Charring *Eucalyptus globulus* as an alternative preparation technique for liquid scintillation counting used in ^{14}C dating with subsequent tritium analysis

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Abstract Dendrochronology helps scientists gain a better understanding about the recent past. However, counting tree rings can only be applied to trees that have distinct rings, such as conifer trees and some hardwood trees. Liquid scintillation counting of radiocarbon of tree samples is one alternative to counting rings. Current standard preparation of wood samples for liquid scintillation counting involves a time-consuming and labor-intensive process of removing non-cellulose components. This study attempted to compare the cellulose preparation technique with a charred wood technique, a less intensive preparation method. Both techniques were used to date samples *Eucalyptus globulus* from the Berkeley hills in California. Preliminary results of radiocarbon dating using accelerator mass spectrometry were unable to provide a reasonable age of the *E. globulus* samples. Machine error was not the cause of these results, thus an unknown environmental factor has caused radiocarbon dating unreliable for this study and has made radiocarbon dating using liquid scintillation counting redundant. Subsequent tritium analysis of the *Eucalyptus globulus* wood samples using liquid scintillation counting were compared to a previous study by Byrne and Sihvola (2007) and results suggest that the addition of CaO may have caused the tritium activity levels to be lower in the study by Byrne and Sihvola. In addition, reconstructed activity levels in both studies generally follow the reported tritium emissions from the National Tritium Labeling Facility from 1969 to 2000.

Introduction

Reconstructing past events, both natural and anthropogenic, helps researchers to understand the past and can also provide insights about what might happen in the future. Dating plays an important role in reconstructing the past because it provides a defined timeline that allows people to create a sequence of events and helps researchers to interpret their findings. Trees can aid in reconstructing the past, as they can live for hundreds of years while adapting to their surroundings, and their rings can provide chronological historical records of the environment. Tree rings have also provided annual information regarding exposure to chemicals in their immediate environment (Nabais et al. 2000; Schaumloffel et al. 1998). This information is becoming increasingly crucial, as it can help researchers understand how anthropogenic influence is shaping the current environment.

Counting tree rings is the easiest and one of the most common ways to date trees. Within one year of a tree's life, a tree will grow earlywood and latewood depending on the season and amount of rainfall, forming a series of light and dark rings caused by varying cell sizes (Grissino-Mayer 2005). Either all the dark rings or all the light rings can be counted, producing an age. Variations in the width of the rings reflect environmental changes such as droughts, floods, or limiting nutrients. However, most tropical trees and certain temperate trees do not have rings; this is due to uniform environmental conditions in which constant precipitation throughout the year allows trees to grow uniformly year round (Byrne and Sihvola 2007). In this situation, alternative methods of dating trees must be applied.

^{14}C dating in trees Radiocarbon dating is one alternative way to date the age of a tree that may not produce visible rings. A study by Hua et al. (1999) compared radiocarbon data from tree rings and radiocarbon data from atmospheric records between the years 1947-1997 and found that the two sets of data are well correlated and produced similar patterns of ^{14}C values, suggesting that radiocarbon dating is an effective method to date trees. Radiocarbon dating utilizes the isotopic properties of carbon to reconstruct past events. ^{12}C, ^{13}C, and ^{14}C are the principle isotopes of carbon that occur naturally (Higham 1999). There are relatively few ^{14}C isotopes compared to ^{12}C in living material. The radiocarbon dating method is based on the beta decay of ^{14}C to ^{14}N when a proton from the carbon decays to a neutron. This decay allows the unstable isotope to decay into a stable isotope. Libby, Anderson and Arnold (1949) were the first to measure the half life of ^{14}C to be 5568 years. Using this information, one can measure

the remaining ^{14}C relative to the amount of ^{12}C in an organic sample and calculate an age of the sample.

Liquid scintillation is one method of radiocarbon dating that measures the radiation from beta-emitting nuclides. Scintillators are organic compounds that can absorb radiant energy in the solid or liquid state. The absorption of this energy results in the formation of excited atoms that release photons and heat when the molecules are returning to normal state (Kobayashi and Maudsley 1974). Liquid scintillation dissolves or suspends samples to be measured in a solvent (or cocktail). The energy from the beta particle emitted from the samples excites the atoms or molecules in the cocktail and when the molecules return to their normal state, photons are emitted and can be detected using photomultiplier tubes (Kobayashi and Maudsley 1974). The output of a liquid scintillation counter is usually reported in counts per minute (cpm) which measures the number of atoms (photon flashes) that are detected to have decayed in one minute in a given quantity of material (Kobayashi and Maudsley 1974).

The current standard methods to prepare wood samples for liquid scintillation counting are the Jayme-Wise and diglym-HCl methods that extract the cellulose for counting (Cullen and MacFarlane 2005). Both methods utilize a series of acid and alkali washes to extract lignins and other labile carbon that may confound the isotopic ratio of carbon in the sample. Similar techniques can be found in Leavitt and Danzer (1993), and Sheu and Chiu (1995).

Although using cellulose for radiocarbon dating is fairly reliable, the pretreatment is considered to be very time-consuming and variations in techniques alter how much labile carbon is removed from the sample. Charring of wood samples is an alternative pretreatment in which a wood sample is heated to a high temperature (ca. 400ºC) without the inflow of oxygen, and becomes charcoal (Turney et al. 2006). This procedure is much faster and requires less manual labor to extract labile carbon. This study attempted to compare the pretreatments of cellulose, charcoal and fresh wood of *E. globulus* for liquid scintillation counting in order to see if charcoal produced different ^{14}C results than that of the standard pretreatment of cellulose. This would suggest that the charcoal treatment is a fast and convenient alternative to extract noncellulose components for radiocarbon dating using a liquid scintillation counter.

Subsequent tritium analysis In addition to the radiocarbon dating analysis of charred wood, tritium activity in the wood was analyzed to act as a check against a previous analysis conducted by Byrne and Sihvola (2007). Tritium is a radionuclide of hydrogen (^{3}H). It is mainly

an anthropogenic byproduct from power plants, nuclear testing, factories, and pharmaceutical companies (Evans 1974). This isotope can substitute any hydrogen atom in a molecule such as hydrogen gas, water and organic compounds (Love 2002). Water allows tritium to move through the hydrologic cycle and make its way into biochemical processes such as photosynthesis (Love 2002). As a result, tritium has been studied to reconstruct tritium release history using tree ring analysis (Kozak et al. 1984; Kalin et al. 1995). Adam Love's dissertation (2002) reconstructed tritium exposure from tree rings using accelerator mass spectrometry to compare his results with results report by the National Tritium Labeling Facility (NTLF) in Berkeley, CA.

Since tritium is able to replace hydrogen in organic molecules, communities near facilities that emit tritium have become concerned about how much tritium is in their drinking water and the effects it may have on their health. Remediation of tritium in water has also become an important issue among community members because if tritium could be taken out of the contaminated water, it would reduce any hazard that the tritium could pose on the community.

Methods

Origin of samples Wood samples were taken from a Tasmanian Blue Gum (*Eucalyptus globulus)* located 70 meters north of the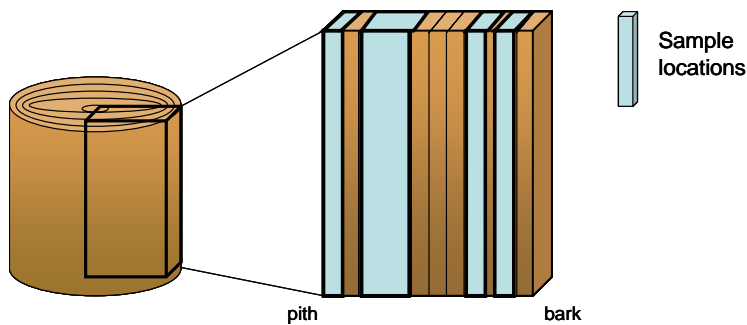 National Tritium Labeling Facility's (Building 75) emission stack near the Lawrence Hall of Science in Berkeley, CA after the upper portion of the tree had fallen down during a windstorm on November 24, 2001. The fallen log was cut into 4 smaller logs and then each smaller log was cut tangentially on either side of the pith to produce rectangular slabs (Figure 1).

Figure 1 Diagram showing radial slab and location of samples within slab.

The slab used in this study measured 12 cm by 31.5 cm by 3.5 cm and 12 columns were cut every 1 cm from the pith to the cambium layer.

Sample Preparation Of the 12 columns, 6 columns were chosen for analysis based on what was available after tests from another project (Fig. 1). Each column was further cut into 9 equal blocks (Fig. 2). Every third block within each column was assigned to be prepared as fresh wood, charcoal, or cellulose for liquid scintillation counting (LSC). The blocks were combined to their respective preparation method within each column to obtain the weight needed for combustion. The samples were dried at 110ºC for 24h and weighed.

Fresh wood treatment – These samples were left untreated.

Charcoal treatment – Removes all components of the sample except stationary carbon. The blocks were placed in a crucible covered in sand to prevent access of oxygen, and were heated to 400ºC for 1 hour and then the samples were weighed.

Figure 2. Diagram of one column and the assigned treatment to each block.

Cellulose treatment – Removes all of the noncellulose components such as lignin of the sample to ensure only stationary carbon content is left in the sample. The samples were pretreated with an acid/alkaline wash as described by Sheu and Chiu (1995).

> a) 4% HCL by weight for 1h at 90ºC
>
> b) 4% NaOH by weight for 1h at 90ºC
>
> c) 4% NaOH by weight for 0.5h at 90ºC
>
> d) 4% HCL by weight for 0.5h at 90ºC

Samples were rinsed with distilled water between each step. The samples were placed in distilled water for 24 hours and then placed in the oven at 90ºC to drive off the water.

Combustion procedure A double-valve, self-sealing 1850 ml capacity oxygen combustion bomb No. 1121 (Parr Instrument Co., Moline, Illinois) plus accessories were used to combust samples into CO_2 and water.

The samples were placed in the ignition cup and a fuse wire was placed to be in contact with the sample. The inlet valve was attached to an oxygen tank and the sealed bomb was charged to 15 atm (Fig. 3). The bomb was fired by an external ignition unit providing the proper electrical
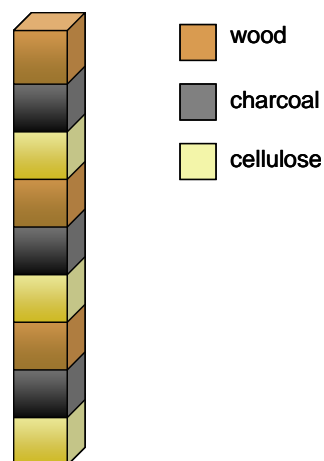
current to burn the sample. Immediately after ignition, the pressure inside the bomb reached 30 atm and then gradually returned back to the original pressure. The bomb was left to cool in a container of ice water for approximately 30 minutes before the outlet valve of the bomb was released. This was to allow maximum condensation of the water inside the bomb for collection. The
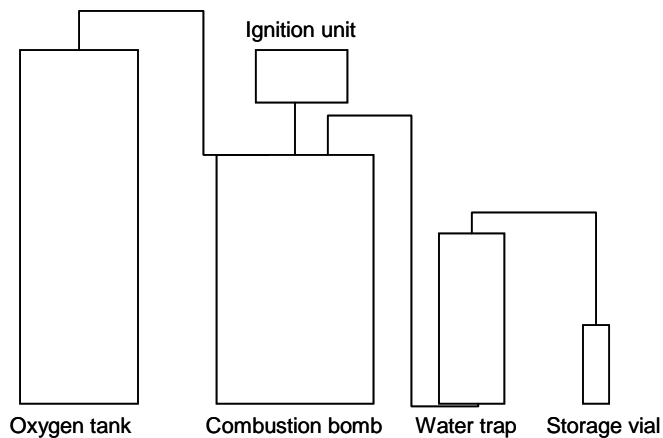

Figure 3. Schematic diagram of experimental setup.

gas from the bomb was released into a water trap to remove any water vapor, then into the storage vial containing 10ml of Carbosorb scintillation cocktail, and then weighed. The water was collected from the bomb in a glass scintillation vial and weighed.

Liquid scintillation counting for ^{14}C analysis A total of 18 samples were prepared to be sent to the Chemical Biology and Nuclear Science Division at Lawrence Livermore National Laboratory and counted on a Perkin Elmer Quantulus Liquid Scintillation Counter using counts per minute as the unit of measurement. However, it was decided to halt further analysis due to preliminary radiocarbon dating that will be discussed in the results section.

Subsequent analysis for tritium using liquid scintillation counting The water collected from the wood samples (6 samples in total) were sent to the DHI Laboratory in Horsholm, Denmark for tritium counting. The counting was 0.5 hours and the mean counting error was +/- 12 percent. The results (Euc-1) were converted to picoCuries per liter (pCi/L) and tritium units (TU), and compared to a previous tritium analysis using another slab from the same Eucalyptus tree (Euc-2). Analysis of Euc-2 was prepared in the same way as Euc-1 except that the 21 samples were neutralized using calcium oxide to reduce the effects acidity may have on the counter, and then were distilled in a microstill to ensure pure water was being counted.

Results

Preliminary radiocarbon dating results for the tritium analysis did not produce dates that were within reason of the age of the *E. globulus* sampled. The samples were chosen to span the

whole age of the tree, but the ages of these samples did not vary. The ages reported for these samples are much too old than the actual age of the tree (Fig. 4).

^{14}C dating of charred wood Analysis was stopped after discovering tree samples using accelerator mass spectrometry as well as liquid scintillation counting gave unreliable dates. These results were not due to machine error, and thus it was decided that analysis of charred wood samples using LSC to test the various techniques would only repeat the AMS results and should not be done.

Figure 4. Percent modern radiocarbon for Euc-2 and study by Love (2002). (Taken from Byrne and Sihvola (2007)

Subsequent tritium analysis The 6 samples from Euc-1 were corrected for the decay rate of tritium (half-life 12.32 years) using the chronology previously established by Love et al. (2003) since radiocarbon dating was unable to produce reliable results (Byrne and Sihvola 2007). The dates of the samples contain a probable error of +/- one year. The Euc-1 results were plotted against Euc-2 tritium results reported by Byrne and Sihvola (2007) to see any trends. The dates and tritium activities are summarized in Figure 5 and Appendix 1.
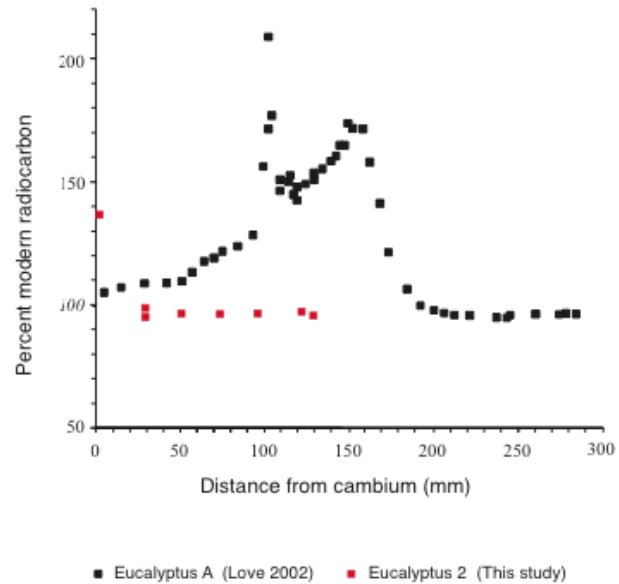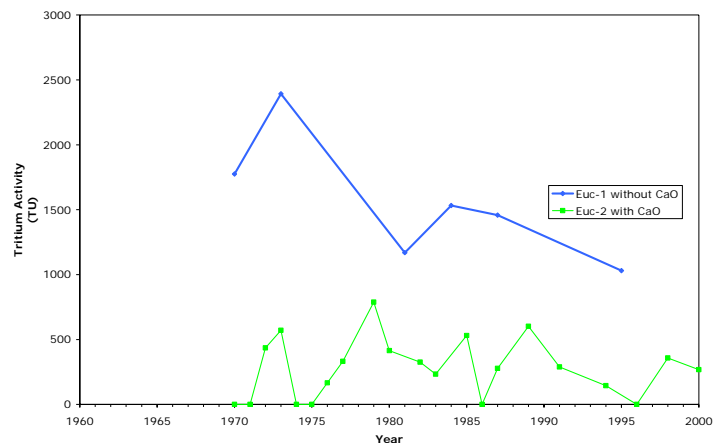

Figure 5. Tritium activity for Euc-1 and Euc-2 from 1969-2000. Activity levels are much higher for Euc-1 than Euc-2 but both have a similar trend.

Discussion

   ^{14}**C dating analysis**   Radiocarbon dating using accelerator mass spectrometry (AMS) is usually quite reliable to date samples (Love 2002). Although ^{14}C AMS dating has been most often used for prehistoric dating, ^{14}C dating within the last 60 years utilizing the ^{14}C bomb spike is becoming a recognized and reliable way of dating samples that can show the bomb spike trend such as trees (Hua et al. 1999). So, it was surprising to see the Euc-2 ages clearly not within an acceptable error range of dates. Figure 4 taken from Byrne and Sihvola (2007) shows the percent modern carbon of Euc-2 compared to study done by Love et al (2003) on Eucalyptus A. It was thought that Euc-2 would follow the same general pattern as Love's in which there would be a sudden increase in percent modern carbon, and then percent carbon would gradually decrease to modern levels as the sample distance from the cambium increases.

   E. globulus in the Berkeley hills were planted near the turn of the twentieth century as a means of making money by producing lumber. Unfortunately *E. globulus* significantly warps and the industry in Berkeley was soon abandoned (Klatt 2006). Ages from AMS dating and LSC were reported to be much older than even the oldest eucalyptus trees planted at the turn of the century. Even the sample taken near the pith where the age is known to be less than 100 years old, the radiocarbon dating gave it an age of 280 +/- 60 years, which is clearly too old (Table 1). Also according to Table 1, all samples with the exception of 2002-5 came back with relatively the same percent modern carbon.

   It is unknown what caused the older ages in the samples. However, since the reliability of AMS is well established, there is most likely a local environmental factor influencing the uptake of

Table 1. Radiocarbon results for Euc-2. (Taken from Byrne and Sihvola (2007)).

Sample number	Distance from cambium (cm)	Lab number	Radiocarbon age BP (before 1950)	Percent modern radiocarbon
2002-1	13.2	Beta – 170839	280 +/- 60 BP	96.53 +/- 0.72
2002-2	7.5	Beta – 170840	170 +/- 60 BP	97.86 +/- 0.73
2002-3	5.2	Beta – 170841	230 +/- 60 BP	97.13 +/- 0.73
2002-4	3	Beta – 170842	70 +/- 50 BP	99.09 +/- 0.62
2002-5	0.1	Beta – 170843	138.06 +/- 0.84 pMC	138.06 +/- 0.84
2007-1	3	CAMS-131372	249 +/- 35 BP	97.05 +/- 0.42
2007-2	9.8	CAMS-131373	235 +/- 35 BP	97.12 +/- 0.37
2007-3	12.5	CAMS-131374	170 +/- 35 BP	97.93 +/- 0.38

carbon dioxide into the tree. Trees can be quite particular about up-taking carbon dioxide from the atmosphere. Stresses such as limited available water or limited nutrients can affect what isotopes of carbon are consumed by the tree. As the availability of water to the tree decreases, the stomatas in the leaves do not open as wide to act as a conservation effort and as a result prefers the smaller CO_2 molecule with ^{12}C instead of ^{14}C (McCarroll and Loader 2004). This would cause the ^{14}C/^{12}C ratio not to reflect the true atmospheric ratio and could explain the lower levels of percent modern carbon.

Anthropogenic emissions of carbon dioxide could be an additional explanation for the older ages. Car exhaust and possible exhaust from near by buildings could have increased the amount of carbon dioxide present near the *E. globulus* and uptake from the tree through photosynthesis would produce cellulose reflecting the carbon ratio of the car exhaust and not necessarily the radiocarbon ratios of the present atmosphere. However, this does not explain why samples ages were mostly uniform throughout the tree.

Given that the AMS and LSC dates for Euc-2 show no ^{14}C trend reflecting the ^{14}C pattern in the atmosphere, the likelihood that LSC dates for Euc-1 would also show no trend in ^{14}C pattern from the atmosphere is high. The goal of this study was to look at how the ^{14}C trends differed among the three preparation methods and whether charcoal could be an alternative treatment to the standard cellulose treatment. However, it would be difficult to make any comparisons among the treatments without a clear ^{14}C trend. Therefore, the Euc-1 CO_2 samples were not counted. In spite of this setback, the water collected from the wood treatment samples were analyzed for tritium activity and yielded useful results.

Subsequent tritium analysis The tritium activity for Euc-1 was analyzed to act as a check against Euc-2 tritium activity from Byrne and Sihvola (2007). This study compared the tritium activity levels of Euc-2 against Adam Love's AMS results to reconstruct the tritium activity emitted near the National Tritium Labeling Facility since 1969. Results of the comparison showed that the reconstruction of the Euc-2 activity follows the general trend with that of Love's Eucalyptus A and with the reported tritium release from the NTFL (Byrne and Sihvola 2007). However, the major difference between the two activity records is that Euc-2 records are 3 orders of magnitude lower in activity than Eucalyptus A from Love's study (Byrne and Sihvola 2007). So the basis of my subsequent tritium analysis was to check the activity levels of Euc-2 and see if the levels are actually that low and that it wasn't due to our preparation technique. Figure 6

compares Euc-1 against Euc-2 and is overlaid on top of the reported tritium emissions record (1969-2000) from the NTLF. Both Euc-1 and Euc-2 follow a similar trend with peaks in 1973 and 1984. Unfortunately, since the resolution of Euc-1 samples is low, clear conclusions cannot be made. Emissions in 1984 from the reported data does show an increase in tritium emissions, however, emissions from 1973 does not show a distinct peak despite high peaks reported for Euc-1, Euc-2, and Love's study.

A possible explanation for the discrepancy among data sets for the year 1973 could be attributed to the lack of instruments that could accurately measure the amount

Figure 6. Tritium activity trend for Euc-1 and Euc-2 plotted against NTLF reported tritium emissions (bars) 1969-2000.

of tritium released from the emission stack. The NTFL was started in 1969, so the facility was still fairly new in 1973 and maybe regulations within the facility about recording emissions hadn't been strictly established or they may not have reported true emissions. Strict enforcement of emissions wasn't in place until 1976 with the Clean Air Act, so it is unclear what was happening at the facility from 1969 to 1976. It is interesting to note that the reported emission following the enforcement of Clean Air Act were much higher than the previous reported emissions suggesting that prior to 1976, the actual emissions from the NTFL were higher than reported.

The tritium record from Euc-1 clearly shows tritium activity in the tree to be much higher than what was reported for Euc-2. Given that both analyses were conducted on the same tree, outside environmental variation cannot be the reason for the different activity levels. The addition of calcium oxide (CaO) to neutralize the water sample for Euc-2 is the only step in the preparation setup that differs from Euc-2. Calcium Oxide, also know as lime, is made from the thermal decomposition of calcium carbonate (O'Neil 2006). When CaO reacts with water, it
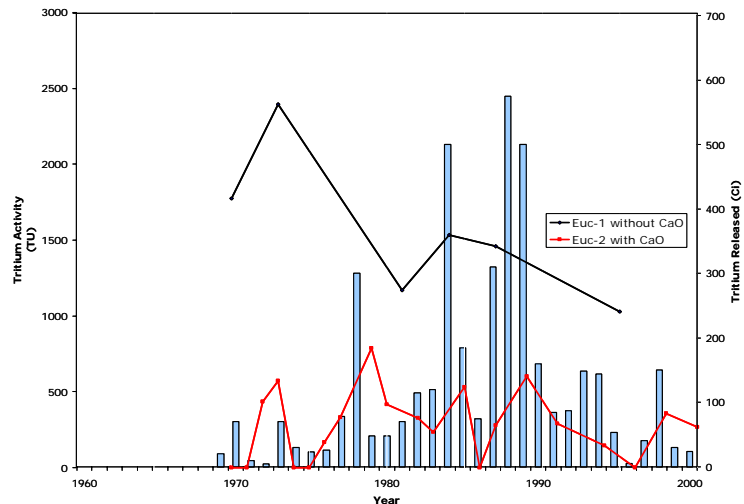
produces slaked lime $Ca(OH)_2$. After the CaO was added to the Euc-2 samples the water was distilled leaving a white precipitate on the bottom of the beaker. This precipitate was probably the slaked lime. Since the tritium replaces one or more of the hydrogen ions in the water molecule, the reaction of the water and lime could produce $Ca(OT)_2$. With the removal of the tritiated slaked lime from the water sample, the overall amounts of tritium in the water sample would be reduced. The removal of $Ca(OT)_2$ by distillation could be the reason why Euc-2 tritium activity was much lower than Euc-1. To test whether this is a reasonable explanation for the lower activity levels in Euc-2, the precipitate from the distillation would need to be analyzed for tritium content.

If future studies look to see if tritium activity is in fact reduced in water when calcium oxide is added, this could have a major impact in water quality. Tritiated water has been a major concern in areas near power plants, factories, and pharmaceutical companies where tritium is a byproduct and is inadvertently or purposely discarded into the nearby waterways. The water may or may not cause major health effects to nearby residents, but if adding calcium oxide does reduce tritium in water, the remediation would have major impacts on the water quality of these areas and would benefit all parties involved.

While looking at the tritium activity in *Eucalyptus globulus* was not the main focus of this project, results suggest that trees are useful in reconstructing environmental histories and calcium oxide may be a potential reagent to remove tritium from water. Although radiocarbon dating of *E. globulus* near the NTLF was unsuccessful for this particular study, it would be reasonable to replicate the study on trees in an isolated area where complication in radiocarbon dating would be reduced.

Acknowledgements

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Appendix 1

Values for Figure 5.

Euc-1

sample number	counting time (s)	dpm	uncorrected pCi/L	assigned year	corrected pCi/L	corrected TU
1	1800	8	706	1970	5663	1775
3	1800	13	1127	1973	7634	2393
4	1800	10	864	1981	3731	1170
5	1800	15	1340	1984	4889	1532
9	1800	17	1511	1987	4654	1459
11	1800	19	1668	1995	3286	1030

Euc-2

sample number	counting time (s)	dpm	uncorrected pCi/L	assigned year	corrected pCi/L	corrected TU
2	3600	6.4	575	2000	853	267
3.5	3600	7.6	687	1998	1140	357
5.5	3600	0.0	0	1996	0	0
7.5	3600	2.5	221	1994	459	144
9.5	3600	4.1	374	1991	920	288
11.5	3600	7.7	697	1989	1919	601
13.5	3600	3.2	287	1987	885	278
15.5	3600	0.0	0	1986	0	0
17.5	3600	5.4	491	1985	1693	531
19.5	3600	2.1	193	1983	745	234
21.5	3600	2.8	254	1982	1038	325
23.5	3600	3.2	290	1980	1323	415
25.5	3600	5.8	520	1979	2514	788
27.5	3600	2.2	196	1977	1057	331
29.5	3600	1.0	92	1976	528	166
31.5	3600	0.0	0	1975	0	0
33.5	3600	0.0	0	1974	0	0
35.5	3600	3.0	269	1973	1823	571
37	3600	2.2	194	1972	1390	436
39.5	3600	0.0	0	1971	0	0
40	3600	0.0	0	1970	0	0