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EDUCATION SERIES
REPORT 06-03

Amphibian Research in the Elwha River Basin

PENINSULA COLLEGE

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Acknowledgements

The Lower Elwha Klallam Tribe, Peninsula College, and the National Science Foundation's REU Program contributed to our efforts associated with this study.

Some of the material in this report is based upon work supported by the National Science Foundation under Grant No. #0443527, awarded to Peninsula College. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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Introduction

The Elwha River is the site of the largest restoration project involving dam removal in US history. A number of studies are being conducted to assess the impact of removal of the Elwha dams on plants and animals and the role of marine-derived nutrients in the ecosystem. As part of a long term, before/after monitoring program, two amphibian projects were conducted during the summer of 2006. The first project addressed the feasibility of using spot pattern as a marking tool for mark-recapture studies of red-legged frogs (Rana aurora). The second was a survey of larval amphibians in ponds and side-channels. As part of a long-term amphibian monitoring program being conducted by the USGS, Peninsula College faculty, and NSF REU student researchers, a number of research sites along the Elwha River have been identified. These sites include locations below the dams, between the dams, and above the dams. At these sites, all pools within 100 meters of the river in a half-kilometer stretch were located and GPS coordinates were obtained. At these pools, larval amphibians are surveyed and tissue samples are taken for future marine-derived nutrient analyses.

Frog Mark-Recapture Methods

A site in the lower Elwha River basin was selected for this study. The study site includes a series of four vernal pools located in a side channel of the river on the Lower Elwha Klallam Reservation. During peak flow, these pools are an active side channel of the river. During late summer, the pools completely dry up. The study was conducted in July and August, when the pools were in the process of drying and visible differences in pool size were evident from day to day. These pools were sampled for larval amphibians earlier in the year, yielding no larvae. The pools were, however, populated by many coho salmon (Oncorhynchus kisutch) which likely consumed any larval amphibians. During larvae surveys, a large number of adult red-legged frogs were encountered and this population was selected for a simple mark-recapture study. Frogs can be marked in a number of ways, including toe clipping, branding, and insertion of a variety of tags. We hoped to test the feasibility of using natural spot patterns to identify individuals and the effectiveness of using this method with researchers with no prior training. Frogs were sampled twice, with two days between sample periods. Frogs were caught by hand. Frogs were weighed to the nearest gram with a Pesola spring scale and snout-vent length (SVL) was measured to the nearest mm with a small ruler. Frogs were then photographed, and released. Photos were taken with a 6.3 mp camera of the dorsal surface of the head and torso (Figures 1 and 2). Photographs, labeled with an identification number, SVL, and mass, were examined by two experienced researchers (Hauge and Epler) to determine if any of the frogs captured in the first sample were recaptured in the second sample. The eleven students were then divided into four teams (2-3 students per team) and each team was presented with the two sets of photographs to determine if they could successfully identify individuals that appeared in both samples. Teams were instructed to identify matches between the samples and indicate their "evidence" for making each match.

Figure 1

Frog Mark-Recapture Results

Figure 2

In all, 30 frogs were captured. The first sample consisted of eight frogs, the second of 22 frogs. The experienced researchers, using spot pattern, SVL, and mass) determined that seven of the frogs from the first sample were recaptured in the second sample. A simple population estimate was calculated using the Peterson Method with the Seber modification to reduce bias (Krebs, 1999). The following formula was used to obtain a point estimate for the population size:

$$N = \frac{(M + 1)(C + 1)}{(R + 1)} - 1$$

Where N is the population estimate, M is the number of marked individuals (number of individuals caught in the first sample = 8), C is the number of individuals caught in the second sample (22), and R is the number of recaptures (7). This yielded a population estimate of 25 frogs. We also calculated 95% confidence intervals using the binomial method (Krebs, 1999). This resulted in an estimated range of 15-62 individuals in the population. It was noted that this method of population estimation assumes a closed population, an assumption which is clearly violated at this particular study site.

When presented with the photographs, the four teams of novice researchers did not successfully identify every individual that appeared in both samples. Two teams failed to recognize one frog, one team failed to recognize two frogs, and one team failed to recognize three frogs. After the experienced researchers demonstrated how they matched frogs from the photos (such as by unique spots distinctive for size, shape, and location on the frog), each team successfully matched all seven recaptures.

Frog Mark-Recapture Conclusions

It was determined that spot pattern could be used for individual identification in a short-term mark-recapture study of R. aurora. We do not know at this time if spot pattern changes markedly over longer periods of time (such as months or years). The population estimate and confidence intervals were calculated as an exercise in methods and, since the population in question is not a closed population, have little practical value. Failure of teams of novice researchers to initially identify each recapture (and success after experienced researchers demonstrated how they did it) indicates that experience and practice play a role in successfully using this method.

Larval Amphibian Survey Methods

Amphibian larvae are sampled using un-baited, collapsible, nylon-mesh minnow traps placed in a manner that allows any adult amphibians captured to obtain air. The number of traps used is a function of pool size, with one trap being placed for approximately every 2-3 meters of shoreline. Traps are removed one day after being set. Water temperature is also recorded for each pool. Students taking part in this portion of the study set traps in four locations, one below the dams (35 traps), two between the dams (10 traps each), and one above the dams (40 traps). Larval amphibians are identified, measured to the nearest mm (SVL), and weighed to the nearest gram. The first specimen for each species at a study site is sacrificed using MS222 and saved. Tissue samples in the form of tail tips are collected from the next 20 subsequent specimens captured at a site. Tissue samples are stored in 95% ethanol in the field, then transferred into distilled water and frozen. Adult amphibians are identified, sexed (where possible), measured (SVL), and weighed. Tissue samples are not collected from adults as only those individuals (in this case, larvae) with a direct connection to the aquatic environment will be used for marine-derived nutrient analyses. In addition to amphibians, these traps capture insects and fish. Insects are discarded, while fish are identified and counted. Finally, the research group conducted a sweep survey of a 0.5 km stretch of the Elwha River (middle river, 14 km from the mouth) to search for pools appropriate for amphibian reproduction.

Larval Amphibian Survey Results

Of the four pools surveyed, only one yielded larval amphibians. This pool, in the lower Elwha drainage 1-1.5 km from the mouth of the river, yielded 24 larval northwestern salamanders (Ambystoma gracile), or 0.69 larvae per trap day. As the survey team who located the pools in this site had already collected a voucher specimen, none of the 24 individuals captured were sacrificed. Tissue samples in the form of tail tips were collected from 20 individuals as per protocol. Average SVL of salamanders captured was 43mm and average mass was 5.16 g. Two adult R. aurora were also captured (0.06 per trap day). In addition to amphibians, two fish species were captured. A total of 105 three-spined sticklebacks (Gasterosteus aculeatus) or 3.0 per trap day and three unidentified lampreys (Lampetra sp.) or 0.09 per trap day were captured. No salmon were captured in this pool. One of the pools in the middle river (17.5-18 km from the mouth) yielded nothing, while the other (18.5-19 km from the mouth) yielded a single adult rough-skinned newt (Taricha granulosa) or 0.1 per trap day. The traps placed above the dams (along the shore of Lake Mills, 20.5-21 km from the mouth) yielded no larval amphibians, but 4 unidentified sculpin (Cottus sp.) or 0.1 per trap day and seven rainbow trout (Onchorhynchus mykiss) or 0.18 per trap day were captured. The sweep survey conducted by the research team located only dried pools, none appropriate for amphibian reproduction.

Larval Amphibian Survey Conclusions

One conclusion reached by this team was that not all pools and ponds along the Elwha River appear to be appropriate habitat for larval amphibians. Some pools which are active side channels of the river during some times of the year are bordered by rocky substrate that may be difficult for adult salamanders to cross, thus they do not lay eggs there. These pools also are frequently populated with trout or salmon. Where trout or salmon are common, few larval amphibians are found. This is probably due to predation by the fish. The presence of three-spined sticklebacks appears to have little effect on larval amphibian populations. Marine-derived nutrient analyses have not yet been conducted, so we do not yet have data regarding the importance of salmon to amphibians. The sweep survey our research team conducted along a 0.5 km stretch of the river demonstrated how difficult such a survey is to do. In just over an hour, the 13 person team searched a 0.5 km long by 50-100 m wide section of uneven ground littered with heavy brush, boulders, downed trees, stinging nettles, and thorny plants. We located no pools appropriate for amphibian reproduction.

Overall Educational Objectives

Elwha High School Students taking part in this REU experience were exposed to the following methods, skills, and learning objectives:

- Teamwork skills
- Learn about the influence of marine-derived nutrients in a river system such as the Elwha
- Capture methods for amphibians, including trap and equipment cleaning protocols to avoid the spread of pathogens between study sites
- Data collection appropriate for field studies of larval and adult amphibians
- Amphibian and fish identification using dichotomous keys and field guides
- How to conduct a mark-recapture study
- A simple method for estimating population size
- Methods for collecting tissue samples from larval amphibians
- How biologists search an area for pools appropriate for amphibian reproduction

Comments made by students in the final reports used to synthesize this paper include:

- "…I have learned a lot of facts and methods I did not know before the Huxley/PC program."
- "Working with the amphibians was an awesome experience for me."
- "The reason we are doing this is because we are part of the river restoration project. The point of the Elwha River Restoration Project is to find and collect data about wildlife, water, and vegetation before the dams are removed and then collect the same data after they are removed and compare the two to determine just how much of an impact the dams are making on the wildlife."
- "I have learned a lot from working out in the field. I learned how to set traps of many kinds. I basically learned what is in my own back yard such as ponds and then what is in them."
- "…I really enjoyed it and learned a lot. Even though there were some parts that were hard for me, but I made it…I am glad that I have been given this opportunity."
- "This part of the job helped me understand how the river is now. We will compare our data after the dam is removed to see if anything is changed."

Literature Cited

Krebs, C.J. 1999. Ecological Methodology. Addison-Wesley Educational Publishers