

TADPOLE – SEDIMENT INTERACTIONS OF THE WESTERN TOAD,
BUFO BOREAS, IN A TEMPERATE-LENTIC SYSTEM

by

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ABSTRACT

Sediment and nutrient loading in freshwater systems are leading causes of aquatic habitat degradation in North America. The impacts of fine-sediment and nutrient additions on the growth and survival of *Bufo boreas* tadpoles and emergent metamorphs was investigated in mesocosm and exclosure experiments. Mesocosm tanks received weekly pulses of fine, organic-rich (8% -9%) sediments to create initial concentrations of 0, 130 and 260 mg/L of sediment and bi-weekly additions of nutrients (N-160 $\mu\text{g/L}$, P-10 $\mu\text{g/L}$) in a factorial design. Within mesocosms, tadpole exclosures allowed for quantification of tadpole grazing pressure on periphyton biomass, chlorophyll *a* and sediment deposition. Tadpoles receiving sediment additions experienced slower growth rates and reduced survival to metamorphosis, though no effects of treatment were detected on metamorphic size or timing. Nutrient additions also lowered survival, but had no impact on other measured parameters. Dissections and gut content analysis revealed that tadpoles ingested sediment in large quantities and scanning electron microscopy showed particles were also found in their gill tissues. Together these results suggest that though organic-rich sediments were readily consumed, tadpoles derived little or no net benefit from these materials. Measures from tiles within the exclosures in the mesocosm experiment demonstrated that tadpoles were able to reduce the standing stocks of periphyton by 35-80% and to clear virtually 100% of all deposited sediment from grazing surfaces. Sediment clearing activities via ingestion acted to restructure the benthic abiotic habitat, but at tadpole densities used in the experiment did not have a beneficial effect on underlying periphyton growth. Under natural conditions, such grazing pressure and sediment removal activities could lead to changes in the algal community and consequent shifts in invertebrate grazers. Together, these results highlight a potential role for *Bufo boreas* tadpoles as ecosystem engineers in temperate pond habitats.

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CO-AUTHORSHIP STATEMENT

Chapter 2 is being prepared for submission in a scholarly journal under the same title as given here. The co-author of this chapter is Dr. John S. Richardson. S.Wood conducted the research, performed all the data analysis and wrote the manuscript. The co-author helped design the study and improve the manuscript.

Chapter 3 is being prepared for submission in a scholarly journal under the same title. The co-author of this chapter is Dr. John S. Richardson. S.Wood conducted the research, performed all the data analysis and wrote the manuscript. The co-author provided funding and improved the manuscript.

CHAPTER ONE: INTRODUCTION

Amphibians, Inputs and The Western Toad

1.1 GLOBAL STATUS OF AMPHIBIANS

There is now general consensus that global amphibian populations are experiencing widespread decline (Alford and Richard 1999, Houlihan et al. 2000, Stuart et al. 2004). Hundreds of species have undergone massive range contractions, population reductions and even extirpation in recent decades (Crump et al. 1992, Pounds and Crump 1994, Fisher and Shaffer 1996, Kiesecker et al. 2001). Currently, nearly one in three amphibian species (31%) is threatened with extinction (IUCN 2004). Although no one stressor has been identified as the sole cause of these declines, 88% of threatened amphibian species are primarily affected by habitat loss and degradation (IUCN 2004). Anthropogenic activities such as land development, urbanization and aquatic pollution have been linked to many of these declines (Wake 1991, Hecnar and M'Closkey 1998).

Larval amphibians in particular have proven to be extremely sensitive to aquatic habitat perturbations. Changes in community composition (Relyea and Werner 1999, Resetarits et al. 2004), water quality (Bishop et al. 1999, Bridges 2000, Smith et al. 2005) and hydroperiod (Tequedo and Reyes 1994, Gray and Smith 2005) in the larval habitat are known to have strong consequences for tadpole growth, morphology and survival. Such effects on larval-stages are likely to have important carry-over effects on terrestrial juveniles, a critical life-stage for population persistence (Vonesh and de la Cruz 2002). Both metamorphic size and emergence date strongly influence adult survivorship, size and fecundity (Smith 1987, Berven 1990, Morey and Reznick 2001).

In the Pacific Northwest, a regional hotspot of amphibian species richness in North America, forest harvesting and logging roads are a wide-spread land-use activity and a major source of disturbance to both terrestrial and aquatic habitats. These harvesting-activities contribute large quantities of sediment and nutrients into adjacent headwater streams and ponds (Swift 1988, Reuss et al. 1997, Ketcheson et al. 1999), increasing turbidity and deposition (Reid and Dunne 1984). The consequences of such inputs to freshwater environments on larval anurans remain largely unknown.

1.2 ALLOCHTHONOUS INPUTS

Flows of energy, materials and nutrients across ecosystems boundaries have recently become recognized as ubiquitous and fundamental linkages between habitats (Polis et al. 1997). Yet, it is not clear how increased sediment erosion and nutrient leaching into adjacent aquatic ecosystems impacts recipient amphibian communities. These inputs can interact with recipient aquatic communities at different trophic levels. Nutrients enter food web at the bottom stimulating primary productivity (Luttenton and Lowe 2006) while sediments affect both primary productivity (Ryan 1991) and higher trophic species if ingested or inspired (Newcombe and MacDonald 1991). As a result, their impacts are likely complex and difficult to predict.

Such allochthonous inputs from terrestrial habitats may act in one of two ways, either as an important supply of energy and nutrients or as a source of habitat degradation. Evidence from investigations of sediment-biota interactions in tropical stream systems suggests that fine, organic-rich sediments represent an important food resource for certain tadpole species (Flecker et al. 1999, Ranvestel et al. 2004, Solomon et al. 2004). In these lotic habitats, organic sediments can act as “resource subsidies” supporting greater tadpole growth and productivity (Flecker et al. 1999). Alternatively, interactions of stream-biota with sediment of a low organic

fraction can negatively affect stream tadpoles by impairing primary productivity, increasing foraging costs by burying periphyton (Gillespie 2002) and reducing refuge availability (Corn and Bury 1989, Lowe et al. 2004). In these circumstances sediment additions behave as a disturbance, contributing to habitat loss and degradation and acting as what might be conversely termed “resource depressants”.

1.3 NATURAL HISTORY AND CONSERVATION OF THE WESTERN TOAD

Bufo boreas (Baird and Girard 1852) is a lake and pond-breeding species with a distribution ranging along the Pacific coast from Baja California up to southern Alaska and east into Colorado and Utah (Stebbins 1985). Although globally secure, the western toad is red-listed by the World Conservation Union (IUCN 2006) as “near threatened” as its populations have undergone extensive range contractions in parts of its southern distribution. In Canada, the western toad is widespread, but listed by COSEWIC (Committee on the Status of Endangered Wildlife in Canada) as a “species of special concern” due to lack of demographic studies. In British Columbia the western toad is yellow-listed in throughout the province (B.C. Conservation Data Center, 2007), but anecdotal evidence points to regional declines over parts of the Lower Mainland of British Columbia and in the southern and central regions of Vancouver Island (Haycock and Knopp 1998, Dupuis 1998, Davis 2000). In British Columbia, breeding sites of this species receive little protection under the Forests and Range Practices Act (Ministry of Forests and Range 2007) due to the small size (< 0.5 ha) of most breeding ponds (Wind and Dupuis 2002). The area around ponds is likely critical for maintaining connectivity between pond and upland habitat for migrating adults and preserving the ecological integrity of the aquatic habitat.

Male and female adult western toads can grow up to 100 mm and 125 mm long, respectively (Green and Campbell 1992), and are often associated with forest-dominated and disturbed habitats. Western toads reach maturity in 4-6 years and can live for as long as 11 years (Olson 1992, Carey 1993). Breeding occurs communally and typically takes place over one or two weeks between January and May depending on latitude and altitude. Along the BC coast, adults have been documented to show a seasonal preference for disturbed habitats, often breeding in clear-cuts (Gyug 1996). Timing of breeding tends to coincide with average daily temperatures reaching above 0°C minimum and 10°C maximum (Gyug 1996). Females lay gelatinous strings of 12 000-16 500 eggs (Blaustein 1988) and the resulting tadpoles are primarily herbivorous and detritivorous feeding on filamentous algae, detritus and settled particulate matter (Wind and Dupuis 2002). Tadpoles take 6 to 8 weeks to complete development (Green and Campbell 1992) during which time spring melts and high rainfall can move large quantities of loose sediment into aquatic habitats, influencing water quality and primary productivity.

As a result of this explosive breeding, anuran tadpoles can make up a large portion of the biomass in ponds, acting as important primary consumers (Dickman 1968, Seale 1980, Loman 2001). *Bufo* tadpoles in particular are highly gregarious, exhibiting schooling behaviour and forming groups ranging from the hundreds to millions (Wassersug 1973). Emergence of larval amphibians into terrestrial habitats represents an important pathway for the transfer of energy and nutrients from aquatic systems into adjacent terrestrial systems (Burton and Likens 1975). Tadpole gregariousness and synchronized emergence of metamorphs is thought to act as a predatory defence mechanism (Arnold and Wassersug 1978, Devito et al. 1998). Although low levels of bufotoxins in the skin make tadpoles and metamorphs unpalatable to most fish species, some aquatic invertebrates (e.g. backswimmers, giant water bugs), garter snakes, amphibians

(Oregon spotted frog), and birds (ravens, ducks, herons) have been observed to feed on them (Olson 1989, Peterson and Blaustein 1992, Davis 2000, Pearl and Hayes 2002).

1.4 DYNAMIC INTERACTIONS OF FINE-SEDIMENTS, NUTRIENTS AND TADPOLES IN LENTIC SYSTEMS

This manuscript-based thesis explores the dynamic interactions of western toad tadpoles, *Bufo boreas*, fine-sediments and nutrient inputs in pond habitats. The research is focused on answering two overarching questions: how do increases in fine sediments loads affect tadpoles in pond systems? and reciprocally, what are the effects of tadpole grazing activities on sediment deposition dynamics? Unlike previous tropical-stream amphibians examined in sediment-biota interactions (above), this study used a temperate zone, pond-breeding species to identify cross-habitat and cross-latitudinal similarities in response, particularly to explore interactions in a temperate pond system with moderately organic-rich sediment inputs. Sediment-biota interactions and its effects on habitat structure in temperate, lentic systems may be quite different than tropical, lotic systems due to the greater residency time of deposited particulate material on the benthos of ponds and lower organic content of sediments.

Chapter two of this thesis is an investigation of the physiological and behavioural impacts of fine-sediment and nutrient additions on tadpoles of the western toad. The objective of this chapter was to quantify the effect of these inputs on tadpole growth, survival and foraging behaviour through a combination of mesocosm experiments and behavioural trials. Possible mechanisms for differences in these parameters were explored using findings from tadpole dissections, scanning electron microscopy and measures of food resource availability.

Chapter three tests the idea of temperate, lentic tadpoles acting as ecosystem engineers and examines the impacts of tadpole foraging activity on deposition dynamics of fine-sediment inputs and its consequent impact for periphyton standing stocks. Through the use of small-scale, tadpole exclosure manipulations within larger mesocosms I documented the ability of tadpoles to affect periphyton standing stocks and sediment accumulation dynamics on foraging surfaces, and discuss a potential role for *Bufo boreas* tadpoles as ecosystem engineers within pond habitats.

1.5 REFERENCES

- Alford, R.A. and S.J. Richards. 1999. Global amphibian declines: A Problem in applied ecology. *Annual Review of Ecology and Systematics* 30: 133-165.
- Arnold, S.J. and R.J. Wassersug. 1978. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*)- social behaviour as a possible defense. *Ecology* 59: 1014-1022.
- Baird, S. and C. Girard. 1852. Appendix C, Reptiles. Pp. 336-355 in H. Stansbury, Exploration and Survey of the valley of the Great Salt Lake of Utah. Lippincott, Grambo & Co. Philadelphia.
- B.C. Conservation Data Centre. 2007. BC Species and Ecosystems Explorer. B.C. Ministry of Environment, Victoria, BC. Available: <http://srmapps.gov.bc.ca/apps/eswp/> (June 28th, 2007)
- Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71: 1599-1608.
- Bishop, C.A., N. A. Mahony, J. Struger, P. Ng and K.E. Pettit. 1999. Anuran development, density and diversity in relation to agricultural activity in the Holland river watershed, Ontario, Canada (1990-1992). *Environmental Monitoring and Assessment* 57: 21-43.
- Blaustein, A.R. 1988. Ecological correlates and potential functions of kin recognition and kin association in anuran larvae. *Behavior Genetics* 18: 449-464.
- Bridges, C.M. 2000. Long-term effects of pesticide exposure at various life stages of the southern leopard frog (*Rana sphenocephala*). *Archives of Environmental Contamination and Toxicology* 39: 91-96.
- Burton, T.M. and G.E. Likens. 1975. Salamander populations and biomass in Hubbard Brook experimental forest, New Hampshire. *Copeia* 3: 541-546.
- Carey, C. 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conservation Biology* 7: 355-362.
- Corn, P.S. and R.B. Bury. 1989. Logging in western Oregon: responses of headwater habitats and stream amphibians. *Forest Ecology and Management* 29: 39-57.
- Crump, M.L., F.R. Hensley and K.L. Clark. 1992. Apparent decline of the Golden Toad: underground or extinct? *Copeia*. 2: 413-420.
- Davis, T.M. 2000. Ecology of the Western Toad (*Bufo boreas*) in forested areas on Vancouver Island. Final Report. Forest Renewal B.C. Ministry of Forests, Victoria, B.C.
- Davis, T.M. 2002. Research priorities for the management of the western toad, *Bufo boreas*, in British Columbia. Wildlife Working Report No. WR-106.

- DeVito, J., D.P. Chivers, J.M. Kiesecker, A. Marco, E.L. Wildy and A.R. Blaustein. 1998. The effects of snake predation on metamorphosis of western toads, *Bufo boreas* (Amphibia, Bufonidae). *Ethology* 104: 185-193.
- Dickman, M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. *Ecology* 49: 1188-1190.
- Dupuis, L.A. 1998. The effect of various silvicultural treatments on amphibian assemblages of the Robert's Creek watershed. Ministry of Forests, Vancouver Region, Interim Report.
- Fisher, R.N. and H.B. Shaffer. 1996. The decline of amphibians in California's Great Central Valley. *Conservation Biology* 10: 1387-1397.
- Flecker A.S., B.P. Feifarek and B.W. Taylor. 1999. Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. *Copeia* 1999: 495-500.
- Gillespie, G.R. 2002. Impacts of sediment loads, tadpole density, and food type on the growth and development of tadpoles of the spotted tree frog *Litoria spenceri*: an in-stream experiment. *Biological Conservation* 106: 141-150.
- Gray, M.J. and L.M. Smith. 2005. Influence of land use on postmetamorphic body size of playa lake amphibians. *Journal of Wildlife Management* 69: 515-524.
- Green, D. and W. Campbell. 1992. Amphibians of British Columbia. Royal B.C. Museum handbook. Victoria.
- Gyug, L. 1996. Part IV Amphibians. *In* Timber harvesting effects on riparian wildlife and vegetation in the Okanagan Highlands of British Columbia. B.C. Environment, Penticton, B.C.
- Haycock, R.D. and D. Knopp. 1998. Amphibian survey with special emphasis on the Oregon Spotted frog (*Rana pretiosa*). Selected wetland sites: Fraser River Lowlands and corridors to the interior Plateau. B.C. Ministry of Environment, Lands and Parks. Draft Report.
- Hecnar, S.J. and R.T. M'Closkey. 1998. Species richness patterns of amphibians in southwestern Ontario ponds. *Journal of Biogeography* 25: 763-772.
- Houlahan J.E., C.S. Findlay, B.R. Schmidt, A.H. Meyer and S.L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. *Nature* 404: 752-755.
- IUCN 2006. 2006 IUCN Red List of Threatened Species. www.iucnredlist.org. Downloaded on 28 June 2007.
- IUCN World Conservation Union. 2004. IUCN Red list of threatened species: A global species assessment. Eds. J.M. Baillie, C. Hilton-Taylor and S.N. Stuart. IUCN publishing services unit. Cambridge, UK.
- Ketcheson, G.L., W.F. Megahan and J.G. King. 1999. "R1-R4" and "BOISED" sediment prediction model tests using forest roads in granitics. *Journal of the American Water Resources Association* 35: 83-98.

- Kiesecker, J.M., A.R. Blaustein and L.K. Belden. 2001. Complex causes of amphibian population declines. *Nature* 410: 681-684.
- Loman, J. 2001. Effects of tadpole grazing on periphytic algae in ponds. *Wetlands Ecology and Management* 9: 135-139.
- Lowe, W.H., K.H. Nislow and D.T. Bolger. 2004. Stage-specific and interactive effect of sediment and trout on a headwater stream salamander. *Ecological Applications* 14: 164-172.
- Luttenton, M.R. and R.L. Lowe. 2006. Response of a lentic periphyton community to nutrient enrichment at low N:P ratios. *Journal of Phycology* 42: 1007-1015.
- Ministry of Forests and Range. 2007. Forest Planning and Practices Regulation. Forest and Range Practices Act. Ministry of Forestry and Range, Victoria, B.C.
- Morey, S. and D. Reznick. 2001. Effects of larval density on postmetamorphic spadefoot toad (*Spea hammondi*). *Ecology* 82: 510-522.
- Newcombe, C.P. and D.D. MacDonald. 1991. Effects of suspended sediments on aquatic ecosystems. *North American Journal of Fisheries Management* 11: 72-82.
- Olson, D.H. 1989. Predation on breeding Western Toads (*Bufo boreas*). *Copeia* 1989: 391-397.
- Olson, D.H. 1992. Ecological susceptibility of amphibians to populations declines. Pages 55-62. In R.R. Harris and D.C. Erman, technical coordinators. Symposium on biodiversity of northwestern California. Report 29, Wildland Resources Center, University of California, Berkeley, California, USA.
- Pearl, C.A. and M.P. Hayes. 2002. Predation by Oregon Spotted frogs (*Rana pretiosa*) on Western Toads (*Bufo boreas*) in Oregon. *American Midland Naturalist* 147: 145-152.
- Peterson, J.A. and A.R. Blaustein. 1992. Relative palatabilities of anuran larvae to natural aquatic insect predators. *Copeia* 2: 577-584
- Polis, G.A., W.B. Anderson and R.D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28: 289-316.
- Pounds, J.M. and M.L. Crump. 1994. Amphibian declines and climate disturbance: the case of the Golden Toad and the Harlequin Frog. *Conservation Biology* 8: 73-85.
- Ranvestel, A.W., K.R. Lips, C.M. Pringle, M.R. Whiles and R.J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology* 49: 274-285.
- Reid, L.M. and T. Dunne. 1984. Sediment production from forest road surfaces. *Water Resources Research* 20: 1753-1761.

- Relyea, R.A. and E.E. Werner. 1999. Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology* 80: 2117–2124.
- Resetarits, W.J. Jr, J.F. Rieger and C.A. Binckley. 2004. Threat of predation negates density effects in larval gray treefrogs. *Oecologia* 138: 532–538.
- Reuss, J.O., R. Stottlemyer and C.A. Troendle. 1997. Effect of clear cutting on nutrient fluxes in a subalpine forest at Fraser, Colorado. *Hydrology and Earth System Sciences* 1: 333–344.
- Ryan, P.A. 1991. Environmental-effects of sediment on New Zealand streams- a review. *New Zealand Journal of Marine and Freshwater Research* 25: 207–221.
- Seale, D.B. 1980. Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology* 61: 1531–1550.
- Smith, D.C. 1987. Adult recruitment in chorus – effects of size and date at metamorphosis. *Ecology* 68: 344–350.
- Smith, G.R., K.G. Temple, D.A. Vaala and H.A. Dingfelder. 2005. Effects of nitrate on the tadpoles of two ranids (*Rana catesbeiana* and *R. clamitans*). *Archives of Environmental Contaminants and Toxicology* 49: 559–562.
- Solomon, C.T., A.S. Flecker, and B.W. Taylor. 2004. Testing the role of sediment-mediated interactions between tadpoles and armored catfish in a neotropical stream. *Copeia* 2004: 610–616.
- Stebbins, R.C. 1985. Western reptiles and amphibians. 2nd Ed. The Peterson Field Guide Series National Audubon Society and National Wildlife Federation. Houghton Mifflin Co., Boston.
- Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L Rodrigues, D.L. Fischman and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1786
- Swift Jr., L.W. 1988. Forest access roads: design, maintenance, and soil loss. *In*: Swank, W.T., and D.A. Crossley Jr. (Eds.), *Forest hydrology and ecology at Coweeta*, Springer-Verlag, New York, pp. 313–324.
- Tejedo, M. and R. Reques 1994. Plasticity in metamorphic traits of Natterjack tadpoles: The interactive effects of density and pond duration. *Oikos* 71: 295–304.
- Vonesh, J.R. and O. De la Cruz. 2002. Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. *Oecologia* 133: 325–333.
- Wake, D.B. 1991. Declining amphibian populations. *Science* 253:860.
- Wassersug, R.J. 1973. *In* *Evolutionary Biology of the Anurans* (ed. Vial, J. L.), University of Missouri Press, pp. 273–297.

Wind, E. and L.A. Dupuis. 2002. COSEWIC status report on the western toad *Bufo boreas* in Canada. *In* COSEWIC assessment and status report on the western toad *Bufo boreas* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-31 pp.

CHAPTER TWO:

Impact of Allochthonous Sediment Inputs on Growth and Survival in Tadpoles of the Western Toad, *Bufo boreas*¹

2.1 INTRODUCTION

Global amphibian populations have undergone massive declines in recent decades (Alford and Richard 1999, Houlahan et al. 2000). Habitat loss and degradation as a result of anthropogenic activities are considered to be the primary and most pervasive threats to amphibian populations (IUCN 2004) and have been linked with several declines and extinctions (Wake 1991, Hecnar and M'Closkey 1998). In freshwater habitats, siltation and sedimentation from adjacent land-use practices are the leading causes of water quality degradation across North America (Environmental Protection Agency 1994). The effects of suspended and deposited sediment on stream biota, particularly fish and invertebrates, have been well documented (reviewed in Newcombe and MacDonald 1991, Waters 1995). However, sediment impacts on amphibian physiology and behaviour, and its contribution to their population declines remain less well understood.

The movement of sediments into aquatic systems is a fundamental process connecting terrestrial and aquatic ecosystems. Terrestrially-derived materials can provide an important source of organic material and nutrients for freshwater systems, supporting higher levels of secondary productivity in these habitats (Wallace et al. 1997, Nakano et al. 1999). Review of previous work on amphibians suggests that the impact of sediment deposition on tadpoles may

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be in part dependent on the relative fraction of organic matter in the sediment. Inputs of highly inorganic sediment contribute to habitat loss by filling interstitial crevices (Corn and Bury 1989, Welsh and Ollivier 1998, Lowe et al. 2004), smothering grazing surfaces (Power 1990) and altering channel morphology (Hassan et al. 2005). Increased inorganic sediment loads in streams have been associated with a reduction in larval anuran abundance (Corn and Bury 1989, Welsh and Ollivier 1998), growth and development rates (Gillespie 2002). Conversely, sediment material with a high organic fraction (9% and above) can be a food resource for tadpoles (Flecker et al. 1999, Ranvestel et al. 2004, Solomon et al. 2004; but see Kupferberg et al. 1994). Indeed, tadpoles exhibit density-dependent growth and faster development in response to the availability of organic-rich sediment (Flecker et al. 1999). In addition to this variable response to sedimentation, how species cope with changes in the rate of sediment inputs in aquatic habitats also remains largely unstudied (but see Shaw and Richardson 2001, Gillespie 2002, Green et al. 2004).

Rates of sedimentation in freshwater habitats can be altered by adjacent land-use practices. In the Pacific Northwest, forestry and road construction are two of the most extensive land-use activities contributing to increased sediment inputs to aquatic habitats. As a consequence of disrupting the topsoil, these activities cause short-term increases of sediment loading (Reid and Dunne 1984, Swift 1988, Ketcheson et al. 1999) and nutrient leaching (Correll et al. 1992, Reuss et al. 1997) into adjacent aquatic habitats. Such fine sediment inputs increase water turbidity (Reid and Dunne 1984, Wemple 1996), decrease primary productivity (Ryan 1991, Power 1990) and lead to benthic deposition (Power 1990). Conversely, nutrient additions from run-off can increase primary productivity and algal standing stocks when aquatic systems are subject to nutrient limitation (Carrick and Lowe 1989, Luttenton and Lowe 2006).

Amphibian tadpoles may be particularly sensitive to alterations in their larval environment caused by changes in the natural fluxes of sediment and nutrients. Their highly specialized feeding apparatuses (Orton 1953, Altig and Johnson 1989) and use of microhabitat niches for foraging and refugia (Heyer 1973, Kopp and Eterovick 2006) make them especially vulnerable to habitat change. As herbivores, tadpoles' fitness is tightly-coupled with primary productivity. Changes to the availability of algal food resources leads to density-dependent effects on tadpole growth, development and survival (Brockleman 1969, Dash and Hota 1980, Skelly and Kiesecker 2001, Gillespie 2002, Mallory and Richardson 2005).

Studies of the impact of sediment loading on tadpole communities have so far focused on stream systems and species, with little attention paid to pond systems. Ponds are critical habitats for many breeding amphibians that require slow or stagnant water, and are generally more widely used for breeding than are streams. Sediment inputs may have markedly different impacts in pond habitats than in streams. In ponds, sediment deposition represents a more permanent disturbance or stressor (Martin and Hartman 1987, Luo et al. 1997) with successive inputs accumulating on the pond benthos rather than being flushed out by continuous flows. Continued inputs over the long term can lead to more rapid pond filling and alteration of hydroperiod (Luo et al. 1997) making affected habitats unsuitable for a number of species.

I investigated the interactive effects of sediment input rates and nutrients on tadpole growth and survival in pond conditions using a combination of mesocosm and behavioural experiments. These experiments were designed to test four key hypotheses; (1) that sediment inputs into ponds would reduce periphyton biomass due to smothering, but (2) that nutrient inputs would mitigate these negative effects on primary productivity, (3) that due to the high fraction of organic material of the inputs applied (~9%), sediment would act as a food resource

for tadpoles enhancing growth and survival rates to metamorphosis, and (4) that the magnitude of these effects would be related to the rate of sediment input.

2.2 METHODS

Study Organism

The western toad, *Bufo boreas*, is a pond and lake-breeding amphibian with a widespread distribution along the west coast of North America from Baja California up to southern Alaska and east into Colorado and Utah (Stebbins 1985). They can be found at elevations ranging from sea level up to 3660 m and are explosive breeders with females laying upwards of 12 000 eggs each. Despite this, in recent decades their populations have undergone unexplained declines and local extinctions across much of their range in the United States (Livo and Loeffler 2003). In Canada, the western toad is listed as a “species of special concern” by COSEWIC (Committee on the Status of Endangered Wildlife in Canada) due to lack of demographic studies, but is thought to be in decline over parts of the Lower Mainland of British Columbia and on Vancouver Island (Haycock and Knopp 1998, Dupuis 1998, Davis 2000). In coastal B.C., adult toads have been noted to show seasonal preference for disturbed forest-habitats, seeking out (Dupuis 1998, Davis 2000, I. Deguise, pers. comm.) and breeding in clear-cuts during the spring (Gyug 1996). Their tadpoles are generally non-selective bottom feeders ingesting filamentous algae and deposited detritus. As a result, tadpoles produced in ponds adjacent to clearcuts and logging roads are likely directly affected by forestry-associated inputs of sediments and nutrients due to the lack of mandatory protection for water bodies < 0.5 ha (Ministry of Forests and B.C. Environment 1995). In the western toad, post-embryonic survival has been identified as a critical life-stage for population maintenance (Vonesh and De la Cruz,

2002) and factors which compromise larval and juvenile survival may have broad consequences for population persistence.

Specimen Collection and Husbandry

Western toad eggs (~3000) were collected on April 26, 2006 from two adjacent communal breeding sites, Edith Lake and Fawn Lake (<1 km apart), each with ~20 pairs in amplexus. The sites were within Alice Lake Provincial Park (N49 46.617, W123 06.476) 13 km north of Squamish, B.C. Eggs were transported back to facilities at the University of British Columbia in plastic containers filled with natal pond water and placed in a cooler. Eggs were allowed to hatch in 40 L aerated aquaria filled with aged tap water (minimum 3 days) containing a 400 ml inoculation of natal pond water. After hatching, tadpoles were reared in aquaria filled with water taken from experimental ponds (pH 8.3, conductivity 198 $\mu\text{S}/\text{cm}$) on UBC South Campus and filtered through a 64 μm screen. Conditions for all tadpoles were consistent during rearing. Water was changed every 3-7 days depending on water clarity by removing and replacing three-quarters of the volume. Tadpoles were raised under natural light conditions at approximately 18-23 °C.

Study Design

I used both mesocosm and behavioural studies to evaluate the effects of sediment and nutrient fluxes on tadpole growth, foraging and periphyton biomass. Mesocosm experiments were designed to address both the impact of sediment and nutrient inputs on tadpole growth and emergence timing as well as on periphyton growth and biomass. Use of mesocosms allows for replication and easy control of extraneous factors of unknown effects by reducing biological and physical complexity (Petersen and Hasting 2001). Additionally, we used a complementary behavioural experiment to quantify the impact of sediment addition on tadpole foraging

behaviour and food intake. Details of the experimental designs for these studies are provided below.

Mesocosm Experiment

The experiment was conducted in 18 pond mesocosms arrayed in 3 spatial blocks (6 tanks each). Mesocosms consisted of 1136 L plastic cattle tanks (Rubbermaid brand) 1.4 m in diameter and 0.5 m deep in an open area bordered by trees and away from traffic. Tanks were arranged in blocks along a North-South axis in a 9 x 2 layout and tanks were spaced 1 m apart on a pavement platform. Two months prior to the experiment, tanks were filled (770 L) with municipal tap water (Ca^{++} 1.3 mg/l, Cond 19 $\mu\text{S}/\text{cm}$, NO_3^- 0.08 mg/l, pH 6.5, TP < 0.0005 mg/l, GVRD 2005), 300 g of dried leaf litter (mostly alder and maple species) and 25 g of rabbit pellets (Hagen ®, Montreal, QC, Canada) to provide substrate and nutrients for periphyton establishment (Barnett and Richardson 2002). Leaf litter provided structural complexity within which tadpoles could forage and seek shelter. Mesocosms also received a 10 L inoculum of local pond water to provide a colonizing community of zooplankton and phytoplankton. The inoculum was filtered through a 64 μm screen to prevent colonization by large predaceous invertebrates (Barnett and Richardson 2002). At two and four weeks after inoculation, 10 L of water was exchanged between adjacent and diagonal tanks respectively to ensure similar planktonic communities (see similar protocols in Relyea and Hoverman 2003). Tanks were covered with 2 x 2 m wooden frames fitted with black fiberglass mesh and heavy construction fencing to prevent predation and ovipositioning by insects. Despite this, many mosquito larvae (Culicidae) and the occasional diving beetle (Dystiscidae) and whirly-gig beetle (Gyrinidae) were noted in tanks during the experiment and removed on site. On May 5th, free-swimming (Gosner stage 26) tadpoles were transferred from aquaria to the mesocosms to complete

development. Tanks were each stocked with 90 tadpoles (58 tadpoles/m^2) at an initial average individual mass of 30.55 ± 0.065 (SD) mg ($n = 40$).

Six treatments were applied to the mesocosms in a factorial, randomized block design. Each block of six tanks received applications of three fine sediment levels crossed by either ambient ($\text{NH}_4\text{-N } 2.6 \pm 4.5 \text{ } \mu\text{g/L}$, $\text{NO}_3\text{-N } 10.5 \pm 1.3 \text{ } \mu\text{g/L}$, $\text{Organic-N } 44.4 \pm 7.9 \text{ } \mu\text{g/L}$, $\text{PO}_4\text{-P } 7.8 \pm 1.9 \text{ } \mu\text{g/L}$) or augmented nutrient levels ($\text{NaNO}_3\text{-N } 160 \text{ } \mu\text{g/L}$, $\text{KH}_2\text{PO}_4\text{-P } 10 \text{ } \mu\text{g/L}$ above background). The experiment ran for 56 days, from May 9th to July 4th. Tanks were pulsed weekly with sediment producing suspended sediment concentrations of 0 mg/L (CONT), 130 mg/L (LOW), or 260 mg/L (HIGH) and half the tanks received NaNO_3 and KH_2PO_4 nutrient additions every other week. These sediment dosing levels were lower than intended due to constraints preparing sediment. Thus applications are modest in comparison with reported values from managed and disturbed landscapes and likely represent naturally occurring conditions in a number of breeding habitats. This created six sediment-nutrient combination treatments hereafter referred to as CONT, CONT+N, LOW, LOW+N, HIGH and HIGH+N. Sediment was collected from the stream bank of Kanaka Creek in Maple Ridge, B.C. and was taken as representative of a typical sample of sediment from riparian habitats. Sediment used in the treatments was sifted wet through a $64 \text{ } \mu\text{m}$ screen resulting in a fine mixture of silt and clay particles for maximum particulate suspension and had 8% - 9% organic content determined by ash-free dry mass (AFDM). Water samples were taken one day following nutrient additions in each of weeks 2, 4, 6 and 8 and analyzed for total $\text{PO}_4\text{-P}$, total nitrogen, organic-N and NH_4^+ . Weekly measures of DO (WTW Oxi330 dissolved oxygen meter, Weilheim, Germany), conductivity (WTW multi 350i +MPP 350 conductivity meter, Weilheim, Germany), and pH (Oakton phtestr® pH meter, Vernon Hills, Illinois, USA) were taken, and hourly temperature readings were recorded ($n = 2/\text{block}$) throughout the experiment (HOBO® H01-001-01 water

temperature data loggers, Pocasset, Massachusetts). Turbidity was measured on a Model 800 Turbidity meter (VWR International, Newark, NJ) daily during the initial 5 weeks to model sediment fall-out patterns after which measures were taken only prior to and following weekly sediment additions. To quantify incident radiation, photosynthetically active radiation (PAR) was measured on each tank under the screen cover at noon on July 7th, 2007 with a LiCor light meter and quantum sensor (Model LI-250; LiCor, Lincoln, NB) to determine if there were any biases in the light environment.

Tadpole Exclusion Experiment

We used small-scale exclosures within our larger mesocosm experiment to measure the impact of sediment on periphyton standing stock and Chl *a* on substrate surfaces. Six weeks before the start of the mesocosm experiment, 8 unglazed ceramic tiles were placed in tanks to provide a substrate for periphyton establishment. Once covered in biofilm, tiles were placed in tadpole exclusion cages and suspended in planter's trays ~5-10 cm below the water's surface at the beginning of the experiment. Exclosures consisted of an aluminum pie plate lined with dark plastic to reduce reflectance, fitted with screened openings along the sides for water circulation and sealed with a removable wire mesh screening on the top. These tiles provided a means of measuring food resources available to tadpoles and the effects of deposited sediment on the periphyton biofilm. In a few instances tadpoles were found in exclusion cages and were immediately removed, tiles were not sampled that week and lack of grazing scars suggested that tadpoles had little effect on periphyton standing stock.

Over the 8 week experiment, tiles were removed from each tank on a weekly basis to sample for periphyton biomass and Chl *a*. Accumulated sediment on tiles was rinsed off with distilled water and the underlying periphyton was then scraped off with a razor and toothbrush.

The resultant slurry was rinsed into a Petri-dish with distilled water and later split into 2 samples for AFDM and Chl *a*. Samples for AFDM were filtered onto pre-ashed Whatman G/F filters, dried for 24 h at 60°C and ashed for 2 h at 550°C. Chl *a* samples were filtered onto un-ashed Whatman filters and frozen for later analysis at which time Chl *a* was extracted from filters in 90% acetone, refrigerated for 24 h, centrifuged for 5 min and measured for uncorrected Chl *a* on a TD-700 Fluorometer (Turner Designs, Sunnyvale, CA) (Standards 18.6-186 µg/L, Turner Designs, Sunnyvale, CA).

Tadpole Growth Rates

Each week 10 tadpoles were removed ($n = 10/\text{tank}$) from each tank and were weighed individually to the nearest 0.01 g using an Ohaus Scout II electronic field scale (Ohaus®, Pine Brook, New Jersey, USA) to provide an index of growth rates leading up to metamorphosis. Tadpoles were collected by random dip net sweeps in tanks, then dabbed dry on tissue paper and placed into a weighed container of water. Tadpoles were out of water for no more than 10 seconds and were returned to their original tank. In one incident 4 tadpoles were accidentally returned to the wrong tank, calculations and analysis of survival to metamorphosis were corrected to make adjustments for differences in changes to the initial numbers of individuals. These four tadpoles represented ~2.02 g of biomass and constituted only a 4% movement of total biomass between donor and recipient tanks. The misplaced tadpoles were thus assumed to have negligible effects on metamorphic parameters or grazing pressure on periphyton in the affected tanks.

Metamorphs

As planter's trays became empty of tiles, their depth in the water column was adjusted to changing water levels to provide surfaces for emerging metamorphosing toads (metamorphs) to

crawl out on. Metamorphs were collected from tanks after reaching Gosner stage 42 (i.e., all four limbs were present and tail reabsorption had begun) (Gosner 1960), and taken into the lab to complete metamorphosis. Metamorphs were housed in plastic cups containing 1 cm of fresh pond water, a piece of metal screening and sphagnum moss until they completed tail reabsorption (Gosner 47) which was recorded as their date of metamorphosis. During the final days of tail reabsorption in the lab, individuals were not fed so as to obtain unbiased mass at emergence and because tadpoles do not typically feed during metamorphosis (Jenssen 1967). The experiment was terminated on August 12th and any remaining tadpoles in tanks ($n = 11$) were captured and released but not included in the results. At metamorphosis, all metamorphs were weighed to the nearest 0.01 g on an electronic balance and measured for snout-urostyle length with callipers to the nearest 0.01 cm. Three measurements of length were taken for repeatability and averaged. After measurements, metamorphs were housed in terrariums filled with moist sphagnum and a ceramic dish containing pond water to provide access to water for hydration and were fed drosophila (fruit flies) and pinhead crickets daily. Metamorphs remained in terrariums no more than 14 days before being released at their natal pond.

Tadpole Dissections

Tadpoles ($n = 2$) from each tank mesocosm were euthanized in week 6 of the experiment in MS222 and preserved in 10% buffered formalin for later dissection. Total length, snout-tail base length and body width at the spiracle were measured with callipers to the nearest 0.01 cm. Wet mass and Gosner stage of each tadpole was recorded. Dissections examined the oral disc and gill tissue for signs of sediment abrasion. Gill tissues were examined for general evidence of sediment in the food sacks of the structures under light microscopy. Scanning electron microscopy (SEM) of gill structures was conducted to determine if sediment particles caused visible damage to the gill tissue or structure. The mid and hind-gut were excised, length

measured, and a visual quantification of gut contents (scale of 1-5 for proportion of sediment content) was made under a light microscope. Pale coloured, particulate sediment was easily identified from dark amorphous detritus and green coloured algae, and allowed for easy estimation of gut content proportions.

Behavioural Trials

Tadpoles from the same stock as the mesocosm experiment were reared under identical aquarium conditions in pond water for behavioural trials. Once tadpoles began feeding (Gosner 26) they were fed a diet of frozen lettuce *ad libitum* and Spirulina algal Tablets (Nutrafin Max, Hagen®, Montreal, Canada) for additional protein. Behavioural trials consisted of placing five tadpoles (stages Gosner 27-36) into a 10 L aerated glass aquarium filled 12 cm deep with filtered pond water (or 9.6 L). Three sides of the tanks were lined with dark plastic to limit influence of neighbouring tanks and minimize visual disturbance. In each aquarium, three ceramic tiles with a pre-established layer of biofilm were then added and tadpoles left to acclimate for 1 h prior to the start of the experiment. Three 20 minute observation periods were conducted at 0 min, 25 min and 24 h post-acclimation during which feeding behaviour was monitored. During each observation period the proportion of tadpoles grazing on tiles or aquarium glass was recorded at one minute intervals for 20 minutes. After the initial 20 min observation period, 2 g of sifted sediment were added to half of the tanks on a random basis producing turbidities of 11-20 NTU, at which point a second observation period was immediately conducted. The disturbance of sediment addition was controlled for by adding an equal amount of water poured in to control aquaria. Tadpoles were left in tanks for 24 h before conducting the final observation period. Following this, tadpoles were dabbed dry, staged and weighed (0.01 g). Individuals from the same trial were placed in a plastic container containing one litre of filtered (0.45 µm filter) pond water and kept for 24 h to collect fecal matter. Feces

were later processed for AFDM to compare organic and inorganic diet composition. Following the completion of all the trials, tadpoles were released to their natal pond.

2.2.1 STATISTICAL ANALYSIS

Mesocosm Analyses

Proportional survivorship data were arcsine, square root-transformed (to convert to unbounded units) and compared in a 2 x 3 ANOVA with blocking. Individual metamorph mass, length and emergence date were analyzed using a 2 x 3 factorial design in PROC MIXED (SAS ver. 9.1) blocking by tank. Only metamorphs with known treatment history were used in analyses (14 metamorphs escaped from temporary cages during transportation from field to lab and couldn't be confidently assigned to a treatment or tank). Average tadpole growth rates were analyzed in a 2 x 3 ANCOVA with blocking. Measures of metamorph mass were regressed on snout-urostyle length and the residuals taken as an index of metamorph condition (Schulte-Hostedde et al. 2005).

Periphyton organic matter and Chl *a* on tiles from exclosures were analyzed in a 2 x 3 repeated measures ANOVA. Organic matter was log-transformed and Chl *a* was ln-transformed to meet assumptions of normality. Dissolved nutrient levels were compared between treatments to detect the effect of nutrient additions in 2 x 3 ANOVAs with blocking. When variances were unequal, a Welch's approximate degrees of freedom ANOVA (Welch 1938) adjustment was applied to ensure the measure of significance was unbiased. Water quality measures (i.e., conductivity, temperature and pH) were analyzed in a 2 x 3 ANOVA for initial conditions and in a repeated measures ANOVA over weeks to assess changes over the course of the experiment. If data failed Mauchly's test of sphericity (Mauchly 1940) in which the variance of

the difference is the same for all possible pairs of observations across time periods (Huynh and Feldt 1970), a more conservative Huynh-Feldt adjustment was applied (Huynh and Feldt 1976).

Tadpole Dissections

Gut length of tadpoles was compared amongst treatments in a 2 x 3 mixed model ANOVA controlling for Gosner stage and body size to evaluate gut length plasticity in response to diet. Visual rank scorings of gut contents of the proportion of sediment in the gut on a scale of 1 to 5 were compared amongst treatments using 2 x 3 ANOVA. Visual observation of presence or absence of sediment particles in gill structures was recorded and later assessed by scanning electron microscopy. SEM images were taken on a representative sample of tadpoles from CONT (n = 2) and HIGH (n = 3) sediment treatments. Gills were examined for evidence of sediment particles on gill tissue and tissue abrasion.

Behavioural Trials

Foraging proportions were arcsine square root-transformed and compared using repeated measures ANOVA for a single fixed factor. The model's variance-covariance structure marginally conformed ($p = 0.08$) to the Type H matrix based on Mauchly's (1940) W-test of sphericity. The more conservative Huynh-Feldt adjustment (Huynh and Feldt 1976) was applied to this analysis to assess significance. A Welch's ANOVA was applied to cases of unequal variance in foraging effort to ensure correct analysis and Tukey-Kramer HSD *post hoc* tests were used to distinguish significance among treatments. To test for differences in diet composition, organic and inorganic AFDM of feces were compared across treatments using two-tailed t-tests. All analyses were conducted in SAS (SAS ver. 9.1) and reported results are means $\pm$ SE (n). A type I error of 0.05 was used to assess significance in all models.

2.3 RESULTS

Mesocosm Experiment

Treatments

Water quality parameters did not vary significantly by treatment or by block at the start of the mesocosm experiment, except for pH which was initially lower in one of the three blocks ($F_{2,15} = 16.33$, $p = 0.001$). Conductivity, pH and temperature in tanks all increased over the course of the experiment, but there were no significant differences attributed to treatments applied (Table 2.1). Spatial blocks along the North-South axis all received equal incoming solar radiation ($F_{2,15} = 1.73$, $p = 0.21$) as measured under the protective screening at mid-day.

Sediment inputs of 0 mg/L (CONT), 130 mg/L (LOW) and 260 mg/L (HIGH) produced initial turbidities of 3.57 ± 0.39 , 21.1 ± 1.04 and 31.47 ± 1.36 NTU respectively, after each weekly addition creating strong distinctions between treatments (Figure 1.1 a-c). Daily measures of turbidity showed a general pattern of sediment fallout over the initial 48 h after addition and an elevated residual turbidity level of ~9-14 NTU, an effect most pronounced in HIGH treatments. Bi-weekly nutrient additions of $\text{NaNO}_3\text{-N}$ and $\text{KH}_2\text{PO}_4\text{-P}$ had little impact on concentrations of nitrogen and phosphorus remaining in the water one day after addition, with a few exceptions. Total PO_4^- was significantly higher in the LOW+N sediment (130 mg/L) treatments receiving nutrient doses than in CONT+N tanks ($F_{5,29.3} = 3.12$, $p = 0.02$), but was not significantly different from any other treatments. No other differences were detected among treatments for NH_4^+ , $\text{NO}_3\text{-N}$ or total organic-N. Across weeks, organic-N increased across all tanks resulting in a significant difference between weeks 2 and 6 ($F_{3,36.7} = 4.26$, $p = 0.011$), but decreased in week 8, likely due to arrested feeding and thus depressed nutrient cycling via

tadpoles. There was also a significant spike of NH_4^+ in week two ($F_{3,36} = 11.49$, $p < 0.0001$) following a cold snap and associated algal die off.

Tadpole Survival and Metamorphs

Of the 1690 tadpoles placed in the tanks, there was an overall survival rate of 78% with 1319 metamorphs emerging by the end of the study. Eleven tadpoles remained in the tanks from CONT and LOW treatments at the termination of the experiment. The analysis of survival was run with and without these remaining tadpoles, and the 11 were dropped as their exclusion had no effect on the results. There was a significant interaction of sediment and nutrient levels on survival to metamorphosis ($F_{2,15} = 5.15$, $p = 0.013$). Sediment reduced survival rates in high sediment treatments from 92% in CONT to 75% in HIGH treatments. Nutrient additions varied in their effect, decreasing survival in CONT+N and LOW+N treatments ($p = 0.09$, $p = 0.054$, respectively), but marginally enhancing survival in HIGH+N treatments (NS, $p = 0.85$) to levels equivalent with CONT+N and LOW+N treatments (Figure 1.2). There was no effect of blocking. A single tank from the LOW sediment treatment had unusually low survivorship to metamorphosis (> 2 SDs below the grand mean) and was removed as an outlier. This removal affected the significance making the sediment-nutrient interaction significant and raising survival in LOW tanks to levels similar with CONT.

Mass, length and date of metamorphosis of metamorphs were pooled across nutrient treatments (NS, $p = 0.66$ to 0.93), but showed no significant difference amongst sediment treatments (all $p > 0.2$) and wide variability ($\text{CV} \approx 0.3$). Calculations of Cohen's d effect sizes (i.e., the difference in the treatment mean from the control mean divided by their pooled standard error) indicated that the $\sim 10\%$ drop in mass and length (Figure 1.3 a,b) in HIGH sediment treatments constituted a relatively large effect size, i.e. greater than 0.8 ($d = 0.87$, $d =$

1.2 respectively). Though a statistical difference may not exist, a 10% decrease in body size in a natural context may have important biological implications for metamorph survival and fitness. A strong relationship existed between metamorphic mass and length ($r = 0.84$, $n = 1306$, $p < 0.001$). Residuals from this regression were used as an index of metamorphic condition. There was a positive relation between mass ($r = 0.35$, $n = 1306$, $p < 0.001$), length ($r = 0.21$, $df = 1307$, $p < 0.001$) and condition ($r = 0.3$, $n = 1306$, $p < 0.001$) with the time of metamorphosis indicating that later emerging metamorphs were larger, i.e. in better condition (Figure 1.4).

Growth Analysis

An ANCOVA of tadpole masses over the first five weeks of the experiment (Figure 1.5) revealed a significant depression of tadpole growth rate with sediment additions ($F_{2,15} = 15.97$, $p < 0.001$), but there was also a significant interaction between block and sediment treatment ($F_{4,13} = 5.1$, $p < 0.001$). Within each block, tadpoles in CONT sediment treatments were always the largest and HIGH sediment treatment tadpoles always smallest. The absolute difference between the mass of the CONT and those of HIGH sediment varied by block, but treatment masses were always ranked in the same relative positions to one another. Measures of tadpoles in week 5 exhibited relatively low variability ($CV \approx 0.12$) providing good estimators of average tadpole masses in each treatment. During the 5 weeks, tadpoles in CONT treatments grew at a rate of 110 ± 5.2 mg/week, while on average LOW sediment tadpoles grew at 94 ± 8.1 mg/week and HIGH sediment tadpoles at only 85 ± 5.9 mg/week (based on extracted regression slopes across blocks). Though LOW sediment tadpoles grew more slowly they reached the same peak mass as CONT tadpoles before entering metamorphosis, while HIGH sediment tadpoles achieved only 83% of this peak mass before metamorphosing.

Periphyton and Chlorophyll *a*

Sediment significantly depressed periphyton standing stocks on tiles ($F_{2,10.2} = 10.96$, $p = 0.003$) from LOW and HIGH treatments (Figure 1.6) and this difference became more pronounced over the course of the experiment ($F_{7,82.6} = 13.92$, $p < 0.0001$). The opposite trend occurred with Chl *a*. Sediment additions in both LOW and HIGH treatments increased the amount of Chl *a* measured on tile surfaces (Figure 1.7) ($F_{2,31.5} = 8.01$, $p = 0.002$) and this difference from CONT increased over the course of the experiment ($F_{3,43.1} = 20.95$, $p < 0.0001$).

Tadpole Dissections

There were no significant differences by treatment in mass, length, body width or Gosner stage between tadpoles randomly selected from tank mesocosms for dissection (all $p > 0.4$). However, gut contents differed significantly between treatments, with CONT tadpoles having a greater proportion of algae in their guts than tadpoles from LOW or HIGH sediment treatments ($F_{2,22} = 25.52$, $p < 0.001$). Nearly all tadpoles from sedimented environments had greater than 70% sediment in their gut contents whereas tadpoles from CONT treatments had none. From qualitative assessment of the presence or absence of sediment in gill tissues it was clear that sediment was present in the gills of tadpoles from both LOW and HIGH sediment treatments. Sediment particles were observed collected in gill pouches or entrained in strings of mucus. Scanning Electron Microscopy revealed the presence of occasional sediment particles in gill tissues (Figures 1.8), but there was no evidence of gill abrasion or strong differences in gill morphology readily discernable from SEM images (Figure 1.9, 1.10).

Foraging Behaviour

A repeated measures ANOVA on observations from the behavioural trials indicated a strong sediment treatment by observation period interaction ($F_{2,54} = 7.24$, $p = 0.002$). Foraging decreased sharply immediately following sediment addition (Figure 1.11). During this period,

tadpoles abandoned foraging activities and swam instead in a fast, erratic manner in what appeared to be a flight or avoidance response. At 24 hours, foraging had recovered to pre-treatment levels.

AFDM of feces collected 24 hours post-trial showed strong differences in the quantity and composition of ingested food. Tadpoles from sediment trials produced on average four times more fecal matter by weight than controls (t-test, $t = 8.38$, $p < 0.001$). Feces from sediment trials showed nearly a nine-fold increase in the amount of inorganic material rising from 1.79 ± 0.29 $\mu\text{g}/\text{animal}$ in controls to 15.64 ± 1.38 $\mu\text{g}/\text{animal}$ in the sediment treatments (t-test, $t = 9.6$, $p < 0.001$) which accounted for the overall differences in the quantity of feces produced (Figure 12-1). Differences in organic content were not as marked, but organic content increased from 3.0 ± 0.24 $\mu\text{g}/\text{animal}$ in control treatments to 3.62 ± 0.24 $\mu\text{g}/\text{animal}$ for sediment treatments (NS, $t = 1.8$, $p = 0.08$), suggesting potential compensatory feeding. Yet, as a proportion of total defecated materials, organic matter made up 63% of fecal content in controls, whereas organic matter contributed only 18% to feces of tadpoles from sediment treatments by weight.

2.4 DISCUSSION

Sediment levels applied in this study were moderate compared to reported conditions in a number of managed or disturbed landscapes, yet still resulted in a number of significant impacts on the aquatic community. Addition and deposition of sediment material significantly depressed periphyton standing stocks. Thus, hypothesis (1) that sediment inputs would reduce periphyton biomass was supported. However, nutrient additions had no effect on periphyton biomass, providing no support for hypothesis (2) that nutrient inputs might mitigate the negative

effects of sediment on primary productivity. Although tadpoles were frequently observed grazing on both periphyton and deposited sediment materials, sediment treatments reduced tadpole growth and survival rates, failing to support hypothesis (3) that sediment would act as a beneficial food resource to tadpoles. These negative impacts on both periphyton and tadpoles increased with the rate of sediment loading applied to tanks, indicating that the magnitude of the effect was dependent on the rate of sediment applied (hypothesis 4).

Contrary to expectations, ingestion of this moderately-rich organic sediment (8% - 9%) did not have a beneficial effect on tadpole growth and survival, but instead supports earlier findings of Ahlgren and Bowen (1991) and Kupferberg et al. (1994) where sediment did not prove to be a nutritional food resource. The moderately high fraction of organic matter in the sediment used in this study is likely due to the high productivity within riparian zones of the temperate rainforest of coastal British Columbia estimated at 1000-1300 g of C/m²/yr (Marczak and Richardson in press). Instead, tadpoles receiving sediment additions had lower growth and survival rates to metamorphosis. These results suggest a cost to foraging in and on these deposited sediments that exceeds any potential nutritional benefit. Possible mechanisms that may lead to an increased energetic burden in sedimented environments that were examined in this study include (i) fewer or poorer quality food resources available to tadpoles, (ii) alteration of behaviour under turbid conditions, or possibly (iii) increased energy expenditure associated with mucus secretion and gill clearing activities to remove congested sediment, or tissue abrasion. These possibilities are discussed below.

(i) Food Quantity and Quality

Patterns of sediment fall-out indicated that the majority of particles in suspension were deposited during the initial 48 h after addition, but that finer particles stayed in suspension over

the intervening week. This implies that although tadpoles may remove settled particles from grazing surfaces directly following additions, algae likely experience continuous shading by suspended particles. Measurements from tiles showed that sediment treatments depressed organic periphyton biomass. This decrease in absolute quantity of periphytic organic matter (algae, bacteria, protozoa etc.) with sediment load does indicate a general decline in the quantity of food resources available in the pond mesocosms. This drop in total quantity of algal resources available per tadpole with sediment additions likely led to greater competition and density-dependent effects. Competition and density are negatively correlated with daily growth and development rates in tadpoles for many amphibian species (Wilbur and Collins 1973, Hota and Dash 1981, Tejedo and Reques 1994, Skelly and Kiesecker 2001, Gillespie 2002) and can lead to fewer (Hota and Dash 1981), smaller (Collins 1979, Hota and Dash 1980) or later emerging metamorphs (Brockelman 1969, Gascon and Travis 1992). Generally, patterns in metamorph size followed those of tadpole growth with HIGH sediment treatments producing smaller (though only marginally) metamorphs. It is likely that the lack of statistical difference in metamorph size with sediment treatment is due to factors affecting mass loss during metamorphosis other than either prior growth rate or mass at initiation of metamorphosis, e.g. metamorphic development rate. Furthermore, wider variability in metamorph mass and length data than in tadpole measures may have made it more difficult to detect overall differences in size ($CV \approx 0.30$ for metamorph parameters versus $CV \approx 0.12$ for tadpole masses). Regardless, competition among tadpoles generated by resource limitation was likely a primary factor contributing to the decline in growth rate and survival of tadpoles seen in sediment treatments.

Deposition of sediment material may have also diluted the nutritional quality of the resources consumed (Gillespie 2002). Fecal matter of tadpoles from sediment treatments was four times greater and contained disproportionately larger quantities of inorganic matter than

controls, though similar levels of organic material. This suggests that tadpoles in highly sedimented environments must consume proportionally more low quality material to ingest sufficient nutritional content, thereby expending more time and energy in foraging. Higher total quantities of fecal material produced by tadpoles in sedimented environments may also suggest that fecal production does not keep pace with ingestion. Tadpoles ingesting sediment are not processing materials efficiently or are digesting them for longer. Although gut length showed no plasticity with respect to sediment or nutrient treatment, increased energy spent processing and extracting nutrients from this poor food resource could contribute to slower growth, but these costs were not directly investigated.

(ii) Behavioural Alterations

Behavioural changes in response to sediment inputs may lead to reductions in growth. Results from the behaviour trials showed that sediment altered behaviour, disrupting foraging under turbid conditions. Immediately following sediment addition, the proportion of tadpoles foraging and their foraging effort declined as they temporarily abandoned feeding activities. The disruption was short-term and normal activity levels were resumed within 24 h. Changes in feeding behaviour, if repeated or chronic (e.g. due to repeated run-off events, predators or human disturbance), may have long term consequences for individual growth and survival (Skelly and Werner 1990, Scrimgeour and Culp 1994, Sinclair and Arcese 1995). Toad tadpoles were observed to be voracious feeders and constant or repeated interruptions to feeding have the potential to slow growth substantially, affecting a tadpole's ability to reach the minimum size and metamorphose before deterioration of larval environment. Such disruptions may be particularly damaging in northern regions, where short summer seasons and ephemeral water bodies impose a limit on development time. In this study, although the tadpoles were not threatened with shortened pond hydro-period, changes in foraging behaviour from sediment

disturbance could have contributed to differences in growth patterns observed between sediment treatments. Again, though these differences in tadpole growth rate did not translate into significant differences in metamorphic size, trends in metamorph data followed tadpole growth patterns and support small differences in size of emerging individuals despite wide measured variability in mass and length.

Sediment addition also triggered fast and erratic swimming behaviour in tadpoles suggestive of a flight or avoidance response. In nature, such responses could expose tadpoles to increased predation pressure as they become more active and abandon refuges. There is also likely an energetic cost to such behavioural responses. In fish, alteration of foraging behaviour such as in an alarm reaction, abandonment of cover or an avoidance response to sediment additions are considered sub-lethal as they relate to feeding reduction, but can lead to increased rates of predation, and slower growth (Newcombe and MacDonald 1996). High levels of turbidity can alternatively provide camouflage and cover for aquatic prey, providing refuge from predators (Gregory 1993).

(iii) Sediment in Gills

Sediment may directly affect growth rates by imposing energetic burdens associated with gill clearing and respiration. Power (1984) demonstrated that the presence of sediment in fish gills can induce coughing, mucus secretion and gill clearing activities that can contribute 10-22% to the daily energetic burden of small, bottom-feeding armoured catfish. Similar energetic burdens may be imposed on developing tadpoles which ingest large quantities of sediment, in turn affecting growth and development rate. Gills are one of the primary locations for gas exchange in larval amphibians prior to the development of lungs (Burggren and West 1982, Boutilier et al. 1992). These fine tissues are delicate structures and directly exposed to

particles passing through the respiratory system. In fish, sediments can abrade delicate gill tissues and lead to increased respiratory stress (Randall and Daxboeck 1984). Decreased efficiency of these tissues in oxygen and carbon dioxide exchange or osmoregulation as a result of physical damage may have an adverse impact on overall respiration and growth of the animal. Visual examination of tadpole gill tissues from animals used in sediment treatments revealed the presence of sediment particles in strings of secreted mucus. The amount of sediment in gills was not quantified, but did not appear to vary with sediment loading rate. SEM images of gills revealed the presence of sediment particles, but there was no evidence of gill abrasion observed. Green et al. (2004) reported similar findings in the gills of Southern Barred frog and Giant Burrowing frog using SEM when exposed to 200 mg/L and 1655 mg/L of fine sediment.

Effects of Nutrients

Contrary to expectations and previously cited work, nutrient additions had little impact on Chl *a*, periphyton organic matter or tadpole functional responses. This lack of response was likely due to the relatively low loading rate applied to tanks compared to other studies and corroborated by the lack of detectable systematic differences in dissolved NO_3^- and PO_4^- concentrations between treatments. However, nutrients did play a significant role in their interactive effects with sediment on rates of tadpole survival to metamorphosis. In CONT and LOW sediment levels, the addition of NO_3^- and PO_4^- had a moderately negative impact on survival, but did not depress survival further in HIGH sediment treatments. The toxic effects of nutrient loading on tadpoles have been extensively studied in laboratory experiments, but only appear to affect tadpoles at levels much greater (>10 mg/L) than those applied here (reviewed in Camargo et al. 2005). Instead, without either a decrease in periphyton biomass or Chl *a* in CONT or LOW sediment treatments receiving nutrient additions, change in survival could be

explained by a nutrient-induced shift in the algal community (Cuker 1983) towards more inedible species, e.g. from nutritious epiphytic diatoms (Kupferberg et al. 1994) to less palatable filamentous green algae or cyanobacteria (Savage 1952, Waringer-Löschenkohl and Schagerl 2001) of which large quantities were observed in tanks at the end of the experiment. A shift in the algal community could have represented a decrease in food quality.

Metamorphs

Although western toads typically exhibit highly synchronous emergence, I documented wide variability in the timing to metamorphosis both across and within mesocosms. The wide variability in timing, length and mass at emergence within treatments suggests that multiple tadpole growth strategies may have been co-occurring within tanks. Depending on the larval environment, some tadpoles may initiate development immediately once they achieve the minimum size limit for metamorphosis, while others may postpone development and continue to accumulate biomass (Wilbur and Collins 1973). According to Wilbur and Collins (1973), initiation of metamorphosis likely depends on recent growth history. Fast-growing tadpoles may delay metamorphosis to capitalize on favourable aquatic conditions (i.e. competitive advantage or abundant resources), while tadpoles experiencing slower growth due to competitive disadvantage, resource limitation or predation may enter metamorphosis immediately upon reaching the minimum critical size in order to escape poor aquatic conditions. This wide variability in growth patterns may explain the positive relationship between metamorph condition and emergence time across treatments, where within tanks a few late emerging individuals were up to three-fold larger than their earlier emerging tank-mates. Despite strong differences in tadpole growth rates throughout the experiment, within-tank variability in metamorph length and mass led to non-significant differences in metamorphic size between treatments, though there was a general trend of decreasing size with sediment loading rate.

This depressive effect of sediment on periphyton biomass likely contributed to a slower growth rate of tadpoles via competition. As well, costs associated with foraging and consuming sediment apparently outweighed any nutritional benefits from ingesting this organic-rich material. Instead, sediment appeared to act as a nutritional sink for tadpoles. A ~10% decrease in metamorphic size of individuals was detected in metamorphs emerging from HIGH sediment treatments, and although this difference was not statistically significant, it may have biologically important consequences for terrestrial fitness. Smith (1987) in a study of natural recruitment among chorus frogs, *Pseudacris triseriata*, documented that metamorphs emerging 20% smaller and later than other conspecifics exhibited lower recruitment as they reached reproductive maturity one year later and original size differences persisted even two years after emergence. As well, Berven (1990) in his 7 year study of breeding wood frogs, *Rana sylvatica*, showed that metamorphs emerging later and only ~10% smaller (similar to size differences seen in this study) exhibited lower survival, later sexual maturity and smaller size at reproduction. More recent studies corroborate these findings that anuran metamorphs are unable to compensate for smaller size at emergence with high post-metamorphic growth (Altwegg and Reyer 2003).

Western toads typically exhibit size assortative mating and size at reproduction has been linked to a male advantage in mating success (Olson et al. 1986). Furthermore, in a number of anuran species, female size is related to clutch size (Howard 1978, Berven 1982, Woolbright 1983) and frequency of reproductive events (Howard 1978). At the moderate sediment dosing levels used in this study, the small decrease in metamorph size in western toads is likely to have negative implications for juvenile survival and future reproduction, while higher dosing levels common in nature have the potential for more marked responses.

Demographic modeling suggests that juvenile survival is the most critical life-stage for population maintenance in the western toad (Vonesh and de la Cruz 2002). Survival of smaller metamorphs may be further compromised when emerging in harsh terrestrial environments such as clear-cuts where their high surface area to volume ratio makes them more vulnerable to desiccation (Livo 1998). Small metamorph size may also lead to greater initial predation pressure as fewer predators are gape-limited during the initial month when metamorphs lack protective bufotoxins in their skin (Benard and Fordyce 2003). Thus, changes in the larval environment which have carry-over effects on later terrestrial survival may have more profound implications for population persistence than previously recognized.

2.5 CONCLUSIONS

The transfer of sediment and nutrient materials into aquatic systems is a vital natural process, but their loading rates can be altered by adjacent land-use practices. Previous studies in tropical stream systems have shown that natural sediment inputs can be important food resources for tadpoles (Flecker et al. 1999), but that elevated levels of inorganic materials can interfere with growth and development (Gillespie 2002). The relatively high organic sediment (8% - 9%) used in this study of temperate pond systems was perceived as a food resource by western toad tadpoles, though it was observed to impose graded costs for growth and survival with loading rate. This study suggests that the loading rate of sediment material into aquatic habitats is a key factor in determining the impact on recipient communities. Though tadpoles were able to cope and experienced few deleterious physiological effects of sediment at low loading rates (130 mg/L), higher loading rates of sediment (260 mg/L) caused slower growth, lower survival and moderately smaller metamorphs. In the long run, the greater impact of

sediment loading on pond-breeding species is likely through pond filling which may alter the hydroperiod and leave little suitable habitat for grazing or refuge.

Elevated levels of sediment loading are widespread across the Pacific Northwest and much of North America in relation to forestry and agricultural land-use practices. Turbidity levels produced in this experiment (~3, 21, 31 NTU) are reflective of typical levels experienced by aquatic life, but were much lower than what is experienced in streams or ponds after large storm events or near agricultural areas where turbidity levels can reach 60-200 NTU (Tucker and Burton 1999, Detenbeck et al. 2002). Thus, the results of this study likely under-estimate the effects of these larger sediment loads frequently observed in disturbed or managed landscapes.

Table 2.1 2 x 3 repeated measures ANOVA of conductivity and pH measured weekly in mesocosms over 8 weeks and temperature measured daily over 75 days. There was no significant effect of blocking.

Factor	DF	F-value	P-value
Conductivity			
Nutrients	1, 10	0.73	0.41
Sediment	2, 10	0.87	0.44
Nutrient x Sediment	2, 10	0.96	0.41
Week	9, 90	188.08	< 0.001*
Nutrient x Week	9, 90	0.38	0.84*
Sediment x Week	18, 90	1.69	0.12*
Nut x Sed x Week	18, 90	0.65	0.75*
Ph			
Nutrients	1, 10	0.50	0.50
Sediment	2, 10	0.55	0.59
Nutrient x Sediment	2, 10	0.93	0.43
Week	9, 90	40.58	< 0.001*
Nutrient x Week	9, 90	0.78	0.53*
Sediment x Week	18, 90	1.00	0.45*
Nut x Sed x Week	18, 90	0.76	0.62*
Temperature			
Nutrients	1, 12	0.23	0.64
Sediment	2, 12	0.33	0.72
Nutrients x Sediment	2, 12	1.17	0.34
Week	6, 72	246.10	< 0.001*
Nutrients x Week	6, 72	0.96	0.41*
Sediment x Week	12, 72	0.56	0.73*
Nut x Sed x Week	12, 72	0.54	0.75*

* failed to meet Mauchly's test of Sphericity and a Huynh-Feldt adjustment was applied

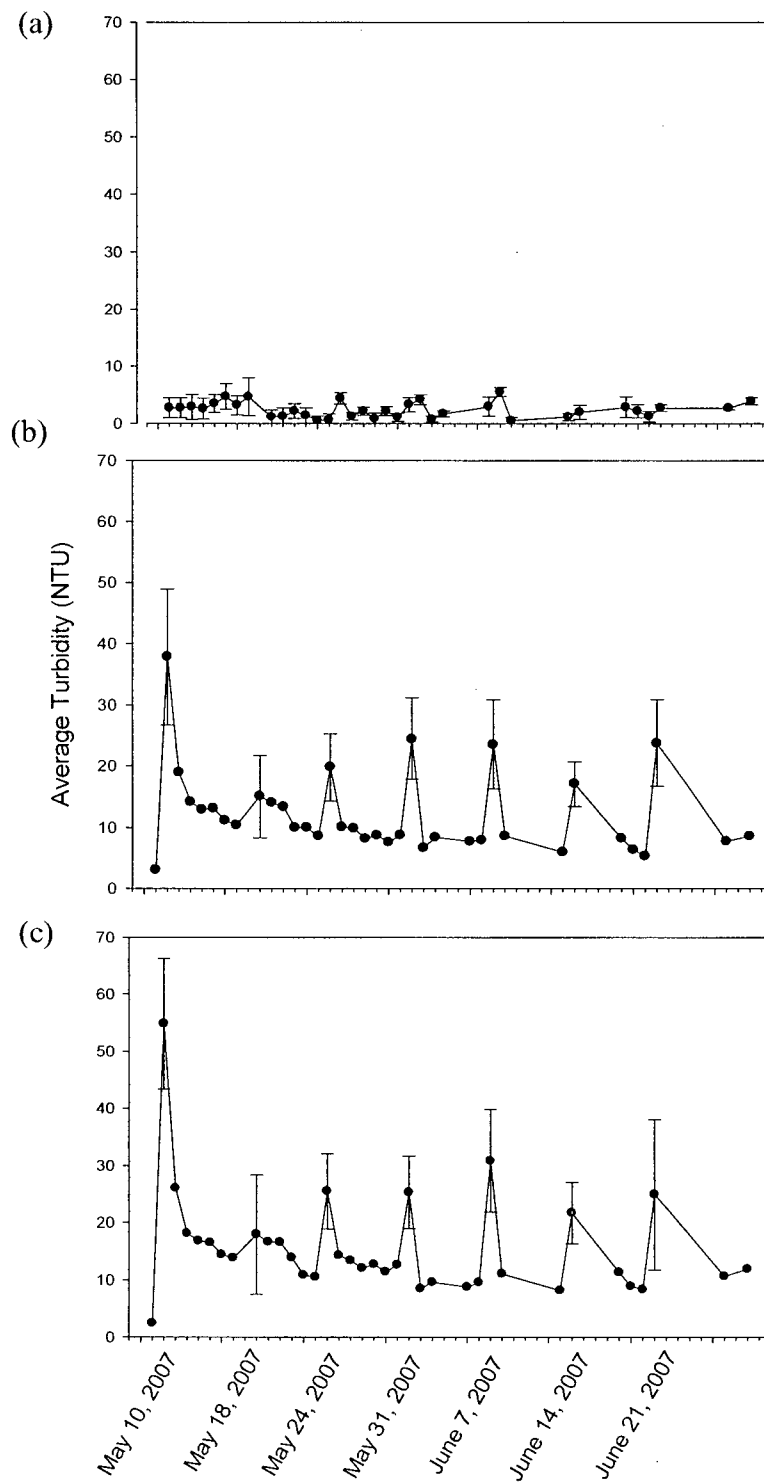


Figure 2.1 (a-c) Average turbidity measures from (a) CONT, (b) LOW, and (c) HIGH sediment treatments illustrating sediment fall-out patterns on a weekly basis. Points with Y-error bars (± 1 SD) are measures from sediment addition days.

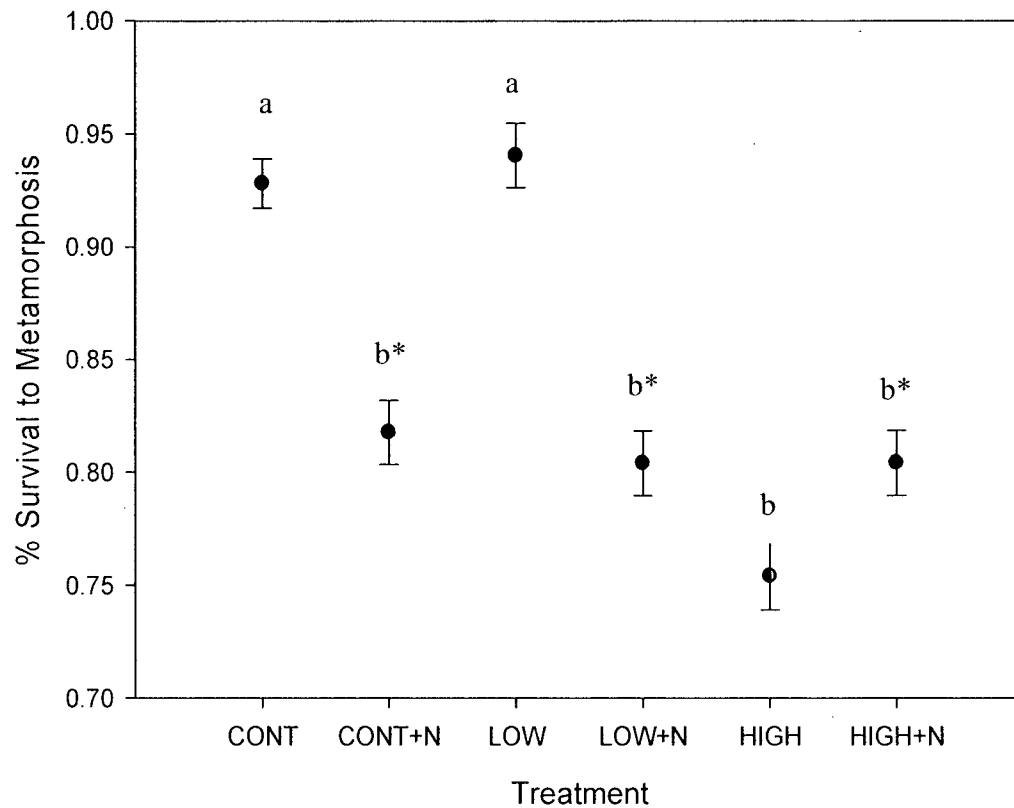
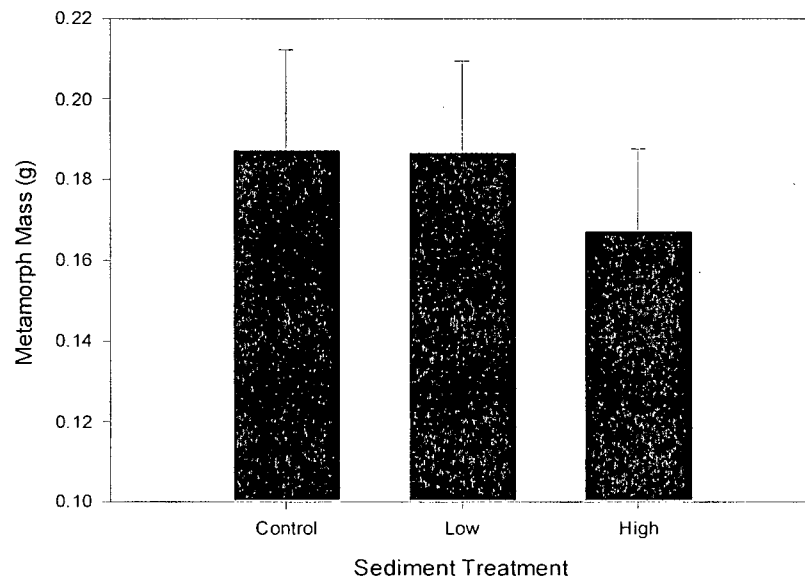


Figure 2.2 Back transformed least squared means for survival to metamorphosis by sediment-nutrient treatment ($n = 3$). Different letters represent significantly different means ($p < 0.05$), b^* indicates nearly significantly different means ($p = 0.053$ to 0.057). Survival in HIGH treatments was significantly lower than in CONT or LOW sediment treatments, while Low+N and High+N treatments were almost significantly lower than CONT and LOW sediment survival. Y-error bars represent SE.

(a)



(b)

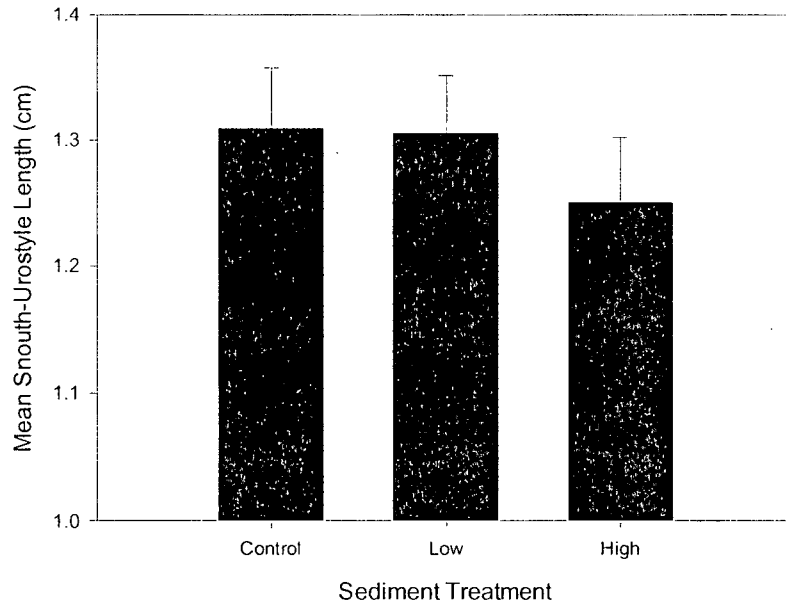


Figure 2.3 (a) Metamorph mass in grams, and (b) snout-urostyle length in cm of individuals by sediment treatment from the mixed model ANOVA controlling for tank ($n = 1306$). No significant difference was observed among treatments. Bars represent means ± 1 SE.

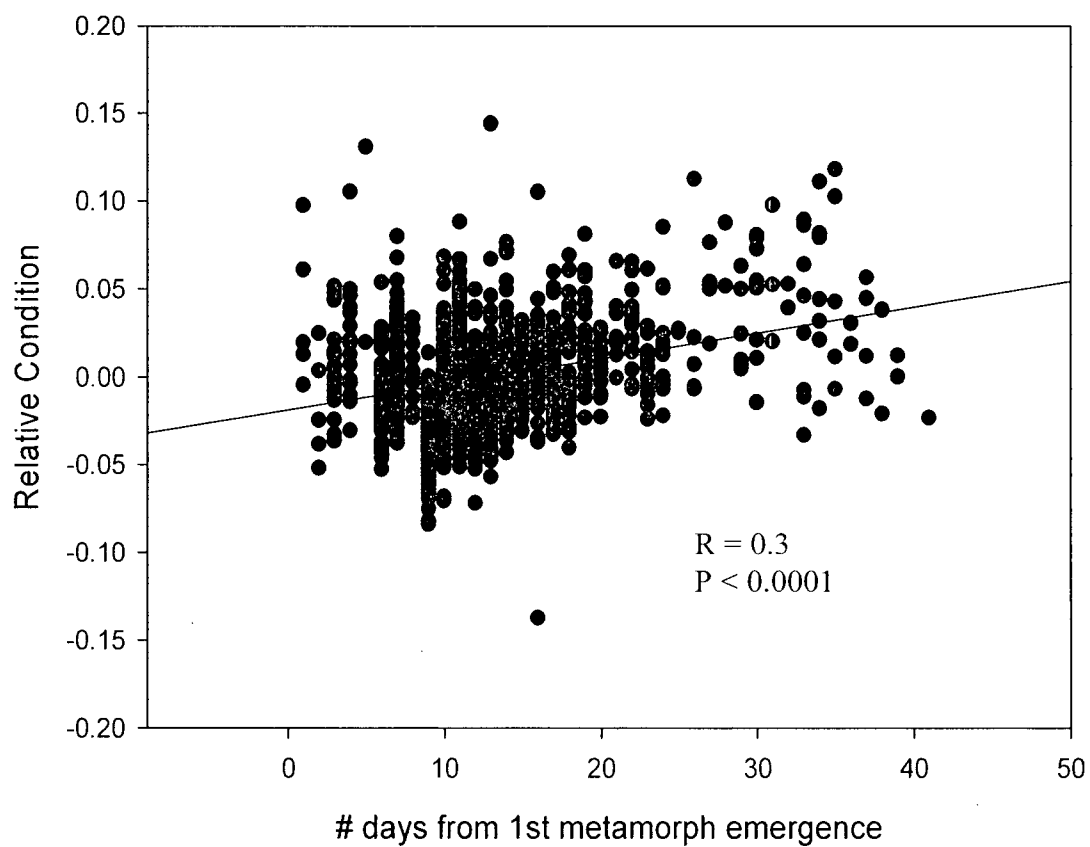


Figure 2.4 Linear regression of metamorph “condition” (i.e. residuals from the regression of mass over length) with the number of days since emergence of the first metamorph on June 17th, 2006.

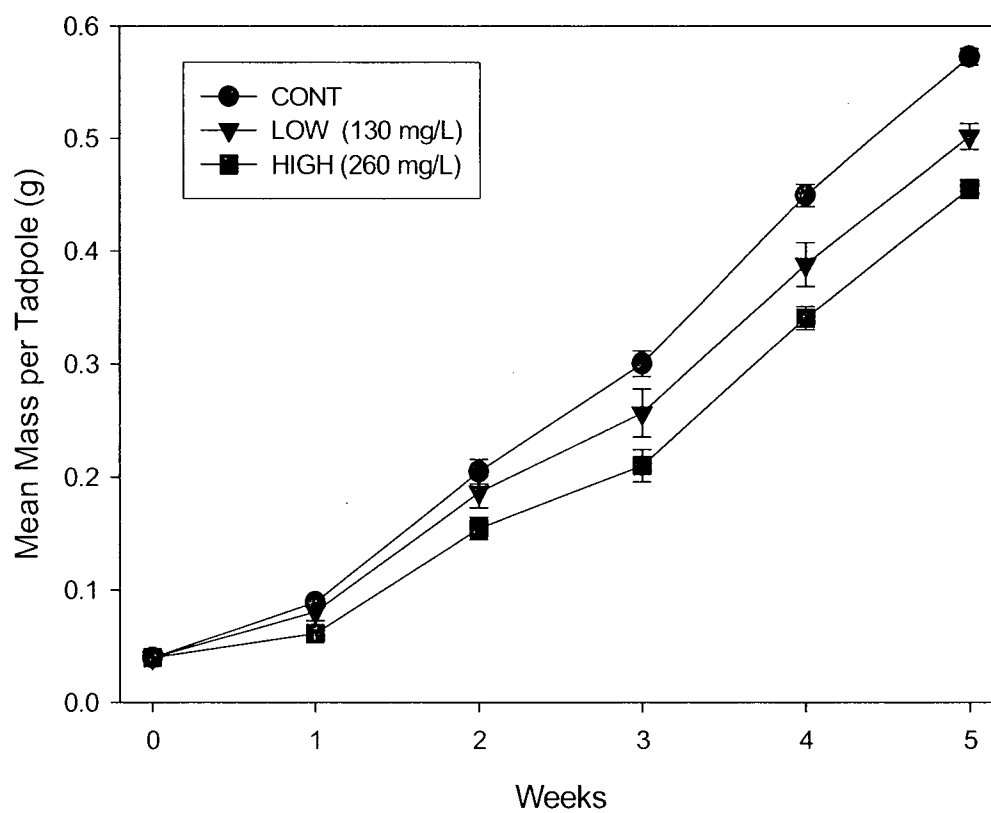


Figure 2.5 Mean mass of tadpoles ($n = 10/\text{tank}$) from CONT, LOW and HIGH sediment treatments ($n = 6$) in weeks 0 through 5 leading up to the initiation of metamorphosis. Symbols represent means ± 1 SE.

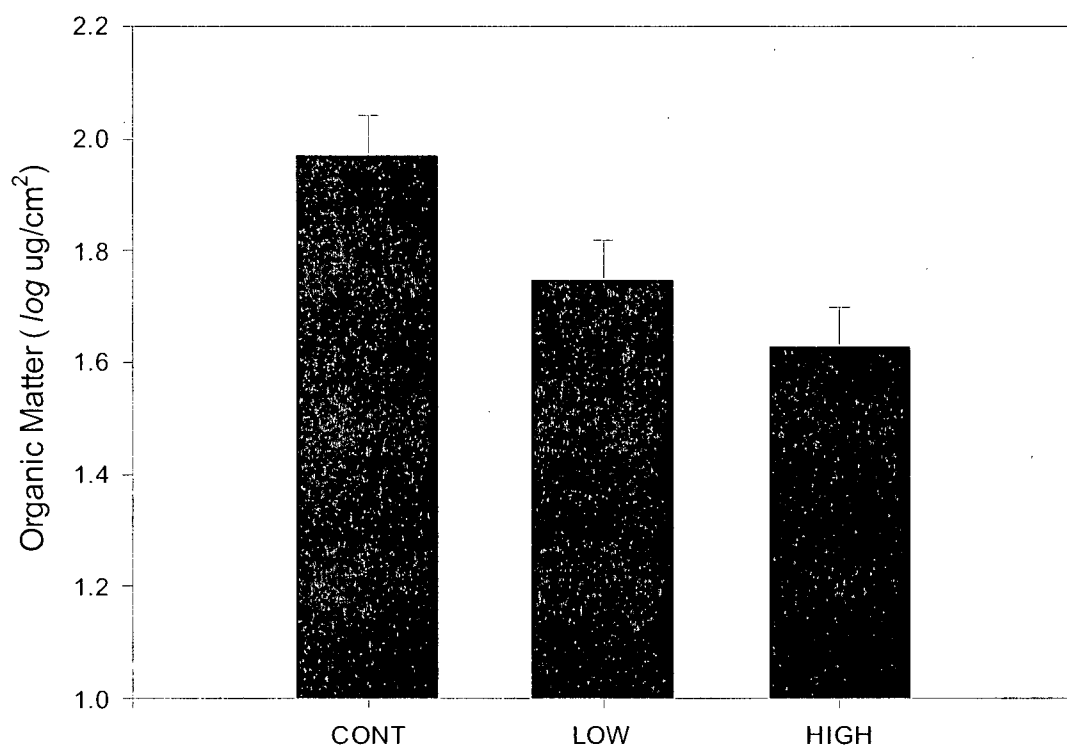


Figure 2.6 Comparison of organic periphyton matter on tiles from tadpole exclosures from CONT, LOW and HIGH sediment treatments. Bars represent least-squared means from repeated measures ANOVA $\pm$ 1 SE. Different letters represent significantly different means ($p < 0.05$).

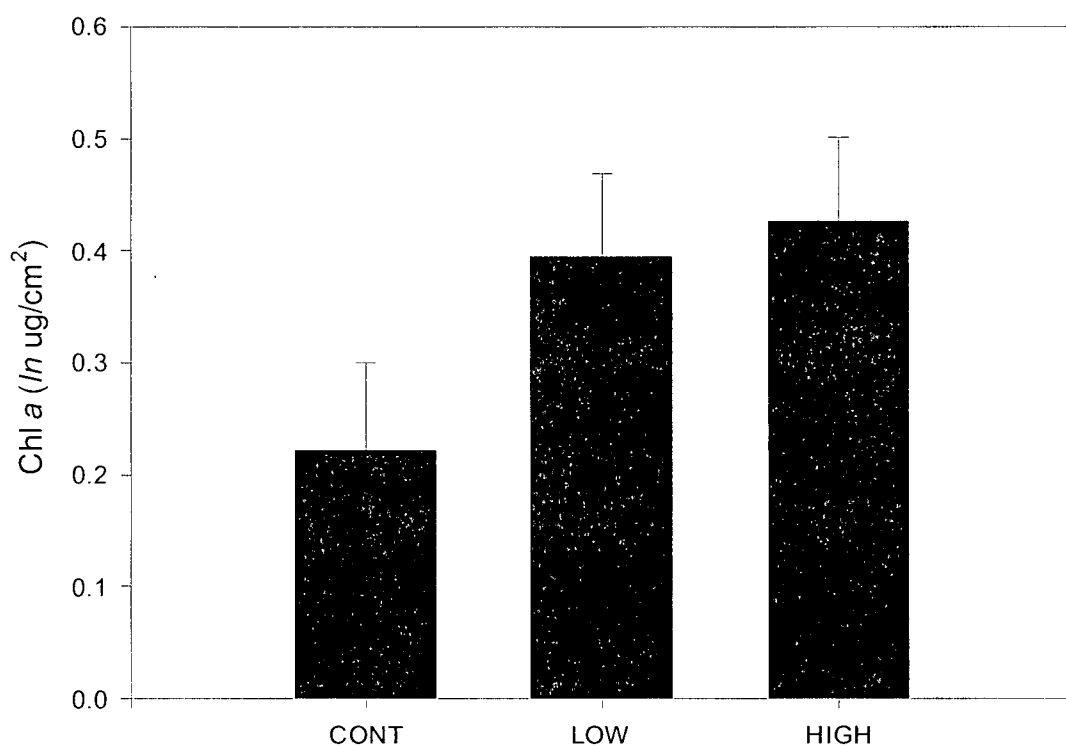


Figure 2.7 Comparison of uncorrected Chl *a* from periphyton on tiles from tadpole exclosures in CONT, LOW and HIGH sediment treatments. Bars represent least-squared means from repeated measure ANOVA ± 1 SE. Different letters represent significantly different means ($p < 0.05$).

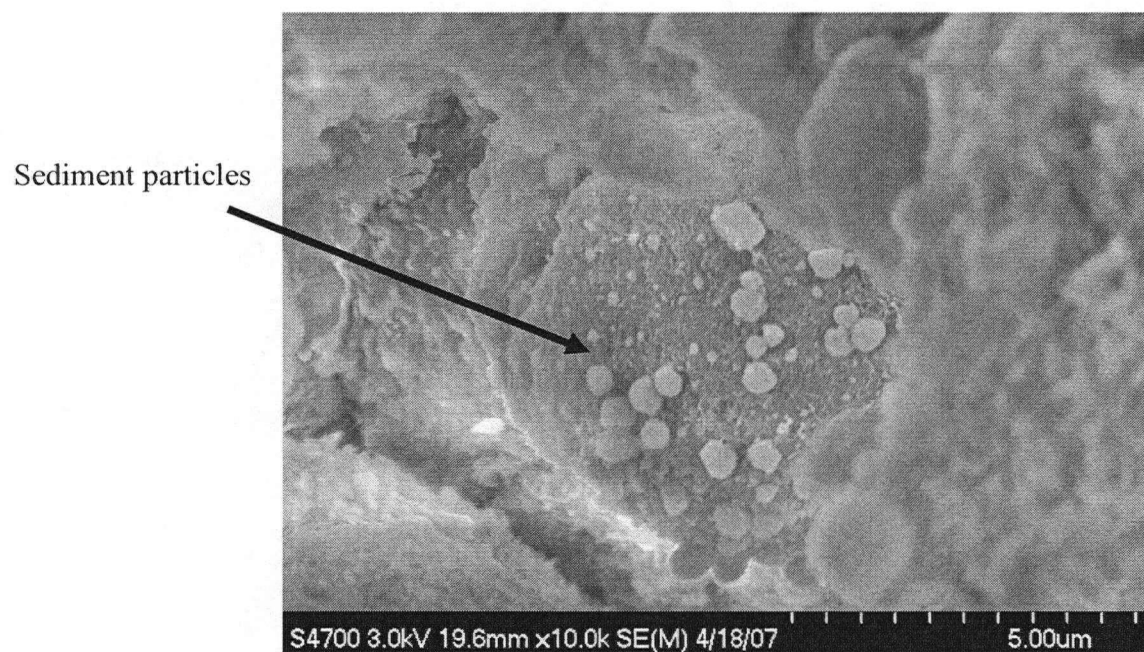
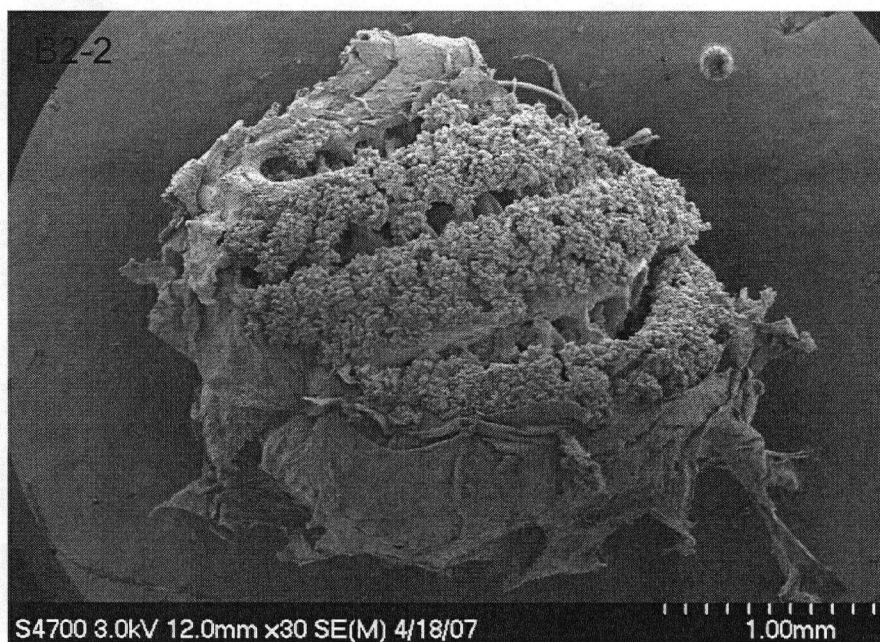


Figure 2.8 SEM of tadpole gill tissue from HIGH sediment treatment. Evidence of sediment particulate on tissue surface.

(a)



(b)

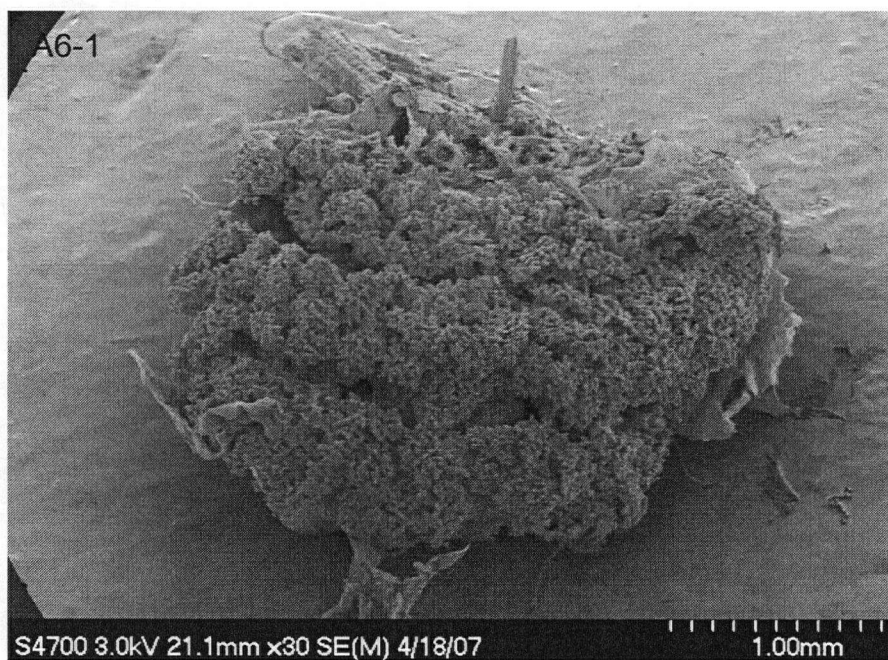


Figure 2.9 SEM images of a whole tadpole gill from (a) HIGH sediment and (b) CONT treatments.

(a)



(b)

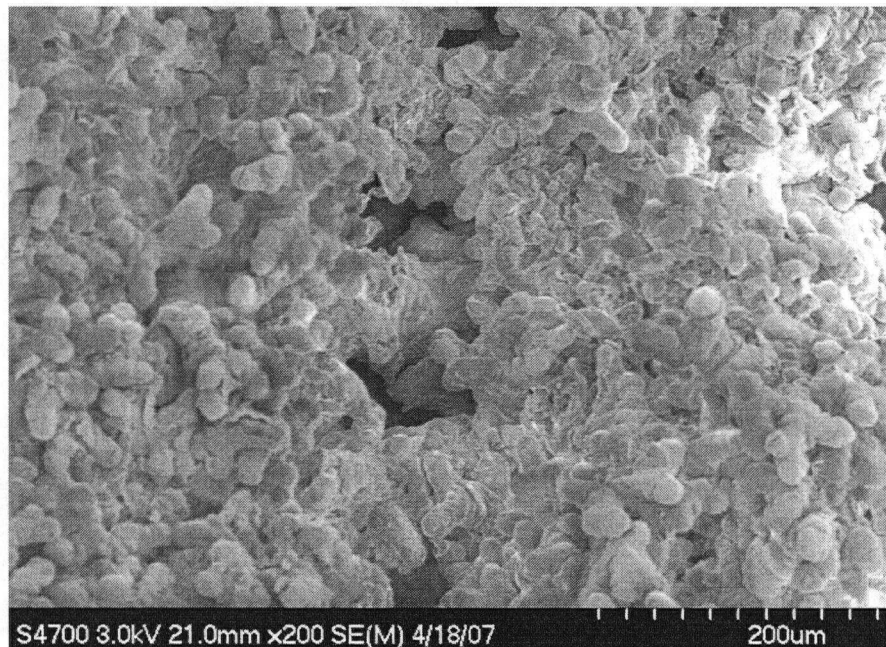


Figure 2.10 SEM of fine gill tuft structure of tadpole gill tissues from (a) HIGH sediment and (b) CONT treatments.

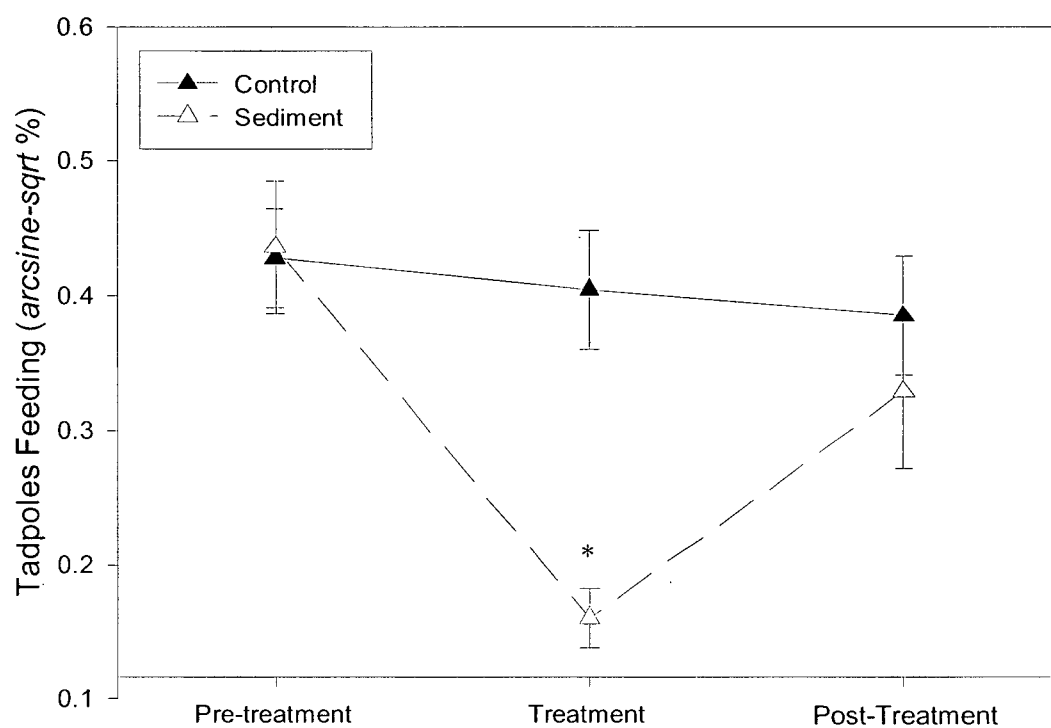


Figure 2.11 The proportion of tadpoles ($n = 5$) feeding at three time periods: pre-treatment (0 h), treatment (+20 min) and post-treatment (+24 h) under control and sediment treatments. Symbols are means ± 1 SE, $p < 0.05$.

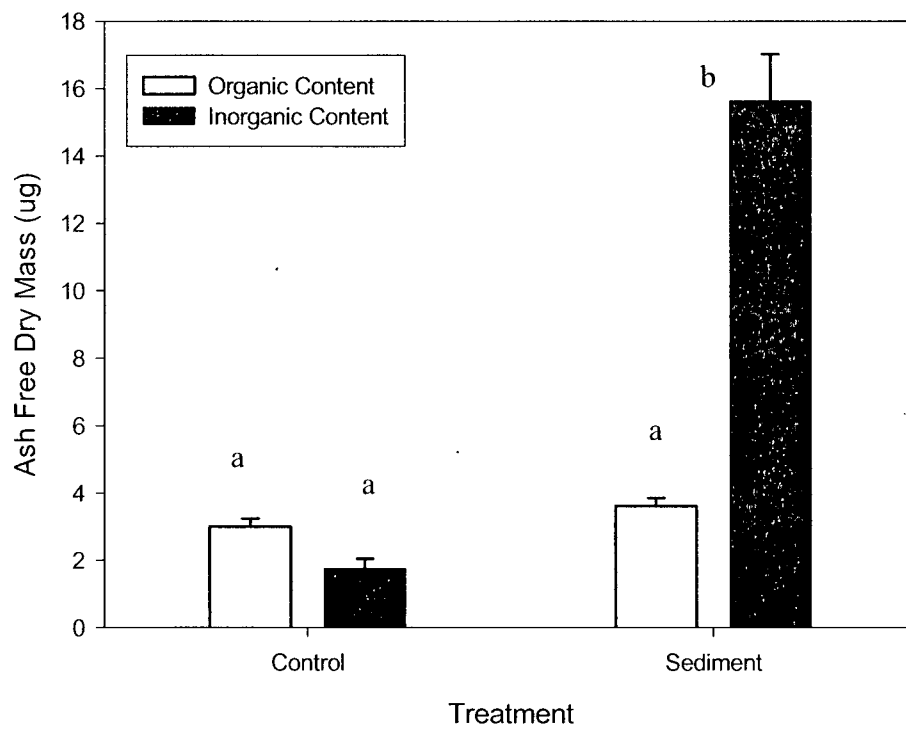


Figure 2.12 AFDM of organic (open bars) and inorganic (dark bars) content from fecal material of tadpoles following behavioural trials from Control ($n = 14$) and Sediment treatments ($n = 15$). Bars represent means ± 1 SE. Bars with different letters indicate statistically significant differences ($p = 0.01$).

2.6 REFERENCES

- Ahlgren, M.O. and S.H. Bowen. 1991. Growth and survival of tadpoles (*Bufo americanus*) fed amorphous detritus derived from natural waters. *Hydrobiologia* 218: 49-51.
- Alford, R.A. and S.J. Richards. 1999. Global Amphibian Declines: A Problem in Applied Ecology. *Annual Review of Ecology and Systematics* 30: 133-165.
- Altig, R. and G.F. Johnson. 1989. Guilds of Anuran Larvae: Relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs* 3: 81-109.
- Altwegg, R. and H-U. Reyer 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57: 872-882.
- Barnett, H.K. and J.S. Richardson. 2002. Predation risk and competition effects on the life-history characteristics of larval Oregon spotted frog and larval red-legged frog. *Oecologia* 132: 436-444.
- Benard, M.F. and J.A. Fordyce. 2003. Are induced defenses costly? Consequences of predator mediated defenses in western toads, *Bufo boreas*. *Ecology* 84: 68-78.
- Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71: 1599-1608.
- Berven, K.A. and D.E. Gill 1983. Interpreting geographic-variation in life-history traits. *American Zoologist* 23: 85-97.
- Boutilier, R.G., D.F. Stiffler and D.P. Toews, 1992. Exchange of respiratory gases, ions and water in amphibious and aquatic amphibians. Pp 81-124 *In* Environmental Physiology of the Amphibians edited by M.E. Feder and W.W. Burggren. The University of Chicago Press, Chicago.
- Brockelman, W.Y. 1969. An analysis of density effects and predation in *Bufo americanus* tadpoles. *Ecology* 50: 632-644.
- Burggren, W.W. and N.H. West. 1982. Changing respiratory importance of gills, lungs, and skin during metamorphosis in the bullfrog, *Rana catesbeiana*. *Respiratory Physiology* 47: 151-164.
- Camargo, J.A., A. Alonso and A. Salamanca. 2005. Nitrate toxicity to aquatic animals: A review with new data for freshwater invertebrates. *Chemosphere* 58: 1255-1267.
- Carrick, H.J. and R.L. Lowe. 1989. Benthic algal response to N and P enrichment along a pH gradient. *Hydrobiologia* 179: 119-127.
- Collins, J.P. 1979. Intrapopulational variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog, *Rana catesbeiana*. *Ecology* 60: 739-749.
- Corn, P.S. and R.B. Bury. 1989. Logging in western Oregon: responses of headwater habitats and stream amphibians. *Forest Ecology and Management* 29: 39-57.

- Correll, D.L., T.E. Jordan and D.E. Weller. 1992. Cross media inputs to eastern US watershed and their significance to estuarine water quality. *Water Science and Technology* 26: 2675-2683.
- Cuker, B.E. 1983. Suspended clays alter trophic interactions in the plankton. *Ecology* 74: 944-953.
- Dash, M.C., and A.K. Hota. 1980. Density effects on the survival, growth rate, and metamorphosis of *Rana tigrina* tadpoles. *Ecology* 61: 1025-1028.
- Davis, T.M. 2000. Ecology of the Western Toad (*Bufo boreas*) in forested areas on Vancouver Island. Final Report. Forest Renewal B.C. Ministry of Forests, Victoria, B.C.
- Detenbeck, N.E., C.M. Elonen, D.L. Taylor, A.M. Cotter, F.A. Puglisi and W.D. Sanville. 2002. Effects of agricultural activities and best management practices on water quality of seasonal prairie pothole wetlands. *Wetlands Ecology and Management* 10: 335-354.
- Dupuis, L.A. 1998. The effect of various silvicultural treatments on amphibian assemblages of the Robert's Creek watershed. Ministry of Forests, Vancouver Region, Interim Report.
- Environmental Protection Agency (EPA). 1994. The quality of our Nation's water: 1992. EPA 841-S-94-002. EPA Office of Water, Washington, D.C.
- Flecker A.S., B.P. Feifarek and B.W. Taylor. 1999. Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. *Copeia* 1999: 495-500.
- Gascon, C. and J. Travis. 1992. Does the spatial scale of the experiment matter? A test with tadpoles and dragonflies. *Ecology* 73: 2237-2243.
- Gillespie, G.R. 2002. Impacts of sediment loads, tadpole density, and food type on the growth and development of tadpoles of the spotted tree frog *Litoria spenceri*: an in-stream experiment. *Biological Conservation* 106: 141-150.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16: 183-190.
- Green, M., M.B. Thompson and F.L. Lemckert. 2004. The effects of suspended sediments on the tadpoles of two stream-breeding and forest dwelling frogs, *Mixophyes balbus* and *Heleioporus australiacus*. Pp 713-720 *In The Conservation of Australia's Forest Fauna* (second edition) 2004, edited by Daniel Lunney. Royal Zoological Society of New South Wales, Mosman, NSW, Australia.
- Gregory, R.S. 1993. Effect of turbidity on the predator avoidance-behaviour of juvenile Chinook salmon (*Oncorhynchus-Tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 50: 241-246.
- Gyug, L. 1996. Part IV Amphibians. *In Timber harvesting effects on riparian wildlife and vegetation in the Okanagan Highlands of British Columbia*. B.C. Environment, Penticton, B.C.

- Hassan, M.A., M. Church, T.E. Lisle, F. Brardinoni, L. Benda and G.E. Grant. 2005. Sediment transport and channel morphology of small, forested streams. *Journal of the American Water Resources Association* p. 853-876.
- Haycock, R.D. and D. Knopp. 1998. Amphibian surveys with special emphasis on the Oregon Spotted frog (*Rana pretiosa*). Selected wetland sites: Fraser River Lowlands and corridors to the interior Plateau. B.C. Ministry of Environment, Lands and Parks. Draft Report.
- Hecnar, S.J. and R.T. M'Closkey. 1998. Species richness patterns of amphibians in southwestern Ontario ponds. *Journal of Biogeography* 25: 763-772.
- Heyer W.R. 1973. Ecological interactions of frog larvae at a seasonal tropical location in Thailand. *Journal of Herpetology* 7: 337-361.
- Hota A.K. and M.C. Dash. 1981. Growth and metamorphosis of *Rana tigrina* larvae- effects of food level and larval density. *Oikos* 37: 349-352.
- Houlahan J.E., C.S. Findlay, B.R. Schmidt, A.H. Meyer and S.L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. *Nature* 404: 752-755.
- Huynh, H. and L.S. Feldt. 1976. Estimation of the Box correction for degrees of freedom from a sample data in a randomized block and split-plot designs. *Journal of Educational Statistics* 1: 69-82.
- Huynh, H., and L.S. Feldt. 1970. Conditions under which mean square ratios in repeated measurements designs have exact F-distributions. *Journal of the American Statistical Association* 65: 1582-1589.
- Ketcheson, G.L., W.F. Megahan and J.G. King. 1999. "R1-R4" and "BOISED" sediment prediction model tests using forest roads in granitics. *Journal of the American Water Resources Association* 35: 83-98.
- Kopp, K. and P.C. Eterovick. 2006. Factors influencing spatial and temporal structure of frog assemblages at ponds in southeastern Brazil. *Journal of Natural History* 40: 1813-1830
- Kupferberg, S.J., J.C. Marks and M.E. Power. 1994. Effects of variation in natural algal and detrital diets on larval anuran (*Hyla regilla*) life history traits. *Copeia* 1994: 446-457.
- Jenssen, T.A. 1967. Food habits of the Green Frog, *Rana clamitans*, before and during metamorphosis. *Copeia* 1967: 214-218.
- Livo, L.J. 1998. Investigations of boreal toad tadpole ecology. *In* Colorado Division of Wildlife Boreal Toad Research Progress Report 1995-1997. Colorado Division of Wildlife, Fort Collins, CO.
- Livo, L.J. and C.Loeffler. 2003. Report on the status and conservation of the Boreal Toad, *Bufo boreas boreas* in the Southern Rocky Mountains 2001-2002. Colorado Division of Wildlife, 6060 Broadway, Denver, CO 80216.

- Lowe, W.H., K.H. Nislow and D.T. Bolger. 2004. Stage-specific and interactive effect of sediment and trout on a headwater stream salamander. *Ecological Applications* 14: 164–172.
- Luo, H.R., L.M. Smith, B.L. Allen, and D. Haukos. 1997. Effects of sedimentation on playa wetland volume. *Ecological Applications* 7: 247-252.
- Luttenton, M.R. and R.L. Lowe. 2006. Response of a lentic periphyton community to nutrient enrichment at low N:P ratios. *Journal of Phycology* 42: 1007–1015.
- Mallory, M.A. and J.S. Richardson. 2005. Complex interactions of light, nutrients and consumer density in a stream periphyton-grazer (tailed frog tadpoles) system. *Journal of Animal Ecology* 74: 1020-1028.
- Martin, D.B. and W.A. Hartman 1987. The effect of cultivation on sediment composition and deposition in prairie potholes. *Water, Air, and Soil Pollution* 34: 45–53.
- Marczak, L.B. and J.S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. *Journal of Animal Ecology* in press.
- Mattson Jr., W.J. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* 11: 119-161.
- Mauchly, J.W. 1940. Significance test for sphericity of a normal n-variate distribution. *Annals of Mathematical Statistics* 11: 204–209.
- Ministry of Forests and B.C. Environment. 1995. Riparian Management Area Guidebook. Forest Practices Code of British Columbia. Ministry of Forests and B.C. Environment, Victoria, B.C.
- Nakano, S., H. Miyasaka and N. Kuhara. 1999. Terrestrial-aquatic linkages: Riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology* 80: 2435-2441.
- Newcombe, C.P. and D.D. MacDonald. 1991. Effects of suspended sediments on aquatic ecosystems. *North American Journal of Fisheries Management* 11: 72-82.
- Newcombe, C.P. and J.O.T. Jensen. 1996. Channel suspended sediment and fisheries: a synthesis for quantitative assessment of risk. *North American Journal of Fisheries and Management* 16: 693-727.
- Orton, G. L. 1953. The systematics of vertebrate larvae. *Systematic Zoology* 2: 63-75.
- Petersen, J.E. and A. Hastings. 2001. Dimensional approaches to scaling experimental ecosystems: designing mousetraps to catch elephants. *American Naturalist* 157: 2498-2508.
- Polis, G.A and D.R. Strong 1996. Food web complexity and community dynamics. *The American Naturalist* 147: 813-846.

- Power M.E. 1984. The importance of sediment in the grazing ecology and size class interactions of an armored catfish, *Ancistrus spinosus*. *Environmental Biology of Fishes* 10: 173–181.
- Power, M.E. 1990. Resource enhancement by indirect effects of grazers: armoured catfish algae and sediment. *Ecology* 71: 897-904.
- Randall, D and C. Daxboeck. 1984. Oxygen and carbon-dioxide transfers across fish gills. *Fish Physiology* 10: 263-314.
- Ranvestel, A.W., K.R. Lips, C.M. Pringle, M.R. Whiles and R.J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology* 49: 274–285.
- Reid, L.M. and T. Dunne. 1984. Sediment production from forest road surfaces. *Water Resources Research* 20: 1753-1761.
- Relyea, R.A. and J.T. Hoverman. 2003. The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. *Oecologia* 134: 596–604.
- Reuss, J.O., R. Stottlemeyer and C.A. Troendle. 1997. Effect of clear cutting on nutrient fluxes in a subalpine forest at Fraser, Colorado. *Hydrology and Earth System Sciences* 1: 333-344.
- Ryan, P.A. 1991. Environmental-effects of sediment on New Zealand streams- a review. *New Zealand Journal of Marine and Freshwater Research* 25: 207-221.
- Savage, R.M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. *Proceedings of the Zoological Society of London* 122: 467-514.
- Schiesari, L. 2006. Pond canopy cover: a resource gradient for anuran larvae. *Freshwater Biology* 51: 412–423.
- Schulte-Hostedde, A.I., B. Zinner, J.S. Millar and G.J. Hickling. 2005. Restitution of mass-size residuals: validating body conditions indices. *Ecology* 86: 155-163.
- Scrimgeour, G.J. and J.M. Culp. 1994. Feeding while evading predators by an lotic mayfly: linking short-term foraging behaviours to long-term fitness consequences. *Oecologia* 100: 128-134.
- Shaw, E.A. and J.S. Richardson. 2001. Direct and indirect effects of sediment pulse duration on stream invertebrate assemblages and rainbow trout (*Oncorhynchus mykiss*) growth and survival. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2213–2221.
- Sinclair, A.R.E. and P. Arcese. 1995. Population consequences of predation-sensitive foraging - the Serengeti wildebeest. *Ecology* 76: 882-891.
- Skelly, D.K. and E.E. Werner. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology* 71: 2313-2322.

- Skelly, D.K. and J.M. Kiesecker. 2001. Venue and outcome in ecological experiments: manipulations of larval anurans. *Oikos* 94: 198-208.
- Smith, D.C. 1987. Adult recruitment in chorus frogs- effects of size and date at metamorphosis. *Ecology* 68: 344-350.
- Solomon, C.T., A.S. Flecker, and B.W. Taylor. 2004. Testing the role of sediment-mediated interactions between tadpoles and armored catfish in a neotropical stream. *Copeia*, 2004: 610–616.
- Stebbins, R.C. 1985. Western Reptiles and Amphibians. 2nd Ed. The Peterson Field Guide Series National Audubon Society and National Wildlife Federation. Houghton Mifflin Co., Boston.
- Swift Jr., L.W. 1988. Forest access roads: design, maintenance, and soil loss. *In*: Swank, W.T., Crossley, Jr., D.A. (Eds.), *Forest Hydrology and Ecology at Coweeta*, Springer-Verlag, New York, pp. 313–324.
- Tejedo, M. and R. Reques 1994. Plasticity in metamorphic traits of Natterjack tadpoles: The interactive effects of density and pond duration. *Oikos* 71: 295-304.
- Tucker, K.A. and G.A. Burton Jr. 1999. Assessment of nonpoint –source runoff in a stream using in situ and laboratory approaches. *Environmental Toxicology and Chemistry* 18: 2797–2803.
- Vonesh, J.R. and O. de la Cruz. 2002. Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. *Oecologia* 133: 325–333.
- Wake, D.B. 1991. Declining amphibian populations. *Science* 253:860.
- Wallace, J.B., S.L. Eggert, J.L. Meyer and J.R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 277: 102-104.
- Waringer-Löschenkohl, A. and M. Schagerl. 2001. Algal exploitation by tadpoles – an experimental approach. *International Review of Hydrobiology* 86: 105–125
- Waters, T.F. 1995. Sediment in streams. Sources, biological effects, and control. American Fisheries Society Monograph 7, Bethesda, Maryland, USA.
- Welch, B.L. 1938. The significance of differences between two means when the population variances are unequal. *Biometrika* 29: 35-362.
- Welsh Jr., H.H. and L.M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's Redwoods. *Ecological Applications* 8: 118-1132.
- Wemple, B.C., J.A. Jones and G.E. Grant. 1996. Channel network extension by logging roads in two basins, Western Cascades, Oregon. *Water Resources Research Bulletin* 32: 1195–1207.

Wilbur, H.M. and J.P. Collins. 1973. Ecological aspects of amphibian metamorphosis. *Science* 182: 1305-1314.

Tadpole-Sediment Dynamics in a Lentic Habitat: Evidence for Ecosystem Engineering by Tadpoles of the Western Toad, *Bufo boreas*²

3.1 INTRODUCTION

Transfers of material across habitat boundaries represent critical linkages in food webs (Polis et al. 1997), and knowledge of their effects in recipient habitats is fundamental to a holistic understanding of ecosystem ecology. The movement of terrestrially-derived matter into aquatic habitats is one of the most obvious and well studied material fluxes. Sediment inputs, once considered a disturbance that negatively affected aquatic biota (Newcombe and MacDonald 1991, Waters 1995), are being increasingly recognized as important elements in sediment-biota interactions and are critical to the structuring and function of freshwater ecosystems (Lamberti et al. 1992, Kupferberg 1997, Flecker et al. 1999, Ranvestel et al. 2004).

Through ingestion and bioturbation of fine particulate matter, benthic organisms restructure the physical environment of the stream benthos. Removal of sediment by so-called “benthic bulldozers” such as shrimp (Pringle et al. 1993), crayfish (Creed and Reed, 2004), mayflies (Moulton et al. 2004), armoured catfish (Power 1990) and tadpoles (Flecker et al. 1999) can facilitate the growth of underlying periphyton (Power 1990). This increase in algal biomass or change in species composition of periphyton as a consequence of sediment removal can have cascading effects on grazer communities (Ranvestel et al. 2004). In such circumstances, the broad, multi-trophic level effects of clearing sediment from important

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periphyton growing surfaces has resulted in these bulldozer species being termed “ecosystem engineers” (Jones et al. 1994, Flecker et al. 1999, Ranvestel et al. 2004).

Amphibian tadpoles can be dominant consumers in freshwater systems with the ability to influence periphyton standing stocks (Dickman 1968), algal community composition (Kupferberg 1997), as well as organic and inorganic sediment dynamics of the stream benthos (Flecker et al. 1999, Ranvestel et al. 2004). Although an ephemeral member of the aquatic community, amphibian larvae can make up a significant portion of the vertebrate biomass in stream and pond environments (Turnipseed and Altig 1975, Cecil and Just 1979). Recent attention has been focused on the role of tadpoles in the regulation of sediment deposition in tropical lotic systems (Flecker et al. 1999, Solomon et al. 2004, Ranvestel et al. 2004). In tropical streams, tadpoles have proven to be important consumers of deposited sediments, ingesting organic-rich sediments (9% organic and above) and exhibiting density-dependent growth and development in response to sediment availability (Flecker et al. 1999). As a consequence of this consumption, tadpoles continuously remove accumulated sediment from grazing surfaces and expose underlying periphyton. In one study, these activities were associated with an increased density of baetid mayflies on the freshly uncovered periphyton surfaces (Ranvestel et al. 2004). Although the engineering effects of tadpoles have so far only been documented in tropical systems, Flecker et al. (1999) suggest that such effects are likely to occur across a broad range of latitudinal gradients and habitats wherever tadpoles are sufficiently abundant.

To date, studies of the sediment-biota dynamics with tadpole grazer communities have focused on lotic systems and species, with little attention paid to sediment-tadpole interactions in lentic systems. The effects of habitat structuring through sediment removal by benthic

feeding species may be even more important in pond habitats, but may be difficult to accomplish as sediment deposits accrue on the benthos rather than being flushed out by strong currents (Martin and Hartman 1987, Luo et al. 1997). Continuous or repeated inputs of sediments over the long-term can smother the benthic environment affecting a broad range of detritivorous and herbivorous species.

Based on evidence of tadpoles acting as engineers via sediment removal in other systems (i.e. tropical streams), I tested this idea in a temperate, pond system with moderately organic-rich sediments. I conducted a tadpole exclosure manipulation within mesocosm ponds to investigate the impact of tadpole foraging on periphyton standing stock and sediment accrual dynamics. Different amounts of sediment were applied to test whether those effects were dependent on the loading rate of sediment in the system. The exclosure manipulation acted to restrict tadpole grazing on a portion of surfaces which then acted as references for sediment accumulation. Based on existing work, I made two hypotheses about tadpole-sediment dynamics; (1) that sediment accrual on grazing surfaces would be reduced as a consequence of tadpole grazing activities (i.e. either intended or accidental ingestion or bioturbation), and (2) that these so-called “ecosystem engineering” effects would have positive effects on periphyton biomass and chlorophyll *a* content.

3.2 METHODS

Study Organism

The western toad, *Bufo boreas*, is a lake and pond-breeding species commonly associated with both forest-dominated and disturbed habitats. During breeding, females lay

strings of up to 12 000 -16 500 eggs (Samolloy 1980, Blaustein 1988). The resulting tadpoles are primarily benthic feeders which graze on diatoms, filamentous algae and settled detritus (Wind and Dupuis 2002). As a result of this explosive breeding, toad tadpoles can make up a large portion of the vertebrate biomass in ponds and can act as important primary consumers (Dickman 1968, Seale 1980, Loman 2001). Toad tadpoles are also highly gregarious, swimming and feeding in large schools ranging from the hundreds to millions of tadpoles (Wassersug 1973). These aggregations are thought to promote growth, as well as greater feeding efficiency via disruptive effects of bioturbation on settled particulate matter (Beiswenger 1975, Katz et al. 1981). Adult western toads are known to breed in ponds within and near clear-cuts (Gyug 1996), likely exposing tadpoles to high rates of sediment inputs associated with timber harvesting activities in the adjacent harvested areas (Swift 1988, Ketcheson et al. 1999).

Specimen Collection and Husbandry

Western toad eggs (~3000) were collected on April 26, 2006 from two communal breeding sites, Edith Lake and Fawn Lake (< 1 km apart), each with ~20 pairs in amplexus, within Alice Lake Provincial Park (N49 46.617, W123 06.476) 13 km north of Squamish, B.C. Eggs were transported back to facilities at the University of British Columbia in plastic containers filled with natal pond water and placed in a cooler. Eggs were allowed to hatch in 40 L aerated aquaria filled with aged tap water (minimum 3 days) containing a 400 ml inoculation of natal pond water. After hatching, tadpoles were reared in aquaria filled with water from experimental ponds (pH 8.3, conductivity 198 μ S/cm) on UBC South Campus filtered through a 64 μ m screen. Tadpoles experienced consistent rearing conditions. Water was changed every 3-7 days depending on clarity by removing and replacing three-quarters of the volume. Tadpoles were raised under natural light conditions at approximately 18-23 °C.

Mesocosms

The experiment was conducted in 18 pond mesocosms arranged in 3 spatial blocks of 6 tanks each. Cattle tanks (1136 L Rubbermaid brand) were used to create mesocosms. Tanks were 1.4 m in diameter and 0.5 m deep, and were filled and conditioned two months prior to the beginning of the experiment (for details see Chapter 2). Six treatments were applied to the mesocosms in a factorial, randomized block design. Each block of six tanks received three levels of fine sediment crossed by either ambient or augmented nutrient levels. The experiment ran over 8 weeks, from May 9th to July 4th. Each week tanks received pulses of sediment to produce initial suspended sediment concentrations of 0 mg/L (Control), 130 mg/L (Low), or 260 mg/L (High) and half the tanks received nutrient additions of NaNO₃-N 160 µg/L and KH₂PO₄-P 10 µg/L on a bi-weekly basis. Sediment was collected from the stream bank of Kanaka Creek in Maple Ridge, B.C. and was used to represent inputs of terrestrial-derived sediment from the coastal temperate rainforest of B.C. Wet sediment was sifted through a 64 µm screen to produce a mixture of fine clay and silt. The sediment contained 8% - 9% organic material as determined by ash-free dry mass (AFDM).

Tadpole Exclusion Experiment

Small-scale tile exclosures within the larger mesocosm experiment were used to measure the impact of tadpole grazing on both periphyton biomass and sediment accumulation on substrate surfaces. On March 24th, six weeks before the start of the experiment, 24 unglazed ceramic tiles (7.5 x 7.5 cm) were placed in each tank to act as a substrate for the establishment of a periphyton biofilm. These tiles provided a means of measuring food resources available to tadpoles, sediment deposition and the effects of tadpoles on both. Tiles were suspended on planter's trays strung approximately 5-10 cm below the water's surface to allow for grazing (personal observation identified this as the level at which most tadpole grazing took place). Of

the 24 tiles, 8 tiles were left open to tadpole grazing. An additional 8 tiles were placed in tadpole the exclusion cages constructed from an aluminium pie plate lined with dark plastic to reduce reflectance, fitted with screened openings along the sides for water circulation and sealed with a removable wire mesh screening on the top. A further 8 tiles were placed in “false” exclusion cages in which tadpoles had access for grazing (i.e. side openings were not screened) to control for the caging effect on periphyton growth. During the experiment, tadpoles were frequently seen grazing on open tiles and entering “false” cages for feeding. In a few instances tadpoles were found in exclusion cages and were immediately removed. Potentially affected tiles were not sampled that week and these tadpoles were assumed to have had little overall effect on periphyton standing stock.

One tile from each caging treatment (i.e. open, caged and false caged) was removed from tanks on a weekly basis to sample for periphyton and sediment accumulation. As tiles were removed an inverted plastic yogurt container (diameter = 7.2 cm) was placed over top of tiles to trap deposited sediment. Exposed corners of tiles were rinsed off with distilled water leaving behind a clear circle of sediment (area = 40.8 cm²). This remaining sediment was then rinsed into a Petri-dish with distilled water and later split into 2 samples for AFDM and chlorophyll *a* (Chl *a*) analysis. The remaining periphyton on tiles was then scraped off with a razor and toothbrush. The resultant slurry was rinsed into a Petri-dish with distilled water and also split into 2 samples for later AFDM and Chl *a* analysis. Periphyton, a matrix of algae, fungi and bacteria was measured as an index of total food availability for tadpoles, while Chl *a* was used to estimate algal biomass specifically.

In the lab, samples for AFDM were filtered onto pre-ashed Whatman G/F filters, dried for 24 h at 60°C and ashed for 2 h at 550°C. Chl *a* from both sediment and periphyton samples

were filtered onto un-ashed Whatman filters and frozen for later analysis at which time Chl *a* was extracted from filters in 90% acetone, refrigerated for 24 h, centrifuged for 5 min and measured on a TD-700 Fluorometer (Turner Designs, Sunnyvale, CA) (Standards 18.6-186 µg/L, Turner Designs, Sunnyvale, CA). Uncorrected Chl *a*, i.e. including phaeophytin, was measured on sediment as well as periphyton samples and the two values added together because algae was observed to be growing between the sediment particles.

3.2.1 STATISTICAL ANALYSES

Periphyton organic matter and inorganic sediment matter from AFDM, as well as Chl *a* were analyzed in a split-split plot design using PROC MIXED in SAS using the Kenward-Roger approximation to determine the appropriate degrees of freedom (Littell et al. 2002). In the model, sediment-nutrient addition combinations were assigned as the whole factor treatments applied to tanks, the tile caging treatments within the tank as the sub-plot, and week sampled as the sub-sub-plot. This design was justified based on critique of repeated measures analysis by Meredith and Stehman (1991). As all parameters were measured on tiles randomly selected from caging treatments over time in a destructive manner, the factor “time” could be randomized to tiles within the sub-plot and the assumption of compound symmetry validated (Potvin et al. 1990). Mixed models were preferred due to the presence of random factors and as the data were unbalanced with occasional data points missing.

Data for organic matter from periphyton were log-transformed, inorganic matter from sediment were square-root transformed, and Chl *a* were ln-transformed to meet assumptions of normality. Dissolved nutrient levels were compared between treatments to detect the effect of nutrient additions in 2 x 3 ANOVAs with blocking. When variances were unequal, a Welch’s

approximate degrees of freedom ANOVA (Welch 1938) adjustment was applied to ensure the accuracy of statistical estimates. In mixed models, blocks were tested for significance by dropping them from the random term and comparing the difference in -2 residual log likelihood of the reduced model to the full model against an χ^2 distribution. All analyses were conducted in SAS (SAS ver. 9.1, SAS Inc, Cary, NC) and reported results are means $\pm$ SE (n).

3.3 RESULTS

Periphyton and Sediment

In statistical analyses, periphyton samples were analyzed for organic matter and sediment samples analyzed for inorganic matter AFDM. Organic matter of biofilm growing on tiles was taken as a measure of the standing stock of periphyton, a food resource for tadpoles. Split-split-plot analyses revealed no significant differences in the amount of organic matter on open tiles and those in “false” cages (adequately controlling for the caging effect), therefore these were pooled and the analysis re-run comparing grazed and ungrazed tiles. Both grazing level and sampling week showed differences in organic matter accrual (Table 2.1), but the interaction between them was also highly significant ($F_{14,406} = 3.59$, $p = 0.001$). Overall, the amount of periphytic organic matter growing on ungrazed tiles in enclosure cages was significantly greater than on tiles open to grazing, and this difference increased over the course of the experiment (2.9 times more at the last sampling point) as organic matter accumulated on ungrazed tiles, but remained constantly at low levels on grazed tiles (Figure 2.1 (a-c)). In control treatments receiving no sediment, tadpoles had a large impact on standing stock of organic matter reducing periphyton on accessible tiles by ~80% compared to that available on adjacent caged tiles within the mesocosm. There was also a negative impact of sediment on periphyton growth ($F_{2,23.8} = 6.84$, $p = 0.045$). A Tukey-Kramer *post hoc* test indicated that high sediment

treatments had significantly less periphyton organic matter than controls; this was especially true for caged tiles lacking grazing pressure.

Several factors governed the accrual of inorganic sediments on the tiles and interactions among factors were observed. The split-split plot analysis of sediment inorganic AFDM revealed a three-way interaction between the sediment-nutrient addition, tile enclosure treatment and sampling week ($F_{59,227} = 1.57$, $p = 0.016$). Within the interaction, sediment treatment had a strong impact on the quantity of inorganic matter accumulating on tiles. Ungrazed caged tiles accumulated more inorganic matter in high sediment treatments than either low sediment or control treatments (Table 2.2). Tadpoles were able to effectively clear sediment from feeding surfaces, leading to similar levels of inorganic matter on grazed, open tiles regardless of sediment treatment. Over the 8 weeks, open tiles from both sediment treatments maintained levels of inorganic matter similar to open tiles in control treatments, meanwhile ungrazed caged tiles in sediment treatments continually accumulated sediment. This difference between open and caged tiles increased over the length of the experiment in sediment treatments as caged tiles accrued increasingly more sediment deposits. Due to inadequate number of samples from false-caged tiles, statistical contrasts were inestimable. Graphing of weekly least-squared means show that false cage tiles were often intermediate in their values between open and caged tiles, but tended to follow similar patterns as open tiles (Figure 2.2 (a-c)). As anticipated, nutrients had no effect on inorganic AFDM levels, thus the sediment treatment drove this part of the interaction.

Chlorophyll *a*

There was a significant effect of tile treatment on Chl *a*. These data show similar trends to those of periphyton organic mass across tile caging treatments. A Tukey-Kramer *post hoc* test revealed that ungrazed caged tiles exhibited significantly greater levels of Chl *a* than tiles open

to tadpole grazing (Figure 2.3), while false-caged tiles generally had intermediate values between the two ($F_{2,211} = 23.11$, $p < 0.001$). There was also a strong interactive effect on Chl *a* of the sediment-nutrient combination and week sampled ($F_{15,198} = 1.96$, $p = 0.02$). Control, low and high sediment treatments all had similar levels of Chl *a* following the initial sediment addition (week 2), but by week 4 both low and high sediment treatments had levels of Chl *a* exceeding controls by nearly an order of magnitude (Figure 2.4). This difference was maintained throughout the rest of the experiment. There was no detectable difference in Chl *a* between low and high sediment treatments. Again nutrients alone had no impact on Chl *a*, and it is assumed that the differences in sediment treatment were driving this part of the interaction.

3.4 DISCUSSION

As in tropical studies, western toad tadpoles treated deposited organic-rich sediments as a food resource and actively ingested deposited material as determined by the feeding scars on tiles. In the process, tadpoles were able to effectively clear sediment from grazing surfaces and reduced periphyton standing stock by up to 80%. The nature of the pulsed sediment additions and subsequent particle fall-out required that tadpoles remove sediment throughout the course of the study. By the end of the experiment, tiles without grazing had accumulated 47 times more inorganic sediment material than tiles open to grazing, supporting hypothesis (1) that sediment accrual on grazing surfaces would be reduced as a consequence of tadpole grazing activities.

Similar sediment clearing results observed in other species of tadpoles have been interpreted as constituting 'ecosystem engineering' activities (Flecker et al. 1999, Ranvestel et al. 2004). In this light, clearing effects seen here in *Bufo boreas* tadpoles could also be interpreted as providing initial support for a potential role as an ecosystem engineer in temperate

pond systems. Modulation of the distribution and impact of sediment deposition through inadvertent consumption and physical displacement by tadpoles may structure the benthic habitat and have broad, multi-trophic level effects on other aquatic community members. Such wider effects still remain to be investigated in this species and substantiated in natural pond systems. The tile grazing manipulation in this study illustrated that tadpoles are able to substantially influence sediment and algal dynamics on grazing surfaces in mesocosm pond systems. Tadpoles actively ingested settled material at a rate sufficient to keep grazing surfaces clear of sediment across repeated input events. Habitat structuring via sediment removal, as seen here, has been shown to alter algal abundance (Power 1990), composition and Chl *a* content (Flecker 1996). In addition, these sediment clearing activities can also have cascading effects on coexisting invertebrate grazer assemblages (Flecker 1992, Ranvestel et al. 2004, De Souza and Moulton 2005).

Despite this evidence in support of an ecosystem engineering role for western toad tadpoles, findings from this study failed to find support for hypothesis (2) that the ecosystem engineering effects of sediment clearing would enhance primary productivity. No positive measurable effect of sediment clearing was observed on periphyton biomass or Chl *a* from grazed surfaces in mesocosms. Previous studies have suggested (Power 1990) that sediment clearing activities could enhance algal growth, producing standing crops of periphyton with larger cells and higher primary productivity, in algae which would be otherwise smothered. In this study, tadpoles consumed not only overlying sediment, but foraged heavily on underlying periphyton as well, reducing periphyton biomass by up to 80% in controls and ~35% in high sediment treatments. The relatively high stocking density of tadpoles, and thus grazing pressure in tanks, may have obscured periphyton enhancement. Lower tadpole density per 'pond' and less intense grazing or longer intervals between repeat foraging bouts could have permitted

greater re-growth of periphyton. Under natural pond conditions where toad tadpole schools move between foraging patches (Sontag et al. 2006), enhanced periphyton re-growth may occur between foraging visits, benefiting both tadpoles and the wider herbivore community.

Measures of algal biomass on tiles showed that while sediment treatments depressed organic periphyton biomass, they simultaneously increased the quantity of Chl *a*. Though counter-intuitive, this change in the ratio of Chl *a* per unit of periphyton biomass could be explained in a number of ways; for instance, by an increase in the amount of Chl *a* per algal cell as a response to sediment shading, i.e. photoacclimation (Thomas et al. 2006), a sediment-induced shift in the algal community to species with greater Chl *a* content (McGowan et al. 2005), or spurred growth of algae from nutrients associated with sediments (Carlton and Wetzel 1988, Hansson 1990) at the expense of other members of the periphyton community. Overestimation of active epipellic chlorophyll on sediments is also highly probable due to my measurement of uncorrected Chl *a* (which includes degraded Chl *a* and phaeophytins) and the layered structure of epipellic algal mats in which new cells overgrow and bury older, senescent layers and attenuate light within the mat (Vadeboncoeur et al. 2006). Due to this ambiguity in changes in Chl *a*, productivity estimates must be interpreted with caution. Recently published work suggests that measurement of uncorrected Chl *a* may be a poor metric of available periphyton biomass in shaded environments (Thomas et al. 2006) and open lakes (Johannsson et al. 1985). Instead, chlorophyll measures may be better used as indicators of changes in the structure of algal communities or algal cell content.

Together, these results from mesocosms provide initial evidence for habitat structuring in pond systems and preliminary support for a general ecological role for amphibian tadpoles as ecosystem engineers when they occur at sufficiently high abundances. Together these highlight

the importance of such species in maintaining freshwater ecosystems function. Habitat structuring by the western toad, a temperate zone, pond breeding species alongside the well recognized role of tropical stream-tadpoles, lends credence to Flecker's et al. (1999) prediction that where tadpoles occur in high abundance, they are likely to influence sediment dynamics. Although this study was conducted in mesocosms and thus the ability to generalize results is limited, there is potential for the western toad to exhibit engineering functions in pond habitats. Their explosive breeding, high tadpole biomass and benthic feeding dictates an important role as primary consumers in pond food webs. Furthermore, because of the gregarious nature of toad tadpoles, schooling behaviours may be particularly beneficial in displacing deposited sediment in order to access buried periphyton (Katz 1981, Beiswenger 1975).

Allochthonous Inputs

Allochthonous inputs such as terrestrially-derived sediments in aquatic systems can also be considered in the light of recent subsidy research. Where such inputs have a beneficial effect on recipient communities they are generally considered to act as "resource subsidies", supplementing the availability of important resources to dependent consumers. If sediment inputs from this experiment are considered in this context, their impacts on primary and secondary growth lead to ambiguous interpretations.

Contrary to results from other studies where highly organic material inputs into aquatic systems have acted as a "resource subsidy", sediments in this experiment had an overall negative impact on the recipient community. Though sediments were perceived as a food resource by toad tadpoles, they had an overall negative effect on tadpole growth and survival (Chapter 2) as well as on periphyton biomass. Previous work has demonstrated that organic, detrital inputs into aquatic habitats provide important food resources for benthic grazers,

shredders and detritivores (Flecker et al. 1999, Wallace et al. 1997, Hall et al. 2000) supporting tadpole growth and greater overall in-stream biomass (Wallace et al. 1999, Hall et al. 2000, Ranvestel et al. 2004). In this experiment, sediment deposits, despite their palatability, reduced periphyton availability, impaired tadpole growth and survival, and altered foraging behaviour (Chapter 2). Rather than resulting in a net positive or neutral effect on ecosystem dynamics and grazer communities, inputs of terrestrially-derived material had a depressive effect, lowering both water quality (via turbidity) and periphyton availability. In such circumstances, sediment inputs may be more aptly described as a “resource depressant” rather than a “resource subsidy”. Thus, it should be remembered that allochthonous inputs have the potential to play disadvantageous as well as beneficial roles in recipient systems. The direction of response in recipient communities to these inputs will likely depend on the role of which these inputs play in community processes and dynamics, and the ability of species to effectively use or regulate these inputs.

3.5 CONCLUSIONS

Similar to a number of amphibian species from tropical stream systems, tadpoles of the western toad exhibited sediment clearing activities in mesocosm ponds that have previously been interpreted as constituting ecosystem engineering functions. These findings support previous suggestions of a general ecological role for tadpoles of habitat structuring through sediment removal and displacement in ponds as well as in streams. Studies quantifying tadpole effects in natural pond systems and across a range of water body sizes is the next step in determining the ecological significance of such habitat structuring for other community members. The effectiveness and consequences of sediment removal are likely dependent on the size of the water body, density and biomass of tadpoles within it and the rate of sediment

deposition. Despite the clearing abilities of tadpoles in pond systems, the negative impacts of sediment deposition are more likely to manifest themselves via pond-filling. Alteration of the hydro-period and eventual filling of all interstitial spaces will likely contribute to overall decline in habitat suitability for a number of pond breeding anurans. Understanding when these types of allochthonous inputs act as resource subsidies or depressants is critical for elucidating important cross-habitat food web linkages and predicting the resilience of systems to anthropogenic disturbances.

Table 3.1 Split-split plot of organic mass from periphyton (log organic AFDM $\mu\text{g}/\text{cm}^2$) on tiles using a mixed model with the Kenward-Rogers denominator degrees of freedom approximation.

Source	Num DF	Den DF	F	P
Sediment	2	23.8	6.84	0.0045
Nutrient	1	23.8	0.03	0.8597
Sed x Nutrient	2	23.8	0.17	0.8409
Graze	1	23.8	51.27	<0.0001
Sed x Nutrient x Graze	5	23.8	1.87	0.1418
Week	7	301	46.77	<0.0001
Graze x Week	7	301	3.59	0.0010
Sed x Nutrient x Week	35	301	1.28	0.1444
Sed x Nutrient x Week x Graze	35	301	1.42	0.0629

Table 3.2 Split-split plot of sediment accumulation (square root-inorganic sediment mg/cm²) on tiles using a mixed model with the Kenward-Rogers denominator degrees of freedom approximation.

Source	Num DF	Den DF	F	P
Sediment	2	8.53	51.95	<0.0001
Nutrient	1	8.58	0.07	0.7984
Sed x Nutrient	2	8.52	0.04	0.9564
Tile Treatment	2	17	102.89	<0.0001
Sed x Nutrient x Tile	10	16.7	6.77	0.0003
Week	6	147	3.39	0.0037
Tile x Week	12	145	11.02	<0.0001
Sed x Nutrient x Week	30	143	1.41	0.0929
Sed x Nutrient x Week x Tile	59	137	1.57	0.0161

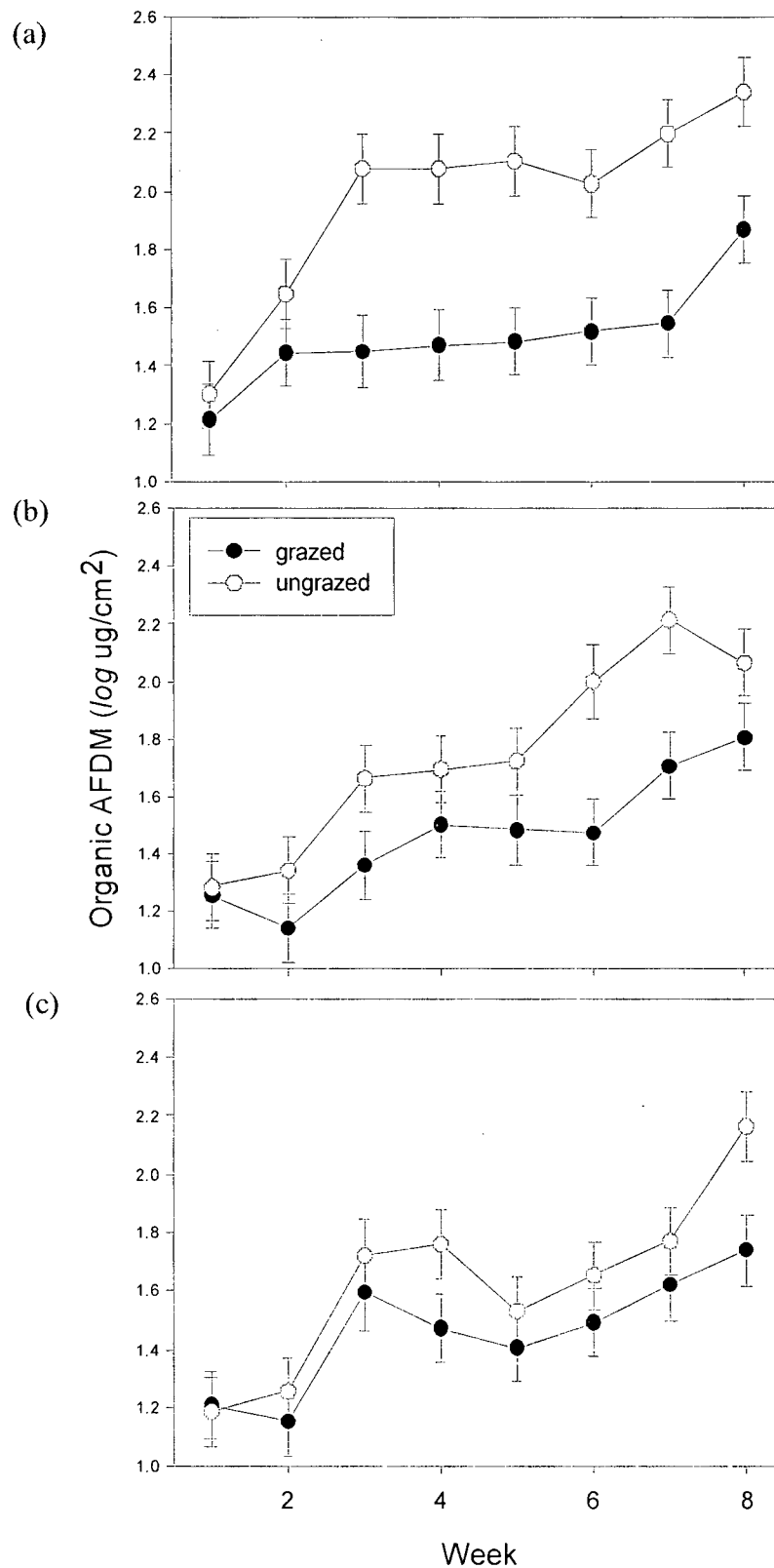


Figure 3.1 (a-c) Organic AFDM of periphyton on tiles from grazed (open and false caged pooled) and ungrazed (caged) tiles in mesocosms exclosures over 8 weeks from (a) CONT, (b) LOW and (c) HIGH sediment treatments (n = 6). Symbols represent means ± 1 SE.

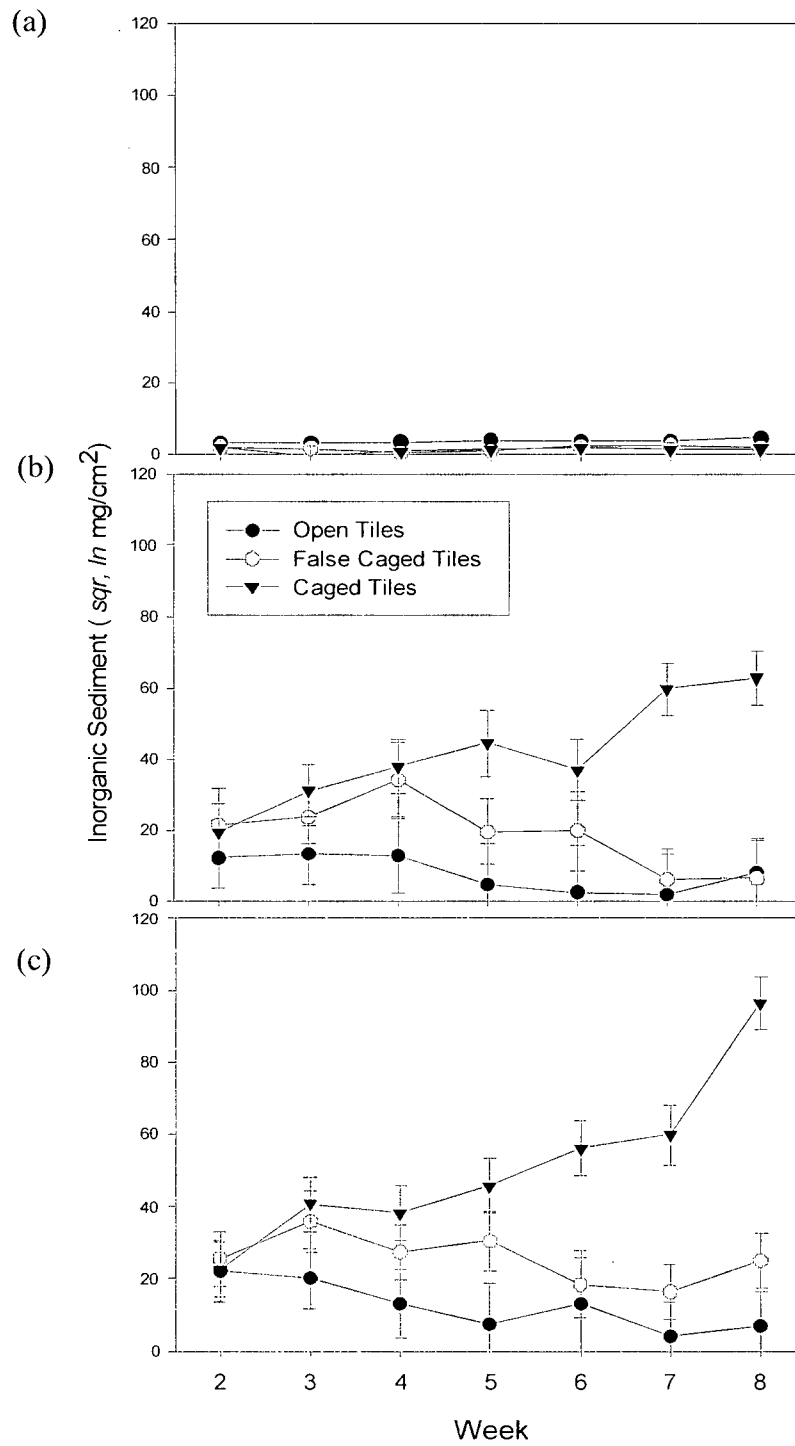


Figure 3.2 (a-c) Inorganic sediment on tiles from open, false and caged exclosures in mesocosms over 7 weeks in (a) CONT, (b) LOW and (c) HIGH sediment retreatments (n = 6). Symbols represent means $\pm$ 1 SE.

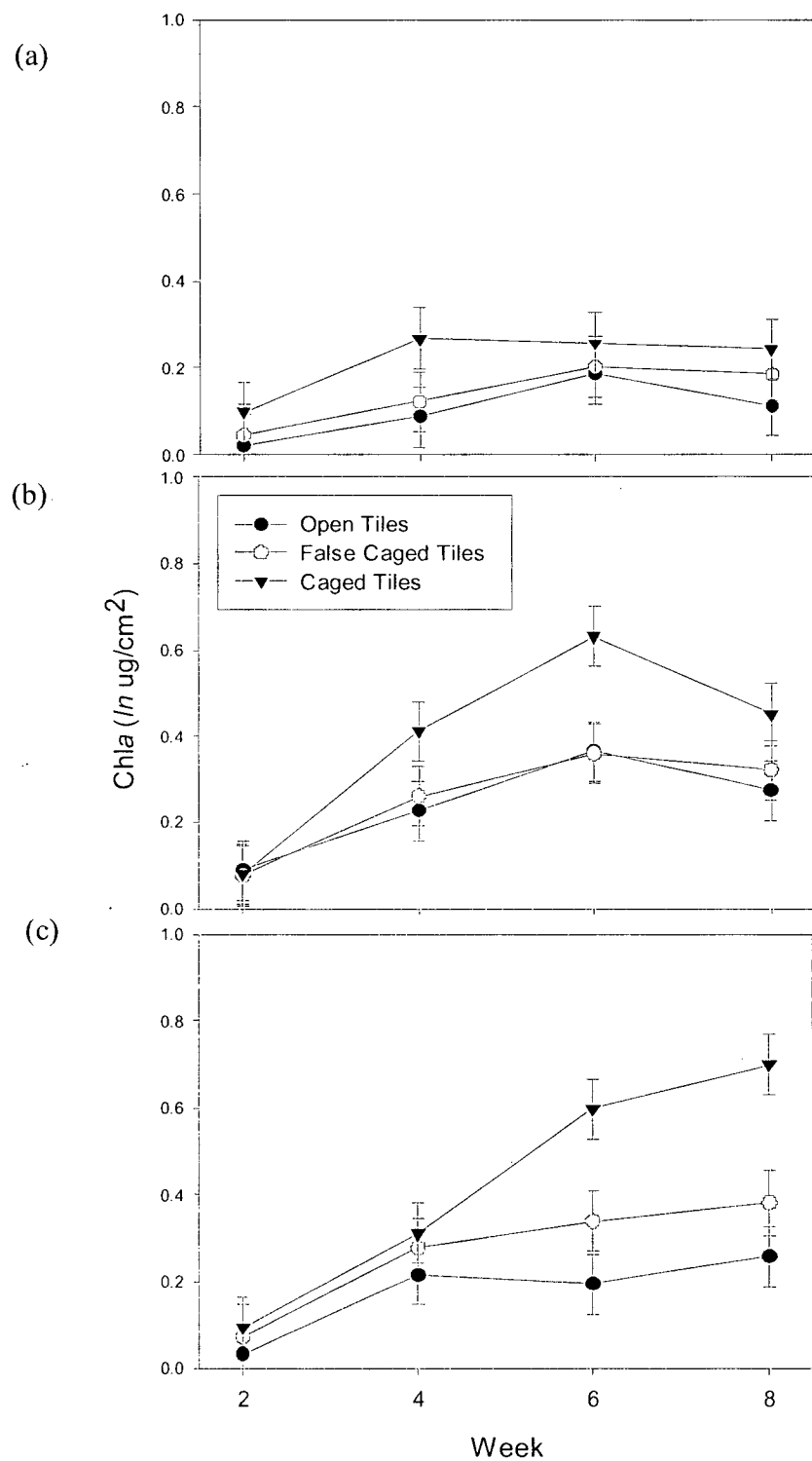


Figure 3.3 (a-c) Comparison of measured Chl *a* levels on tiles from the exclosure treatment in (a) CONT, (b) LOW and (c) HIGH sediment treatments. Symbols represent means ± 1 SE.

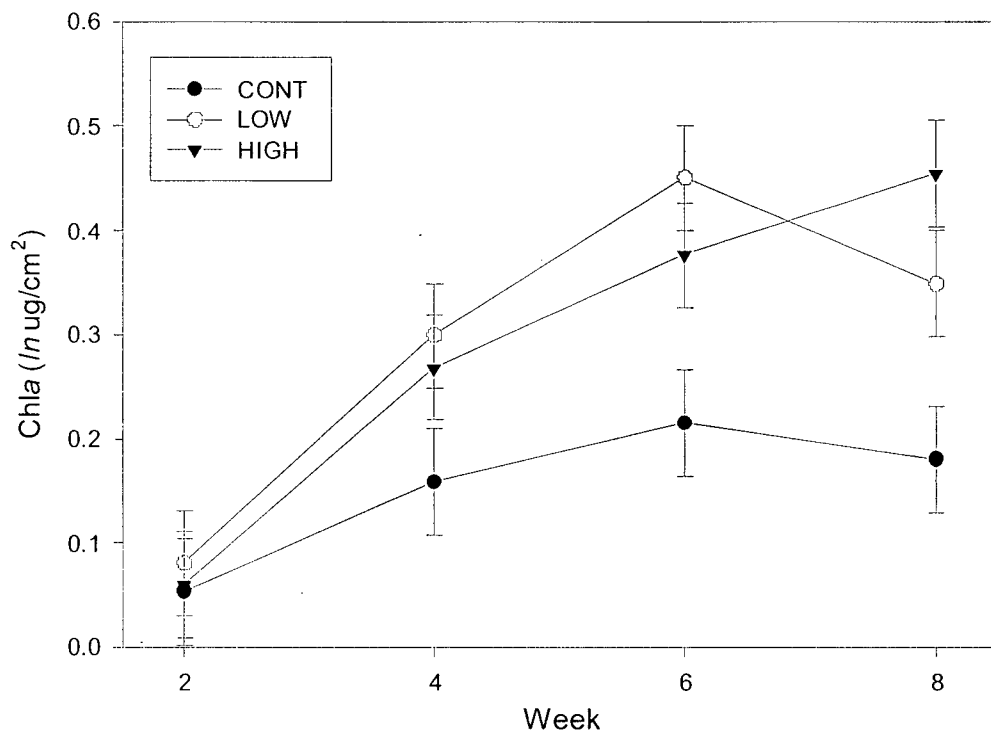


Figure 3.4 Uncorrected Chl *a* in CONT, LOW and HIGH sediment treatments across weeks. Chl *a* levels were consistently lower in CONT treatments ($p < 0.05$) but were not different among LOW and HIGH treatments. Symbols represent means $\pm$ 1 SE.

3.6 REFERENCES

- Beiswenger, R.E. 1975. Structure and function in aggregations of tadpoles of the American toad, *Bufo americanus*. *Journal of Herpetology* 31:222-233.
- Blaustein, A.R. 1988. Ecological correlates and potential functions of kin recognition and kin association in anuran larvae. *Behavior Genetics* 18: 449-464.
- Carlton, R.G. and R.G. Wetzel. 1988. Phosphorus flux from lake sediments: effect of epipellic algal oxygen production. *Limnology and Oceanography* 33:562-570.
- Cecil, S.G and J.J. Just. 1979. Survival rate, population density and development of a naturally occurring anuran larvae (*Rana catesbeiana*). *Copeia* 1979: 447-453.
- Creed Jr., R.P. and J.M. Reed. 2004. Ecosystem engineering by crayfish in a headwater stream community. *Journal of North American Benthological Society* 23: 224-236.
- De Souza, M.L. and T.P. Moulton. 2005. The effects of shrimps on benthic material in a Brazilian island stream. *Freshwater Biology* 50: 592-602.
- Dickman, M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. *Ecology* 49: 1188-1190.
- Flecker, A.S. 1992. Fish trophic guild and the structure of a tropical stream: weak direct versus strong indirect effects. *Ecology* 73: 927-940.
- Flecker, A.S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology* 77: 1845-1854.
- Flecker, A.S., B.P. Feifarek and B.W. Taylor. 1999. Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. *Copeia* 1999: 495-500.
- Gyug, L. 1996. Part IV Amphibians. In *Timber harvesting effects on riparian wildlife and vegetation in the Okanagan Highlands of British Columbia*. B.C. Environment, Penticton, B.C.
- Hall, R.O., J.B. Wallace, and S.L. Eggert. 2000. Organic matter flow in stream food webs with reduced detrital resource base. *Ecology* 81: 3445-3463.
- Hansson, L.-A. 1990. Quantifying the impact of periphytic algae on nutrient availability for phytoplankton. *Freshwater Biology* 24: 256-274.
- Johannsson, O.E., R.M. Dermott, R. Feldkamp and J.E. Moore. 1985. Lake Ontario long term biological monitoring program: Report for 1981 and 1982. Canadian Technical Report for Fisheries and Aquatic Sciences, 1414.
- Jones, C.G., J.H. Lawton and M. Shachak. 1994. Organism as ecosystem engineers. *Oikos* 69: 373-386.

- Katz, L.C., M.J. Potel and R. J. Wassersug. 1981. Structure and mechanisms of schooling in tadpoles of the clawed frog, *Xenopus laevis*. *Animal Behaviour* 29: 20–33.
- Ketcheson, G.L., W.F. Megahan and J.G. King. 1999. "R1-R4" and "BOISED" sediment prediction model tests using forest roads in granitics. *Journal of the American Water Resources Association* 35: 83-98.
- Kupferberg S. 1997. Facilitation of periphyton production by tadpole grazing: functional differences between species. *Freshwater Biology* 37: 427–439.
- Lamberti G.A., S.V. Gregory, C.P. Hawkins, R.C. Wildman, L.R. Ashkenas and D.M. Denicola. 1992. Plant-herbivore interactions in streams near Mount St. Helens. *Freshwater Biology* 27: 237–247.
- Littell R.C., W.W. Stroup, and R.J. Freund. 2002. SAS for linear models. SAS Institute, Cary, N.C.
- Loman, J. 2001. Effects of tadpole grazing on periphytic algae in ponds. *Wetlands Ecology and Management* 9: 135–139.
- Luo, H.R., L.M. Smith, B.L. Allen and D. Haukos. 1997. Effects of sedimentation on playa wetland volume. *Ecological Applications* 7: 247-252.
- Martin, D.B. and W.A. Hartman 1987. The effect of cultivation on sediment composition and deposition in prairie potholes. *Water, Air, and Soil Pollution* 34: 45–53.
- McGowan, S., P.R. Leavitt, R.I. Hall, N.J. Anderson, E. Jeppensen and B.V. Ogaard. 2005. Controls of algal abundance and community composition during ecosystem state change. *Ecology* 86:2200-2211.
- Meredith, M. P. and S.V. Stehman. 1991. Repeated measures experiments in forestry: focus on analysis of response curves. *Canadian Journal of Forest Research* 21: 957–965.
- Moulton, T.P., M.L. de Souza, R.M.L. Silveira and F.A.M. Kruslovic. 2004. Effects of ephemeropterans and shrimps on periphyton and sediments in a coastal stream (Atlantic forest, Rio de Janeiro, Brazil). *Journal North American Benthological Society* 3: 868–881.
- Newcombe, C.P. and D.D. MacDonald. 1991. Effects of suspended sediments on aquatic ecosystems. *North American Journal of Fisheries Management* 11: 72-82.
- Polis, G.A., W.B. Anderson, and R.D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28: 289-316.
- Potvin, C., Lechowicz, M.J., and S. Tardiff. 1990. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. *Ecology* 71:1389-1400.

- Power, M.E. 1990. Resource enhancement by indirect effects of grazers: armoured catfish algae and sediment. *Ecology* 71: 897-904.
- Pringle, C.M., G.A. Blake, A.P. Covich, K.M. Buzby and A. Finley. 1993. Effects of omnivorous shrimp in a montane tropical stream: sediment removal, disturbance of sessile invertebrates, and enhancement of understory algal biomass. *Oecologia* 93: 1-11.
- Ranvestel, A.W., K.R. Lips, C.M. Pringle, M.R. Whiles and R.J. Bixby. 2004 Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology* 49: 274-285.
- Samollow, P.B. 1980. Selective mortality and reproduction in a natural population of *Bufo boreas*. *Evolution* 34: 18-19.
- Seale, D.B. 1980. Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology* 61: 1531-1550.
- Solomon, C.T., A.S. Flecker and B.W. Taylor. 2004. Testing the role of sediment-mediated interactions between tadpoles and armored catfish in a neotropical stream. *Copeia* 2004: 610-616.
- Sontag, C., D. Sloan-Wilson and R.S. Cox. 2006. Social foraging in *Bufo americanus* tadpoles. *Animal Behaviour* 72:1451-1456
- Swift Jr., L.W. 1988. Forest access roads: design, maintenance, and soil loss. *In*: Swank, W.T., Crossley, Jr., D.A. (Eds.), *Forest Hydrology and Ecology at Coweeta*, Springer-Verlag, New York, pp. 313-324.
- Thomas, S., E.E Gaiser and F.A. Tobias. 2006. Effects of shading on calcareous benthic periphyton in a short-hydroperiod oligotrophic wetland (Everglades, FL, USA). *Hydrobiologia* 569: 209-221.
- Turnipseed; G. and R. Altig. 1975. Population density and age structure of three species of Hylid tadpoles. *Journal of Herpetology* 9: 287-291.
- Vadeboncoeur, Y., J. Kalff, K. Christoffersen and E. Jeppesen. 2006. Substratum as a driver of variation in periphyton chlorophyll and productivity in lakes. *Journal of the North American Benthological Society* 25: 379-392.
- Wallace, J.B., S.L. Eggert, J.L. Meyer, and J.R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* 277: 102-104.
- Wassersug, R.J. 1973. *In* *Evolutionary Biology of the Anurans* (ed. Vial, J. L.), University of Missouri Press, pp. 273-297.
- Waters, T.F. 1995. Sediment in streams. Sources, biological effects, and control. American Fisheries Society Monograph 7, Bethesda, Maryland, USA.

Welch, B.L. 1938. The significance of differences between two means when the population variances are unequal. *Biometrika* 29: 35-362.

Wind, E. and L.A. Dupuis. 2002. COSEWIC status report on the western toad *Bufo boreas* in Canada. *In* COSEWIC assessment and status report on the western toad *Bufo boreas* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-31 pp.

CHAPTER FOUR:

Summary and Future Directions

This thesis set out to investigate the interactive and dynamic relation between tadpoles of the western toad, *Bufo boreas*, and sediment-nutrient inputs. Based on previous studies, I expected to see an increase in growth and survival of tadpoles as a result of moderately organic sediment (8% - 9%) added and consequent differences in metamorphic size and/or timing. Furthermore, due to their high biomass and benthic feeding, I anticipated that *Bufo* tadpoles had the potential to structure the benthic habitat via sediment removal, thereby potentially acting as ecosystem engineers in pond systems.

Results from the pond mesocosm and behavioural trials in Chapter Two showed that contrary to expectations, sediment additions reduced growth and survival of tadpoles to metamorphosis. Furthermore, these larval differences did not translate into strong differences in size or mass of metamorphs, nor differences in their timing of metamorphosis. Tadpole dissections and fecal analysis revealed that tadpoles perceived sediment as a food resource and consumed deposited particulate in large quantities, but apparently derived little nutritional or energetic benefit. Instead, sediment appeared to act as a 'resource depressant' reducing algal food resources, stimulating costly behavioural responses and/or potentially increasing energetic costs associated with gill maintenance.

Exclosure manipulations in Chapter Three revealed that through voracious feeding activity, tadpoles were able to significantly reduce the quantity of both periphyton and deposited sediment on foraging surfaces. Removal of sediment did not result in an enhancement of underlying periphyton. This was likely due to the heavy and constant foraging pressure exerted

by tadpoles in tanks. Regardless, this enclosure-mesocosm experiment offers preliminary evidence in support of a role for *Bufo boreas* as an ecosystem engineer within their larval habitat and more generally for tadpoles across habitats and temperate-tropical ecosystems.

Future Directions

This work was carried out in mesocosms, which are simplifications of natural environments and thus may not accurately encompass all important ecological interactions. For broader application of the findings of sediment and nutrients on tadpoles, similar manipulations need to be carried out in natural settings to ensure that mesocosm results are representative of real responses of tadpoles to sediment-nutrient loading. Furthermore, the wider effects of sediment clearing activities on other members of the aquatic community need to be investigated in order to better ascertain whether such activities in *Bufo boreas* and other pond breeding amphibians may be considered ecosystem engineering.

Also, due to logistical limitations, application of sediment and nutrient additions only created turbidity levels similar to background levels experienced in watersheds in the Pacific Northwest. Yet, amphibians may come into contact with much higher elevations of suspended sediments (2 - 6 times higher) in heavily managed landscapes. How they cope with these conditions remains poorly documented and needs to be investigated to adequately understand the impacts of habitat modification on such species.

Lastly, the observed reduction in growth and size from sediment additions may have implications for juvenile survival, especially in harsh environments. Adult toads in coastal British Columbia show preference for breeding in clear-cut habitats, but the consequences for tadpoles growing in and emerging into such environments has not been thoroughly documented.

Conditions leading to smaller size at metamorphosis may be harmful to juvenile survival due to a higher vulnerability to desiccation and higher incidence of predation in these open habitats.

Field experiments monitoring summer survival and growth of metamorphs in clear-cuts would help to elucidate whether these habitats acts as sinks for breeding toads or are viable habitats for juvenile recruitment and population persistence.