AQUATIC AND TERRESTRIAL MOVEMENTS OF TAILED FROGS (*ASCAPHUS TRUEI*) IN RELATION TO TIMBER HARVEST IN COASTAL BRITISH COLUMBIA

by

TANYA RHODA WAHBE

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M.Sc., The University of British Columbia, 1996

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Department of Forest Science

The University of British Columbia Vancouver, Canada

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ABSTRACT

In British Columbia, Oregon, and California, coastal *Ascaphus* populations are designated 'at risk.' Local extirpation is a concern because recolonization may be slow, even if post-logging habitats recover quickly. In coastal BC streams flowing through clearcuts, second growth, and old growth, I investigated movements of larval *Ascaphus* and associations with stream parameters using area-constrained stream surveys. In old growth, larvae moved seven times farther than in clearcuts. Embedded logs, abundant in clearcut streams, may explain shorter larval movements.

Using pitfall traps, I examined terrestrial movements of *Ascaphus* in clearcuts and old growth. More juveniles were trapped in clearcuts but more mature adults were trapped in old growth, suggesting fewer effective migrants in clearcuts. Many frogs moved at least 100 m from streams, and exhibited weaker stream affinity compared to inland *Ascaphus* that moved at least 12 m. In fall, I trapped frogs farther from streams in old growth than in clearcuts, and more frogs were trapped within 25 m of streams in clearcuts. Long distance overland movements appear more likely where forested stands are present.

Using RAPDs, I examined population genetic structure of *Ascaphus* in an old growth and clearcut stream. In the clearcut, larvae were less diverse than in the old growth and exhibited no relationship between physical distance and genetic relatedness. In the old growth, larvae decreased in genetic similarity with increasing physical distance. Lower heterozygosity in the clearcut suggests that *Ascaphus* may be less able to adapt to environmental fluctuations and more susceptible to disease than larvae in the old growth.

Maintaining viable populations throughout the range of *Ascaphus* is an underlying assumption of my thesis. My results suggest reduced recolonization potential and lower genetic variation where forest cover is absent. Aggregations of *Ascaphus* at individual streams may not represent distinct populations, and should not be managed as distinct units. Connectivity between multiple streams within a watershed will be a more meaningful unit of management than individual streams with forested buffers. Conservation measures more likely to promote long-term population persistence should be considered, such as the retention of a partial forest matrix between streams.

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CHAPTER ONE
General Introduction

Forests are structurally complex ecosystems that tend to contain greater vertebrate species richness than other terrestrial biomes (Wilson 1988; Bunnell and Kremsater 1990). Timber harvest can significantly alter the structure and function of forests, and in the temperate zone, habitat loss and forest fragmentation are major threats to species (Soulé 1991; Caughley and Gunn 1996). Amphibians are particularly susceptible to removal of forest canopy because of their physiological requirements for cool, moist conditions. In many forests, amphibians are the most abundant vertebrate group (Burton and Likens 1975*a*; Crisafulli and Hawkins 1998), and some workers argue they may play a key role in ecosystem dynamics (deMaynadier and Hunter 1995). Although little experimental evidence for the role of amphibians in forest ecosystems exists, indirect evidence suggests amphibians may play a significant role in nutrient cycling (Burton and Likens 1975*b*; Hairston 1987) and food web dynamics (Jaeger 1972; Burton and Likens 1975*b*; Fraser 1976). Thus, forest management practices that modify the density and distribution of amphibians may have important implications for forest function and productivity (Bormann and Likens 1979).

Early signs of ecosystem dysfunction usually appear at the level of populations, affecting species with narrow ecological tolerances (Odum 1992). Numerous correlative studies have shown that the abundance of terrestrial and aquatic amphibian species is lower in harvested forests compared to mature forests in both western (Bury 1983; Bury and Corn 1988*a*; Dupuis and Bunnell 1999; Grialou *et al.* 2000) and eastern (Pough *et al.* 1987; Ash 1988; Waldick 1997) North America. Because of their biphasic life histories (water and land), specific habitat requirements (Blaustein *et al.* 1994), longevity, and strong site fidelity compared with most vertebrates, amphibians are especially sensitive to environmental changes in ecosystems and may serve as valuable indicators of ecosystem dysfunction.

There is limited knowledge about the ecological role, population ecology and life history of amphibians in Pacific Northwest forest systems. For some species, the effects of forest management have begun to be addressed, but for many species with complex life histories, we know little about them and the potential effects of timber management. For my

research, I selected the tailed frog (*Ascaphus truei* Stejneger) as the focal species for three reasons.

1) *Ascaphus truei* has specialized habitat requirements and is of considerable conservation interest. They are considered "vulnerable" in coastal British Columbia (BC Conservation Data Centre 2001), Oregon (Oregon Natural Heritage Program 2001), and California (California Natural Heritage Program 2001).
2) Current recommendations for protection of the species with riparian forest buffers is based on larval habitat needs, but data on the effects of timber harvest on larval population abundance and density are contradictory or dependent on geographic location. Also, available evidence from inland populations indicates that adults are long lived and have strong site fidelity, remaining within a narrow range of 0–20 m around streams (Daugherty and Sheldon 1982*a*). Movement potential appears low (Daugherty and Sheldon 1982*a*; Bury and Corn 1988*a*). Research on post-metamorphic *Ascaphus* is sparse, and management strategies fail to incorporate half of the life history. Thus, management efforts to protect *Ascaphus* populations in areas subject to timber harvest may not be effective.
3) Species richness in riparian areas of small streams is usually higher than upslope areas (Bunnell and Dupuis 1994), hence other species could benefit from protection of *Ascaphus* habitat were that found to be important to continued survival of *Ascaphus* populations.

Ascaphus is endemic to the Pacific Northwest of North America. It is the only representative of the family Ascaphidae (Nussbaum *et al.* 1983), and is a highly distinct lineage, perhaps a sister group to other anurans (Ford and Cannatella 1993; Jamieson *et al.* 1993). It is believed to be the most primitive frog living due to the retention of many primitive morphological features that were lost in most extant anuran lineages (Ford and Cannatella 1993). Primitive features include the possession of ribs, short arms on sternum, tail-wagging muscles, and nine vertebrae in front of the sacrum. The only other living frogs as primitive as *Ascaphus* are three species in the genus *Leiopelma* (family Leiopelmatidae, terrestrial-breeding frogs) of New Zealand (Green and Cannatella 1993), but the two groups may not be closely related (Green *et al.* 1989; Green and Campbell 1992).

Ascaphus range from British Columbia (BC) to western Montana, between the Pacific Coast and the Rocky Mountains, and southward to northern California (Corkran and Thoms 1996). *Ascaphus* are found in mountainous, coniferous forests in cool, fast-flowing, perennial streams that usually lack fish (e.g., Figure 1.1). The larvae (Figure 1.2) have a

large suctorial mouth to cling to rocks, an adaptation for their stream environment (Bury 1988; Welsh 1990). *Ascaphus* range in elevation from near sea level to above 2,140 m (Nussbaum *et al.* 1983; Leonard *et al.* 1993; Corkran and Thoms 1996) but, in coastal BC, *Ascaphus* occurs from near sea level to subalpine zones (30–1,100 m; Sutherland 2000).

Habitat structure is reported to influence the occurrence and abundance of terrestrial (deMaynadier and Hunter 1995; Aubry 1997) and stream amphibians (Welsh and Ollivier 1998; Sutherland and Bunnell 2001). Several researchers have documented negative effects of forest harvesting activities on population density and relative abundance of *Ascaphus* larvae (Bury 1983; Bury *et al.* 1991; Corn and Bury 1989; Welsh and Lind 1991; Dupuis and Steventon 1999). *Ascaphus* are believed to be sensitive to habitat alteration because of their lengthy larval period of 1–5 years (Wahbe 1996; Wallace and Diller 1998; Bury and Adams 1999), small clutch size of 30–70 eggs (Metter 1967; Leonard *et al.* 1993), biennial reproduction at high elevation, inland sites (Rocky Mountains, Metter 1967; reproduction may be annual elsewhere, Bury *et al.* 2001), low recolonization potential (with the exception of Crisafulli and Hawkins 1998), and specialized characteristics of their habitats (Bury and Corn 1988*a*; Hawkins *et al.* 1988). They also have a low desiccation tolerance (Claussen 1973) and one of the lowest and narrowest temperature tolerances among anurans (Brattstrom 1963; de Vlaming and Bury 1970). Also, recent metamorphs are more vulnerable to desiccation and predation than adults (Jones and Raphael 1998).

Figure 1.1. Stream flowing through old-growth forest. Photo: T. Wahbe.

Figure 1.2. Larval and adult male *Ascaphus*. Photo: T. Wahbe.

Ascaphus in the Rocky Mountains may live 15 to 20 years (Daugherty and Sheldon 1982*b*) but do not reach reproductive maturity until they are seven or eight years of age (Daugherty and Sheldon 1982*b*; Brown 1990). *Ascaphus* breed in the fall (Metter 1964*a*;

Nussbaum *et al.* 1983). However, unlike most North American frogs, breeding migrations of *Ascaphus* (i.e., from stream to stream, or upland to stream) have not been reported. *Ascaphus* differ from most Pacific Northwest frogs in that fertilization is internal. Male *Ascaphus* possess an extended cloaca (misnamed the tail; Figure 1.2), which allows internal fertilization of the eggs. No other anuran is known to engage in copulation (Stebbins and Cohen 1995). Females (Appendix III) can retain sperm through winter (Metter 1964*b*). In mid-summer (after spring runoff), female *Ascaphus* attach strings of eggs to the undersides of rocky substrate (Brown 1975; Adams 1993; Karraker and Beyersdorf 1997; Bury *et al.* 2001). *Ascaphus* eggs are slow to develop, averaging six weeks to hatching (Brown 1975). Free-swimming larvae emerge from late August to September (Metter 1964*a*; Adams 1993), often overwinter at the nest site (Metter 1964*a*), and feed on the yolk sac (Brown 1990) until their first spring when a suctorial mouth fully develops (Metter 1964*a*). In general, time to metamorphosis varies with geographic location (Bury and Adams 1999) and elevation. Larvae in most populations metamorphose after 2–3 years (Metter 1967), but may take 4–5 years in high elevation or northern locales (Brown 1990; Wahbe 1996), or only one year in coastal areas in the southernmost portion of their range (Wallace and Diller 1998; Bury and Adams 1999). These life history traits suggest that *Ascaphus* are slow to reach maturity and best persist in relatively stable habitats (i.e., old-growth forests).

Many amphibian species live in habitats that are altered or fragmented by human activities. Because of their permeable skin, most amphibians move relatively short distances and have limited dispersal capabilities compared to other vertebrates (Sinsch 1990). Some authors (e.g., Welsh 1990) have argued that recolonization of logged sites is critical to sustaining productive amphibian populations, but movement may be impeded in altered habitats. Blaustein *et al.* (1994) suggested that recolonization of sites following local extinction may be difficult for amphibians because: 1) physiological constraints limit amphibians to cool, moist habitats, 2) many amphibians move only short distances, and 3) many amphibian species show extreme site fidelity. Thus, when local amphibian populations become extinct, they may be less likely to recover than are other tetrapods.

Movement patterns can indicate the dispersal ability of individuals (Daugherty and Sheldon 1982*a*), and the potential for recolonization of disturbed areas (Kramer *et al.* 1993). Information on the movement rates of organisms also is critical for predicting extinction thresholds (Diffendorfer 1998; Fahrig 2001). In partially harvested watersheds, recolonization by *Ascaphus* will be important for continued regional persistence. However, habitat fragmentation may create barriers to their dispersal. Molecular studies suggest that

gene flow among *Ascaphus* populations is low (Pauken and Metter 1971; Ritland *et al.* 2000; Neilson *et al.* 2001), but processes governing genetic structure render interpretations of small spatial scales from available genetic results uncertain (Wahbe *et al.* 2001). Direct measures of movement could be more revealing. Understanding the movement patterns and dispersal abilities of *Ascaphus* could lead to improved conservation actions for this and other riparian obligates, especially in managed forest landscapes.

In the following chapters, I use three different approaches to examine the recolonization potential of *Ascaphus* in managed and unmanaged forests. In Chapter 2, I examine the instream movements of larval *Ascaphus*. It is unknown how stream-dwelling larvae may contribute to the dispersal of *Ascaphus*. However, downstream movements would be energy-efficient, because they can be passive with the stream current. I ask how movement rates of larvae differ between managed and unmanaged forests, and explore the relationship between stream and site parameters and dispersal ability among three treatments: old-growth forests, mature second-growth stands, and clearcuts.

Ascaphus movements important in dispersal may occur laterally and overland. In Chapter 3, I examine terrestrial movements of juvenile and adult *Ascaphus*. I begin by addressing differences in abundance and proportions of each developmental stage in clearcuts and old growth. I ask how stream affinity and patterns of movement differ between clearcuts and old growth, compare findings with evidence from inland populations, and explore differences in movement patterns among developmental stages and sexes.

Habitat fragmentation can play a significant role in limiting dispersal and gene flow. In Chapter 4, I use RAPDs to examine population genetic structure of *Ascaphus* in two streams within a single watershed. I use larval *Ascaphus* tissue to compare genetic variation in streams flowing through a clearcut and an old-growth forest. Also, I examine the relationship between genetic relatedness and physical distances within each stream.

Maintaining viable populations throughout the range of *Ascaphus* is an underlying assumption of my thesis. The general context for my research is the issue of fragmentation via timber harvesting and whether it reduces recolonization potential of *Ascaphus*. In the final chapter, I synthesize the components of my thesis and summarize my major findings. Based on available data, and recognizing that uncertainties exist, I discuss some implications to conservation, provide habitat protection options that may better protect *Ascaphus* populations and other species dependent on headwater stream habitats, and recommend directions for future research.

CHAPTER TWO

Movements of Larval *Ascaphus*

INTRODUCTION

Recolonization of habitats vacated by local extinction is problematic for amphibians because of their physiological constraints and movement behaviors (Blaustein *et al.* 1994). Many factors can influence the recolonization potential of amphibians. For example, the mountain yellow-legged frog (*Rana muscosa*) has become extinct at many high elevation sites in the Sierra Nevada Mountains of California. Bradford (1991) suggested that recolonization might never occur because streams connecting extant populations are inhabited by introduced fish that eat tadpoles. Forest practices (e.g., clearcut harvest) often have negative impacts on adult and larval *Ascaphus* (Metter 1968; Bury 1988; Welsh 1990) due to a reduction in soil moisture and increases in temperature, sedimentation, and solar radiation. Amphibians require moist, cool conditions to avoid desiccation, thus the removal of forest cover may lead to a reduction in the recolonization potential of disturbed habitats for some species. Corn and Bury (1989) reported no detection or low numbers of *Ascaphus* in streams 14–40 years after clearcut harvest. At inland sites, Daugherty and Sheldon (1982*a*) found that 50% of reproductively mature *Ascaphus* remained in the same 20–m area of their previous capture and concluded that adult *Ascaphus* exhibit extreme site fidelity. Given the long larval stage of *Ascaphus*, movements by larvae may be particularly important to recolonization of managed forests. After clearcutting, however, small streams often contain logging debris and sedimentation that could impede movement and recolonization by *Ascaphus* larvae.

Few laboratory studies have examined the movements of larval amphibians. Anholt *et al.* (2000) reported reduced larval ranid frog activity with increased predator density and food availability, and greater activity among larger animals. In stream-breeding salamander larvae, Sih *et al.* (2000) reported ineffective anti-predator behavior (high emergence rate from refugia, and high activity while out of refuge) and high predation by sunfish. I found no published studies addressing movements of larval amphibians in streams in western North America.

In this chapter, I investigate larval *Ascaphus* movement rates and assess influences of stream parameters on instream movements in managed and unmanaged forests. I test hypotheses based on three predictions: (1) I predicted that larval movement rates in recently harvested forests are lower than in unmanaged forests; (2) Because stream gradient, which is related to flow rate, may influence larval movements, I predicted that larval movements are less in streams where logjams are abundant; and (3) Post-harvest volumes of woody debris in

streams have been reported to increase three times over pre-harvest levels (Baillie *et al.* 1999). Because embedded logs may influence larval movement rates, I predicted that larval movements are greater in high gradient streams.

STUDY AREA

I conducted my study near Squamish in southwestern British Columbia (49° N, 122° W) within forests dominated by western hemlock (*Tsuga heterophylla*). Sites were distributed within four basins that drain into Howe Sound (Ashlu, Elaho, Mamquam, and Squamish Rivers; see Appendix III), and included reaches transecting old-growth forest (250+ years old), mature second-growth forest (60–80 years since logging) and recent clearcuts (~5 years since logging). These sites are hereafter referred to in this thesis as old-growth streams, second-growth streams, and clearcut streams. In reaches designated as 'old growth,' the predominant forest type in the watershed upstream was old-growth forest. In reaches designated as 'second growth,' the predominant forest type in the watershed upstream was mature second-growth forest. And, in reaches designated as 'clearcut,' the predominant forest type in the watershed upstream was clearcut (5–10 years since logging). Reaches within each basin were located on three separate tributaries, with predominant forest cover type being old growth, mature second growth, or clearcut. In 1996, the Squamish clearcut site was replaced with the Ashlu clearcut site due to loss of road access. Three replicates of each forest cover type were selected. The nine streams, approximately 3 m in wetted width, were selected on the basis of larval presence, and were chosen upstream (except Ashlu and Mamquam clearcut sites) from logging roads.

METHODS

Stream Surveys and Parameters

I determined larval movement patterns of *Ascaphus* using area-constrained stream surveys (adapted from Bury and Corn 1991 and Shaffer *et al.* 1994) together with mark-recapture techniques. Three 5–m sections (reaches) per stream were selected 25 m apart. Location of the first downstream reach was randomly selected. Larvae were marked in these three reaches. To maximize number of recaptures, two additional 10–m reaches were added directly below the second and third 5–m reaches (Figure 2.1). A 10–m reach was not added below reach 1 because I sought to maintain a distance of 50 m from logging roads or clearcut edges where appropriate. Area-constrained searches were conducted in all stream reaches by scanning the stream for surface-active larvae, then slowly moving up the stream turning and brushing undersides of rocks and capturing larvae with dipnets as they became dislodged. Stream searches were conducted between 0700–2200 hrs, taking three people roughly 3 hrs per stream, with an additional 3 hrs to process larvae and record stream parameters.

Initial stream surveys were conducted in June and early July of 1995 and 1996. Each stream was resurveyed once in July and once in August (approximately 20 days apart), and yielded information on the extent of larval movements. Three sizes of dipnets each with 1–mm mesh were used for sampling; appropriate net size (width of net: small, medium, large) was based on dominant stream substrate size. No seines were placed at the lower end of stream reaches.

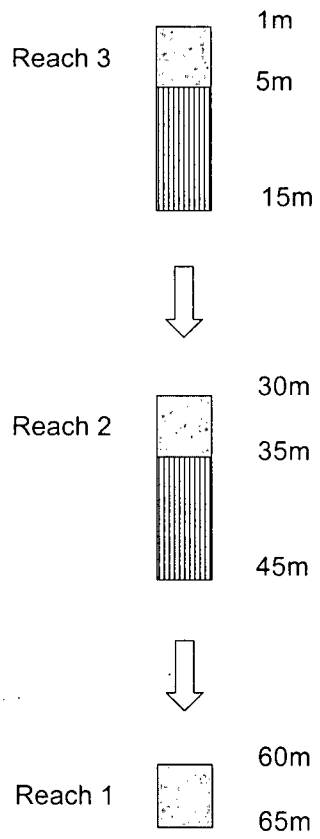

Figure 2.1. Sampling scheme: Initial marking occurred in three 5–m reaches (shaded) separated by 25 m; sampling for recaptures included two additional 10–m reaches (hatched). In the text, recaptures noted for reaches 2 and 3 include the contiguous 10–m reaches. Arrows indicate direction of stream flow.

In July of 1995, 1996, and 1999, I measured stream parameters I thought could influence the movement of larvae: stream gradient (using a clinometer), logs embedded in the stream (logjams; visually estimated and ranked low, medium or high), stream wetted width (meter tape), and canopy closure (visually estimated as percent cover).

Mark-Recapture

Larvae captured in the three 5–m reaches were marked by fin clipping with a code unique-to-reach (e.g., two notches for individuals captured in reach two). Taking special care not to cut into the central axis of caudal muscle, the dorsal fin of the tail was marked with "V"-shaped notches following Turner (1960). Notches were visible for at least one month and allowed for identification of recaptures by reach in subsequent stream surveys within that period. After a reach had been searched, I marked larvae, recorded the number of notches given, measured the distance from the upstream end of the reach, replaced disturbed rocks, and released larvae less than one meter upstream from the reach of capture.

I kept 25 marked larvae (three notches each) in a lab enclosure to evaluate potential impacts of marking on larval survival. Lab conditions closely mimicked field conditions: 6–hr light : 18–hr dark cycle (using timer and fluorescent lamp). An aquarium of fresh stream water and algae-covered rocks, collected weekly from known *Ascaphus* streams, was maintained at 5 ^{o}C and oxygenated with an air pump.

Statistical Analyses

Each of the nine streams was treated as an experimental unit for statistical analyses. I used the statistics package SPSS® for Windows® (SPSS Inc., Chicago, Illinois, U.S.A.) to run analyses on mean values per experimental unit (i.e., nine treatment means) for the 737 *Ascaphus* larvae captured during three years. I evaluated potential patterns in initial capture data using Kendall's coefficient of concordance, where a coefficient of 1.0 indicates complete concordance among ranks. I used a two-way analysis of variance (ANOVA) to test differences in distance moved by larvae among old-growth, second-growth, and clearcut streams. The randomized complete block design included three rivers (as blocks; 2 degrees of freedom; Elaho, Squamish, Mamquam), and three forest cover types (2 degrees of freedom; old growth, mature second growth, clearcut). The design is unbalanced because of two missing observations (no recapture data in Squamish and Elaho watersheds), leaving a total of 2 degrees of freedom for experimental error. These data, however, could be evaluated by the General Linear Model Factorial Procedure (Norusis 1993). To explain variation in larval movement rates and recapture rates, I used the forward selection method for multiple linear regression analyses. To evaluate the effect of logjams, I used Spearman's rank correlation procedure (Spearman's rho; Zar 1984). All statistics were tested against a preset significance level of $\alpha = 0.05$. The $\pm$ values reported indicate the standard error of the mean.

RESULTS

Mortality Due to Marking

During the first six-week period, no mortality was observed in larvae given three tailfin notches and kept in the laboratory. In week seven, three larvae (12%) were found dead in the aquarium. The remaining 22 larvae lived for another five weeks. I could not determine whether their death was related to tailfin notching, handling stress, disease or unnatural living conditions. However, time to any mortality appeared long enough that marking itself would not have influenced recapture estimates.

Recapture Rates

During the initial searches in June and July, I captured and marked 737 larvae. During July and August, I recaptured 52 larvae (7% recapture rate). Although recapture numbers were low, larval movements differed by forest cover type with greater movement rates in old growth than in clearcuts (Table 2.1). Only distances of 0 to 15 m, 30 to 45 m or 60 to 65 m could be detected because of the two stretches (15 m each) of unsampled stream between reaches (Figure 2.1).

Captures of larvae were unevenly distributed across stream reaches. This had the potential to bias results if, for example, most larvae in clearcut streams were initially captured in reach 1. However, within any forest cover type, I failed to detect a significant difference in the proportion of initial captures across reaches 1 through 3 (Kendall's coefficient of concordance = 0.86; asymptotic significance = 0.051). Consistent with movement from upper to lower reaches, rates of recapture differed among reaches (Kendall's coefficient of concordance = 0.149; asymptotic significance = 0.719). In streams flowing through old growth and second growth, recapture rates in the two lower reaches combined (1 and 2 of Figure 2.1) were 12.6% and 10.7%, respectively; recapture rates in reach 3 were 2.5% and 2.4%, respectively. Conversely, in clearcuts where movements were shorter, the recapture rate in reach 3 was 10.5% but only 3.7% for the lower two reaches (consistent with less drift from the upper reaches).

Table 2.1. Larval *Ascaphus* movements (m/day) and stream parameters in southwestern British Columbia. 1995–1996 and 1999. Means are in parentheses. Logjams are ranked as 1 = low, 2 = medium, and 3 = high.

Forest Cover[1]	River	Movement Rate (m/day)		Larval Density (#/m^2)		Stream Gradient (°)		Stream pH		Stream Temp (° C)		Logs		% Canopy		Wetted Width (m)	
OG	Squamish	0.34		0.43		18.0		7.55		10.0		2.0		60		3.1	
OG	Elaho	1.32	(0.92)	0.74	(1.29)	6.0	(10.0)	7.48	(7.34)	9.7	(8.5)	2.0	(1.7)	35	(40)	2.6	(2.8)
OG	Mamquam	1.10		2.70		6.0		7.00		5.7		1.0		25		2.7	
SG	Squamish	0.36		0.85		20.0		7.45		11.5		3.0		90		2.1	
SG	Elaho	1.06	(0.53)	0.58	(0.67)	11.0	(12.0)	7.16	(7.37)	9.8	(9.8)	2.5	(2.7)	85	(90)	6.6	(3.6)
SG	Mamquam	0.16		0.59		5.0		7.50		8.1		2.5		95		2.2	
CC	Ashlu	0.19		0.52		8.5		7.65		9.5		3.0		0		2.5	
CC	Elaho	0.06	(0.13)	0.36	(0.99)	10.0	(9.2)	7.16	(7.36)	11.8	(10.0)	2.5	(2.8)	0	(0)	3.0	(2.4)
CC	Mamquam	0.15		2.08		9.0		7.28		8.8		3.0		0		1.6	

[1] OG = old growth; SG = second growth; CC = clearcut.

Movement Rates

Although larvae in old-growth streams moved about 7.1 times as far as larvae in streams flowing through clearcuts (Table 2.1), distances moved did not differ significantly among forest cover types ($F_{(2,2)} = 1.935$; $P = 0.341$). Maximum movement rates also reflected forest cover type associations. Over the average 20–day period, larvae moved up to 3.76 m/day in old-growth streams, up to 1.94 m/day in mature second-growth streams, and up to 0.30 m/day in clearcut streams. My sampling design could not detect upstream movements <15 m. However, I did observe short upstream movements of 15–30 cm during area-constrained searches.

Neither movements per day nor rate of recapture showed any relation with larval densities, which were highly variable (Table 2.1). I examined the potential influence of logjams on movement rates because all clearcuts contained abundant logjams that could have constrained movements. A multiple linear regression analysis was conducted with four stream variables (stream gradient, logjams, stream wetted width, percent canopy cover). Using the forward selection method, the only variable that was selected and was significant was logjams, which explained 13% of the variation in movement rates ($P = 0.009$). None of the other stream variables added significantly to the amount of variation in larval movement rates that was explained by logjams. Abundance of logjams was ranked higher in clearcuts than in old-growth streams. Large amounts of logs in streams found mainly in clearcuts (Figure 2.2a) were associated with lower rates of movement (Spearman's rho (r_s) = -0.507; $P = 0.001$: Figure 2.2b).

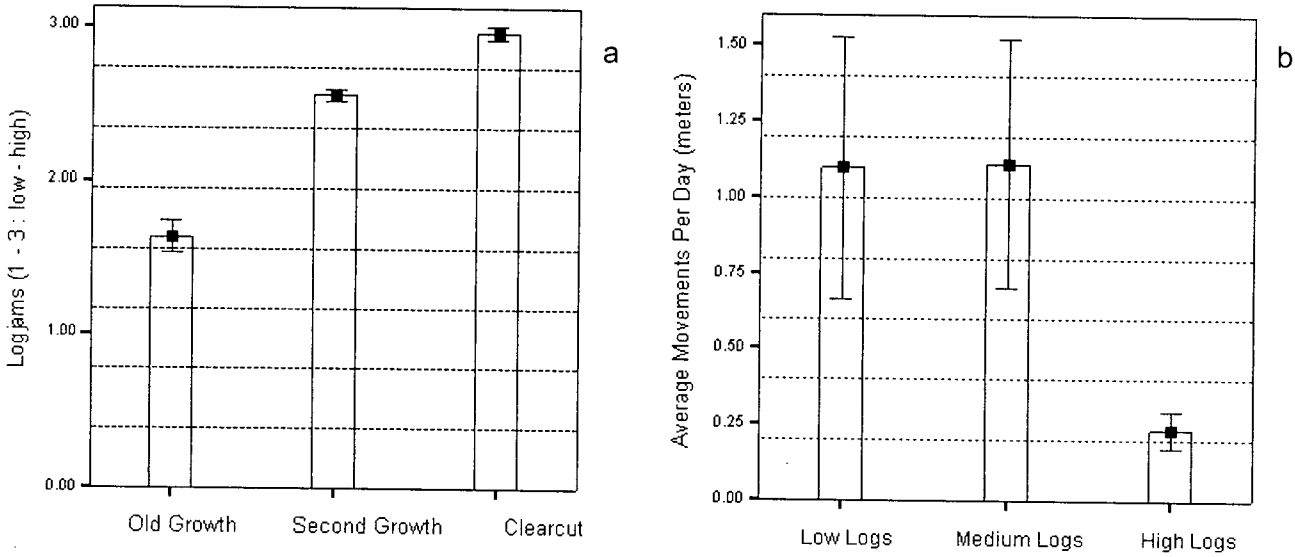

Figure 2.2. Logjams and larval movements. a) Logjams estimated in each forest cover type: old growth, second growth, and clearcut, b) Larval movements per day (m) in streams with low, medium, and high levels of logjams. Values of 2.5 (between medium and high rankings) were lumped with values of 3 because movement values were similar. Error bars represent standard error of the mean.

Because stream gradients tended to vary among treatments (Table 2.1), I tested for the effect of gradient on movement rate. Assuming a linear relationship, there was no effect of stream gradient on distance moved per day ($r^2 = 0.01$, $P = 0.643$), and the extremes of movement tended to be higher at lower gradients (Figure 2.3). Given the possibility of a factor-ceiling distribution *sensu* Thomson *et al.* (1996), there was relatively little power to test the relationship between movement per day and gradient. This inability may be due to insufficient sampling at the upper threshold rather than the result of weak relationships. Wider streams (i.e., large wetted width) were correlated with higher rates of movement (Spearman's rho, $r_s = 0.420$; $P = 0.002$). There was no relationship between percent canopy closure and larval movement rates.

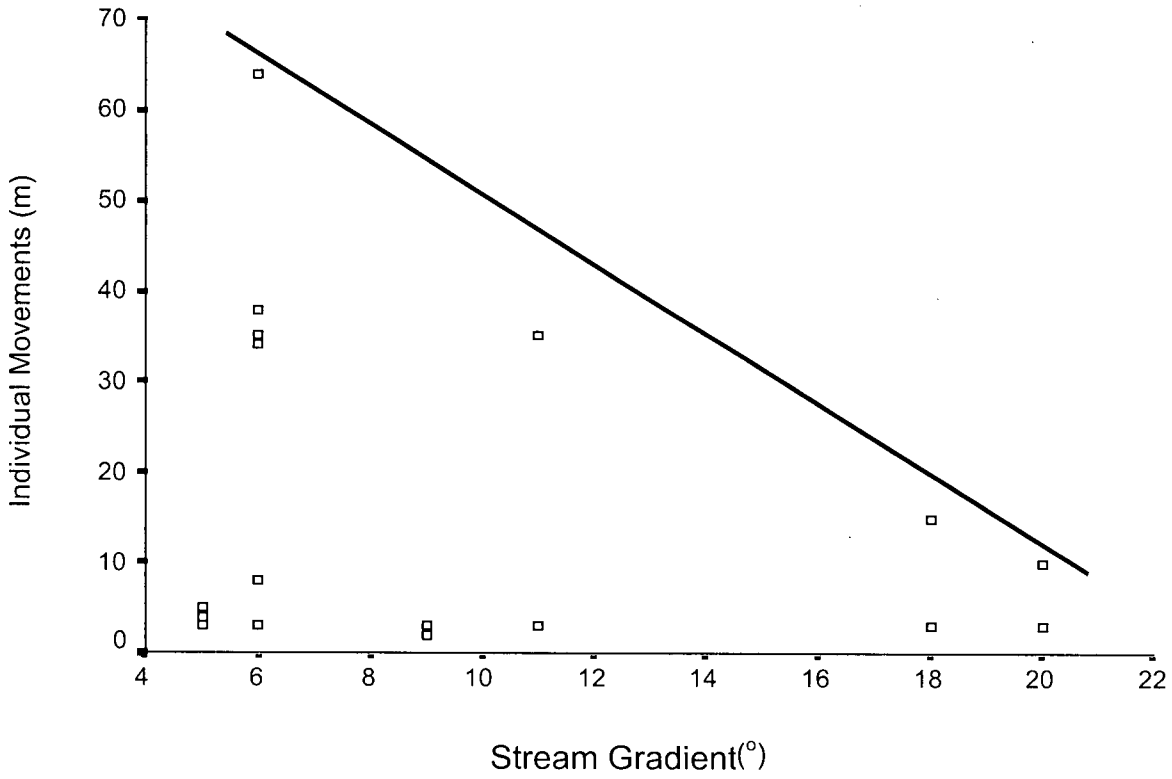

Figure 2.3. Individual movements of larvae in relation to stream gradient (some points overlap). Solid line above extreme movements represents a potential factor ceiling *sensu* Thompson *et al.* (1996).

DISCUSSION

Stream Gradient, Logjams, and Movement Rates

I detected considerable downstream movements by *Ascaphus* larvae. Larval movements were associated with forest age class, stream wetted width, gradient, and logjams. Logjams explained 13% of the variation in movement rates. Though not statistically significant, I found larval movement to be much greater in old-growth streams regardless of gradient over a range of 4 to 44%. On the basis of stream gradient, second-growth streams should have shown the greatest movements per day, followed by old growth, then clearcuts. That pattern was not apparent, and maximum movement rates tended to be inversely, but not significantly, related to gradient. These results suggest that movements by *Ascaphus* larvae are active movements rather than passive drift.

The sampled movement rates could be low because larvae moved outside the 65–m range of distances sampled. I believe that is unlikely for three reasons. First, the recapture rate is not uncommonly low (see review of dispersal studies of Sutherland *et al.* 2000). Second, at least some amphibians are documented to show strong site fidelity (Martof 1953; Stebbins 1954; Bellis 1965; Kleeberger and Werner 1982; Ovaska 1988). Third, the relative movement rates among forest cover types suggest that other factors are involved. Clearcut streams all contained abundant debris and logjams, sufficiently embedded in the stream that they could have constrained movements by serving as dispersal barriers. I found a negative relationship between distance moved and amount of logjams estimated in a stream. In some studies, post-harvest volumes of woody debris have been reported to increase three-fold on average over pre-harvest levels (Baillie *et al.* 1999). Logjams may serve to reduce drift rates and possibly the recolonization potential of *Ascaphus* larvae.

Algal Productivity and Movement Rates

Alternatively, greater biomass of algae in clearcut streams may influence rates of larval movement. *Ascaphus* are commonly found in oligotrophic streams (Brown 1990; Rosenfeld 1997), and some data suggest that larvae are food limited (Kiffney and Richardson 2001). Primary production is often directly related to stream gradient and incident radiation (McIntyre 1966). The greater influx of solar radiation in clearcut streams generally increases biomass of periphyton (see Kiffney and Bull 2000). A superior food supply in clearcut streams could result in lower movement rates of larvae. The extreme movements (potential factor ceiling of Figure 2.3) are consistent with the pattern expected from associations of primary production with gradient (shorter movements with steeper gradients and potentially greater food supply). Hawkins *et al.* (1983) suggested that the higher autotrophic production following canopy removal (or in naturally open stream sections) is responsible for higher abundances of invertebrates and stream vertebrates. When larval anurans are more active, they encounter both more food and more predators, thus activity rates mediate a trade-off between growth rates and predation risk (Anholt *et al.* 2000). Shorter movements per day in clearcuts, where incident radiation was higher and periphyton likely more abundant, also are consistent with the concept of lower food supply encouraging movement. My analyses indicate that larval movements were shorter in streams transecting clearcuts, and that these streams contained more woody debris. However, algal productivity in headwater streams transecting clearcuts and old-growth forests requires investigation.

CHAPTER THREE

Terrestrial Movements of Juvenile and Adult *Ascaphus*

INTRODUCTION

Available data on the effects of forest practices on larval population abundance and density are contradictory or vary by geographic location. While most studies report negative influences of forest harvest on larval *Ascaphus*, others have shown no effect or increases in abundance in some areas (e.g., in maritime locations). Still, relatively little is known about the larval ecology of *Ascaphus*, and even less is known about the transformed frogs, especially in terrestrial habitats. To determine influences of habitat disturbance and to better conserve *Ascaphus* populations, an understanding of both aquatic and terrestrial life stages is necessary.

Patterns of *Ascaphus* movements may vary among regions due to inherent geographic differences. Rainfall is plentiful and desiccation risks for *Ascaphus* are less in coastal forests of the Pacific Northwest with maritime climates than for drier, inland locales. In coastal areas, some juvenile and adult *Ascaphus* have been found >100 m from streams during wet weather (e.g., Welsh and Reynolds 1986; Bury and Corn 1988*a*), but movements after metamorphosis are poorly documented. *Ascaphus* are afforded some protection in BC, but guidelines are based on larval habitat requirements (Ministry of Forests and Ministry of Environment, Lands and Parks 1999). Understanding the movement patterns of metamorphosed *Ascaphus* will help in the development of effective conservation actions, especially in managed forest landscapes.

In a species like *Ascaphus*, which may have low vagility or dispersal controlled by abiotic factors (e.g., climate, wind or water currents), source-sink dynamics are expected to be common (Diffendorfer 1998). Despite the linear nature of streams and the ease with which movements can occur near water, *Ascaphus* may disperse laterally and overland. Larval movements downstream would have to be followed by extensive movements upstream to arrive at stream reaches that are cooler and less disturbed than lower reaches. However, these movements would represent a downstream drift – upstream movement cycle, not dispersal. Thus, to arrive in new habitats (streams) and introduce new members to the gene pool, terrestrial movements by *Ascaphus* may be critical to population persistence.

My goal in this chapter was to examine the colonization potential of juvenile and adult *Ascaphus* in clearcuts and old-growth forests. I tested hypotheses based on three predictions: (1) To avoid desiccation, amphibians require moist habitats, so I predicted that frogs in clearcuts remain closer to streams than frogs in old-growth forest; (2) Some researchers (Bury and Corn 1987, 1988*b*) recorded juveniles moving at least 75 m from streams during fall. Others reported

reduced movement at the onset of reproductive maturity (Daugherty and Sheldon 1982*a*), so I predicted that juveniles undertake most upland movements, while adults move along streams; and (3) Precipitation and temperature strongly influence amphibian activity. Coastal populations should experience less thermal stress than inland populations, and I predicted that *Ascaphus* in coastal regions move greater distances than in inland regions.

STUDY AREA

I conducted research near Squamish in southwestern BC (49° N, 122° W), 60 km N of Vancouver in Coastal Western Hemlock (CWH) and lower portions of Mountain Hemlock (MH) biogeoclimatic zones (Meidinger and Pojar 1991). Both zones are relatively moist with annual precipitation ranging 1000–4400 mm in CWH and 1700–5000 mm in MH (Meidinger and Pojar 1991). Sites were located in four adjacent river basins (Figure 3.1) that flow into Howe Sound.

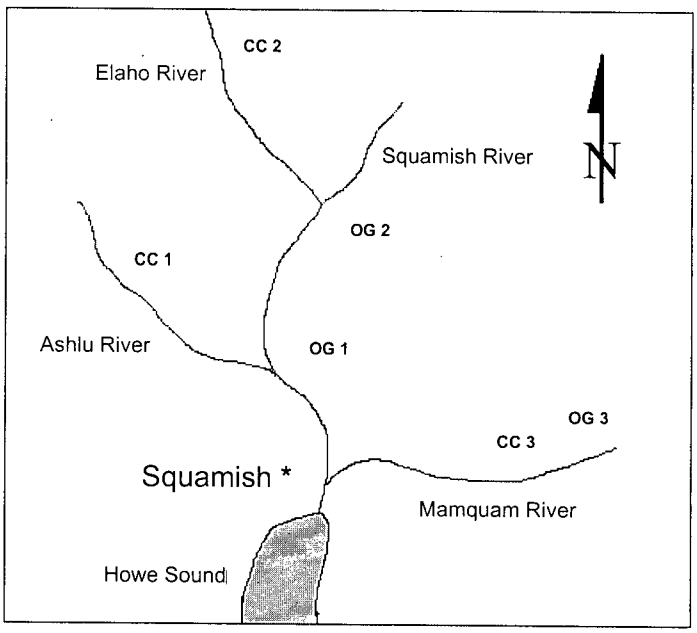

Figure 3.1. Schematic map of study area showing relative positions of the four river basins, and the three replicates of each treatment: old growth (OG) and clearcut (CC).

In the upper headwaters of watersheds, I selected rocky streams in old-growth forests (250+ years old; n = 3) and recent clearcuts (five years since logging; n = 3) using 20–min stream searches to establish presence of larval *Ascaphus*. All streams were 1–3 m wide and fishless. Each site covered about 2.25 hectares (Figure 3.2), adjacent to which was a surrounding 'buffer' of at least 50 m of contiguous forest. For example, a trapping grid in old growth was surrounded by at least 50 m of old-growth forest, beyond which a clearcut, road, or different forest cover type may be present. Mamquam and Elaho clearcuts were 200 and 500 m

downstream from old growth, respectively, while the Ashlu clearcut was 300 m downstream from a rocky, unforested ridge. Selected streams were 200–400 m from any adjacent streams. Vegetation cover in clearcuts consisted mainly of an open canopy of sparsely distributed shrubs and small trees (< 1 m tall). About 1/3 of the Mamquam and Elaho clearcuts contained a closed canopy of shrubs and small trees (< 2 m tall).

METHODS

Pitfall Trap and Drift Fence Arrays

To evaluate frog movements, I used streamside and upland arrays of pitfall traps with clear, plastic drift fences, adapted from prior designs (Corn and Bury 1990; Corn 1994). Arrays each consisted of four pitfall traps and one or five drift fences (Figure 3.2). Traps were constructed of white, smooth-walled polyvinyl chloride (PVC) sewer pipe, 15 cm in diameter and 40 cm deep. Streamside arrays were designed to detect movements up and down the stream bank. Each array was constructed with a 5–m long drift fence with two traps at each end, installed perpendicular to the stream as close to the water's edge as possible (Appendix III). Upland arrays detected movements towards and away from streams, and included five 5–m long drift fences installed in zigzag formation with one trap at each elbow (adapted from suggestions by R.B. Bury; Appendix III). Arrays were 20 m in length and parallel to the stream.

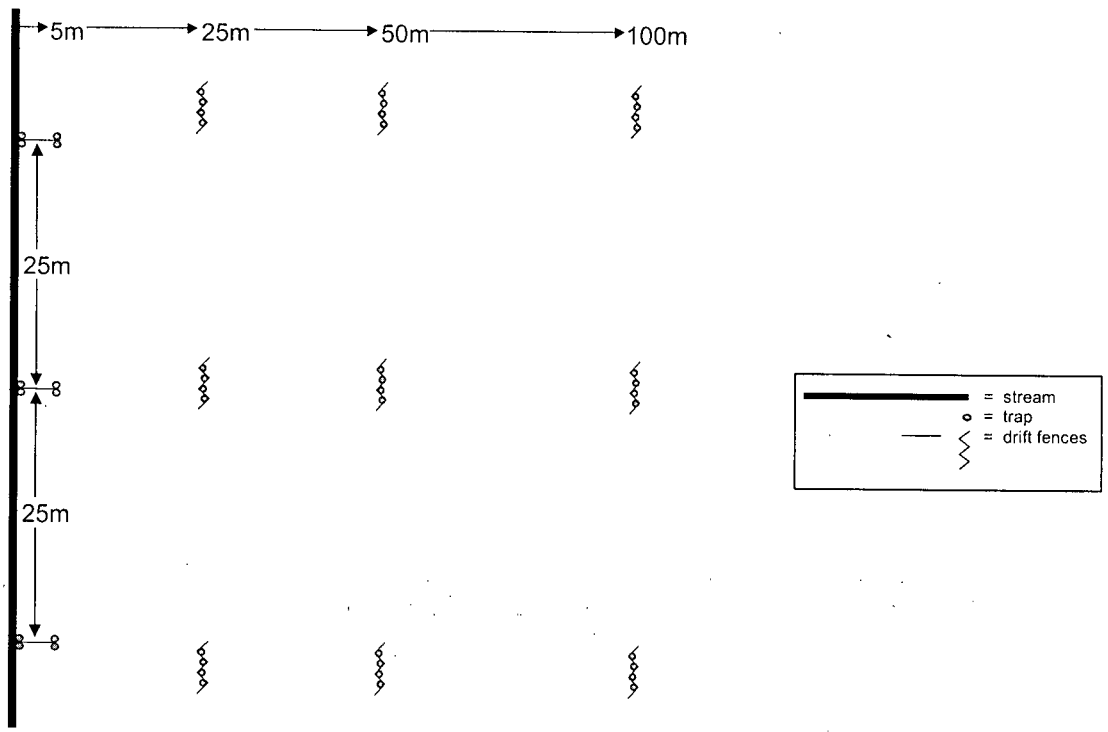

Figure 3.2. A trapping grid (representing one experimental unit) showing arrays of streamside and upland pitfall traps and drift fences. Not to scale.

At each site, I installed 48 pitfall traps and 48 m of drift fence (total = 288 traps and 240 m of fence for all sites). Selection of the first streamside array was random, while all others were systematic. The minimum distance of the first array from the nearest logging road was 50 m. In most cases, the first array was upstream from a logging road. In the Ashlu and Mamquam clearcuts, the first arrays were established downstream from logging roads because upstream forests were not the appropriate size or forest cover type.

I placed a bottomless margarine container at the trap opening, forming a funnel, to keep frogs from hopping or climbing out and checked traps every 2–3 days for 1–4 week periods. To reduce small mammal mortality, I facilitated escape by placing sisal rope in traps (see Appendix III), securing it in soil beside the trap (adapted from Wind 1996). Insertion of ropes significantly reduces small mammal mortality (Karraker 2001) while *Ascaphus* are retained in traps. Small mammal mortality during three field seasons is given in Table A-1.1 (Appendix I). I inclined a cedar shingle against the fence over each trap opening (see Appendix III) to protect animals from direct sunlight, predation, and rainfall that can flood traps. The cover also may serve to attract frogs. To prevent desiccation of animals, I placed wet moss in traps, and added fresh water on every visit to maintain moisture.

During rainy periods (mid-September through November), when captures were expected to be high (Bury and Corn 1987), grids were operated almost continuously and traps were visited frequently. I operated traps Sep 18–30 and Oct 4–13 in 1998, Jul 18–25, Aug 8–17, and Sep 10–Nov 1 in 1999, and Sep 1–Nov 9 in 2000. Unlike fall trapping, grids were not operated continuously in the summer of 1999: 2–3 times during Jul (eight days total), Aug (10 days), and early Sep (seven days). At 0 m and 100 m from stream, soil temperature at 15 cm depth (digital thermometer: $\pm$ 0.1°C) and air temperature in the shade 15 cm above the soil surface (mercury thermometer: $\pm$ 0.5°C), and a relative measure of soil moisture at 15 cm depth (conductivity meter: $\pm$ 0.5 units) were recorded on each site visit. Soil moisture also was recorded at each capture location. My conductivity meter had a scale from zero (dry) to 10 (wet), and Thomson *et al.* (1996) reported that this device provides stable, reproducible readings.

Mark-Recapture

I measured, individually marked, and released frogs on the opposite side of the drift fence where they were captured, assuming that was their movement direction. Frogs were classified into two developmental stages based on snout-vent length (SVL) and morphological features: "juveniles," most with residual tail present (16.3–27.9 mm SVL) and "adults," most with secondary sexual characteristics present (28.0–50.1 mm SVL).

I marked frogs using Visual Implant Fluorescent Elastomer Tags (VIE; Northwest Marine Technologies 2002; see Appendix III). VIE has no reported effects on mortality or behavior of amphibians (Anholt *et al.* 1998; Jung *et al.* 2000; Davis and Ovaska 2001) and is preferable to toe clipping (Clarke 1972). To avoid potentially harmful chemical anaesthetics (e.g., tricaine methanosulfonate, aka MS–222), hyperactive frogs were submerged briefly in ice water to reduce their activity. This likely caused minimal or no stress as some species of terrestrially hibernating frogs endure freezing of extra-cellular body fluids (Churchill and Storey 1993), and *Ascaphus* are cold-adapted frogs.

Directions of frog movement were categorized as upstream, downstream, upslope, or downslope. Upstream movements were parallel to the stream, against stream flow, and within 5 m of the edge of stream. Downstream movements were parallel to stream, in the direction of stream flow. Upslope movements away from stream were roughly perpendicular to stream and within 100 m of stream edge. Downslope movements towards the stream were roughly perpendicular.

All animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. I held the required certificate from the University of British Columbia Committee on Animal Care (#A0–0110), and the British Columbia provincial permit (#C088692) necessary for fieldwork on a "blue-listed" species.

Statistical Analyses

Each trapping grid was treated as an experimental unit for statistical analyses. I installed one trapping grid at each of the six sites (three old growth, three clearcut), for a total of six experimental units. I used the statistics package SPSS® for Windows® (SPSS Inc., Chicago, Illinois, U.S.A.) to run one-way analyses of variance (ANOVAs) on mean values per experimental unit (i.e., six treatment means) for the 254 *Ascaphus* captured during three years (1998–2000).

In a separate analysis, interactions among the two treatments, three watersheds, and three years were tested using an ANOVA based on a randomized complete block design with a split-plot year effect. In this mixed-model ANOVA, year and forest cover were fixed factors and watershed was a random factor. Because interactions among forest cover, year, and watershed were not significant when analyzing mean distances, I pooled watershed and year interaction degrees of freedom into experimental error degrees of freedom to obtain a more precise estimate of variance (Hicks 1982). When differences among multiple means were statistically significant, I used a multiple comparison test (Bonferroni) to evaluate which means differed. All reported *P*-values were obtained using statistics tested against a preset significance level of $\alpha = 0.05$. The $\pm$ values reported indicate the standard error of the mean. When analyzing distance

categories, tests for independence (chi-square, χ^2) were performed on numbers of frogs captured. Cochran-corrected tests for independence (χ_c^2; Zar 1984) were used for 2x2 contingency tables. Numbers of frogs captured were converted to numbers of frogs per 100 trap nights (TN), or catch per unit effort (CPUE), to address unequal trapping effort. Although CPUE are effectively percentage data, CPUE values are presented only in text and tables, thus arc sine transformation was not employed. A trap night is the number of traps in operation × the number of nights in operation. Traps full of water, pushed above ground (due to rising water table), or broken were deemed inoperable and deleted from trap night totals.

To assess body condition of individual frogs in old growth and clearcuts, I used a body condition index (BCI). I calculated BCI using observed mass divided by expected mass. Expected mass (predicted Y) was based on residuals derived from a regression equation of mass (Y) against snout-vent length (X). This calculation incorporates length-corrected mass and has been used in studies of whiptail lizards (Dickinson and Fa 2000), terrestrial salamanders (Dupuis *et al.* 1995), and bears (Cattet *et al.* 2002). BCI calculations were restricted to males and non-gravid females to avoid bias created by differences in reproductive status (Stamps 1983).

RESULTS

Catch Per Unit Effort

I recorded 281 *Ascaphus* from 1998–2000: 254 captured, 27 incidental encounters (Table 3.1). Other amphibians that were encountered, trapped, and recaptured are summarized in Tables A-I.2 and A-I.3 (Appendix I). Of 254 *Ascaphus* captured, 142 were in clearcuts and 112 were in old growth. Catch per unit effort (CPUE) was 1.3 times greater in clearcuts than in old-growth forests (Table 3.2) but was not statistically significant. Distributions of developmental stages differed significantly between clearcuts and old-growth forests ($\chi_{(3)}^2 = 52.30$; $P < 0.001$) with 2.9 times more juveniles in clearcuts than in old growth, and 2.3 times more adults in old growth than in clearcuts (Table 3.2). Juvenile and adult captures also were unevenly distributed across the three watersheds and across the three years. The Mamquam watershed yielded three times more adult captures than the Ashlu watershed, and nearly twice as many juveniles were captured in the Elaho watershed compared with Ashlu and Mamquam watersheds (Figure 3.3a). The most adults and juveniles were captured in 1999, and the least were captured in 1998 (Figure 3.3b). It later becomes more apparent that relations with forest cover, watershed, and year result from different distributions in developmental stages captured among watersheds and years (Figure 3.3).

Table 3.1. Number of nights of trapping and number of *Ascaphus* recorded (trapped and encountered. Southwestern British Columbia. 1998–2000.

Year	# of Nights	Number of Frogs		
		Trapped	Incidental Encounters	Total Recorded
1998	21	32	13	45
1999	63	128	8	136
2000	70	94	6	100

Recapture Rate

In three years, I recaptured only 17 frogs (6.7%) of the 254 marked animals. None were recaptured in 1998. Recapture rates in 1999 and 2000 were 6.3% and 9.6%, respectively. Recaptured frogs included 10 juveniles and seven adult frogs captured 4–400 days after initial capture. In old growth, recaptures included four adults (three males, one female) and one juvenile female. Recaptures in clearcuts included one adult male, two adult females, and nine juvenile males. Recapture rates were 4.5% in old growth and 8.5% in clearcuts.

Adult males in old growth were recaptured 25–70 m from their initial capture site, about one year after initial capture (345–420 days; $n = 3$). One adult male was recaptured in a clearcut 330 days after initial capture, 85 m from the initial capture site. Two adult females were recaptured in a clearcut after five and 360 days, 90 m and 100 m from initial capture sites, while one adult female was recaptured in old growth after five days, 50 m from initial capture site. Nine juvenile males were recaptured in clearcuts after 5–14 days, in the same trap or up to 50 m away from initial capture site. One juvenile female was recaptured in old growth after four days, 30 cm from initial capture site.

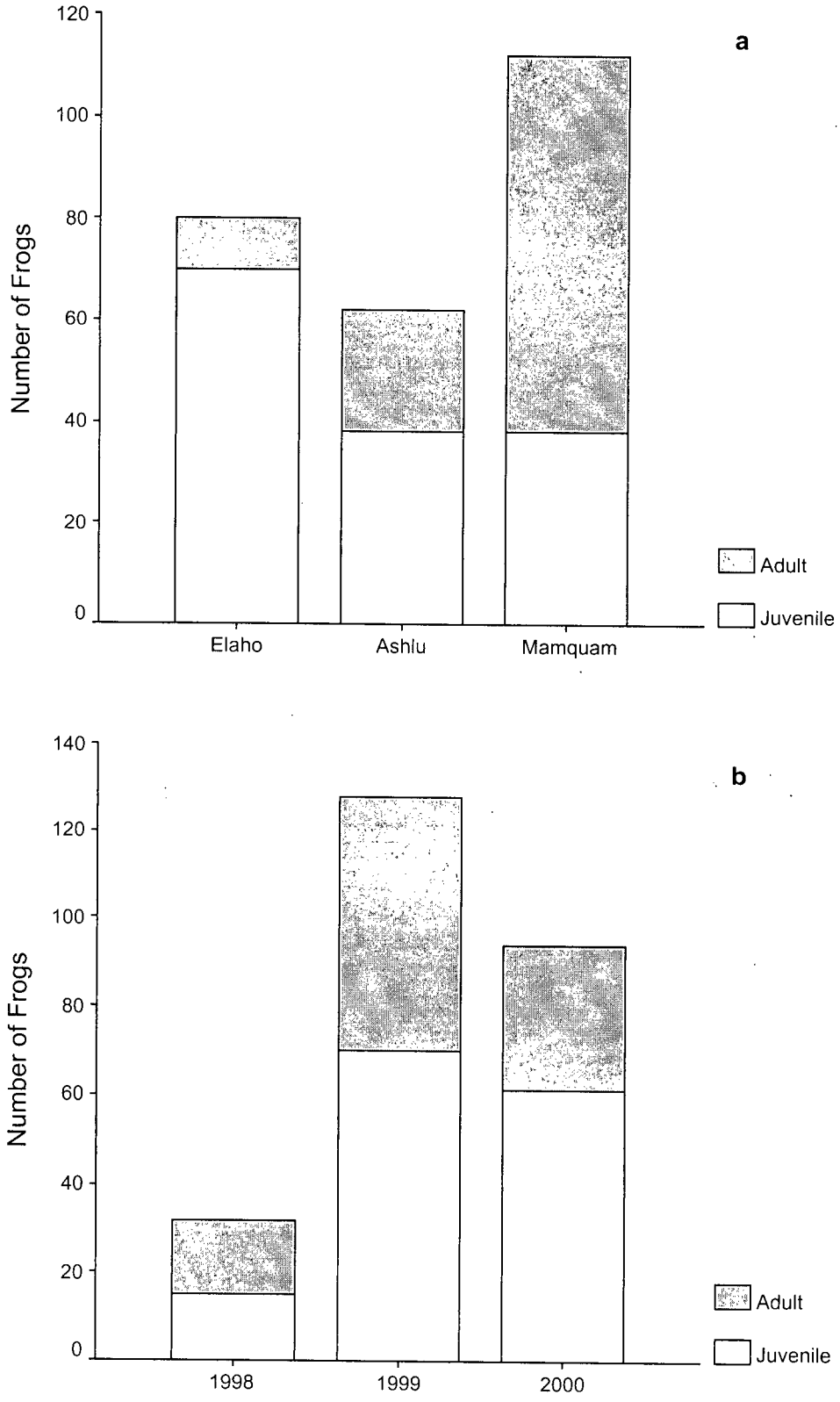

Figure 3.3. Number of juvenile and adult *Ascaphus* trapped: a) differences among three watersheds, b) differences among three years.

Table 3.2. Total captures, catch per unit effort (CPUE: number of frogs per 100 trap nights), mean (± $S_{\bar{x}}$) distance from stream, mean snout-vent length, individual mass, and total mass by site for juvenile and adult *Ascaphus* at six sites. 1998–2000.

	Juvenile		*Adult*		*Total*	
	Clearcut	**Old Growth**	**Clearcut**	**Old Growth**	**Clearcut**	**Old Growth**
Captures	109	37	33	75	142	112
Catch Per Unit Effort (#/100TN)	0.70	0.24	0.21	0.48	0.91	0.72
Distance (m)	14.36 ± 3.05	20.01 ± 9.12	37.25 ± 7.22	20.57 ± 9.98	25.81 ± 6.20	20.29 ± 6.05
	[$F_{(1,4)} = 0.346$; $P = 0.588$] [1]		[$F_{(1,4)} = 1.835$; $P = 0.247$]		[$F_{(1,4)} = 0.037$; $P = 0.857$]	
Snout-vent Length (mm)	19.18 ± 0.31	19.92 + 0.02	37.18 ± 4.48	38.12 ± 1.17	28.18 ± 4.50	29.02 ± 4.10
	[$F_{(1,4)} = 5.738$; $P = 0.075$]		[$F_{(1,4)} = 0.041$; $P = 0.850$]		[$F_{(1,4)} = 2.405$; $P = 0.196$]	
Individual Mass (g)	0.73 ± 0.08	0.84 ± 0.02	5.26 ± 1.65	5.72 ± 0.25	2.99 ± 1.25	3.29 ± 1.10
	[$F_{(1,4)} = 2.098$; $P = 0.221$]		[$F_{(1,4)} = 0.078$; $P = 0.793$]		[$F_{(1,4)} = 2.015$; $P = 0.229$]	
Total Mass [2] (g)	24.08 ± 6.44	9.35 ± 2.75	73.92 ± 35.96	122.83 ± 72.02	98.00 ± 29.64	132.18 ± 74.75
	[$F_{(1,4)} = 4.428$; $P = 0.103$]		[$F_{(1,4)} = 0.369$; $P = 0.576$]		[$F_{(1,4)} = 0.181$; $P = 0.693$]	

[1] All statistical analyses of between-subjects effects were conducted using one-way ANOVAs.
[2] Total mass of *Ascaphus* for each developmental stage and forest cover type.

Movement Patterns

Distance from Stream

I quantified colonization potential of *Ascaphus* by evaluating their mean distances from streams in old-growth forests and clearcut sites. I also evaluated distribution of captures across distance categories. I recognized two trapping periods: summer (Jul–Aug) and fall (Sep–Nov). Summer trapping occurred in 1999 and yielded 13 frogs. During the three years of fall trapping, no frogs were captured in November during snowfall. I captured the most frogs (n = 241) between Sep 26–Oct 23. In summer, I captured six frogs in streamside arrays and seven in upland arrays; in fall, 134 were streamside, 107 were in upland arrays.

I found a significant difference in the proportion of frog captures across distance categories among old growth and clearcut ($\chi_{(3)}^2$ = 23.16; P <0.001). The number of frogs captured within 25 m of the stream was not independent of forest cover type ($\chi_{c'(1)}^2$ = 8.04; P <0.005) with more frogs captured within this distance in clearcuts. However, the proportion of frogs captured at distance categories 25–100 ($\chi_{(2)}^2$ = 2.69; P >0.250) and 50–100 ($\chi_{c'(1)}^2$ = 1.70; P >0.100) were independent of forest cover type.

I failed to detect a difference when examining mean distances from streams in old-growth forests and clearcuts (mixed-model ANOVA: $F_{(1,2)}$ = 1.810; P = 0.311). However, I detected a watershed (mixed-model ANOVA: $F_{(2,8)}$ = 8.440; P = 0.011) and year ($F_{(2,8)}$ = 7.270; P = 0.016) effect on distance from stream. Mean distances from streams in the Ashlu (15.15 ± 4.80 m) and Mamquam (31.53 ± 9.13 m) watersheds were 2–4 times greater than in the Elaho watershed (7.72 ± 6.13 m), and the difference between Mamquam and Elaho watersheds was statistically significant (P = 0.013). During fall, mean distances from streams in 1999 (19.56 ± 9.12 m) and 2000 (28.22 ± 8.59 m) were 3–4 times greater than in 1998 (6.62 ± 4.16 m), and the difference between 1998 and 2000 was statistically significant (P = 0.019).

Potential relations with forest cover may have been obscured by variation in spatial and temporal conditions. Site variables, however, did not appear different among forest cover types. Mean elevation of old-growth sites (717 m) was similar to that of clearcuts (752 m). Mean gradient (hillslope relative to stream) in old-growth sites (12°) was equal to that in clearcuts (12°). Mean aspect of streams in old-growth sites (235°) was also similar to that in clearcuts (272°). Relative soil moisture at increasing distances from stream also was not informative for explaining patterns of frog movements (Figure A-I.1, Appendix I).

Mean distances from streams differed in summer and fall. During summer, frogs were captured in only one clearcut and two old-growth sites, but were 8.8 times farther from the clearcut stream (55.00 m versus 6.25 ± 4.09 m). In fall, frogs in old growth (21.59 ± 9.15 m; n = 3) were captured 1.3 times farther from streams than in clearcuts (17.00 ± 3.93 m; n = 3).

Different trapping periods between years also sampled different developmental stages of frogs. For example, a greater proportion of juveniles were captured in fall (Figure 3.4*a*) and these were found farther from streams in old growth (Table 3.2). Because the uneven distribution of developmental stages had a dominating influence on relations with forest cover (Table 3.2), I examined differences in distance from stream separately for juveniles and adults (Figure 3.5*a*). On average, adult distance from stream (28.91 $\pm$ 6.65 m) was farther than juvenile distance from stream (17.19 $\pm$ 4.48 m; $F_{(1,210)} = 2.296$; $P = 0.029$). Juveniles were captured 1.4 times farther from streams in old growth (20.01 $\pm$ 9.12 m) than in clearcuts (14.36 $\pm$ 3.05 m; $F_{(1,4)} = 0.346$; $P = 0.588$). Adults were captured 1.8 times farther from streams in clearcuts (37.25 $\pm$ 7.22 m) than in old growth (20.57 $\pm$ 9.98 m; $F_{(1,4)} = 1.835$; $P = 0.247$). When distance from stream was examined separately by forest cover type, I found no difference between mean juvenile distance (20.01 $\pm$ 9.12 m) and adult distance (20.57 $\pm$ 9.98 m) in old growth ($F_{(1,4)} = 0.002$; $P = 0.969$). However, I found a significant difference between mean juvenile distance (14.36 $\pm$ 3.05 m) and adult distance (37.25 $\pm$ 7.22 m) in clearcuts ($F_{(1,4)} = 8.527$; $P = 0.043$).

I captured 72 females and 180 males during the three years. Mean distances from streams were 23.29 $\pm$ 7.80 m for females and 16.81 $\pm$ 3.90 m for males. I found that 29% of mature females and 61% of mature males were captured within 25 m of the stream (Figure 3.5*b*). At streamside, I captured 4.5 times more males than females. Males and females were differentially represented in fall when I captured significantly more males than females (Figure 3.4*b*). Captures of females during the coldest two-week interval (Oct 24–Nov 6) were similar to captures of females during the driest months.

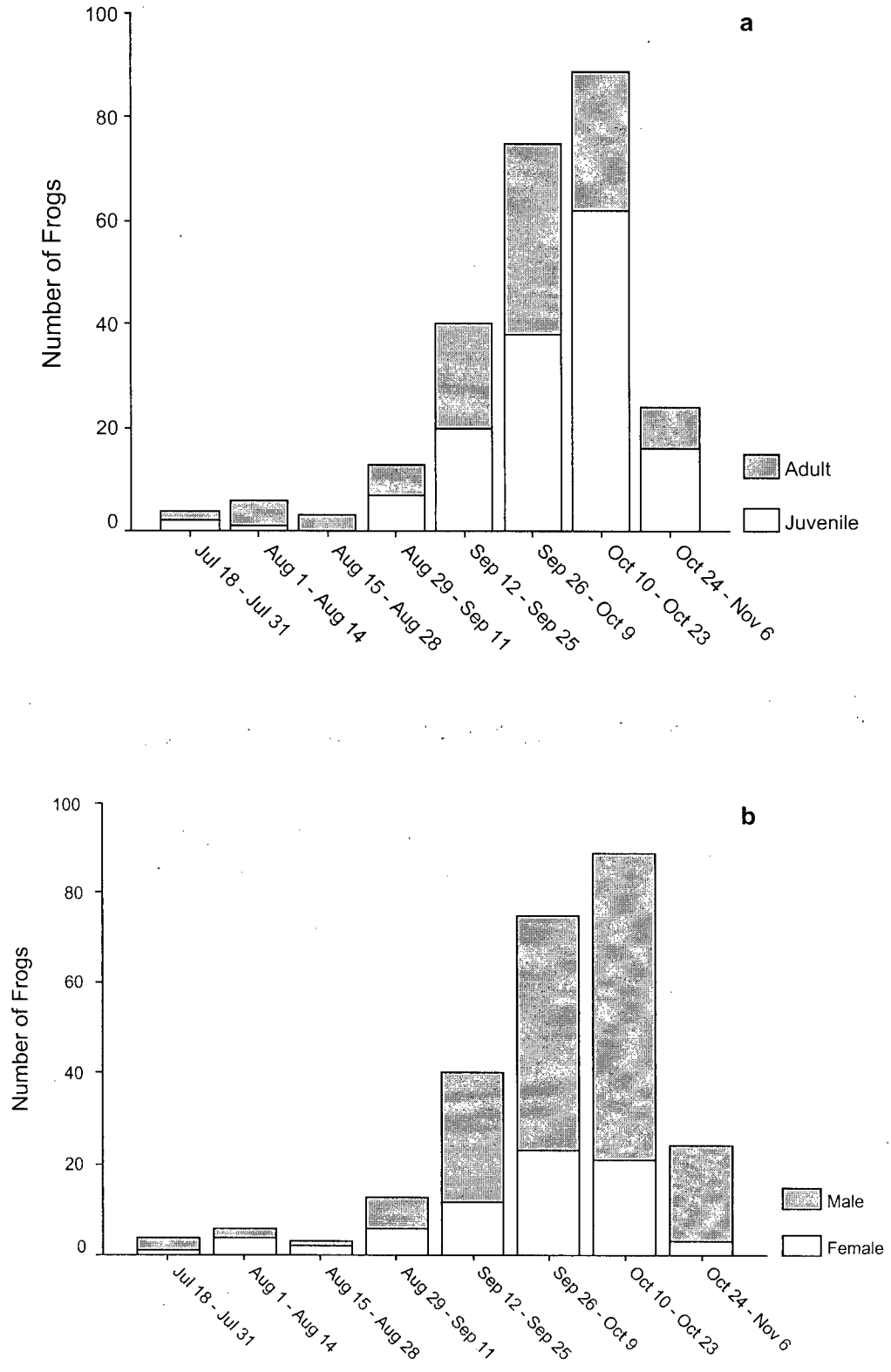

Figure 3.4. Number of *Ascaphus* trapped during each two-week period: a) juveniles and adults, b) females and males (juveniles and adults combined).

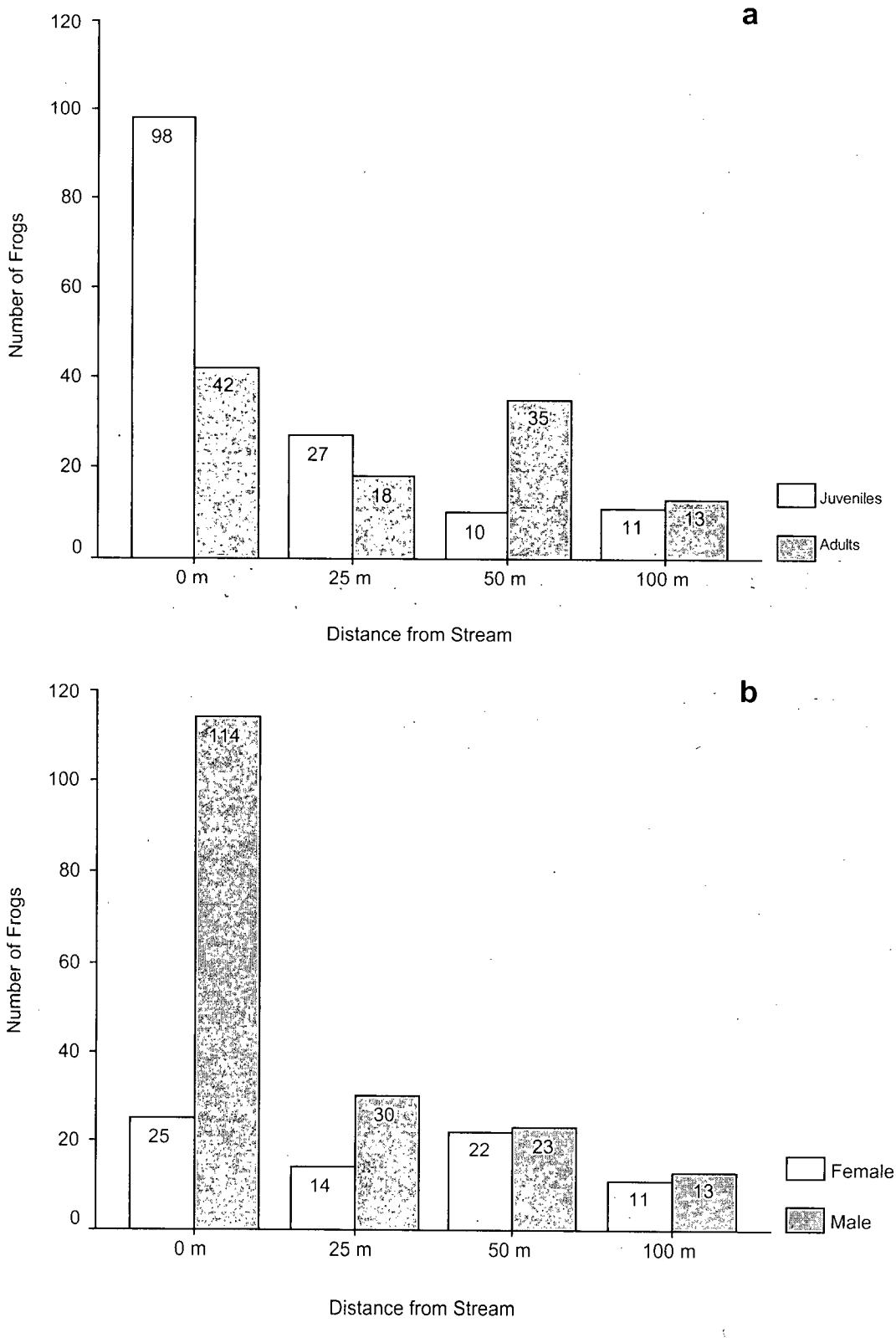

Figure 3.5. Number of *Ascaphus* trapped at each distance from stream: a) juveniles and adults, b) females and males (juveniles and adults combined). Values within bars represent sample sizes.

Movement Rates and Directions

Although sample size was small, I estimated movement rates based on distances between initial and subsequent capture. Frogs recaptured in old growth moved an average 2.09 m/day (n = 5), and frogs in clearcuts moved 3.36 m/day (n = 12). Nine of 12 frogs recaptured in clearcuts were juveniles. Also, more males than females were recaptured in old growth, and males were captured nearer streams, on average.

There appeared to be no difference in direction of movement between sexes. Although almost no downstream movement was recorded for females, they were responsible for most downslope movement. For all years combined, directions of movement in the fall tended to differ between juvenile and adult *Ascaphus* in old-growth forest and clearcut sites (Figure 3.6). Adult movement appeared little affected by forest cover type. However, juveniles exhibited stronger stream affinity in clearcuts. For both forest cover types combined, upstream movements constituted 57% of all juvenile movement, and 28% of all adult movement. Fifteen percent of all juvenile movement and 42% of all adult movement was downslope. The apparent differences between forest cover types results primarily from the proportion of developmental stages captured in each forest cover type.

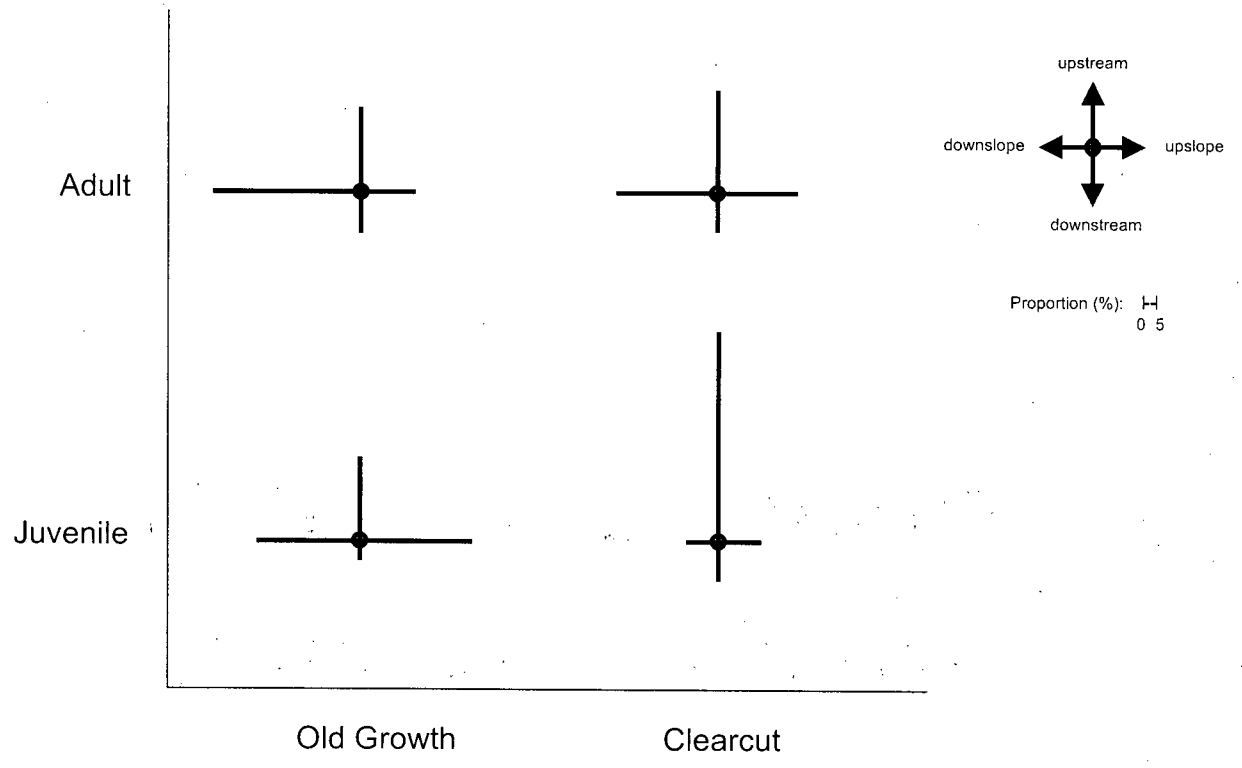

Figure 3.6. Relative proportion of *Ascaphus* moving in four directions relative to stream: upstream, downstream, downslope (towards stream), and upslope (away from stream). Legend shows 5% scale for each direction. Fall data only.

Body Size and Condition

I evaluated variation in frog body size between old growth and clearcuts. Differences were small and none were statistically significant (Table 3.2). When stages were analyzed separately, juvenile and adult frogs both tended to be longer and weighed more in old growth (Table 3.2). Total mass of frogs captured in clearcuts (294 g; n = 142 frogs) appeared lower than in old-growth forests (396 g; n = 112), primarily because there were more adults in old growth (Table 3.2). Evaluating stages separately, mean total mass of juveniles in clearcuts (n = 109) was 2.5 times greater than in old growth (n = 37). Mean total mass of adults in old growth (n = 75) was 1.7 times greater than in clearcuts (n = 33). Juvenile BCI did not differ between clearcuts (0.999 $\pm$ 0.001) and old growth (1.040 $\pm$ 0.020; $F_{(1,4)} = 4.064$; $P = 0.114$). Adult body condition in clearcuts (0.999 $\pm$ 0.002) also did not differ from old growth (1.001 $\pm$ 0.001; $F_{(1,4)} = 1.143$; $P = 0.345$).

DISCUSSION

Catch Per Unit Effort

Research on the effects of timber harvesting on *Ascaphus* habitat has focused on impacts to streams and riparian zones, and often failed to distinguish different responses by developmental stage (Corn and Bury 1989; Welsh 1990; Dupuis and Steventon 1999; Welsh and Lind 2002). Welsh and Lind (2002) reported more *Ascaphus* in streams in late seral forest compared with streams in younger forests. In Oregon, Biek *et al.* (2002) reported *Ascaphus* densities (primarily adults) of 0.11/m^2 in streams transecting clearcuts and 0.21/m^2 in old growth. In contrast, CPUE of frogs in clearcuts tended to be greater than in old growth for my study. However, I also captured 2.9 times more juveniles in clearcuts than in old growth, and 2.3 times more adult frogs in old growth than in clearcuts (Table 3.2).

The capture of more juveniles in clearcuts may be linked to greater primary productivity (Murphy and Hall 1981; Hawkins *et al.* 1983), related to increased solar radiation reaching streams in clearcuts (reviewed by Beschta *et al.* 1987). Wahbe (1996) and Kim (1999) reported larger (length and weight) *Ascaphus* larvae in stream reaches through clearcuts compared with those through old growth in southwestern BC. Greater in-stream productivity and warmer temperatures could increase larval growth rates and, in turn, survival, which appears low (Sutherland 2000). However, if stream temperature rises too high, *Ascaphus* may be excluded as eggs die above 18.5° C (Brown 1975). At least two southwestern BC datasets revealed greater densities of larvae in streams flowing through clearcuts (Richardson and Neill 1998; Wahbe *unpubl. data*). Furthermore, data from within my study area suggest faster larval growth and earlier metamorphosis in clearcuts (3–4 year larval period) compared to a 4–5 year larval period in old-growth streams (Wahbe 1996). Therefore, *Ascaphus* in clearcut streams may exist

at higher densities and have a shorter larval period, which together can lead to the emergence of more metamorphic juveniles compared with streams in old-growth forests. I captured significantly more juveniles in clearcuts than in old growth, and 94% of those captures were new metamorphs. Thus, there would be an increased number of juvenile frogs and catch per unit effort in clearcuts. However, I captured fewer adult frogs in clearcuts, which may suggest lower survival of young frogs. Also, larval movement tended to be greater in old growth than in clearcut streams (Chapter 2). This may result in a more densely distributed larval population closer to the headwaters in clearcut streams, which could translate into the emergence of more metamorphs from a given area, compared with old growth where larvae are likely more spread along the length of the stream. In old growth, some metamorphs may be missed in my trapping efforts if the older individuals (which also take longer to reach metamorphic stage compared with larvae in clearcuts) have moved further downstream. However, this possibility is difficult to assess without sampling lower sections of the stream.

Recapture Rate

My recapture rates were low (4.5–8.5%) compared to other studies. Daugherty and Sheldon (1982*a*) reported rates up to 33% for juveniles and up to 73% for adult *Ascaphus* captured during summer at inland sites. Landreth and Ferguson (1967) reported a 37.5% recapture rate, also during summer at inland sites. No developmental stages were reported for either study. These higher rates of recapture may result from summer sampling when *Ascaphus* aggregate near streams, increasing captures. I operated traps mainly during fall, and away from streamside into adjacent forest where frogs are less abundant. Among studies, there were also different sampling designs and intensities of effort. My lower recapture rates may reflect capturing mostly young frogs, which have a higher rate of mortality (Jones and Raphael 1998), or high vagility in moist, coastal areas. Furthermore, compared with old growth, recapture rate tended to be greater in clearcuts where I captured more juveniles than adults. Juveniles were found closer to streams than adults and were shorter distances between captures.

Movement Patterns

Distance from Stream

My results were consistent with the prediction that frogs in clearcuts would remain closer to streams than frogs in old growth. I found that there were more frogs captured within 25 m of streams in clearcuts than in old growth. This result may suggest stronger stream affinity of frogs in clearcuts where the most favorable microclimatic conditions are likely near streams. I captured frogs 1.3 times farther from streams in old growth compared with clearcut sites,

although no differences were significant when using mean distances (Table 3.2). In a radio-telemetry study of Pacific giant salamanders (*Dicamptodon tenebrosus*) in BC, stream affinity, longer refuge duration, and smaller home ranges were reported in clearcuts compared with those in old-growth forests (Johnston and Frid 2002). Developmental stage was not reported, however mean distance from streams was four times greater in old-growth forests, where refuge duration was two days shorter, and home range size was 10 times greater than that in clearcuts. Being less restricted by microclimate constraints, stream-breeding amphibians in old-growth forests may move greater distances from streams for foraging purposes or dispersal to adjacent streams.

Although number of *Ascaphus* captured at distance categories of 25 m or greater was independent of forest cover type, influences of forest cover on distance from stream varied with developmental stage, and, to a lesser extent, sex of the frogs. Adult frogs were captured 2.6 times farther from streams than juveniles in clearcuts. Because desiccation is more likely, juveniles may remain closer to streams in clearcuts. In old growth, I recorded juveniles 1.4 times farther from streams than in clearcuts. Although patterns are consistent with forest cover reducing anticipated adverse microclimate effects, my measurements of microclimate showed little difference with distance from the streams. Other site variables measured (elevation, gradient, aspect) did not explain differences in distances frogs moved away from streams.

Movement Rates and Directions

Rates of movement estimated by successive recaptures are difficult to interpret because frogs likely did not move consistently in the same direction, but these data provide an index of relative vagility. Other difficulties in interpretation stem from the low recapture rate, a small sample size, and a large variation in time between captures (4–400 days) that may represent daily movement or in some cases, dispersal. I observed movement rates that were slightly higher in clearcuts (3.36 m/day) than in old growth (2.09 m/day). The majority of recaptures in clearcuts were juveniles, which may be a more exploratory life stage than adults. There also tended to be more male recaptures than females in old growth (although sample size is small), and males tended to be found shorter distances from streams, suggesting less movement, than for females. Frogs may move more often (but shorter distances from streams) in clearcuts because of increased physiological stress. Maxcy (2000) reported greater mean daily movement rates for *Ascaphus* in forested sites (12.27 $\pm$ 3.48 m/day) compared with buffered sites (8.53 $\pm$ 5.01 m/day) in southwestern BC. During summer in western Montana (mean annual precipitation = 635 mm; BC: ~2500 mm), Daugherty and Sheldon (1982*a*) evaluated streamside movements and reported mean distances between successive captures of 0.34 m/day (within years) and 0.75 m/day (between years). I did not recapture any frogs during

summer, but during fall, I recorded a mean distance between successive captures of 3.0 m/day. Daugherty and Sheldon (1982*a*) reported a maximum streamside movement of 360 m by an immature female over 12.5 months, a movement rate of 0.96 m/day.

My results were inconsistent with predictions that younger developmental stages would perform most overland movements and dispersal, while adults would move primarily along streams (having left the natal area when younger). Juveniles were found to move predominantly upstream (Figure 3.6), and adults were twice as far from streams as juveniles (Table 3.2, Figure 3.5*a*). In contrast, Bury and Corn (1987, 1988*b*) captured many recently metamorphosed *Ascaphus* in fall $\geq$ 75 m from streams in pitfall traps set in forested stands. Also, in summer, Daugherty and Sheldon (1982*a*) showed a reduction in movement at the onset of reproductive maturity, with greater movements in pre-reproductive frogs and extreme site fidelity in reproductively mature frogs.

Considerable downstream movements by *Ascaphus* larvae occurred over distances up to 64 m in old-growth forests and 3 m in clearcut sites within a few weeks (Chapter 2). Frogs may compensate for these movements by moving predominantly upstream following metamorphosis. I found that directional movements in clearcut sites differed from those in old-growth forests. While most frogs in clearcut sites moved upstream, most frogs in old-growth forests moved towards streams (downslope). The closest adjacent stream was 200 m, and movement patterns of frogs in old-growth forests may represent dispersal from adjacent streams. In both old-growth forests and clearcut sites, upstream movements constituted 57% of all juvenile movements, and 28% of all adult movements. Juveniles clearly showed stronger stream affinity in clearcut sites, but adults appeared little affected by forest cover type. Metter (1964*a*) observed fewer frogs during summer sampling and speculated that frogs moved upstream for "protection," presumably in more shaded stream reaches. In clearcut sites, there may be a tendency to move upstream because higher elevation sites or steeper gradients in upper portions of streams are often less disturbed due to historical patterns of logging starting at valley bottoms. These upstream movements may also represent movements towards breeding or oviposition sites. Within-stream movements by adults are believed by some to be critical for survival in clearcut sites (Adams and Frissell 2001).

Movements toward streams may be dispersal events, breeding migrations, movements associated with foraging, or searches for oviposition, overwintering or over summering sites. Breeding migrations in *Ascaphus* are unreported but hypothesized (Landreth and Ferguson 1967; Brown 1975; Wahbe *et al.* 2001). Males do not vocalize (Schmidt 1970), and aggressive or territorial behavior in *Ascaphus* has not been detected. Frogs aggregate near streams during summer or fall for breeding. During summer, marked *Ascaphus* adults in Montana did not demonstrate any movement associated with breeding or oviposition (Daugherty and Sheldon

1982*a*). However, my data (Figure 3.6) indicate upstream movements and movements towards streams by adult frogs during the breeding season, which supports speculations by Brown (1975) that frogs move upstream to breed in headwaters. Some authors suggest that frogs move downstream to mate (Landreth and Ferguson 1967) or overwinter (Adams and Frissell 2001), but these may be condition-specific responses (e.g., behavioral thermoregulation as suggested by Adams and Frissell 2001).

The variation in distance from streams and movement directions between males and females may be explained by migrations during the breeding season. I observed stream affinity and upstream movements in mature males during breeding season and believe these frogs aggregated in search of mature females. I recorded almost no downstream movement for females, but females were responsible for most of the downslope movement. While some females may remain beside streams to breed or move towards streams to deposit eggs (some were gravid), others may leave the site and disperse to breed or locate suitable oviposition sites in neighboring streams. All three gravid females I captured along streams were moving upstream. Of 15 gravid females captured upslope, 73% moved towards streams. These data may provide the first evidence suggesting breeding migrations (stream to stream or upland to stream movements) in *Ascaphus*. However, some females may not breed every year (Metter 1964*a*). Because females may store sperm for a year (Metter 1964*b*), they may move through forest uplands and later return to the same stream for oviposition.

Amphibian capture rates vary within and among years because patterns of precipitation and temperature strongly influence surface activity and likelihood of detection (Bury and Corn 1987; deMaynadier and Hunter 1998; Aubry 2000). I expected movement patterns to be similarly influenced, and hypothesized that *Ascaphus* in wet regions (coastal BC) would show longer movements than frogs in drier regions (e.g., Idaho). My results were consistent with this prediction. *Ascaphus* in coastal BC moved at least 100 m from streams in both old growth and clearcuts. Few studies of inland populations have sampled *Ascaphus* away from streams. In southeastern Washington and northern Idaho, *Ascaphus* moved at least 12 m from streams (Metter 1964*a*). Sampling took place during wet and dry seasons for both studies. However, it is difficult to make a valid comparison because although 100 m was the maximum trapping distance for my study, the area sampled was not reported for the inland study. For inland populations, high site fidelity in *Ascaphus* has been attributed to low summer precipitation and warm conditions of interior regions that restrict movements of amphibians, and suggests low recolonization potential for inland populations (Daugherty and Sheldon 1982*a*).

Ascaphus should experience less thermal stress on the coast than in the interior Rocky Mountains (Diller and Wallace 1999; Welsh and Lind 2002). Climatic conditions likely favorable for *Ascaphus* along the coast (e.g., high humidity, extended periods of rain) may enable adults

to occupy larger home ranges or move longer distances than in inland populations. I recorded fall movement rates that were 8.8 times greater than those reported by Daugherty and Sheldon (1982*a*) in summer. Available data suggest that affinity for streams is much greater in dry regions compared to wet regions. Although coastal conditions are not as restrictive to movements, a greater proportion of *Ascaphus* in clearcuts were captured within 25 m of streams compared to *Ascaphus* in old growth, which suggests greater stream affinity in clearcuts.

Body Size and Condition

Despite evidence of larger-sized larvae in stream reaches flowing through clearcuts (Wahbe 1996; Kim 1999), in this study juveniles and adults in clearcuts did not differ in size from those in old growth. Size at metamorphosis and length of larval period are important to amphibians because they influence larval and juvenile survival and the potential level of adult fecundity (Travis 1984). A shorter larval period is expected when there is more food early in larval development, while less food at this stage will lengthen the larval period (Wilbur and Collins 1973). A smaller size at metamorphosis may lead to reduced survivorship, because body size has a major influence on an animal's energetic requirements, its potential for resource exploitation and its susceptibility to natural enemies (Werner and Gilliam 1984). Body size in amphibians is also directly related to their rehydration and desiccation rates (Ray 1958; Spotila 1972) and to their ability to withstand food deprivation. I found more juvenile frogs in clearcuts, but both juveniles and adults were in slightly reduced condition in clearcuts compared with old growth. My findings suggest that while the aquatic environment in clearcut sites initially may be beneficial to larvae, the terrestrial habitat may not be conducive to long-distance movements by juveniles. Some alternatives may be that clearcuts provide poor habitat cover and reduced foraging opportunities, increased competition, or shorter active seasons due to the increases in temperature and decline in relative humidity.

CHAPTER FOUR

Population Genetic Structure of *Ascaphus* at the Watershed Scale

INTRODUCTION

Timber harvesting may reduce habitat patch size and habitat connectivity, thus reducing dispersal among fragments and increasing the probability of local extinction (e.g., Sjögren 1991; Bunnell *et al.* 1992; Fahrig and Merriam 1994). Connectivity between habitat patches is believed to be key to metapopulation persistence (Sjögren 1991) because it allows dispersal between populations (Taylor 1990; Hanski and Gilpin 1991). *Ascaphus* populations may exist in a metapopulation structure (Metter and Pauken 1969; Daugherty and Sheldon 1982*a*; Ritland *et al.* 2000). Because direct estimates of dispersal can be problematic, Vos *et al.* (2001) recommended using genetic techniques to determine influences of landscape connectivity on animal dispersal.

A primary goal of conservation genetics is to estimate the level and distribution of genetic variation within and among populations of rare and endangered taxa (Fritsch and Rieseberg 1996). Research examining gene flow among *Ascaphus* populations has focused on genetic differences among watersheds across portions of the species' range (i.e., genetic comparisons based on geographically extensive samples). Having estimated relationships among populations and inferring evolutionary processes, Nielson *et al.* (2001) suggested recognition of inland populations of BC, Idaho, Montana, Washington, and Oregon as a distinct species, *Ascaphus montanus* (following the epithet of Mittleman and Myers 1949). However, Ritland *et al.* (2000) reported that although inland populations in British Columbia were distinct, their genetic distance from other groups (north coast, mid coast, and south coast) was equal to that expected from isolation by geographic distance alone, as opposed to taxonomic differentiation. Nonetheless, both studies report strong genetic differences (i.e., low gene flow) among *Ascaphus* populations, suggesting a complex history of restrictions to geographic refugia and range expansions.

Genetic structure among *Ascaphus* populations at large (range-wide) spatial scales is now better understood. However, we know little about possible metapopulation genetic structure. Gene flow among populations is necessary for long-term persistence. This information is critical to our understanding of the effects of forest management on *Ascaphus* populations.

Population genetic structure can be inferred using a variety of genetic markers. Isozyme electrophoresis is traditionally the most popular and cost-effective method (Fritsch and Rieseberg 1996). Other techniques include microsatellites, sequencing of variable regions of mitochondrial DNA, and randomly amplified polymorphic DNAs (RAPDs). All of these techniques have been used to examine population genetic structure in amphibians (e.g., Daugherty 1979; Green *et al.* 1989; Rowe *et al.* 1999; Ritland *et al.* 2000; Neilson *et al.* 2001). DNA sequencing is useful for studies at the species level, but it is not relevant for studies of populations at a small spatial scale. Although microsatellite markers are useful for population studies, they are not yet developed for *Ascaphus*. Isozymes require a large sample size and a large amount of tissue, which were not possible for this study. Isozymes also provide highly biased genomic sampling and generate too few loci (a maximum of 40 loci). I selected RAPD markers to examine population genetic structure in *Ascaphus*.

For conservation genetics studies, RAPDs provide robust data for estimating levels and patterns of genetic variation (Fritsch and Rieseberg 1996). Compared with other techniques, the RAPD technique can generate essentially unlimited numbers of loci, provides a more random sample of the genome, requires only a small amount of genomic DNA, is economical, and requires simple and relatively fast procedures. Because *Ascaphus* populations are at risk and protected from destructive sampling in British Columbia, I was limited by the amount of tissue I could collect. I was also limited by the number of larvae available for tissue sampling in each stream. Therefore, the RAPD technique was well suited for my analysis of *Ascaphus* population genetic structure.

My goal in this chapter was to examine the population genetic structure of *Ascaphus* in one clearcut stream and one old-growth stream in a single watershed. Also, I examine patterns of spatial distribution among larvae within each stream along a 180–m transect. In Chapter 2, I recorded larval movement rates that were lower in clearcut streams than in old-growth streams. In Chapter 3, I reported stronger stream affinity, suggesting lower colonization potential, of juvenile frogs in clearcuts. The purpose of this study was to determine whether I could infer fragmentation impacts from differences in genetic structure. In this chapter, I test hypotheses based on two predictions: (1) I captured twice as many breeding adults in old-growth forests as in clearcuts so I predicted that *Ascaphus* larvae in the clearcut stream would be less diverse than larvae in the old-growth stream; and (2) Female *Ascaphus* deposit eggs in the upper headwaters of streams and I recorded considerable downstream movements by larvae, so I predicted that larvae would exhibit lower genetic relatedness with increasing physical distance along a 180–m stream transect.

STUDY AREA

Ascaphus tissue samples were collected from two streams within the Mamquam River drainage in the south coast of British Columbia's Coast Mountains, near the city of Squamish (Figure 4.1). I selected one stream flowing through a clearcut site and a second stream flowing through old-growth forest. These sites were about 1.6 km apart. Aside from forest age and stream aspect, stream and site attributes did not differ substantially between the two sites (Table 4.1).

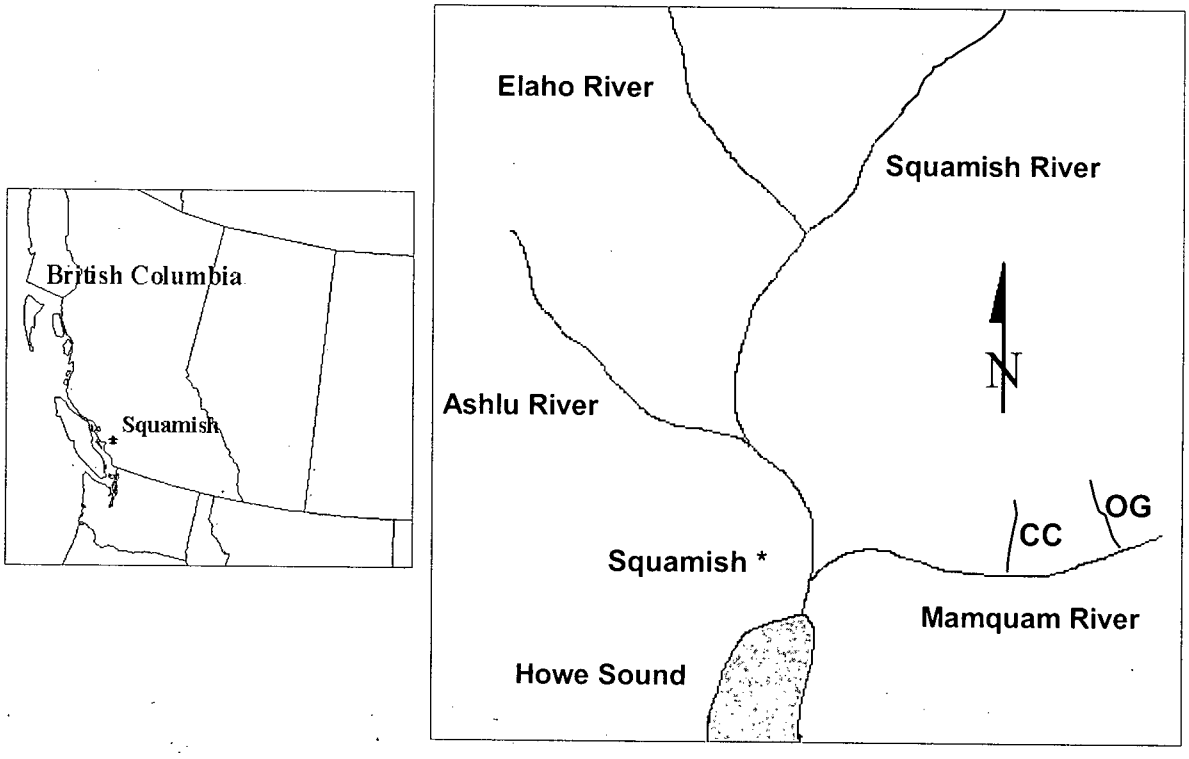

Figure 4.1. Map showing location of study area in south coastal British Columbia and schematic map showing relative positions of river basins near collection sites of *Ascaphus truei*. The streams flowing through the clearcut (CC) and old-growth (OG) sites are tributaries of the Mamquam River. Sites are approximately 1.6 km apart.

Table 4.1. Site locations and stream attributes of *Ascaphus truei* tissue collection sites. Sample sizes are given at left of table.

Forest Cover Type	Forest Age (years)	Site Locations			Stream Attributes		
		Latitude	Longitude	Site Elevation (meters)	Gradient (°)	Aspect	Width (meters)
Clearcut (*n* = 87)	10	49° 38' 01"	123° 03' 58"	840	9	NW	1.6
Old Growth (*n* = 63)	250 +	49° 37' 50"	123° 03' 50"	935	6	SE	2.7

METHODS

Tissue Sampling Design

Ten reaches were established within each stream for tissue sampling positioned 20 m apart starting at 0 m moving downstream to 180 m (Figure 4.2). I attempted to sample a minimum of 100 *Ascaphus* larvae per stream, but fewer were found. In all, I collected 63 individuals from the old-growth forest and 87 individuals from the clearcut. Tissue was collected by clipping two or three 2–mm notches (approximately 5 mg) from the tails of larval *Ascaphus*. Samples were preserved in 95% ethanol and stored at -70° C until DNA extraction. I used all 150 individual *Ascaphus* larvae from two streams residing in one watershed for subsequent DNA assays.

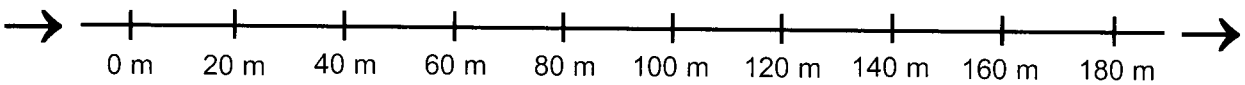

Figure 4.2. Schematic showing 10 stream reaches (vertical bars) sampled for larval *Ascaphus*. Arrows indicate direction of stream flow. The 0 m reach is at the head of the stream sampling area, and the 180 m reach is closest to the Mamquam River. This sampling scheme was established in both the clearcut and the old growth.

Isolation of DNA

DNA extraction from clippings of larval *Ascaphus* tailfins followed Johnson *et al.* (1994). Clippings were air-dried briefly before being subjected to proteinase-K treatment. Samples were then incubated in 360 μl STE buffer (pH 8.0), 40 μl of 10% SDS, and 4 μl of 20 mg/μl proteinase K at 55° C for 16–24 hours. To increase volumes, 100 μl of STE buffer was then added to samples. Samples were extracted twice with phenol – chloroform – isoamyl alcohol (25:24:1). DNA was precipitated by the addition of 0.15 M NaCl ($\leq$ 7.5 μl) and two volumes of -20° C absolute (100%) ethanol, and was pelleted at 10 000 x g in a microcentrifuge. Pelleted DNA was frozen for a minimum of two hours at -20° C and centrifuged at 10 000 x g for 15 min at 4° C. The pellet was then washed twice with -20° C 70% ethanol to remove excess salt. DNA was recovered by centrifugation, dried with a speed vacuum, and resuspended in 50 μl distilled water for a minimum of 48 hours at 4° C. DNA was quantified and yield was estimated using a spectrophotometer (Pharmacia Biotech, Ultrospec® 3000) and electrophoresed on a 0.8% agarose gel. On average, 10 mg of tail clippings yielded 100 ng of DNA.

Assay for RAPD Markers

An assay for RAPD markers (Williams *et al.* 1990) was performed using 10.0 ng DNA in the presence of reaction buffer (50 mM Tris pH 7.6, 2.1 mM $MgCl_2$, 10 mM KCl; Roche Diagnostics, Germany), 0.2 mM of each dNTP (Invitrogen Life Technologies, Canada), 0.3 μM decamer RAPD primer (Nucleic Acids and Protein Service Unit, UBC, Canada), and 0.13 U Taq DNA polymerase (Roche Diagnostics, Germany). The polymerase chain reaction (PCR) cycling conditions for the RAPD reactions were: two minutes at 94° C, followed by 45 cycles of one minute at 94° C, one minute at 36° C, and one minute at 72° C. A final extension of 10 min at 72° C was performed to ensure complete amplification. All PCR reactions were performed on the same Programmable Thermal Controller (PTC 100, MJ Research, Inc., USA).

Following PCR, the products were electrophoresed on 2% agarose gel in 1x TBE buffer at 140 V for 3.5 hours. Gels were then stained with ethidium bromide and photographed using ultraviolet light. Images were electronically saved, and light levels were standardized across all images using Photoshop (Version 6.0) prior to scoring. RAPD bands were manually sized and scored as presence versus absence of banded phenotypes. I performed all assays at the University of British Columbia (UBC) Genetic Data Centre. To ensure consistency, I scored all gels. Two gel images demonstrating a RAPD pattern obtained with primer 213 are presented in Figure 4.3. All data used in analyses of six primers and 61 loci are given in Table A-II.1 (Appendix II). I established gel schemes that would evenly sample individuals from both clearcut and old-growth sites, and from multiple sampling distances (see Table A-II.2, Appendix

II). However, an equal number of individuals from both clearcut and old-growth sites were not always possible because sample size was slightly smaller for clearcuts.

Screening of Primers

Among 16 primers screened against a subset of individuals, six primers were used for the full analysis (UBC primers 211, 213, 221, 268, 352, and 400). The sequences are viewable at http://www.biotech.ubc.ca/frameset.html (Services, Nucleic Acids and Protein Service Unit, Primer Kits, Primers.pdf). Overall, the six primers yielded 97 zones of RAPD band activity.

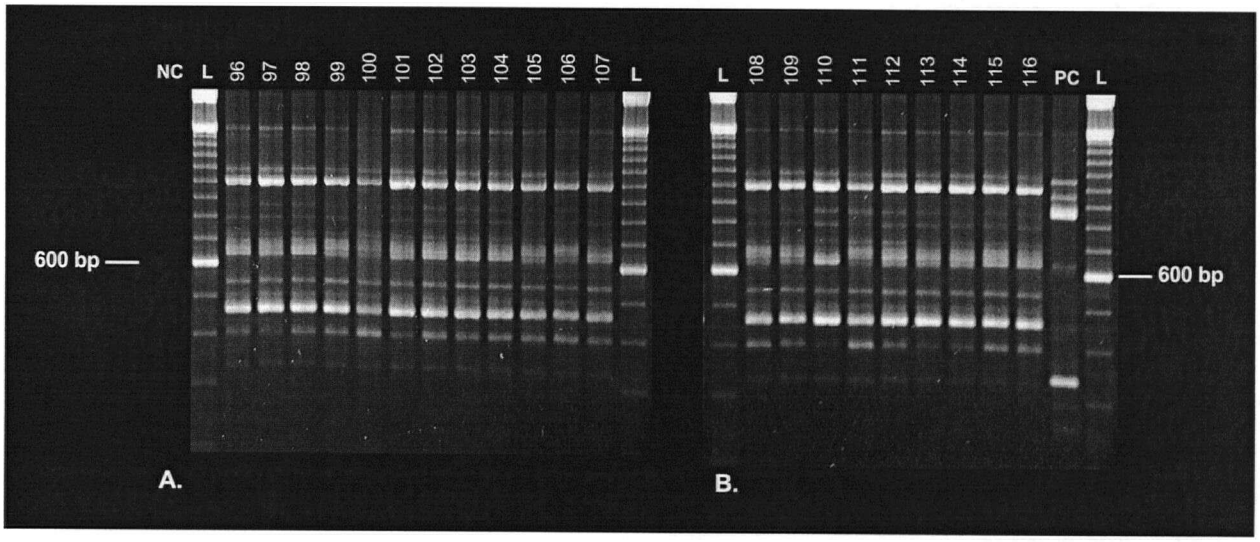

Figure 4.3. RAPD pattern obtained with primer 213. Lanes labelled "L" are size marker DNA fragments. The brightest middle band is 600 base pairs in molecular weight. Lane "NC" is the negative control, and "PC" is the positive control. Samples, sites (CC = clearcut, OG = old growth), and sampling distances from left to right are: A) 96 = CC–0 m; 97–99 = CC–20 m; 100 = OG–60 m; 101–103 = CC–60 m; 104–107 = CC–80 m, B) 108–109 = CC–80 m; 110–115 = CC–100 m; 116 = OG–120 m.

Scoring of Diploid RAPD Markers

To score RAPD markers as presence and absence data, I made the following assumptions (following Lynch and Milligan 1994):

1) Each scorable band per primer (different molecular weights) represented an independent locus and was treated as a two-allele system;
2) Marker alleles from different loci did not comigrate to the same position on a gel; and
3) The interpretation of banding patterns on gels was accomplished in a completely unambiguous manner (i.e., the investigator was unbiased and accurate in matching bands from different lanes within and among gels).

I visually scored monomorphic and polymorphic RAPD markers once from the electronically saved gel images. A blind scoring method was used, in which I was unaware of the identity or origin of the individuals (e.g., old growth, 60 m). Regardless of intensity, if any bands were present in negative control lanes, those loci were omitted from the dataset. Only relatively easy to score bands (i.e., usually bright and discrete) were considered. Presence of a band was scored as a one and absence as a zero. Images were grouped by primer, and all possible markers for one primer were scored prior to scoring markers generated from a different primer.

Statistical Analyses

Allele frequencies of loci scored in the old growth and clearcut were calculated using the program TFPGA (Tools For Population Genetic Analysis; Miller 1997). Genetic distances between stream reaches were calculated using Nei's genetic distance (Nei 1978) included in the program TFPGA. A one-way ANOVA was used to test differences in heterozygosity estimates obtained from the old growth and clearcut. All reported *P*-values were obtained using statistics tested against a preset level of significance, $\alpha = 0.05$.

F_{ST} values, which measure the degree of population divergence, were obtained using the program TFPGA by applying Weir and Kockerham's (1984) methods of calculating Wright's (1931) *F*-statistics. Assumptions of Hardy-Weinberg (H-W) equilibrium are made by TFPGA when calculating F_{ST} values. Standard errors of F_{ST} estimates were calculated using jackknifing where replicates were obtained from sequential elimination of loci. Confidence intervals (95%) of F_{ST} estimates were determined by the bootstrap method, where individuals within populations were resampled to create replicated data sets (1000 iterations).

Such genetic equilibrium assumed when using *F*-statistics can only occur under conditions of zero selection and mutation, no emigration or immigration, random mating, and a large population size. *Ascaphus* populations are probably not at equilibrium, because of the

relatively recent recolonization, and colonization of northern habitats that may still be occurring (Ritland *et al.* 2000). Therefore, when using dominant markers, Φ_{ST} (phi_{ST}), an analog to F_{ST} that does not rely on H-W equilibrium, may be an appropriate alternative. I used the program WINAMOVA (Excoffier 1996) to perform an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) and calculate Φ_{ST}. Φ_{ST}, like its analog, F_{ST}, indicates genetic differentiation. AMOVA is essentially a multivariate analysis of variance (MANOVA), but it uses the pairwise distance matrix as input, rather than the raw Y-vectors themselves (Smouse *et al.* 2001). The program AMOVA-PREP (Miller 1998) was used to prepare the group, distance, and population input files for use with WINAMOVA.

The band-sharing coefficient is the main index of similarity used to describe DNA fingerprints from two individuals (Bruford and Beaumont 1998). It gives the probability that a band will be shared for a certain molecular weight range within the fingerprint. Band sharing coefficients were calculated using a program written in Fortran by Kermit Ritland. Shared bands are those that have comigrated (within 0.5 mm) and have no more than two-fold intensity difference (i.e., the difference between a homozygote and heterozygote; Bruford and Beaumont 1998). The coefficient describes behavior, or how the phenotypic difference changes with distance (behavior in spatial pattern), and calculates the frequency of shared dominant phenotypes. Standard errors of the estimates were obtained by bootstrapping, where 100 repeated random samples were selected from the data and the model is estimated from each one (Norusis 1993). The relationships between genetic relatedness and physical distance were analyzed using Pearson correlation coefficients (Norusis 1993).

RESULTS

Polymorphic Loci and Heterozygosity

I scored a total of 97 RAPD loci. However, loci that were faint or not present on all gels were omitted. The total number of RAPD loci scored with high confidence was 61, and these loci were used in subsequent analyses. No loci were unique to differentiate between *Ascaphus* larvae from either sampling location (old growth or clearcut), but streams were too close for this to be possible. However, there were 72% (44/61) polymorphic loci present in old growth, and 61% (37/61) in clearcut (Table 4.2).

Table 4.2. Mean heterozygosity (*H*) and percentage of polymorphic loci at each stream reach station for old growth and clearcut. Mean values for old growth and clearcut were calculated using estimates obtained for each of the 61 loci; these are not based on means for each stream reach station. The total number of samples used to calculate estimates is given in the last row of the Average *n* column.

	H[1]	$S_{\bar{x}}$	% Polymorphic Loci[2]	Average *n*
Old Growth				
0m	0.2396		49.18	9.80
20m	0.1493		31.15	10.00
40m	0.1169		24.59	6.80
60m	0.1818		37.70	10.00
80m	0.0000		0.00	1.00
100m	0.0486		9.84	1.97
120m	0.2620		57.38	5.90
140m	0.2578		57.38	6.66
160m	0.1994		47.54	4.41
180m	0.1908		40.98	4.00
Mean	0.3109	0.0263	72.13	60.54
Clearcut				
0m	0.0575		13.11	10.00
20m	0.0673		14.75	9.67
40m	0.0849		16.39	3.62
60m	0.1643		36.07	9.64
80m	0.1781		36.07	9.97
100m	0.1679		34.43	9.98
120m	0.2130		44.26	10.00
140m	0.1288		26.23	5.00
160m	0.1323		27.87	10.00
180m	0.1965		42.62	7.30
Mean	0.2275	0.0259	60.66	85.18

[1] Nei's (1978) unbiased heterozygosity

[2] Estimates based on 95% criterion (gives percentage of loci that are not fixed for one allele)

Mean heterozygosity that is expected under random mating (H) for old growth and clearcut is given in Table 4.2. In old growth, the estimate at 80 m is zero because only one *Ascaphus* larva was available for the calculation. Based on mean heterozygosity, larvae in the clearcut were genetically less diverse, or more closely related (0.2275 $\pm$ 0.0259) than larvae in old growth (0.3109 $\pm$ 0.0263; $F = 4.259$; $P = 0.041$).

Frequency of Dominant Alleles

Allele frequencies were estimated based on Lynch and Milligan's (1994) Taylor expansion estimate, which takes into account the dominance of RAPD markers and the statistical bias introduced by small sample size. The distribution of RAPD marker allele frequencies (Figure 4.4) showed the frequency of the dominant allele ranged from 0.01 to 1.00 (fixed). Fewer loci were nearly fixed for the dominant allele in the old growth (15) compared with the clearcut (22). These loci contribute little information about population structure and genetic differentiation, as none are specific to either site. Furthermore, most loci were not sufficiently variable to be informative.

Population Differentiation

Genetic differentiation estimates between "populations" (forest cover types) and among "subpopulations" (stream reaches), based on F_{ST} values, are provided in Table 4.3. The degree of differentiation was high among stream reaches ($F_{ST} = 0.3228 \pm 0.0211$), but low between forest cover types ($F_{ST} = 0.0677 \pm 0.0169$). Forest cover types also were analyzed separately, revealing that the level of differentiation among stream reaches in the clearcut appeared to be lower ($F_{ST} = 0.2285 \pm 0.0185$) than among stream reaches in the old growth ($F_{ST} = 0.3136 \pm 0.0232$; Table 4.3). The AMOVA (Table 4.4) confirmed that most of the genetic diversity (97.11%) was found among reaches within each stream ("within population"). Genetic variation between the two populations (old growth and clearcut: "among populations") was small (2.89%).

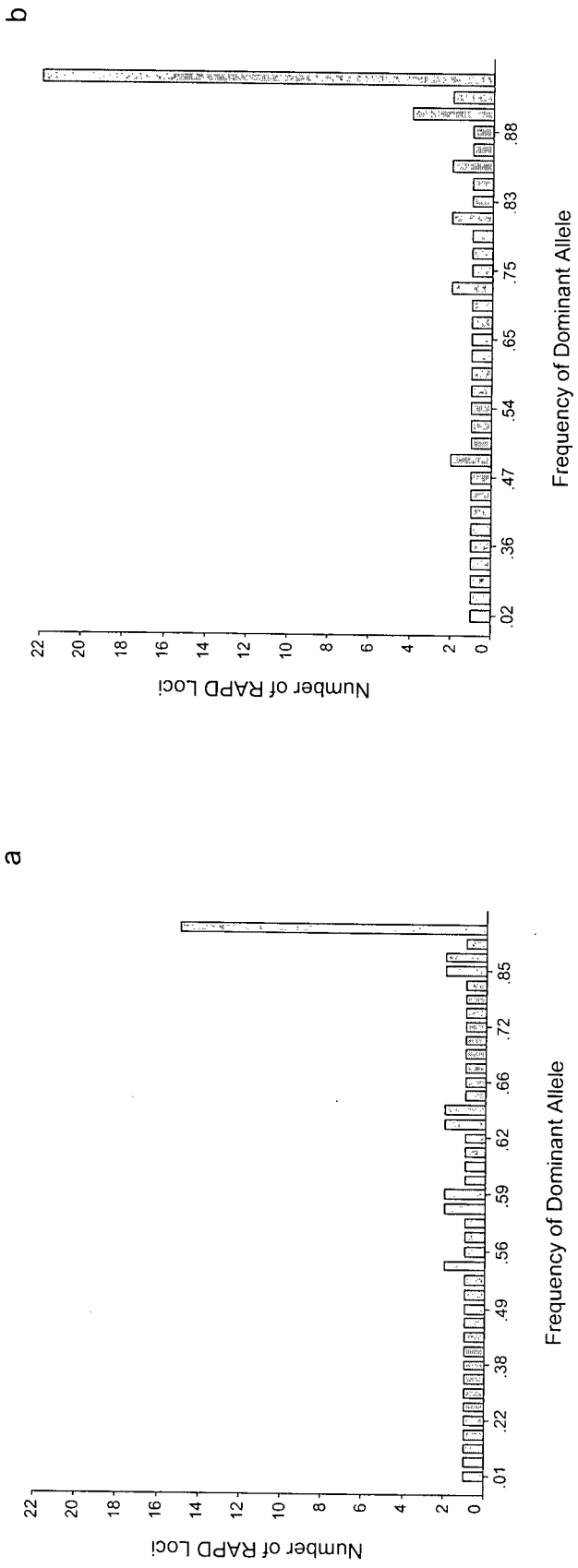

Figure 4.4. Frequency distributions of the dominant allele among the 61 RAPD loci scored: a) old growth, and b) clearcut.

Table 4.3. Genetic differentiation between old growth and clearcut ("population"), among stream reaches ("subpopulations") pooled within old growth and clearcut, and among stream reaches analyzed separately within old growth and within clearcut, as given by F_{ST} values.

Unit	F_{ST}	SE_E[1]	95% Confidence Intervals[2]	
			Lower	Upper
Population	0.0677	0.0169	0.0383	0.1034
Subpopulation	0.3228	0.0211	0.2835	0.3658
Old Growth	0.3136	0.0232	0.2689	0.3613
Clearcut	0.2285	0.0185	0.1945	0.2632

[1] Based on jackknifing over loci
[2] Based on bootstrapping over loci (1000 iterations)

Table 4.4. Hierarchical analysis of molecular variance: between old growth and clearcut ("among populations") and among stream reaches ("within populations") pooled within old growth and clearcut, as given by Φ_{ST} values.

Variance Component	Observed Partition		P[1]	Φ_{ST}
	Variance	% Total		
Among Populations	0.123	2.89	0.024	
Within Populations	4.135	97.11	0.008	0.029

[1] Probability of having a more extreme variance component and Φ_{ST} than the observed values by chance alone

Genetic Relatedness Along Stream Transects

Similarity of RAPD bands within and among stream reach stations are given in Table 4.5; the smaller the value, the less band sharing (i.e., lower genetic similarity). For both forest cover types, larvae sampled from the furthest upstream reach (0 m) were more genetically similar than larvae sampled from furthest downstream reach (180 m). Frequency of RAPD band sharing was averaged for individuals at increasing distances from one another and summarized in Table 4.6. These values are for distance categories (e.g., 0–m apart, 20–m apart) and do not represent stations. On average, frequency of band sharing was greater for larvae sampled in the clearcut stream compared with the old-growth stream, regardless of their physical distance (Table 4.6). In the old-growth stream, larvae sampled within one reach station had a high band similarity frequency, while larvae sampled at stations of increasing distances from one another showed a low band similarity frequency. This pattern was not seen in the clearcut stream.

Table 4.5. Similarity of RAPD bands within and among stream reach stations in a) clearcut and b) old growth. Reach 0 m is the top of the stream sampling area, and 180 m is the bottom. Values in the first row indicate similarities among individuals within each station. The rows that follow indicate similarities between stations of different distances from one another. For example, row four of the 80–m station in the clearcut indicates that individuals sampled at the 80–m and 140–m stations have a band-sharing frequency of 0.925.

Clearcut Site

a.

Station	0 m	20 m	40 m	60 m	80 m	100 m	120 m	140 m	160 m	180 m
	0.921	0.934	0.928	0.917	0.916	0.917	0.912	0.918	0.922	0.914
	0.940	0.953	0.944	0.929	0.931	0.936	0.924	0.927	0.934	
	0.927	0.946	0.927	0.920	0.922	0.923	0.918	0.919		
	0.938	0.948	0.937	0.924	0.925	0.926	0.914			
	0.948	0.956	0.948	0.929	0.933	0.933				
	0.946	0.954	0.945	0.930	0.927					
	0.940	0.947	0.934	0.929						
	0.963	0.964	0.957							
	0.968	0.978								
	0.968									

Old-Growth Site

b.

Station	0 m	20 m	40 m	60 m	80 m	100 m	120 m	140 m	160 m	180 m
	0.876	0.892	0.905	0.890	0.885	0.887	0.852	0.835	0.830	0.856
	0.836	0.843	0.851	0.850	0.842	0.815	0.823	0.817	0.810	
	0.835	0.848	0.859	0.853	0.856	0.858	0.812	0.821		
	0.862	0.870	0.883	0.863	0.841	0.851	0.855			
	0.875	0.902	0.911	0.908	0.915	0.931				
	0.890	0.918	0.930	0.920						
	0.914	0.935	0.942	0.926						
	0.928	0.952	0.954							
	0.922	0.945								
	0.902									

Table 4.6. Mean frequency of RAPD band sharing among individuals sampled within the same stream reach station (0 m) and among individuals sampled at increasing distances from one another (20–m apart, 40–m apart, etc.). Categories indicate distances between stream reach stations. Standard errors for the parameter estimates (SE_E) were obtained with bootstrapping in which 100 repeated random samples were selected from the data and the model is estimated from each one (Norusis 1993).

Forest Cover	Stream Reach Distance Categories									
	0 m	20 m	40 m	60 m	80 m	100 m	120 m	140 m	160 m	180 m
Clearcut	0.937	0.936	0.934	0.934	0.935	0.934	0.936	0.936	0.937	0.921
$\pm$ SE_E	*0.014*	*0.013*	*0.014*	*0.014*	*0.014*	*0.015*	*0.014*	*0.015*	*0.017*	*0.023*
Old Growth	0.889	0.885	0.882	0.876	0.876	0.868	0.863	0.861	0.864	0.876
$\pm$ SE_E	*0.017*	*0.017*	*0.018*	*0.017*	*0.018*	*0.018*	*0.017*	*0.018*	*0.021*	*0.026*

Figure 4.5 summarizes the relationship between genetic distance and physical distance. The between-individual mean band sharing frequency was from 0.921 ± 0.023 to 0.937 ± 0.017 in the clearcut, and 0.861 ± 0.018 to 0.889 ± 0.017 in the old-growth site (Table 4.6). Overall, larvae were more genetically similar in the clearcut but less similar in old growth (Figure 4.5). In the clearcut, larvae 0–m to 160–m apart showed no decrease in genetic relatedness (Figure 4.5). In old growth, however, larvae 0–m to 140–m apart decreased in genetic similarity (Figure 4.5). Values at 160–m and 180–m stream reaches in old growth, and at the 180–m reach in the clearcut (Figure 4.5) have high standard errors of the estimate (Table 4.6) and may represent outliers. Pearson correlations revealed a low negative correlation between genetic relatedness and physical distance of individuals in the clearcut site (r = -0.475, P = 0.165), but a high negative correlation between genetic relatedness and physical distance in old growth (r = -0.786, P = 0.007; Figure 4.5).

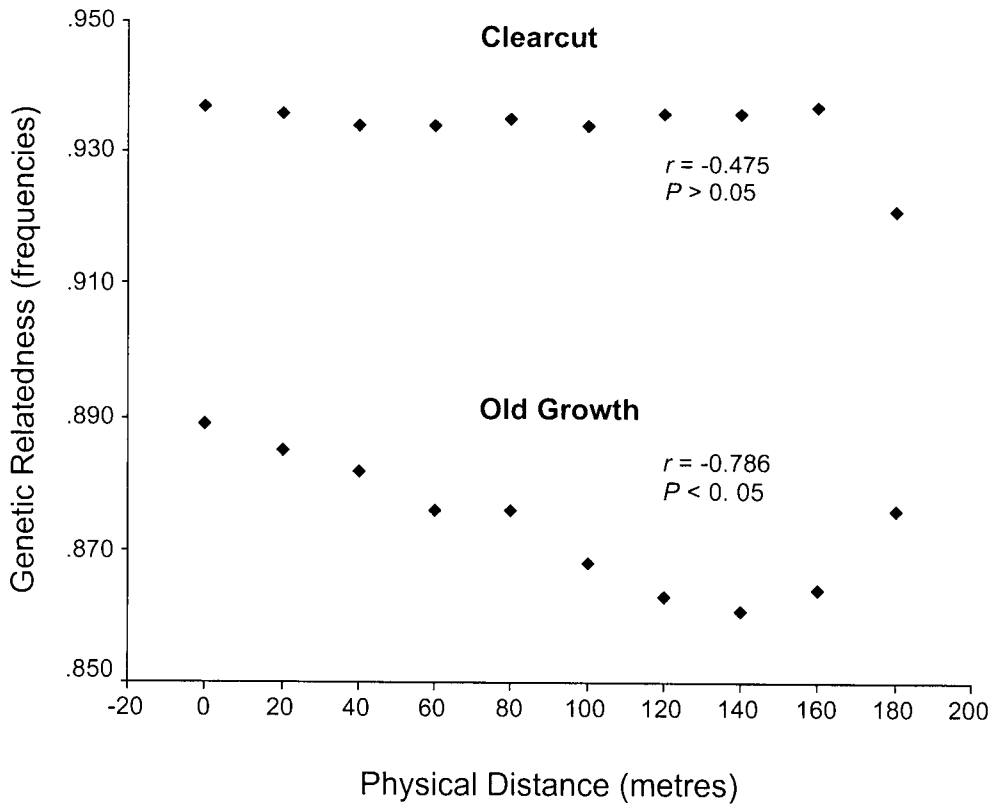

Figure 4.5. Scatterplot of genetic relatedness (frequency of RAPD band sharing) and physical distance (distance between stream sampling stations; meters apart) for individuals sampled from the clearcut and old-growth site.

DISCUSSION

Heterozygosity

In my RAPD study, *Ascaphus* had lower genetic variation in the clearcut ($H = 0.23 \pm 0.03$) than in the old growth ($H = 0.31 \pm 0.03$), based on mean heterozygosity and percentage of polymorphic loci (72% and 61%, respectively). Using RAPD data, Ritland *et al.* (2000) reported a mean heterozygosity of 0.21 ± 0.02 for *Ascaphus* in a clearcut stream. *Ascaphus* tissue samples were collected from the same clearcut site in each study, and their estimate corresponds closely with that obtained in my RAPDs study.

I found no studies examining differences in genetic structure between populations in old growth and clearcuts using RAPD techniques. However, some results based on allozyme data are noteworthy. Green *et al.* (1989) reported a mean heterozygosity of 0.096 for *Ascaphus* (six frogs; coastal Oregon). Hitchings and Beebee (1997) reported lower genetic variation ($H = 0.06$; 42% polymorphic loci) in urban populations compared with rural populations ($H = 0.07$; 50% polymorphic loci) of *Rana temporaria*, a common pond-breeding frog. Nevo and Beiles (1991) reported decreases in genetic diversity with decreasing ecological heterogeneity, from terrestrial and arboreal to aquatic and subterranean habitats (i.e., with decreasing niche breadth, or with increasing ecological stability and predictability). However, the terrestrial genus *Leiopelma* generally had the lowest levels of heterozygosity (0.01–0.09) among aquatic, terrestrial, and arboreal frogs (Green *et al.* 1989; Nevo and Beiles 1991). Reported heterozygosity of other amphibian taxa included 0.05 for tree frogs (Hylidae), 0.08 for true frogs (Ranidae), and 0.11 for true toads (Bufonidae; Nevo and Beiles 1991). Because these data are based on allozymes, they cannot be directly compared with results from RAPD assays. However, trends in genetic variation among anurans can be evaluated and may provide the basis for predictions when using other molecular markers.

For some species, heterozygosity is positively correlated with fitness components such as survival, growth rates, and fertility (Mitton and Grant 1984; Reed and Frankham 2003). If this is true for *Ascaphus* my data may suggest that individuals in the clearcut are less capable of adapting to environmental fluctuations (e.g., increases in temperature and ultra-violet radiation). Equally important is their increased susceptibility to disease (e.g., pathogenic fungi such as chytrids or *Saprolegnia* water molds). My results suggest lower population persistence for *Ascaphus* in the clearcut.

A number of factors could be responsible for the observed pattern of lower genetic variation in the clearcut than in the old growth. Harvested 10 years ago, the clearcut may now have few individuals entering the population. Dispersal may be limited in clearcuts due to this organism's physiological constraints. Recall that *Ascaphus* have extremely low desiccation tolerance (Claussen 1973), and one of the lowest and narrowest ranges of temperature

tolerance among anurans (Brattstrom 1963; de Vlaming and Bury 1970). Although it is possible that genetic variation may have been low originally (i.e., few founding members prior to logging), it is unlikely as the heterozygosity estimate for the nearby old growth is higher. Another factor that may affect estimates of genetic variation is the number of mating individuals. Over three years, I captured 33 mature adults in clearcuts and 75 mature adults in old-growth forests (Chapter 3). During this time, I captured an equal number of gravid females in both forest cover types across the three years. However, with less than half the number of reproductively mature adults contributing to the gene pool in clearcuts, estimates of genetic variation were expected to be lower in clearcuts than in old growth. Populations in harsher habitats (e.g., clearcuts, urban settings) are expected to show reduced genetic diversity due to lower effective population sizes and temporary contractions of population size (Ritland *et al.* 2000).

Population Differentiation

I estimated large F_{ST} among stream reaches (F_{ST} = 0.32), indicating a high degree of genetic differentiation among stream reaches (old growth and clearcut combined). When sites were analyzed separately, the degree of genetic differentiation among reaches within old growth was still high (F_{ST} = 0.31). However, F_{ST} in the clearcut was low (0.23). The lower genetic differentiation among reaches in the clearcut and lower heterozygosity are consistent with a smaller effective population size. These results suggest that the larval population in the clearcut stream is less stable than that in the old-growth stream. I estimated a small F_{ST} value between old growth and clearcut (F_{ST} = 0.07), indicating a low degree of genetic differentiation among streams. Prior to forest harvesting activities in that portion of the watershed (10 years ago), it may be possible that adults moved between the two streams, which are 1.6 km apart. This could explain the currently small degree of differentiation between the two streams. *Ascaphus* in the clearcut stream may have reduced in numbers since logging (recall the capture of fewer mature frogs in clearcuts than in old growth; Chapter 3), resulting in a less stable population genetic structure than in the old growth.

Using RAPDs, Ritland *et al.* (2000) reported an F_{ST} of 0.04 among five populations in the south coast of BC, near the city of Squamish. Samples in their study were collected from sites too distant from one another for gene flow to occur. However, the authors suggested that the low values for F_{ST} and H, and a lack of association between genetic and geographic distance, were the result of more recent colonization. Based on allozymes, genetic differentiation among populations for other anurans include: *Bufo japonicus* (F_{ST} = 0.24), *Bufo bufo* (F_{ST} = 0.49), and *Rana temporaria* (F_{ST} = 0.32; summarized by Hitchings and Beebee 1997). Using RAPDs, a high degree of within-population differentiation among *Rana pipiens* also was reported by Kimberling *et al.* (1996). Based on allozyme data, Hitchings and Beebee (1997) reported a high

degree of genetic differentiation (F_{ST} = 0.35) among urban populations of *Rana temporaria*, and a low degree of differentiation (F_{ST} = 0.11) among rural populations. The authors attributed the high degree of urban subpopulation differentiation to the increasingly inhospitable terrain of the urban environment.

Because *F*-statistics rely on assumptions of H-W equilibrium, I thought it appropriate to use an alternative estimator of population differentiation that did not rely on this assumption. There were a number of reasons why I chose to explore alternative statistics. H-W equilibrium is rare and probably does not characterize *Ascaphus* populations. For example, in the fall, reproductively mature adults congregate at the upper headwaters of streams, and there is evidence that adults have strong site fidelity (Daugherty and Sheldon 1982*a*). In my study, of 17 total recaptures, five reproductively mature adults (29% of recaptures) were recaptured at the same stream in consecutive years (Chapter 3) suggesting some adults may return to their natal stream. Therefore, random mating may not occur. Also, *Ascaphus* population size is unknown; however there is evidence that it is small. I captured only 254 frogs (many were metamorphs) at six sites over three years (Chapter 3). Furthermore, because of the relatively recent and continuing colonization of northern habitats suggested by Ritland *et al.* (2000), some degree of immigration and emigration likely exists.

Based on AMOVA, estimates confirmed genetic differentiation among old-growth and clearcut streams was small (2.89%; Φ_{ST} = 0.03), and most genetic differentiation (97%) was found within streams. A major disadvantage to using the program WINAMOVA is that it cannot analyze a dataset containing missing observations, thus 26 samples (17 from old growth and nine from clearcut) were omitted. Using a small sample size when calculating estimates for genetic differentiation means that some rare alleles may be missed, which may under- or over-estimate F_{ST} values (and Φ_{ST}), which are directly related to estimates of heterozygosity. However, the reduction in sample sizes due to omission of those data is probably not critical (C. Ritland *pers com*). In another study of *Ascaphus*, AMOVA indicated that both coastal (88.7%) and inland populations (82.7%) exhibited significant genetic differentiation (Neilson *et al.* 2001). Significant differentiation also occurred within a group encompassing 14 populations (Φ_{ST} = 0.571; Neilson *et al.* 2001). The long generation time in *Ascaphus* (6–8 years) and low metabolic rate may result in a slow evolutionary rate, which can reduce genetic divergence (Martin and Palumbi 1993).

Geographic Distance and Genetic Relatedness

Based on band-sharing frequencies, individuals in the clearcut were more related to one another than were individuals in the old growth. This is consistent with heterozygosity and F_{ST} estimates. Both streams had more genetically similar individuals at the top of the stream than at the bottom. This observation suggests that larval drift from upper reaches influenced the patterns of genetic relatedness as larvae move further downstream. The difference was greater in old growth. Results are not surprising, as siblings are more likely to be found within close proximity of one another at the upper reaches, closer to where egg masses are deposited. However, in the clearcut, there was a high degree of genetic relatedness among individuals, and geographic distance did not appear to have any influence on relatedness of larvae up to 160 meters away. Individuals in old growth, however, showed a decrease in genetic relatedness with increasing geographic distance between sampled reaches. These findings may suggest that more movement of larvae across reaches occurs in the clearcut stream. However, that would be inconsistent with results of Chapter 2, in which larval *Ascaphus* in clearcut streams moved shorter distances (up to 3 m) compared with larvae found in old-growth streams (up to 65 m). Stream and site parameters have the potential to influence larval movement within streams. I measured stream gradient, wetted width, and site aspect (Table 4.1), however none appear significantly different to have caused a response in larval movement patterns (also see Chapter 2). An alternative to more movement is the possibility that many larvae in the clearcut are siblings. The entire sample may be offspring from as few as two mating pairs, or from closely related parents. This is a plausible explanation for the inconsistency in results. Mating pairs that were restricted to the clearcut stream since logging may explain the greater genetic similarity of larvae in the clearcut stream compared with those in the old-growth stream.

CHAPTER FIVE
Summary and Conservation Implications

INTRODUCTION

Timber harvesting reduces habitat patch size, increases habitat fragmentation (increasing probabilities of local extinction), and removes habitat connectivity, thus reducing dispersal among patches (e.g., Sjögren 1991; Bunnell *et al.* 1992; Fahrig and Merriam 1994). Welsh and Lind (2002) believed *Ascaphus* populations would continue to decline in the Pacific Northwest in response to anthropogenic disturbance regimes. If *Ascaphus* movements through upland habitats are restricted in recently harvested forests, connectivity among populations will be lower and recolonization of streams from which they have been extirpated may be more difficult (Aubry 2000).

A metapopulation is a collection of smaller subpopulations occupying habitat patches, connected by dispersal, and balanced by extinction and colonization (Levins 1969; Hanski and Simberloff 1997). The subpopulations can each be characterized in terms of genetic variation and demographic parameters (Galbraith 1997). In many respects, amphibian spatial dynamics resemble classical metapopulation models. This is particularly evident where subpopulations in breeding ponds blink in and out of existence over time, and extinction and colonization rates are functions of pond spatial arrangement (Marsh and Trenham 2001). The extent of individual movement is related to the potential for gene flow among populations (Lande 1988). Breeding ponds form discrete habitat patches for many anurans, but the connected nature of *Ascaphus* streams may not result in discrete populations. Also, aggregations of amphibians at individual streams or ponds may not represent distinct populations. Many amphibian species regularly disperse between ponds (Marsh and Trenham 2001), and the same may be true of stream-breeding amphibians.

I investigated the role of forest cover in the movement patterns and colonization potential of *Ascaphus*. I examined differences in movement patterns and dispersal abilities of larval, juvenile, and adult *Ascaphus*, and explored genetic variation in *Ascaphus* populations in managed and unmanaged forests. I reviewed reasons for concern about the likelihood of *Ascaphus* to be extirpated locally and then recolonize managed forests, and indicated existing information gaps in Chapter 1. In Chapter 2, I evaluated larval movements of *Ascaphus* and discussed associations with stream and site parameters. In Chapter 3, I examined terrestrial movements of juvenile and adult *Ascaphus*, and the multiple factors that can confound responses to timber harvesting. I conducted a preliminary examination of the population genetic

structure (using RAPDs) of *Ascaphus* in two streams within a single watershed in Chapter 4. Here, I present a synthesis of results among investigations. I discuss the implications of those results to *Ascaphus* conservation goals, and I propose options to develop more effective management for *Ascaphus* and other species that rely on forested headwater streams.

SUMMARY OF RESULTS

Maximum daily movement rates of larvae in streams flowing through old-growth forests (3.8 m/day) appeared to be greater than rates in clearcuts (0.3 m/day) and mature second-growth forests (1.9 m/day). Stream gradient differed among forest cover types (ranging from 4 to 44%), and larval movement rates had a tendency to be greater in low gradient streams, suggesting active movement versus passive drift. Higher rates of larval movement were observed in streams with larger wetted widths. Neither larval density nor percent canopy closure influenced larval movement rates. Logjams had a significant role and explained 13% of the variation in larval movement rates. Logs embedded in the stream are features that can create physical barriers to larval movement. All clearcut sites contained abundant logjams, and larval movement rates were greater in streams with lower levels of logjams. Alternatively, lower movement rates in clearcuts may be a response to greater instream primary productivity (related to less canopy and more solar insolation), which could encourage faster growth and reduced movement of larvae.

More juvenile and adult frogs were captured in clearcuts (0.91/100 TN) than in old-growth forests (0.72/100 TN). Developmental stages were differentially represented with 3X more juveniles in clearcuts (0.70/100 TN; 91% of these were metamorphs) than in old growth (0.24/100 TN), but 2X more adults in old growth (0.48/100 TN) than in clearcuts (0.21/100 TN). There appeared to be more recaptures (8.5%) and higher movement rates (3.36 m/day; n = 12) in clearcuts than in old-growth forests (4.5%; 2.09 m/day; n = 5), but sample sizes were small. Many (n = 24) of these coastal *Ascaphus* moved at least 100 m from streams compared to at least 12 m from streams reported for inland populations (Metter 1964*a*; Landreth and Ferguson 1967). However, in the fall, frogs were captured 1.3X farther from streams in old-growth forests (21.59 $\pm$ 9.15 m) than in clearcuts (17.00 $\pm$ 3.93 m), and there were more frogs captured within 25 m of streams in clearcuts. Adults were captured farther (2X) from streams than were juveniles. Females were captured twice as far as males, and movement patterns appear to suggest breeding migrations (upland to streams). Reproductively mature frogs were captured twice as far from streams in old growth compared with clearcuts. Most frogs in clearcuts were moving upstream, and most frogs in old-growth forests were moving towards streams. Adult movement directions appeared little affected by forest cover type. Juveniles, however, exhibited stronger stream affinity in clearcuts than in old-growth forests, and moved mainly upstream.

Juveniles and adults in clearcuts tended to be slightly smaller and had a lower body condition index (BCI) than frogs in old-growth forests.

In both the old growth and clearcut, there were no unique loci to differentiate between larvae from either forest cover type. Fewer loci were nearly fixed for the dominant allele in the old growth (n = 15) than in the clearcut (n = 22). Forty-four (72%) polymorphic loci were present in the old growth, and 37 (61%) polymorphic loci were present in the clearcut. Based on mean heterozygosity, larvae in the clearcut were less diverse, or more closely related (H = 0.23 $\pm$ 0.03), than larvae in the old growth (H = 0.31 $\pm$ 0.03). The degree of genetic differentiation was high among stream reaches (F_{ST} = 0.32 $\pm$ 0.02), but low between forest cover types (F_{ST} = 0.07 $\pm$ 0.02). When forest cover types were analyzed separately, the level of differentiation among stream reaches in clearcut was lower (F_{ST} = 0.23 $\pm$ 0.02) than among stream reaches in old growth (F_{ST} = 0.31 $\pm$ 0.02). AMOVA confirmed that most of the genetic diversity (97%) was found within streams, and genetic variation between the old-growth and the clearcut site was small (3%). For both the old growth and the clearcut, larvae sampled from the furthest upstream reach (0 m) were more genetically similar than larvae sampled from furthest downstream reach (180 m). Frequency of band sharing (genetic relatedness) was greater for larvae sampled in the clearcut compared with the old growth, regardless of their physical distance. In the old growth, larvae sampled within the same stream reach had high band similarity (high genetic relatedness), and larvae sampled at stream reaches of increasing distances from one another had lower band similarity. Thus, geographic distance and genetic similarity were negatively correlated in the old growth (r = -0.786, P = 0.007). Larvae in the clearcut showed no pattern in genetic relatedness with increasing geographic distance (r = -0.475, P = 0.165).

IMPLICATIONS OF MY RESULTS TO *ASCAPHUS* CONSERVATION GOALS

Conservation has long been an important part of wildlife management. One goal of conservation biologists is to maintain natural levels of biodiversity and reduce factors driving species to extinction (Caughley and Gunn 1996). *Ascaphus* is a taxonomically unique endemic of the Pacific Northwest of North America, and is believed to be the most primitive frog in the world (Ford and Cannatella 1993). Maintaining representative viable populations throughout the range of *Ascaphus* is an underlying assumption of my thesis. Here, I outline how my research on *Ascaphus* contributes to the overall conservation goal. I also provide some management recommendations and directions for future research.

1. My research revealed a tendency for shorter larval movements in streams containing many embedded logjams. Embedded logs were abundant in clearcut streams and may reduce the recolonization potential of *Ascaphus* larvae. Stream ecologists use the term 'drift' to describe the downstream movement of stream organisms either passively with floods or actively as in behavioral drift. Müller (1974) hypothesized that drift would eventually wash entire populations out of streams unless organisms actively moved upstream to compensate for drift. He coined the term "colonization cycle" to describe the maintenance of stream populations through a dynamic interplay between downstream drift and upstream movement.

   It appears that *Ascaphus* may follow a colonization cycle. Larvae exhibit downstream movements, and my terrestrial data suggest that many post-metamorphic *Ascaphus* move upstream, compensating for larval movements. Reduced larval movements in clearcuts may be problematic in maintaining the colonization cycle. Logging activities resulting in a major increase in the density or embeddedness of large wood in stream channels should be avoided. Falling and yarding away from streams will be necessary to maintain slash-free channels (Chapter 2; Dupuis and Steventon 1999).

   My recommendations for future research on larval *Ascaphus* include: 1) estimation of algal production among headwater streams transecting clearcuts and old growth; 2) assessment of larval movements along a continuous stream sampling area (e.g., 30–50 m in length), more regular sampling (e.g., every 2–3 days), and the placement of a weir at the base of the lower sampling reach to increase recapture rates; and 3) sampling of larvae along an elevational gradient to determine dispersion of larvae in streams transecting clearcuts and old-growth forests.

2. My research suggests that long distance overland movement is more likely when forested stands are present. Terrestrial habitat use by *Ascaphus* (particularly juvenile frogs) during fall appears more spatially reduced in clearcuts than in old-growth forests. Recent metamorphic wood frogs (*Rana sylvatica*) prefer closed canopies (deMaynadier and Hunter 1999), and I captured juvenile *Ascaphus* farther from streams in old growth than in clearcuts. Adult frogs are considered the most evolutionarily effective migrants (*sensu* MacArthur and Wilson 1967) because their reproductive value is high, especially for gravid females. I recorded adult *Ascaphus* undertaking more long distance overland movements than juveniles. Also, in old growth, I captured twice as many reproductively mature frogs as in clearcuts. Therefore it appears there are fewer

evolutionarily effective migrants available to disperse through clearcuts to adjacent streams. The capture of fewer mature frogs in clearcuts may result from poor survival of juveniles.

These results have implications to population persistence of *Ascaphus*. Limited movement might lead to increased population subdivision and isolation (Nijhuis and Kaplan 1998) due to a reduction in gene flow, and increase the chance of local extinction by demographic or environmental stochasticity. Amphibians are known to benefit from retention of riparian habitats during logging (Gomez and Anthony 1996; Dupuis and Steventon 1999; Maxcy 2000). Large riparian buffers may ensure moist and less variable microclimatic conditions, particularly in times of drought. Despite reports of strong stream fidelity for inland populations of *Ascaphus*, I found that BC frogs could move at least 100 m from streams into the upland forest in rainy weather.

Maintaining some gene flow between populations is important and *Ascaphus* movement is more likely where forest cover is present. A forest buffer on both sides of streams appears to be a useful approach to maintaining *Ascaphus* populations (Welsh and Lind 2002). However, an effective width is not clear from my results and will require further investigation and monitoring. Adjusting zone widths depending on local conditions (e.g., stream wetted width, gradient, intensity of adjacent forest harvest) may be an appropriate option (deMaynadier and Hunter 1995). Windthrow damage is estimated to amount to 4% of the provincial annual allowable cut (Mitchell 1995), thus buffer integrity should be maintained by ensuring that harvested edges of buffers are windfirm.

My recommendations for future research include: 1) investigation of daily movements of juveniles and adults using passive integrated transponders (PITs) or radio transmitters for tracking individuals among clearcuts and old growth; 2) long-term mark-recapture studies to reveal stream-to-stream movements where different buffer widths are maintained; 3) summer assessment of juvenile and adult abundance (CPUE) along an elevational gradient to determine dispersion along clearcut and old-growth streams; and 4) daily tracking of gravid females (using PITs or radio transmitters) in the summer and fall to evaluate possible breeding migrations, reproductive cycle (e.g., number of clutches per year), and determine oviposition site selection.

3. My results revealed lower heterozygosity (genetic variation) for larvae captured in the clearcut site. In some species (including amphibians), heterozygosity is positively correlated with fitness components such as survival, growth rates, and fertility (Mitton and Grant 1984; Reed and Frankham 2003). If this is true for *Ascaphus*, then my data may suggest that individuals in the clearcut site will be less capable of adapting to environmental fluctuations and will be more susceptible to factors such as disease. The capture of fewer breeding adults and the lower genetic variation recorded in the clearcut is consistent with a smaller effective population size, which may mean clearcut populations are less stable (i.e., lower population persistence) than in old growth. Small effective population size and a lack of dispersal between sites may limit mate choice, which leads to breeding with close relatives.

Results also suggest *Ascaphus* populations exist in a metapopulation structure, but further investigation is required. Through monitoring of genetic diversity within and among *Ascaphus* populations, metapopulation dynamics may be revealed. Riparian buffers likely serve as movement corridors for juvenile and adult *Ascaphus*, but large openings in adjacent forests may prevent frogs from reaching nearby streams via overland movements. A partial forest matrix between streams could be retained within each watershed to provide habitat pathways for dispersing frogs. When connectivity between streams cannot be maintained, habitat conservation strategies for *Ascaphus* could be improved by including riparian management areas on multiple adjacent headwaters in areas favoring *Ascaphus*. Reducing distances between mature forest patches would improve the chances of *Ascaphus* moving through harvested watersheds. To encourage gene flow among populations, I recommend monitoring *Ascaphus* populations where a partial forest matrix is retained between streams. Studies could focus on varying the level of harvest within the matrix. My RAPD study in two *Ascaphus* streams provides some understanding of how habitat fragmentation can influence population genetic structure. The examination is incomplete, but signals the need for further research on this topic.

My recommendations for future research include: 1) increasing studies to include at least three replicates and larger sample sizes (e.g., >30 larvae/sampling location; >100 larvae/stream); 2) evaluating population genetic structure in *Ascaphus* using additional molecular markers (e.g., microsatellites when these become available for *Ascaphus*); 3) analysis of genetic relatedness among mature frogs and larvae in streams transecting clearcuts and old growth to examine site fidelity in *Ascaphus*; 4)

examination of population genetic structure and possible metapopulation structuring of *Ascaphus* populations within multiple adjacent watersheds and multiple streams within each watershed; and 5) molecular monitoring of genetic diversity within and among *Ascaphus* populations where a partial forest matrix is retained between streams.

My results, though not all significant statistically, are internally consistent and suggest reduced recolonization potential and lower genetic variation in *Ascaphus* populations where forest cover has been removed. Aggregations of *Ascaphus* at individual streams may not represent distinct populations, and should not be managed as distinct units. *Ascaphus* may regularly disperse between streams. Thus, connectivity between multiple streams within a watershed will be a more meaningful unit of management than individual streams with forested buffers. Promoting some connectivity between streams may be the single most important issue to address when defining *Ascaphus* conservation strategies. Riparian buffers alone may not be effective for promoting long-term persistence of *Ascaphus* populations, but when no other habitat protection is provided, buffers should be retained along streams as a first step towards *Ascaphus* protection. Conservation measures that are more likely to promote long-term population persistence should be considered, such as the retention of a partial forest matrix between streams.

Whitlock and McCauley (1999) suggest that movement of individuals is usually more relevant to our understanding of dispersal than gene flow alone. However, direct estimates of dispersal are difficult (Vos *et al.* 2001), and there are additional limitations to ecological studies such as confounding factors (e.g., watershed, year, developmental stage; see Chapter 3). Using ecological or molecular techniques alone to conduct monitoring of *Ascaphus* populations can be problematic because each technique has limitations. I recommend that any future *Ascaphus* research include both ecological and molecular tools. By coupling ecological and molecular tools, conservation biologists and forest managers may be better able to answer questions about species at risk, and with reduced uncertainty. For example, long-term mark-recapture studies (or when technologies are improved, tracking individuals using radio transmitters to evaluate long-distance overland movements) and molecular monitoring of genetic diversity over at least one generation would constitute an effective monitoring program for *Ascaphus*. Monitoring populations within different management scenarios and evaluating the success of management will allow us to determine if we are indeed achieving our conservation goals.

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Table A-I.1. Small mammal mortalities: forest cover type, watershed, year, month, and distance from stream (m). All shrew mortalities occurred in traps where escape ropes were not properly secured (either not touching bottom of trap, or rope missing due to bear disturbances).

Small Mammal[1]	Forest Cover	Watershed	Year	Month	Distance from Stream (m)
shrew	Clearcut	Mamquam	1998	August	5
shrew	Clearcut	Mamquam	1998	August	5
mole	Old Growth	Ashlu	1999	July	5
mole	Old Growth	Ashlu	1999	August	100
mole	Old Growth	Ashlu	1999	August	0
mole	Old Growth	Ashlu	1999	August	0
mole	Old Growth	Ashlu	1999	August	0
mole	Clearcut	Mamquam	1999	September	25
mole	Old Growth	Ashlu	1999	October	25
mole	Old Growth	Mamquam	1999	October	50
shrew	Old Growth	Ashlu	1999	September	100
shrew	Old Growth	Mamquam	1999	September	25
mole	Old Growth	Ashlu	2000	September	0
mole	Old Growth	Ashlu	2000	October	25
shrew	Clearcut	Elaho	2000	September	25
shrew	Clearcut	Elaho	2000	September	25
shrew	Clearcut	Mamquam	2000	September	25
shrew	Clearcut	Ashlu	2000	October	50
shrew	Clearcut	Ashlu	2000	October	50
shrew	Clearcut	Ashlu	2000	October	50
shrew	Old Growth	Mamquam	2000	October	50

[1] Moles (shrew-mole, *Neurotrichus gibbsii*) were identified by Dr. Kevin Campbell, UBC. Shrews (*Sorex* spp.) were not identified to species.

Table A-I.2. The number of amphibians trapped, encountered, and recaptured during the *Ascaphus* field season. Southwestern British Columbia. 1998–2000.

Year	Month	Numbers Trapped[RC] (Encountered)[1]							Total
		AMGR	AMMA	ENES	PLVE	BUBO	RAAU	HYRE	
1998	July	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	August	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (1)	0 (0)	0 (2)
	September	8 (2)	8 (2)	5 (0)	4 (1)	4 (0)	0 (0)	0 (2)	29 (7)
	October	13 (0)	10 (0)	4 (0)	6 (2)	4 (1)	0 (0)	0 (0)	37 (3)
1999	July	2^{1} (0)	0 (0)	0 (0)	0 (0)	11 (0)	0 (0)	0 (1)	13^{1} (1)
	August	6 (0)	2 (0)	2 (0)	1 (0)	10^{1} (0)	0 (0)	0 (1)	21^{1} (1)
	September	4 (0)	4 (0)	2 (0)	4 (0)	9 (0)	0 (0)	0 (0)	23 (0)
	October	22^{3} (0)	18^{1} (0)	6 (0)	5 (0)	8 (0)	2 (0)	0 (1)	61^{4} (1)
2000	September	28^{3} (0)	11 (0)	4 (0)	6^{1} (0)	43 (2)	1 (0)	0 (1)	93^{4} (3)
	October	22 (0)	9 (0)	4 (0)	4 (0)	13^{1} (0)	0 (0)	0 (0)	52^{1} (0)
	November	4 (0)	2 (0)	2 (0)	3^{1} (0)	0 (0)	0 (0)	0 (0)	11^{1} (0)

[1] Recaptures were included in counts, and reported as superscripts; AMGR = *Ambystoma gracile*, AMMA = *Ambystoma macrodactylum*, ENES = *Ensatina eschscholtzi*, PLVE = *Plethodon vehiculum*, BUBO = *Bufo boreas*, RAAU = *Rana aurora*, HYRE = *Hyla regilla*.

Table A-I.3. The relative abundance (number per 100 trap nights) of amphibians trapped during the *Ascaphus* field season. Southwestern British Columbia. 1998–2000.

Year	Month	Relative Abundance (#/100 TN)[1]						
		AMGR	AMMA	ENES	PLVE	BUBO	RAAU	HYRE
1998	September	0.31	0.31	0.19	0.15	0.15	0.00	0.00
	October	0.52	0.40	0.16	0.24	0.16	0.00	0.00
1999	July	0.08	0.00	0.00	0.00	0.84	0.00	0.00
	August	0.35	0.12	0.12	0.06	0.53	0.00	0.00
	September	0.08	0.08	0.04	0.08	0.18	0.00	0.00
	October	0.22	0.20	0.07	0.06	0.09	0.00	0.00
2000	September	0.37	0.16	0.06	0.07	0.63	0.00	0.00
	October	0.30	0.12	0.05	0.05	0.16	0.00	0.00
	November	0.28	0.14	0.14	0.14	0.00	0.00	0.00

[1] Incidental encounters and recaptures not included; AMGR = *Ambystoma gracile*, AMMA = *Ambystoma macrodactylum*, ENES = *Ensatina eschscholtzi*, PLVE = *Plethodon vehiculum*, BUBO = *Bufo boreas*, RAAU = *Rana aurora*, HYRE = *Hyla regilla*.

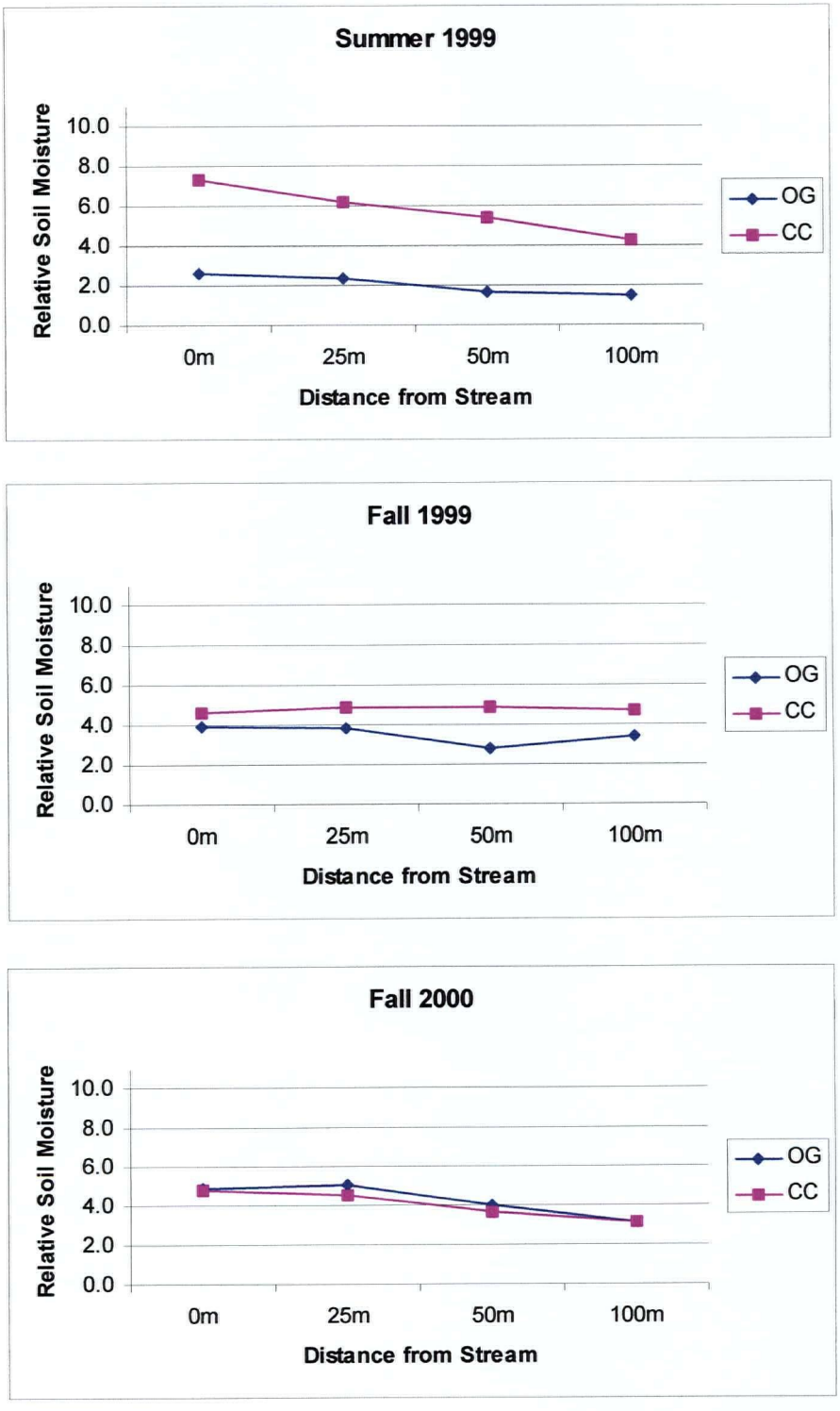


Figure A-I.1. Relative soil moisture at four distances from stream in old growth (OG) and clearcut (CC): 0 m, 25 m, 50 m, and 100 m. a) summer 1999, b) fall 1999, and c) fall 2000.

Table A-II.1. Six primers, and 61 loci scored with high confidence. Lab sample number, tadpole DNA sample number, treatment (TR: OG = Old Growth, CC = Clearcut), and distance (stream sampling station, in meters).

Primer 211				Loci (bp)										
Sample #	DNA #	TR	Distance (m)	1250	1050	975	925	875	840	825	780	650	525	500
1	36	OG	0	1	1	0	1	0	0	1	1	0	1	1
2	37	OG	0	1	0	0	1	0	0	1	1	0	0	1
3	38	OG	0	0	0	0	1	0	0	1	1	1	0	0
4	31	CC	0	1	0	0	1	0	0	1	1	0	0	1
5	32	CC	0	1	0	0	1	0	0	1	1	1	0	1
6	33	CC	0	1	0	0	0	0	0	1	1	0	0	1
7	40	OG	20	1	1	0	1	0	0	1	0	0	0	0
8	46	OG	20	0	0	0	1	0	1	1	1	0	1	1
9	47	OG	20	1	1	0	1	0	0	1	1	0	1	1
10	192	CC	20	1	1	0	1	0	0	1	1	0	1	1
11	193	CC	20	1	1	0	1	0	0	1	1	0	1	1
12	194	CC	20	1	1	0	1	0	0	1	1	1	1	1
13	50	OG	40	0	0	0	1	0	0	1	1	0	0	0
14	141	OG	40	1	0	1	1	0	0	1	1	0	0	0
15	142	OG	40	1	0	1	0	0	0	1	1	0	1	1
16	202	CC	40	1	1	0	1	0	0	1	1	0	1	1
17	203	CC	40	1	1	0	1	0	0	1	1	0	0	1
18	204	CC	40	1	1	0	1	0	0	1	1	0	1	1
19	147	OG	60	1	1	0	1	0	0	1	1	0	0	0
20	148	OG	60	1	1	0	1	0	0	1	1	0	1	1
21	149	OG	60	1	1	1	1	0	0	1	1	1	1	1
22	207	CC	60	1	1	1	1	0	0	1	1	1	1	1
23	208	CC	60	1	1	1	1	0	0	1	1	1	1	1
24	209	CC	60	1	1	1	1	0	0	1	1	1	1	1
25	157	OG	120	1	1	0	1	0	0	1	0	0	0	1
26	158	OG	120	1	1	1	1	0	0	1	0	0	0	1
27	159	OG	120	1	1	1	1	0	0	1	1	0	0	1
28	237	CC	120	1	1	1	1	0	0	1	0	1	0	1
29	238	CC	120	1	1	1	1	0	0	1	1	1	0	1
30	239	CC	120	1	1	1	1	1	0	1	1	1	1	1
31	167	OG	140	1	1	1	1	0	0	1	1	1	1	1
32	168	OG	140	1	1	1	1	0	0	1	1	1	1	1
33	169	OG	140	1	1	1	1	0	0	1	0	0	1	1
34	247	CC	140	1	1	1	1	0	0	1	1	1	1	1
35	248	CC	140	X	X	X	X	X	X	X	X	X	X	X
36	249	CC	140	1	1	1	1	0	0	1	1	0	0	1
37	174	OG	160	1	1	1	1	0	0	1	0	1	1	1
38	175	OG	160	1	1	1	1	0	0	1	1	1	1	1
39	176	OG	160	1	1	0	0	0	0	1	1	0	1	1
40	250	CC	160	1	1	1	1	0	0	1	1	0	1	1
41	251	CC	160	1	1	1	1	0	0	1	1	0	1	1
42	252	CC	160	1	1	1	1	0	0	1	1	0	1	1
43	11	OG	180	1	1	1	1	0	0	1	1	1	1	0
44	12	OG	180	1	1	1	1	0	0	1	1	0	1	0
45	13	OG	180	1	1	1	1	0	0	1	1	0	1	0
46	39	OG	0	1	1	1	0	0	0	0	1	0	0	0
47	34	CC	0	X	X	X	X	X	X	X	X	X	X	X
48	35	CC	0	1	0	0	0	0	0	0	1	0	0	0
49	189	CC	0	1	0	0	0	0	0	0	1	0	1	0
50	48	OG	20	1	1	0	1	1	0	0	0	0	0	0

Table A-II.1. Continued.

Primer 211				Loci (bp)										
Sample #	DNA #	TR	Distance (m)	1250	1050	975	925	875	840	825	780	650	525	500
51	49	OG	20	1	1	0	0	0	0	1	1	0	0	0
52	195	CC	20	1	1	0	0	0	0	1	1	0	0	0
53	196	CC	20	1	0	1	0	0	0	0	1	0	0	0
54	197	CC	20	1	1	0	0	0	0	1	1	0	0	0
55	143	OG	40	1	0	0	0	0	0	1	1	0	0	0
56	144	OG	40	0	1	0	0	0	0	0	0	0	0	0
57	145	OG	40	1	0	0	1	0	0	0	1	0	0	0
58	205	CC	40	1	0	0	0	0	0	0	1	0	1	1
59	206	CC	40	1	0	0	0	1	0	1	1	0	0	0
60	150	OG	60	0	0	1	0	0	0	0	0	0	1	0
61	151	OG	60	0	0	0	0	0	0	0	0	1	0	0
62	152	OG	60	0	0	0	0	0	0	0	0	0	1	0
63	210	CC	60	0	0	0	0	0	0	0	0	0	0	0
64	211	CC	60	1	0	0	0	0	1	1	1	0	0	1
65	212	CC	60	1	0	0	0	0	0	0	1	0	0	1
66	154	OG	80	1	0	0	0	0	0	0	0	0	0	1
67	155	OG	80	1	0	0	0	0	0	0	1	0	0	0
68	217	CC	80	1	0	0	0	0	0	0	1	0	1	0
69	218	CC	80	1	1	0	0	0	0	0	1	0	1	0
70	219	CC	80	1	1	0	1	0	0	0	1	0	0	0
71	156	OG	100	1	0	0	1	0	0	0	1	0	1	1
72	226	CC	100	1	0	0	1	0	0	1	1	0	0	1
73	227	CC	100	1	0	0	0	0	0	1	1	0	0	1
74	228	CC	100	1	1	0	1	0	0	1	1	0	1	1
75	160	OG	120	1	1	0	0	0	0	0	1	0	0	1
76	161	OG	120	1	1	1	1	0	0	1	1	0	1	0
77	162	OG	120	1	1	0	1	0	0	0	1	0	1	0
78	240	CC	120	X	X	X	X	X	X	X	X	X	X	X
79	241	CC	120	X	X	X	X	X	X	X	X	X	X	X
80	242	CC	120	1	1	1	1	0	0	1	1	1	1	1
81	170	OG	140	1	X	X	X	X	X	X	1	X	X	X
82	171	OG	140	1	1	0	1	0	0	1	1	0	1	1
83	172	OG	140	1	1	0	1	0	0	1	1	0	1	1
84	260	CC	140	1	1	1	1	0	0	1	1	0	1	1
85	177	OG	160	1	1	1	1	0	0	1	1	1	1	1
86	178	OG	160	1	1	0	1	0	0	1	1	0	0	1
87	179	OG	160	1	1	0	1	0	0	1	1	1	1	1
88	253	CC	160	1	1	0	1	0	0	1	1	0	1	1
89	254	CC	160	1	1	1	1	0	0	1	1	0	1	1
90	255	CC	160	X	X	X	X	X	X	X	X	X	X	X
91	190	CC	0	1	1	1	1	0	0	1	1	0	1	1
92	198	CC	20	1	1	1	1	0	0	1	1	1	1	1
93	199	CC	20	1	1	1	1	0	0	1	1	0	1	1
94	200	CC	20	1	1	1	1	0	0	1	1	0	1	1
95	153	OG	60	1	X	X	1	X	X	1	1	1	X	X
96	213	CC	60	1	1	1	1	0	1	1	1	0	1	1
97	214	CC	60	1	1	1	1	0	0	1	1	1	1	1
98	215	CC	60	1	1	1	1	0	0	1	1	0	1	1
99	220	CC	80	1	1	1	1	0	0	1	1	0	1	1
100	221	CC	80	1	1	1	1	0	0	1	1	0	1	1
101	222	CC	80	1	1	1	1	0	0	1	1	0	1	1

Table A-II.1. Continued.

Primer 211				Loci (bp)										
Sample #	DNA #	TR	Distance (m)	1250	1050	975	925	875	840	825	780	650	525	500
102	223	CC	80	1	1	1	1	0	0	1	1	0	0	1
103	224	CC	80	1	1	1	1	0	0	1	1	1	1	1
104	225	CC	80	1	1	1	1	1	0	1	1	1	0	1
105	229	CC	100	1	1	1	1	1	0	1	1	1	1	1
106	230	CC	100	1	1	1	1	0	0	1	1	0	1	1
107	231	CC	100	1	1	1	1	0	0	1	1	1	1	1
108	232	CC	100	1	1	1	1	0	0	1	1	0	1	1
109	233	CC	100	1	1	1	1	0	0	1	1	0	1	1
110	235	CC	100	1	1	1	1	0	0	1	1	0	1	1
111	163	OG	120	1	1	1	1	0	0	1	1	0	1	1
112	164	OG	120	1	1	1	1	0	0	1	1	0	1	1
113	165	OG	120	1	1	0	1	0	0	1	1	0	1	1
114	243	CC	120	1	1	1	1	0	0	1	1	1	1	1
115	244	CC	120	1	0	1	1	0	0	1	1	0	1	1
116	245	CC	120	1	1	1	1	0	0	1	1	1	1	1
117	173	OG	140	1	1	0	1	0	0	1	1	0	1	1
118	180	OG	160	1	0	0	1	0	0	1	1	0	1	1
119	181	OG	160	1	1	0	1	0	0	1	1	0	1	1
120	182	OG	160	1	1	1	1	0	0	1	1	1	1	1
121	256	CC	160	1	1	1	1	0	0	1	1	0	1	1
122	257	CC	160	1	1	1	1	0	0	1	1	0	1	1
123	14	OG	180	1	0	0	1	0	0	1	1	0	1	1
124	15	OG	180	1	1	1	1	0	0	1	1	0	1	1
125	184	OG	180	1	1	0	1	0	0	1	1	0	1	1
126	185	OG	180	1	1	1	1	0	0	1	1	1	1	1
127	186	OG	180	1	1	1	1	0	0	1	1	0	1	1
128	16	CC	180	1	1	1	1	0	0	1	1	1	1	1
129	17	CC	180	1	1	1	1	0	0	1	1	1	1	1
130	18	CC	180	1	0	1	1	0	0	1	1	0	1	1
131	19	CC	180	1	0	1	1	0	0	1	1	0	1	1
132	20	CC	180	1	0	1	1	0	0	1	1	0	1	1
133	261	CC	180	1	1	1	1	0	0	1	1	0	1	1
134	262	CC	180	1	1	1	1	0	0	1	1	0	1	1
135	263	CC	180	1	1	1	1	0	0	1	1	1	1	1
136	191	CC	0	1	1	0	1	0	1	1	1	1	1	1
137	201	CC	20	1	1	1	1	0	0	1	1	0	1	1
138	146	OG	40	1	1	0	1	0	1	1	1	0	0	0
139	216	CC	60	1	1	0	1	0	0	1	1	0	0	1
140	234	CC	80	1	1	0	1	0	0	1	1	0	1	1
141	236	CC	100	1	1	1	1	0	1	1	1	0	0	1
142	166	OG	120	1	1	0	1	0	0	1	1	1	1	1
143	246	CC	120	1	1	0	1	0	1	1	1	0	1	1
144	183	OG	160	1	1	1	1	0	1	1	1	0	1	1
145	258	CC	160	1	1	0	1	0	0	1	1	0	1	1
146	259	CC	160	1	1	0	1	0	1	1	1	0	0	1
147	187	OG	180	1	1	0	1	0	0	1	1	1	0	0
148	188	OG	180	1	1	0	1	0	0	1	1	0	1	0
149	264	CC	180	1	1	0	1	0	0	1	1	0	1	1
150	265	CC	180	1	1	0	1	0	0	1	1	1	1	1

Table A-II.1. Continued.

Primer 213				Loci (bp)								
Sample #	DNA #	TR	Distance (m)	1500	1000	850	775	710	675	500	400	340
1	36	OG	0	1	1	1	1	1	1	1	1	1
2	37	OG	0	1	1	1	1	1	1	1	1	1
3	38	OG	0	1	1	1	0	1	1	1	1	1
4	31	CC	0	1	1	1	0	1	0	1	1	1
5	32	CC	0	1	1	1	1	1	1	1	1	1
6	33	CC	X	X	1	X	X	X	X	1	1	1
7	40	OG	20	1	1	0	0	0	0	1	1	1
8	46	OG	20	1	1	0	0	0	0	1	1	1
9	47	OG	20	1	1	1	0	1	1	1	1	1
10	192	CC	20	1	1	1	1	1	1	1	1	1
11	193	CC	20	1	1	1	0	1	1	1	1	1
12	194	CC	20	1	1	1	0	1	1	1	1	1
13	50	OG	40	0	1	1	0	0	1	1	1	1
14	141	OG	40	1	1	1	0	1	1	1	1	1
15	142	OG	40	1	1	1	0	1	1	1	1	1
16	202	CC	40	1	1	1	1	1	1	1	1	1
17	203	CC	40	1	1	1	1	1	1	1	1	1
18	204	CC	40	1	1	1	1	1	1	1	0	1
19	147	OG	60	X	1	X	1	1	1	1	1	1
20	148	OG	60	1	1	1	1	1	1	1	1	1
21	149	OG	60	0	1	1	0	1	1	1	1	1
22	207	CC	60	1	1	1	1	1	0	1	1	1
23	208	CC	60	1	1	1	1	1	0	1	1	1
24	209	CC	60	1	1	1	1	1	1	1	1	1
25	157	OG	120	1	1	0	0	0	1	1	1	1
26	158	OG	120	1	1	0	0	0	1	1	1	1
27	159	OG	120	1	1	1	1	1	1	1	1	0
28	237	CC	120	1	1	1	0	0	1	1	1	1
29	238	CC	120	1	1	1	0	1	0	1	1	1
30	239	CC	120	1	1	1	0	0	0	1	1	1
31	167	OG	140	1	1	1	1	1	1	1	1	1
32	168	OG	140	1	1	1	1	1	1	1	1	1
33	169	OG	140	0	1	1	0	1	1	1	1	1
34	247	CC	140	1	1	1	1	1	1	1	1	1
35	248	CC	140	1	1	1	0	0	0	1	1	1
36	249	CC	140	1	1	1	1	1	1	1	1	1
37	174	OG	160	1	1	1	1	1	1	1	1	1
38	175	OG	160	1	1	1	1	1	0	1	1	1
39	176	OG	160	1	1	1	1	1	1	1	1	1
40	250	CC	160	1	1	1	1	1	1	1	1	1
41	251	CC	160	1	1	1	1	1	0	1	1	1
42	252	CC	160	1	1	1	1	1	1	1	1	1
43	11	OG	180	1	1	1	1	1	1	1	1	1
44	12	OG	180	1	1	1	1	1	1	1	1	1
45	13	OG	180	1	1	1	1	1	1	1	1	1
46	39	OG	0	1	1	0	0	1	1	1	0	0
47	34	CC	0	X	1	X	X	X	X	X	X	X
48	35	CC	0	1	1	0	0	0	1	1	0	0
49	189	CC	0	1	1	0	0	0	0	1	1	0
50	48	OG	20	1	1	0	0	0	0	1	1	0

Table A-II.1. Continued.

Primer 213				Loci (bp)								
Sample #	DNA #	TR	Distance (m)	1500	1000	850	775	710	675	500	400	340
51	49	OG	20	1	1	0	0	0	0	1	1	0
52	195	CC	20	1	1	0	0	0	0	1	0	0
53	196	CC	20	1	1	0	0	0	0	1	0	0
54	197	CC	20	1	1	0	0	0	0	1	0	0
55	143	OG	40	0	1	0	0	0	1	1	0	0
56	144	OG	40	1	1	0	0	0	0	1	0	0
57	145	OG	40	0	1	0	0	0	0	1	0	0
58	205	CC	40	1	1	0	0	0	0	1	1	0
59	206	CC	40	1	1	0	0	0	0	1	0	0
60	150	OG	60	1	1	1	1	1	1	1	1	0
61	151	OG	60	1	1	1	1	1	1	1	1	0
62	152	OG	60	1	1	1	1	1	0	1	0	0
63	210	CC	60	1	1	1	1	0	1	1	0	0
64	211	CC	60	1	1	1	0	0	0	1	0	0
65	212	CC	60	1	1	0	0	0	1	1	1	1
66	154	OG	80	1	1	1	1	1	1	1	1	0
67	155	OG	80	0	1	0	0	1	1	1	1	1
68	217	CC	80	1	1	1	0	1	0	1	1	0
69	218	CC	80	1	1	1	0	1	0	1	0	0
70	219	CC	80	1	1	1	0	0	1	1	1	0
71	156	OG	100	1	1	1	0	0	1	1	1	0
72	226	CC	100	1	1	1	0	0	1	1	1	0
73	227	CC	100	1	1	1	0	0	0	1	0	0
74	228	CC	100	1	1	1	0	0	0	1	0	0
75	160	OG	120	1	1	1	1	1	1	1	1	1
76	161	OG	120	1	1	1	1	1	1	1	1	1
77	162	OG	120	1	1	1	0	1	1	1	1	1
78	240	CC	120	1	1	1	0	1	0	1	0	0
79	241	CC	120	1	1	1	0	1	0	1	1	0
80	242	CC	120	1	1	0	0	1	0	1	0	0
81	170	OG	140	1	1	1	1	1	1	1	1	1
82	171	OG	140	1	1	1	1	1	1	1	1	1
83	172	OG	140	1	1	1	1	1	1	1	1	1
84	260	CC	140	1	1	1	1	1	0	1	1	1
85	177	OG	160	1	1	1	1	1	1	1	1	1
86	178	OG	160	1	1	1	1	1	1	1	1	1
87	179	OG	160	0	1	1	1	1	0	1	1	1
88	253	CC	160	1	1	1	1	1	1	1	1	1
89	254	CC	160	X	1	X	X	X	1	1	1	1
90	255	CC	160	X	1	X	X	X	X	1	1	1
91	190	CC	0	1	1	1	1	1	1	1	1	1
92	198	CC	20	1	1	1	1	1	1	1	1	1
93	199	CC	20	1	1	1	1	1	1	1	1	1
94	200	CC	20	1	1	1	1	1	1	1	1	1
95	153	OG	60	1	1	0	0	1	1	1	1	1
96	213	CC	60	1	1	1	1	1	1	1	1	1
97	214	CC	60	1	1	1	1	1	1	1	1	1
98	215	CC	60	1	1	1	1	1	1	1	1	1
99	220	CC	80	1	1	1	1	1	1	1	1	1
100	221	CC	80	1	1	1	1	1	1	1	1	1
101	222	CC	80	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 213				Loci (bp)								
Sample #	DNA #	TR	Distance (m)	1500	1000	850	775	710	675	500	400	340
102	223	CC	80	1	1	1	1	1	1	1	1	1
103	224	CC	80	1	1	1	1	1	1	1	1	1
104	225	CC	80	1	1	1	1	1	1	1	1	1
105	229	CC	100	1	1	1	1	1	0	1	1	1
106	230	CC	100	1	1	1	1	1	1	1	1	1
107	231	CC	100	1	1	1	1	1	1	1	1	1
108	232	CC	100	1	1	1	1	1	1	1	1	1
109	233	CC	100	1	1	1	1	1	1	1	1	1
110	235	CC	100	1	1	1	1	1	1	1	1	1
111	163	OG	120	1	1	1	1	1	1	1	1	1
112	164	OG	120	1	1	1	1	1	1	1	1	1
113	165	OG	120	1	1	1	0	1	1	1	1	1
114	243	CC	120	1	1	1	1	1	1	1	1	1
115	244	CC	120	1	1	0	1	1	1	1	1	1
116	245	CC	120	1	1	0	0	1	1	1	1	1
117	173	OG	140	1	1	1	1	1	1	1	1	1
118	180	OG	160	1	1	0	1	1	1	1	1	1
119	181	OG	160	1	1	1	1	1	1	1	1	1
120	182	OG	160	1	1	1	1	1	1	1	1	1
121	256	CC	160	1	1	1	1	1	1	1	1	1
122	257	CC	160	1	1	1	1	1	1	1	1	1
123	14	OG	180	1	1	1	0	1	1	1	1	1
124	15	OG	180	1	1	1	1	1	1	1	1	1
125	184	OG	180	1	1	1	1	1	1	1	1	1
126	185	OG	180	1	1	1	1	1	1	1	1	1
127	186	OG	180	1	1	1	1	1	1	1	1	1
128	16	CC	180	1	1	1	1	1	1	1	1	1
129	17	CC	180	1	1	1	1	1	1	1	1	1
130	18	CC	180	1	1	1	1	1	1	1	1	1
131	19	CC	180	1	1	1	1	1	1	1	1	1
132	20	CC	180	1	1	1	1	1	1	1	1	1
133	261	CC	180	1	1	1	1	1	1	1	1	1
134	262	CC	180	1	1	1	1	1	1	1	1	1
135	263	CC	180	1	1	1	1	1	1	1	1	1
136	191	CC	0	1	1	1	1	1	1	1	1	1
137	201	CC	20	1	1	1	1	1	1	1	1	1
138	146	OG	40	0	1	0	0	1	1	1	1	1
139	216	CC	60	1	1	1	1	1	1	1	1	1
140	234	CC	80	1	1	1	1	1	1	1	1	1
141	236	CC	100	1	1	0	0	1	1	1	1	1
142	166	OG	120	1	1	1	0	1	0	1	1	1
143	246	CC	120	1	1	1	1	1	1	1	1	1
144	183	OG	160	1	1	1	1	1	1	1	1	1
145	258	CC	160	1	1	1	1	1	1	1	1	1
146	259	CC	160	1	1	1	1	1	1	1	1	1
147	187	OG	180	1	1	0	0	1	1	1	1	1
148	188	OG	180	1	1	0	0	1	1	1	1	1
149	264	CC	180	1	1	1	1	1	1	1	1	1
150	265	CC	180	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 221				Loci (bp)									
Sample #	DNA #	TR	Distance (m)	1380	1020	975	810	775	650	480	420	360	325
1	36	OG	0	1	1	1	1	1	1	1	1	1	1
2	37	OG	0	1	1	1	1	1	1	1	1	1	1
3	38	OG	0	1	1	1	1	1	1	0	1	1	1
4	31	CC	0	1	1	1	1	1	1	0	1	1	1
5	32	CC	0	1	1	1	1	1	1	0	1	1	1
6	33	CC	0	0	1	1	1	1	0	0	1	1	1
7	40	OG	20	1	1	1	1	1	1	0	1	1	1
8	46	OG	20	1	1	1	0	1	0	0	1	1	1
9	47	OG	20	1	1	1	1	1	1	0	1	1	1
10	192	CC	20	1	1	1	1	1	1	1	1	1	1
11	193	CC	20	1	1	1	1	1	1	1	1	1	1
12	194	CC	20	1	1	1	1	1	1	1	1	1	1
13	50	OG	40	1	1	1	0	1	0	0	1	1	1
14	141	OG	40	1	1	1	1	1	1	0	1	1	1
15	142	OG	40	1	1	1	1	1	1	0	1	1	1
16	202	CC	40	1	1	1	1	1	1	1	1	1	1
17	203	CC	40	1	1	1	1	1	1	1	1	1	1
18	204	CC	40	1	1	1	1	1	1	1	1	1	1
19	147	OG	60	X	1	1	X	1	X	X	1	1	1
20	148	OG	60	1	1	1	1	1	1	1	1	1	1
21	149	OG	60	1	1	1	1	1	1	0	1	1	0
22	207	CC	60	1	1	1	1	1	1	1	1	1	1
23	208	CC	60	1	1	1	1	1	1	1	1	1	1
24	209	CC	60	1	1	1	1	1	1	1	1	1	1
25	157	OG	120	1	1	1	1	1	1	1	1	1	1
26	158	OG	120	1	1	1	1	1	1	1	1	1	1
27	159	OG	120	1	1	1	1	1	1	1	1	1	1
28	237	CC	120	1	1	1	1	1	1	1	1	1	1
29	238	CC	120	1	1	1	1	1	1	1	1	1	1
30	239	CC	120	1	1	1	1	1	1	1	1	1	1
31	167	OG	140	1	1	1	1	1	1	1	1	1	1
32	168	OG	140	1	1	1	1	1	1	1	1	1	1
33	169	OG	140	1	1	1	1	1	1	0	1	1	1
34	247	CC	140	1	1	1	1	1	1	1	1	1	1
35	248	CC	140	1	1	1	X	1	X	X	1	1	1
36	249	CC	140	1	1	1	1	1	1	1	1	1	1
37	174	OG	160	1	1	1	1	1	1	1	1	1	1
38	175	OG	160	1	1	1	1	1	1	1	1	1	1
39	176	OG	160	1	1	1	1	1	1	1	1	1	1
40	250	CC	160	1	1	1	1	1	1	1	1	1	1
41	251	CC	160	1	1	1	1	1	1	1	1	1	1
42	252	CC	160	1	1	1	1	1	1	1	1	1	1
43	11	OG	180	1	1	1	1	1	1	1	1	1	1
44	12	OG	180	1	1	1	1	1	1	1	1	1	1
45	13	OG	180	1	1	1	0	1	1	1	1	1	1
46	39	OG	0	1	1	1	1	1	1	1	1	1	1
47	34	CC	0	1	1	1	1	1	1	1	1	1	1
48	35	CC	0	1	1	1	1	1	1	1	1	1	1
49	189	CC	0	1	1	1	1	1	1	1	1	1	1
50	48	OG	20	1	1	1	1	1	0	0	1	1	1

Table A-II.1. Continued.

Primer 221				Loci (bp)									
Sample #	DNA #	TR	Distance (m)	1380	1020	975	810	775	650	480	420	360	325
51	49	OG	20	1	1	1	1	1	0	1	1	1	1
52	195	CC	20	1	1	1	1	1	1	1	1	1	1
53	196	CC	20	1	1	1	1	1	1	1	1	1	1
54	197	CC	20	1	1	1	1	1	1	1	1	1	1
55	143	OG	40	1	1	1	1	1	1	1	1	1	1
56	144	OG	40	1	1	1	1	1	1	1	1	1	1
57	145	OG	40	1	1	1	1	1	1	1	1	1	1
58	205	CC	40	1	1	1	1	1	1	1	1	1	1
59	206	CC	40	1	1	1	1	1	1	1	1	1	1
60	150	OG	60	1	1	1	1	1	0	0	1	1	1
61	151	OG	60	1	1	1	1	1	0	0	1	1	1
62	152	OG	60	1	1	1	1	1	0	0	1	1	1
63	210	CC	60	1	1	1	1	1	1	1	1	1	1
64	211	CC	60	1	1	1	1	1	1	1	1	1	1
65	212	CC	60	1	1	1	1	1	1	0	1	1	1
66	154	OG	80	1	1	1	1	1	1	1	1	1	1
67	155	OG	80	1	1	1	1	1	1	1	1	1	1
68	217	CC	80	1	1	1	1	1	1	1	1	1	1
69	218	CC	80	1	1	1	1	1	1	1	1	1	1
70	219	CC	80	1	1	1	1	1	1	1	1	1	1
71	156	OG	100	1	1	1	1	1	1	1	1	1	1
72	226	CC	100	1	1	1	1	1	1	1	1	1	1
73	227	CC	100	1	1	1	1	1	1	1	1	1	1
74	228	CC	100	1	1	1	1	1	1	1	1	1	1
75	160	OG	120	1	1	1	1	1	1	1	1	1	1
76	161	OG	120	1	1	1	1	1	1	1	1	1	1
77	162	OG	120	1	1	1	1	1	1	1	1	1	1
78	240	CC	120	1	1	1	1	1	1	1	1	1	1
79	241	CC	120	1	1	1	1	1	1	1	1	1	1
80	242	CC	120	1	1	1	1	1	1	1	1	1	1
81	170	OG	140	1	1	1	1	1	1	1	1	1	1
82	171	OG	140	1	1	1	1	1	1	1	1	1	1
83	172	OG	140	1	1	1	1	1	1	1	1	1	1
84	260	CC	140	1	1	1	1	1	1	1	1	1	1
85	177	OG	160	1	1	1	1	1	1	1	1	1	1
86	178	OG	160	1	1	1	1	1	1	1	1	1	1
87	179	OG	160	1	1	1	1	1	1	1	1	1	1
88	253	CC	160	1	1	1	1	1	1	1	1	1	1
89	254	CC	160	1	1	1	1	1	1	1	1	1	1
90	255	CC	160	1	1	1	1	1	1	1	1	1	1
91	190	CC	0	1	1	1	1	1	1	1	1	1	1
92	198	CC	20	1	1	1	1	1	1	1	1	1	1
93	199	CC	20	1	1	1	1	1	1	1	1	1	1
94	200	CC	20	1	1	1	1	1	1	1	1	1	1
95	153	OG	60	1	1	1	1	1	X	X	1	1	X
96	213	CC	60	1	1	1	1	1	1	1	1	1	1
97	214	CC	60	1	1	1	1	1	1	1	1	1	1
98	215	CC	60	1	1	1	1	1	1	1	1	1	1
99	220	CC	80	1	1	1	1	1	1	1	1	1	1
100	221	CC	80	1	1	1	1	1	1	1	1	1	1
101	222	CC	80	1	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 221				Loci (bp)									
Sample #	DNA #	TR	Distance (m)	1380	1020	975	810	775	650	480	420	360	325
102	223	CC	80	1	1	1	1	1	1	1	1	1	1
103	224	CC	80	1	1	1	1	1	1	1	1	1	1
104	225	CC	80	1	1	1	1	1	1	1	1	1	1
105	229	CC	100	1	1	1	1	1	1	1	1	1	1
106	230	CC	100	1	1	1	1	1	1	1	1	1	1
107	231	CC	100	1	1	1	1	1	1	1	1	1	1
108	232	CC	100	1	1	1	1	1	1	1	1	1	1
109	233	CC	100	1	1	1	1	1	1	1	1	1	1
110	235	CC	100	1	1	1	1	1	1	1	1	1	1
111	163	OG	120	1	1	1	1	1	1	1	1	1	1
112	164	OG	120	1	1	1	1	1	1	1	1	1	1
113	165	OG	120	1	1	1	1	1	0	0	1	1	0
114	243	CC	120	1	1	1	1	1	1	1	1	1	1
115	244	CC	120	1	1	1	1	1	1	1	1	1	1
116	245	CC	120	1	1	1	1	1	1	1	1	1	1
117	173	OG	140	1	1	1	1	1	1	1	1	1	1
118	180	OG	160	1	1	1	1	1	1	1	1	1	0
119	181	OG	160	1	1	1	1	1	1	1	1	1	1
120	182	OG	160	1	1	1	1	1	1	1	1	1	1
121	256	CC	160	1	1	1	1	1	1	1	1	1	1
122	257	CC	160	1	1	1	1	1	1	1	1	1	1
123	14	OG	180	1	1	1	1	1	0	0	1	1	0
124	15	OG	180	1	1	1	1	1	1	1	1	1	1
125	184	OG	180	1	1	1	1	1	1	1	1	1	1
126	185	OG	180	1	1	1	1	1	1	1	1	1	1
127	186	OG	180	1	1	1	1	1	1	1	1	1	1
128	16	CC	180	1	1	1	1	1	1	1	1	1	1
129	17	CC	180	1	1	1	1	1	1	1	1	1	1
130	18	CC	180	1	1	1	1	1	1	1	1	1	1
131	19	CC	180	1	1	1	1	1	1	1	1	1	1
132	20	CC	180	1	1	1	1	1	1	1	1	1	1
133	261	CC	180	1	1	1	1	1	1	1	1	1	1
134	262	CC	180	1	1	1	1	1	1	1	1	1	1
135	263	CC	180	1	1	1	1	1	1	1	1	1	1
136	191	CC	0	1	1	1	1	1	1	1	1	1	1
137	201	CC	20	1	1	1	1	1	1	1	1	1	1
138	146	OG	40	1	1	1	1	1	0	0	1	1	0
139	216	CC	60	1	1	1	1	1	1	1	1	1	1
140	234	CC	80	1	1	1	1	1	1	1	1	1	1
141	236	CC	100	1	1	1	1	1	0	1	1	1	1
142	166	OG	120	1	1	1	1	1	1	1	1	1	1
143	246	CC	120	1	1	1	1	1	1	1	1	1	1
144	183	OG	160	1	1	1	1	1	1	1	1	1	1
145	258	CC	160	1	1	1	1	1	1	1	1	1	1
146	259	CC	160	1	1	1	1	1	1	1	1	1	1
147	187	OG	180	1	1	1	1	1	0	0	1	1	0
148	188	OG	180	1	1	1	1	1	0	1	1	1	1
149	264	CC	180	1	1	1	1	1	1	1	1	1	1
150	265	CC	180	1	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 268				Loci (bp)													
Samp #	DNA #	TR	Distance (m)	1500	1350	1250	1050	1000	875	760	640	610	590	510	490	400	350
1	36	OG	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	37	OG	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	38	OG	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1
4	31	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
5	32	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
6	33	CC	0	X	X	1	X	1	1	1	X	X	X	1	X	1	X
7	40	OG	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	46	OG	20	1	1	1	0	1	1	1	0	1	0	0	0	1	0
9	47	OG	20	1	1	1	1	1	1	1	1	1	1	1	1	1	0
10	192	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	193	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	0
12	194	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	50	OG	40	1	1	1	0	1	1	1	1	0	0	1	0	1	0
14	141	OG	40	1	1	1	1	1	1	1	1	1	1	1	0	1	0
15	142	OG	40	1	1	1	1	1	1	1	1	1	1	1	0	1	1
16	202	CC	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	203	CC	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	204	CC	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	147	OG	60	1	X	1	X	X	1	X	X	X	X	X	X	X	X
20	148	OG	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	149	OG	60	1	1	1	1	1	1	1	0	1	1	1	0	1	1
22	207	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	208	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	209	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	157	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	158	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	159	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	237	CC	120	1	1	1	1	1	1	1	1	1	0	1	1	1	1
29	238	CC	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	239	CC	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	167	OG	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32	168	OG	140	1	1	1	0	1	1	1	1	1	1	1	1	1	1
33	169	OG	140	1	1	1	0	1	1	1	1	1	0	1	1	1	1
34	247	CC	140	1	1	1	1	1	1	1	1	1	1	1	0	1	1
35	248	CC	140	1	1	1	1	1	1	1	1	1	1	1	0	1	1
36	249	CC	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	174	OG	160	1	1	1	1	1	1	1	1	1	1	1	0	1	0
38	175	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	176	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	250	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41	251	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
42	252	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
43	11	OG	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
44	12	OG	180	1	1	1	1	1	1	1	1	1	0	1	1	1	0
45	13	OG	180	1	1	1	1	1	1	1	1	1	1	1	0	1	1
46	39	OG	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1
47	34	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
48	35	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
49	189	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
50	48	OG	20	X	X	1	X	X	1	1	X	X	X	X	X	1	X

Table A-II.1. Continued.

Primer 268				Loci (bp)													
Samp #	DNA #	TR	Distance (m)	1500	1350	1250	1050	1000	875	760	640	610	590	510	490	400	350
51	49	OG	20	X	1	1	X	X	1	1	X	X	X	X	X	1	X
52	195	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
53	196	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
54	197	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
55	143	OG	40	1	0	1	1	1	1	1	0	1	1	1	1	1	1
56	144	OG	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
57	145	OG	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
58	205	CC	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
59	206	CC	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1
60	150	OG	60	1	0	1	0	1	1	0	1	1	1	0	0	1	0
61	151	OG	60	X	X	1	X	1	1	X	1	X	X	X	X	1	X
62	152	OG	60	X	X	1	X	1	1	1	1	1	1	X	X	1	1
63	210	CC	60	1	1	1	1	1	1	1	1	1	1	0	1	1	1
64	211	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
65	212	CC	60	1	0	1	1	1	1	1	1	1	1	1	1	1	1
66	154	OG	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
67	155	OG	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
68	217	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
69	218	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
70	219	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
71	156	OG	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
72	226	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
73	227	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
74	228	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
75	160	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
76	161	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
77	162	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
78	240	CC	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
79	241	CC	120	1	1	1	1	1	1	1	1	1	1	0	1	1	1
80	242	CC	120	1	1	1	1	1	1	1	1	1	1	0	1	1	1
81	170	OG	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
82	171	OG	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
83	172	OG	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
84	260	CC	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
85	177	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
86	178	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
87	179	OG	160	1	1	1	1	1	1	1	1	1	1	0	1	1	1
88	253	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
89	254	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
90	255	CC	160	1	1	1	1	1	1	1	1	1	1	0	1	1	1
91	190	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
92	198	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
93	199	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
94	200	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
95	153	OG	60	1	X	1	X	X	1	1	1	1	1	X	1	1	1
96	213	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
97	214	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
98	215	CC	60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
99	220	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
100	221	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
101	222	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 268				Loci (bp)													
Samp #	DNA #	TR	Distance (m)	1500	1350	1250	1050	1000	875	760	640	610	590	510	490	400	350
102	223	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
103	224	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
104	225	CC	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
105	229	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
106	230	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
107	231	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
108	232	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
109	233	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
110	235	CC	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
111	163	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
112	164	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
113	165	OG	120	1	1	1	0	1	1	1	1	1	1	0	1	1	1
114	243	CC	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
115	244	CC	120	1	1	1	1	1	1	1	1	1	1	0	1	1	1
116	245	CC	120	1	1	1	1	1	1	1	1	1	1	0	1	1	1
117	173	OG	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1
118	180	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
119	181	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
120	182	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
121	256	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
122	257	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
123	14	OG	180	1	1	1	0	1	1	1	0	1	1	0	1	1	1
124	15	OG	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
125	184	OG	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
126	185	OG	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
127	186	OG	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
128	16	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
129	17	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
130	18	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
131	19	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
132	20	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
133	261	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
134	262	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
135	263	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
136	191	CC	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
137	201	CC	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
138	146	OG	40	X	X	1	X	X	1	1	X	X	X	X	X	1	1
139	216	CC	60	1	1	1	1	1	1	1	0	1	1	1	1	1	1
140	234	CC	80	0	1	1	1	0	1	1	0	1	1	1	1	1	1
141	236	CC	100	1	1	1	0	1	1	1	0	1	1	1	1	1	1
142	166	OG	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
143	246	CC	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1
144	183	OG	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
145	258	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
146	259	CC	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1
147	187	OG	180	X	X	1	X	X	1	1	X	X	X	X	1	X	1
148	188	OG	180	1	0	1	0	0	1	1	0	1	1	0	1	0	1
149	264	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1
150	265	CC	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 352				Loci (bp)							
Sample #	DNA #	TR	Distance (m)	1375	1350	1180	925	875	650	480	450
1	36	OG	0	1	1	1	1	1	1	1	1
2	37	OG	0	0	1	0	1	0	1	0	0
3	38	OG	0	0	1	0	1	0	1	1	0
4	31	CC	0	0	0	0	1	1	1	0	0
5	32	CC	0	0	0	1	1	1	1	0	1
6	33	CC	0	X	X	X	X	X	1	X	X
7	40	OG	20	X	X	X	1	X	1	X	X
8	46	OG	20	1	1	X	1	1	1	1	1
9	47	OG	20	1	1	1	1	X	1	X	1
10	192	CC	20	0	1	1	1	1	1	1	1
11	193	CC	20	0	1	1	1	1	1	1	1
12	194	CC	20	0	1	1	1	1	1	1	1
13	50	OG	40	1	1	1	1	1	1	0	1
14	141	OG	40	1	1	1	1	1	1	0	1
15	142	OG	40	1	1	1	1	1	1	1	1
16	202	CC	40	1	1	1	1	1	1	1	0
17	203	CC	40	1	1	1	1	1	1	1	1
18	204	CC	40	1	1	1	1	1	1	1	1
19	147	OG	60	1	1	1	1	1	1	0	1
20	148	OG	60	1	1	1	1	1	1	1	1
21	149	OG	60	X	X	X	1	1	1	X	X
22	207	CC	60	1	0	1	1	1	1	1	1
23	208	CC	60	1	1	1	1	1	1	1	1
24	209	CC	60	1	1	1	1	1	1	1	1
25	157	OG	120	0	1	1	1	1	1	0	1
26	158	OG	120	1	1	1	1	1	1	0	0
27	159	OG	120	1	1	1	1	1	1	1	1
28	237	CC	120	1	1	1	1	1	1	0	1
29	238	CC	120	1	1	1	1	1	1	0	1
30	239	CC	120	1	1	1	1	1	1	0	1
31	167	OG	140	1	1	1	1	1	1	1	1
32	168	OG	140	0	1	1	1	1	1	0	1
33	169	OG	140	X	1	1	1	1	1	X	X
34	247	CC	140	0	0	1	1	1	1	1	1
35	248	CC	140	X	X	X	X	X	1	X	X
36	249	CC	140	0	1	1	1	1	1	1	1
37	174	OG	160	1	1	1	1	1	1	1	1
38	175	OG	160	1	1	1	1	1	1	1	1
39	176	OG	160	0	1	1	1	1	1	1	1
40	250	CC	160	0	1	1	1	1	1	1	1
41	251	CC	160	1	0	1	1	1	1	1	1
42	252	CC	160	1	0	1	1	1	1	1	1
43	11	OG	180	1	1	1	1	1	1	1	1
44	12	OG	180	1	1	1	1	0	1	0	1
45	13	OG	180	1	1	1	X	X	1	X	1
46	39	OG	0	0	1	0	1	1	1	0	1
47	34	CC	0	1	1	1	1	1	1	1	1
48	35	CC	0	1	1	1	1	1	1	1	1
49	189	CC	0	1	1	1	1	1	1	1	1
50	48	OG	20	X	X	X	1	1	1	X	X

Table A-II.1. Continued.

Primer 352				Loci (bp)							
Sample #	DNA #	TR	Distance (m)	1375	1350	1180	925	875	650	480	450
51	49	OG	20	X	1	X	1	1	1	X	1
52	195	CC	20	1	1	1	1	1	1	1	1
53	196	CC	20	0	1	1	1	1	1	1	1
54	197	CC	20	1	1	1	1	1	1	1	1
55	143	OG	40	1	1	0	1	1	1	0	1
56	144	OG	40	1	1	1	1	1	1	1	1
57	145	OG	40	1	0	0	1	1	1	0	1
58	205	CC	40	1	1	1	1	1	1	1	1
59	206	CC	40	0	1	1	1	1	1	1	1
60	150	OG	60	0	0	0	1	1	1	0	0
61	151	OG	60	0	0	0	1	1	1	0	0
62	152	OG	60	0	0	0	1	1	1	0	0
63	210	CC	60	0	1	1	1	1	1	1	1
64	211	CC	60	1	1	1	1	1	1	1	1
65	212	CC	60	1	1	1	1	1	1	1	1
66	154	OG	80	1	1	1	1	1	1	1	1
67	155	OG	80	1	1	X	1	1	1	X	1
68	217	CC	80	0	1	1	1	1	1	1	1
69	218	CC	80	1	1	1	1	1	1	1	1
70	219	CC	80	X	1	1	1	1	1	1	1
71	156	OG	100	0	1	1	1	1	1	0	1
72	226	CC	100	0	1	1	1	1	1	0	1
73	227	CC	100	1	1	1	1	1	1	1	1
74	228	CC	100	0	1	1	1	1	1	1	1
75	160	OG	120	1	1	1	1	1	1	1	1
76	161	OG	120	0	1	1	1	1	1	1	1
77	162	OG	120	1	0	1	1	1	1	0	1
78	240	CC	120	0	1	1	1	1	1	1	1
79	241	CC	120	1	1	1	1	1	1	0	1
80	242	CC	120	1	1	1	1	1	1	1	1
81	170	OG	140	0	1	1	1	1	1	0	1
82	171	OG	140	1	1	1	1	1	1	0	1
83	172	OG	140	0	1	1	1	1	1	0	1
84	260	CC	140	0	1	1	1	1	1	1	1
85	177	OG	160	0	1	1	1	1	1	0	1
86	178	OG	160	0	1	1	1	1	1	0	1
87	179	OG	160	0	1	1	1	1	1	0	1
88	253	CC	160	1	1	1	1	1	1	1	1
89	254	CC	160	1	1	1	1	1	1	1	1
90	255	CC	160	0	1	1	1	1	1	0	1
91	190	CC	0	1	0	1	1	1	1	1	1
92	198	CC	20	1	0	1	1	1	1	1	1
93	199	CC	20	0	1	1	1	1	1	1	1
94	200	CC	20	1	1	1	1	1	1	1	1
95	153	OG	60	X	X	X	X	1	1	1	X
96	213	CC	60	1	1	1	1	1	1	1	1
97	214	CC	60	1	1	1	1	1	1	1	1
98	215	CC	60	0	1	1	1	1	1	0	1
99	220	CC	80	1	1	1	1	1	1	1	1
100	221	CC	80	0	1	1	1	1	1	1	1
101	222	CC	80	0	0	1	1	1	1	0	1

Table A-II.1. Continued.

Primer 352				Loci (bp)							
Sample #	DNA #	TR	Distance (m)	1375	1350	1180	925	875	650	480	450
102	223	CC	80	0	1	1	1	1	1	1	1
103	224	CC	80	1	1	1	1	1	1	1	1
104	225	CC	80	1	1	1	1	1	1	1	1
105	229	CC	100	0	1	1	1	1	1	1	1
106	230	CC	100	1	X	1	1	1	1	X	1
107	231	CC	100	1	0	1	1	1	1	1	1
108	232	CC	100	0	1	1	1	1	1	1	1
109	233	CC	100	1	1	1	1	1	1	1	1
110	235	CC	100	1	1	1	1	1	1	1	1
111	163	OG	120	1	1	1	1	1	1	0	1
112	164	OG	120	1	1	1	1	1	1	0	1
113	165	OG	120	0	1	1	1	1	1	0	0
114	243	CC	120	0	1	1	1	1	1	1	1
115	244	CC	120	0	0	1	1	1	1	0	0
116	245	CC	120	1	0	1	1	1	1	0	0
117	173	OG	140	0	1	1	1	1	1	1	1
118	180	OG	160	1	0	1	1	1	1	0	0
119	181	OG	160	1	1	1	1	1	1	0	1
120	182	OG	160	1	1	1	1	1	1	0	1
121	256	CC	160	1	0	1	1	1	1	1	1
122	257	CC	160	1	1	1	1	1	1	1	1
123	14	OG	180	1	0	1	1	1	1	0	0
124	15	OG	180	1	1	1	1	1	1	1	1
125	184	OG	180	0	1	1	1	1	1	0	1
126	185	OG	180	0	1	1	1	1	1	1	1
127	186	OG	180	0	1	1	1	1	1	0	1
128	16	CC	180	0	1	1	1	1	1	0	1
129	17	CC	180	0	1	1	1	1	1	0	1
130	18	CC	180	0	0	1	1	1	1	0	1
131	19	CC	180	0	1	1	1	1	1	0	1
132	20	CC	180	0	0	1	1	1	1	1	1
133	261	CC	180	0	1	1	1	1	1	1	1
134	262	CC	180	1	1	1	1	1	1	1	1
135	263	CC	180	0	1	1	1	1	1	1	1
136	191	CC	0	1	1	1	1	1	1	1	1
137	201	CC	20	1	1	1	1	1	1	1	1
138	146	OG	40	X	X	X	1	1	1	X	X
139	216	CC	60	0	0	0	1	1	1	0	1
140	234	CC	80	0	0	1	1	1	1	0	1
141	236	CC	100	0	0	0	1	1	1	0	0
142	166	OG	120	0	1	1	1	1	1	0	1
143	246	CC	120	1	1	1	1	1	1	1	1
144	183	OG	160	0	1	1	1	1	1	1	1
145	258	CC	160	1	1	1	1	1	1	1	1
146	259	CC	160	1	1	1	1	1	1	1	1
147	187	OG	180	1	1	1	1	1	1	0	0
148	188	OG	180	0	0	0	1	1	1	0	0
149	264	CC	180	0	0	1	1	1	1	1	1
150	265	CC	180	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 400				Loci (bp)								
Samp #	DNA #	TR	Dist (m)	1180	1020	980	900	780	760	675	390	350
1	36	OG	0	1	1	1	1	1	1	1	1	1
2	37	OG	0	1	1	1	1	0	1	1	1	1
3	38	OG	0	0	0	1	1	1	0	0	1	1
4	31	CC	0	1	1	1	1	1	1	1	1	1
5	32	CC	0	1	1	1	1	0	1	1	1	1
6	33	CC	0	X	X	1	X	1	X	1	1	1
7	40	OG	20	1	0	1	1	0	0	1	1	1
8	46	OG	20	0	0	1	0	0	0	0	1	1
9	47	OG	20	1	1	1	1	1	1	1	1	1
10	192	CC	20	1	1	1	1	1	1	1	1	1
11	193	CC	20	1	1	1	1	1	1	1	1	1
12	194	CC	20	1	1	1	1	1	1	1	1	1
13	50	OG	40	X	X	1	1	1	X	1	1	1
14	141	OG	40	1	1	1	1	1	0	0	1	1
15	142	OG	40	1	1	1	1	1	0	1	1	1
16	202	CC	40	1	1	1	1	0	1	1	1	1
17	203	CC	40	1	1	1	1	1	1	1	1	1
18	204	CC	40	1	1	1	1	1	1	1	1	1
19	147	OG	60	X	X	X	X	X	X	1	1	1
20	148	OG	60	1	1	1	1	1	1	1	1	1
21	149	OG	60	1	0	1	1	0	0	1	1	0
22	207	CC	60	1	1	1	1	0	1	1	1	1
23	208	CC	60	1	1	1	1	1	1	1	1	1
24	209	CC	60	1	1	1	1	1	1	1	1	1
25	157	OG	120	1	1	1	1	1	1	1	1	1
26	158	OG	120	1	1	1	1	0	1	1	1	1
27	159	OG	120	1	1	1	1	1	1	1	1	0
28	237	CC	120	1	1	1	1	1	1	1	1	1
29	238	CC	120	1	1	1	1	1	1	1	1	1
30	239	CC	120	1	1	1	1	1	1	1	1	1
31	167	OG	140	1	1	1	1	1	0	1	1	1
32	168	OG	140	1	1	1	1	1	1	1	1	1
33	169	OG	140	1	0	1	1	1	0	1	1	0
34	247	CC	140	1	1	1	1	1	1	1	1	1
35	248	CC	140	X	1	1	1	1	X	1	1	1
36	249	CC	140	1	1	1	1	1	1	1	1	1
37	174	OG	160	1	1	1	1	1	1	1	1	1
38	175	OG	160	1	1	1	1	1	1	1	1	1
39	176	OG	160	1	1	1	1	1	1	1	1	0
40	250	CC	160	1	1	1	1	1	1	1	1	1
41	251	CC	160	1	1	1	1	1	1	1	1	1
42	252	CC	160	1	1	1	1	0	1	1	1	1
43	11	OG	180	1	1	1	1	1	1	1	1	1
44	12	OG	180	1	1	1	1	1	0	1	1	1
45	13	OG	180	1	1	1	1	1	0	1	1	1
46	39	OG	0	1	0	1	1	0	1	1	1	1
47	34	CC	0	1	1	1	1	1	1	0	1	0
48	35	CC	0	1	1	1	1	1	1	1	1	1
49	189	CC	0	1	1	1	1	1	1	1	1	1
50	48	OG	20	0	0	1	1	0	1	1	1	1

Table A-II.1. Continued.

Primer 400				Loci (bp)								
Samp #	DNA #	TR	Dist (m)	1180	1020	980	900	780	760	675	390	350
51	49	OG	20	0	0	1	1	0	1	1	1	0
52	195	CC	20	1	1	1	1	1	1	1	1	0
53	196	CC	20	1	1	1	1	1	1	1	1	0
54	197	CC	20	1	1	1	1	1	1	1	1	0
55	143	OG	40	0	0	1	1	0	1	0	1	1
56	144	OG	40	1	1	1	1	1	1	0	1	0
57	145	OG	40	1	0	1	1	1	1	0	1	0
58	205	CC	40	1	1	1	1	0	1	0	1	1
59	206	CC	40	1	1	1	1	0	1	1	1	1
60	150	OG	60	1	1	1	1	0	1	1	1	1
61	151	OG	60	1	0	1	1	0	0	0	1	1
62	152	OG	60	1	X	1	1	X	1	X	1	1
63	210	CC	60	1	1	1	1	0	1	1	1	1
64	211	CC	60	1	1	1	1	0	1	0	1	1
65	212	CC	60	1	1	1	1	0	1	1	1	1
66	154	OG	80	1	1	1	1	0	1	1	1	1
67	155	OG	80	1	1	1	1	0	1	1	1	1
68	217	CC	80	1	1	1	1	1	0	1	1	1
69	218	CC	80	1	1	1	1	1	1	1	1	1
70	219	CC	80	1	1	1	1	0	1	1	1	1
71	156	OG	100	1	1	1	1	1	1	1	1	1
72	226	CC	100	1	1	1	1	0	1	1	1	1
73	227	CC	100	1	1	1	1	0	1	1	1	1
74	228	CC	100	1	1	1	1	0	1	0	1	1
75	160	OG	120	1	1	1	1	0	1	1	1	1
76	161	OG	120	1	1	1	1	0	1	1	1	1
77	162	OG	120	1	1	1	1	0	1	1	1	1
78	240	CC	120	1	1	1	1	0	1	0	1	1
79	241	CC	120	1	1	1	1	0	1	1	1	1
80	242	CC	120	1	1	1	1	0	1	1	1	1
81	170	OG	140	1	1	1	1	0	1	0	1	1
82	171	OG	140	1	1	1	1	0	1	1	1	1
83	172	OG	140	1	1	1	1	0	1	1	1	0
84	260	CC	140	1	1	1	1	0	1	0	1	0
85	177	OG	160	1	1	1	1	0	1	1	1	1
86	178	OG	160	1	1	1	1	0	1	1	1	1
87	179	OG	160	1	1	1	1	0	1	1	1	1
88	253	CC	160	1	1	1	1	0	1	1	1	1
89	254	CC	160	1	1	1	1	0	1	1	1	1
90	255	CC	160	1	1	1	1	0	1	1	1	1
91	190	CC	0	1	1	1	1	1	1	1	1	1
92	198	CC	20	1	1	1	1	1	1	1	1	1
93	199	CC	20	1	1	1	1	1	1	1	1	1
94	200	CC	20	1	1	1	1	1	1	1	1	1
95	153	OG	60	X	X	1	1	X	X	1	1	1
96	213	CC	60	1	1	1	1	1	1	1	1	0
97	214	CC	60	1	1	1	1	1	1	1	1	1
98	215	CC	60	1	1	1	1	1	1	1	1	1
99	220	CC	80	1	1	1	1	1	1	1	1	1
100	221	CC	80	1	1	1	1	1	1	1	1	1
101	222	CC	80	1	1	1	1	1	1	1	1	1

Table A-II.1. Continued.

Primer 400				Loci (bp)								
Samp #	DNA #	TR	Dist (m)	1180	1020	980	900	780	760	675	390	350
102	223	CC	80	1	1	1	1	1	1	1	1	0
103	224	CC	80	1	1	1	1	0	1	1	1	1
104	225	CC	80	1	1	1	1	1	1	1	1	1
105	229	CC	100	1	1	1	1	1	1	1	1	1
106	230	CC	100	1	1	1	1	1	1	1	1	1
107	231	CC	100	1	1	1	1	1	1	1	1	1
108	232	CC	100	1	1	1	1	1	1	1	1	1
109	233	CC	100	1	1	1	1	1	1	1	1	1
110	235	CC	100	1	1	1	1	1	1	1	1	1
111	163	OG	120	1	1	1	1	1	1	1	1	1
112	164	OG	120	1	1	1	1	1	1	1	1	1
113	165	OG	120	1	1	1	1	1	0	1	1	1
114	243	CC	120	1	1	1	1	0	1	1	1	0
115	244	CC	120	1	1	1	1	1	1	1	1	0
116	245	CC	120	1	1	1	1	1	1	1	1	1
117	173	OG	140	1	1	1	1	1	1	1	1	1
118	180	OG	160	1	1	1	1	1	1	1	1	1
119	181	OG	160	1	1	1	1	1	1	1	1	1
120	182	OG	160	1	1	1	1	1	1	1	1	0
121	256	CC	160	1	1	1	1	1	1	1	1	1
122	257	CC	160	1	1	1	1	1	1	1	1	1
123	14	OG	180	1	1	1	1	1	1	1	1	0
124	15	OG	180	1	1	1	1	1	1	1	1	1
125	184	OG	180	1	1	1	1	1	1	1	1	1
126	185	OG	180	1	1	1	1	0	1	1	1	1
127	186	OG	180	1	1	1	1	0	1	1	1	1
128	16	CC	180	1	1	1	1	1	1	1	1	1
129	17	CC	180	1	1	1	1	1	1	1	1	1
130	18	CC	180	1	1	1	1	0	0	1	1	1
131	19	CC	180	1	1	1	1	1	1	1	1	1
132	20	CC	180	1	1	1	1	0	1	1	1	1
133	261	CC	180	1	1	1	1	0	1	1	1	1
134	262	CC	180	1	1	1	1	1	1	1	1	1
135	263	CC	180	1	1	1	1	1	1	1	1	1
136	191	CC	0	1	1	1	1	1	1	1	1	1
137	201	CC	20	1	1	1	1	1	1	1	1	1
138	146	OG	40	X	X	1	1	X	1	1	1	X
139	216	CC	60	1	1	1	1	0	1	1	1	1
140	234	CC	80	1	1	1	1	1	1	1	1	1
141	236	CC	100	0	1	1	1	1	1	1	1	1
142	166	OG	120	1	1	1	1	0	1	1	1	1
143	246	CC	120	1	1	1	1	1	1	1	1	1
144	183	OG	160	1	1	1	1	1	1	1	1	1
145	258	CC	160	1	1	1	1	1	1	1	1	1
146	259	CC	160	1	1	1	1	1	1	1	1	1
147	187	OG	180	0	1	1	1	0	1	1	1	1
148	188	OG	180	0	1	1	1	0	1	1	1	1
149	264	CC	180	1	1	1	1	0	1	1	1	1
150	265	CC	180	1	1	1	1	0	1	1	1	1

Table A-II.2. Gel scheme used for all samples. Gel scheme for samples 141–158 provided skipped wells for the repeats necessary when samples gave no reaction or bands showed weak signals.

Upper Wells *Samples 1-47*

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample			1		2	3	4	5	6	7	8	9	10	11	12	13		14	15	16	17	18	19	20	21	22	23			
Forest	*skip*	*skip*	**NC**	**L**	OG	OG	OG	CC	CC	CC	OG	OG	OG	CC	CC	CC	**L**	OG	OG	OG	CC	CC	CC	OG	OG	OG	**PC**	**L**	*skip*	*skip*
Distance					0	0	0	0	0	0	20	20	20	20	20	20		40	40	40	40	40	40	60	60	60				

Lower Wells

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample				24	25	26	27	28	29	30	31	32	33	34	35		36	37	38	39	40	41	42	43	44	45	46	47		
Forest	*skip*	*skip*	**L**	CC	CC	CC	OG	OG	OG	CC	CC	CC	OG	OG	OG	**L**	CC	CC	CC	OG	OG	OG	CC	CC	CC	OG	OG	OG	**L**	*skip*
Distance				60	60	60	120	120	120	120	120	120	140	140	140		140	140	140	160	160	160	160	160	160	180	180	180		

Upper Wells *Samples 48-94*

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample			48		49	50	51	52	53	54	55	56	57	58	59	60		61	62	63	64	65	66	67	68	69	70			
Forest	*skip*	*skip*	**NC**	**L**	OG	CC	CC	CC	OG	OG	CC	CC	CC	OG	OG	OG	**L**	CC	CC	OG	OG	OG	CC	CC	CC	OG	**PC**	**L**	*skip*	*skip*
Distance					0	0	0	0	20	20	20	20	20	40	40	40		40	40	60	60	60	60	60	60	80				

Lower Wells

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample				71	72	73	74	75	76	77	78	79	80	81	82		83	84	85	86	87	88	89	90	91	92	93	94		
Forest	*skip*	*skip*	**L**	OG	CC	CC	CC	OG	CC	CC	CC	OG	OG	OG	CC	**L**	CC	CC	OG	OG	OG	CC	OG	OG	OG	CC	CC	CC	**L**	*skip*
Distance				80	80	80	80	100	100	100	100	120	120	120	120		120	120	140	140	140	140	160	160	160	160	160	160		

Table A-II.2. Continued.

Upper Wells *Samples 95-141*

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample			95		96	97	98	99	100	101	102	103	104	105	106	107		108	109	110	111	112	113	114	115	116	117			
Forest	*skip*	*skip*	**NC**	**L**	CC	CC	CC	CC	OG	CC	CC	CC	CC	CC	CC	CC	**L**	CC	CC	CC	CC	CC	CC	CC	CC	OG	**PC**	**L**	*skip*	*skip*
Distance					0	20	20	20	60	60	60	60	80	80	80	80		80	80	100	100	100	100	100	100	120				

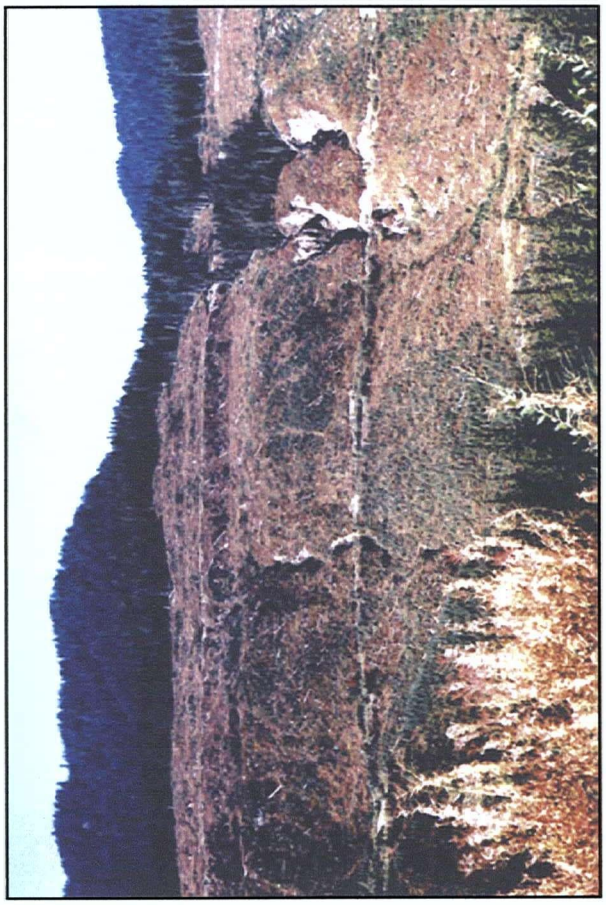
Lower Wells

Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample				118	119	120	121	122	123	124	125	126	127	128	129		130	131	132	133	134	135	136	137	138	139	140	141		
Forest	*skip*	*skip*	**L**	OG	OG	CC	CC	CC	OG	OG	OG	OG	CC	CC	OG	**L**	OG	OG	OG	OG	CC	CC	CC	CC	CC	CC	CC	CC	**L**	*skip*
Distance				120	120	120	120	120	140	160	160	160	160	160	180		180	180	180	180	180	180	180	180	180	180	180	180		

Upper Wells *Samples 142-158*

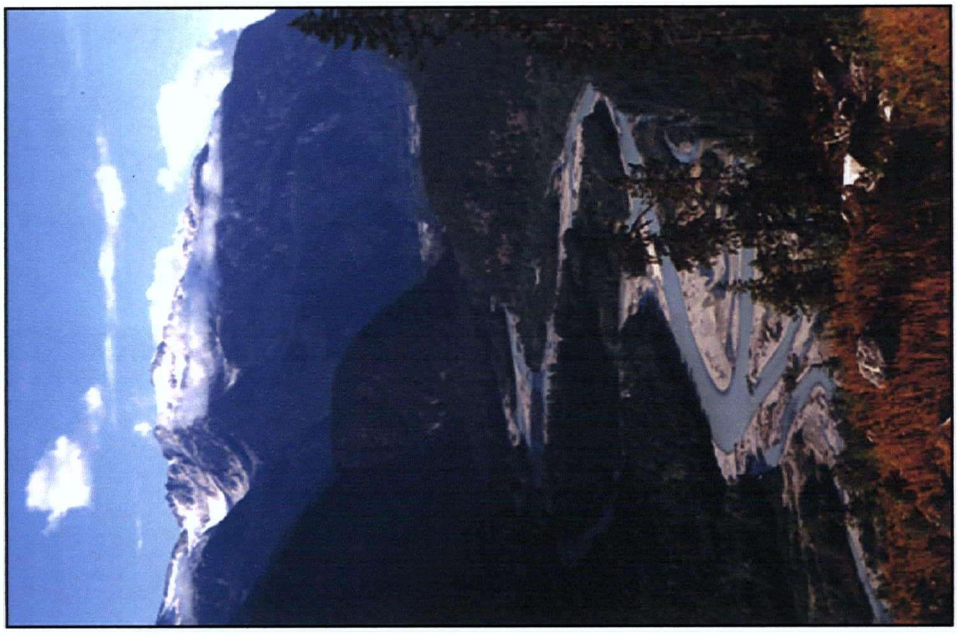
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Sample			142		143	144	145	146	147	148	149	150		151	152	153	154	155	156	157	158									
Forest	*skip*	*skip*	**NC**	**L**	CC	CC	OG	CC	CC	CC	OG	CC	**L**	OG	CC	CC	OG	OG	CC	CC	**PC**	**L**	*skip*	*skip*	*skip*	*skip*	*skip*	*skip*	*skip*	*skip*
Distance					0	20	40	60	80	100	120	120		160	160	160	180	180	180	180										

Figures A-III. Photo plates of *Ascaphus*, trapping arrays, rivers, and marking technique.



Mamquam watershed. Clearcut showing bank failures around several streams and roads.

Photo credit: Tanya Wahbe



Squamish River.

Photo credit: Leigh Burrows

Adult female *Ascaphus*.

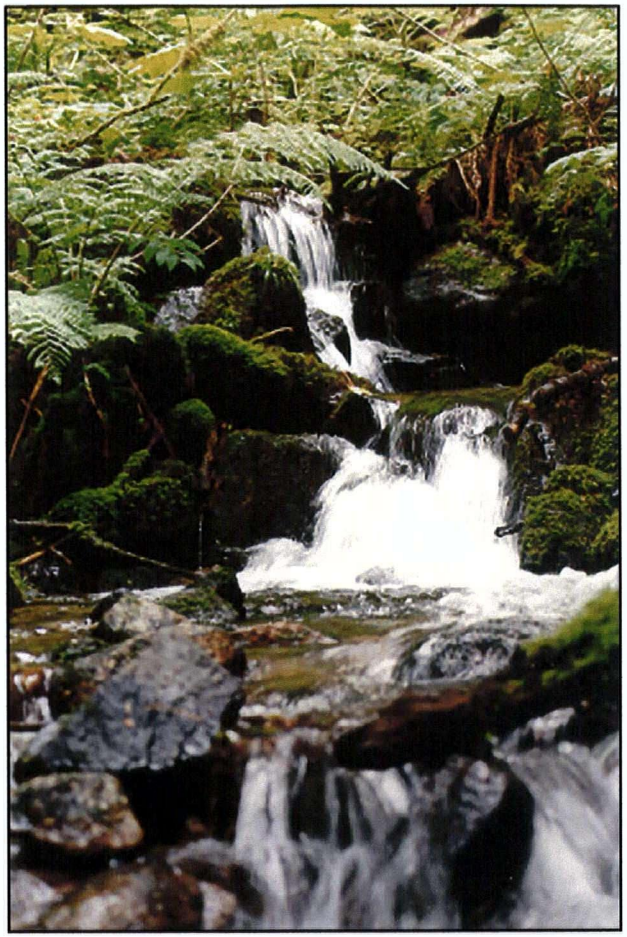
Photo credit: Tanya Wahbe

Adult male *Ascaphus*.

Photo credit: Tanya Wahbe

Upland drift fences with pitfall traps. One hundred metres from stream. Site: Branch 100 Old Growth.

Photo credit: Debbie Higgins

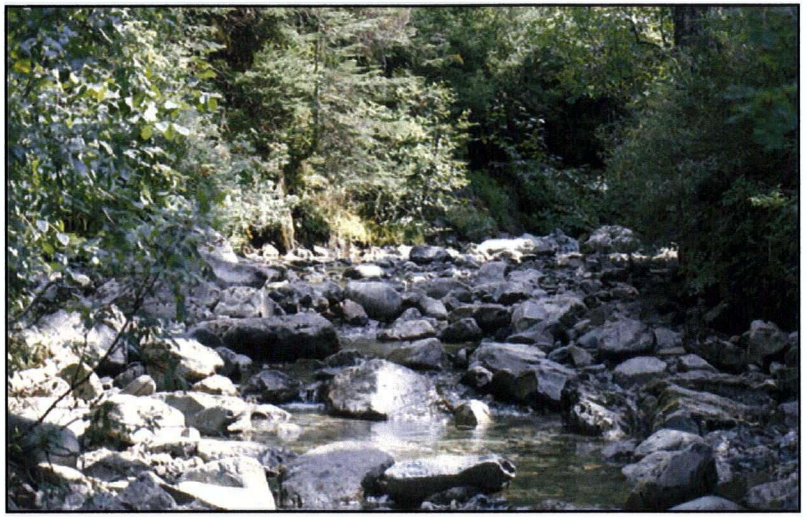


Mamquam watershed. Stream flowing through a second-growth forest.

Photo credit: Tanya Wahbe

Streamside pitfall traps showing one end of a 5–m drift fence. Site: Ashlu clearcut.

Photo credit: Tanya Wahbe



Mamquam watershed. Stream flowing through an old-growth forest.

Photo credit: Tanya Wahbe

Ventral surface of two adult female *Ascaphus* showing green (left) and red (right) visual implant fluorescent elastomer marks in thigh.

Photo credits: Tanya Wahbe