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Submergence of black ash logs to control emerald ash borer and preserve wood for American Indian basketmaking

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Abstract

1 Indigenous artisans in the Great Lakes region rely on the ring-porous property of black ash *Fraxinus nigra* Marshall (Oleaceae), which allows annual layers of xylem to be easily separated to make baskets that are important economic resources and vessels of culture.
2 The emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is threatening North America's ash resource, including black ash and this centuries-old art form, resulting in grave concern about the availability of black ash trees for basketmaking and about movement of black ash (along with *A. planipennis*) from areas where it is cut to lands where it is pounded and split to make baskets.
3 We evaluated the traditional practice of storing black ash logs submerged in water as a possible method for killing within-tree life stages of *A. planipennis* at the same time as preserving the wood's value for basketmaking.
4 Black ash trees infested with overwintering *A. planipennis* larvae were felled and cut into 60-cm bolts in 2010 and in 2011. These were submerged in a river for different lengths of time and, after treatment, placed into rearing tubes to determine survival and adult emergence, or dissected within 24 h to determine larval mortality, and then pounded and peeled into splints to assess colour and pliability.
5 In 2010, all *A. planipennis* larvae had died and no adults emerged from logs submerged in spring for 10 weeks or longer, whereas some larvae survived and adults emerged from logs submerged for up to 9 weeks.
6 In 2011, submergence for 18 weeks during winter or 14 weeks in spring resulted in complete mortality of *A. planipennis* larvae and no emergence of adults at the same time as still preserving wood quality for basketmaking.

Keywords *Agrilus planipennis*, basketmaking, black ash, emerald ash borer, *Fraxinus nigra*, submergence.

Introduction

Black ash *Fraxinus nigra* Marshall (Oleaceae) has special importance for American Indian and First Nations peoples in the Great Lakes region and northeastern North America. It has ring porous wood that allows layers of xylem to be easily separated (U.S. Department of Agriculture Natural Resources Conservation Service, 2014) and, for centuries, has been used for making baskets. The wood of black ash is also used for lacrosse sticks, pipe stems, fish weirs and other purposes (Benedict, 2001; Mundell, 2008; Benedict & David, 2003). Today, black ash basketmaking is central to a cultural renaissance among tribes in the region and to the household economies of skilled Native artisans. The emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is threatening North America's ash resource, including black ash (Poland & McCullough, 2006), and the indigenous cultures and traditions that rely on it.

Submergence in water is a traditional method of holding black ash logs from the time they are cut until they are needed for basketmaking. Research has shown that submergence of logs for

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at least 1 month also is effective in killing buprestid beetle larvae (Gardiner, 1962).

In the present collaborative study, research scientists partnered with American Indian basket makers to determine whether submergence of black ash logs infested with *A. planipennis* would kill the insect at the same time as preserving wood properties sought for basketmaking.

Background

Agrilus planipennis was first detected in North America near Detroit, Michigan, in 2002 (Haack *et al*., 2002; Poland & McCullough, 2006). This phloem-feeding insect, native to Asia (Yu, 1992), has killed tens of millions of ash trees and continues to spread throughout the range of ash in North America (EAB Info, 2014). All North American ash species that have been encountered by EAB thus far are susceptible (Anulewicz *et al*., 2008; EPPO, 2013). Black, green and white ash are highly vulnerable (Klooster *et al*., 2014), although white ash is somewhat less preferred (Anulewicz *et al*., 2007). Adults feed on leaves throughout their lifetime, causing negligible damage. Females oviposit in bark cracks and crevices, and larvae feed in the phloem and cambial region, killing trees within 2–3 years of infestation (Bauer *et al*., 2004; Poland & McCullough, 2006). After development, which can take 1–2 years (Tluczek *et al*., 2011), adults chew out through the bark leaving D-shaped exit holes. Adults may fly up to a few kilometers (Taylor *et al*., 2010) and are transported long distances through human-assisted movement of infested ash firewood, nursery stock, logs and wood products.

Although efforts to collect black ash seed may help to preserve genetic material for future restoration (Benedict & Richard, 2000; Benedict & David, 2003; Agricultural Research Service, 2014), there is grave concern about the availability of large black ash trees for basketmaking in the near future with respect to passing on cultural traditions. In addition, there is concern about movement of infested or potentially infested black ash from areas where it is cut to lands, including tribal lands, where it is pounded and split to make baskets. Submergence of black ash logs may allow preservation of the wood for basketmaking in the near future and may also be an effective treatment for killing *A. planipennis* and preventing the artificial spread of this devastating invasive species.

Black ash trees are cut for basketmaking year round, although some harvesters have preferred times. Because basket-grade trees frequently are found in wetlands, access may be easiest in winter, when the ground is frozen (Benedict, 2001). Others find spring, when the buds begin to swell, to be the optimal time for cutting black ash trees for basketmaking. At this time, moisture content in the sapwood of the tree is high, which loosens the bark and the annual growth rings (Severn, 2010). Often, trees are harvested one at a time, as the need arises and processed immediately or within 1 week. However, there are circumstances under which it makes sense to cut several black ash logs and bury or submerge them to retain moisture and provide material to work with throughout the year (Severn, 2010). Hence, logs are often submerged for periods of a few weeks to a year. Prolonged submergence for periods longer than 1 year may result in wood decay (Wetherbee, 1985).

A common method used for processing black ash for basketmaking is to systematically pound debarked, whole logs to delaminate the growth rings from one another using a heavy wooden mallet or the back of an axe with rounded edges (Benedict, 2001; Mundell, 2008; M. Davis, Gun Lake Band of Potawatomi Indians, personal communication). Logs are pounded in an overlapping pattern along the entire length. The axe blade or a knife is used to score a splint along the true grain of the tree. Each splint or strip of separated ring is pulled from the log then rolled up. There is some variation in black ash processing techniques and tools from tribe to tribe and individual to individual. Black ash logs sometimes are cut into vertical sections before pounding. A variety of hand and small shop-made mechanical devices are used by some individuals to separate growth rings. Splints intended for later use may be coiled in an air-tight container for further processing before being woven into baskets.

Entomologists partner with basket makers

A small but growing body of literature is demonstrating the value of partnerships between traditional ecological knowledge and western science for natural resources research and management (Kimmerer & Lake, 2001; Houde, 2007; Mason *et al*., 2012; Emery *et al*., 2014). Traditional ecological knowledge has been defined as a ‘cumulative body of knowledge, practice, and belief, evolving by adaptive processes and handed down through generations by cultural transmission, about the relationship of living beings (including humans) with one another and with their environment’ (Berkes, 1999).

The challenge of responding to invasive species such as *A. planipennis* at the same time as preserving indigenous cultural traditions requires information provided by both traditional ecological knowledge and western science. The former provides a basis for understanding the values of natural resources and actual practices related to them and the latter a systematic way of evaluating questions that arise from these relationships.

In 2010, U.S. Department of Agriculture Forest Service and Animal and Plant Health Inspection Service scientists partnered with basket makers from the Match-e-be-nash-she-wish Band of Potawatomi Indians of Michigan (referred to hereafter as the Band) to test whether infested black ash logs could be submerged to kill within-tree life stages of *A. planipennis* and retain the wood colour and pliability needed for basket making. The Band's environmental specialist explained the practices frequently used in the region to choose a tree for basketmaking. These include looking for a tall, straight tree, growing in moist soil or marshy conditions, preferably with at least 2–3 m of straight, clear lower bole that is fairly large in diameter. Spiritual guidance also may be sought in choosing a tree. An axe is used to cut a chink out of likely trees to assess their rings. The ideal wood for black ash basketmaking is creamy white, with growth rings approximately the thickness of a U.S. nickel or quarter coin (approximately 2 mm). Wood also must be sufficiently pliable that it does not break when woven (M. Davis, Gun Lake Band of Potawatomi Indians, personal communication).

The Band basketmakers come from a long line of master basketmakers and ensured all research methods respected traditional practices from design through evaluation of the suitability of submerged logs for basketmaking. We chose trees for the study using traditional Band methods and submerged logs according to Band practices in water that was sufficiently deep to completely cover the logs and with a running current to minimize wood rot that may occur in stagnant water. After submergence and dissection, logs were transported to Band lands, where they were processed respecting traditional practices for basketmaking.

Materials and methods

In previous research reported by Siegert *et al*. (2014), infested black ash logs were submerged in tubs of water and held in a laboratory for several weeks. Within 9 weeks, *A. planipennis* larval mortality was observed; however, the logs began to decay in the stagnant water (Siegert *et al*., 2014). Our objectives were to evaluate mortality of *A. planipennis* under natural conditions that reflect actual cultural practices. As a result, treatments were developed in accordance wth Band guidelines, with logs submerged in a stream of running water at different times of the year and for various periods of time. Furthermore, measures were added to determine the effects of submergence on wood splint colour and properties.

2010 spring submergence study

In 2010, five black ash trees (mean ± SE diameter at breast height 24.1 ± 0.5 cm) infested with overwintering *A. planipennis* larvae were felled in southern Michigan in late April 2010 and cut into logs (mean ± SE length 64.4 ± 1.4 cm, diameter 18.2 ± 0.9 cm). Logs were randomly divided among five treatments with eight logs per treatment: (i) unsubmerged control logs; or logs submerged for (ii) 1 week, (iii) 4 weeks, (iv) 10 weeks or (v) 16 weeks. Logs were submerged on 10 May in the Red Cedar River, in Okemos, Ingham County, Michigan (42.713755 N, −84.431734 W). Eye-bolts were screwed into both ends of each log. A cinder block was tied with rope to the eye-bolt at one end of each log before placing the logs in the river. A long rope was tied to the eye-bolt at the other end. Logs were placed approximately 1–2 m out from the bank of the river and allowed to drop to the bottom of the river. The long ropes were tied to trees along the bank of the river. Water depth, temperature and surface flow rate were measured periodically throughout the experiment. Water depth was measured adjacent to the groups of submerged logs by recording the length of wet surface after inserting a long wooden pole into the water until it touched the bottom. Temperature was measured using a thermometer held under water near the submerged logs for several minutes. A stick was thrown into the centre of the river and allowed to float downstream. The time it took to travel 300 m (measured along the bank) was recorded with a stop watch. This procedure was repeated three times and averaged to determine the surface flow rate of the river.

On 11–12 May, half of the control logs were dissected and the number of live and dead *A. planipennis* pupae and larvae of each developmental instar were tallied. The length and mean diameter (mean of the diameters of the two cut ends of the log) of each log were measured to calculate surface area and determine larval and pupal density. Dissected logs were then pounded and peeled into splints to assess colour and pliability. One side of each splint was shaved with a sharp knife to remove any rough wood fibres, resulting in a bright smooth surface. Log pounding and splint peeling and shaving was performed in accordance with traditional Band techniques. The remaining control logs were placed in rearing tubes and held in the laboratory at room temperature to assess *A. planipennis* survival and emergence. Rearing tubes were checked daily to collect emerged adults until no additional adults emerged for a period of at least 1 week. Similarly, for logs that were submerged, half of the logs were dissected within 24 h after treatment to determine larval and pupal mortality, then pounded and peeled into splints to assess splint colour and pliability. Larvae from dissected logs were held in Petri dishes at room temperature for 24 h and observed periodically for movement; if movement was observed, they were considered to be alive. The remaining logs were placed into rearing tubes to determine survival and adult emergence.

Splint properties were assessed visually and by measuring spectral reflectance curves. Within 1 h of peeling, each splint was examined to determine whether the wood was intact and pliable (i.e. could be rolled into a 5-cm diameter coil without cracking or breaking) or if it began to crack, crumble or decay. The colour of each splint was assessed after splints had dried, at least 4 weeks after peeling, by measuring its spectral reflectance curve using a FieldSpec Pro full range spectrophotometer (Analytical Spectral Devices Inc., Boulder, Colorado). The FieldSpec Pro FR uses a 1-m fibre optic bundle for light collection and covers the range from 350 to 2500 nm. The visible and near-infrared portion of the spectrum (350–1000 nm) was measured by a 512-channel silicon photodiode array. The spectral resolution of this array is 3 nm in the visible and near-infrared region based on the full-width-half-maximum response of the spectrophotometer. Spectral readings were recorded as relative reflectance. To calculate the reflectance, a white reference reading was taken on a spectralon panel before each sample was scanned. A square area of $2 \times 2\ cm^2$ was scanned on the shaved side and unshaved side of each sample.

2011 winter submergence study

In 2011, we conducted two experiments (winter submergence and spring submergence) to evaluate the efficacy of log submergence at different times of the year with a different water temperature and to refine the period of time required for complete *A. planipennis* mortality. Nine infested black ash trees (mean ± SE diameter at breast height 22.0 ± 1.0 cm) were felled in February 2011 and cut into logs. For the winter submergence experiment, infested logs (mean ± SE length 58.9 ± 0.8 cm, diameter 17.4 ± 0.9 cm) were submerged in the Red Cedar River during the first week of March when the water temperature was at freezing (0 °C). We hypothesized that longer periods of submergence may be required at colder temperatures because larvae would remain dormant and would not attempt to feed and be affected by the saturated conditions. We also aimed to evaluate the effects of prolonged submergence [i.e. a full year (52 weeks) and a year-and-a-half (78 weeks)] on splint quality. Therefore, we selected longer time periods for the treatments of this experiment. Logs were randomly divided among six submergence time treatments with six to eight replicates per treatment: (i) unsubmerged control; or submerged for (ii) 10 weeks, (iii) 18 weeks, (iv) 26 weeks, (v) 52 weeks or (vi) 78 weeks. The outer bark had begun to decay and fall off logs that were submerged for 26, 52 or 78 weeks; therefore, they could not be dissected to assess larval survival. Control logs and all logs submerged for 10 or 18 weeks were dissected within 24 h of removal from the river. Larvae dissected from logs were held for 48 h at room temperature and observed periodically for movement. All logs for every submergence treatment duration were pounded and peeled into splints to assess the impact of prolonged submergence on splint properties by measuring spectral reflectance as described above.

2011 spring submergence study

For the spring submergence experiment, infested logs (mean ± SE length 58.8 ± 0.7 cm, diameter 13.1 ± 0.3 cm) were submerged in the Red Cedar River on 12 May 2011, when the water temperature was 5.5 °C. We aimed to refine our estimate based on 2010 results indicating that some larvae were alive after 4 weeks of submergence but all were dead after 10 weeks of submergence. Therefore, we selected weekly time periods between 4 and 10 weeks. Initially, we assigned eight logs per treatment with four logs designated for dissection and four logs for rearing each week. However, by week 6, when we still found little mortality of larvae during log dissection, we decided to prolong the experiment by pulling only four logs for dissection each week and foregoing rearing, allowing those logs to remain submerged for up to 14 weeks. At 14 weeks, the final set of four logs was placed in rearing tubes to determine adult survival and emergence. As a result, submergence treatment times varied for dissected logs and logs that were reared. Because logs had originally been randomly assigned to treatments with four logs to be dissected and four logs to be reared, the reassignment of groups of four logs to new treatments (submergence times and dissection or rearing) was still considered to be random. For dissected logs, there were eight treatments: (i) unsubmerged control; or submerged for (ii) 4 weeks, (iii) 5 weeks, (iv) 6 weeks, (v) 7 weeks, (vi) 8 weeks, (vii) 9 weeks or (viii) 13 weeks, with four logs per treatment. For logs that were reared for adult emergence there were five treatments: (i) unsubmerged control; or submerged for (ii) 4 weeks, (iii) 5 weeks, (iv) 6 weeks or (v) 14 weeks with four logs per treatment.

Statistical analysis

Although initial *A. planipennis* attack densities undoubtedly varied between trees and among logs within each tree, the logs were randomly assigned to treatments and replicated with four to eight logs per treatment to avoid any confounding or bias of results. For each experiment, we verified that differences in initial attack densities were random with no significant differences among treatments that could confound or bias results. Initial attack densities were calculated as the density of all *A. planipennis* life stages (dead and alive) in dissected logs and were compared among treatments (duration of log submergence) for each experiment by a general linear mixed model (PROC GLIMMIX) because assumptions of analysis of variance were violated (PROC UNIVARIATE). Duration of submergence was tested as a fixed effect and degrees of freedom were determined using the ddfm KR method. The response distribution was lognormal with the identity link function.

On finding no significant difference in overall initial attack density among treatments for any experiment that could confound results, we then compared the density of adults that emerged after submersion and the density of immature life stages and percentage mortality within logs after submersion among treatments for each experiment to determine the effects of the submergence treatments on survival. The densities of adults emerged from logs placed in rearing tubes were transformed by $\ln(y+1)$ then tested for normality and homoscedasticity (PROC UNIVARIATE). Transformed data were compared among treatments by analysis of variance (PROC GLM) followed by Tukey's honestly significant difference multiple comparison procedure. The densities of immature life stages in dissected logs were analyzed separately from adult emergence data from logs in rearing tubes because the samples were from different logs and, in the case of the spring 2011 experiment, the submergence treatment times were different for adult rearing logs compared with dissected logs. The densities in dissected logs of live and dead larvae, prepupae, pupae and all life stages combined, as well as the percentages of dead *A. planipennis* of all life stages, were compared among treatments by a general linear mixed model (PROC GLIMMIX) because assumptions of analysis of variance were violated (PROC UNIVARIATE). Duration of submergence was tested as a fixed effect and degrees of freedom were determined using the ddfm KR method. The response distribution for comparing the density of *A. planipennis* lifestages was lognormal with the identity link function. The response distribution for comparing the percentage of dead *A. planipennis* was specified as beta with the link function set as logit. Differences among treatments were tested using the Tukey–Kramer means comparison procedure. All analyses were conducted using SAS, version 9.4 (SAS Institute, 2012) with an α-level of 0.05. The reflectance recorded at each wavelength across the spectrum from 350 to 2500 nm was averaged for splint samples collected from logs of the same submergence treatment. Mean reflectance curves were plotted by submergence treatment.

Results

2010 spring submergence study

Logs randomly assigned to different periods of submergence did not differ in initial attack density among treatments (mean ± SE = 44.9 ± 7.1 *A. planipennis* per cm^2; $F = 1.22$; d.f. = 4,17; $P = 0.3$). The density of *A. planipennis* adults that emerged from logs decreased significantly as duration of log submergence increased. Some adults emerged from logs submerged for 1 or 4 weeks, although none emerged from logs submerged for 10 or 16 weeks (Fig. 1A). Similarly, the density of live larvae and prepupae decreased significantly with an increasing duration of submergence and there were no live larvae or prepupae found in dissected logs after 10 or 16 weeks of submergence (Table 1). On the other hand, the density of dead larvae and prepupae increased significantly with the duration of submergence (Table 1). The percentage of *A. planipennis*

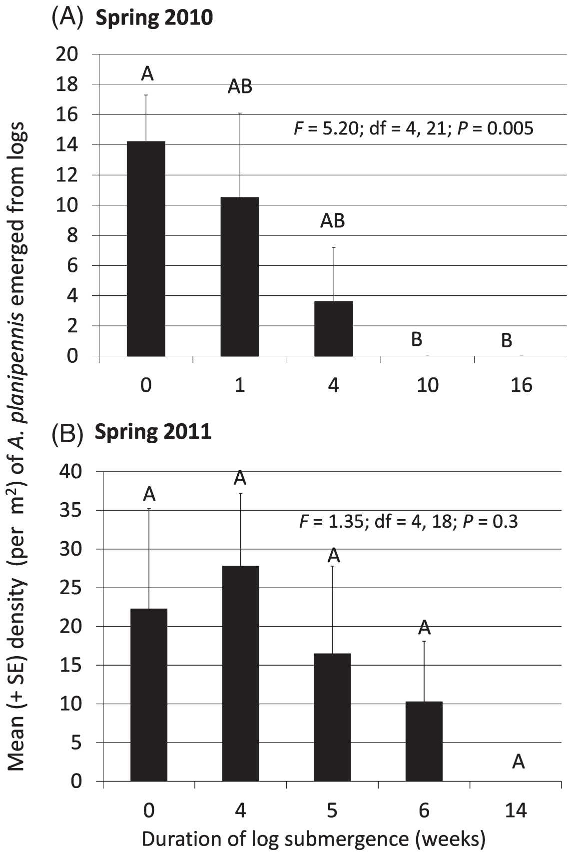

Figure 1 Mean ± SE number of *Agrilus planipennis* adults emerging from black ash logs that had been submerged for different periods of time in (A) spring 2010 and (B) spring 2011. Columns with different uppercase letters are significantly different, Tukey's honestly significant difference multiple comparison procedure, $P < 0.05$ ($n = 4$ logs per treatment).

larvae, prepupae and pupae that were dead increased from 7% in control logs to 35% after 1 week and 100% after 10 or 16 weeks of submergence (Table 1). It appeared that some larvae actually continued to develop in the submerged logs during the first 4 weeks of submergence because no live or dead pupae were found during dissection of unsubmerged control logs or logs submerged for 1 week; however, some live pupae were found in logs submerged for 4 weeks and dead pupae were found in logs submerged for 10 or 16 weeks (Table 1).

Water in the river was approximately 1.5 m deep with a flow rate of 0.88–1.05 m/s and mean temperature of 13.5 °C during the first 4 weeks of the study. The temperature increased to 21.1 °C, whereas flow rate and depth decreased to 0.33 m/s and 36 cm, respectively, over the next 12 weeks.

The spectral reflectance patterns for shaved and unshaved black ash splints were similar, regardless of the duration of submergence (data not shown). There were peaks in reflectance at approximately 850, 1100, 1300 and 1650 nm for ash splints submerged for each period of time. Although there was some variation in the mean reflectance for splints submerged for different lengths of time, the overall pattern of the reflectance curve was very similar and reflectance did not increase or decrease consistently with the duration of submergence. In general, the shaved side of the splints (rough wood fibres shaved off resulting in a brighter and smoother surface) had a slightly higher reflectance at low wavelengths between 550 and 700 nm. Pliability of splints did not decrease after 16 weeks of submergence and no cracking, splitting or decay of splints was observed.

2011 winter submergence study

Logs randomly assigned to different periods of submergence did not differ in initial attack density among treatments (mean ± SE = 39.2 ± 6.9 *A. planipennis* per cm^2; $F = 1.59$; d.f. = 2,16; $P = 0.2$). The density of live *A. planipennis* larvae and prepupae did not decrease after 10 weeks of logs submergence; however, there was a significant decrease after 18 weeks of submergence and all larvae and prepupae had died (Table 2). Few dead larvae were found during dissection and the density of dead larvae did not increase over time. However, the density of dead prepupae increased significantly between 10 and 18 weeks of log submergence (Table 2). No live or dead pupae were found during dissection. Overall, the percentage of dead *A. planipennis*

Table 1 Mean ± SE density (per m^2) of live and dead larvae, prepupae, and pupae or all immature *Agrilus planipennis* (EAB) life stages combined and percentage of all immature *A. planipennis* life stages that were dead found in logs that had been submerged for different periods of time in spring 2010 and then dissected ($n = 4$ logs per treatment)

	Within-tree density (per m^2)								
Duration submerged (weeks)	Live larvae	Live prepupae	Live pupae	Live EAB	Dead larvae	Dead prepupae	Dead pupae	Dead EAB	Percent dead (%)
0	28.6 ± 9.7a	15.5 ± 5.0^{a}	0 ± 0	44.2 ± 7.5^{a}	0.4 ± 0.4^{b}	0.4 ± 0.4^{b}	0 ± 0	0.8 ± 0.8^{b}	7.1 ± 7.1^{c}
1	29.2 ± 13.3^{a}	5.2 ± 2.3^{a}	0 ± 0	35.7 ± 14.5^{ab}	18.4 ± 9.4^{ab}	2.8 ± 1.2^{b}	0 ± 0	21.2 ± 9.9^{ab}	35.4 ± 12.4^{b}
4	1.4 ± 1.4^{a}	11.3 ± 4.7^{a}	2.8 ± 1.9	15.5 ± 7.4^{ab}	49.4 ± 22.5^{a}	0.6 ± 0.6^{b}	0 ± 0	49.9 ± 22.1^{a}	69.4 ± 15.9^{ab}
10	0 ± 0^{b}	0 ± 0^{b}	0 ± 0	0 ± 0^{b}	10.6 ± 5.4^{ab}	4.6 ± 0.4^{b}	0.7 ± 0.7	15.8 ± 5.5^{ab}	100 ± 0^{a}
16	0 ± 0^{b}	0 ± 0^{b}	0 ± 0	0 ± 0^{b}	20.5 ± 10.9^{ab}	12.2 ± 3.3^{a}	1.5 ± 0.9	34.1 ± 13.8^{a}	100 ± 0^{a}
F	3.10	2.85	1.98	5.87	3.04	10.17	2.43	3.09	10.8
d.f.	4,17	4,17	4,17	4,17	4,17	4,17	4,17	4,17	4,17
P	0.04	0.05	0.1	0.004	0.04	0.0002	0.08	0.004	0.0002

Means within a column followed by different superscript letters are significantly different; Tukey–Kramer multiple comparison procedure, $P < 0.05$.

Table 2 Mean ± SE density (per m^2) of live and dead larvae, prepupae or all immature *Agrilus planipennis* (EAB) life stages combined and percentage of all immature *A. planipennislife* stages that were dead found in logs that had been submerged for different periods of time in winter 2011 and then dissected ($n = 4$ logs per treatment)

	Within-tree density (per m^2)						
Duration submerged (weeks)	Live larvae	Live prepupae	Live EAB	Dead larvae	Dead prepupae	Dead EAB	Percent dead (%)
0	26.8 ± 9.7^{a}	24.4 ± 12.3	51.2 ± 16.3^{a}	3.4 ± 3.4^{a}	1.6 ± 1.6^{ab}	4.9 ± 3.3^{a}	5.2 ± 3.5^{b}
10	33.7 ± 6.4^{a}	11.8 ± 3.9	45.5 ± 6.4^{a}	1.4 ± 0.7^{ab}	0.4 ± 0.4^{b}	1.7 ± 0.9^{a}	3.0 ± 1.6^{b}
18	0 ± 0^{b}	0 ± 0	0 ± 0^{b}	0.5 ± 0.2^{b}	13.8 ± 6.6^{a}	14.3 ± 6.5^{a}	100 ± 0^{a}
F	7.85	3.31	9.95	7.24	3.96	2.90	21.8
d.f.	2,16	2,16	2,16	2,16	2,16	2,16	2,16
P	0.04	0.06	0.002	0.04	0.04	0.08	< 0.0001

Means within a column followed by different superscript letters are significantly different; Tukey–Kramer multiple comparison procedure, $P < 0.05$.

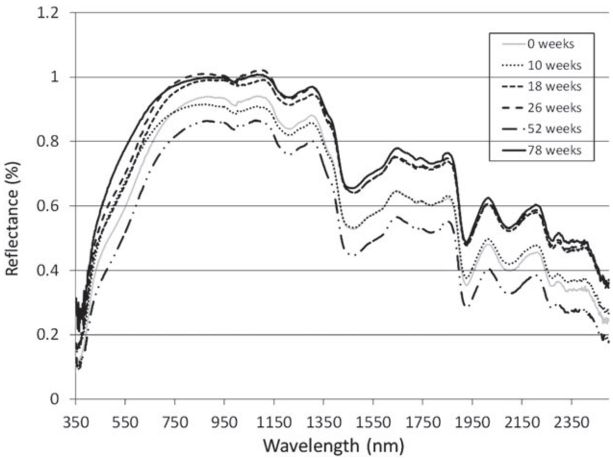

Figure 2 Average reflectance spectra of shaved black ash splints separated and peeled from logs that had been submerged for different periods of time in winter 2011. Means are based on one or two splints sampled per log and four logs from each submergence period ($n = 4–8$).

larvae and prepupae increased from 5% in unsubmerged logs to 100% in logs submerged for 18 weeks (Table 2). The bark and 1 or 2 outer rings of sapwood had begun to decay on logs submerged for 26, 52, or 78 weeks. Most of the bark had fallen off the logs and large pieces of decaying bark fell off the logs when they were removed from the river. Therefore, we were unable to dissect the logs to recover dead larvae, prepupae and pupae and, undoubtedly, they had all died.

Water in the river was 2 m deep and 0 °C with a surface flow rate of 0.54 m/s during the first week of March when logs were submerged. After 4 weeks, the water temperature remained at freezing (0 °C) and was 1.8 m deep with a flow rate of 0.57 m/s. Water temperature increased to 5.5 °C by 10 weeks, 14.4 °C by 14 weeks, 15.5 °C by 18 weeks, 21.1 °C by 22 weeks and 25.5 °C by 26 weeks of submergence and then gradually decreased back to freezing by 52 weeks of submergence. Water levels peaked in June 2011, 14 weeks after submergence, at 2.5 m deep and a flow rate of 1.2 m/s. The river was at its lowest at the end of July, 22 weeks after submergence, and was 25 cm deep with a flow rate of 0.16 m/s.

The inner rings of sapwood were sound and we pounded and peeled splints from the logs submerged for 26, 52 and 78 weeks in the same manner as the logs dissected after 0, 10 or 18 weeks of submergence. The spectral reflectance pattern was similar for splints peeled from logs, regardless of the duration of submergence between 0 and 78 weeks (Fig. 2). Similar to the splints from logs submerged for up to 16 weeks in 2010, there were peaks in reflectance at approximately 850, 1100, 1300 and 1650 nm for ash splints submerged for each period of time. Although there was some variation in the mean reflectance for splints submerged for different lengths of time, the overall pattern of the reflectance curve was very similar and reflectance did not increase or decrease consistently with the duration of submergence. In general, the shaved side of the splints (Fig. 2) had a slightly higher reflectance at low wavelengths between 550 and 700 nm compared with the unshaved side of the splints (data not shown). Although the outer 1–3 rings of sapwood began to decay on logs submerged for 26 weeks or longer, the colour and pliability of splints from the inner rings of sapwood did not decrease even after 78 weeks of submergence and no cracking, splitting or decay of splints was observed. Logs submerged for 26 weeks or longer also had a mild pungent odour that completely dissipated when the bark and outer rings of sapwood were removed and the inner rings were exposed to air for a few hours.

2011 spring submergence study

Logs randomly assigned to different periods of submergence did not differ in initial attack density among treatments (mean ± SE = 33.2 ± 5.3 *A. planipennis* per cm^2; $F = 1.95$; d.f. = 7,23; $P = 0.1$). The density of live *A. planipennis* larvae, prepupae and pupae tended to decrease with the duration of submergence from 0 to 13 weeks for logs submerged in spring 2011 and there were no live *A. planipennis* of any life stage in logs submerged for 13 weeks, although differences among submergence periods were not significant (Table 3). The density of dead *A. planipennis* larvae and prepupae was somewhat variable. However, the percentage of dead *A. planipennis* increased from 5% in unsubmerged control logs to 26% after 1 week and 100% after 13 weeks of submergence (Table 3). No live or dead pupae were found during dissection.

Similarly, some live adults emerged from logs that had been submerged for up to 6 weeks. No logs were placed in rearing tubes between 7 and 13 weeks after submergence. No adults emerged from logs submerged for 14 weeks (Fig. 1B).

Table 3 Mean ± SE density (per m^2) of live and dead larvae, prepupae, or all *Agrilus planipennis* (EAB) life stages combined and percentage of all immature *A. planipennis* life stages that were dead found in logs that had been submerged for different periods of time in spring 2011 and then dissected ($n = 4$ logs per treatment)

	Within-tree density (per m^2)						
Duration submerged (weeks)	Live larvae	Live prepupae	Live EAB	Dead larvae	Dead prepupae	Dead EAB	Percent dead (%)
0	26.8 ± 9.7	24.4 ± 12.3	51.2 ± 16.3	3.4 ± 3.4	1.6 ± 1.6	4.9 ± 3.3	5.1 ± 3.5^{b}
4	13.7 ± 6.8	18.5 ± 3.4	32.2 ± 3.6	11.2 ± 4.3	3.2 ± 1.9	14.4 ± 5.9	26.5 ± 1.0^{ab}
5	7.4 ± 4.6	16.1 ± 7.2	23.4 ± 11.1	1.2 ± 1.2	8.4 ± 3.0	9.6 ± 3.8	33.6 ± 8.7^{ab}
6	9.5 ± 9.5	15.2 ± 6.8	24.6 ± 7.9	4.9 ± 3.6	4.4 ± 1.7	9.3 ± 4.5	29.8 ± 8.6^{ab}
7	6.9 ± 4.1	8.1 ± 3.1	14.9 ± 3.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0^{b}
8	8.7 ± 5.9	20.6 ± 7.4	29.3 ± 10.8	14.1 ± 7.5	4.0 ± 1.8	18.2 ± 6.9	40.1 ± 4.4^{ab}
9	0.9 ± 0.9	5.8 ± 5.8	6.8 ± 6.8	2.2 ± 1.2	0 ± 0	2.2 ± 1.2	66.7 ± 33.3^{a}
13	0 ± 0	0 ± 0	0 ± 0^{c}	3.9 ± 3.9	0 ± 0	3.9 ± 3.9	100 ± 0^{a}
F	1.53	1.57	2.23	1.68	1.61	2.05	3.25
d.f.	7,23	7,23	7,23	7,23	7,23	7,23	7,23
P	0.2	0.2	0.06	0.1	0.2	0.09	0.01

Means within a column followed by different superscript letters are significantly different; Tukey–Kramer multiple comparison procedure, $P < 0.05$.

Discussion

Prolonged submersion of black ash logs was effective at killing all *A. planipennis* larvae and prepupae; however, the duration of submersion required for complete mortality varied between experiments and likely depended on the time of year, water temperature and other factors. Although all *A. planipennis* larvae had died and no adults emerged from logs submerged for 10 weeks in spring 2010, some larvae survived and adults emerged from logs submerged during winter for 10 weeks and during spring for up to 9 weeks, and complete mortality was not found until 13 weeks of submergence in spring or 18 weeks of submergence in winter 2011. It is possible that greater larval survival was found in 2011 because larvae were observed over a 48-h period after dissection rather than only 24 h as in 2010, allowing them more time to recover and move after removal from the submerged logs. Johnson (1967) found that recovery time of Douglas-fir beetles *Dendroctonus pseudostugae* Hopkins (Coleoptera: Curculionidae: Scolytinae) was related to time submerged and it took almost 8 h for beetles to recover after 1024 h (6 weeks) of submergence. Different temperature conditions during the different experiments may also have influenced *A. planipennis* survival. In spring 2010, logs were submerged when the water temperature was 13.5 °C and it increased to 21.2 °C within 12 weeks, whereas, in winter 2011, logs were submerged when water temperatures remained near 0 °C for 4 weeks and, in spring 2011, the water temperature was only 5.5 °C when logs were submerged but warmed to 21.1 °C within 12 weeks. Similarly, Johnson (1967) found that *D. pseudostugae* adults were able to survive immersion longer at colder temperatures and were able to withstand up to 1000 h (5.9 weeks) at 4.5 °C but only 256 h (1.5 weeks) at room temperature. Larvae and prepupae in logs submerged in winter 2011 were cold-acclimated and dormant. Maintaining dormancy for the first 4 weeks when the water temperatures remained near freezing may have prolonged the survival of *A. planipennis* in logs submerged in winter 2011. On the other hand, *A. planipennis* in logs submerged in spring 2010 and 2011 were likely breaking diapause and may have had higher metabolic activity and appeared to be actively feeding and developing for the first several weeks in submerged logs. Logs cut and submerged earlier in winter would experience longer periods of cold water temperatures during which overwintering prepupal larvae remain dormant; therefore, they may require longer periods of submersion for complete mortality. By contrast, logs cut and submerged in spring or summer when larvae are actively feeding may require less time for complete mortality. Siegert *et al*. (2014) found that *A. planipennis* larvae feeding in the phloem when logs were harvested experienced higher mortality after shorter periods of submersion than those in prepupal chambers.

Insects exhibit a remarkable suite of adaptations that allow them to survive extreme conditions such as hypoxia and immersion for periods of time (Hoback & Stanley, 2001). Adaptations include switching from aerobic to anaerobic metabolic pathways, reducing basal metabolism and entering a quiescent state, altered behaviours and enlarged tracheal system volumes (Hoback *et al*., 1998; Hoback & Stanley, 2001). In the present study, some *A. planipennis* larvae and prepupae survived and were able to complete development and emerge from black ash logs submerged in a river for several weeks. Other terrestrial insects, including several species of tiger beetles (Coleoptera: Cincindelidae; Hoback & Stanley, 2001; Brust *et al*., 2005) and subterranean termites (Isoptera: Rhinotermitidae; Forschler & Henderson, 1995), have been found to be able to survive several hours or days of submersion when individuals were immersed directly in water. Enclosure within a substrate may provide some protection from direct contact with water and allows prolonged survival. Termites immersed in soil arenas survived longer than termites that were immersed directly in water as a result of air pockets that frequently surrounded termites in the soil and provided reservoirs of oxygen (Forschler & Henderson, 1995). Siegert *et al*. (2014) found that moisture content of the outer 1.3 cm of ash sapwood doubled from 42% before submergence to 81% at 12 weeks and 83% at 24 weeks after submergence. In addition to preventing complete saturation, the structure of phloem, comprising fibres and sieve tubes that have a rapid sealing mechanism in response to stress or wounding (Cronshaw, 1981), may provide some protection from direct immersion and total saturation.

Soaking cut logs by sprinkling with water has been used to reduce bark beetle and ambrosia beetle (Scolytinae) infestation and brood survival. The film of water over the surface of the log forms a physical barrier to beetle attack and reduces the emission of ethanol that attracts bark and ambrosia beetles (Wylie *et al*., 1999). In addition, brood survival is reduced for various bark beetles when the phloem moisture level is elevated (Beanlands, 1967; Webb & Franklin, 1978) and serves as the basis for the recommendation of sprinkling water on cut logs to reduce bark beetle infestation (McMullen & Betts, 1982; Syme & Saucier, 1995).

The overall pattern of spectral reflectance of splints did not change significantly with the duration of submergence and remained within the ranges desired by basketmakers. Reflectance was higher at the lower wavelengths on the shaved side of splints compared with the unshaved side with rough fibres. It is traditional practice to shave rough fibres from the surface of splints, resulting in a smoother, brighter surface. Even after 78 weeks of submergence, splints form all but the 1–3 outermost rings of sapwood exhibited a bright, light colour suitable for artistic baskets. The splints from inner rings also remained pliable after prolonged submergence, with no visible splits, crumbling or decay, although the outer few rings began to decay after 26 weeks of submergence. Similarly, Zare-Maivan and Shearer (1988) found that blocks of ash sapwood submerged in freshwater for 26 weeks experienced less fungal decay than blocks of ash bark, and blocks of ash decayed more slowly than blocks of cottonwood. In addition, logs that escaped rafts during transportation to sawmills in the late 19th and early 20th Centuries and remained submerged under lakes and rivers for many decades still retain quality wood properties and are very valuable (Tenenbaum, 2004). Although the colour may change slightly, the wood remains almost the same as recently cut timber. This may be because some sugars leach out of submerged logs, effectively seasoning the wood and preserving the quality that makes it highly desirable for musical instruments (Tenenbaum, 2004).

Based on our 2 years of data, submergence of infested black ash logs during winter or spring and holding underwater for 13 weeks after water temperatures have warmed above 10–13 °C should ensure complete mortality of *A. planipennis* larvae and no emergence of adults. These submergence times and conditions preserve wood properties for basketmaking and are compatible with traditional cultural practices.

Conclusions

With the spread of *A. planipennis*, we risk losing the North American ash resource. Such a loss has profound potential implications for American Indian and First Nations communities. Black ash basketmaking is a key cultural practice that links living members of families with each other, as well as their ancestors and their environment. The act of choosing and harvesting black ash trees, as well as processing their wood and making baskets, brings together multiple generations who are able to share time and exchange knowledge. In the immediate term, loss of access to black ash compromises the livelihoods of artisans and their communities. So too does the potential to hasten the spread of *A. planipennis* if infested logs are transported to tribal lands and other sites where the insect has not yet arrived. Ultimately, loss of black ash for basketmaking would mean the loss of a suite of important practices for preserving indigenous culture and identity.

The research described in the present study combines the traditional cultural knowledge of members of the Match-e-be-nash-she-wish Band of Potawatomi Indians of Michigan (Gun Lake) with entomological science to explore a potential response to *A. planipennis* infestation. Our findings demonstrate that the traditional practice of submerging black ash logs can achieve the immediate goals of providing wood for basketmaking at the same time as killing *A. planipennis* before it emerges and spreads. However, the length of time required to completely eliminate *A. planipennis* at all life stages appears to vary as a function of time of year of log harvest, water temperature and length of submergence.

Our results may be useful to black ash harvesters and basketmakers with respect to establishing guidelines that help to reduce the inadvertent spread of *A. planipennis*, with all the threats that entails for their lives and livelihoods. The process and results described in the present study also may help inform *A. planipennis* response planning at local to state and regional levels. It demonstrates that a well-designed programme, especially when undertaken in collaboration with tribes and basketmakers, offers the opportunity to reduce vectors of spread at the same time as supporting a key cultural practice over the short term and, in parallel, the exploration and development of long-term integrated management strategies, including seed preservation, biological control and host resistance, to preserve ash as a component of the landscape for future generations.

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