THE INFLUENCE OF RED-BACKED SALAMANDERS (*PLETHODON CINEREUS*) ON NUTRIENT CYCLING IN APPALACHIAN HARDWOOD FORESTS

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Abstract—The use of amphibians as biological indicators of ecosystem health has received considerable attention because of the increasing importance placed upon maintaining biodiversity in forested ecosystems. In this study, we imposed three different eastern red-backed salamander (*Plethodon cinereus*) treatments: 1) low (n = 4; added 0 salamanders to each mesocosm), 2) medium (n = 4; added 3 salamanders each mesocosm) and 3) high (n = 4; added 6 salamanders to each mesocosm), into 3-m^2 *in situ* field enclosures to monitor the potential effects of salamander abundance on the availability of nitrogen (N). Cationic and anionic exchange membranes were placed horizontally under the forest floor and vertically within the A-horizon to index N-availability through time. There was significantly more nitrate ($p < 0.001$) under the forest floor in the low and medium salamander density treatments than the high density treatments. No consistent treatment by time interaction trends were observed for any measures of inorganic N. At this juncture of the experiment, we would reject the research hypothesis that nutrient availability increases as salamander abundance increases.

INTRODUCTION

The role of amphibians in aquatic and terrestrial ecosystems has been the subject of many ecological studies. Of all vertebrate species, amphibians may be the best biological indicators of ecosystem health because of their sensitivity to environmental change (Vitt and others 1990). Although numerous studies have attempted to determine causes of declines of amphibian populations (Alford and Richards 1999), fewer studies have examined the potential implications to ecosystem processes associated with amphibian declines.

The potential top-down trophic effects of amphibian species on leaf litter decomposition, invertebrate communities and nutrient cycling dynamics has been the central theme of many studies (Wyman 1998, Beard and others 2002, Beard and others 2003, Walton 2005, Walton and Steckler 2005, Walton and others 2006). In general, it is considered that small vertebrate predators such as salamanders constitute too little biomass and therefore their impact on nutrient cycling and leaf litter decomposition in terrestrial ecosystems would be minimal at the ecosystem scale (Schlesinger 1997). However, in Appalachian forests, salamanders do constitute a significant portion of the vertebrate biomass (Burton and Likens 1975a, 1975b). In addition, several studies suggest that certain frog and salamander species do regulate certain nutrient cycling processes and impart top-down effects on detrital food webs (Wyman 1998 Beard and others 2002, Beard and others 2003, Walton 2005, Walton and Steckler 2005, Walton and others 2006). Furthermore, biomass alone is not a sufficient indicator of the ecological importance of these vertebrate predators because it ignores waste production and population turnover, which may be important fluxes of nutrients (Beard and others 2002).

The forests of the Appalachian Mountains contain more salamander species than any other temperate region in the world. Salamander species in this region are also the most dominant vertebrate predators (Hairston 1987), suggesting they undoubtedly affect certain trophic food webs. Terrestrial salamanders of the family Plethodontidae are the most abundant forest vertebrate fauna in Appalachia, and red-backed salamanders (*Plethodon cinereus)* are common throughout the eastern United States (Hairston 1949, Burton and Likens 1975a, Burton and Likens 1975b, Hairston 1987). Densities of the eastern red-backed salamander reportedly vary across their range, from 4.0 individuals/m^2 in Virginia (Jaeger 1980), to 0.9/m^2 in Michigan hardwoods (Heatwole 1962), to 0.5/m^2 in hardwood forests of south-central New York (Wyman 1988), and 0.3/m^2 in the Hubbard Brook Experimental Forest of New Hampshire (Burton and Likens 1975b). Due to the extensive geographic range of the red-backed salamander, many *in situ* and laboratory based experiments have used this species to determine and enhance our understanding of the ecological importance of salamanders (Test and Bingham 1948, Hairston 1949, Taub 1961, Heatwole 1962, Burton and Likens 1975a, Burton and Likens 1975b, Jaeger 1980, Hairston 1987, Petranka and others 1993, Wyman 1998, Rooney and others 2000, Welsh and Droege 2000, Davic and Welsh 2004, Morneault and others 2004, Walton 2005, Walton and Steckler 2005, Walton and others 2006). The focus of these studies has ranged from the effects of eastern red-backed salamanders on invertebrate communities, leaf litter decomposition, and nutrient cycling. The sensitivity of plethodontid salamanders to changes in the soil microclimate make them ideal candidates for studying direct and indirect effects on soil processes (Welsh and Droege 2000).

Reported top-down effects of salamanders and other amphibians on decomposition processes and nutrient cycling have varied depending on experimental methodologies, leaf litter substrate, and geographic location. Several studies have hypothesized that salamanders may indirectly affect leaf litter decomposition processes by regulating detritivore prey (Burton and Likens 1975a, Hairston 1987, Davic and Welsh 2004). Wyman (1998) demonstrated with field enclosures containing red-backed salamanders that leaf litter decomposition rates were significantly lower in field enclosures with high densities of salamanders

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compared to enclosures with no salamanders in a forest primarily dominated by American beech (*Fagus grandifolia*). Wyman (1998) attributed the slower decomposition rates to the increased consumption of macrofaunal detritivores responsible for fragmenting leaf litter. In contrast, others have hypothesized that control of invertebrate populations by salamanders would cause decomposition rates to increase because of a reduction in bacterial and fungal consumers, thus releasing microbes (Hairston 1987). Beard (2002, 2003) found that the terrestrial frog, *Eleutherodactylus coqui* (coquì), enhanced nutrient cycling processes and increased leaf litter decomposition rates in a Puerto Rican subtropical wet forest. Beard (2002) concluded that coquìs enhance nutrient cycling processes and accelerate leaf litter decomposition by converting recalcitrant arthropod tissue into more decomposable frog feces and other waste products. Finally, Walton and Steckler (2005) reported no salamander-mediated effects on leaf litter decomposition rates in a laboratory microcosm experiment.

Variation in the top-down effects of amphibians on invertebrate communities, leaf litter decomposition and nutrient cycling warrants additional research that focuses on one or more of the aforementioned ecological processes. Therefore, the primary objective of our research experiment was to determine if variations of eastern red-backed salamander abundance affects nitrogen (N) availability directly under the forest floor and within the A-horizon. Our research hypothesis is that as salamander density increases, N availability will increase.

MATERIALS AND METHODS

Site Description

This study was installed on the Jefferson National Forest approximately 8 km west of Blacksburg, VA in Montgomery County (37°17’38” N, 80°27’27” W). The elevation is approximately 670 m and the average annual precipitation is approximately 101.5 cm. Average annual temperature is 10.8 °C, ranging from 0.3 °C in the winter to 20.7 °C in the summer. The forest cover type is predominately white oak (*Quercus alba* L.) and scarlet oak (*Quercus coccinea* Muenchh.). The study area is in the Ridge and Valley physiographic region, which is comprised of shale, sandstone and limestone sedimentary rocks. Many forest soils in this physiographic region are derived from a combination of sandstone and/or shale in residuum or colluvial parent materials. The soil at the study site is classified as a Clymer soil series (coarse-loamy, siliceous, active, mesic typic Hapludults). The majority of forests in the southern Appalachian Mountains are second growth forests approximately 80 to 100 years in age because of extensive clearcutting and high-grading practices during the late 19th and early 20th centuries.

Experimental Design

Field enclosures (mesocosms) were established randomly using a 5 by 5 grid containing 20 by 20-m cells. Twelve cells were randomly selected for mesocosm locations and treatments were assigned randomly. There were three treatments consisting of red-backed salamanders: 1) low (n = 4; added 0 salamanders to each mesocosm), 2) medium (n = 4; added 3 salamanders each mesocosm) and 3) high (n = 4; added 6 salamanders to each mesocosm). The primary purpose of adding salamanders to the mesocosms was to ‘press’ the system so that we could examine how above average increases in salamander abundance affect certain ecosystem processes. Each mesocosm was 1.2 by 1.5 m (3 m^2) in size and were 0.25 m tall. Vinyl flashing was buried along the perimeter of each mesocosm to 40 cm. In addition, vinyl flashing was also attached perpendicular to the top of each mesocosm so that approximately 16.5 cm of flashing overhung both within and outside the mesocosms. The purpose of the vinyl flashing was to restrict salamanders from migrating in and out of the mesocosms. Three 30 by 20 by 5 cm rough-cut yellow-poplar (*Liriodendron tulipfera* L.) artificial cover objects (i.e. cover boards) were placed in each mesocosm to monitor salamanders. Cover boards were sampled at least monthly to recapture salamanders.

Following the construction of the mesocosms and placement of the cover boards but before the addition of salamanders, there was a two week acclimation period. During this adjustment period, cover boards were periodically checked and all salamanders found underneath were removed from the mesocosms. We captured 36 adult salamanders outside of the mesocosms and marked them using visible implant fluorescent elastomer (VIE) (Northwest Marine Technology Inc., Shaw Island, Washington, United States). Unique combinations of red, orange and yellow VIE were injected on the ventral side of each salamander. Two injections located posterior to the front legs and two more located anterior to the hind legs were administered. Each color combination was recorded so that we could monitor activity of individual salamanders and identify any salamanders that we did not add (i.e. salamanders with no VIE marks). We removed any captured unmarked salamanders from the mesocosms. All unmarked salamanders captured within the mesocosms were assumed to be within the enclosures at study establishment, but because salamanders remain below the soil surface for extended periods, some were likely unaccounted for during the acclimation period.

Indexing Nutrient Availability

Cation and anion exchange membranes (Ionics Inc., Watertown, MA, United States) were used to index the amount of available ammonium (NH_4^+) and nitrate (NO_3^-) within each mesocosm. Each membrane type (anion and cation) had a surface area of 6.45 cm^2 and was placed directly underneath the forest floor in a horizontal position and within the A-horizon in a vertical position, resulting in a total of 12 membranes for each mesocosm; three anion membranes and three cation membranes for each orientation (horizontal and vertical). Cation and anion membranes were extracted monthly and were replaced with a newly charged set once extracted. To minimize the disturbance to the mesocosms, each enclosure was divided into 189, 25.8-cm^2 cells (i.e. 21 rows consisting of 9 cells per row). Each month, 12 cells were randomly selected for each replacement batch of freshly charged membranes. After a cell was used, it was then disregarded from the randomization procedure and could no longer be selected until all other cells had been used (i.e. each mesocosm contains enough cells for

15 months). All cells that contained a cover board were not used because it could potentially interfere with the monthly salamander sampling since salamanders are sensitive to habitat disturbance. Extracted membranes were placed in individual centrifuge tubes containing 25 ml of 1M KCl. Tubes were shaken for 1 hour and subsequently poured through Whatman #2 filter papers (Whatman International Ltd.). Solutions were analyzed using a Bran and Leubbe TRACCS 2000 Auto-Analyzer (SPX Corporation). Values were converted from parts per million to mg of NO_3^- or NH_4^+ per m^2 of membrane per day (i.e. mg/m/d). In essence, the reported values are an index that represents the average amount of inorganic N available for plant uptake on a daily basis.

Statistical Analysis

One-way repeated measures analysis of variance (SAS 9.0 Institute Inc., Cary, NC) was used to detect potential differences in nutrient availability between the three salamander density treatments through time ($\alpha = 0.05$). There were five extraction periods from July 2006 to December 2006 included in the analysis. Each extraction period was examined individually to analyze the data for treatment by time interactions. When significant differences were detected, we used the Tukey-Kramer differences of least square means test to examine how treatments differed. Three variance-covariance structures (compound symmetry, unstructured and autoregressive) were examined to determine the best model for the data. The compound symmetry variance-covariance model was used for the nutrient data because it had the lowest overall fit statistics, which is the primary determinant for choosing the best model. The selected variance-covariance model was used independently for each response variable: 1) mg-NO_3^-/m^2/d for the vertical and horizontal anion membranes and 2) mg-NH_4^+ /m^2/d for the vertical and horizontal cation membranes.

RESULTS

Effects of Salamander Treatments

The only significant treatment effect across the entire sampling period from July 2006 to December 2006 for available nitrogen was for NO_3^- directly under the forest floor (i.e. horizontally oriented anion membranes; $p < 0.001$; table 1; fig. 1). The low and medium salamander density treatments had significantly more available NO_3^- than the high salamander treatment. Overall nutrient availability values within the horizontally oriented anion membranes also had lower variability than the other membrane orientations. Available NO_3^- within the A-horizon (table 1; fig. 2) and available NH_4^+ under the forest floor (table 1; fig. 3) and within the A-horizon (table 1; fig. 4) were more variable within treatments, resulting in relatively higher p-values.

Effects of Salamander Treatment by Time

Significant treatment by time interactions occurred for each membrane type and orientation across the entire sampling period (table 1). However, because of the disorderly nature of the data (figs. 1-4), treatment by time interactions by each sampling period was examined to discern any potential trends. No significant treatment by time interactions were observed for the July 2006 and August 2006 sampling periods (table 2). Significant time by treatment interactions were observed for available NO_3^- during the September, November and December 2006 sampling periods for the anionic membranes. However, specific treatment differences for the low-, medium-, and high-density treatments varied tremendously for each of the three significant treatment by time interactions (figs. 1 and 2). No specific trends could be discerned except for the steady increase in available NO_3^- from September through December for the low salamander density treatment (figs. 1 and 2). In regards to available NH_4^+ for the cationic membranes, specific treatment by time interactions occurred during September 2006 (fig. 3) for the horizontally orientated cation membranes and during November 2006 (fig. 4) for the vertically oriented cation membranes.

DISCUSSION

Analysis of the effects of salamanders on nutrient cycling of inorganic N, indicates that as salamander abundance increases that there is a decrease in NO_3^- availability directly under the

Table 1—Salamander density treatment results from repeated-measures Analysis of Variance

	p-values	
Response Variable	treatment	treatment x time
Horizontal Anion Membrane	<0.001	<0.001
Vertical Anion Membrane	0.3981	<0.001
Vertical Cation Membrane	0.1283	0.025
Horizontal Cation Membrane	0.7362	0.006

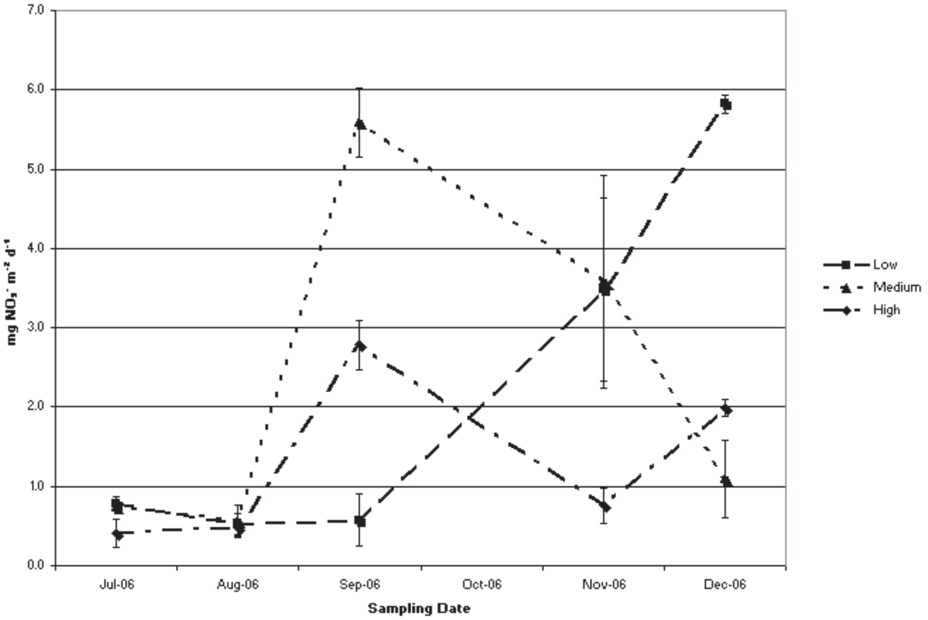

Figure 1—Horizontal anion exchange membrane nutrient index values by sampling date, showing patterns in field enclosures with varying densities of salamanders, Jefferson National Forest, Montgomery County, VA.

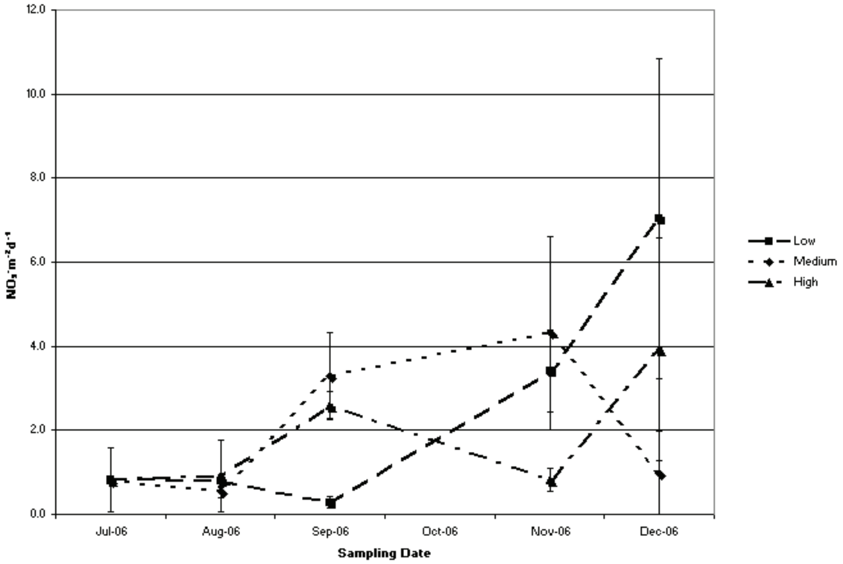

Figure 2—Vertical anion exchange membrane nutrient index values by sampling date, showing patterns in field enclosures with varying densities of salamanders, Jefferson National Forest, Montgomery County, VA.

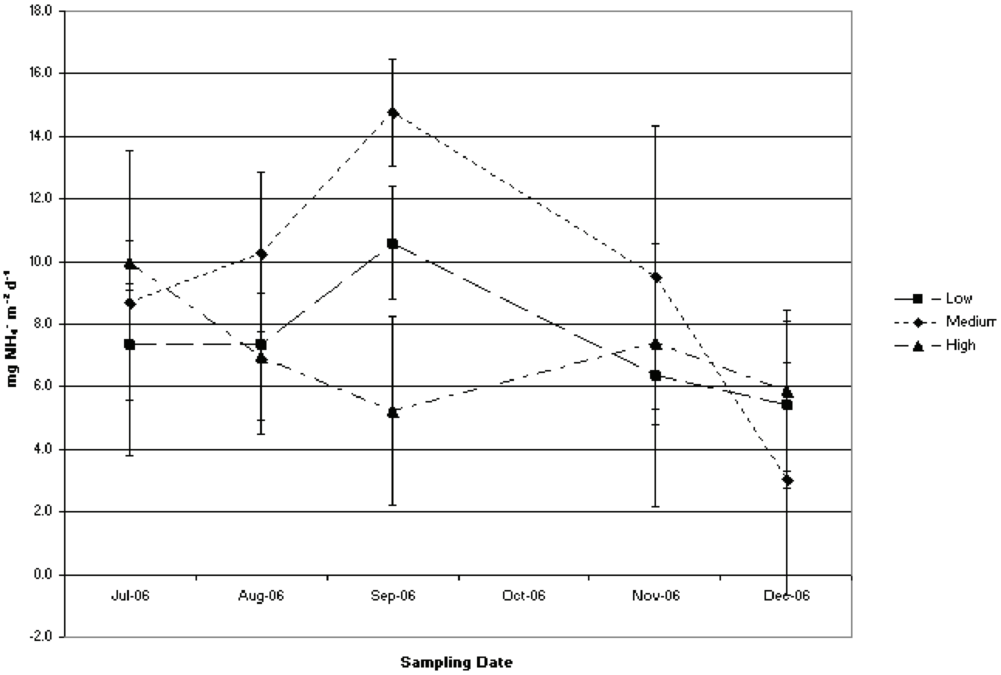

Figure 3—Horizontal cation exchange membrane nutrient index values by sampling date, showing patterns in field enclosures with varying densities of salamanders, Jefferson National Forest, Montgomery County, VA.

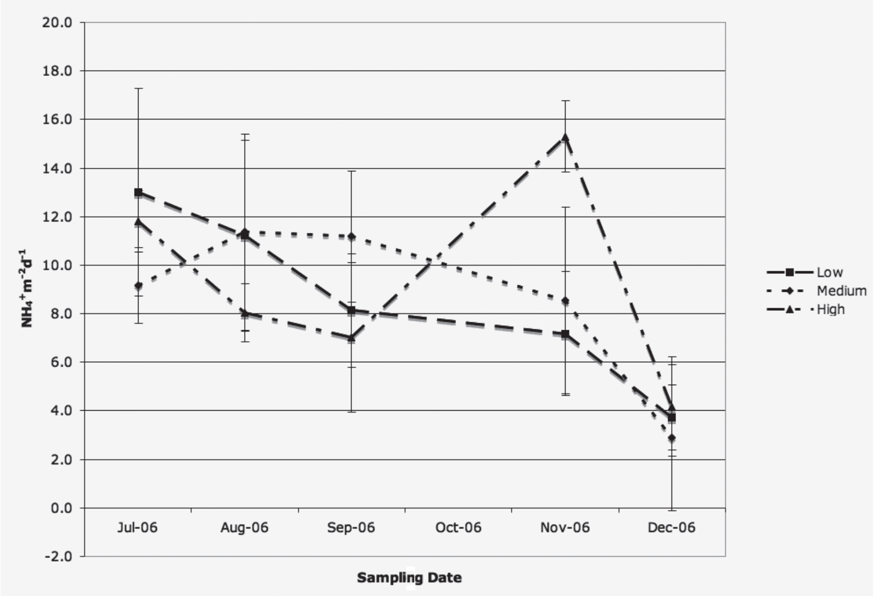

Figure 4—Vertical cation exchange membrane nutrient index values by sampling date, showing patterns in field enclosures with varying densities of salamanders, Jefferson National Forest, Montgomery County, VA.

forest floor (table 1; fig. 1). Forested ecosystems typically adsorb more NH_4^+ than NO_3^- because there are usually higher quantities of NH_4^+ compared to NO_3^- . However, because there is a net cation exchange capacity in Appalachian hardwood forests, NO_3^- is extremely mobile and often leaches through the soil profile. During this process, exchangeable cations such as magnesium, calcium and potassium often bind to the NO_3^- ion as it leaves the system. Therefore, these results potentially suggest that higher salamander abundance could increase base cation retention in these ecosystems.

Some trends have begun to develop as the lower salamander density treatments have steadily increased over the medium and high density treatments from September to December 2006 for available NO_3^- indexed by the anionic membranes (figs. 1 and 2), whereas no specific trends have developed for available NH_4^+ in the forest floor and A-horizon (ts. 3 and 4). However, the early results (5 months) of this relatively long-term experiment (18 months) are not conclusive because of the large amount of variability of NO_3^- and NH_4^+ within the three salamander treatments. In addition, measurements during the spring and summer of 2007 may reveal more consistent treatment effects opposed to the disorderly nature of the data collected and analyzed thus far.

The first two sampling periods (July and August 2006) showed no significant treatment by time interaction for any measure of nutrient availability (table 2). There are two potential reasons for this trend: 1) July and August in the Ridge and Valley Physiographic region in Virginia are exceptionally dry and this area received considerably less than average rainfall during the summer of 2006 or 2) a longer acclimation period may have been required following the addition of the salamander density treatments before measurements began. During the spring months, there is an increase in microbial and insect activity and populations as temperatures rise and as rainfall increases. Spring 2007 nutrient data may indicate higher amounts of available NO_3^- and NH_4^+ as increased salamander pressure could cause significant top-down trophic effects on nutrient availability, invertebrate communities and/or leaf litter decomposition.

At this juncture of the experiment, we reject our research hypothesis that increased salamander densities increase nutrient availability of N. Leaf litter decomposition rates and measures of invertebrate populations and diversity may alter these results once the data are analyzed. There have been conflicting results with some studies involving plethodontid salamanders in regards to the effects these amphibians have on leaf litter decomposition rates, nutrient availability and invertebrate communities. Whether plethodontid salamanders decrease (Wyman 1998) or increase decomposition rates (Hairston 1987) of leaf litter and/or increase the amount of microbial detritivores (Rooney and others 2000) is yet to be fully assessed in this experiment. Concurrent research is examining the effects of red-backed salamanders on invertebrate communities and rates of litter decomposition within these mesocosms.

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Table 2—Salamander density treatment by time interaction results from repeated measures Analysis of Variance

	Treatment x time p-values			
Extraction period	Horizontal anion membrane	Vertical anion membrane	Horizontal cation membrane	Vertical cation membrane
July 2006	0.537	0.999	0.470	0.155
August 2006	0.975	0.931	0.245	0.183
September 2006	<0.001*	0.017*	<0.001*	0.113
November 2006	<0.001*	0.006*	0.323	<0.001*
December 2006	<0.001*	<0.001*	0.370	0.807

* significant differences detected at α <0.05

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