Characterization of Larval Amphibian Populations Present in Vernal Ponds of the Ottawa National Forest Prior to Large-Scale Logging of the Surrounding Forest.

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Abstract

Previous studies have shown that amphibian populations are more sensitive than other terrestrial environmental fluctuations, due to their highly permeable skin and biphasic mode of development. Furthermore, amphibians have been shown to have differential responses to the same environmental changes, according to their region and species. With this in mind, it is clear that an understanding of the way in which amphibian populations respond to environmental change may make them useful as a measure of the overall health of an ecosystem. This would allow harmful environmental changes to be recognized and corrected before other vertebrate species were significantly impacted. However, for the data to be applicable, direct measurements must be collected for the region of interest, rather than generalizing the response of species in other regions. This study used populations of larval amphibians as an indicator of overall amphibian species diversity within the Ottawa National Forest in the Upper Peninsula of Michigan. Four vernal ponds were selected for study in the Ottawa National Forest, two in a region slated to be clear-cut and two in a patch to be selectively thinned. Logging had not yet occurred at either site. Additionally, three vernal ponds on the adjoining University of Notre Dame Environmental Research Center (UNDERC), where no logging is scheduled to occur, were selected as controls. Physical data was obtained about both the vernal pond water environment and the surrounding forest stand over two separate time frames, one in June 2001 and the other in July 2001. Water depth and overall reach of the vernal ponds decreased dramatically over the course of the study, with two vernal ponds in the Ottawa becoming completely dry. Larval amphibian density dropped for the clear-cut and selective thinning ponds from June to July, and increased for the control plots. However, the total number of amphibians decreased from June to July for each of the treatment types. The variability in species number and density was not highly correlated with physical and chemical properties of their respective vernal ponds. However, there was not a significant change in the physical and chemical environment over the course of the study. Therefore, the lack of correlation does not necessarily indicate that these amphibian species were unresponsive to fluctuating environmental conditions. Likely, changes in the rate of desiccation, UV-B intensity, and the introduction of habitat fragmentation as a result of forest clearing will exert the most profound effects on populations of amphibians in the Upper Peninsula of Michigan. But, further study is required before definitive conclusions can be made.

Introduction

By ordinance of the National Forest Management Act of 1976, national forests in the United States are required to maintain viable numbers of all vertebrate species within a given forest territory (Mitchell et al.). Initial investigations of species richness and diversity have focused on mammalian species, with only a recent growing interest in the effects of ecological change on the diversity and richness of amphibian species (Mitchell et al.).

The proliferation of amphibian-focused experiments is important for predictive reasons. Experimental data has shown that amphibians are more sensitive than other terrestrial vertebrates to environmental fluctuations, due to their highly permeable skin and biphasic developmental scheme (Alford and Richards). Thus, by observing changes in highly-susceptible amphibian populations to certain habitat alterations, forest managers might be able to detect and correct the disturbances before they become severe enough to impact larger vertebrate species.

In many past amphibian studies, the focus of the investigation has been on how particular aspects of the environment impact particular amphibian species. In one such study, total amphibian species density varied with characteristics of the forest floor microhabitat and the availability and proximity of water (Mitchell et al.). This study also showed that total amphibian species richness had no statistically significant correlation with estimated stand age, total number of canopy trees, tree diversity, or frequency of underground rocks.

Additional data relevant to the understanding of variability within amphibian populations has been provided by the studies of Kolozsvary and Swihart and Delis, Mushinsky, and McCoy. In both of these studies, there is an affirmation of the findings of Mitchell et al. that amphibian species density is positively associated with certain aspects of the environment, while unaffected by others. Furthermore, these studies made clear that individuals of different species of amphibians are not equally impacted by the same environmental changes.

In the Kolozsvary and Swihart study, the

> Occurrence of redback salamanders (*Plethodon cinereus*) was positively associated with the area of a forest patch. Occurrence of ranid frogs was positively associated with proximity of wetlands for three of four species, and occurrences of smallmouth salamanders (*Ambrystoma texanum*), spring peepers (*Pseudacris crucifer*), and western chorus frogs (*Pseudacris tristerata*) were related to the degree of wetland permanency.

Thus, while each of the studied species was positively associated with some environmental variable, there was clearly not a uniform response between different species individuals to the same environmental conditions.

In a comparison study of a residential development and a nearby, undisturbed park, Delis, Mushinsky, and McCoy showed that four anuran species (*Bufo quercicus, Scaphiophus h. holbrookii, Hyla femoralis*, and *H. gratiosa*) were negatively impacted by changes in surrounding water habitat. One interesting result of the study was that "three species of ranids, *Rana utricularia, R. grylio,* and *R. catesbeiana* were [actually] found in higher abundances at the residential development than at the park" (Delis, Mushinsky, and McCoy). From this data, it is again evident, as it was in the study by Kolozsvary and Swihart, that species variation cannot be described by universal rules. This is to say that it is fallacious to assume that environmental changes that cause one species of amphibians to decline in one region will necessarily be indicative of the response that another amphibian species will undergo to the same environmental change in the same or different region.

Past studies have reinforced the points that amphibian decline occurs in a regional and species-specific fashion (Kolozsvary and Swihart). Hence, to develop an effective, biologically based management scheme for conserving amphibians within the Midwest, data must be obtained regarding the specific responses of each endemic species to a particular series of environmental changes (Semlitsch). "Although it [was] desirable to monitor all the plants and animals in [this] region (alpha richness), the number of species and the cost associated with developing a statistically appropriate sampling frame [renders] this impractical" (Welsh Jr. and Droege). Thus, relying on the observation of past experimenters that "temporary ponds serve as important breeding sites for many amphibians" (Dodd and Cade), this study is utilizing populations of larval amphibian species within vernal ponds as an indicator of overall species diversity and richness within the Ottawa National Forest ecosystem. Future changes in the populations of larvae should reflect changes within the parent species populations due to a particular environmental influence or combination of influences (Welsh Jr. and Droege).

As in prior studies, it is hypothesized that not all amphibian species will respond identically to the same environmental changes because each of these species is not equally impacted by the same environmental elements. But, it is hoped that general trends that help govern species richness and diversity in the Midwest can be elucidated by comparing population changes in experimentally logged areas of the Ottawa to larvae population changes within control vernal ponds in adjoining property of the University of Notre Dame Environmental Research Center (UNDERC).

Materials and Methods

In early June 2001, sites for larval amphibian sampling were chosen within two areas of the Ottawa National Forest in the Upper Peninsula of Michigan (elevation 550m). Two (OTT2 and OTT 3) were located in an aspen dominated forest set to be clear-cut and two others (OTT5 and OTT6) in a sugar maple dominated forest set for selective thinning by the US Forest Service. Control vernal ponds were chosen near-by, on the property of the University of Notre Dame Environmental Research Center, where no logging is scheduled to occur. These vernal ponds were selected on the basis of their physical similarity to those of the Ottawa National Forest and the known presence of amphibian larvae.

Before sampling began, the physical properties and the makeup of the vegetation surrounding each vernal pond were determined so that future habitat changes may be tracked. First, a Garmin 12 global positioning system (GPS) device was used to determine the exact latitude/longitude and UTM coordinates of each pond using the "NAD27 Central" datum. Analysis of the vernal pond water involved quantitative measurement of dissolved oxygen (D.O.) concentration, conductivity, maximum depth, ambient temperature, and water temperature. D.O. and water temperature measurements were made using a YSI 55 D.O. meter (YSI Incorporated); conductivity was measured with a HI 9033 multi-range conductivity meter (Hanna Instruments); pH was recorded using a pHep 3 with ATC meter (Hanna Instruments); and ambient temperature was recorded using a standard alcohol thermometer. In addition to quantitative measurements, qualitative data was taken for water color, water turbidity, primary substrate, and the percent margin containing emergent vegetation.

The forest surrounding each vernal pond was characterized by noting the wood density, vegetation present, and canopy cover at each of four sites, one north, south, east, and west of each vernal pond. Wood density was measured by scoring trees, determined to be within a variable radius plot using wedge dispersion prism, by distance away from the center of the plot and by diameter, measured using a measuring tape that corrects the circumference by a factor of 2π. Undergrowth and tree species were identified visually and canopy cover was measured using a spherical densiometer.

Each vernal pond was divided into plots one meter wide, along the circumference of the pond, and two meters out into the pond. These plots were further divided into one by one meter square plots, one adjacent to shore and one toward the middle of the pond. In preparation for sampling, wooden stakes, approximately 40cm in length, were placed in ten-meter intervals around the circumference of each vernal pond in order to establish a frame of reference for locating sampling sites within each pond. Specific sampling sites were located by anchoring a tape measure to either end of two adjacent dowels. Each meter interval on the measuring tape corresponded to a boundary between sampling sites. One-third of the total number of 1x2m plots at each vernal pond were randomly chosen for sampling using the random number function on Microsoft Excel. A coin was flipped to determine whether each plot would be sampled at the near (a) or far (b) 1x1m plot.

Initial sampling was carried out on June 12, 2001 in the Ottawa National Forest and on June 26, 2001 at UNDERC. Late summer sampling was carried out on July 9 and 10, 2001. A modified (bottom was removed) 31-gallon Rubbermaid© bin, measuring .876m long, .508m wide, and .425m deep, was used as the sampling apparatus. The bin was placed into the water within a specified 1x1m sampling site and held firmly against the bottom of the vernal pond so that no amphibian larvae could escape. A 12.5cm x 15cm fish-tank net was then swept across the interior of the bin and the net contents were inspected for presence of larval amphibians. This was done an average of fifteen times per sampling site. Captured larvae were separated into site-labeled, gallon Zip-Lock™ sandwich bags and brought back to the lab for identification, measurement, and preservation. The amphibian larvae were analyzed under a stereomicroscope and identified to species primarily by examining mouthpart structure, size, and tail length-to-body length ratio, and secondarily by using leg structure and coloration. Following identification, the larval amphibians were measured by length using a standard 30cm ruler and one specimen from each species from each vernal pond was preserved in 95% EtOH for future reference. All other larvae were returned to their original vernal ponds.

Results

Preliminary sampling for amphibian larvae was carried out in two vernal ponds each for two areas of the Ottawa National Forest slated to be either clear-cut or selectively thinned in the near future. Three vernal ponds on the UNDERC property were also sampled as controls, where no logging would occur during the duration of the study. Sampling was carried out once during the early summer, in June, and once during the middle to late summer, in July. All of the vernal ponds were of significant size in the early summer, but half of the vernal ponds in the Ottawa, OTT3 and OTT6, had completely dried out by July, while the other two had dropped significantly in water level. The vernal ponds at UNDERC had not dried out by July, but ND-CR was much smaller and would have been dry within the week.

The major focus of this study was to establish which species of amphibians were present at each vernal pond and in what numbers. Figure 1 shows the results of comparisons between vernal ponds based on species richness. Two amphibian species were present at three of the four vernal ponds located in the Ottawa National Forest at the first sampling, the other, OTT3, did not contain any amphibian larvae.

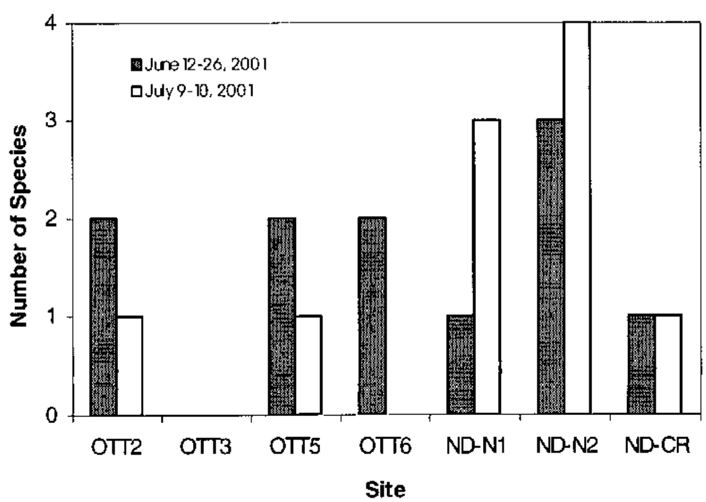

Figure 1 AMPHIBIAN SPECIES RICHNESS BY SITE AND DATE.

The control ponds were more varied in the results. Two ponds had only one species, and one had three. The samples taken in July showed changes in species richness at all of the vernal ponds that actually contained amphibian larvae. Vernal ponds OTT3 and OTT6 had completely dried up by the second sampling and thus contained no

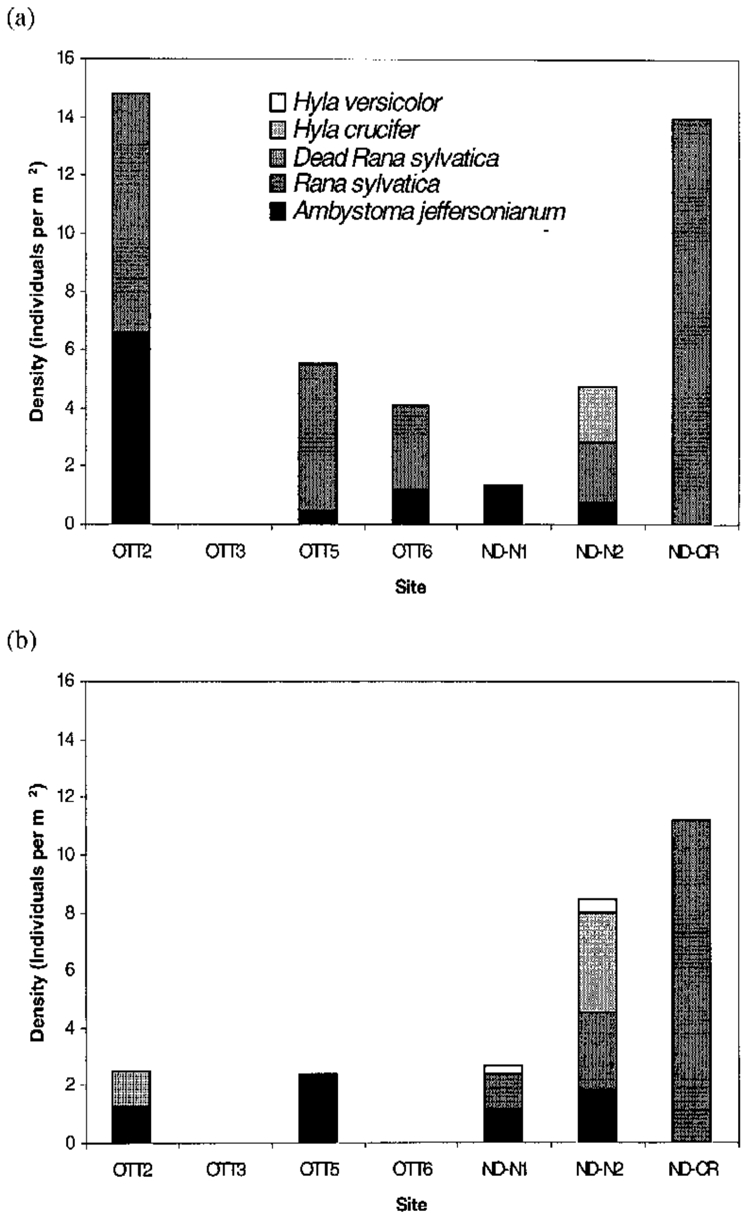

Figure 2 LARVAL AMPHIBIAN DENSITY BY SPECIES AT EACH SITE: (a) as measured on June 12 - 26, 2001 and (b) July 9 - 10, 2001.

amphibian larvae. Interestingly, each of the experimental ponds showed a reduction in species richness, while the control ponds increased or remained constant.

Figure 2 shows which species were found at each vernal pond in June and July as well as their densities. Only two species were found in the Ottawa sites in June, *Ambystoma jeffersonianum and Rana sylvatica.* Those same species plus *Hyla crucifer* were found in the control ponds during June. In July, only *A. jeffersonianum* was present in the Ottawa, while each of the original three species plus *H. versicolor* were present in the control ponds. The *R. sylvatica* larvae were absent in the Ottawa showing that they had either metamorphosed or died, as was the case with many in OTT2. The amphibian larvae densities in the Ottawa decreased overall from June to July while only the smallest vernal pond, ND-CR, showed a decrease in the control ponds, with the other two increasing. This suggests a difference in the time of egg-laying between the Ottawa vernal ponds and the UNDERC vernal ponds. It may also be that species of frog that mate later in the summer, such as the Hylids are present in the UNDERC vernal ponds. Figure 3

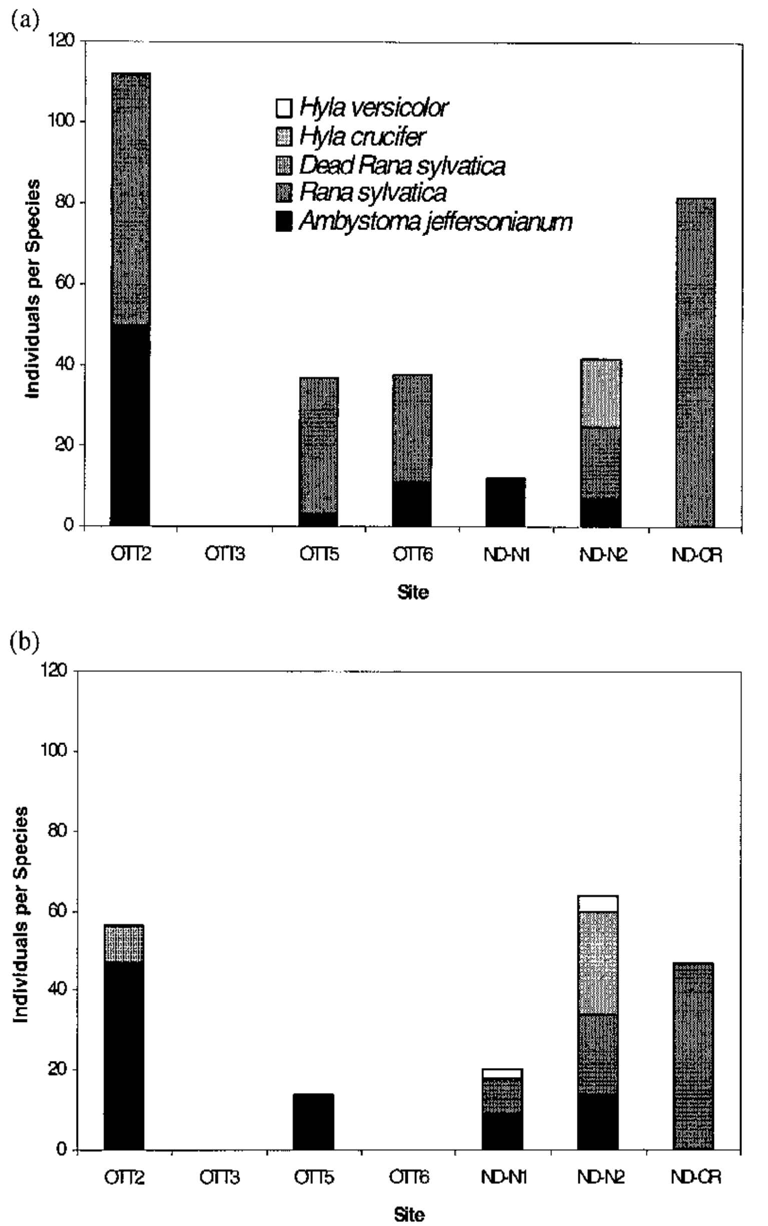

Figure 3 TOTAL LARVAL AMPHIBIAN COUNT BY SPECIES AT EACH SITE: (a) as measured on June 12 – 26, 2001 and (b) July 9 – 10, 2001.

shows the total number of each species of amphibian larvae found at each vernal pond. OTT2 contained the most amphibians in June, with ND-CR second. In July, OTT2 declined by about half, probably due to the fact that no living *R. sylvatica*, the in June, were present in July. No living *R. sylvatica* were present in any of the Ottawa vernal ponds in July, but were present in all of the UNDERC vernal ponds.

When the treatment sites as a whole are considered, trends become more visible. In general, the overall amphibian densities dropped for the clear-cut and selective thinning ponds from June to July, and increased for the control plots. (Figure 4) However, the total number of amphibians decreased from June to July for each of the three treatment types. (Figure 5) The density of *R. sylvatica* in each treatment type decreased with time, while that of *A. jeffersonianum* increased, as did *H. crucifer* and *H. versicolor* in the control ponds. (Figure 4) The total number of *A. jeffersonianum* remained relatively constant from June to July at each treatment type, while *R. sylvatica* decreased at each treatment type. Both *H. crucifer* and *H. versicolor* increased in

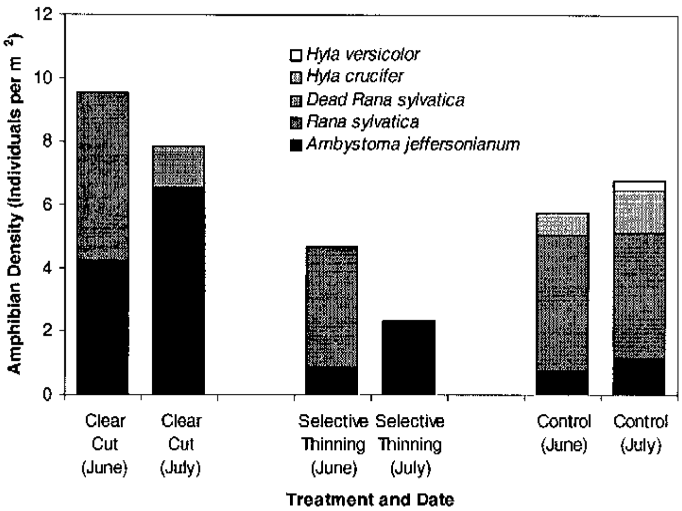

Figure 4 LARVAL AMPHIBIAN DENSITY BY SPECIES, TREATMENT TYPE, AND DATE. Vernal ponds OTT2and OTT3 made up the clear-cut sites, OTT5 and OTT6 made up the selective thinning sites, and ND-N1, ND-N2, and ND-CR made up the control sites. Both OTT3 and OTT6 were dry in July and were not sampled.

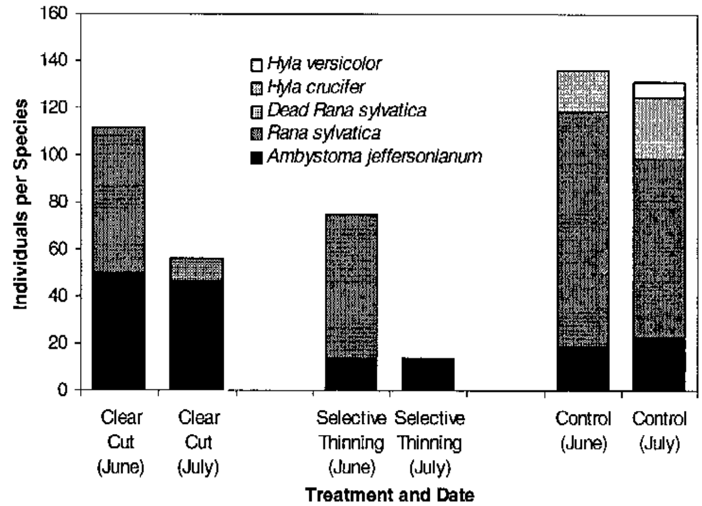

Figure 5 TOTAL LARVAL AMPHIBIAN COUNT BY SPECIES, TREATMENT TYPE, AND DATE. Vernal ponds OTT2 and OTT3 made up the clear-cut sites, OTT5 and OTT6 made up the selective thinning sites, and ND-N1, ND-N2, and ND-CR made up the control sites. Both OTT3 and OTT6 were dry in July and were not sampled.

number in the control ponds from June to July. (Figure 5) The majority of the larvae found increase in *A. jeffersonianum* density at each treatment type was most probably due to the shrinking of the vernal ponds as they dried.

The amphibian density and the total number of amphibians at each vernal pond were compared with the physical and chemical properties of their respective vernal pond. Water temperature, pH, dissolved O_2 concentration, conductivity, length, and maximum depth were measured for each vernal pond. No significant trends were found in the data, but conductivity seemed to show the greatest trend with amphibian densities and total number decreasing with increasing conductivity. Water temperature also showed a comparatively strong trend showing that, counter to what would be expected, amphibian density and number decrease with increasing temperature. It may be that these amphibian species are adapted to the colder temperatures of the early summer, or that these species simply have a higher fecundity. (Figure 6, 7)

The size-range for each species of amphibian larvae was measured for each vernal pond in June and July. The results show that, in general each species increased in average size from June to July. It is assumed that the loss of *R. sylvatica* from OTT2 and OTT5 occurred

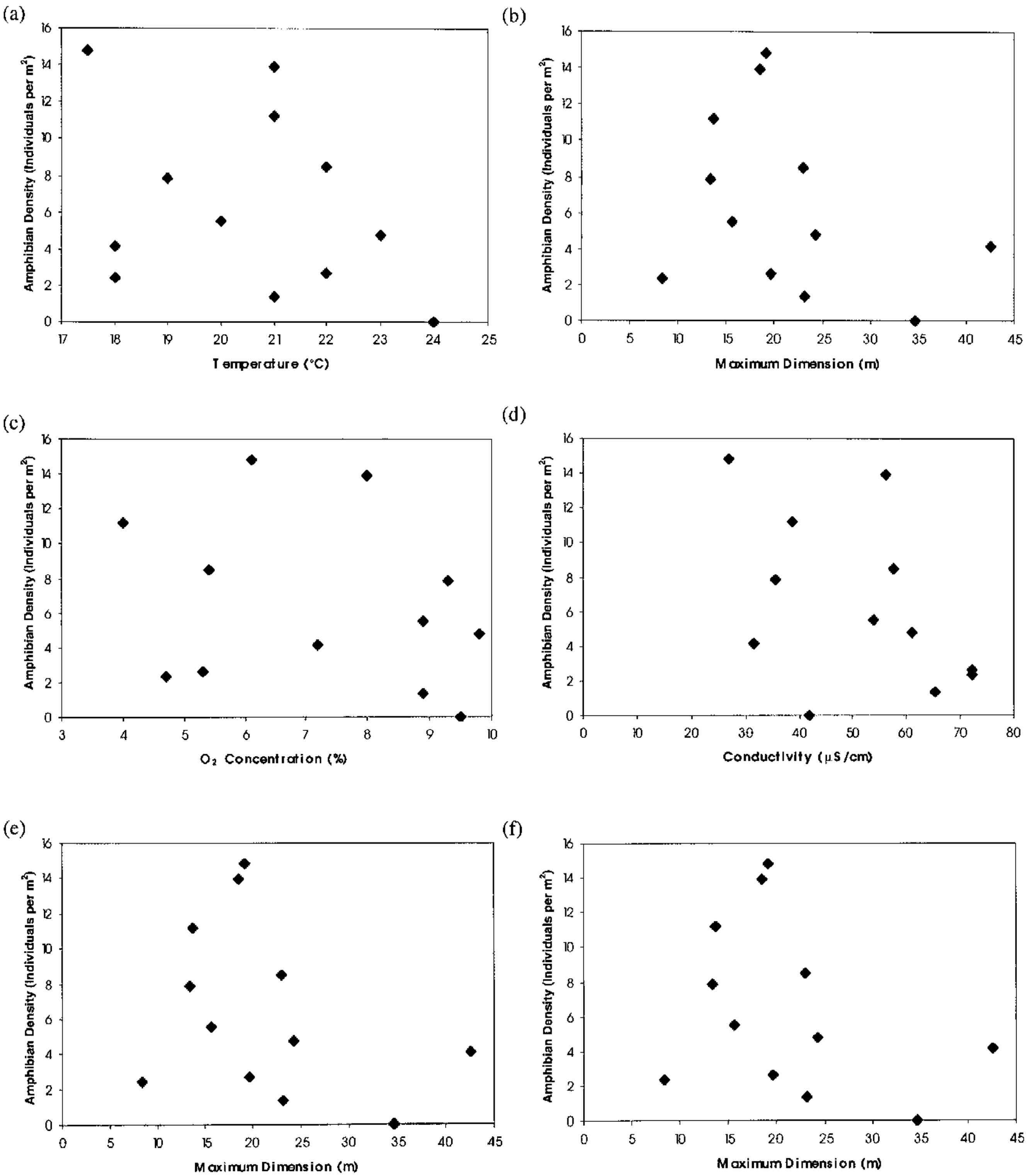

Figure 6 LARVAL AMPHIBIAN DENSITY BY: (a) water temperature (R^2 = 0.1069), (b) pH (R^2 = 0.0196), (c) O_2 concentration (R^2 = 0.078), (d) conductivity (R^2 = 0.2058), (e) maximum dimension (R^2 = 0.1103), (f) and maximum depth (R^2 = 0.0127).

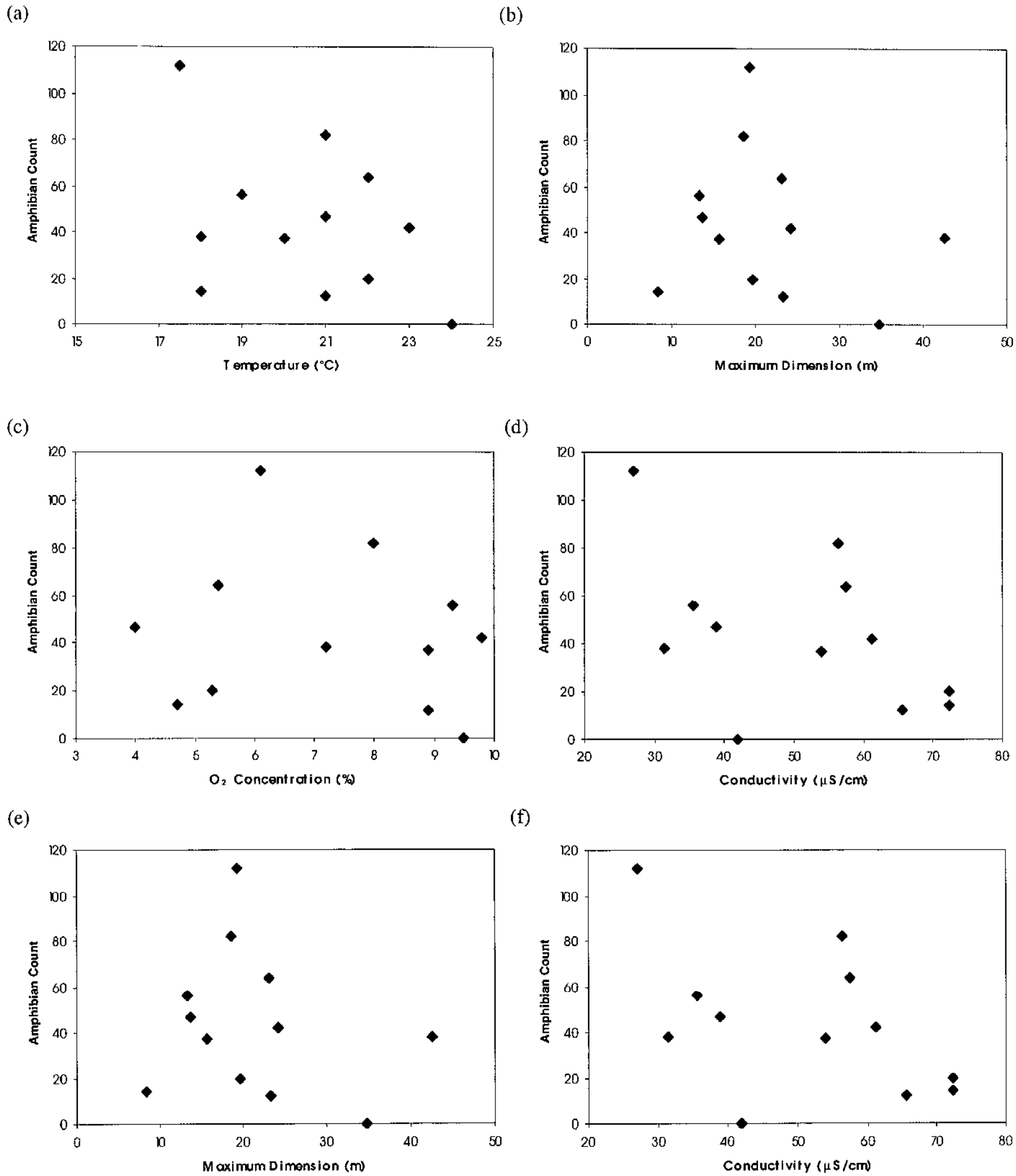

Figure 7 TOTAL LARVAL AMPHIBIAN COUNT BY: (a) water temperature ($R^2 = 0.1568$), (b) pH ($R^2 = 0.1203$), (c) O_2 concentration ($R^2 = 0.0258$), (d) conductivity ($R^2 = 0.2323$), (e) maximum dimension ($R^2 = 0.0385$), (f) and maximum depth ($R^2 = 0.0442$).

Table 1 Amphibian size range by site

(a) ***Ambystoma jeffersonianum***

Site	Date June 11-26, 2001	July 9-10, 2001
OTT2	1.4 - 2.8 (cm)	2.9 - 5.0 (cm)
OTT5	1.9 - 3.6	3.1 - 5.4
OTT6	1.8 - 3.6	-
ND-N1	1.8 - 2.3	3.0 - 3.6
ND-N2	1.9 - 3.5	2.3 - 3.4
ND-CR	-	-

(b) ***Rana sylvatica***

Site	Date June 11-26, 2001	July 9-10, 2001
OTT2	2.5 - 4.7 (cm)	5.1 - 5.8 (cm) **
OTT5	3.7 - 4.4	-
OTT6	4.2 - 4.9	-
ND-N1	-	5.2 - 6.0
ND-N2	4.1 - 4.9	4.3 - 5.8
ND-CR	4.5 - 6.2	4.9 - 5.5

(c) ***Hyla crucifer***

Site	Date June 11-26, 2001	July 9-10, 2001
ND-N1	-	-
ND-N2	1.9 - 2.6 (cm)	2.1 - 3.1 (cm)

(d) ***Hyla versicolor***

Site	Date June 11-26, 2001	July 9-10, 2001
ND-N1	-	2.1 - 3.5 (cm)
ND-N2	-	1.5 - 3.7

**Data represents dead *R. sylvatica* larvae.

because all individual larvae had metamorphosed into adult frogs, or, as in the case of OTT2, had become density limited by some resource, such as food, and died. (Table 1)

Discussion

Past studies have shown that deforestation "eliminates shade, increases surface temperature, disrupts soil structure, and reduces soil moisture, making these areas inhospitable or less suitable for many [amphibian] species" (Semlitsch). These consequences of deforestation will, likely, have similar negative effects on species density and diversity within the logged areas of the Ottawa National Forest.

A key physical character shared by all vernal ponds surveyed in the Ottawa was shallow depth (max depth $\leq$ 0.56 m). By eliminating the shade of the canopy, evaporation will occur much more quickly from the surface of the ponds, resulting in earlier desiccation. The onset of drought was shown to be quickest in vernal ponds OTT 6 and OTT 3, which had the highest surface to volume ratio of all vernal ponds sampled in the Ottawa. No amphibian species were ever found in OTT 3 (Figures 1,2, and 3), however, so this early-onset desiccation would not affect total count or species number in any way.

Because amphibians breed at different times, two pronounced effects will likely be observed among larval amphibians in the Ottawa, depending on the timing of desiccation. If the vernal pond desiccation occurs at the beginning of the breeding season, there will be little, if any, population growth across all species. If, however, the drying occurs in the latter stages of the breeding season (i.e., in July), there will be a reduction in total number of species, but not necessarily a reduction in total count of amphibians. If the latter scenario prevails, larval *Hylid* and *Ambystoma jeffersonianum* species, which had their greatest respective number of individuals during the July sampling period (Figure 3b) will be severely reduced, if not completely eliminated. However, larval *Rana sylvatica,* which were only found alive in the Ottawa during the June sampling period (Figure 3a), will, overall, be unaffected by early-onset drought. During, the July sampling, wherein all identified *R. sylvatica* were dead, *A. jeffersonianum* greatly outnumbered *R. sylvatica* in OTT 2 (Figure 3b). Thus, it may have been the case that, due to a lack of suitable fooe, the carnivorous *A. jeffersonianum* larvae killed *R. sylvatica* larvae, but could not consume them affectively. The likely case is that some aspect of the water chemistry changed between June and July, and those *R. sylvatica* larvae that had not metamorphosed could not tolerate the new conditions and died.

Drought is not the only factor that may cause a decline in the larval amphibian populations of the Ottawa as a result of deforestation. Increases in UV-B radiation, which would occur if tree canopy around the ponds were removed, have been shown to reduce survival, hatching success, development, and growth of some amphibians (Blaustein et al.). Furthermore, increased levels of UV-B may cause photoactivation of chemical contaminants in the vernal ponds, may reduce the pH, and may make the surviving larvae more susceptible to disease and pathogens (Semlitsch). Reduction of pH would probably be the easiest variable for this study to monitor in the Ottawa. As Figure 7b shows, most amphibian growth in the sampled ponds occurred in the range of pH 5.9-6.2. At pH of 5.5, the total amphibian count was 0 and at pH of 5.4, the total count was still low. This data is in accord with previous investigations, which have shown that acidic breeding sites contain lower densities of amphibian assemblages than sites with higher pH (Alford and Richards). In addition to density effects, lower pH has been shown to have sublethal effects, including "reduced larval body size, disturbed swimming behavior, and slower growth rates resulting from reduced response to, and capture of, prey" (Alford and Richards). These sublethal effects were not observed, but may appear in future years of the study.

The increase in UV-B is likely to be largest in vernal pond OTT 2 because it is slated to be clear-cut and, thus, will have direct exposure to the sun. This possibility is especially troubling because this site was home to the greatest number of species (2) and the greatest total number of individuals (Figure 3) of any vernal site in the Ottawa during both sampling periods. So, if detrimental effects do result from increased exposure to UV-B, the largest amphibian sink in the proposed logging region will be affected. Direct measurement of UV-B intensity before and after the logging, as well as continuing research as to the effects of UV-B light on larval amphibian viability, should be conducted because this variable could have a dramatic effect on overall amphibian survival.

Habitat fragmentation is another important consideration in planning the logging of portions of the Ottawa National Forest. de Maynadier and Hunter showed that

> for amphibians that breed in temporary pools, juvenile emigration is an important life-history movement linking the aquatic habitat of larvae to the surrounding upland habitats occupied by maturing animals and adults. [And, furthermore, that] juvenile wood frogs [(*R. sylvatica*)] showed an emigration preference for closed-canopy habitat immediately upon metamorphosis, with the highest capture rates occurring in microhabitats characterized by dense foliage in both the understory and canopy layers (1999).

The findings that amphibians migrate to surrounding areas and are positively connected with upland and wetland forests have been confirmed by other studies, such as that of Knutson et al. (1999). These results show that amphibian conservation is not a simple matter of preserving the tree stand immediately around the vernal ponds. Rather, there must be a large tract of trees preserved that extends away from the vernal ponds. Amphibians would be most viable if this preserve extended into other wetland areas and consisted of mature hardwoods (Mitchell et al.). Additionally, the path that the loggers take must not interfere with the preserved area, so as not to disrupt emigration patterns.

The objective of this study is to provide data that will enable a sound biologically based management strategy to be developed for the logging project in the Ottawa National Forest. While predictions and recommendations have been made in this report using proven data from other experiments, it is important to continue to monitor the vernal pond sites in the Ottawa and collect accurate information. For, as previous studies have shown, environmental changes that cause one species of amphibians to decline in one region will not necessarily be indicative of the response that another amphibian species will undergo to the same environmental change in the same or different region. By monitoring the changes specific for species in the Ottawa, not only will we improve decisions for this particular project, but we will also be in a better position to make recommendations for other projects in the Midwest, which share the same kinds of species.

Acknowledgements

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