A REVIEW OF BEST MANAGEMENT PRACTICES AND THE MITIGATION OF STREAM-BREEDING SALAMANDERS IN THE EASTERN DECIDUOUS FOREST

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Abstract.—Timber harvest has been implicated as a causative factor in the decline of amphibian populations and diversity in many areas of the world. The adoption of best management practices (BMPs) is intended to minimize the impacts of timber harvest on the biotic community, including amphibians and their habitat requirements. Herein, we synthesize the current scientific literature on the impact and effectiveness of BMPs in preventing population declines of stream-breeding salamanders and associated habitat loss in the eastern deciduous forests of North America. We frequently found sedimentation to be a suspected cause of population declines; many studies also described a correlation between basal area and salamander abundances. In addition to summarizing research, we offer recommendations to improve the efficacy and implementation of BMPs in the central and eastern United States. We also make suggestions for further research, such as increased testing of BMP methods and an increase in long-term studies.

INTRODUCTION

In the past half century, numerous amphibian species have been experiencing significant declines, a worldwide phenomenon that has been interpreted as indicative of a global decline in ecosystem function (Blaustein and Bancroft 2007, Houlahan et al. 2000). Chief among the primary threats to amphibian populations is habitat loss and degradation, especially among species associated with freshwater wetlands or riparian areas (Lannoo 2005, Semlitsch 2000). Timber harvest has been implicated in the degradation of amphibian habitat, including freshwater habitats (Corn and Bury 1989, Vesely and McComb 2002).

Over the past few decades, a greater awareness of the negative effects of timber harvests has resulted in numerous efforts to improve regulations pertaining to logging. Usually termed best management practices (BMPs), these regulations have been repeatedly revised to incorporate new areas of concern such as freshwater fisheries protection or wildlife habitat, but also to reform and target specific practices that have the most demonstrated negative ecosystem impacts.

Streamside management zones (SMZs) are an example of a BMP strategy to help maintain the integrity of freshwater riparian and headwater stream habitats, which are generally more susceptible to damage from timber harvest than terrestrial ones (Chizinski et al. 2010). Although originally designed to protect water quality, the goal of many of these regulations has been expanded to incorporate both abiotic and biotic ecosystem components. These management zones rely on use of riparian buffer strips or stringers to reduce sedimentation into streams, help reduce postharvest increases in stream temperature and light intensity, and provide both core riparian habitat and

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corridors for forest-dwelling species. Consequently, SMZs have become one of the most important components of BMPs used to prevent or reduce expected post-timber harvest declines of aquatic biota, including stream salamanders, defined as those species that require freshwater streams for part of their life cycle (Maigret 2013).

Of all the biota present in headwater streams, stream salamanders can be considered among the most important for ecosystem function. In many deciduous forests they are the most abundant vertebrates, with a biomass equal to or exceeding that of other vertebrate taxa including birds and mammals (Burton and Likens 1975, Petranka and Murray 2001). Stream salamanders also play important roles in nutrient cycling and energy flow (Petranka 1998); as predators of detritivores, they can influence leaf litter accumulation rates and potentially carbon cycling (Wyman 1998). Additionally, stream salamanders have been suggested to be suitable bioindicators of ecosystem integrity (Welsh and Ollivier 1998).

TIMBER HARVEST IMPACTS ON STREAMSIDE SALAMANDER POPULATION DYNAMICS

Throughout the past few decades, several studies have sought to determine how timber harvest affects stream salamander populations. Using a research design framework, we present a review of these research findings from the eastern deciduous forests of North America, most of which were focused on the family Plethodontidae.

Stand Age Studies

One method commonly used in studies evaluating timber harvest impacts on salamanders involves determining the relationship between timber stand age (time since harvest) and resident salamander populations. This method has many benefits, including a streamlined logistical structure, ease of replication, and the convenience of omitting the installation of a treatment midway through data collection. However, the statistical inferences drawn can be more tenuous than from studies using a control-impacted (CI) or before-after control-impacted (BACI) design.

Over the past few decades, many studies have evaluated stream salamander populations in stands of assorted ages (Connette and Semlitsch 2013, Crawford and Semlitsch 2008, Ford et al. 2002, Lowe and Bolger 2002, Lowe et al. 2004); all found that stand age was positively associated with stream salamander abundance. Lowe and Bolger (2002) studied populations of *Gyrinophilus porphyrititcus* in first-order streams of a mixed softwood-hardwood forest in New Hampshire by using a stepwise multiple regression analysis. Their best supported model for predicting *G. porphyrititcus* abundance contained the following factors: (1) the presence of brook trout (*Salvelinus fontalis*), (2) a variable representing a combination of years since harvest and substrate embeddedness by sediment, and (3) landscape configuration, defined as whether the streams were paired (a stream with a confluent first-order stream) or isolated. It is also worth noting that they found years since harvest and substrate embeddedness to be highly autocorrelated. Substrate embeddedness was defined by Lowe et al. (2004) as "a measure of fine sediment accumulation among the larger substrate particles of the streambed."

Ford et al. (2002) calculated abundance, species richness, and a Shannon's diversity index for salamanders in Appalachian cove hardwood stands of northern Georgia. Using an analysis of covariance, they found species richness and Shannon's diversity to be higher in stands ≥85 years old than in stands aged 15, 25, or 50 years. Abundance was positively correlated with stand age in *Desmognathus aeneus* and *D. ocoee*. Stand age was found to be significantly associated with abundances of *Eurycea bislineata*, but abundances peaked in 25-year-old stands. Species richness, Shannon's diversity, and abundances of *D. ocoee* and *D. quadramaculatus* were also found to be positively correlated with basal area although abundances of *E. bislineata* were negatively correlated with basal area.

Lowe et al. (2004) sought to investigate stage-specific effects of logging-associated sedimentation and *S. fontinalis* presence on *G. porphyrititcus* in mixed hardwood-softwood forests of New Hampshire. Using multiple regression analysis, they determined that larvae abundance was negatively related to the presence of *S. fontinalis*, but unrelated to substrate embeddedness. Conversely, adult abundance was primarily associated with substrate embeddedness. They concluded that in streams where fish are absent and with high levels of salamander larvae, salamander populations may be buffered from logging-associated sedimentation. In streams with fish and corresponding low larval abundances, the reduction in adult survival associated with sediment inputs from logging may threaten salamander persistence.

Crawford and Semlitsch (2008) examined salamander populations of riparian forests adjacent to headwater streams in western North Carolina and found abundances of *E. wilderae* to be significantly reduced in stands ≤40 years old compared to stands 41-80 years old and those ≥81 years old; this was true for both night and daytime sampling. Additionally, they found that terrestrial habitat usage was significantly greater with increased stand age for *D. monticola*, *D. ocoee*, and *E. wilderae*. Out of numerous covariates, only leaf litter depth was found to have a significant association with salamander abundance.

Connette and Semlitsch (2013) examined populations of *D. ocoee* and *E. wilderae* in streams with different histories of timber harvest. In accordance with previous research, they established a positive relationship between timber stand age and salamander abundance. A negative association between abundance and distance to forest edge was found as well. Additionally, it was determined that the populations increased faster in younger regenerating forest stands than in forest stands closer to climax. Salamander abundance was estimated to reach a peak at 100 years postharvest.

Paired Analyses

Other research has been designed to reflect paired watershed analyses, whereby separate watersheds or other land divisions with different logging treatments are studied. These watersheds often incorporate different SMZ characteristics and include comparative control watersheds within a CI study design. A CI study design presents the advantage of generating comparisons dependent on treatment, but requires the assumption that population levels have not fluctuated temporally due to an uncontrolled variable. Research using a CI design to investigate timber harvest effects has also been widely practiced (Knapp et al. 2003, Moseley et al. 2008, Peterman and Semlitsch 2009, Peterman et al. 2011).

Knapp et al. (2003) conducted research in mixed mesophytic forests of southwestern Virginia and southern West Virginia and found that gravid *D. ochrophaeus* females weighed more in uncut treatments than in treatments subjected to timber harvest. However, no differences in number of gravid females or in number of eggs per gravid female were found.

Moseley et al. (2008) found that abundance of *Desmognathus* spp. was affected negatively by increased removal of timber volume, and was positively associated with increased time since disturbance in a northeastern West Virginia mixed mesophytic hardwood forest. They further concluded that higher percentages of imbedded substrate were associated with lower abundances of *Desmognathus* spp. Their conclusion is shared by Peterman and Semlitsch (2009), who focused on *E. wilderae* and *D. quadramaculatus* in forests of western North Carolina. In that study, larval *E. wilderae* abundances in streams with unharvested forest buffer widths of 0 m, 9 m, and 30 m were compared along with unharvested control streams. Abundances in the streams with a 9-m buffer were not significantly different from 0-m buffer streams, and abundances in streams prescribed a 30-m buffer were not significantly different from the control. However, the 9-m buffer was found to have significantly fewer salamanders than the 30-m buffer and the control. A corresponding pattern in sedimentation was noted, and suggested as the mechanism of decline due to the filling of interstitial spaces used as refugia for larval salamanders.

In examining five western North Carolina forest headwater streams, Peterman et al. (2011) found significantly higher densities of adult and juvenile *D. monticola* and *D. quadramaculatus* at a treatment without a stream buffer, and substantially higher densities at sites with a 9-m unharvested buffer in comparison to unharvested controls. They considered their findings to be attributable to evacuation, characterized by the abandonment of logged areas by stream salamanders in search of more suitable habitat.

Before-After Control-Impacted Design

A BACI study approach is often used to determine the impact of perturbation events on one or more biological or nonbiological entities. These studies are frequently preferred to CI designs because BACI analysis can alleviate the chance that unmeasured covariates are influencing observed effects (McDonald et al. 2000). Considering that salamander populations are often distributed unevenly on a landscape (Wyman 1988), an assumption of pretreatment site homogeneity could potentially weaken experimental conclusions (deMaynadier and Hunter 1995). However, the design can be subject to flaws: the life histories of different salamander species can dictate how much time must have passed between the installation of a treatment and data collection to ensure the integrity of conclusions applied to diverse age classes.

The BACI approach has been used in several studies of timber harvest impacts on stream salamander populations (Maigret 2013, Perkins and Hunter 2006, Williams et al. 2002). Statistical inference from these studies can often be improved over other designs, but they typically are more expensive and time consuming (deMaynadier and Hunter 1995) and often require timber harvests to be tailored to specific study designs. Consequently, BACI projects can be difficult to implement and maintain over long study periods and across multiple funding and data-collecting entities.

Using a BACI study design, Williams et al. (2002) found that a population of *D. brimleyorum* was significantly lower after a removal of 0.04 ha of vegetation from a stream bank in the Ouachita Mountains of eastern Oklahoma. They also noted a large increase in the amount of sediment in the stream but did not measure it.

Perkins and Hunter (2006) conducted a study in a northern hardwood to mixed-conifer-hardwood Maine forest, and found abundance of *E. bislineata* in a partial timber cutting to be more similar to unharvested control streams than clearcuts mitigated by 25- to 35-m stream buffers. The similarity was not significant, however.

Conducting research in ephemeral headwater streams of a mixed mesophytic forest of southeastern Kentucky, Maigret (2013) used a BACI study design with three different treatments: a no-harvest control, a set of watersheds where the current State BMP requirements for SMZ application were applied, and a set of watersheds where intermittent stream SMZ requirements were applied to ephemeral streams under augmented requirements. Maigret (2013) found lower abundances of *Desmognathus* spp. in watersheds subjected to current State BMP requirements for SMZ application, and further found that the treatment where an augmented SMZ was applied was not statistically different from the control. However, these results were found only in a treatment versus control watershed comparison. When preharvest data were compared to postharvest, the abundances followed similar patterns but lacked statistical significance. Similar patterns were found when analyzing *E. bislineata* abundances, but they were not significant in CI or BACI comparisons.

Identifying Cause-Specific Sources of Stream Salamander Declines

Of the studies we reviewed, only a surprisingly small variety of likely causes of stream salamander population decline from timber harvest were described. Logging-associated sedimentation was one factor in many of these studies that was found or hypothesized to be negatively associated with salamander abundance (Crawford and Semlitsch 2008, Lowe and Bolger 2002, Lowe et al. 2004, Moseley et al. 2008, Peterman and Semlitsch 2009). Stand age was identified as being associated with higher abundances of salamanders (Crawford and Semlitsch 2008, Ford et al. 2002, Lowe and Bolger 2002,) as well as higher Shannon's diversity and species richness (Ford et al. 2002), and with increased terrestrial habitat usage (Crawford and Semlitsch 2008). Stand age is a factor that was demonstrated to be associated with numerous other microhabitat factors important to stream salamander populations, including leaf litter depth and soil moisture (Crawford and Semlitsch 2008, Lowe and Bolger 2002). Therefore, timber stand age could be interpreted as a proxy for these more specific microhabitat features.

EVALUATING POTENTIAL SOLUTIONS

Timber harvests can potentially influence habitat characteristics in two important ways for stream salamanders: by increased sedimentation and filling of interstitial spaces in the substrate (Swank et al. 2001), and by degradation of the surrounding terrestrial habitat to the point that it interferes with the terrestrial component of the life cycles of many stream salamander species (Petranka et al. 1993). The preservation of forest buffer strips attempts to address both of these suspected proximate causes of stream salamander population declines.

Stream management zones are designed to help prevent or reduce soil erosion and associated sedimentation in two ways: by the restriction of unimproved stream crossings, roads, or landings within the SMZ, and by preservation of standing timber within buffer strips. Forest roads, including haul roads and skid trails, have been found to be the main source of sediment deposited in headwater streams during and immediately after harvest (Bowker 2013). Studying the same harvests at the same location as Maigret (2013), Witt et al. (2013) found that truck use of improved crossings over headwater streams reduced levels of total suspended solids by 86 percent. Furthermore, the preservation of a buffer strip, even if improved crossings are not mandated, has been shown to reduce sedimentation by physically obstructing sediment paths (Bowker 2013).

The preservation of some standing timber is likely the most effective, most practical, and perhaps the only solution for preserving the required quality and quantity of microhabitat factors necessary for salamanders and other stream-dwelling species to persist. For example, Peterman and Semlitsch (2009) found that a 30-m SMZ buffer and control did not differ in *E. wilderae* abundance. Likewise, Maigret (2013) found that retention of even the nearest streamside canopy tree was associated with increased *Desmognathus* spp. And although many have suggested a more liberal underharvested or unharvested buffer (Crawford and Semlitsch 2007), the amount of forest buffer required to maintain species viability on short and longer temporal scales is likely to be highly dependent on forest type, local hydrology, geology, and climate, as well as the salamander species and their prey base. Until we have a better understanding of the various spatial and compositional aspects of SMZs required to maintain stream salamander populations and other aquatic organisms, we argue that maintaining a precautionary approach to design and implementation of stream buffers would be wise. If a canopy retention requirement is included, some loggers may give the stream more buffer than the legal minimum to ensure not violating the requirement. This behavior was noted during the harvests associated with Maigret (2013), Bowker (2013), and Witt et al. (2013).[2]

To summarize these potential solutions, we propose the following list of minimum requirements for SMZ regulations in deciduous forests of the eastern United States. These should not be interpreted as the only regulations which may be of benefit, or as regulations in full detail, but rather as general ideas which would likely prove to benefit stream salamander populations during and after timber harvests.

- The SMZ regulations must apply to streams which flow perennially, intermittently, and ephemerally.
- The buffer widths mandated by SMZ regulations should increase with increasing bank slope.
- The buffer widths mandated by SMZ regulations should require at least some canopy retention.
- Within each buffer:
  - Use of heavy equipment should be prohibited.
  - Construction of roads, landings, or trails should be prohibited.
  - Use of improved crossings should be mandated.
- Dumping of materials into streams, whether perennial, intermittent, or ephemeral, should be prohibited.

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FUTURE RESEARCH

Specific and tactical studies instead of broad, homogenous ones may prove more informative to development of strategies for preserving stream salamander populations while maintaining or modifying contemporary timber harvest practices. For example, Lowe et al. (2004) showed what stage-specific differences can exist in *G. porphyrititcus.* Few stage-specific studies have been conducted on other stream salamander species (but see Peterman and Semlitsch 2009), and therefore for many salamander species, we remain largely ignorant of the habitat requirements that may extend well beyond SMZ boundaries. Aside from life stages, vast differences in behavior, spatial ecology, and reproduction occur between species which are often grouped together as stream salamanders (Petranka 1998). For example, the finding by Ford et al. (2002) that basal area and abundances have a strong positive relationship for *D. ocoee* and *D. quadramaculatus* but a negative correlation for *E. bislineata* suggests that such differences likely make some species more susceptible than others to the effects of certain types of timber harvest.

Investigations of specific aspects of timber harvesting are few. Many details about tree selection and road construction remain untested, and may prove to be disproportionately informative to SMZ design and policy considering the small investments of money and time typically required to conduct these studies. Comparing, for example, different types of improved stream crossing techniques used by logging machinery may prove more worthwhile than studying improved crossings versus unimproved crossings in general.

A major concern for the forest industry is the cost of increased regulations to protect the biotic and abiotic resources that might be jeopardized by timber harvests. In many states, the application of BMPs is nonbinding and entirely at the discretion of loggers. Therefore, in-depth economic analyses of the costs of measures to ensure the protection of natural resources, including stream salamander populations, may be warranted to show how the adoption of such measures can be balanced with profitability. For example, Bowker (2013) videotaped the installation and removal of improved stream crossings and found that using a reusable skidder bridge or steel culvert to cross headwater streams was more time efficient (and therefore more cost efficient in labor and fuel) than using an unimproved ford. An analysis determining the value of timber left behind to satisfy SMZ canopy preservation guidelines may also be useful. Highlighting the potential to remove trees of high value and leave trees of low value while still retaining sufficient canopy cover, may help persuade logging companies to implement BMPs in states where they are not required. Although companies may be by definition profit driven, public image is important to many businesses in the forest industry, as shown by the popularity of Forest Stewardship Council and similar accreditations. In any event, providing evidence that complying with regulations designed to protect stream salamander species can be done without damaging profitability may be key to the implementation of regulations that help maintain the biological integrity of forest streams.

LITERATURE CITED

Blaustein, A.R.; Bancroft, B.A. 2007. **Amphibian population declines: evolutionary considerations.** BioScience. 57: 437-444.

Bowker, D.W. 2013. **Forest harvest equipment movement and sediment delivery to streams.** Lexington, KY: University of Kentucky, College of Agriculture. 197 p. M.S. thesis.

Burton, T.M.; Likens, G.E. 1975. **Salamander populations and biomass in the Hubbard Brook experimental forest, New Hampshire.** Copeia. 197: 541-546.

Chizinski, C.J.; Vondrace, B.; Blinn, C.R. [et al.]. 2010. **The influence of partial timber harvesting in riparian buffers on macroinvertebrate and fish communities in small streams in Minnesota, USA.** Forest Ecology and Management. 259: 1946-1958.

Connette, G.M.; Semlitsch, R.D. 2013. **Life history as a predictor of salamander recovery rate from timber harvest in southern Appalachian forests, U.S.A.** Conservation Biology. 27: 1399-1409.

Corn, P.S.; Bury, R.B. 1989. **Logging in western Oregon: responses of headwater habitats and stream amphibians.** Forest Ecology and Management. 29: 39-57.

Crawford, J.A.; Semlitsch, R.D. 2007. **Estimating core terrestrial habitat for stream-breeding salamanders and delineation of riparian buffers for protection of biodiversity.** Conservation Biology. 21: 152-158.

Crawford, J.A.; Semlitsch, R.D. 2008. **Post-disturbance effects of even-aged timber harvest on stream salamanders in southern Appalachian forests.** Animal Conservation. 11: 369-376.

deMaynadier, P.G.; Hunter, M.L. 1995. **The relationship between forest management and amphibian ecology: a review of the North American literature.** Environmental Reviews. 3: 230-261.

Ford, W.M.; Chapman, B.R.; Menzel, M.A.; Odom, R.H. 2002. **Stand age and habitat influences on salamanders in Appalachian cove hardwood forests.** Forest Ecology and Management. 155: 131-141.

Houlahan, J.E.; Findlay, C.S.; Schmidt, B.R.; Meyer, A.H.; Kuzmin, S.L. 2000. **Quantitative evidence for global amphibian population declines.** Nature. 404: 752-755.

Knapp, S.M.; Haas, C.A.; Harpole, D.N.; Kirkpatrick, R.L. 2003. **Initial effects of clearcutting and alternative silvicultural practices on terrestrial salamander abundance.** Conservation Biology. 17: 752-762.

Lannoo, M.J., ed. 2005. **Amphibian declines: the conservation status of United States species.** Berkeley, CA: University of California Press. 1094 p.

Lowe, W.H.; Bolger, D.T. 2002. **Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams.** Conservation Biology. 16: 183-193.

Lowe, W.H.; Nislow, K.H.; Bolger, D.T. 2004. **Stage-specific and interactive effects of sedimentation and trout on a headwater stream salamander.** Ecological Applications. 14: 164-172.

Maigret, T.A. 2013**. Effects of streamside management zone timber harvest on salamander communities in Robinson Forest.** Lexington, KY: University of Kentucky, College of Agriculture. 66 p. M.S. thesis.

McDonald, T.L.; Erickson, W.P.; McDonald, L.L. 2000. **Analysis of count data from before-after control-impact studies.** Journal of Agricultural, Biological, and Environmental Statistics. 5: 262-279.

Moseley, K.R.; Ford, W.M.; Edwards, J.W.; Schuler, T.M. 2008. **Long-term partial cutting impacts on *Desmognathus* salamander abundance in West Virginia headwater streams.** Forest Ecology and Management. 254: 300-307.

Perkins, D.W.; Hunter, M.L. 2006. **Effects of riparian timber management on amphibians in Maine.** Journal of Wildlife Management. 70: 657-670.

Peterman, W.E.; Crawford, J.A.; Semlitsch, R.D. 2011. **Effects of even-aged timber harvest on stream salamanders: support for the evacuation hypothesis.** Forest Ecology and Management. 262: 2344-2353.

Peterman, W.E.; Semlitsch, R.D. 2009. **Efficacy of riparian buffers in mitigating local population declines and the effects of even-aged timber harvest on larval salamanders.** Forest Ecology and Management. 257: 8-14.

Petranka, J.W. 1998. **Salamanders of the United States and Canada.** Washington, DC: Smithsonian Institution Press. 587 p.

Petranka, J.W.; Eldridge, M.A.; Haley, K.E. 1993. **Effects of timber harvesting on southern Appalachian salamanders.** Conservation Biology. 7: 363-370.

Petranka, J.W.; Murray, S.S. 2001. **Effectiveness of removal sampling for determining salamander density and biomass: a case study in an Appalachian streamside community.** Journal of Herpetology. 35: 36-44.

Semlitsch, R.D. 2000. **Principles for management of aquatic breeding amphibians. Journal of Wildlife Management.** 64: 615-631.

Swank, W.T.; Vose, J.M.; Elliott, K.J. 2001. **Long-term hydrologic and water quality responses following commercial clearcutting of mixed hardwoods on a southern Appalachian catchment.** Forest Ecology and Management. 143: 163-178.

Vesely, D.G.; McComb, W.C. 2002. **Salamander abundance and amphibian species richness in riparian buffer strips in the Oregon coast range.** Forest Science. 48: 291-297.

Welsh, H.H., Jr.; Ollivier, L.M. 1998. **Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods.** Ecological Applications. 8: 1118-1132.

Williams, L.R.; Crosswhite, D.L.; Williams, M.G. 2002. **Short-term effects of riparian disturbance on *Desmognathus brimleyorum* (Plethodontidae) at a natural spring in Oklahoma.** The Southwestern Naturalist. 47: 611-613.

Witt, E.L.; Barton, C.D.; Stringer, J.W.; Bowker, D.W.; Kolka, R.K. 2013. **Evaluating best management practices for ephemeral stream protection following forest harvest in the Cumberland Plateau.** Southern Journal of Applied Forestry. 37: 36-44.

Wyman, R.L. 1988. **Soil acidity and moisture and the distribution of amphibians in five forests of southcentral New York.** Copeia. 1988, 394-399.

Wyman, R.L. 1998. **Experimental assessment of salamanders as predators of detrital food webs: effects on invertebrates, decomposition and the carbon cycle.** Biodiversity and Conservation. 7: 641-650.