6.40	0.750315367
274	6.43	0.74689339
275	6.46	0.743502485
276	6.49	0.740142231
277	6.51	0.736812213
278	6.54	0.733512025
279	6.57	0.730241269
280	6.60	0.726999552
281	6.63	0.723786489
282	6.63	0.724004833
283	6.63	0.724223308
284	6.63	0.724441916
285	6.62	0.724660655
286	6.62	0.724879527
287	6.62	0.72509853
288	6.62	0.725317666
289	6.62	0.725536935
290	6.61	0.725756336
291	6.61	0.72597587
292	6.61	0.726195537
293	6.61	0.726415337
294	6.63	0.724514085
295	6.64	0.722622759
296	6.66	0.720741282
297	6.68	0.718869578
298	6.69	0.717007569
299	6.71	0.715155181
300	6.73	0.71331234
301	6.75	0.711478972
302	6.76	0.709655004
303	6.73	1.475657813
304	6.75	1.47186545
305	6.76	1.468092529
306	6.78	1.464338902
307	6.80	1.46060442
308	6.82	1.456888938
309	6.83	1.453192311
310	6.85	1.449514396
311	6.87	1.445855051
312	6.85	1.450823073
313	6.82	1.455825354
314	6.80	1.460862249
315	6.77	1.465934118
316	6.75	1.471041327
317	6.73	1.476184247
318	6.70	1.481363254
319	6.68	1.486578728

320	6.66	1.491831057
321	6.63	1.497120632
322	6.61	1.502447851
323	6.59	1.507813117
324	6.57	1.512603762
325	6.54	1.517424946
326	6.52	1.522276962
327	6.50	1.527160107
328	6.48	1.53207468
329	6.46	1.537020987
330	6.44	1.541999335
331	6.42	1.547010038
332	6.43	1.057554281
333	6.41	1.060996369
334	6.39	1.064460937
335	6.37	1.067948205
336	6.35	1.071458397
337	6.33	1.07499174
338	6.30	1.078548464
339	6.28	1.082128802
340	6.25	1.088052227
341	6.22	1.094040856
342	6.18	1.100095773
343	6.15	1.106218084
344	6.11	1.112408922
345	6.08	1.118669441
346	6.04	1.125000827
347	6.01	1.131404289
348	5.98	1.137881065
349	5.94	1.14443242
350	5.91	1.151059652
351	5.87	1.157764085
352	5.84	1.164547077
353	5.80	1.171410016
354	5.72	1.188925509
355	5.63	1.206972753
356	5.55	1.225576336
357	5.46	1.244762388
358	5.38	1.264558697
359	5.29	1.284994851
360	5.21	1.306102379
361	5.12	1.32791492
362	5.08	0.534657171
363	4.99	0.543819117
364	4.91	0.553300538
365	4.82	0.56311844
366	4.73	0.573291058
367	4.65	0.583837971
368	4.56	0.594780223
369	4.48	0.606327341
370	4.39	0.618331689
371	4.30	0.630820974
372	4.22	0.643825185
373	4.13	0.657376839
374	4.04	0.671511247
375	3.95	0.686266825
376	3.87	0.701685441

377	3.78	0.717812813
378	3.69	0.73469896
379	3.61	0.752398721
380	3.52	0.770972351
381	3.43	0.790486204
382	3.35	0.811013528
383	3.29	0.824713447
384	3.24	0.838884167
385	3.18	0.85355038
386	3.12	0.868738537
387	3.07	0.884477005
388	3.01	0.900796245
389	2.96	0.91772901
390	2.92	0.473060589
391	2.86	0.482257202
392	2.80	0.49181848
393	2.75	0.501766553
394	2.69	0.512125376
395	2.64	0.522920925
396	2.58	0.534181413
397	2.53	0.545937536
398	2.53	0.545419308
399	2.53	0.544902064
400	2.53	0.544385799
401	2.54	0.543870512
402	2.54	0.5433562
403	2.54	0.542842859
404	2.54	0.542330487
405	2.55	0.541819082
406	2.55	0.54130864
407	2.55	0.540799159
408	2.55	0.540290636
409	2.55	0.539783069
410	2.56	0.539276454
411	2.56	0.53877079
412	2.56	0.538266073
413	2.61	0.529327995
414	2.65	0.520681908
415	2.69	0.512313734
416	2.73	0.504210284
417	2.78	0.496359193
418	2.82	0.488748853
419	2.87	0.432988036
420	2.91	0.426549895
421	2.95	0.420300408
422	3.00	0.414231402
423	3.04	0.40833517
424	3.08	0.402604439
425	3.13	0.397032335
426	3.17	0.391612364
427	3.21	0.386373417
428	3.25	0.381272792
429	3.30	0.376305081
430	3.34	0.371465157
431	3.38	0.366748152
432	3.43	0.362149441
433	3.47	0.357664629

434	3.51	0.353289538
435	3.56	0.349020189
436	3.60	0.344852794
437	3.64	0.340783744
438	3.68	0.336809599
439	3.73	0.332927077
440	3.77	0.329133045
441	3.83	0.32428926
442	3.88	0.319585978
443	3.94	0.315017172
444	4.00	0.310577156
445	4.05	0.30626056
446	4.11	0.302062309
447	4.16	0.297977602
448	4.16	1.691059214
449	4.22	1.668487646
450	4.28	1.646510694
451	4.33	1.625105166
452	4.39	1.604249063
453	4.44	1.583921498
454	4.50	1.564102631
455	4.56	1.544773604
456	4.61	1.526024336
457	4.67	1.507724738
458	4.73	1.489858825
459	4.78	1.472411361
460	4.84	1.455367816
461	4.89	1.438714322
462	4.95	1.422437642
463	5.01	1.406525129
464	5.06	1.390964698
465	5.12	1.37574479
466	5.17	1.36085435
467	5.23	1.346282792
468	5.29	1.332019983
469	5.34	1.318056213
470	5.40	1.304048744
471	5.46	1.290335869
472	5.51	1.276908391
473	5.57	1.263757493
474	5.63	1.250874716
475	5.69	1.238251944
476	5.74	1.225881383
477	5.80	1.208740424
478	5.86	1.196967813
479	5.91	1.18542231
480	5.97	1.174097407
481	6.03	1.16298684
482	6.09	1.152084582
483	6.14	1.141384829
484	6.20	1.130881991
485	6.26	1.120570682
486	6.31	1.110445709
487	6.37	1.100502067
488	6.43	1.090734928
489	6.48	1.081139634
490	6.54	1.071711689

491	6.60	1.062446753
492	6.66	1.053340634
493	6.71	1.044389285
494	6.77	1.035588791
495	6.83	1.026935372
496	6.88	1.018473615
497	6.94	1.010150164
498	7.00	1.001961658
499	7.00	1.002143487
500	6.99	1.002325383
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504	6.99	1.003053626
505	6.99	1.003235852
506	6.99	0.998704478
507	6.99	0.998885971
508	6.98	0.999067531
509	6.98	0.999249156
510	6.98	0.999430848
511	6.98	0.999612606
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516	6.97	1.00066274
517	6.97	1.000891756
518	6.97	1.001120877
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521	6.97	1.00180887
522	6.96	1.002038411
523	6.96	1.002268057
524	6.96	1.002497809
525	6.96	1.002727666
526	6.96	1.002957629
527	6.96	0.998453102
528	6.96	0.99864035
529	6.95	0.998827668
530	6.95	0.999015056
531	6.95	0.999249391
532	6.95	0.999483835
533	6.95	0.999718389
534	6.95	0.999953054
535	6.94	1.000187829
536	6.94	1.000422713
537	6.94	1.000657709
538	6.94	1.000892814
539	6.94	1.001128031
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541	6.93	1.001598795
542	6.93	1.001834343
543	6.93	1.002070002
544	6.93	1.002305772
545	6.93	1.002541653
546	6.93	0.998022787
547	6.93	0.998257759

548	6.92	0.998539872
549	6.92	0.998822145
550	6.92	0.999104577
551	6.92	0.999387169
552	6.92	0.999669921
553	6.91	0.999952832
554	6.91	1.000235904
555	6.91	1.000519137
556	6.91	1.00080253
557	6.91	1.001038813
558	6.90	1.001275208
559	6.90	1.001511714
560	6.90	1.001748333
561	6.90	1.001985063
562	6.90	1.002221905
563	6.90	1.002458859
564	6.89	0.997919033
565	6.89	0.998202291
566	6.89	0.99848571
567	6.89	0.99876929
568	6.89	0.999053031
569	6.88	0.999336933
570	6.88	0.999620997
571	6.88	0.999905222
572	6.88	1.000189609
573	6.88	1.000474158
574	6.88	1.000758869
575	6.87	1.001043741
576	6.87	1.001328776
577	6.87	1.001613974
578	6.87	1.001851762
579	6.87	1.002089664
580	6.86	1.002327678
581	6.86	0.997766905
582	6.86	0.998051425
583	6.86	0.998336108
584	6.86	0.998620953
585	6.86	0.998905961
586	6.85	0.999191132
587	6.85	0.999476465
588	6.85	0.999761962
589	6.85	1.000047621
590	6.85	1.000333444
591	6.84	1.000619431
592	6.84	1.00090558
593	6.84	1.001191894
594	6.84	1.001478372
595	6.84	1.001765013
596	6.83	1.002051819
597	6.83	1.002290949
598	6.91	0.985644305
599	7.00	0.974095221
600	7.08	0.96281365
601	7.16	0.951790405
602	7.24	0.941016713
603	7.32	0.930484194
604	7.41	0.92018484

605	7.49	0.910110993
606	7.57	0.900255327
607	7.65	0.89061083
608	7.73	0.881170787
609	7.82	0.871928765
610	7.90	0.862878598
611	7.98	0.854014373
612	8.06	0.845330417
613	8.14	0.836821288
614	8.23	0.828481758
615	8.31	0.816317818
616	8.28	0.818910363
617	8.26	0.821519429
618	8.23	0.824145172
619	8.20	0.826787754
620	8.18	0.829447338
621	8.15	0.832124087
622	8.12	0.834818169
623	8.10	0.837529752
624	8.07	0.840259007
625	8.05	0.843006108
626	8.02	0.845771231
627	7.99	0.848554553
628	7.97	0.851356254
629	7.94	0.854176518
630	7.91	0.857015529
631	7.89	0.859873475
632	7.86	0.858548961
633	7.84	0.861412804
634	7.81	0.864295818
635	7.78	0.867198194
636	7.76	0.870120128
637	7.73	0.87306182
638	7.71	0.876023469
639	7.68	0.87900528
640	7.65	0.882007459
641	7.63	0.885030217
642	7.60	0.888073764
643	7.57	0.891138316
644	7.55	0.894224092
645	7.52	0.897331313
646	7.50	0.900460202
647	7.47	0.903610989
648	7.36	2.053046778
649	7.36	2.052222065
650	7.37	2.051398015
651	7.37	2.050574626
652	7.37	2.049751897
653	7.38	2.048929829
654	7.38	2.04810842
655	7.38	2.047287669
656	7.38	2.046467576
657	7.39	2.045648139
658	7.39	2.044829359
659	7.39	2.044011233
660	7.40	2.043193762
661	7.40	2.042376945

662	7.40	2.041560781
663	7.40	2.040745268
664	7.41	2.039930407
665	7.41	2.039116196
666	7.52	0.564974952
667	7.55	0.562831808
668	7.58	0.560704863
669	7.61	0.558593932
670	7.64	0.556498836
671	7.67	0.554419398
672	7.69	0.552355442
673	7.72	0.550306796
674	7.75	0.54827329
675	7.78	0.546254757
676	7.81	0.544251033
677	7.84	0.542261955
678	7.87	0.540287363
679	7.89	0.5383271
680	7.92	0.536381009
681	7.95	0.534448938
682	7.98	0.530515279
683	8.01	0.528596523
684	7.99	0.529975404
685	7.97	0.531361498
686	7.95	0.532754861
687	7.93	0.534155551
688	7.91	0.535563626
689	7.88	0.536979144
690	7.86	0.538402164
691	7.84	0.539832746
692	7.82	0.541270951
693	7.80	0.54271684
694	7.78	0.544170474
695	7.76	0.545631916
696	7.74	0.547101229
697	7.72	0.548578477
698	7.70	0.550063724
699	7.56	0.940102352
700	7.46	0.953265716
701	7.36	0.966802943
702	7.40	0.960996593
703	7.44	0.95525957
704	7.49	0.949590638
705	7.53	0.943988594
706	7.58	0.93845226
707	7.62	0.932980486
708	7.67	0.927572151
709	7.71	0.922226156
710	7.76	0.916941431
711	7.80	0.911716928
712	7.84	0.906551623
713	7.89	0.901444517
714	7.93	0.89639463
715	7.98	0.891401008
716	8.02	0.886462713
717	8.15	1.183759415
718	8.30	1.161937775

719	8.46	1.140906102
720	8.64	1.116587112
721	8.82	1.093283227
722	9.01	1.070932189
723	9.19	1.04947673
724	9.38	1.028864079
725	9.56	1.009045533
726	9.74	0.989976069
727	9.93	0.971614005
728	10.11	0.953920696
729	10.30	0.93686026
730	10.48	0.920399339
731	10.67	0.904506876
732	10.85	0.889153926
733	11.03	0.874300795
734	11.27	0.414089699
735	11.45	0.407425162
736	11.64	0.400971751
737	11.82	0.394719589
738	11.97	0.389741322
739	12.12	0.384887065
740	12.27	0.38015224
741	12.43	0.375532494
742	12.58	0.371023681
743	12.73	0.366621854
744	12.88	0.362323248
745	13.03	0.358124276
746	13.18	0.354021513
747	13.33	0.350011689
748	13.48	0.346091683
749	13.63	0.34225851
750	13.76	0.339217333
751	13.88	0.336229725
752	13.85	1.411836902
753	13.97	1.399487389
754	14.10	1.387352048
755	14.22	1.375425356
756	14.31	1.366879367
757	14.40	1.358438921
758	14.48	1.350102075
759	14.57	1.341866932
760	14.66	1.333731643
761	14.75	1.325694402
762	14.84	1.317753448
763	14.93	1.309907061
764	15.02	1.302153562
765	15.11	1.29449131
766	15.20	1.286918704
767	15.28	1.279434181
768	15.35	1.274138089
769	15.41	1.268885662
770	15.44	1.467664878
771	15.51	1.461652104
772	15.57	1.455688395
773	15.63	1.449773154
774	15.58	1.454821331
775	15.53	1.459904787

776	15.47	1.465023893
777	15.42	1.470179025
778	15.36	1.475370565
779	15.31	1.4805989
780	15.25	1.485864422
781	15.20	1.49116753
782	15.15	1.496508628
783	15.09	1.501888125
784	15.04	1.507306437
785	15.03	1.50775162
786	15.03	1.508197067
787	15.02	1.508642778
788	15.05	1.284351996
789	15.05	1.284730937
790	15.04	1.285110103
791	15.04	1.285489492
792	15.11	1.279628087
793	15.18	1.273819891
794	15.25	1.268064184
795	15.32	1.262360257
796	15.38	1.256707413
797	15.45	1.251104971
798	15.52	1.245552259
799	15.59	1.240048618
800	15.66	1.2345934
801	15.73	1.229185969
802	15.80	1.2238257
803	15.72	1.230054829
804	15.64	1.236347692
805	15.56	1.242705275
806	15.51	1.031539978
807	15.43	1.036887934
808	15.35	1.042291632
809	15.27	1.047751948
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811	15.04	1.06416729
812	14.92	1.072569371
813	14.80	1.081105185
814	14.68	1.089777949
815	14.56	1.098590986
816	14.45	1.107547728
817	14.33	1.116651717
818	14.21	1.125906616
819	14.09	1.135316208
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821	13.83	1.157213197
822	13.68	1.169810408
823	13.53	1.182684899
824	13.50	0.312800288
825	13.35	0.316289271
826	13.20	0.319856965
827	13.05	0.323506063
828	12.92	0.3268452
829	12.78	0.330253987
830	12.65	0.333734626
831	12.52	0.337289413
832	12.38	0.340920744

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834	12.12	0.348423141
835	11.98	0.352299542
836	11.85	0.356263168
837	11.72	0.360316996
838	11.58	0.364464141
839	11.48	0.367637881
840	11.38	0.37086738
841	11.28	0.37415412
842	11.11	1.039980365
843	11.01	1.049425788
844	10.91	1.059044356
845	10.81	1.068840874
846	10.73	1.07732164
847	10.64	1.085938064
848	10.56	1.094693428
849	10.47	1.103591119
850	10.39	1.112634637
851	10.30	1.121827596
852	10.22	1.131173731
853	10.13	1.140676903
854	10.04	1.150341103
855	9.96	1.160170459
856	9.87	1.170169242
857	9.82	1.176336447
858	9.77	1.182569005
859	9.72	1.188867957
860	9.71	0.801311443
861	9.65	0.805608702
862	9.60	0.809952301
863	9.50	0.818859998
864	9.39	0.827965804
865	9.29	0.837276402
866	9.18	0.84679878
867	9.08	0.856540247
868	8.98	0.866508451
869	8.87	0.876711402
870	8.77	0.887157491
871	8.66	0.897855514
872	8.56	0.908814696
873	8.45	0.920044719
874	8.35	0.931555747
875	8.36	0.930070691
876	8.38	0.928590362
877	8.39	0.927114738
878	8.39	1.112905072
879	8.40	1.111138891
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881	8.46	1.103319297
882	8.51	1.097326113
883	8.55	1.091397686
884	8.60	1.085532973
885	8.64	1.079730952
886	8.69	1.073990624
887	8.74	1.068311009
888	8.78	1.062691149
889	8.83	1.057130106

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892	8.97	1.040790791
893	8.94	1.043949222
894	8.91	1.04712688
895	8.89	1.050323943
896	8.84	1.223795201
897	8.82	1.227560973
898	8.79	1.231349991
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900	8.75	1.23752527
901	8.73	1.239897124
902	8.71	1.242278088
903	8.70	1.244668213
904	8.68	1.247067553
905	8.66	1.249476162
906	8.65	1.251894093
907	8.63	1.2543214
908	8.61	1.256758138
909	8.60	1.259204362
910	8.58	1.261660127
911	8.60	1.258571789
912	8.62	1.255498534
913	8.69	0.638930967
914	8.72	0.637387754
915	8.74	0.635851979
916	8.76	0.634323586
917	8.72	0.636909082
918	8.69	0.639515741
919	8.65	0.642143824
920	8.62	0.644793596
921	8.58	0.647465328
922	8.54	0.650159292
923	8.51	0.652875768
924	8.47	0.655615039
925	8.44	0.658377393
926	8.40	0.661163123
927	8.37	0.663972528
928	8.33	0.66680591
929	8.28	0.67092208
930	8.23	0.675089384
931	8.14	1.085632619
932	8.09	1.09248837
933	8.04	1.09943126
934	7.99	1.10646296
935	7.97	1.109548112
936	7.95	1.112650517
937	7.92	1.115770319
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939	7.88	1.122062707
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956	7.54	1.310862168
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972	8.35	0.505842105
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983	9.48	0.445425746
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1181	8.36	0.451818579
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1186	7.99	1.446872664
1187	7.95	1.453749869
1188	7.91	1.460692764
1189	7.87	1.467702293
1190	7.84	1.474779421
1191	7.80	1.48192513
1192	7.76	1.489140423
1193	7.72	1.496426319
1194	7.68	1.503783861
1195	7.65	1.511214111
1196	7.61	1.518718152
1197	7.66	1.508584111
1198	7.71	1.498584418
1199	7.76	1.488716419
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1201	7.89	1.097958527
1202	7.94	1.090894443
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1204	8.02	1.080318286
1205	8.05	1.076739762
1206	8.08	1.073184867
1207	8.10	1.069653369
1208	8.13	1.066145036
1209	8.16	1.062659641
1210	8.18	1.059196961
1211	8.21	1.055756774
1212	8.23	1.052338861
1213	8.26	1.048943008
1214	8.29	1.045569
1215	8.33	1.040547242
1216	8.37	1.035573491
1217	8.41	1.030647062
1218	8.46	0.893087233
1219	8.50	0.888884183
1220	8.54	0.884720508
1221	8.58	0.880595658
1222	8.59	0.879402542
1223	8.60	0.878212654
1224	8.61	0.877025982
1225	8.63	0.875842513
1226	8.64	0.874662233
1227	8.65	0.873485131
1228	8.66	0.872311192
1229	8.67	0.871140404
1230	8.68	0.869972755
1231	8.70	0.868808232

1232	8.71	0.867646822
1233	8.64	0.874287725
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1235	8.51	0.887879244
1236	8.42	1.214558062
1237	8.35	1.224178422
1238	8.28	1.233952402
1239	8.22	1.242955028
1240	8.16	1.252089981
1241	8.10	1.261360201
1242	8.04	1.270768714
1243	7.98	1.280318639
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1245	7.86	1.29985567
1246	7.80	1.309849499
1247	7.74	1.319998192
1248	7.68	1.330305377
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1250	7.56	1.351410308
1251	7.53	1.35699152
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1253	7.47	1.368293395
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1256	7.40	1.05069889
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1259	7.37	1.055451583
1260	7.36	1.057045383
1261	7.35	1.058644005
1262	7.34	1.060247468
1263	7.32	1.061855797
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1265	7.30	1.065087137
1266	7.29	1.066710193
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1271	7.27	1.06997119
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1274	7.30	0.677148002
1275	7.29	0.678180593
1276	7.27	0.679216337
1277	7.26	0.680255251
1278	7.25	0.681297347
1279	7.24	0.682342641
1280	7.23	0.683391147
1281	7.22	0.684442881
1282	7.21	0.685497857
1283	7.20	0.68655609
1284	7.19	0.687617595
1285	7.17	0.688682388
1286	7.16	0.689750484
1287	7.13	0.693407506
1288	7.09	0.697103514

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1292	6.93	0.80151328
1293	7.03	0.790613405
1294	7.12	0.78000601
1295	7.22	0.769679478
1296	7.31	0.7596228
1297	7.41	0.749825536
1298	7.50	0.740277774
1299	7.60	0.730970105
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1302	7.89	0.704400378
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1304	8.08	0.687734919
1305	8.20	0.677667117
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1307	8.42	0.897562621
1308	8.54	0.884946423
1309	8.66	0.872679977
1310	8.78	0.860748937
1311	8.78	0.860966699
1312	8.77	0.86118457
1313	8.77	0.861402553
1314	8.77	0.861620645
1315	8.77	0.861838848
1316	8.76	0.862057162
1317	8.76	0.862275586
1318	8.76	0.862494121
1319	8.76	0.862712766
1320	8.76	0.862931523
1321	8.75	0.86315039
1322	8.75	0.863369369
1323	8.78	0.860797973
1324	8.80	0.858241848
1325	8.84	0.728948556
1326	8.87	0.726799432
1327	8.89	0.724662943
1328	8.92	0.722538978
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1339	8.92	0.722538978
1340	8.91	0.723036754
1341	8.91	0.723535217
1342	8.90	0.724034368
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1344	8.75	2.259061605
1345	8.75	2.260647114

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1362	9.94	0.759928101
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1366	10.12	0.746248754
1367	10.17	0.742986619
1368	10.21	0.73975288
1369	10.26	0.736547168
1370	10.30	0.73336912
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1372	10.39	0.727094595
1373	10.44	0.723997424
1374	10.48	0.720926527
1375	10.52	0.71788157
1376	10.58	0.714111524
1377	10.64	0.710380869
1378	10.69	0.706688991
1379	10.77	0.458672162
1380	10.83	0.456318642
1381	10.88	0.453989151
1382	10.89	0.453711092
1383	10.90	0.453433374
1384	10.90	0.453155996
1385	10.91	0.452878957
1386	10.92	0.452602257
1387	10.92	0.452325894
1388	10.93	0.452049869
1389	10.94	0.451774181
1390	10.94	0.451498828
1391	10.95	0.451223811
1392	10.96	0.450949129
1393	10.96	0.450674781
1394	10.84	0.455942197
1395	10.71	0.4613342
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1397	10.38	1.198625684
1398	10.26	1.213429635
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1433	9.96	0.71418815
1434	10.04	0.708163952
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1437	10.13	0.701750577
1438	10.16	0.699638522
1439	10.19	0.697539142
1440	10.23	0.695452323
1441	10.26	0.693377953
1442	10.29	0.691315921
1443	10.32	0.689266117
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1445	10.38	0.685202762
1446	10.41	0.683188997
1447	10.36	0.686118428
1448	10.32	0.68907309
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1450	10.22	0.848341153
1451	10.17	0.852047874
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1457	10.03	0.86432251
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1459	9.99	0.867784526

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1461	9.95	0.871274387
1462	9.93	0.873029865
1463	9.91	0.874792431
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1466	9.86	0.879330061
1467	9.84	0.880720593
1468	9.78	1.37173636
1469	9.76	1.373923021
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1472	9.84	1.36306442
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1476	10.03	1.337688893
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1482	10.31	1.301348994
1483	10.40	1.289394011
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1485	10.65	0.751440417
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1487	10.84	0.738189885
1488	10.93	0.731738327
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1499	10.93	0.731738327
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1523	12.71	1.311582233
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1547	11.08	0.641555395
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1549	10.99	0.647265392
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1553	10.79	0.658995835
1554	10.72	0.66350481
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1572	9.67	2.228890684
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1576	9.48	0.281300185
1577	9.38	0.284165097
1578	9.29	0.287088965
1579	9.22	0.289304313
1580	9.14	0.291554117
1581	9.07	0.293839186
1582	9.00	0.296160358
1583	8.93	0.298518493
1584	8.86	0.300914482
1585	8.79	0.303349244
1586	8.72	0.305823728
1587	8.65	0.308338914
1588	8.58	0.310895814
1589	8.50	0.313495475
1590	8.53	0.3125971
1591	8.55	0.31170386
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1593	8.60	0.361852742
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1602	8.40	0.370179104
1603	8.36	0.372147438
1604	8.32	0.374136815
1605	8.27	0.376147577
1606	8.23	0.378180068
1607	8.18	0.380234643
1608	7.99	0.389220433
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1610	7.62	0.408529347
1611	7.39	0.962737418
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1645	7.42	1.28702469
1646	7.43	1.286255442
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1649	7.51	0.354957894
1650	7.52	0.354748183
1651	7.78	0.342689819
1652	8.04	0.331424267
1653	8.31	0.320875825
1654	8.57	0.310978135
1655	8.84	0.301672779
1656	9.10	0.292908129
1657	9.37	0.284638389
1658	9.63	0.276822789
1659	9.90	0.26942492
1660	10.16	0.262412164
1661	10.42	0.255755212
1662	10.65	0.250364606
1663	10.87	0.245196548
1664	11.10	0.240237534
1665	11.18	1.530865125
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1674	13.15	1.300882359
1675	13.37	1.279757675
1676	13.59	1.259308105
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1682	14.82	1.154820605
1683	15.15	0.234726905
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1686	15.66	0.226986418
1687	15.79	0.225102008

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1689	16.06	0.221425519
1690	16.19	0.219631945
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1692	16.45	0.216130576
1693	16.58	0.214421424
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1695	16.84	0.211082954
1696	16.97	0.209452401
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1733	16.93	1.702344053
1734	16.94	1.70189675
1735	16.94	1.701449683
1736	17.01	1.332801765
1737	17.01	1.332453105
1738	17.02	1.332104627
1739	17.02	1.331756331
1740	17.11	1.325025956
1741	17.19	1.318363266
1742	17.28	1.311767246
1743	17.37	1.305236899
1744	17.45	1.29877125

1745	17.54	1.292369342
1746	17.62	1.286030237
1747	17.71	1.279753015
1748	17.80	1.273536776
1749	17.88	1.267380633
1750	17.97	1.26128372
1751	18.06	1.255245187
1752	17.88	1.267426698
1753	17.71	1.279846956
1754	17.66	0.566153958
1755	17.49	0.571771961
1756	17.32	0.577502579
1757	17.14	0.58334923
1758	16.92	0.590933077
1759	16.70	0.598716711
1760	16.48	0.606708129
1761	16.26	0.614915766
1762	16.04	0.623348516
1763	15.82	0.63201577
1764	15.60	0.640927448
1765	15.38	0.650094036
1766	15.16	0.659526631
1767	14.94	0.669236983
1768	14.72	0.679237542
1769	14.50	0.689541517
1770	14.29	0.699804265
1771	14.08	0.710377118
1772	13.92	0.287266551
1773	13.71	0.291722307
1774	13.50	0.296318465
1775	13.29	0.30106177
1776	13.10	0.305440273
1777	12.91	0.309948014
1778	12.71	0.3145908
1779	12.52	0.319374792
1780	12.33	0.324306531
1781	12.14	0.32939297
1782	11.95	0.334641503
1783	11.76	0.340060004
1784	11.57	0.345656865
1785	11.38	0.35144104
1786	11.19	0.357422093
1787	11.06	0.361586497
1788	10.93	0.365849085
1789	10.80	0.370213372
1790	10.59	1.151628941
1791	10.46	1.165813011
1792	10.34	1.180350835
1793	10.21	1.195255814
1794	10.02	1.217792143
1795	9.83	1.24119464
1796	9.64	1.265514219
1797	9.45	1.290805865
1798	9.26	1.317129047
1799	9.07	1.344548188
1800	8.88	1.373133186
1801	8.70	1.402960016

1802	8.51	1.434111398
1803	8.32	1.466677567
1804	8.13	1.500757144
1805	8.07	1.512334201
1806	8.00	1.524091261
1807	7.94	1.536032554
1808	7.93	0.953100636
1809	7.86	0.960642276
1810	7.80	0.968304218
1811	7.74	0.976089364
1812	7.72	0.978900832
1813	7.70	0.981728544
1814	7.67	0.984572639
1815	7.65	0.987433262
1816	7.63	0.990310555
1817	7.61	0.993204666
1818	7.58	0.996115742
1819	7.56	0.999043933
1820	7.54	1.00198939
1821	7.52	1.004952266
1822	7.50	1.007932717
1823	7.53	1.002879212
1824	7.57	0.997876129
1825	7.61	0.992922715
1826	7.62	1.282330413
1827	7.66	1.276009277
1828	7.70	1.269750154
1829	7.74	1.263552136
1830	7.90	1.237217507
1831	8.07	1.211958186
1832	8.23	1.187709629
1833	8.40	1.164412357
1834	8.56	1.142011468
1835	8.73	1.120456205
1836	8.89	1.099699571
1837	9.06	1.079697989
1838	9.22	1.060410997
1839	9.38	1.041800972
1840	9.55	1.023832886
1841	9.63	1.015042395
1842	9.71	1.006401566
1843	9.80	0.997906611
1844	9.94	0.379971852
1845	10.02	0.376836667
1846	10.11	0.373752797
1847	10.18	0.370890087
1848	10.26	0.368070897
1849	10.34	0.365294242
1850	10.42	0.362559167
1851	10.50	0.359864743
1852	10.57	0.357210073
1853	10.65	0.354594282
1854	10.73	0.352016523
1855	10.81	0.349475971
1856	10.89	0.346971828
1857	10.96	0.344503316
1858	11.04	0.34206968

1859	11.17	0.338257183
1860	11.29	0.334528733
1861	11.41	0.33088158
1862	11.50	0.676295566
1863	11.62	0.669054858
1864	11.75	0.661967551
1865	11.88	0.654416449
1866	12.02	0.647035675
1867	12.15	0.639819531
1868	12.29	0.63276257
1869	12.43	0.625859581
1870	12.56	0.619105581
1871	12.70	0.612495796
1872	12.83	0.606025658
1873	12.97	0.599690786
1874	13.10	0.593486982
1875	13.24	0.587410221
1876	13.38	0.58145664
1877	13.49	0.576570779
1878	13.60	0.571766344
1879	13.72	0.567041316
1880	13.70	1.494001369
1881	13.81	1.481744216
1882	13.93	1.469686548
1883	13.89	1.473447356
1884	13.86	1.47722746
1885	13.82	1.481027009
1886	13.79	1.484846155
1887	13.75	1.488685048
1888	13.71	1.492543843
1889	13.68	1.496422694
1890	13.64	1.500321759
1891	13.61	1.504241196
1892	13.57	1.508181164
1893	13.54	1.512141827
1894	13.50	1.516123346
1895	13.53	1.513382754
1896	13.55	1.510652053
1897	13.58	1.472798516
1898	13.60	1.470151514
1899	13.63	1.467514009
1900	13.65	1.464885951
1901	13.71	1.458476446
1902	13.77	1.452122786
1903	13.83	1.445824243
1904	13.89	1.439580103
1905	13.95	1.433389666
1906	14.01	1.42725224
1907	14.07	1.421167148
1908	14.13	1.415133723
1909	14.19	1.40915131
1910	14.25	1.403219266
1911	14.31	1.397336955
1912	14.37	1.391503756
1913	14.39	1.389570164
1914	14.41	1.387641938
1915	14.42	1.463822552

1916	14.44	1.461795354
1917	14.46	1.459773763
1918	14.48	1.457757756
1919	14.45	1.461345066
1920	14.41	1.464950075
1921	14.38	1.468572915
1922	14.34	1.472213718
1923	14.30	1.475872618
1924	14.27	1.47954975
1925	14.23	1.483245252
1926	14.20	1.48695926
1927	14.16	1.490691914
1928	14.13	1.494443355
1929	14.09	1.498213726
1930	14.06	1.502003169
1931	13.89	1.51956956
1932	13.73	1.537551701
1933	13.72	0.453608308
1934	13.55	0.459045905
1935	13.39	0.464615449
1936	13.23	0.470321802
1937	13.24	0.470070941
1938	13.24	0.469820348
1939	13.25	0.469570021
1940	13.26	0.469319961
1941	13.26	0.469070168
1942	13.27	0.46882064
1943	13.28	0.468571377
1944	13.29	0.468322379
1945	13.29	0.468073646
1946	13.30	0.467825177
1947	13.31	0.467576971
1948	13.32	0.467164114
1949	13.33	0.466751985
1950	13.34	0.466340583
1951	13.32	0.73409774
1952	13.33	0.733450111
1953	13.34	0.732803624
1954	13.22	0.739454436
1955	13.10	0.746227077
1956	12.98	0.753124925
1957	12.86	0.760151486
1958	12.74	0.767310395
1959	12.62	0.774605428
1960	12.50	0.782040504
1961	12.38	0.789619695
1962	12.26	0.797347232
1963	12.14	0.805227514
1964	12.02	0.813265113
1965	11.90	0.82146479
1966	11.77	0.830614742
1967	11.64	0.839970824
1968	11.51	0.849540082
1969	11.43	0.369281257
1970	11.30	0.373565176
1971	11.17	0.377949654
1972	11.19	0.377424272

1973	11.20	0.376900347
1974	11.22	0.376377876
1975	11.23	0.37585685
1976	11.25	0.375337266
1977	11.26	0.374819116
1978	11.28	0.374302394
1979	11.30	0.373787095
1980	11.31	0.373273214
1981	11.33	0.372760743
1982	11.34	0.372249677
1983	11.36	0.371740011
1984	11.52	0.366434528
1985	11.69	0.361278354
1986	11.85	0.356265273
1987	11.85	1.787664977
1988	12.01	1.763188527
1989	12.17	1.73937328
1990	12.36	1.713415552
1991	12.54	1.688221197
1992	12.73	1.66375703
1993	12.91	1.63999176
1994	13.10	1.616895859
1995	13.28	1.594441441
1996	13.47	1.572602146
1997	13.65	1.551353038
1998	13.83	1.530670514
1999	14.02	1.51053221
2000	14.20	1.490916925
2001	14.39	1.471804545
2002	14.54	1.456729774
2003	14.69	1.441960674
2004	14.97	0.534537468
2005	15.12	0.529272052
2006	15.26	0.524109358
2007	15.41	0.519046408
2008	15.48	0.516736781
2009	15.55	0.514447618
2010	15.62	0.512178648
2011	15.69	0.509929604
2012	15.76	0.507700226
2013	15.83	0.505490256
2014	15.90	0.503299442
2015	15.96	0.501127537
2016	16.03	0.498974296
2017	16.10	0.49683948
2018	16.17	0.494722853
2019	16.24	0.492624184
2020	16.36	0.488878025
2021	16.49	0.485188411
2022	16.47	1.376515071
2023	16.59	1.366190375
2024	16.72	1.356019408
2025	16.84	1.345998762
2026	16.97	1.335950301
2027	17.09	1.32605076
2028	17.22	1.316296854
2029	17.35	1.306685391

2030	17.47	1.297213275
2031	17.60	1.287877497
2032	17.73	1.278675133
2033	17.85	1.269603346
2034	17.98	1.260659374
2035	18.11	1.251840536
2036	18.23	1.243144224
2037	18.36	1.234567901
2038	18.32	1.237458084
2039	18.27	1.240361831
2040	18.21	1.354469486
2041	18.17	1.357666307
2042	18.13	1.360878254
2043	18.08	1.364105434
2044	17.99	1.370893877
2045	17.90	1.377750222
2046	17.81	1.384675494
2047	17.72	1.391670738
2048	17.63	1.39873702
2049	17.54	1.405875426
2050	17.46	1.413087068
2051	17.37	1.420373077
2052	17.28	1.42773461
2053	17.19	1.435172848
2054	17.10	1.442688995
2055	17.14	1.439135189
2056	17.18	1.435598847
2057	17.22	1.432079843
2058	17.35	0.960791232
2059	17.39	0.958458369
2060	17.43	0.956136808
2061	17.47	0.953826466
2062	17.44	0.95577162
2063	17.40	0.957724723
2064	17.37	0.959685825
2065	17.33	0.961654974
2066	17.29	0.963632222
2067	17.26	0.965617616
2068	17.22	0.967611209
2069	17.19	0.969613051
2070	17.15	0.971623193
2071	17.12	0.973641686
2072	17.08	0.975668584
2073	16.90	0.986188859
2074	16.72	0.996938479
2075	16.53	1.007925027
2076	16.35	1.032872349
2077	16.17	1.044513029
2078	15.99	1.056419085
2079	15.80	1.068599697
2080	15.64	1.079835441
2081	15.47	1.091309973
2082	15.31	1.103030984
2083	15.15	1.115006503
2084	14.98	1.127244911
2085	14.82	1.139754961
2086	14.65	1.152545797

2087	14.49	1.16562698
2088	14.32	1.17900851
2089	14.16	1.192700852
2090	14.00	1.20671496
2091	13.81	1.222830612
2092	13.63	1.23938254
2093	13.44	1.256388701
2094	13.30	0.918676601
2095	13.12	0.931591801
2096	12.94	0.944875315
2097	12.75	0.958543127
2098	12.65	0.965820908
2099	12.56	0.973210048
2100	12.46	0.980713123
2101	12.37	0.988332789
2102	12.27	0.996071784
2103	12.17	1.003932933
2104	12.08	1.011919152
2105	11.98	1.02003345
2106	11.89	1.028278933
2107	11.79	1.036658807
2108	11.69	1.045176385
2109	11.68	1.046615583
2110	11.66	1.04805875
2111	11.65	1.049505903
2112	11.71	0.379596111
2113	11.69	0.38011821
2114	11.68	0.380641746
2115	11.54	0.385187445
2116	11.40	0.389843028
2117	11.26	0.394612527
2118	11.12	0.399500175
2119	10.99	0.404510419
2120	10.85	0.409647928
2121	10.71	0.414917614
2122	10.57	0.420324645
2123	10.44	0.425874461
2124	10.30	0.431572793
2125	10.16	0.437425685
2126	10.02	0.44343951
2127	9.88	0.44972201
2128	9.74	0.456185085
2129	9.60	0.462836634
2130	9.44	0.658839375
2131	9.30	0.668753251
2132	9.16	0.678970043
2133	9.06	0.68713418
2134	8.95	0.695497042
2135	8.84	0.704065975
2136	8.73	0.712848691
2137	8.62	0.72185329
2138	8.51	0.73108829
2139	8.40	0.740562647
2140	8.29	0.750285789
2141	8.18	0.760267645
2142	8.08	0.770518682
2143	7.97	0.781049936

2144	7.86	0.791873056
2145	7.80	0.798193737
2146	7.73	0.804616133
2147	7.67	0.811142718
2148	7.52	2.002372315
2149	7.46	2.019077859
2150	7.40	2.036064492
2151	7.40	2.034232603
2152	7.41	2.032404008
2153	7.42	2.030578697
2154	7.42	2.028756662
2155	7.43	2.026937894
2156	7.44	2.025122384
2157	7.44	2.023310124
2158	7.45	2.021501104
2159	7.46	2.019695315
2160	7.46	2.017892751
2161	7.47	2.016093401
2162	7.48	2.014297257
2163	7.56	1.991798942
2164	7.64	1.969797657
2165	7.85	0.367870909
2166	7.94	0.363956235
2167	8.02	0.360123998
2168	8.10	0.356371624
2169	8.14	0.355008783
2170	8.17	0.353656326
2171	8.20	0.352314135
2172	8.23	0.350982094
2173	8.26	0.349660086
2174	8.29	0.348348001
2175	8.32	0.347045725
2176	8.35	0.34575315
2177	8.38	0.344470168
2178	8.42	0.343196673
2179	8.45	0.341932558
2180	8.48	0.340677722
2181	8.49	0.340142865
2182	8.50	0.339609684
2183	8.49	0.707039402
2184	8.50	0.705930522
2185	8.51	0.704825115
2186	8.53	0.703723165
2187	8.53	0.702990169
2188	8.54	0.702258698
2189	8.55	0.701528748
2190	8.56	0.700800314
2191	8.57	0.700073391
2192	8.58	0.699347975
2193	8.59	0.69862406
2194	8.60	0.697901642
2195	8.61	0.697180717
2196	8.61	0.69646128
2197	8.62	0.695743326
2198	8.63	0.695026851
2199	8.55	0.701484458
2200	8.47	0.708063188

2201	8.33	1.548020712
2202	8.25	1.562939607
2203	8.17	1.578148859
2204	8.09	1.593657027
2205	8.01	1.608135509
2206	7.94	1.622879478
2207	7.87	1.637896305
2208	7.80	1.653193635
2209	7.72	1.668779401
2210	7.65	1.684661839
2211	7.58	1.700849501
2212	7.50	1.717351271
2213	7.43	1.734176381
2214	7.36	1.751334428
2215	7.29	1.768835393
2216	7.34	1.757045633
2217	7.38	1.745411996
2218	7.43	1.733931402
2219	7.54	1.002648957
2220	7.58	0.996185355
2221	7.63	0.989804555
2222	7.68	0.983504975
2223	7.69	0.982651742
2224	7.70	0.981799988
2225	7.70	0.98094971
2226	7.71	0.980100903
2227	7.72	0.979253564
2228	7.72	0.978407688
2229	7.73	0.977563273
2230	7.74	0.976720314
2231	7.74	0.975878808
2232	7.75	0.97503875
2233	7.76	0.974200137
2234	7.73	0.977281282
2235	7.71	0.980381979
2236	7.68	0.983502414
2237	7.66	0.928120579
2238	7.64	0.931091869
2239	7.61	0.934082245
2240	7.59	0.937091892
2241	7.58	0.937641747
2242	7.58	0.938192248
2243	7.58	0.938743396
2244	7.57	0.939295192
2245	7.57	0.939847637
2246	7.56	0.940400732
2247	7.56	0.940954478
2248	7.55	0.941508877
2249	7.55	0.942063929
2250	7.54	0.942619637
2251	7.54	0.943176
2252	7.47	0.95243963
2253	7.39	0.961887035
2254	7.32	0.971523739
2255	7.24	1.074254362
2256	7.17	1.085247087
2257	7.09	1.096467112

2258	7.02	1.107921561
2259	7.08	1.097841584
2260	7.15	1.087943372
2261	7.21	1.07822205
2262	7.28	1.068672919
2263	7.34	1.059291445
2264	7.41	1.05007325
2265	7.47	1.041014109
2266	7.54	1.03210994
2267	7.60	1.0233568
2268	7.66	1.014750879
2269	7.73	1.006288494
2270	7.85	0.991182971
2271	7.96	0.976524242
2272	8.08	0.962292774
2273	8.24	0.431340955
2274	8.36	0.425263652
2275	8.48	0.419355219
2276	8.60	0.413608716
2277	8.77	0.405299563
2278	8.95	0.397317687
2279	9.12	0.389644125
2280	9.30	0.382261353
2281	9.48	0.375153148
2282	9.65	0.368304473
2283	9.83	0.36170137
2284	10.00	0.355330863
2285	10.18	0.349180874
2286	10.36	0.343240148
2287	10.53	0.337498184
2288	10.71	0.331807609
2289	10.89	0.326305751
2290	11.08	0.320983374
2291	11.24	0.494393462
2292	11.42	0.48657048
2293	11.60	0.478991213
2294	11.63	0.477800963
2295	11.66	0.476616614
2296	11.68	0.475438122
2297	11.71	0.474265444
2298	11.74	0.473098536
2299	11.77	0.471937356
2300	11.80	0.470781862
2301	11.83	0.469632013
2302	11.86	0.468487767
2303	11.89	0.467349083
2304	11.92	0.466215921
2305	11.94	0.465088241
2306	11.97	0.464224232
2307	11.99	0.463363426
2308	12.01	0.462505807
2309	11.90	1.62528972
2310	11.92	1.622257987
2311	11.94	1.619237544
2312	11.91	1.622861249
2313	11.89	1.626501209
2314	11.86	1.630157535

2315	11.83	1.633830336
2316	11.81	1.637519725
2317	11.78	1.641225813
2318	11.75	1.644948715
2319	11.73	1.648688545
2320	11.70	1.652445419
2321	11.67	1.656219454
2322	11.65	1.660010767
2323	11.62	1.663819478
2324	11.64	1.661594419
2325	11.65	1.659375303
2326	11.67	1.657162107
2327	11.62	2.165964351
2328	11.64	2.163068712
2329	11.65	2.160180804
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2331	11.65	2.160180804
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2339	11.65	2.160180804
2340	11.65	2.160180804
2341	11.65	2.160180804
2342	11.63	2.163894184
2343	11.61	2.167620353
2344	11.75	0.85132901
2345	11.73	0.852781004
2346	11.71	0.85423796
2347	11.69	0.855699903
2348	11.82	0.8457289
2349	11.96	0.835987594
2350	12.10	0.826468138
2351	12.24	0.817163039
2352	12.38	0.808065137
2353	12.51	0.799167587
2354	12.65	0.790463844
2355	12.79	0.781947644
2356	12.93	0.773612989
2357	13.06	0.765454136
2358	13.20	0.757465581
2359	13.34	0.749642046
2360	13.34	0.749642046
2361	13.34	0.749642046
2362	13.39	0.381742962
2363	13.39	0.381742962
2364	13.39	0.381742962
2365	13.39	0.381742962
2366	13.27	0.385259871
2367	13.14	0.388842185
2368	13.02	0.392491743
2369	12.90	0.396210457
2370	12.78	0.400000313
2371	12.66	0.403863371

2372	12.53	0.407801772
2373	12.41	0.411817743
2374	12.29	0.415913598
2375	12.17	0.420091745
2376	12.04	0.424354688
2377	11.92	0.428705036
2378	11.74	0.435301031
2379	11.56	0.442103169
2380	11.40	0.311963607
2381	11.21	0.316988723
2382	11.03	0.322178379
2383	10.85	0.327540791
2384	10.67	0.333154339
2385	10.49	0.338963658
2386	10.30	0.34497917
2387	10.12	0.351212052
2388	9.94	0.357674302
2389	9.76	0.364378819
2390	9.57	0.371339486
2391	9.39	0.378571269
2392	9.21	0.386090322
2393	9.02	0.39391411
2394	8.84	0.402061541
2395	8.81	0.403481632
2396	8.78	0.404911791
2397	8.75	0.406352124
2398	8.56	2.25861511
2399	8.53	2.266856617
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2401	8.47	2.283521392
2402	8.57	2.255695243
2403	8.68	2.228539087
2404	8.78	2.202029015
2405	8.88	2.176142241
2406	8.99	2.150857039
2407	9.09	2.126152679
2408	9.20	2.102009374
2409	9.30	2.078408227
2410	9.41	2.055331178
2411	9.51	2.032760961
2412	9.62	2.010681061
2413	9.77	1.9791206
2414	9.92	1.9485356
2415	10.08	1.918881524
2416	10.35	0.68700734
2417	10.50	0.676978909
2418	10.66	0.667239042
2419	10.81	0.657775459
2420	10.83	0.65673544
2421	10.84	0.655698705
2422	10.86	0.654665238
2423	10.88	0.653635023
2424	10.90	0.652608046
2425	10.91	0.651584291
2426	10.93	0.650563743
2427	10.95	0.649546386
2428	10.96	0.648532207

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2438	11.22	0.613686601
2439	11.24	0.612958629
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2449	11.21	0.614296849
2450	11.07	0.622313855
2451	10.92	0.630542882
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2453	10.50	1.947147758
2454	10.36	1.97431002
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2456	10.05	2.034562888
2457	9.90	2.066089811
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2459	9.59	2.132168588
2460	9.44	2.166818759
2461	9.28	2.202613745
2462	9.13	2.239611234
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2464	8.82	2.317464528
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2468	8.45	2.418598268
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2471	8.50	0.803111217
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2473	8.46	0.806486883
2474	8.46	0.806911314
2475	8.45	0.807336193
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2477	8.44	0.808187294
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2482	8.42	0.810322918
2483	8.42	0.810751398
2484	8.41	0.811180331
2485	8.41	0.811394485

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2487	8.42	0.580254491
2488	8.42	0.580407449
2489	8.42	0.580560488
2490	8.42	0.580713608
2491	8.42	0.580559798
2492	8.42	0.580406071
2493	8.42	0.580252424
2494	8.43	0.580098859
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2512	7.15	0.559737259
2513	7.02	0.56983791
2514	6.89	0.580309798
2515	6.77	0.591173776
2516	6.64	0.602452282
2517	6.51	0.614169505
2518	6.39	0.626351549
2519	6.26	0.639026635
2520	6.13	0.652225311
2521	6.14	0.651212313
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2523	6.13	1.086586219
2524	6.14	1.084899135
2525	6.15	1.083217282
2526	6.16	1.081540636
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2571	8.29	0.938052585
2572	8.42	0.923693444
2573	8.55	0.909767276
2574	8.65	0.899016942
2575	8.75	0.888517705
2576	8.86	0.87826087
2577	9.01	0.313431245
2578	9.11	0.309913953
2579	9.21	0.306474726
2580	9.30	0.303690436
2581	9.38	0.30095628
2582	9.46	0.298270916
2583	9.55	0.295633051
2584	9.63	0.293041434
2585	9.72	0.29049486
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2589	10.06	0.280736299
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2591	10.22	0.27609882
2592	10.30	0.273955321
2593	10.38	0.271844847
2594	10.37	1.200186336
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2611	11.44	1.087365881
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2615	11.57	1.459842657
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2652	12.09	1.089428017
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2667	10.82	0.780436227
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2673	10.68	0.790679644
2674	10.67	0.791011472
2675	10.67	0.791343578
2676	10.67	0.791675964
2677	10.66	0.792008629
2678	10.66	0.792341574
2679	10.65	0.792674799
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2683	10.70	0.706284672
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2778	10.43	0.468845468
2779	10.38	0.470957765
2780	10.33	0.473089181
2781	10.29	0.475239978
2782	10.24	0.47741042
2783	10.19	0.479600777
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2788	9.93	0.492291479
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2798	9.09	1.17301382
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2825	10.39	0.919785643
2826	10.48	0.911401823
2827	10.56	1.114297947

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2835	10.63	1.106841644
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2837	10.59	1.111022965
2838	10.57	1.113125494
2839	10.55	1.115235997
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2842	10.43	1.12782892
2843	10.35	1.136302169
2844	10.28	1.102495182
2845	10.20	1.110900905
2846	10.12	1.119435788
2847	10.05	1.12810283
2848	10.06	1.126358254
2849	10.08	1.124619065
2850	10.09	1.122885239
2851	10.11	1.121156751
2852	10.12	1.119433576
2853	10.14	1.11771569
2854	10.15	1.116003068
2855	10.17	1.114295687
2856	10.19	1.112593522
2857	10.20	1.110896549
2858	10.22	1.109204745
2859	10.23	1.107518086
2860	10.26	1.104639036
2861	10.29	1.101774914
2862	10.28	1.404827417
2863	10.31	1.401192818
2864	10.34	1.397576978
2865	10.36	1.393979752
2866	10.31	1.400588201
2867	10.26	1.407259605
2868	10.22	1.413994868
2869	10.17	1.420794913
2870	10.12	1.427660679
2871	10.07	1.434593121
2872	10.02	1.441593218
2873	9.97	1.448661963
2874	9.92	1.455800373
2875	9.87	1.463009481
2876	9.82	1.470290343
2877	9.78	1.477644036
2878	9.70	1.488452232
2879	9.63	1.499419707
2880	9.68	0.298419863
2881	9.61	0.300624775
2882	9.54	0.302862512
2883	9.46	0.305133813
2884	9.45	0.305703573

2885	9.43	0.306275466
2886	9.41	0.306849501
2887	9.39	0.307425693
2888	9.38	0.308004053
2889	9.36	0.308584593
2890	9.34	0.309167325
2891	9.32	0.309752263
2892	9.31	0.310339418
2893	9.29	0.310928803
2894	9.27	0.311520431
2895	9.26	0.311969002
2896	9.24	0.312418866
2897	9.23	0.31287003
2898	9.12	1.33954406
2899	9.11	1.341503973
2900	9.10	1.34346963
2901	9.08	1.345441055
2902	9.06	1.34840981
2903	9.04	1.351391695
2904	9.02	1.354386797
2905	9.00	1.357395205
2906	8.98	1.360417008
2907	8.96	1.363452294
2908	8.94	1.366501156
2909	8.92	1.369563683
2910	8.90	1.372639968
2911	8.88	1.375730104
2912	8.86	1.378834185
2913	8.81	1.386830669
2914	8.76	1.394920444
2915	8.71	1.403105154
2916	8.74	0.534164918
2917	8.68	0.537308758
2918	8.63	0.540489823
2919	8.58	0.543708779
2920	8.47	0.551172452
2921	8.35	0.55884389
2922	8.23	0.566731891
2923	8.12	0.574845755
2924	8.00	0.583195325
2925	7.88	0.591791024
2926	7.77	0.600643896
2927	7.65	0.60976566
2928	7.54	0.619168753
2929	7.42	0.628866396
2930	7.30	0.638872649
2931	7.30	0.638929512
2932	7.30	0.638986386
2933	7.30	0.63904327
2934	7.25	1.379776614
2935	7.25	1.379900371
2936	7.25	1.38002415
2937	7.29	1.371191943
2938	7.34	1.362472069
2939	7.39	1.3538624
2940	7.43	1.345360859
2941	7.48	1.336965423

2942	7.53	1.328674116
2943	7.57	1.320485014
2944	7.62	1.312396239
2945	7.67	1.304405957
2946	7.71	1.296512382
2947	7.76	1.288713767
2948	7.81	1.28100841
2949	7.76	1.288712106
2950	7.71	1.29650902
2951	7.67	1.304400853
2952	7.63	1.223781725
2953	7.58	1.231316036
2954	7.53	1.238943692
2955	7.53	1.238943692
2956	7.53	1.238943692
2957	7.53	1.238943692
2958	7.53	1.238943692
2959	7.53	1.238943692
2960	7.53	1.238943692
2961	7.53	1.238943692
2962	7.53	1.238943692
2963	7.53	1.238943692
2964	7.53	1.238943692
2965	7.53	1.238943692
2966	7.53	1.238943692
2967	7.61	1.22663989
2968	7.68	1.21457806
2969	7.76	1.202751134
2970	7.90	0.357325037
2971	7.98	0.353939917
2972	8.05	0.350618333
2973	8.17	0.345375601
2974	8.30	0.340287346
2975	8.42	0.33534684
2976	8.54	0.33054774
2977	8.66	0.32588406
2978	8.78	0.321350149
2979	8.91	0.316940664
2980	9.03	0.312650552
2981	9.15	0.308475032
2982	9.27	0.304409572
2983	9.40	0.300449877
2984	9.52	0.296591873
2985	9.59	0.294460942
2986	9.66	0.292360414
2987	9.68	0.780732931
2988	9.75	0.775215273
2989	9.82	0.769775058
2990	9.88	0.764410666
2991	9.91	0.762324396
2992	9.94	0.760249482
2993	9.97	0.758185833
2994	9.99	0.756133357
2995	10.02	0.754091963
2996	10.05	0.752061562
2997	10.07	0.750042066
2998	10.10	0.748033387

2999	10.13	0.746035437
3000	10.15	0.744048132
3001	10.18	0.742071386
3002	10.21	0.740105116
3003	10.24	0.737668546
3004	10.28	0.735247966
3005	10.26	1.190858974
3006	10.30	1.186959182
3007	10.33	1.183084849
3008	10.28	1.189224007
3009	10.22	1.195427212
3010	10.17	1.20169547
3011	10.12	1.20802981
3012	10.06	1.214431283
3013	10.01	1.220900961
3014	9.96	1.227439941
3015	9.90	1.234049342
3016	9.85	1.240730307
3017	9.80	1.247484006
3018	9.74	1.254311632
3019	9.69	1.261214406
3020	9.64	1.268193576
3021	9.65	1.26664663
3022	9.66	1.265103453
3023	9.63	1.754418771
3024	9.64	1.752276206
3025	9.65	1.750138867
3026	9.66	1.747605166
3027	9.68	1.745078791
3028	9.69	1.74255971
3029	9.71	1.74004789
3030	9.72	1.737543302
3031	9.73	1.735045914
3032	9.75	1.732555694
3033	9.76	1.730072612
3034	9.78	1.727596638
3035	9.79	1.72512774
3036	9.80	1.722665889
3037	9.82	1.720211054
3038	9.78	1.726071934
3039	9.75	1.731972888
3040	9.72	1.737914328
3041	9.73	1.306307186
3042	9.69	1.310799887
3043	9.66	1.315323598
3044	9.65	1.316838903
3045	9.64	1.318357703
3046	9.63	1.319880011
3047	9.61	1.321405839
3048	9.60	1.322935198
3049	9.59	1.324468102
3050	9.58	1.326004562
3051	9.57	1.327544591
3052	9.56	1.329088202
3053	9.55	1.330635406
3054	9.54	1.332186217
3055	9.53	1.333740646

3056	9.47	1.341879963
3057	9.41	1.350119231
3058	9.44	0.423749013
3059	9.38	0.426358779
3060	9.32	0.429000889
3061	9.27	0.431675949
3062	9.28	0.431262197
3063	9.28	0.430849236
3064	9.29	0.430437066
3065	9.30	0.430025683
3066	9.31	0.429615086
3067	9.32	0.429205273
3068	9.33	0.42879624
3069	9.34	0.428387987
3070	9.35	0.42798051
3071	9.36	0.427573807
3072	9.36	0.427167877
3073	9.37	0.426762717
3074	9.34	0.428489403
3075	9.30	0.430230119
3076	9.26	0.456069549
3077	9.22	0.457937934
3078	9.18	0.459821691
3079	9.14	0.46172101
3080	9.12	0.463005103
3081	9.09	0.464296358
3082	9.07	0.465594835
3083	9.04	0.466900596
3084	9.02	0.468213702
3085	8.99	0.469534214
3086	8.97	0.470862195
3087	8.94	0.47219771
3088	8.92	0.473540822
3089	8.89	0.474891597
3090	8.87	0.4762501
3091	8.84	0.477616398
3092	8.86	0.476728168
3093	8.87	0.475843235
3094	8.87	0.726746153
3095	8.88	0.72539875
3096	8.90	0.724056333
3097	8.90	0.723774138
3098	8.91	0.723492163
3099	8.91	0.723210408
3100	8.91	0.722928872
3101	8.92	0.722647555
3102	8.92	0.722366456
3103	8.92	0.722085577
3104	8.93	0.721804916
3105	8.93	0.721524472
3106	8.93	0.721244247
3107	8.94	0.720964239
3108	8.94	0.720684449
3109	9.03	0.713461662
3110	9.12	0.706382214
3111	9.21	0.699441879
3112	9.24	1.419414068

3113	9.33	1.405639067
3114	9.42	1.39212886
3115	9.51	1.378875886
3116	9.52	1.377266644
3117	9.61	1.364293816
3118	9.70	1.351563096
3119	9.79	1.339067769
3120	9.88	1.326801367
3121	9.97	1.314757656
3122	10.06	1.302930625
3123	10.15	1.29131448
3124	10.24	1.279903629
3125	10.33	1.268692679
3126	10.42	1.257676421
3127	10.51	1.24711312
3128	10.60	1.236725785
3129	10.69	1.226510055
3130	10.77	1.333138886
3131	10.85	1.322293339
3132	10.94	1.311622832
3133	11.03	1.301123162
3134	11.12	1.290790258
3135	11.21	1.28062018
3136	11.31	1.269148427
3137	11.41	1.257880377
3138	11.51	1.246810651
3139	11.61	1.235934059
3140	11.71	1.225245591
3141	11.81	1.214740408
3142	11.92	1.204413834
3143	12.02	1.194261355
3144	12.12	1.184278603
3145	12.20	1.176600794
3146	12.28	1.169021897
3147	12.37	1.055217839
3148	12.45	1.04851427
3149	12.53	1.041895336
3150	12.61	1.035359445
3151	12.69	1.028905042
3152	12.76	1.022530614
3153	12.83	1.017217569
3154	12.90	1.01195945
3155	12.96	1.006755412
3156	13.03	1.001604624
3157	13.10	0.996506273
3158	13.16	0.991459562
3159	13.23	0.986463711
3160	13.30	0.981517954
3161	13.36	0.976621542
3162	13.43	0.97177374
3163	13.43	0.97177374
3164	13.43	0.97177374
3165	13.43	0.97177374
3166	13.51	0.378297801

Table S.6. Calculation of event indices from CHAR data at Pyramid Lake using the 100-yr running mean

166	7.75	1.032702246
167	7.75	1.032702246
168	7.78	1.0284539
169	7.81	1.024240364
170	7.84	1.020061212
171	7.87	1.015916026
172	7.91	1.011804393
173	7.94	1.007725907
174	7.97	1.003680169
175	8.00	0.999666786
176	8.06	0.694904043
177	8.13	0.688524596
178	8.21	0.682261214
179	8.26	0.678075562
180	8.31	0.673940954
181	8.36	0.669856463
182	8.41	0.665821183
183	8.46	0.66183423
184	8.51	0.65789474
185	8.56	0.654001871
186	8.57	1.166951926
187	8.57	1.16640747
188	8.58	1.165863521
189	8.58	1.165320079
190	8.55	1.17022937
191	8.51	1.175180199
192	8.47	1.180173097
193	8.44	1.185208601
194	8.40	1.190287261
195	8.37	1.195409632
196	8.33	1.200576281
197	8.34	0.639488372
198	8.35	0.638875541
199	8.36	0.638263885
200	8.40	0.635223085
201	8.44	0.632211121
202	8.48	0.629227586
203	8.52	0.626272078
204	8.56	0.623344205
205	8.60	0.620443581
206	8.64	0.617569826
207	8.63	1.111969116
208	8.65	1.109912136

209	8.67	1.107862752
210	8.65	1.110425667
211	8.63	1.113000468
212	8.61	1.115587237
213	8.59	1.118186058
214	8.57	1.120797016
215	8.55	1.123420195
216	8.53	1.126055682
217	8.51	1.128703563
218	8.45	1.51419559
219	8.43	1.517786567
220	8.43	1.519227732
221	8.42	1.520671636
222	8.41	1.522118287
223	8.40	1.523567694
224	8.39	1.525019863
225	8.39	1.526474803
226	8.38	1.527932522
227	8.39	1.525019863
228	8.41	1.522118287
229	8.45	1.230866345
230	8.47	1.228539932
231	8.50	1.223913389
232	8.53	1.219321562
233	8.56	1.21476406
234	8.59	1.210240501
235	8.63	1.205750507
236	8.66	1.201293706
237	8.65	1.20296114
238	8.63	1.204633209
239	8.62	1.206309934
240	8.65	0.739941424
241	8.64	0.740969438
242	8.69	0.736233961
243	8.75	0.731558628
244	8.80	0.726942301
245	8.86	0.722383868
246	8.92	0.717882248
247	8.97	0.713436386
248	9.07	0.705398256
249	9.18	0.697539236
250	9.25	1.038361694
251	9.35	1.027006464
252	9.39	1.021904714
253	9.44	1.0168534
254	9.49	1.011851779
255	9.53	1.00689912
256	9.58	1.001994707
257	9.63	0.99713784
258	9.63	0.996723728
259	9.64	0.996309959
260	9.68	0.620119428
261	9.68	0.619863168
262	9.68	0.619863168
263	9.68	0.619863168
264	9.68	0.619863168
265	9.68	0.619863168

266	9.68	0.619863168
267	9.68	0.619863168
268	9.68	0.619863168
269	9.65	0.621919194
270	9.60	0.749722322
271	9.57	0.752228823
272	9.54	0.75475214
273	9.50	0.757930192
274	9.46	0.761135121
275	9.42	0.76436727
276	9.38	0.767626987
277	9.34	0.770914625
278	9.30	0.774230545
279	9.26	0.777575114
280	9.24	0.778921046
281	9.21	0.955321813
282	9.20	0.956984046
283	9.18	0.958652072
284	9.16	0.960325924
285	9.15	0.962005631
286	9.15	0.962005631
287	9.15	0.962005631
288	9.15	0.962005631
289	9.15	0.962005631
290	9.15	0.962005631
291	9.19	0.957817333
292	9.16	1.698204796
293	9.19	1.692292856
294	9.22	1.686421935
295	9.26	1.680591609
296	9.29	1.674801457
297	9.32	1.669051066
298	9.35	1.663340027
299	9.38	1.657667938
300	9.42	1.652034402
301	9.42	1.652034402
302	9.47	1.055792778
303	9.53	1.049587157
304	9.58	1.04345406
305	9.64	1.037392222
306	9.70	1.031400408
307	9.75	1.025477412
308	9.81	1.019622055
309	9.86	1.013833186
310	9.92	1.008109678
311	10.01	0.998845774
312	10.11	0.949784535
313	10.20	0.941217478
314	10.26	0.936078002
315	10.31	0.93099435
316	10.37	0.925965615
317	10.42	0.920990914
318	10.48	0.91606938
319	10.54	0.911200165
320	10.59	0.906382439
321	10.64	0.902632675
322	10.68	0.898913809

323	10.73	0.820011593
324	10.78	0.816663226
325	10.80	0.814546648
326	10.83	0.812441013
327	10.86	0.810346236
328	10.89	0.808262233
329	10.92	0.806188922
330	10.94	0.80412622
331	10.97	0.802074047
332	10.98	0.801197747
333	11.00	0.80032336
334	11.01	0.799450879
335	11.02	0.798290526
333	11.02	0.943434258
337	11.04	0.942066907
338	11.06	0.940703514
339	11.07	0.939344061
340	11.09	0.937988532
341	11.10	0.936636909
342	11.12	0.935289176
343	11.08	0.866738892
344	11.02	0.870792176
345	10.97	0.874883549
346	10.96	0.875947928
347	10.95	0.8770149
348	10.93	0.878084474
349	10.92	0.87915666
350	10.91	0.880231468
351	10.89	0.881308908
352	10.88	0.882388988
353	10.87	1.398887437
354	10.91	1.393472683
355	10.95	1.388099686
356	10.97	1.386074413
357	10.98	1.384055041
358	11.00	1.382041545
359	11.01	1.380033898
360	11.03	1.378032076
361	11.05	1.376036053
362	11.06	1.374045804
363	11.08	1.371566075
364	11.14	1.041458843
365	11.16	1.039592131
366	11.22	1.034031934
367	11.28	1.028530897
368	11.34	1.023088082
369	11.40	1.017702568
370	11.46	1.012373455
371	11.52	1.007099863
372	11.58	1.001880928
373	11.64	0.996715804
374	11.71	0.990926005
375	11.79	0.848160436
376	11.85	0.844150973
377	11.90	0.84017924
378	11.96	0.836244705
379	12.01	0.83234685

380	12.07	0.828485162
381	12.13	0.824659142
382	12.18	0.820868298
383	12.24	0.817112146
384	12.29	0.813390212
385	12.35	0.842362938
386	12.40	0.838559399
387	12.44	0.835863549
388	12.41	0.838018839
389	12.38	0.840185273
390	12.35	0.842362938
391	12.31	0.84455192
392	12.28	0.846752308
393	12.25	0.848964193
394	12.23	0.850630704
395	12.20	0.852303771
396	12.14	1.171517465
397	12.12	1.173838067
398	12.14	1.171903595
399	12.16	1.169975487
400	12.18	1.168053714
401	12.20	1.166138244
402	12.22	1.164229046
403	12.24	1.162326089
404	12.20	1.165755904
405	12.16	1.16920602
406	12.15	0.95440085
407	12.12	0.95723612
408	12.08	0.960088286
409	12.07	0.961187575
410	12.05	0.962289385
411	12.04	0.963393724
412	12.03	0.964500601
413	12.01	0.965610024
414	12.00	0.966722002
415	12.02	0.964938226
416	12.00	1.299601806
417	12.03	1.297204677
418	12.05	1.294816375
419	12.07	1.292436851
420	12.09	1.290066057
421	12.10	1.289273735
422	12.11	1.288482386
423	12.11	1.287692007
424	12.12	1.286902598
425	12.13	1.286114156
426	12.16	1.183724548
427	12.19	1.181448922
428	12.21	1.179182029
429	12.24	1.176923818
430	12.26	1.17467424
431	12.28	1.172433245
432	12.29	1.17121788
433	12.31	1.170005033
434	12.32	1.168794695
435	12.33	1.167586858
436	12.34	1.166759539

437	12.35	1.165933392
438	12.43	0.579180169
439	12.44	0.578773019
440	12.45	0.578366441
441	12.46	0.577960434
442	12.47	0.577554997
443	12.48	0.577150128
444	12.48	0.576745826
445	12.48	0.576835521
446	12.48	0.576925244
447	12.44	0.578787919
448	12.36	0.938843543
449	12.32	0.941905316
450	12.28	0.944987124
451	12.24	0.948089164
452	12.19	0.951211638
453	12.15	0.954354747
454	12.11	0.957518696
455	12.07	0.960703694
456	12.03	0.963909951
457	12.01	0.965886575
458	11.99	0.967871322
459	11.94	1.157470837
460	11.91	1.159863522
461	11.89	1.162266119
462	11.86	1.164678691
463	11.84	1.167101299
464	11.82	1.169534007
465	11.79	1.171976877
466	11.77	1.174429974
467	11.70	1.180916504
468	11.63	1.188566584
469	11.55	1.196316425
470	11.48	1.204167992
471	11.41	1.081334073
472	11.34	1.088516038
473	11.26	1.095794043
474	11.19	1.103170026
475	11.11	1.110645981
476	11.04	1.118223953
477	10.97	1.124674969
478	10.91	1.131200849
479	10.85	1.137802902
480	10.77	1.145617613
481	10.70	1.153540412
482	10.64	1.059942478
483	10.56	1.06736806
484	10.49	1.074898419
485	10.41	1.082535787
486	10.34	1.090282462
487	10.27	1.098140808
488	10.19	1.106113255
489	10.19	1.106330334
490	10.19	1.106547497
491	10.18	1.107654381
492	10.17	1.108763482
493	10.16	1.109874806

494	10.15	1.110988359
495	10.15	1.005635992
496	10.14	1.006645925
497	10.13	1.007657888
498	10.12	1.008671888
499	10.06	1.014102324
500	10.01	1.019591549
501	9.96	1.025140523
502	9.90	1.030750226
503	9.85	1.036039074
504	9.80	1.041382476
505	9.75	1.046781282
506	9.70	1.052236358
507	9.66	0.945947217
508	9.61	0.950923198
509	9.56	0.955951806
510	9.49	0.963281243
511	9.41	0.970723941
512	9.34	0.978282545
513	9.27	0.985959783
514	9.19	0.993758472
515	9.10	1.004116561
516	9.00	1.014692853
517	8.91	1.025494315
518	8.83	0.914195113
519	8.73	0.924126295
520	8.64	0.934275619
521	8.54	0.94465035
522	8.46	0.953592796
523	8.38	0.962706165
524	8.30	0.971995406
525	8.22	0.981465658
526	8.17	0.987117207
527	8.13	0.992834219
528	8.08	0.998617838
529	8.03	1.004469235
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532	7.90	0.885828095
533	7.87	0.889925275
534	7.83	0.894060532
535	7.79	0.8982344
536	7.76	0.902447422
537	7.72	0.90670015
538	7.83	0.893635578
539	7.95	0.880942151
540	8.06	0.868604275
541	8.18	0.755727616
542	8.29	0.74544196
543	8.41	0.735432525
544	8.52	0.725688333
545	8.63	0.716198977
546	8.75	0.706091686
547	8.88	0.696265701
548	9.00	0.68670944
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552	10.11	0.611662772
553	10.38	0.630640114
554	10.66	0.614297443
555	10.93	0.598780398
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558	11.77	0.5560961
559	12.06	0.542868001
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562	12.85	0.509529025
563	13.06	0.501130327
564	13.28	0.493004016
565	13.51	0.320652911
566	13.73	0.315624669
567	13.94	0.310751689
568	14.16	0.306026892
569	14.39	0.301219698
570	14.61	0.296561195
571	14.84	0.292044589
572	15.06	0.287663495
573	15.29	0.283411903
574	15.56	0.278457523
575	15.83	0.273673384
576	16.07	0.475105994
577	16.35	0.467198485
578	16.62	0.459549887
579	16.89	0.452147689
580	17.16	0.444980173
581	17.44	0.437768006
582	17.73	0.430785898
583	18.01	0.424023013
584	18.29	0.417469187
585	18.46	0.413706581
586	18.62	0.410011194
587	18.79	0.40638124
588	18.81	1.199541033
589	18.97	1.189023821
590	19.14	1.17868943
591	19.31	1.168533133
592	19.48	1.158063809
593	19.66	1.147780417
594	19.83	1.137678046
595	20.01	1.127751959
596	20.18	1.117997581
597	20.36	1.108031091
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601	20.94	1.80645096
602	21.12	1.790922968
603	21.30	1.775659653
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605	21.65	1.746486825
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607	22.01	1.71825708

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611	23.14	1.634026741
612	23.50	1.305002594
613	23.78	1.289439085
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616	24.66	1.243782035
617	24.96	1.228546887
618	25.27	1.213680455
619	25.57	1.199169513
620	25.88	1.185001462
621	26.18	1.171272728
622	26.49	1.157858457
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624	27.05	1.304161639
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626	27.65	1.275550176
627	27.92	1.263202579
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629	28.46	1.239210929
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631	29.00	1.216113627
632	29.34	1.202395614
633	29.67	1.188983634
634	30.00	1.175867557
635	30.44	0.776376356
636	30.78	0.768028449
637	31.11	0.759858152
638	31.44	0.751859856
639	31.62	0.747540189
640	31.80	0.743269874
641	31.98	0.73904807
642	32.16	0.734873955
643	32.30	0.731816271
644	32.43	0.728783926
645	32.57	0.725776608
646	32.70	0.722794006
647	32.83	0.741219055
648	32.96	0.738197186
649	33.10	0.735199855
650	33.08	0.735603952
651	33.06	0.736008493
652	33.04	0.736413479
653	33.02	0.736818912
654	33.01	0.73722479
655	32.91	0.739499191
656	32.80	0.741787668
657	32.70	0.744090353
658	32.49	1.074296158
659	32.39	1.077662818
660	32.29	1.081050645
661	32.19	1.08445984
662	32.09	1.087890605
663	32.06	1.088908632
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668	32.21	1.083674332
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670	32.46	1.075499705
671	32.58	1.063938626
672	32.71	1.059956089
673	32.83	1.056003256
674	32.95	1.052079796
675	33.03	1.049647219
676	33.10	1.047225865
677	33.18	1.044815657
678	33.21	1.043957555
679	33.23	1.043100861
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681	33.29	1.041391684
682	33.26	1.224679482
683	33.28	1.223675948
684	33.31	1.222674057
685	33.34	1.221673806
686	33.48	1.216432706
687	33.62	1.211236384
688	33.77	1.206084268
689	33.91	1.200975797
690	33.95	1.199560879
691	33.99	1.198149291
692	34.03	1.196741022
693	34.12	1.055127757
694	34.16	1.053892212
695	34.20	1.052659557
696	34.24	1.051429781
697	34.28	1.050202876
698	34.31	1.049191906
699	34.35	1.048182881
700	34.38	1.047175794
701	34.41	1.046170641
702	34.44	1.045158221
703	34.48	1.044147758
704	34.51	1.043139247
705	34.63	0.798970882
706	34.66	0.79820252
707	34.69	0.797435634
708	34.73	0.79667022
709	34.66	0.798335131
710	34.58	0.800007015
711	34.51	0.801685916
712	34.44	0.803371879
713	34.37	0.805064948
714	34.37	0.805064948
715	34.37	0.805064948
716	34.37	0.805064948
717	34.21	1.254162824
718	34.21	1.254162824
719	34.21	1.254162824
720	34.21	1.254162824
721	34.21	1.254162824

722	34.22	1.254073964
723	34.22	1.253985117
724	34.22	1.253896283
725	34.22	1.253807462
726	34.10	1.25834229
727	33.98	1.26291004
728	33.90	1.120873811
729	33.78	1.124966353
730	33.66	1.12908889
731	33.53	1.133241753
732	33.41	1.137425278
733	33.22	1.143722291
734	33.04	1.150089416
735	32.86	1.156527829
736	32.67	1.163038736
737	32.62	1.164853738
738	32.57	1.166674415
739	32.52	1.168500792
740	32.57	0.848443133
741	32.52	0.849771259
742	32.47	0.851103549
743	32.42	0.852440024
744	32.42	0.852535647
745	32.41	0.852631291
746	32.41	0.852726957
747	32.41	0.852822645
748	32.40	0.852918353
749	32.56	0.848727382
750	32.72	0.844577394
751	32.88	0.840467794
752	33.04	0.837322775
753	33.20	0.833287705
754	33.36	0.829291339
755	33.52	0.825333123
756	33.77	0.819385243
757	34.01	0.813522479
758	34.25	0.807743015
759	34.50	0.80204509
760	34.53	0.80119346
761	34.57	0.800343637
762	34.61	0.799495615
763	34.64	0.798649389
764	34.61	1.008756567
765	34.64	1.007688876
766	34.68	1.006623442
767	34.72	1.00556026
768	34.60	1.008924427
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771	34.25	1.01915336
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773	34.62	1.008235676
774	34.81	1.002864083
775	34.99	0.997549424
776	35.31	0.632563718
777	35.49	0.62925837
778	35.68	0.625987385

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780	36.15	0.617863541
781	36.38	0.61388018
782	36.62	0.609947851
783	36.85	0.606065581
784	36.76	0.607494344
785	36.68	0.608929861
786	36.59	0.610372177
787	36.37	0.97982854
788	36.28	0.982168967
789	36.20	0.984520601
790	36.11	0.986883523
791	36.13	0.986419961
792	36.14	0.985956833
793	36.16	0.985494141
794	36.18	0.985031882
795	36.19	0.984570058
796	36.22	0.983779364
797	36.25	0.982989939
798	36.28	0.98220178
799	36.15	1.438547371
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803	36.26	1.433943346
804	36.29	1.432805913
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810	36.67	0.854573697
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812	36.72	0.853332446
813	36.75	0.852713171
814	36.77	0.852094795
815	36.73	0.853156424
816	36.68	0.854220703
817	36.63	0.855287639
818	36.59	0.856357245
819	36.73	0.853135307
820	36.87	0.849937522
821	37.00	0.84676362
822	36.84	1.66811982
823	36.98	1.661886413
824	37.12	1.655699419
825	37.26	1.64955832
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831	38.41	1.599999991
832	38.37	1.601668
833	38.33	1.603339491
834	38.61	0.759728123
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837	38.49	0.762096703
838	38.32	0.765538123
839	38.14	0.769010765
840	37.97	0.772515056
841	37.80	0.77605143
842	37.63	0.779620331
843	37.70	0.778041226
844	37.78	0.776468506
845	37.85	0.77490213
846	37.92	0.805555792
847	37.99	0.803936753
848	38.07	0.802324208
849	38.15	0.800718119
850	38.06	0.802554176
851	37.97	0.804398671
852	37.89	0.806251665
853	37.72	0.809742633
854	37.56	0.813263963
855	37.40	0.816816053
856	37.23	0.820399309
857	37.07	0.824014141
858	36.91	0.821866065
859	36.74	0.825519345
860	36.58	0.829205249
861	36.62	0.828224152
862	36.67	0.827245374
863	36.71	0.826268906
864	36.75	0.825294741
865	36.82	0.823841183
866	36.88	0.822392737
867	36.95	0.820949374
868	37.01	0.81951107
869	36.89	1.320706687
870	36.96	1.31838941
871	37.02	1.316080251
872	37.09	1.313779167
873	36.85	1.322205329
874	36.62	1.330740275
875	36.38	1.339386124
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878	35.46	1.374276761
879	35.15	1.386314464
880	34.84	1.398564913
881	34.84	0.526260842
882	34.53	0.530953249
883	34.22	0.535730088
884	33.91	0.54059366
885	33.93	0.540381204
886	33.94	0.540168915
887	33.99	0.53939836
888	34.04	0.53863
889	34.09	0.537863825
890	34.13	0.537099828
891	34.18	0.536337998
892	34.23	0.535578325

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894	34.08	1.269796093
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896	34.18	1.266193164
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913	35.03	1.018104448
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916	35.65	1.060818567
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919	36.29	1.042136381
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926	36.73	0.834866403
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931	37.05	0.827798325
932	37.41	0.819685416
933	37.78	0.811729986
934	38.26	0.801577816
935	38.74	0.791676452
936	39.21	0.782016713
937	39.66	0.861910868
938	40.14	0.851635693
939	40.62	0.84160262
940	41.09	0.831803192
941	41.57	0.822229343
942	42.05	0.812873371
943	42.53	0.803727923
944	42.76	0.799421689
945	42.72	0.800102134
946	42.69	0.800783739
947	42.65	0.801466506
948	42.61	0.802150439
949	42.59	0.766975929

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951	42.52	0.768287821
952	42.48	0.768945451
953	42.45	0.769604208
954	42.49	0.768885121
955	42.53	0.768167377
956	42.57	0.767450971
957	42.60	0.766735901
958	42.80	0.763153428
959	43.00	0.759604278
960	43.20	0.756087986
961	43.40	0.752604099
962	43.42	1.189362746
963	43.62	1.183908848
964	43.82	1.178504741
965	44.02	1.173149746
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967	44.37	1.163711862
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969	44.60	1.157789604
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971	44.70	1.155277738
972	44.71	1.230124837
973	44.76	1.228792326
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986	46.86	1.412339858
987	47.15	1.403553862
988	47.41	1.395910689
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990	47.93	1.380871382
991	48.19	1.3734726
992	48.44	1.366152683
993	48.49	1.364889094
994	48.53	1.36362784
995	48.84	0.811489907
996	48.89	0.810745481
997	48.93	0.810002419
998	48.98	0.809260718
999	49.02	0.808520375
1000	49.08	0.807532031
1001	49.14	0.8065461
1002	49.20	0.805562574
1003	49.26	0.804581443
1004	49.32	0.8036027
1005	49.32	0.803652074
1006	49.32	0.803701455

1007	49.31	0.803750842
1008	49.15	1.132566372
1009	49.15	1.132636203
1010	49.14	1.132706042
1011	49.14	1.13277589
1012	49.14	1.132845746
1013	48.95	1.137306377
1014	48.75	1.141802274
1015	48.56	1.146333857
1016	48.32	1.151940808
1017	48.09	1.157602878
1018	47.85	1.163320883
1019	47.75	0.893633501
1020	47.51	0.898079467
1021	47.27	0.902569893
1022	47.04	0.90710545
1023	46.77	0.912342548
1024	46.50	0.91764047
1025	46.23	0.92300028
1026	45.96	0.928423071
1027	45.69	0.933909957
1028	45.43	0.939161297
1029	45.18	0.944472027
1030	44.92	0.94984316
1031	44.66	0.955275733
1032	44.24	1.356378057
1033	43.98	1.364256478
1034	43.72	1.372226956
1035	43.36	1.383850877
1036	42.99	1.39567341
1037	42.62	1.407699689
1038	42.26	1.419935025
1039	41.96	1.429809356
1040	41.67	1.439821983
1041	41.38	1.449975831
1042	41.09	1.460273909
1043	41.01	0.942866439
1044	40.72	0.949623804
1045	40.43	0.956478726
1046	40.40	0.957102895
1047	40.37	0.95772788
1048	40.35	0.958353681
1049	40.32	0.958980301
1050	40.29	0.959607741
1051	40.27	0.960236002
1052	40.30	0.959542795
1053	40.33	0.958850589
1054	40.36	0.95815938
1055	40.45	0.800143836
1056	40.48	0.799568762
1057	40.51	0.798994513
1058	40.53	0.79842109
1059	40.40	0.801014007
1060	40.27	0.803623821
1061	40.14	0.806250697
1062	40.01	0.808894803
1063	39.93	0.810521592

1064	39.85	0.812154938
1065	39.77	0.81379488
1066	39.73	0.704719484
1067	39.65	0.706146685
1068	39.57	0.707579678
1069	39.49	0.709018499
1070	39.54	0.70812737
1071	39.59	0.707238477
1072	39.64	0.706351814
1073	39.69	0.705467371
1074	39.60	0.707070706
1075	39.51	0.708681345
1076	39.42	0.710299339
1077	39.33	0.711924738
1078	39.23	0.750903853
1079	39.14	0.75263071
1080	39.05	0.754365528
1081	38.96	0.756108362
1082	38.87	0.757859268
1083	38.60	0.763029181
1084	38.34	0.768270114
1085	38.08	0.773583541
1086	37.94	0.776252412
1087	37.81	0.778939763
1088	37.68	0.781645785
1089	37.48	0.987288956
1090	37.35	0.990749754
1091	37.21	0.994234901
1092	37.08	0.997744653
1093	36.95	1.001279273
1094	37.04	0.999050858
1095	37.12	0.996832341
1096	37.20	0.994623654
1097	37.28	0.992424733
1098	37.42	0.988888167
1099	37.55	0.985376717
1100	37.68	0.981890117
1101	37.82	0.978428103
1102	37.89	1.122759888
1103	38.03	1.118823174
1104	38.16	1.11491397
1105	38.29	1.111031988
1106	38.49	1.105363859
1107	38.69	1.099753271
1108	38.88	1.094199351
1109	39.06	1.089208007
1110	39.24	1.084261994
1111	39.42	1.079360696
1112	39.60	1.074503511
1113	39.72	1.199221886
1114	39.90	1.193866631
1115	40.08	1.188558993
1116	40.26	1.183298338
1117	40.48	1.176814075
1118	40.70	1.170400489
1119	40.92	1.164056433
1120	40.96	1.163014374

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1123	41.07	1.159899364
1124	41.25	0.816245571
1125	41.28	0.815520589
1126	41.32	0.814796894
1127	41.36	0.814074482
1128	41.39	0.81335335
1129	41.41	0.812918904
1130	41.44	0.812484923
1131	41.48	0.811701364
1132	41.52	0.810919315
1133	41.56	0.810138772
1134	41.60	0.80935973
1135	41.64	0.808582185
1136	41.54	1.129135787
1137	41.58	1.128049668
1138	41.62	1.126965637
1139	41.66	1.125883687
1140	41.63	1.126842583
1141	41.59	1.127803115
1142	41.56	1.128765285
1143	41.49	1.130578946
1144	41.42	1.132398446
1145	41.36	1.134223811
1146	41.29	1.136055071
1147	41.22	1.137892253
1148	41.11	1.264992294
1149	41.04	1.267047172
1150	40.97	1.269108737
1151	40.91	1.271177022
1152	40.84	1.273252059
1153	40.72	1.277070773
1154	40.58	1.281534255
1155	40.43	1.286029047
1156	40.29	1.290555479
1157	40.15	1.295113888
1158	40.01	1.299704612
1159	39.89	1.258148329
1160	39.74	1.26263781
1161	39.60	1.267159446
1162	39.46	1.271713584
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1167	38.66	1.298029437
1168	38.52	1.302624124
1169	38.39	1.307251453
1170	38.44	0.823879101
1171	38.30	0.82681247
1172	38.16	0.829766802
1173	38.03	0.832742322
1174	37.89	0.83573926
1175	37.89	0.835665744
1176	37.90	0.835592241
1177	37.90	0.835518752

1178	38.02	0.832848512
1179	38.14	0.830195285
1180	38.27	0.82755891
1181	38.37	0.871920822
1182	38.49	0.869168139
1183	38.61	0.866432783
1184	38.73	0.863714589
1185	38.85	0.861013397
1186	38.98	0.858329047
1187	38.97	0.858569352
1188	38.95	0.858809792
1189	38.87	0.860630808
1190	38.79	0.862459562
1191	38.71	0.864296105
1192	38.62	0.866140487
1193	38.57	0.786374735
1194	38.49	0.78805866
1195	38.41	0.789749813
1196	38.33	0.79144824
1197	38.24	0.793153988
1198	38.16	0.794867104
1199	38.03	0.797654045
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1202	38.09	0.796283473
1203	38.12	0.795827661
1204	38.16	0.743340896
1205	38.18	0.742916096
1206	38.20	0.742491782
1207	38.22	0.742067951
1208	38.24	0.741644605
1209	38.27	0.741221741
1210	38.31	0.740447741
1211	38.35	0.739675357
1212	38.11	0.744239293
1213	37.88	0.7488599
1214	37.64	0.75353824
1215	37.41	0.758275401
1216	37.11	0.916097849
1217	36.88	0.921939196
1218	36.64	0.927855514
1219	36.41	0.933848255
1220	36.17	0.93991891
1221	36.12	0.941219896
1222	36.07	0.942524488
1223	36.00	0.944436496
1224	35.93	0.946356277
1225	35.85	0.948283878
1226	35.78	0.950219348
1227	35.71	0.952162735
1228	35.52	1.290036354
1229	35.44	1.292694404
1230	35.37	1.29536343
1231	35.30	1.2980435
1232	35.21	1.301395221
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1234	35.03	1.308150861

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1237	35.57	1.288091128
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1239	36.01	1.073902333
1240	36.19	1.068506697
1241	36.37	1.063165009
1242	36.55	1.057876464
1243	36.73	1.052640272
1244	36.95	1.046570768
1245	37.16	1.040570855
1246	37.37	1.034639345
1247	37.59	1.028775073
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1259	39.68	1.365469203
1260	39.83	1.360482098
1261	39.97	1.35553129
1262	40.39	0.660204671
1263	40.54	0.657835728
1264	40.68	0.655483725
1265	40.83	0.65314848
1266	40.97	0.650829815
1267	41.06	0.649417745
1268	41.15	0.648011789
1269	41.24	0.646611908
1270	41.42	0.643853765
1271	41.59	0.641119051
1272	41.77	0.638407471
1273	41.97	0.580497023
1274	42.15	0.578063764
1275	42.32	0.575650819
1276	42.50	0.573257935
1277	42.68	0.570884862
1278	42.85	0.568531355
1279	42.91	0.567756505
1280	42.97	0.566983764
1281	43.10	0.565309399
1282	43.23	0.563644894
1283	43.35	0.561990163
1284	43.48	0.560345118
1285	43.33	1.191583399
1286	43.46	1.188093965
1287	43.59	1.184624909
1288	43.72	1.181176052
1289	43.84	1.177747218
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1292	44.44	1.161942304
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1294	44.49	1.160549496
1295	44.52	1.159854343
1296	44.55	1.159160023
1297	44.57	1.158466534
1298	44.62	1.105565167
1299	44.65	1.104904873
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1302	44.42	1.110633557
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1311	44.47	0.964873428
1312	44.49	0.964557945
1313	44.78	0.95831726
1314	45.07	0.95215681
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1316	45.43	0.944610848
1317	45.61	0.940882542
1318	45.79	0.937183551
1319	45.97	0.933513531
1320	46.06	1.121784333
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1342	47.09	1.243315256
1343	47.07	0.878113463
1344	46.88	0.881666625
1345	46.69	0.885248658
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1365	45.74	0.97644347
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1376	45.72	0.977051879
1377	45.85	0.808972719
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1379	45.97	0.806935421
1380	46.02	0.805920615
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1383	46.06	0.805289644
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1385	46.04	0.80567129
1386	45.84	0.80910641
1387	45.65	0.812570947
1388	45.45	0.816065282
1389	45.30	0.721123299
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1391	44.91	0.727400326
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1394	44.49	0.734172394
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1397	44.42	0.735329233
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1469	42.05	0.800674564
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1478	40.86	1.201521926
1479	40.59	1.209550971
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1482	39.90	0.91060985
1483	39.63	0.916841896
1484	39.36	0.923159832
1485	39.09	0.929565445
1486	38.82	0.936060574
1487	38.73	0.938155292
1488	38.64	0.940259406
1489	38.56	0.94237298
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1491	38.57	0.942062
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1493	38.48	1.209648852
1494	38.48	1.209448831
1495	38.49	1.209248876
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1497	38.50	1.208849165
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1502	38.50	1.209058505
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1509	38.56	0.812624754
1510	38.60	0.81164243
1511	38.65	0.810662478
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1540	40.69	0.974207351
1541	40.62	0.975682541
1542	40.56	0.977162207
1543	40.50	0.978646367
1544	40.34	0.982616415
1545	40.17	0.986618806
1546	40.01	0.990653934
1547	39.85	0.994722205
1548	39.68	0.998824027
1549	39.54	1.002498595
1550	39.39	1.006200299
1551	39.25	1.009929441
1552	39.11	0.980035168
1553	38.97	0.983693238
1554	38.82	0.987378719
1555	38.68	0.99109192
1556	38.53	0.994833154
1557	38.54	0.994661063
1558	38.55	0.994489032
1559	38.55	0.99431706
1560	38.56	0.994145148
1561	38.46	0.99673009
1562	38.36	0.999328509
1563	38.26	1.001940512
1564	37.99	1.454939934
1565	37.89	1.45877987
1566	37.79	1.462640128
1567	37.69	1.46652087
1568	37.51	1.473534542
1569	37.33	1.480615623
1570	37.15	1.487765088
1571	36.97	1.494983934
1572	36.79	1.502273175
1573	36.65	1.508260702
1574	36.50	1.514296149
1575	36.35	1.520380093
1576	36.29	1.295079371

1577	36.15	1.300312716
1578	36.00	1.305588529
1579	35.94	1.307646914
1580	35.89	1.309711799
1581	35.83	1.311783216
1582	35.77	1.313861196
1583	35.72	1.31594577
1584	35.66	1.318036969
1585	35.76	1.31447362
1586	35.85	1.310929486
1587	35.95	1.307404412
1588	36.21	0.833430954
1589	36.31	0.831212181
1590	36.41	0.82900519
1591	36.41	0.828922397
1592	36.41	0.828839621
1593	36.42	0.828756861
1594	36.42	0.828674118
1595	36.43	0.828591391
1596	36.44	0.828205551
1597	36.46	0.827820071
1598	36.48	0.827434949
1599	36.48	0.877309586
1600	36.49	0.876901617
1601	36.51	0.876494027
1602	36.53	0.876086815
1603	36.56	0.875367848
1604	36.59	0.87465006
1605	36.62	0.873933448
1606	36.65	0.87321801
1607	36.68	0.872503741
1608	36.65	0.873145808
1609	36.62	0.87378882
1610	36.60	0.87443278
1611	36.67	0.581688376
1612	36.65	0.582116449
1613	36.62	0.582545152
1614	36.59	0.582974487
1615	36.40	0.586119613
1616	36.20	0.58929886
1617	36.00	0.592512784
1618	35.81	0.595761957
1619	35.61	0.599046962
1620	35.37	0.603214852
1621	35.12	0.607441145
1622	34.87	0.611727077
1623	34.59	0.713021856
1624	34.35	0.718129697
1625	34.10	0.723311247
1626	33.86	0.728568114
1627	33.69	0.732099985
1628	33.53	0.735666267
1629	33.37	0.739267463
1630	33.20	0.742904089
1631	33.18	0.743494434
1632	33.15	0.744085717
1633	33.12	0.744677941

1634	33.10	0.745271109
1635	32.92	1.215145127
1636	32.89	1.216119106
1637	32.87	1.217094648
1638	32.84	1.218071756
1639	32.98	1.212833989
1640	33.12	1.207641075
1641	33.26	1.202492439
1642	33.19	1.205215296
1643	33.11	1.207950511
1644	33.04	1.210698169
1645	32.96	1.213458356
1646	32.88	1.257281901
1647	32.80	1.260162591
1648	32.72	1.263056512
1649	32.65	1.265963755
1650	32.56	1.269593049
1651	32.46	1.273243211
1652	32.37	1.276914424
1653	32.28	1.280606868
1654	32.34	1.27815884
1655	32.40	1.275720153
1656	32.46	1.273290754
1657	32.52	1.270870591
1658	32.64	1.091719282
1659	32.70	1.089655694
1660	32.77	1.087599892
1661	32.83	1.085551833
1662	33.00	1.080008827
1663	33.16	1.07452214
1664	33.33	1.069090919
1665	33.50	1.063714327
1666	33.76	1.055427925
1667	34.03	1.047269628
1668	34.29	1.039236488
1669	34.55	1.031325648
1670	34.87	0.879541117
1671	35.13	0.872955635
1672	35.39	0.866468037
1673	35.66	0.860076156
1674	35.89	0.854570948
1675	36.12	0.849135768
1676	36.34	0.843769288
1677	36.45	0.841377139
1678	36.55	0.838998515
1679	36.65	0.836633302
1680	36.76	0.834281388
1681	36.72	1.208010483
1682	36.83	1.204620993
1683	36.93	1.20125047
1684	37.03	1.197898756
1685	37.14	1.194565693
1686	37.09	1.196176146
1687	37.04	1.197790947
1688	36.99	1.199410114
1689	36.87	1.203314033
1690	36.75	1.207243449

1691	36.63	1.211198611
1692	36.72	0.617202464
1693	36.60	0.61922581
1694	36.48	0.621262467
1695	36.36	0.623312564
1696	36.24	0.625376237
1697	36.11	0.627685293
1698	35.98	0.630011463
1699	35.84	0.632354938
1700	35.71	0.634715913
1701	35.54	0.63769204
1702	35.38	0.640696208
1703	35.21	0.643728816
1704	34.89	1.094357968
1705	34.72	1.099610764
1706	34.56	1.104914229
1707	34.39	1.110269099
1708	34.22	1.115676126
1709	34.11	1.119263764
1710	34.00	1.122874549
1711	33.89	1.126508707
1712	33.87	1.12723433
1713	33.85	1.127960888
1714	33.83	1.128688384
1715	33.81	1.129416818
1716	33.69	1.41394881
1717	33.67	1.414865092
1718	33.65	1.415782561
1719	33.62	1.416701222
1720	33.60	1.417621075
1721	33.63	1.416445919
1722	33.66	1.415272711
1723	33.69	1.414101444
1724	33.50	1.421981014
1725	33.31	1.429948889
1726	33.13	1.43800656
1727	33.07	1.058477442
1728	32.88	1.064486696
1729	32.69	1.070564572
1730	32.51	1.076712252
1731	32.32	1.082930946
1732	32.00	1.093884664
1733	31.67	1.105062237
1734	31.35	1.116470599
1735	31.03	1.128116972
1736	30.86	1.134232207
1737	30.69	1.140414103
1738	30.52	1.146663753
1739	30.43	0.920263728
1740	30.26	0.925351011
1741	30.09	0.930494852
1742	29.92	0.9356962
1743	29.97	0.934144811
1744	30.02	0.932598557
1745	30.07	0.931057413
1746	30.12	0.929521355
1747	30.17	0.927990356

1748	30.29	0.924517728
1749	30.40	0.921070992
1750	30.51	0.917649861
1751	30.66	0.804538635
1752	30.77	0.801575588
1753	30.89	0.798634286
1754	31.00	0.795714491
1755	30.96	0.79678936
1756	30.92	0.797867136
1757	30.87	0.798947833
1758	30.83	0.80003146
1759	30.59	0.806307833
1760	30.35	0.812683463
1761	30.11	0.819160722
1762	29.78	1.123229675
1763	29.54	1.13235413
1764	29.30	1.141628043
1765	29.06	1.151055116
1766	28.82	1.160639175
1767	28.49	1.174268192
1768	28.16	1.188221094
1769	27.82	1.202509567
1770	27.49	1.217145863
1771	27.15	1.232142839
1772	26.82	1.247513994
1773	26.60	1.257818624
1774	26.59	0.451261481
1775	26.37	0.45502074
1776	26.15	0.458843159
1777	25.93	0.462730342
1778	25.84	0.464401731
1779	25.75	0.466085237
1780	25.65	0.467780993
1781	25.56	0.469489134
1782	25.47	0.471209795
1783	25.37	0.472943115
1784	25.28	0.474689234
1785	25.19	0.476419633
1786	24.94	1.108194822
1787	24.84	1.112357919
1788	24.75	1.116552412
1789	24.66	1.120778659
1790	24.63	1.121840224
1791	24.61	1.122903802
1792	24.59	1.123969399
1793	24.56	1.12503702
1794	24.54	1.126106671
1795	24.52	1.127178358
1796	24.49	1.128252087
1797	24.47	1.129327863
1798	24.27	1.400923956
1799	24.13	1.408945923
1800	23.99	1.41706029
1801	23.86	1.425268662
1802	23.75	1.431560678
1803	23.65	1.437908493
1804	23.54	1.444312854

1805	23.44	1.450774519
1806	23.33	1.457294261
1807	23.23	1.463872867
1808	23.12	1.470511138
1809	23.21	0.610902247
1810	23.11	0.613673905
1811	23.00	0.616470828
1812	23.10	0.61397984
1813	23.10	0.613834886
1814	23.11	0.613689999
1815	23.11	0.613545181
1816	23.12	0.613400431
1817	23.13	0.61325575
1818	23.13	0.613111137
1819	23.14	0.612966592
1820	23.14	0.612822115
1821	23.15	0.612677706
1822	23.15	0.612533366
1823	22.98	1.116927998
1824	22.92	1.119763102
1825	23.08	1.112176173
1826	23.23	1.104691362
1827	23.39	1.097306621
1828	23.55	1.090019957
1829	23.70	1.082829429
1830	23.86	1.075733146
1831	24.02	1.068729269
1832	24.17	1.061816003
1833	24.33	1.054991601
1834	24.49	1.048254362
1835	24.48	1.054507651

Table S.7. Calculation of event indices from CHAR data at Summit Lake using the 100-yr running mean

Events per 100-yr period

200-yr window

	Little Monon Lake	Pyramid Lake	Summit Lake
0-99	0	1	0
100-199	2	1	1
200-299	1	1	5
300-399	0	2	3
400-499	0	1	5
500-599	1	0	1
600-699	1	2	6
700-799	1	3	4
800-899	3	4	3
900-999	2	1	3
1000-1999	2	5	3
1100-1199	6	2	5
1200-1299	2	2	4
1300-1399	5	2	5
1400-1499	3	2	4
1500-1599	2	3	4
1600-1699	2	1	4
1700-1799	3	3	5
1800-1899	2	2	2
1900-1999	1	2	
2000-2099	6	4	
2100-2199	1	1	
2200-2299	4	1	
2300-2399	0	3	
2400-2499	3	1	
2500-2599		1	
2600-2699		3	
2700-2799		2	
2800-2899		4	
2900-2999		1	
3000-3999		3	
3100-3199		3	
	53	67	67

Table S.8. Events per 100-yr period BP based on the 200-yr running mean

<u>Events per 100-yr period</u>

150-yr window

	Little Monon Lake	**Pyramid Lake**	**Summit Lake**
0-99	0	1	0
100-199	2	1	1
200-299	1	1	5
300-399	0	2	3
400-499	1	1	5
500-599	1	0	2
600-699	0	2	6
700-799	1	2	4
800-899	3	4	3
900-999	2	2	3
1000-1999	3	5	2
1100-1199	7	2	5
1200-1299	3	3	5
1300-1399	5	2	3
1400-1499	3	2	5
1500-1599	1	2	4
1600-1699	2	2	4
1700-1799	3	3	6
1800-1899	2	2	2
1900-1999	1	2	
2000-2099	4	4	
2100-2199	2	1	
2200-2299	4	1	
2300-2399	3	3	
2400-2499	3	1	
2500-2599		1	
2600-2699		3	
2700-2799		2	
2800-2899		4	
2900-2999		2	
3000-3999		3	
3100-3199		3	
	57	69	68

Table S.9. Events per 100-yr period BP based on the 200-yr running mean

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