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THE ECOLOGY OF *Gammarus lacustris* (CRUSTACEA: AMPHIPODA) IN LAKES OF THE CANADIAN ROCKY MOUNTAINS

by

Frank Michael Wilhelm ©

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL BIOLOGY AND ECOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

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DATED this 30 day of SEPTEMBER 1999.

> "We are ignorant, terribly, immensely ignorant. And our work is to learn. To observe, to experiment, to tabulate, to induce, to deduce. Biology was never a clearer or more inviting field for fascinating, joyful, hopeful work."
>
> Vernon L. Kellogg 1907

Kellogg, V. L. 1907. Darwinism To-Day. London: George Bell and Sons.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for the acceptance, a thesis entitled "The Ecology of *Gammarus lacustris* (Crustacea: Amphipoda) in Lakes of the Canadian Rocky Mountains" submitted by Frank Michael Wilhelm in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the field of Environmental Biology and Ecology.

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DATE: TUES. SEPT. 14/99

ABSTRACT

I used comparative and experimental approaches to examine the ecology of *Gammarus lacustris* (Crustacea: Amphipoda) from ten lakes along an elevation gradient from prairie to alpine (736 to 2320 m a.s.l.) in western Canada. The duration of the ice-free season, mid-summer surface water temperature, and fecundity decreased with elevation, while life cycle length, age at maturity, growth rate, lipid levels, egg size, and size of young at hatching increased with elevation. The reproductive investment and size of mature females varied independently of elevation.

The development time of eggs was negatively related to temperature and large eggs required longer incubation than small eggs. Young from large eggs were larger than young from small eggs. Under starvation conditions, large young survived longer at cold temperatures than small young, indicating that the production of fewer but larger eggs and young at cold temperatures is adaptive.

My findings suggest that if lakes warm as a result of climate-induce warming, *G. lacustris* populations in currently cold montane and alpine lakes should shift their life history strategies to reflect those presently found in warmer lakes, while populations in prairie lakes may become extirpated because temperatures already approach the upper lethal limit.

Size-selective predation by bull trout was a selective pressure against large *G. lacustris* in Harrison Lake and explained the smaller size and younger age at maturity compared to other alpine lakes. The co-existence of *G. lacustris* with bull trout in Harrison Lake is mediated by the diet switching of bull trout among seasonally abundant alternative prey species.

In a mesocosm experiment in Snowflake Lake, *G. lacustris* influenced the plankton community structure in a manner similar to planktivorous fish. At medium and high *G. lacustris* densities, *Daphnia* were eliminated from mesocosms and phytoplankton biomass increased. The experimentally measured release of phosphorus by *G. lacustris* showed that the amphipod was a net source of sediment-derived phosphorus to the pelagic zone. Therefore, the increase of phytoplankton biomass was not only facilitated by the direct removal of grazers but also indirectly through the addition of nutrients. These results show that *G. lacustris* is an important link between multiple trophic levels and habitats in aquatic ecosystems.

PREFACE

The structure of this thesis follows the paper format outlined by the Faculty of Graduate Studies and Research, University of Alberta, 1999. The research is presented in seven manuscripts, chapters 2 through 8. The introductory and concluding chapters are intended to outline the research, summarize the findings and present areas of future research. I have acknowledged individuals that have contributed their ideas or time in the collection of data by way of authorship and used the plural throughout the data chapters. Although I have made an effort to keep repetition to a minimum, common aspects that underlie the research are unavoidably repeated. Below is a list of manuscripts resulting from the seven chapters as they have been, or I expect them to be published.

Chapter 2. Wilhelm, F. M., Witt, J. and Schindler, D. W. Life history strategies of *Gammarus lacustris* (Crustacea: Amphipoda) from ten lakes along an elevation gradient in western Canada.

Chapter 3. Wilhelm, F. M. and Schindler, D. W. In press. Reproductive strategies of *Gammarus lacustris* (Crustacea: Amphipoda) along an elevation gradient. *Functional Ecology*.

Chapter 4. Wilhelm, F. M., Parker, B. R., Schindler, D. W., and Donald, D. B. In press. Seasonal food habits of bull trout in a small alpine lake in the Canadian Rocky Mountains. *Transactions of the American Fisheries Society*.

Chapter 5. Wilhelm, F. M., and Schindler, D. W. 1999. Effects of *Gammarus lacustris* (Crustacea: Amphipoda) on plankton community structure in an alpine lake. *Canadian Journal of Fisheries and Aquatic Sciences 56: 1401-1408.*

Chapter 6. Wilhelm, F. M., and Schindler D. W. The influence of experimental scale on estimating the predation rate of *Gammarus lacustris* (Crustacea: Amphipoda) on *Daphnia* in an alpine lake. Submitted to *Journal of Plankton Research*.

Chapter 7. Wilhelm, F. M., Hudson, J. J. and Schindler, D. W. 1999. Contribution of *Gammarus lacustris* to phosphorus recycling in a fishless alpine lake. *Canadian Journal of Fisheries and Aquatic Sciences 56: 1679-1686.*

Chapter 8. Wilhelm, F. M., Schindler D. W., Arts, M. T. Lipid levels in *Gammarus lacustris* from lakes along an elevation gradient.

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Many people provided stimulating and challenging criticisms of my science during my stay in the department. I thank all of them, especially my committee, B. Parker, A. S. McNaught, P. J. Curtis, and R. Vinebrooke.

Alpine research in the remote backcountry of Banff National Park presented interesting challenges. Above all, not much can be accomplished alone. I thank all the summer staff J. Clare, S. Majewski, C. Wilson, R. Whittington, S. Clare, T. Johnston, and D. Oickle; and fellow graduate students and post-docs B. Parker, R. Vinebrooke, A. K. Hardie, N. McMaster, A. S. McNaught, and J. Hudson for their help and company out of our base at Scotch Camp. August snowstorms and hailstorms at the lakes made me wonder why we were pursuing research in such a desolate place; however, warm cinnamon buns at camp and sunshine made me realize that we were in a special place, and I wouldn't have had it any other way.

David Donald contributed unpublished fish data from Harrison Lake, mid- to late-70's amphipod samples from all over the National Parks and plenty of discussion focussed on fish and amphipods in lakes of the Rockies. I thank him for willingly parting with data and samples.

I thank Dr. Michael Arts for allowing me to join his lab at NHRI in Saskatoon to learn the micro-method for lipid analysis. He contributed his time, expertise and reference collection to bring me up to speed.

Special recognition must be given to Parks Canada, especially Banff and Jasper National Parks for providing permits and allowing this research to progress unhindered. Although not without prior human influence, the lakes and ponds in the parks are a national treasure and provide an unequalled opportunity for ecological research.

The Department of Biological Sciences contributed in many ways. Not only did it provide shelter and space, but also opportunities and experiences other than academic studies including committee participation, conference organizing and a social atmosphere.

I am grateful for the financial support of the following: The Challenge Grants in Biodiversity Program (University of Alberta and Alberta Conservation Association); the Canadian Circumpolar Institute (University of Alberta); the Friends of Banff National Park; the Natural Sciences and Engineering Research Council of Canada (operating grant to D. W. S.); Faculty of Graduate Studies and Department of Biological Sciences travel grants; and NSERC, Izaak Walton Killam, University of Alberta Dissertation, Walter H. Johns and Andrew Stewart scholarships.

Finally, I would like to thank my wife, Jennifer Hiebert, for her support of this project and her dedication to our relationship despite both of our theses.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. General Introduction

Communities are structured through the interaction of numerous biotic and abiotic factors. The multi-dimensional space defined by these factors in combination with the genetic variability of organisms expressed through morphology, physiology and life history strategies govern which species will be present in a community, their interactions and their population dynamics. Community ecologists seek to understand the interplay between these factors in structuring biological communities.

In this thesis, I first examine the life history strategies of *Gammarus lacustris*, a crustacean amphipod, from lakes along an elevation gradient from prairie to alpine (736 to 2320 m a.s.l.) in western Canada in relation to altitude (decreasing water temperature with increasing elevation) and fish predation. I then focus on its role in aquatic ecosystems by examining its effect on the structure of plankton communities through predation on zooplankton and the cycling of nutrients. Due to the heavy ice-scouring of the steep stallus shorelines at ice-out, it is difficult to distinguish littoral and pelagic zones in high alpine lakes. For this reason, I examined *Gammarus*/zooplankton/nutrient release interactions in the pelagic zone.

Gammarus lacustris is an ideal study species for the examination of life history strategies. It has a wide latitudinal and altitudinal distribution in the northern hemisphere (Bousfield 1958; Zhadin 1963; Ökland 1969). During the day, amphipods usually occur in dense aggregations along peaty shorelines or in macrophytes and are easy to collect. Unlike other aquatic invertebrates, it does not have a resting stage and individuals are present year-round. Females carry their young in a ventral brood pouch which allows fecundity, egg size and weight to be determined accurately. While the species is omnivorous, it can complete its life cycle on a diet of detritus or algae (Anderson and Raasveldt 1974; Moore 1977), so that its development does not depend on lake productivity alone. Although the biology of the species has been investigated in prairie lakes (Menon 1969; Biette 1969) and some Norwegian lakes (Bjerknes 1974), its biology in arctic and alpine lakes has not been thoroughly examined.

Temperature is one of the most important abiotic factors affecting organisms because it has an overriding effect on rates of biological processes (Beisner et al. 1997). For example, fecundity may be constrained by small body size at maturity in cold environments due to reduced growth rates and/or a shorter growing season (e.g. Blanckenhorn 1997; Johansson and Rowe 1999). The slower development of eggs at cold versus warm temperatures may require that more energy is allocated per egg (Clarke and Gore 1992). Thus, a change in ambient temperature has the potential to influence life history strategies and population dynamics (Beisner et al. 1996; Blanckenhorn 1997). Given the anticipated effects of global warming on aquatic systems (e.g. Schindler et al. 1990; Schindler et al. 1996), it is important to understand the life history strategies and population dynamics of species at different temperatures to predict the fate of populations and communities

Natural temperature gradients occur with changes in latitude and altitude. These gradients have been used to examine the life history strategies in a number of species such as dung flies (Blanckenhorn 1997), lizards (Grant and Dunham 1990; Smith et al. 1994; Rohr 1997; Tracy 199); crickets (Mousseau and Roff 1995) and land snails (Baur and Baur

1997). However, few aquatic ecologists have made use of altitudinal gradients to examine the effect of water temperatures on life history strategies of aquatic organisms (but see Hancock et al. 1997). Recently, it has been suggested that these gradients serve as a basis for predicting the response of populations to increases in temperature predicted from current global warming scenarios (Whittaker and Tribe 1996; Schindler 1997). I used a comparative approach to examine the life history strategy of ten populations of *Gammarus lacustris* (Chapter 2) along an elevation gradient from prairie to alpine. Specifically, I examined life cycle length, growth rate, age and size at maturity, reproductive investment, fecundity, and size of young at hatching.

The success of a species depends on the production of viable offspring. Given that most organisms have a limited amount of energy that can be invested in reproduction, a trade-off exists between the number and size of offspring that can be produced (Smith and Fretwell 1974; Elgar 1990; Roff 1992; Stearns 1992). I noticed that females from alpine lakes produced fewer but larger eggs than females from prairie lakes. Therefore, I examined the relationship between fecundity, egg size and weight in detail for four of the study populations spanning the entire elevation gradient (Chapter 3). I also examined whether young from large eggs were larger than young from small eggs. I then used starvation experiments to test the hypothesis that large young would survive better than small young at cold temperatures (Chapter 3).

Predation is of central importance in ecosystems. Consumers influence the distribution and abundance of prey and vice versa (Begon et al. 1986). In aquatic ecosystems, fish predators exert a large influence through size-selective predation on the species composition and size-structure of organisms at other trophic levels (e.g. Brooks and Dodson 1965; Carpenter and Kitchell 1993). Generally, planktivores reduce the mean size of zooplankton communities by selectively consuming large bodied species that are replaced by small bodied species that are less vulnerable to fish predation (Brooks and Dodson 1965; Galbraith 1967; Anderson 1972; Vonder Brink and Vanni 1993; Wellborn 1994). Thus, the age and size at maturity of species with plastic life history traits would be expected to mature earlier and at a smaller size. This would reduce the probability of mortality incurred by large size (Roff 1992; Stearns 1992).

In Harrison Lake, a small alpine lake, *G. lacustris* co-exist with bull trout. I hypothesized that as a result of predation by fish, *G. lacustris* would mature at a smaller size than in nearby fishless Snowflake Lake. To confirm that bull trout predation accounted for the difference in *G. lacustris* size, I examined the stomach contents of live fish in 1996 (Chapter 4). The results supported my prediction; however, I was surprised to find that *G. lacustris* was one of three main prey items, occurring in nearly 70% of all fish sampled. It was apparent that the *G. lacustris* population could not remain viable at such a rate of predation. Therefore, in 1997 I determined if predation on *G. lacustris* varied seasonally (Chapter 4). I hypothesized that bull trout would switch their diet, preying on seasonally abundant prey items. Although *G. lacustris* are present year round, other prey items should be selected if they are more abundant or easier to obtain.

Previous studies (Anderson and Raasveldt 1974; Kortelainen 1990) showed that *G. lacustris* in turn, was a predator of zooplankton. Therefore, I hypothesized that *G. lacustris* would affect the structure of the plankton community in fishless lakes by selectively preying on large zooplankton in a manner similar to planktivorous fish and other invertebrate predators such as *Mysis* (e.g. Lasenby and Fürst 1981). However, it was unrealistic to extrapolate the earlier results to estimate the impact of *G. lacustris* on zooplankton at a whole-lake scale because the experiments were conducted in small (100 mL and 1 L) chambers. Cautions about extrapolating from inappropriately scaled experiments to whole-lake ecosystems are frequent in the literature (e.g. de Lafontaine and Leggett 1987; Piercey and Maly 1998; Schindler 1998; May 1999). For this reason, I used large 2800-L mesocosms in which I manipulated the density of *G. lacustris* to examine the effect of predation by *G. lacustris* on the plankton community structure in Snowflake Lake (Chapter 5).

The lack of agreement between zooplankton densities in Snowflake Lake and the mesocosms prompted me to examine if the results of the predation experiments were influenced by the scale of the experiment (Chapter 6). I compared predation rates of *G. lacustris* from experiments in 20-L carboys, the 2800-L mesocosms and Snowflake Lake to examine the influence of experimental scale on the predation rate of *G. lacustris*. To determine the predation rate in Snowflake Lake, I analyzed the gut contents of *G. lacustris* collected at six hour intervals during three 24 h surveys in 1995. Predation rates were calculated from the number of *Daphnia* ingested per *G. lacustris* divided by the time the amphipods spent in the water column.

Organisms that migrate into the water column can transport materials vertically and horizontally within a lake. This can affect the net supply rate of nutrients to certain regions of the lake (e.g. Dini et al. 1987; Schindler 1992). Invertebrates such as *Mysis relicta* and *Diporeia hoyi* that migrate into the water column at night to prey on zooplankton (Marzolf 1965; Wells 1968; Lasenby and Fürst 1981) release and redistribute material ingested from epibenthic feeding during the day, including nutrients (Madeira et al. 1982), metals (Van Duyn-Henderson and Lasenby 1986) and organic contaminants (Landrum and Fisher 1998). In fishless alpine lakes *G. lacustris* undergoes diurnal migrations similar to *Mysis* and *Diporeia* (Anderson and Raasveldt 1974; Wilhelm pers. obs.). Because of the nutrient-poor waters in alpine lakes (McNaught et al. 1999, D. W. Schindler unpub. data), I hypothesized that *G. lacustris* was an important net source of sediment-derived nutrients to the pelagic region. To investigate the importance of *G. lacustris* in the regeneration of nutrients from the benthic to the pelagic zone in Snowflake Lake, I experimentally measured the release of phosphorus by *G. lacustris* at night, after its migration into the water column (Chapter 7). I used published P values for *Daphnia* and the average number of *Daphnia* ingested by each amphipod per night to calculate the P removed from the water column. I then put the net P regeneration rate of *G. lacustris* in context by comparing it to the whole-water planktonic regeneration rate (Hudson and Taylor 1996).

Organisms must acquire a minimum supply of energy to satisfy basic metabolic processes before energy can be allocated to growth, reproduction or storage. In highly variable environments, organisms tend to store large amounts of energy, usually as lipids, during favourable periods. These energy reserves are then used during unfavourable periods to fuel metabolism, growth and reproduction (e.g. Clark 1977; Gardner et al. 1985; Gardner et al. 1990; Vanderploeg et al. 1992). At cold environmental temperatures organisms also require high lipid levels to maintain membrane fluidity, intracellular transport processes and overall cell metabolism (e.g. Adams 1998; Olsen 1998). Given the large differences in water temperature between lakes at different elevations, I hypothesized that populations of

G. lacustris from high elevation lakes would have higher lipid levels than those at low elevations (Chapter 8). I used a micro-method to analyse and compare the lipid reserves of individual *G. lacustris* from lakes at different elevations. I also examined patterns of lipid use and accumulation in juveniles, immature males and immature females.

The research in this thesis is a start to understanding the ecology of *G. lacustris* in relation to biotic and abiotic factors. Only by understanding the basic ecological relations between organisms and their environment can ecologists hope to undertake meaningful experiments and provide competent predictions regarding the responses of individuals and communities to altered conditions in the future. The work shows that investigations of organisms along natural gradients, such as the temperature-elevation gradient examined here, will provide an excellent knowledge base to help predict the direction and magnitude of population responses that may ensue from changes in environmental temperature caused by global climatic warming.

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2. LIFE HISTORY STRATEGIES OF *Gammarus lacustris* (CRUSTACEA: AMPHIPODA) FROM TEN LAKES ALONG AN ELEVATION GRADIENT IN WESTERN CANADA

Introduction

The response of aquatic organisms to global warming is dependent on the magnitude of warming (Beisner et al. 1997) and the sensitivity of those species to increasing temperatures (Baur and Raboud 1988; Adolph and Porter 1996; Rahel et al. 1996). Here, we use the negative relationship between elevation and water temperature (e.g. Lamontagne et al. 1994) to examine the influence of temperature on the life history strategies of *Gammarus lacustris*, an amphipod. The direction and rate of response of species to increased temperatures is a product of the intrinsic physiological abilities of the individuals and the amount of relevant genetic variation present that permits the population to respond and deal with the changes (Hairston 1996). Environmentally mediated selective pressures can lead to either rapid evolution or the expression of alternative phenotypes (Spitze and Sadler 1996). If the former occurs, they are regarded as specialists adapted for a specific set of biotic and abiotic conditions. Species that adopt alternative phenotypes are viewed as generalists (Stearns 1992).

Phenotypic plasticity is the ability of a genotype to exhibit alternative phenotypes in alternative environments (Bradshaw 1965; Stearns 1989). Within each phenotype, the morphology, physiology and life history are integrated to function under a specific set of ecological conditions (Stearns 1989). Examples include predator-induced morphological defences (Havel 1987) such as the development of helmets, long tailspines and neckteeth in *Daphnia* in the presence of *Chaoborus* (Havel and Dodson 1984; Spitze 1992; Tollrian 1993) and the bent-over growth of the barnacle *Chathamalus anisopoma* in the presence of a predatory snail *Acanthina angelica* (Lively 1986). *Daphnia* morphs with melanized carapaces (Hessen 1996) and highly pigmented copepods (Hairston 1976) in environments with high UV exposure are examples of phenotypes that differ primarily in their physiology.

Alterations in morphology and physiology may carry an associated energetic cost. Therefore, we may expect trade-offs in processes that compete for a limited amount of energy or are constrained by morphology. For example, the production of a bent-over body for protection against predation by snails in the barnacle *Chathamalus anisopoma* decreases its fecundity (Lively 1986). Similarly, fecundity is decreased under size-selective predation on large individuals which acts to reduce the size and age at maturity (e.g. Reznick et al. 1990; Wellborn 1994; Wilhelm et al. in press). Polar marine invertebrates produce fewer but larger eggs than conspecifics from lower latitudes (Clarke and Gore 1992) because more energy is required to produce viable young at colder temperatures. Thus, highly plastic life history strategies are adaptations to buffer against biotic and abiotic environmental variation and to improve the dispersal and establishment of populations in new habitats (e.g. Morgan 1980).

Individual species have different impacts in ecosystems and the trajectory of any change in populations or communities as a result of altered environments may result in an altered ecosystem (Morgan 1980; Spencer et al. 1991; Hairston 1996). *Gammarus* *lacustris* is a key species in lake and pond ecosystems (Wilhelm and Schindler 1999) and changes in its population are expected to affect several trophic levels. In fishless alpine lakes it is a predator of zooplankton (Wilhelm and Schindler 1999), structuring plankton communities in a manner similar to planktivorous fish. It is also important in the recycling of nutrients such as phosphorus from lake sediments to the pelagic zone (Wilhelm et al. in press a). In lakes with fish, it is an important food item for fish (Donald et al. 1980). To predict the response of *G. lacustris* populations to increased water temperatures requires an understanding of the species' life history strategies. We used the natural variation in water temperature of lakes along an elevation gradient from prairie to alpine (736 to 2320 m above sea level) to examine the life history strategy of ten populations of *G. lacustris*. Specifically, we examined life cycle length, growth rate, age and size at maturity, reproductive investment, fecundity, and size of young at hatching in relation to elevation (hence water temperature).

Materials and Methods

Study lakes

In this section we give detailed descriptions of the study lakes. Lakes were chosen for their elevation, ease of access, and the availability of historic data.

Cooking Lake is a large, shallow, hyper-eutrophic prairie lake located ~25 km east of Edmonton, in Alberta, Canada (Table 2-1) (Mitchell and Prepas 1990). The lake is isothermal and warms to a maximum temperature of 20 to 25°C in mid- to late-July. The ice-free season usually spans from the end of April to the middle of November. After freeze-up, the water becomes anoxic and remains unoxygenated until break-up. The lake does not contain any sportfish, but some brook stickleback survive in some years. The biotic community is dominated (biomass) by chironomids, followed by amphipods, *Gammarus lacustris* and *Hyalella azteca* (Mitchell and Prepas 1990). Large blooms of *Daphnia* occur in some years shortly after ice-out (Wilhelm pers. obs.), but only last for a period of one to two weeks. Large numbers of waterfowl use the lake as a staging area during spring and fall migration.

Sauer Lake is a small, mesotrophic prairie lake located ~40 km west of Edmonton, Alberta, Canada (Table 2-1). The lake stratifies during the open-water season with surface temperatures reaching up to 23°C, while bottom waters remain near 5°C (Mitchell and Prepas 1990). Intensive sampling in 1982 and 1983 showed that oxygen was depleted to less than 1 $mg \cdot L^{-1}$ below 6 m from the beginning of June through August, and it is thought that in years when the onset of ice cover is rapid the lake does not mix completely and the bottom water remains anoxic all year. The anoxic conditions in the hypolimnion lead to a high (138 to 164 $\mu g \cdot L^{-1}$) concentration of phosphorus, some of which is mixed into surface waters before and after the lake stratifies (Mitchell and Prepas 1990) leading to moderate concentrations of chlorophyll *a* in spring. The entire water column was anoxic by mid-February in 1983. Such conditions result in the frequent winterkill of fish. The lake is stocked annually with rainbow trout for a put-and-take fishery.

Benthic invertebrates in Sauer Lake have not been studied. *Gammarus lacustris* and *H. azteca* were the most conspicuous invertebrates when the near-shore area was

Table 2-1. Summary of physical and chemical parameters for ten lakes from which populations of *Gammarus lacustris* were studied.

Lake	Elev. m (a.s.l.)	Region	Location[g] Lat.	Long.	Area ha	Max. Depth m	Mean Depth m	Break-up	Freeze-up	Total phosphorus $\mu g \cdot L^{-1}$	Chl *a* $\mu g \cdot L^{-1}$	Fish
Cooking Lake[a]	736	Prairie	113°03'W	53°25'N	3 600	4.6	1.7	mid-Apr.	mid-Nov.	232-270	80-85	BS[j]
Sauer Lake[a]	740	Prairie	114°05'W	53°37'N	8.5	14.0	4.2	mid-Apr.	mid-Nov.	30-40	4.5-6.2	BS, RD, RT[j,k]
Mildred Lake[b]	1033	Montane	118°03'W	52°53'N	6.0	3.5	---	mid-Apr.	mid-Nov.	20-25[h]	1.0-10	none
Edith Lake[c]	1024	Montane	118°03'W	52°54'N	50	18.0	5.1	late-Apr.	early-Nov.	4.5-5.5[h]	0.8-1.9	RT, LC
Pyramid Lake[c]	1186	Montane	118°05'W	52°55'N	127.4	19.0	8.7	mid-May	mid-Nov.	6.3-8.7[h]	0.7-1.0	RT, LT, LC, LS
Cutt Lake[d]	1455	Montane	118°20'W	52°53'N	15.5	7.5	3.7	mid-May	late-Oct.	6.9-7.3[h]	0.7-1.3	None
Maligne Lake[e]	1675	Sub-alpine	117°33'W	51°36'N	2 066	96.0	38.3	early-Jun.	late-Nov.	3.5-6.8[h]	0.5-0.9	RT, EBT
Harrison Lake[f]	2243	Alpine	115°48'W	51°32'N	8.4	10.7	5.4	late-Jun.	early-Oct.	6.0[i]	0.82	BT
Pipit Lake[f]	2217	Alpine	115°51'W	51°35'N	10.6	21.0	12.6	early-Jul.	early-Oct.	5.0[i]	0.83	None
Snowflake Lake[f]	2320	Alpine	115°50'W	51°35'N	7.1	12.5	6.1	early-Jul.	early-Oct.	4.0[i]	0.83	None

[a] Mitchell and Prepas 1990; [b] Anderson 1970a; [c] Anderson and Donald 1978a; [d] Anderson and Donald 1980; [e] Donald and Anderson 1978; [f] Anderson and Donald 1978b; [g] Ward 1974 (for mountain lakes); [h] values from fall 1996 and spring 1997 samples (total phosphorus and Chl *a*); [i] Leavitt et al. 1994; D.W. Schindler unpub. data. (total phosphorus and Chl *a*); [j] Lake winterkills frequently; [k] stocked annually for put-and-take fishery; Fish Species: BS-brooks stickleback; RT-rainbow trout; EBT-eastern brook trout; LT-lake trout; BT-bull trout; LS-longnose sucker; LC-lake chubb; RD-red belly dace.

surveyed for this study. Zygoptera and Plecoptera larvae and corixids were also collected. While live invertebrates were collected from the lake just after the spring thaw had started in the spring of 1996, many dead brook stickleback and redbelly dace were observed near shore indicating winterkill conditions while the lake was ice-covered.

Mildred Lake is a small, shallow montane lake in Jasper National Park (Table 2-1). There are no inflows to the lake and the small outflow to the Athabasca River has a control structure on it. Before the construction of this structure, the lake was connected to the river during times of high flow. Although the lake is only 3.5 m deep, Anderson (1970a) found that it was stratified when he sampled it in August 1968. The surface water temperature was 17°C while the bottom temperature was near 7°C. The lake is well sheltered which explains why it stratifies. Macrophytes are abundant throughout the entire lake and in winter the decay of this biomass leads to anoxic conditions throughout the entire water column (Anderson 1970a). For this reason fish are absent from the lake. When the lake was surveyed in 1968 no *G. lacustris*, *H. azteca* or Zygoptera larvae were reported (Anderson 1970a). These organisms were the most abundant species when the lake was sampled for this study. The lake is frequented by large numbers of waterfowl in spring and fall.

Edith Lake is a large, oligotrophic montane lake in Jasper National Park (Table 2-1). It is considered to have one of the most intensely developed (cottages) shorelines of all the lakes in the mountain national parks (Anderson and Donald 1978a). Although the lake is 17 m deep, it remains nearly isothermal down to 14 m throughout the open-water season because of its orientation along the direction of prevailing winds (Anderson and Donald 1978a). Both the inlet and outlet are intermittent, thus the major source of water is from snowmelt and rain during the spring and summer. Because of the sandy and rocky bottom few macrophytes are present in the lake. Anderson and Donald (1978a) cited this lack of macrophytes for the low abundance of benthic invertebrates including *G. lacustris* and *H. azteca*. However, both of these species occur in high densities in depressions filled with organic debris near shore (F. M. Wilhelm pers. obs.) and were the most abundant invertebrates sampled during this study.

A wide range of fish species were stocked into Edith Lake beginning as early as 1917. Cutthroat trout were stocked for two years starting in 1917, followed by a single stocking of Atlantic salmon in 1920. Brook trout were stocked for two years starting in 1927 while rainbow trout were first stocked in 1933 and continued to be stocked for 42 years. Splake were stocked for two years starting in 1953. Lake chubb were also introduced, probably from anglers using them as bait fish. The number of fish stocked each year is summarized in Anderson and Donald (1978a; Table 1). Of the species stocked, rainbow trout and lake chubb are the only species that remain today (D. B. Donald, Environment Canada, Regina, Canada, pers. comm.). In 1958 the lake was treated with rotenone as part of a reclamation project (Anderson and Donald 1978a).

Pyramid Lake is a large, oligotrophic montane lake located in Jasper National Park, Alberta, Canada (Table 2-1). The lake stratifies in summer with the thermocline near 4 to 6 m. In 1972 the surface water reached 18°C in August while the bottom remained just under 8°C (Anderson and Donald 1978a). Macrophytes are absent from the nearshore area, but large beds of submerged macrophytes occur approximately 15-20 m offshore in some areas. In these beds macroinvertebrates are abundant. When the lake was sampled for this study, *G. lacustris*, and *H. azteca* were the most abundant macroinvertebrates. *Mysis relicta*, leeches, Zygoptera and chironomid larvae were also common.

Native fish species in Pyramid Lake included longnose sucker and Rocky Mountain whitefish (Ward 1974). Pyramid Lake was stocked with fish starting in 1917 (Anderson and Donald 1978a). Lake trout and Atlantic salmon were stocked for 22 and seven years, respectively, starting in 1917. In 1918, a one-time stocking of landlocked Atlantic salmon (Ouananiche) was undertaken. Rainbow trout were stocked for 21 years starting in 1933. Beginning in 1961, 1964 and 1968, splake, brook trout, and cutthroat trout were stocked for seven, two and one years, respectively. Similar to Edith Lake, lake chubb were introduced by anglers using them as bait fish. Today, longnose sucker, rainbow trout, lake trout and lake chubb remain (D. B. Donald, Environment Canada, Regina, Canada, pers. comm.).

Cutt Lake is a small, oligotrophic montane lake in Jasper National Park, Alberta, Canada (Table 2-1). The lake stratifies during the open-water season with maximum surface temperatures near 17°C in August, while bottom temperatures remain near 9°C (Anderson and Donald 1980). The ice-free season lasts from the middle of May to late-October. Large flat rocks are present along the east shore of the lake near the outflow while the rest of the shoreline is marshy. Beyond a depth of 0.5 to 0.75 m the bottom substrate is a rich organic silt that seems "bottomless". In the area where *G. lacustris* was collected, it was numerically the most abundant macroinvertebrate. Zygoptera and chironomid larvae also occurred with *G. lacustris*. Anderson and Donald (1980) reported Zygoptera and chironomid larvae as the most abundant macroinvertebrates when they sampled the deepest spot in the lake in 1978. The lake was originally fishless, but was stocked with cutthroat trout for 6 years starting in 1960 (Anderson and Donald 1980). The trout did not reproduce in the lake due to the lack of suitable spawning habitat and the lake is now considered to be fishless again (last sampling in mid-1980's D. B. Donald, Environment Canada, Regina, Canada, pers. comm.).

Maligne Lake is a large, oligotrophic sub-alpine lake in Jasper National Park, Alberta, Canada (Table 2-1). The lake stratifies weakly during the open water season which lasts from mid-June to early-November. Surface water temperatures reach a maximum of 10 to 13°C in summer (Donald and Anderson 1978), while bottom waters remain near 4°C. Fish were not present in the Maligne River watershed before 1928. Brook trout were stocked into Maligne Lake for 13 years starting in 1928 (Donald and Anderson 1978) and rainbow trout were stocked for three years starting in 1971. Chironomid larvae were numerically the most abundant (>1000 ind.$\cdot$m^{-2}) macroinvertebrates sampled in 1976 and 1977 (Donald and Anderson (1978), while *G. lacustris* occurred at only 3 ind.$\cdot$m^{-2}. However, amphipods are locally abundant, because areas with >30 ind.$\cdot$m^{-2} were sampled in 1996-98 (Wilhelm unpub. data). Trout are still present in Maligne Lake and are known to prey on *G. lacustris*. However, given the size of the lake, I consider this predation pressure to be too weak to influence *G. lacustris* size at maturity (cf. Wilhelm et al. in press b). In addition, our samples contained large amphipods similar in size to those collected from the other fishless study lakes, which also suggests that fish predation has had little influence on amphipod size in Maligne Lake.

Harrison Lake is a small, oligotrophic alpine lake in the Front Ranges of the Rocky Mountains in Banff National Park, Alberta, Canada (Table 2-1). Harrison Lake is bowl shaped with a shallow ice-scoured shelf to a depth of ~ 1 m which extends 1 to 5 m from shore. The ice-free season lasts approximately 100 days from late-June until late-September to mid-October. Maximum surface water temperatures reach ~12 °C in August (Anderson and Donald 1978b, D. W. Schindler unpub. data). The lake stratifies weakly; temperature differences between the surface and bottom are usually less than 3 °C. Three small streams, one on the west side and two on the southwest side direct snowmelt runoff to the lake. Inflow volume decreases as the catchment snowpack diminishes throughout the summer. A single shallow outflow is located on the north shore of the lake. A small waterfall approximately 20 m downstream of the outlet prevents immigration of fish from the stream. Macrophytes are absent from the lake and chironomid larvae, cyclopoid copepods (mostly *Diacyclops bicuspidatus thomasi*), *Daphnia pulex var.*, and the amphipod *G. lacustris* are the most common invertebrates. Bull trout, *Salvelinus confluentus*, is the only fish species present in Harrison Lake, determined from fishing records earlier this century, gill net surveys in 1977, and continued monitoring in 1996, 1997 and 1998. The bull trout population is considered unexploited because of the lake's remote location. Bull trout prey selectively on the largest *G. lacustris* (Wilhelm et al. in press b).

Pipit Lake is a small, oligotrophic, alpine lake in the Front Ranges of the Rocky Mountains in Banff National Park, Alberta, Canada (Table 2-1). The ice-free season usually lasts from early-July until late-September. In some years the lake stratifies weakly, but stratification can be broken at any time during the summer by high winds or snowfall. There are no macrophytes in the lake and an ice-scoured shelf is present to a depth of 1 m, extending up to 5 m from shore in some locations. The west end of the lake is bordered by a steep cliff and the shoreline, composed mainly of scree, drops steeply to the maximum depth. *Gammarus lacustris* is abundant in interstitial spaces formed by the rocks, which makes it difficult to sample them. Chironomid larvae and large *Daphnia middendorffiana* are the most abundant invertebrates in the profundal region of the lake (D. B. Donald, unpub. data; Wilhelm et al. 1998). From 1960 to 1966, rainbow trout were stocked into Pipit Lake in an attempt to create a recreational fishery. However, the fish did not reproduce and the lake is now fishless again.

Snowflake Lake is a small, oligotrophic alpine lake in the Front Ranges of the Rocky Mountains in Banff National Park, Alberta, Canada (Table 2-1). Ice-out usually occurs between the end of June and mid-July. The lake stratifies weakly in late summer when surface temperatures reach a maximum of 9-12 °C. Freeze-up occurs between late-September and early-October (Anderson 1970b; D. W. Schindler unpub. data).

The biotic community of Snowflake Lake has been affected several times by fish and zooplankton introductions. The lake was originally fishless, but was stocked with brook trout, cutthroat trout and rainbow trout between 1960 and 1966. The presence of fish altered the invertebrate community. *Gammarus lacustris* became rare, and *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* were replaced by *Diacyclops* and rotifers (Anderson 1972; McNaught et al. 1999). Fish did not reproduce in the lake and declined gradually, with the last fish caught in 1984. Once the lake was devoid of fish, *G. lacustris* and *D. middendorffiana* populations recovered (McNaught et al. 1999), but *H. arcticus*, remained absent. *Hesperodiaptomus arcticus* was reintroduced on July 30, 1992, but did not become abundant in the plankton until late in 1996. *Hesperodiaptomus arcticus* reintroduction has caused the decline in abundance or elimination of all cyclopoid copepod and rotifer species (Schindler unpub. data).

Collection of physical data

Surface water temperature was recorded with an alcohol thermometer at the time of sampling. Whole water-column temperature profiles at 1 m intervals were taken in Pipit, Snowflake, and Harrison Lakes with a Flett Mark II thermistor temperature probe because these lakes are part of a suite of lakes sampled regularly for other purposes. The mean surface water temperature (0-2 m) was calculated for these lakes for July and August of each year in which at least 2 or more temperature profiles were taken per month. We compared the mean surface water temperature from the profiles to the mid-summer surface water temperature (MSSWT, 0-2 m) calculated from the temperature-elevation relationship of Lamontagne et al. (1994). This relationship was used to calculate the surface temperature for lakes which were not sampled in mid-summer. To compare growth rates versus temperature, we calculated mean water temperatures for Cooking, Harrison, Pipit and Snowflake Lakes for sampling intervals used to calculate growth rates (see below). Total phosphorus and chlorophyll *a* concentrations were obtained from 500 mL water samples collected 0.1 m below the surface at the time when amphipods were sampled. For details of analysis methods see McNaught et al. (1999).

Field collections of G. lacustris

Gammarus lacustris were collected from the study lakes between 1994 and 1998. Sampling occurred primarily during the open-water season, but collections were also made while the lakes were ice-covered. When the lakes were ice-free, amphipods were obtained by scraping a 500-µm-mesh dip net over the bottom substrate in water 0.5 m to 1.3 m deep. In Snowflake and Pipit Lakes, amphipods were also collected at night by vertical hauls of a 64-µm-meshed Wisconsin-style plankton net hauled from 1 m above the lake bottom to the surface over the deepest spot of the lake. Various efficiencies have been reported for such a net (25-40% Anderson and Donald 1978b, Anderson 1970c). However, because *G. lacustris* represents a much larger particle than zooplankton and is not easily influenced by bow waves, we believe the sampling efficiency is high. When the lakes were ice-covered, amphipods were obtained through a hole in the ice using a long handled dip net to reach the sediment in shallow lakes, or by using a bottom closing net (Lasenby and Sherman 1991) in deep lakes e.g. Snowflake and Pipit. Sweeps or hauls were conducted until approximately 30 individuals of each cohort (identified by subjectively by eye in the field) were collected. Amphipods were sorted from the debris while alive using the method of Wilhelm and Hiebert (1996) and were placed into clean lake water at ambient temperature in which they were held until processed in the lab. Amphipods from the prairie and montane lakes usually arrived back at the lab on the day they were collected, and were analyzed immediately. Amphipods from the alpine lakes were either taken to our base camp and analyzed immediately, or transported back to the lab if collections occurred close to a crew change, usually within 2 to 3 days.

Measurement and identification of G. lacustris *life stages*

Amphipods were sacrificed in groups of ten in 4% formalin. After movement ceased amphipods were transferred back to native lake water for measuring and stage determination under a Wild M5 Stereo microscope fitted with an ocular micrometer. Lengths of all individuals were measured from the tip of the rostrum to the tip of the telson after straightening with fine forceps (Wilhelm and Lasenby 1998). Amphipods were classified into the following stages: young-of-the-year (YOY), juveniles, immature females, immature males, mature females, mature males and spent/resting females. Individuals released in spring or mid-summer were classified as YOY until December 31 of the year in which they were released, or until secondary sexual characteristics could be distinguished (if before December 31). Individuals were identified as juveniles from Jan. 01 following the year of their release until secondary sexual characteristics could be observed (montane and alpine populations only). Immature females were distinguished from the time oostegites developed on the inside of coxal gills until the eggs were deposited in the brood pouch. Individuals were classified as immature males if genital papillae were present on the 7th peraeonic segment but calceoli on the flagellum of the second antenna were absent. Mature females were classified as those individuals that had setose oostegites and eggs in the brood pouch. Mature males were distinguished by the presence of genital papillae and calceoli on the flagellum of the second antenna (after Menon 1966, Bousfield 1989). Spent or resting females were individuals that had distended oostegites with setae, or distended oostegites without setae (after Steele 1967). Life stages and size-frequency histograms were used to identify cohorts. For some samples from alpine populations with multiple cohorts it was necessary to use counts of segments on the flagellum of the second antenna and dry weights to distinguish individuals belonging to different cohorts. The mean size of cohorts was plotted versus sampling date for lakes for which multiple samples per year were collected.

Growth rates

We examined the relationship between growth rate and water temperature for Cooking, Harrison and Snowflake Lakes. Growth rate was calculated as:

$$G = \frac{L_2 - L_1}{T_2 - T_1}$$

where G is the growth rate (mm·day^{-1}); L_1 and L_2 are body length (mm) at time one (T_1) and time two (T_2), respectively. Sampling intervals varied among lakes and can be determined from dates when life history samples were taken (see results). Growth rates were calculated for the YOY to immature life stage interval. Because this represents a relatively small span of the life cycle, we chose to use the linear instead of the exponential model to estimate growth. Maturing individuals were not included to avoid underestimating the growth rate during maturation when energy is diverted from growth in size to reproduction. Relationships between growth rate and water temperature were examined using the method of least squares regression. To determine if the growth rate differed between populations, we compared populations using analysis of covariance (ANCOVA), with growth rate as the response variable, water temperature as the covariate and lake as the grouping variable. Differences among populations were detected with a Tukey post hoc test (Sokal and Rohlf 1981).

Reproductive investment, fecundity and size of young at hatching

Mature females were sacrificed individually so that any eggs or young dislodged from the brood pouch could be assigned to the female from which they were released. To determine if the size of reproductive females differed among lakes, we used a one-way ANOVA followed by a Tukey post hoc test to compare the size of reproductive females among lakes and years. Because the data set was too unbalanced (i.e. mature females from one year only for some lakes), we could not analyse it with a two-way ANOVA using lake and year as factors.

The reproductive investment for each female was calculated as:

$$RI\ (\%) = \frac{EW}{FW} \times 100$$

where RI is the reproductive investment (%); EW is the dry weight of eggs (mg) and FW is the dry weight of the female (mg; without eggs). Because eggs may be displaced from the brood pouch as egg volume increases with development and females may lose weight while carrying the developing young, only females with stage 1 or 2 eggs were included to avoid biassing the calculation. Again, because of the unbalanced data set we were restricted to analysing RI among lakes and years with a one-way analysis of variance (ANOVA) followed by a Tukey test to detect means that differed.

All eggs or young from each female were counted, staged following the description of Sheader and Chia (1970), and transferred to a pre-weighed drying boat. Females were transferred to a second pre-weighed drying boat and all were dried at 55°C for 24 to 48 h. Dried eggs, young and females were then placed in a desiccator for 24 h before the dry weight was determined on a Cahn C-31 Electrobalance, for eggs and young, or a Mettler AT261 DeltaRange balance for females. Lake-specific fecundity - female size relationships were established for females with stage 1 or 2 eggs to calculate the number of eggs for a standardized (11 mm) female from each population. This calculated fecundity was then regressed versus elevation using the method of least squares.

The size of young at hatching was obtained from females that had hatched young (stage 6) in their brood pouch. Although this restricted the data set considerably, it was necessary to ensure that we only included newly hatched young. We used a one-way ANOVA followed by a Tukey post hoc test to examine if the size of young differed among lakes and years.

Results

Variation of water temperature among lakes

The ice-free season was shorter in lakes at high elevations than in lakes at low elevations, ranging from ~230 days in Cooking Lake to ~ 90-120 days in Snowflake Lake (Table 2-1, and 2-2). This difference in the ice-free season resulted in large differences in water temperature between lakes at different elevations. The estimated mid-summer surface temperature was negatively related to elevation and ranged from 22.5°C in Cooking Lake to 7.9°C in Snowflake Lake. Although the MSSWT temperature-elevation relationship predicted the temperature of low elevation lakes fairly accurately, at high elevations,

Table 2-2. Summary of summer water temperatures, dates of ice-out and life history characteristics of *Gammarus lacustris* from ten lakes in western Canada.

Lake	Year	MSSWT[a] °C	Ice-out[b]	May Surface Temp. °C	Jul. Mean Surface Temp. °C	Aug. Mean Surface Temp. °C	Age at reproduction (Years)	Cohort[c]	Size of mature females mm±SE	No. of Eggs[d]	Reproductive investment[e] %±SE	Size of young at hatching mm±SE
Cooking Lake	1996	22.5	May 02[b]	5.0	16		1	C95	12.8±0.10	38	23.0±1.08	2.12±0.015
	1997		Apr. 27[b]	5.0			1	C96	12.2±0.07	35	22.4±0.73	1.95±0.003
	1998		Apr. 10[b]	6.0			1	C97	12.7±0.15	39	20.0±1.18	2.07±0.007
Sauer Lake	1996	22.5	May 10[b]	10.0			1	C95	14.7±0.16	52	16.2±1.07	2.13±0.008
	1997		Apr. 10-15	10.0			1	C96	15.1±0.39	55	26.3±0.78	2.00±0.012
Mildred Lake	1996	19.8					1	C95	16.0±0.19	40	18.8±1.55	2.21±0.036
	1997			14.0			1	C96	14.5±0.19	40	21.3±2.64	
Edith Lake	1997	19.8		13.0			1-2	C96/95	10.8±0.16	18	19.8±1.01	
Pyramid Lake	1997	18.4		9.5			1-2	C96/95	9.6±0.14	16	17.5±1.24	
Cutt Lake	1997	15.9		11.0			2	C95	10.9±0.15	19	21.7±1.87	2.01±0.007
	1998			10.0			2	C96	12.3±0.24	26	18.7±1.35	2.10±0.014
Maligne Lake	1997	13.8	Moated May 30	4.0			2	C95	13.5±0.13	31	27.5±1.74	
	1998		Open to Narrows Jun. 15	4.0			2	C96	11.4±0.52	20	10.7±1.18	2.28±0.015
Harrison Lake	1995	8.6	Jun. 10-15				2	C93	9.7±0.09	14	22.1±0.94	2.62±0.010[f]
	1996		Jul. 05[b]		9.5	11.5	2	C94	10.8±0.13	18	25.2±0.93	2.24±0.017
	1997		Jun. 10-15		8.8	12.1	2	C95	10.2±0.16	16	18.6±1.62	2.64±0.024[f]
Pipit Lake	1995	8.8			5.1	6.5	3	C92	13.6±0.18	19	15.8±1.71	2.45±0.014

Table 2-2 Continued

Lake	Year	MSSWT[a] °C	Ice-out[b]	May Surface Temp. °C	Jul. Mean Surface Temp. °C	Aug. Mean Surface Temp. °C	Age at reproduction (Years)	Cohort[c]	Size of mature females mm±SE	No. of Eggs[d]	Reproductive investment[e] %±SE	Size of young at hatching mm±SE
Snowflake	1994	7.9	Jun. 22[b]		11.2	12.1	2	C92	13.7±0.57	16		2.36±0.014
Lake	1995		Jul. 04[b]	2.0	7.6	7.3	3	C92	14.4±0.12	24	26.0±2.02	2.33±0.007
							2	C93	12.2±0.10	16	24.7±1.77	
	1996		Jul. 06[b]		6.8	9.5	3	C93	13.4±0.17	19	22.2±1.45	2.26±0.030
							2	C94	10.7±0.18	11	18.6±1.75	
	1997		Jun. 15		8.5	10.7	3	C94	10.4±0.10	12	16.3±1.21	2.45±0.026

[a]Mid-summer surface water temperature calculated using temperature-elevation model from Lamontagne et al. (1994); [b]observed ice-out dates, others estimated; [c]data for reproductive cohorts are identified by year in which they were released (e.g. C94 was born in 1994); [d]number of eggs for an average sized reproductive female estimated from lake-specific fecundity/ female size relationships; [e]reproductive investment calculated as [(dry weight of eggs / dry weight of female)× 100] for females with stage 1 and 2 eggs; [f]YOY collected from lake sediment after emerging from brood pouch.

particularly in Snowflake Lake, the actual temperature exceeded the predicted temperature in three out of 4 years (Table 2.2). Thus, the temperature-elevation relationship provides a general overall relationship that should be augmented by temperature lake-specific temperature data where possible. The between-year time of ice-out varied by as much as three weeks in individual lakes at all both low or high elevation (Table 2-2). It is unlikely that this difference in ice-out times had a large but inconsistent influence on the maximum summer water temperature in low elevation lakes. However, in the alpine lakes the difference in ice-out time had a large influence on water temperature in summer. The early ice-out in 1994 in Snowflake Lake combined with extended periods without cloud cover resulted in the warmest July and August surface water temperatures recorded in the lake during this study. In contrast, the late ice-out in 1995 combined with long periods of cloud cover, rain and snow throughout July resulted in the lowest August surface temperature (7.3°C, Table 2-2).

Variation of life cycle length of G. lacustris *with elevation*

The life cycle length of *G. lacustris* increased with elevation from one year in prairie lakes to greater than four years in alpine lakes (Figures 2-1 to 2-11). In all lakes, young were released in the open-water season after spring break-up. Mature male and female *G. lacustris* formed precopula pairs as early as the middle of November in Mildred Lake, the beginning of February in Cooking Lake and the middle of March in Snowflake Lake (F. M. Wilhelm pers. obs.). After females had extruded their eggs into the ventral brood pouch formed by the oostegites and males had fertilized them, the pairs separated and females carried the developing embryos until the young hatched and emerged. The development of young within broods and populations was highly synchronous.

Gammarus lacustris reached sexual maturity in one year in Cooking, Sauer, Mildred, Edith and Pyramid Lakes (Figures 2-1 to 2-5). In these lakes, mature females released multiple broods during the open-water season. For example, in Cooking Lake, young from the first brood were >5 mm on July 6th, 1997 when mature females were incubating the second brood (Figure 2-1G). Similarly, the size frequency histograms for Mildred Lake indicated that females may have released up to three broods of young in 1995 (Figure 2-3A). Growth in length during the ice-covered period was minimal, as indicated by similar size-frequency distributions in Cooking Lake for samples collected in late fall and while the lake was ice-covered (Figures 2-1C to 2-1E, and 2-11A). In Edith and Pyramid Lakes, mature females also released multiple broods during the open-water season. Young released early in the open-water season matured and bred the following spring (Figure 2-4, and 2-5), while young released late in summer failed to reach maturity in one year and may have required a second year of growth (Figure 2-4B, and 2-5B). It may be possible that those young that did not mature in the first year matured in the following spring and released young late in the summer.

Gammarus lacustris in Cutt, Maligne and Harrison Lakes required two years to reach maturity and females only produced a single brood during the open-water season (Figure 2-6 to 2-8). In these lakes, a portion of the mature individuals continued to live after reproducing in their second year to breed a second time in their third year (Figure 2-7C, 2-8D, and 2-8G). In Maligne Lake, a portion of the cohort that was released in 1997 matured and released young after just one year (Figure 2-7B, and 2-7C). Mature individuals in

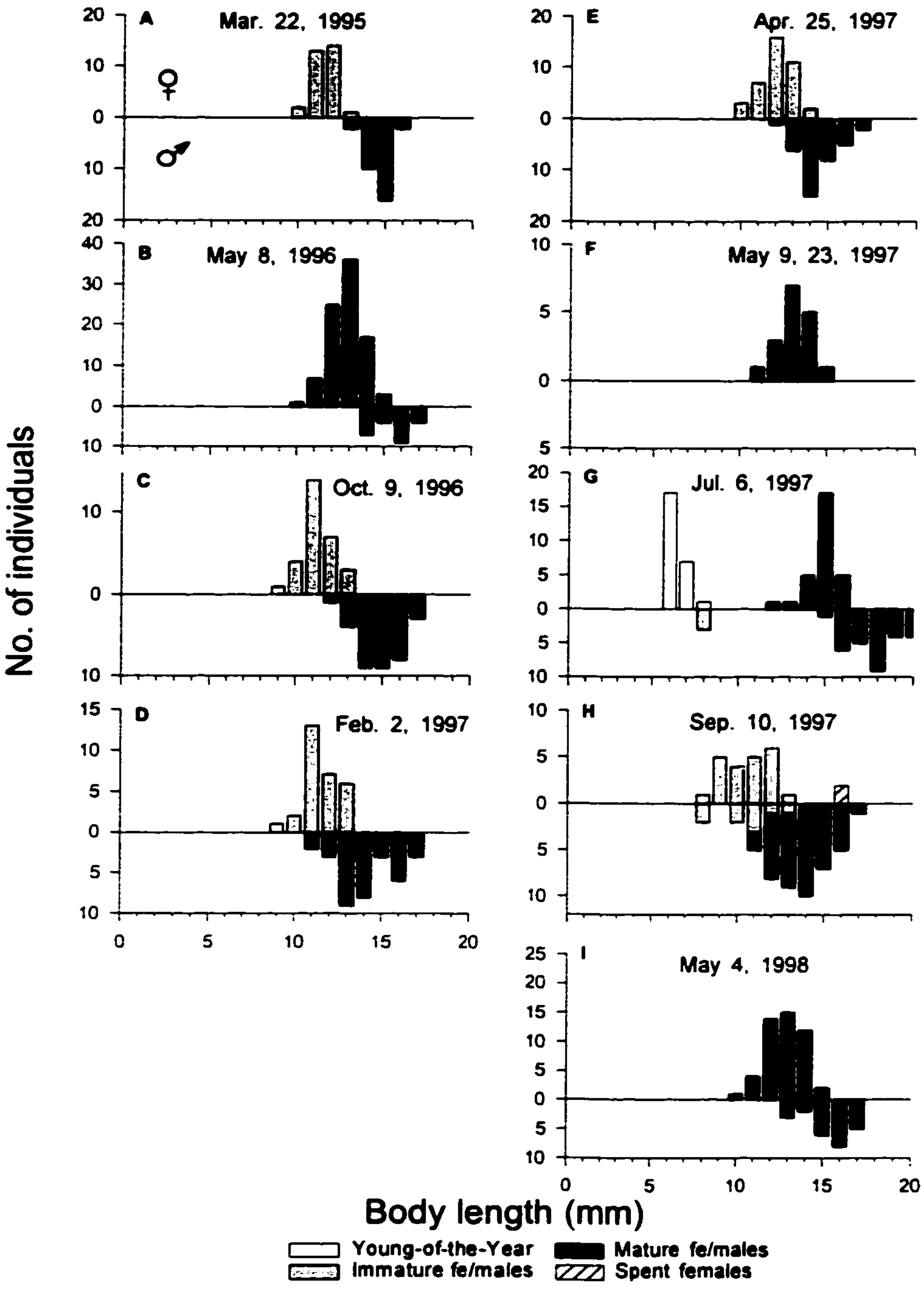

Figure 2-1. Length-frequency histograms of *Gammarus lacustris* in Cooking Lake from 1995 to 1998.

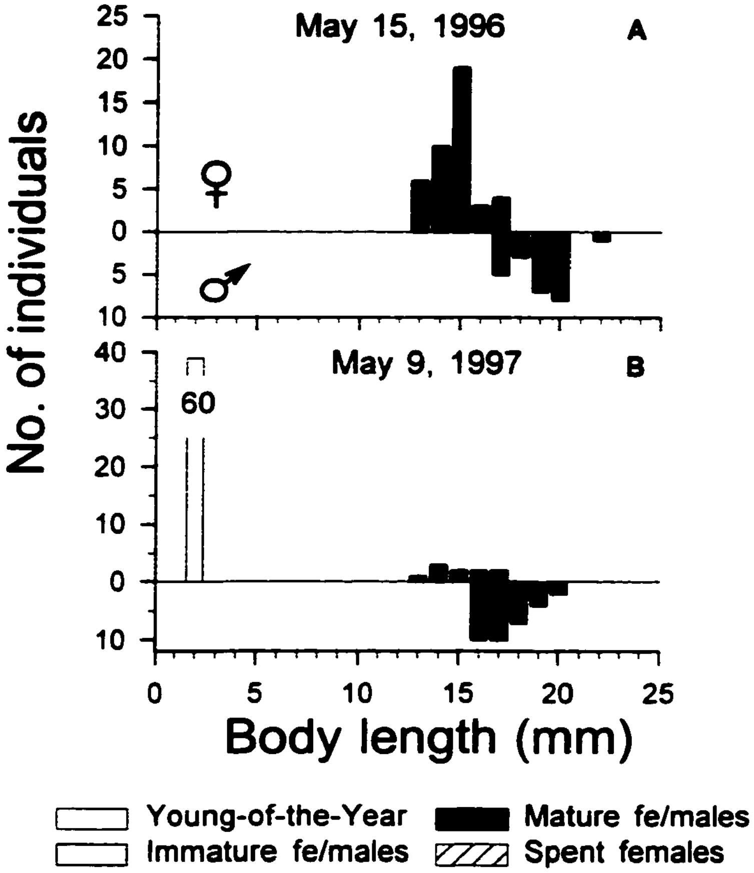

Figure 2-2. Length-frequency histograms of *Gammarus lacustris* in Sauer Lake for 1996 and 1997.

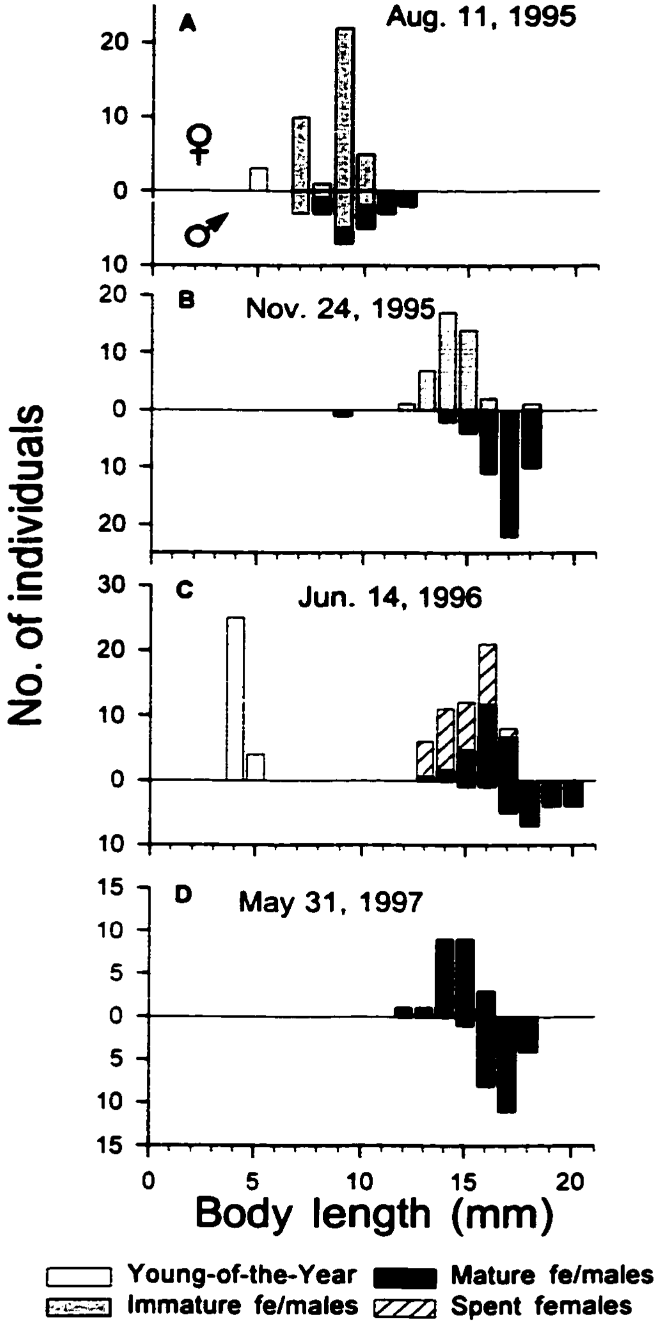

Figure 2-3. Length-frequency histograms of *Gammarus lacustris* in Mildred Lake from 1995 to 1997.

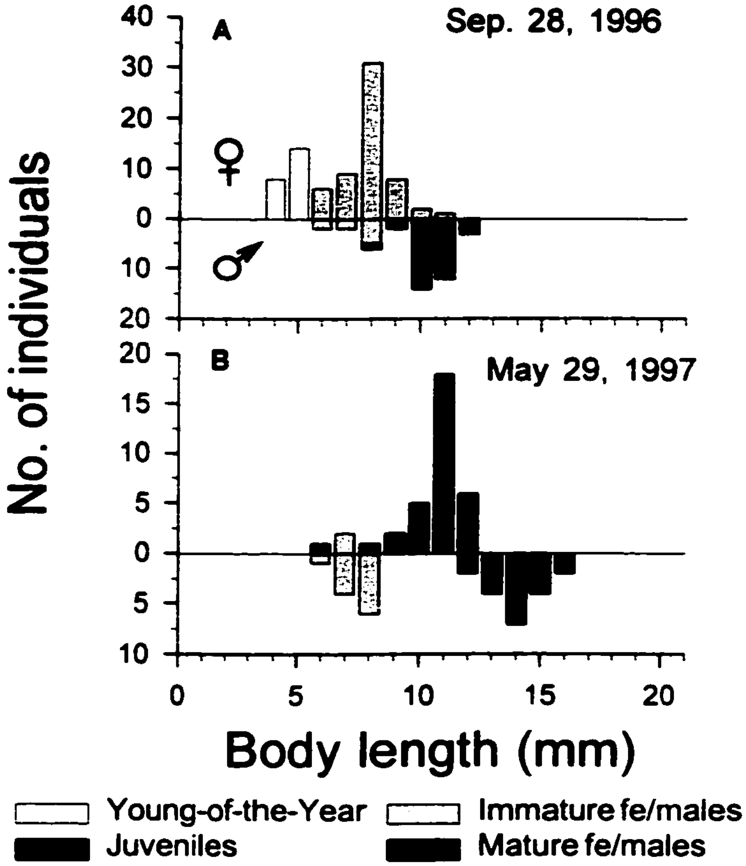

Figure 2-4. Length-frequency histograms of *Gammarus lacustris* in Edith Lake for 1996 and 1997.

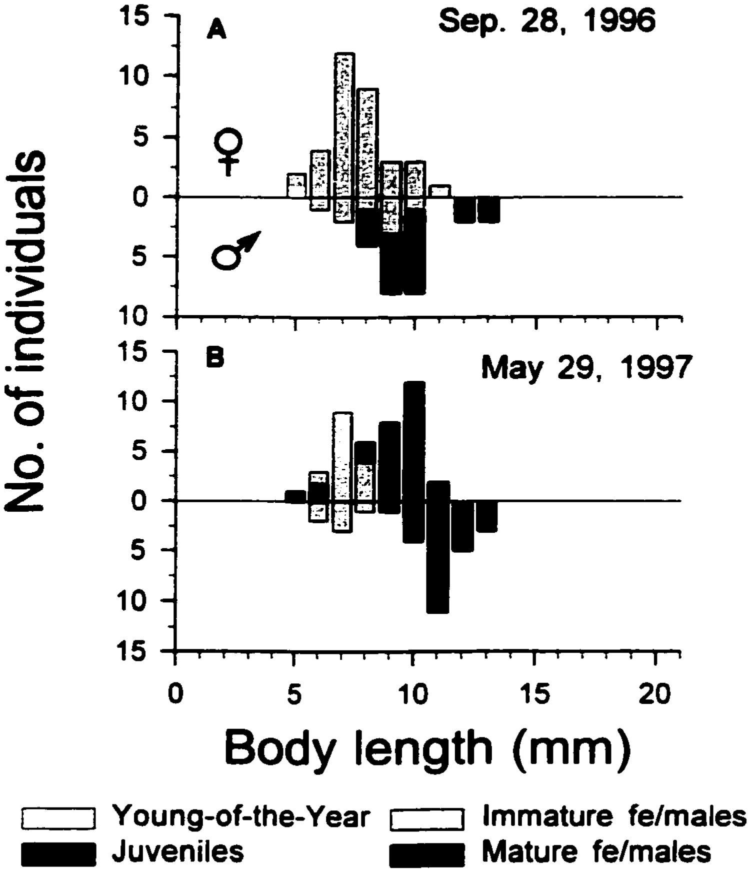

Figure 2-5. Length-frequency histograms of *Gammarus lacustris* in Pyramid Lake for 1996 and 1997.

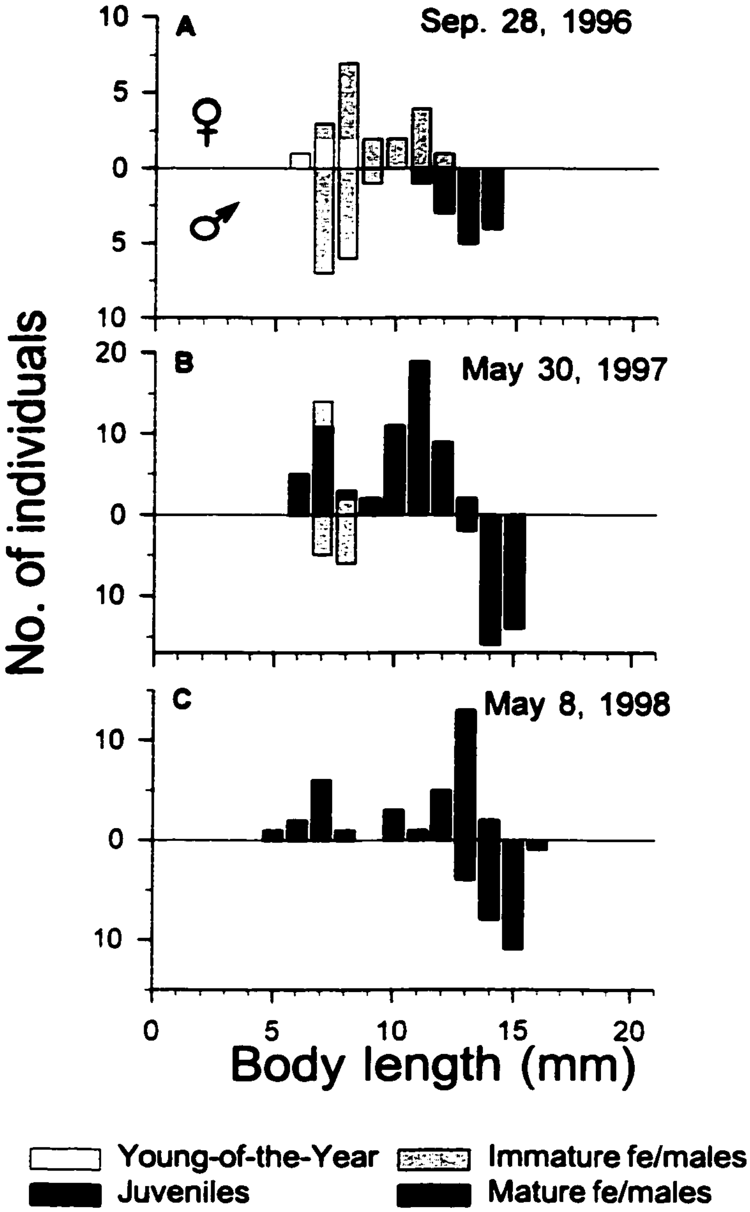

Figure 2-6. Length-frequency histograms of *Gammarus lacustris* in Cutt Lake from 1996 to 1997.

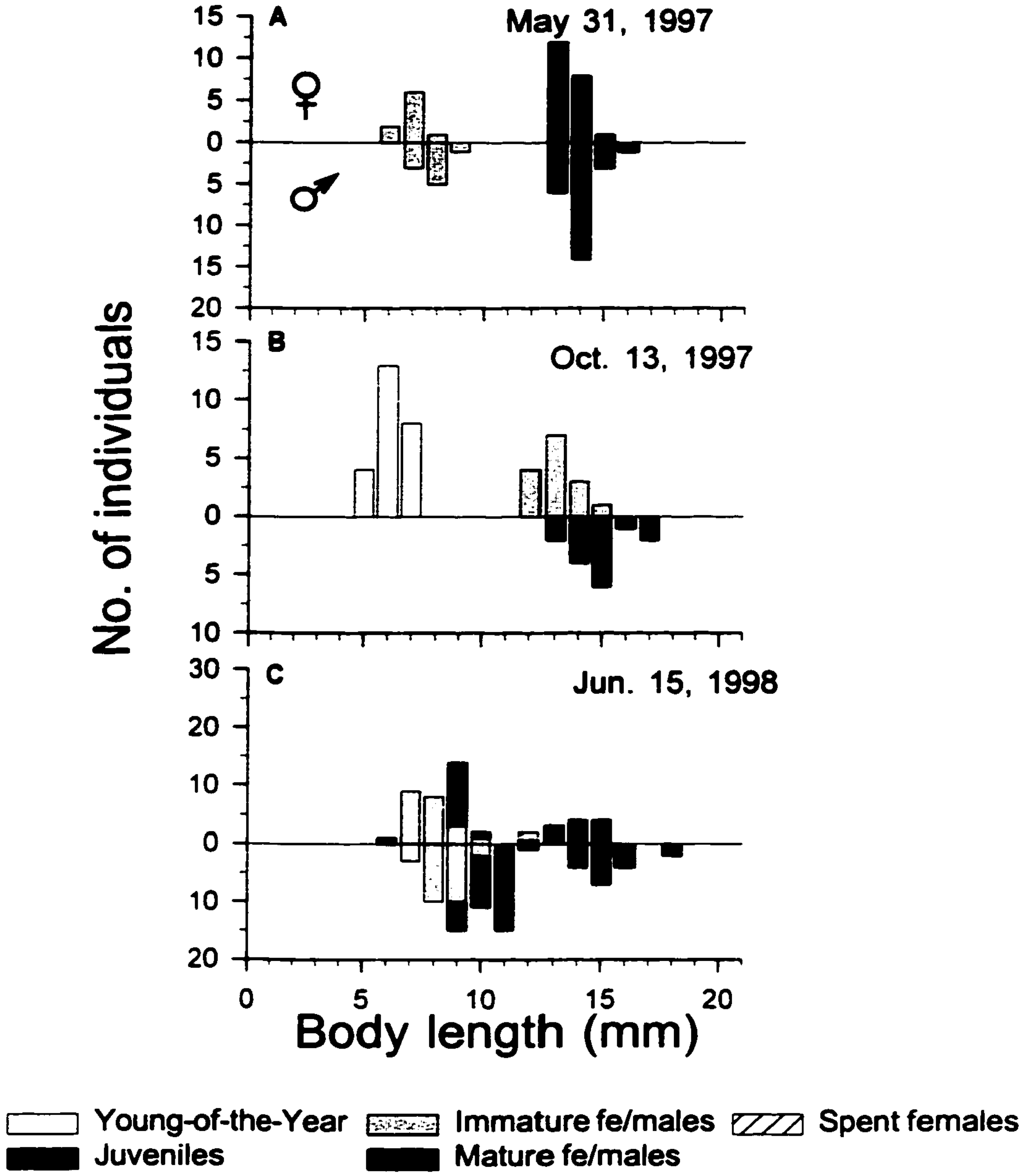

Figure 2-7. Length-frequency histograms of *Gammarus lacustris* in Maligne Lake from 1997 to 1998.

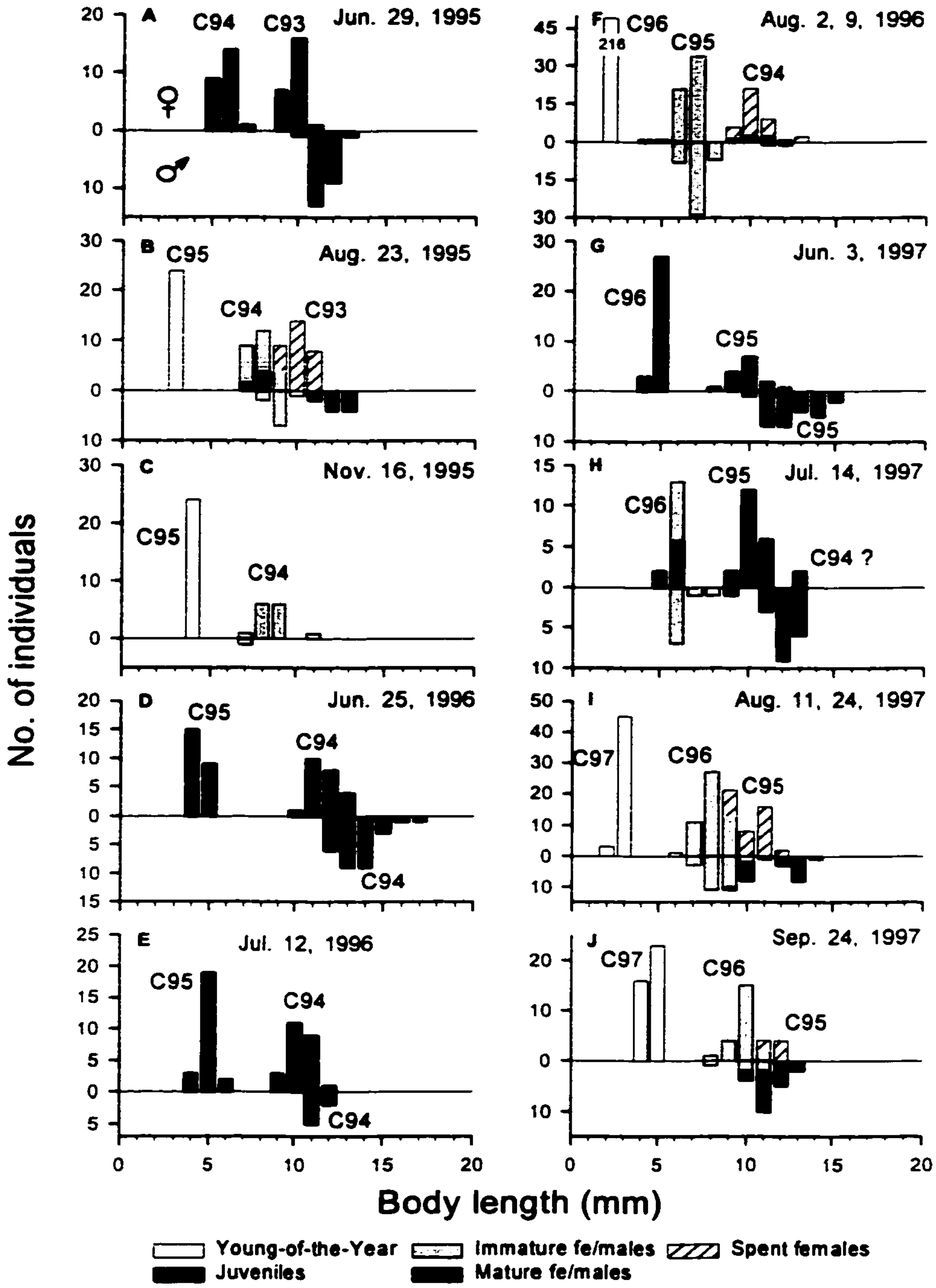

Figure 2-8. Length-frequency histograms of *Gammarus lacustris* in Harrison Lake from 1995 to 1997.

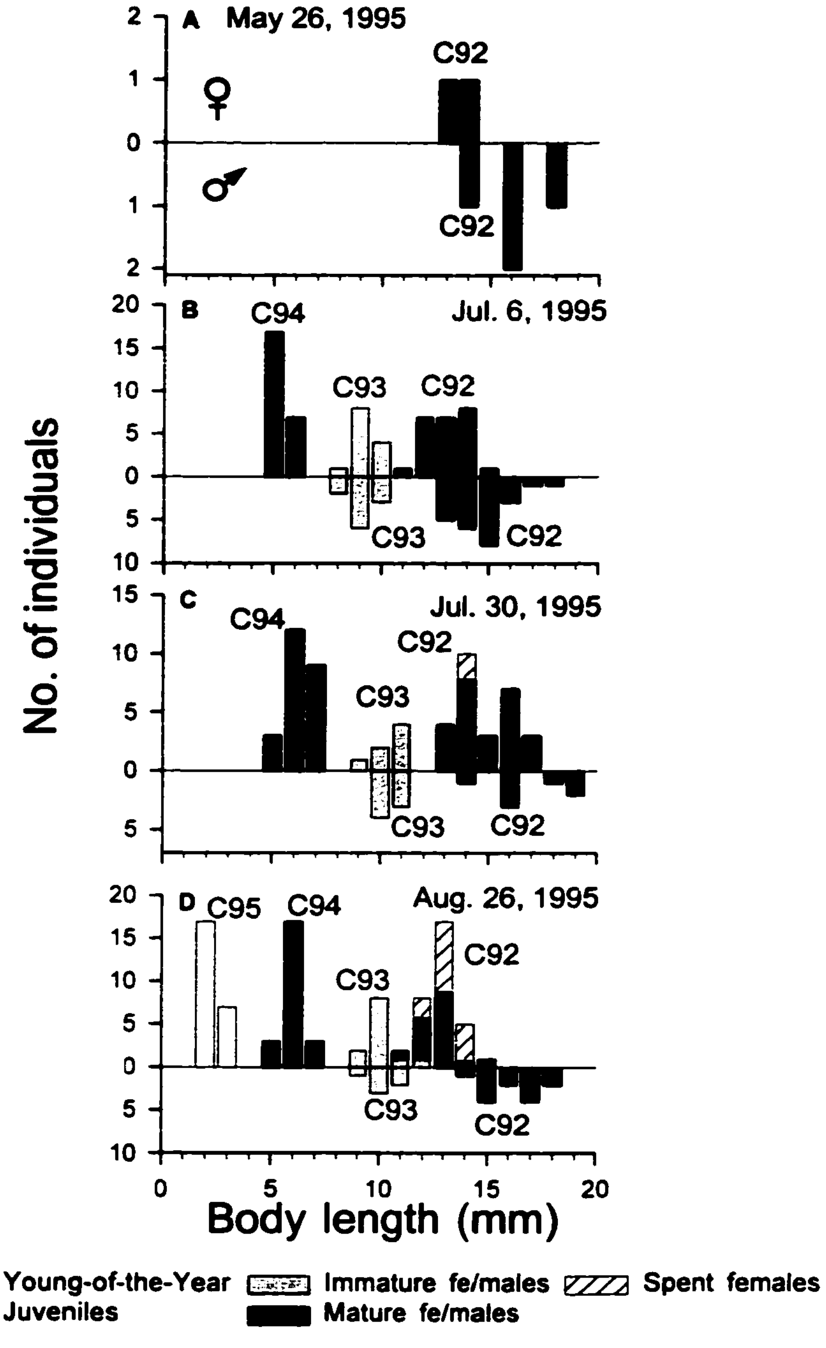

Figure 2-9. Length-frequency histograms of *Gammarus lacustris* in Pipit Lake for 1995.

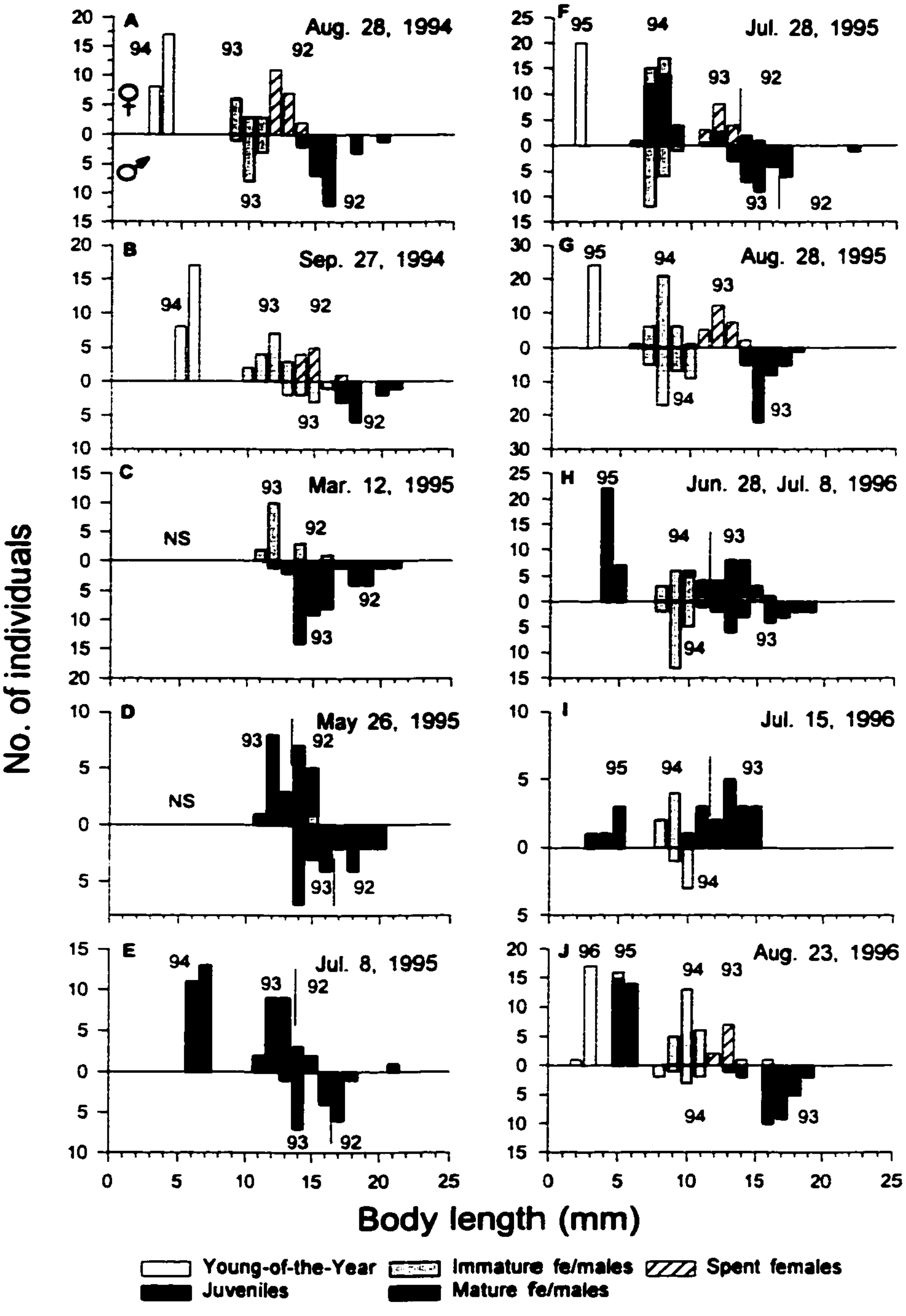

Figure 2-10. Length-frequency histograms of *Gammarus lacustris* in Snowflake Lake from 1994 to 1997. Vertical lines and numbers indicate cohorts. Cohorts are identified by the year in which they were born. NS indicates no sample taken.

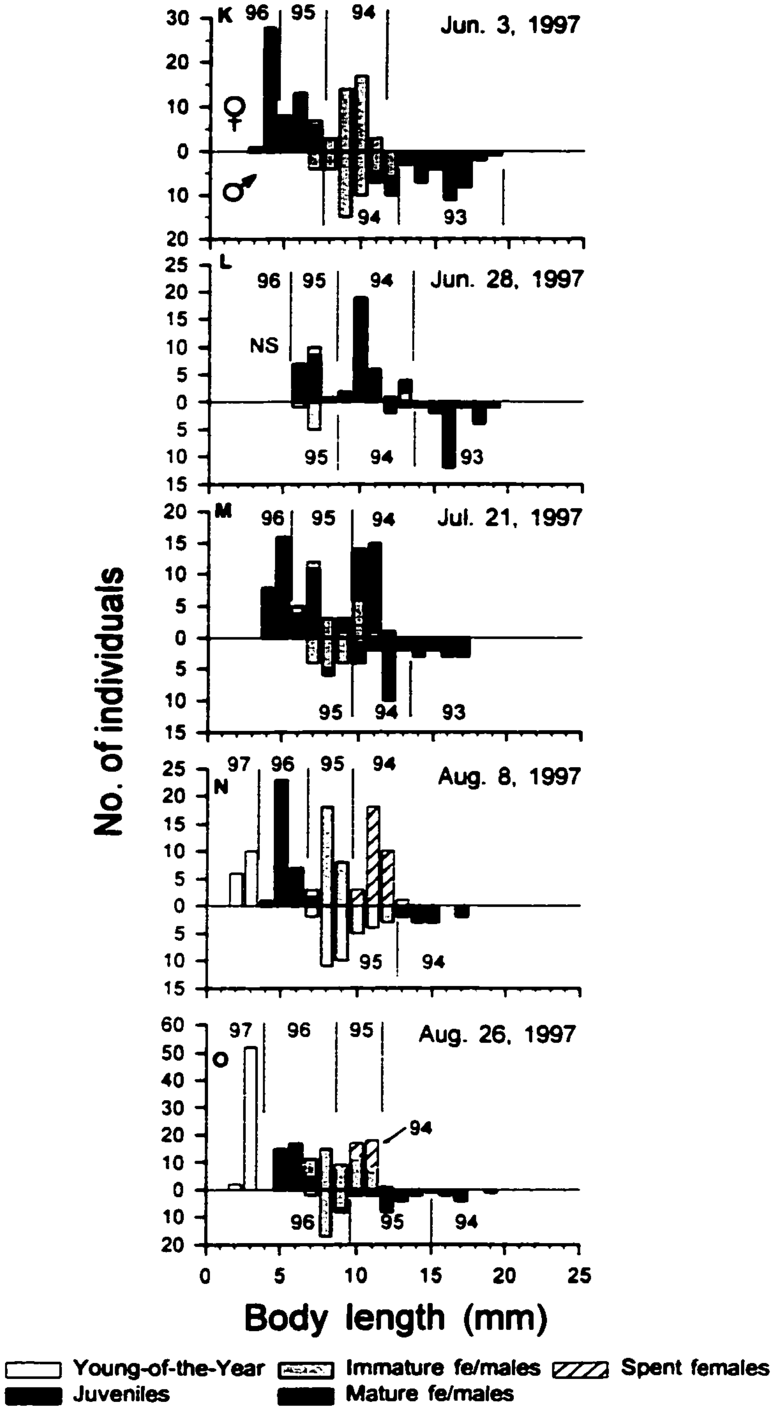

Figure 2-10. Continued.

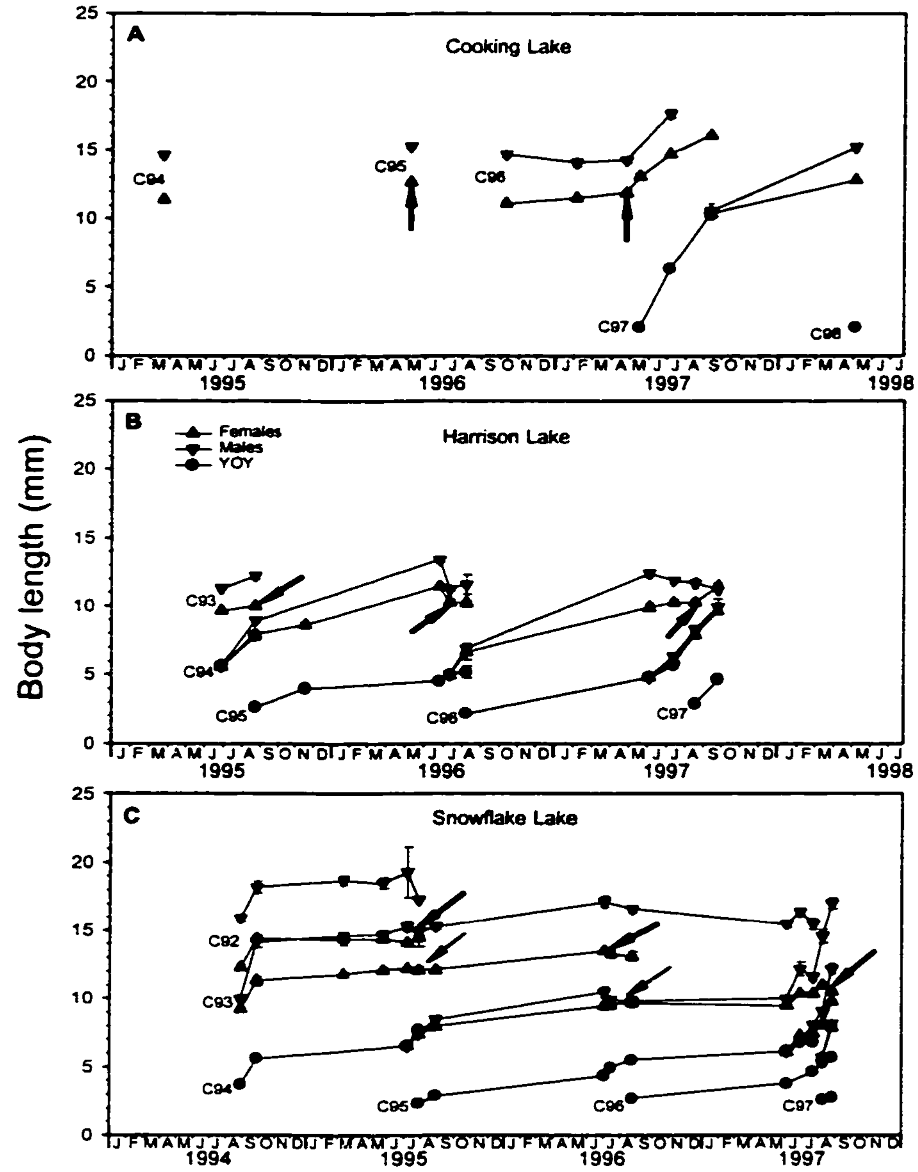

Figure 2-11. Seasonal growth curves for *Gammarus lacustris* from A) Cooking Lake, B) Harrison Lake and C) Snowflake Lake. Symbols represent means±SE; thin arrows indicate partial reproduction, while broad arrows indicate full reproduction in a cohort.

Maligne Lake were between 1 to 4 mm larger than in Harrison Lake, suggesting that predation pressure from fish in Maligne Lake was weaker (cf Chapter 4).

In Pipit Lake *G. lacustris* required three years to reach maturity. Mature females only produced a single brood per year (Figure 2-9). In 1995, the release of young did not occur until the latter part of August, as a result of the cold water temperature (Table 2-2). In addition, the growth rate of the juvenile and immature cohorts was low. Unlike in other years, juveniles did not show any signs of maturing by late August (Figure 2-9D). Although Pipit and Snowflake Lakes are at similar altitudes, females in Snowflake Lake released young earlier than in Pipit Lake because the water temperature in Pipit Lake is approximately 2 °C colder than in Snowflake Lake due to the larger volume of water and greater depth in Pipit Lake.

The time required by *G. lacustris* to reach maturity in Snowflake Lake varied between two and three years (Figure 2-10). Mature females only produced one brood of young per year. In August 1994, three cohorts could be distinguished, indicating that individuals had matured in 2 years (Figure 2-10A). Mature females from cohort 92 reproduced for a second time in 1995 (Figure 2-10D to 2-10F, and 2-11C), while mature females from cohort 93 reproduced for the first time in 1995 at two years of age (Figure 2-10D to 2-10F). Juveniles (cohort 94) grew slowly during 1995 when water temperatures were cold, but most developed to the immature stage by the end of August (Figure 2-10G). The largest individuals (n = 5) of cohort 94 reproduced for the first time in 1996 at age 2 along with mature females from cohort 93 which reproduced for the second time. However, the majority of cohort 94 did not reach maturity in 1996, but required an additional year of growth, reproducing for the first time at age three in 1997 (Figure 2-10L to 2-10N, and 2-11C). Males of cohort 93 were exceptionally long-lived, and were still present in the population in 1997 (Figure 2-10, 2-11C). The occasional occurrence of extremely large individuals such as the 21 and 22 mm female and male sampled on July 8, 1995 and July 28, 1995, respectively (Figure 2-10E, and 2-10F) suggests that individuals may live for more than five years. In 1992, a 38 mm male was sampled (D. W. Schindler unpub. data).

Growth rate

Growth rate was positively related to water temperature in Cooking, Harrison and Snowflake Lakes (Figure 2-12). The assumption of homogeneity of slopes was met (ANCOVA interaction term: $F_{2,32} = 0.65$, $P = 0.53$), so the interaction term was removed. After adjusting for temperature, the growth rate in Snowflake Lake was higher ($F_{2,34} = 4.92$, $P = 0.013$) than in Cooking Lake, while the growth rate in Harrison Lake was intermediate and did not differ from that in Cooking or Snowflake Lakes ($P = 0.44$, $P = 0.084$, respectively).

Size of reproductive females

The size of mature females ranged from 9.6±0.14 to 16.0±0.19 mm and varied significantly among lakes and between years within lakes (ANOVA $F_{20,884} = 86.24$, $P < 0.001$; Figure 2-13A). The size of mature females was not related to life cycle length (e.g. the largest females were obtained from Mildred Lake in 1996 where *G. lacustris* had a one-year life cycle. However, in 1997 females from Mildred Lake were similar in size to females from Snowflake Lake in 1995 where *G. lacustris* had a two year life cycle). For

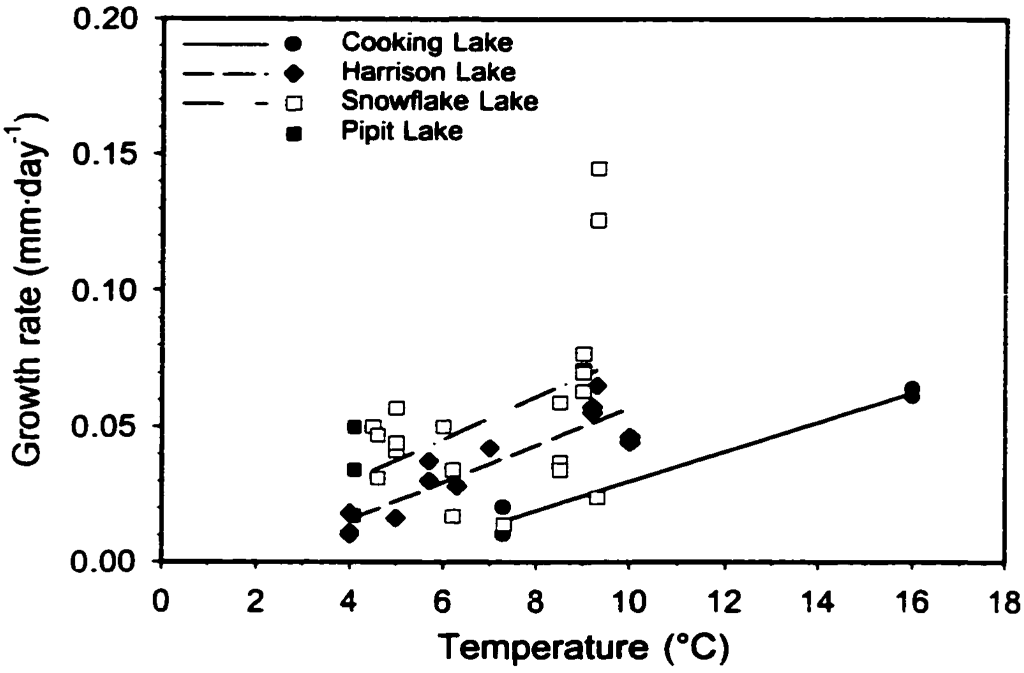

Figure 2-12. The relationship between growth rate of *Gammarus lacustris* and water temperature for three populations. Linear regression equations are in the form $y = mx$ (±95% CI) + b(±95% CI): Cooking Lake $y = 0.005x$ (0.003) - 0.024 (0.019), $R^2 = 0.98$; Harrison Lake $y = 0.007x$ (0.002) - 0.012 (0.013), $R^2 = 0.85$; Snowflake Lake $y = 0.008x$ (0.008) - 0.001 (0.056), $R^2 = 0.21$. Data for Pipit Lake shown for comparison.

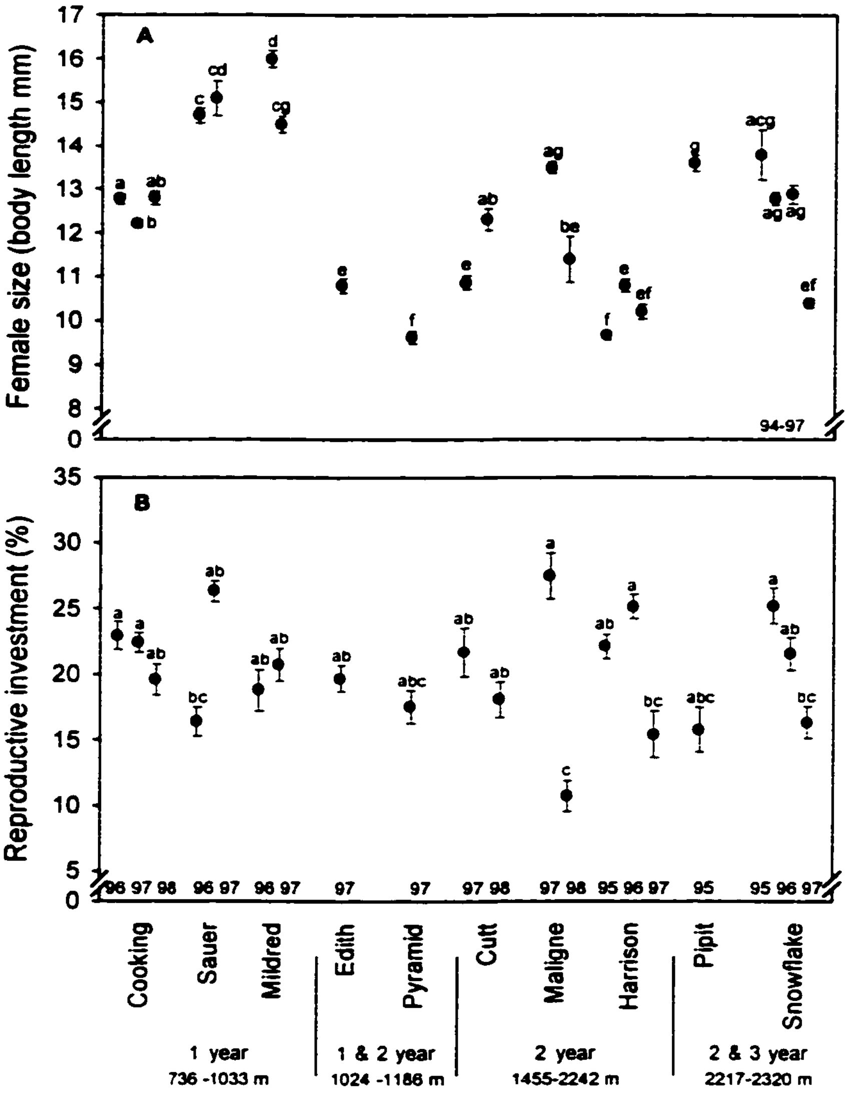

Figure 2-13. The size (A) and reproductive investment (B) of mature females of *Gammarus lacustris* from ten lakes in western Canada. All mature females were included in the size analysis, while only females with stage 1 and 2 eggs were included in the reproductive investment analysis. Reproductive investment was calculated as egg weight/female weight (without egg weight) ×100. Means±SE are presented and letters indicate similar pairs of means determined with a Tukey post hoc test. Age at maturity and elevation range for lakes is indicated at bottom.

three of four lakes with data for both 1996 and 1997, the size of mature females within lakes was smaller in 1997 than in 1996 (Figure 2-13A).

Reproductive investment

On average, mature females of *G. lacustris* invested between 15 to 25% of their body weight in eggs (Figure 2-13B). Reproductive investment varied significantly between lakes and within-lakes between years (ANOVA $F_{19,395} = 7.97$, $P < 0.001$; Figure 2-13B). The lowest and highest reproductive investment 10.7 and 27.5 %, respectively, occurred in Maligne Lake in consecutive years suggesting it was not under genetic control. In Snowflake Lake, the reproductive investment of females declined from a high of 25.2% in 1995 to 16.3% in 1997 (Figure 2-13B).

Fecundity

Fecundity was highly variable between lakes and between years within lakes ranging from a mean of 11 to 55 eggs per female (Table 2-2). Part of this variation was due to variation in female size, because fecundity was positively related to female size in all populations, except in Mildred Lake where fecundity was not related to body size. The fecundity for a standardized 11 mm female was negatively related to elevation ($P = 0.018$, Figure 2-14).

Size of young at hatching

The size of newly hatched young ranged from 1.95 to 2.45 mm and varied significantly among lakes and between years within lakes (ANOVA $F_{1,14} = 31.3$, $P < 0.001$; Figure 2-15). The smallest young were produced in prairie lakes while the largest young were produced in alpine lakes (Figure 2-15). The size of young at hatching was positively related to elevation (Size of young (mm) = $0.0192 \cdot 10^{-3} \cdot$elevation (m a.s.l.) + 0.1907, $R^2 = 0.69$, $P < 0.001$). Young also appeared progressively later during the open-water season in high elevation lakes than low elevation lakes. In prairie lakes, young were present at the end of May, while in alpine lakes young appeared at the end of July or in early August.

Discussion

The length of the ice-free season and water temperature decreased with increasing altitude in the study lakes. In general, the mid-summer surface water temperature was 13.7 to 14.6°C lower in the alpine lakes than in the prairie lakes. The between-year differences in ice-out dates led to less predictable environmental conditions and hence higher variability in high-altitude lakes because the variation in ice-out times represented a greater fraction of the average ice-free period than in low-altitude lakes.

These differences in temperature conditions were accompanied by large differences in life history traits among populations of *G. lacustris*. Two of the most prominent features were the increase in life cycle length and age at maturity with elevation, ranging between one to four-plus years and one to three years, respectively (Table 2-2, Figure 2-11). Variation in the age at maturity of *G. lacustris* has been noted in earlier studies (e.g. Menon 1966, Biette 1969, Mayhood 1978; Bjerknes 1974; de March 1981), however, only single

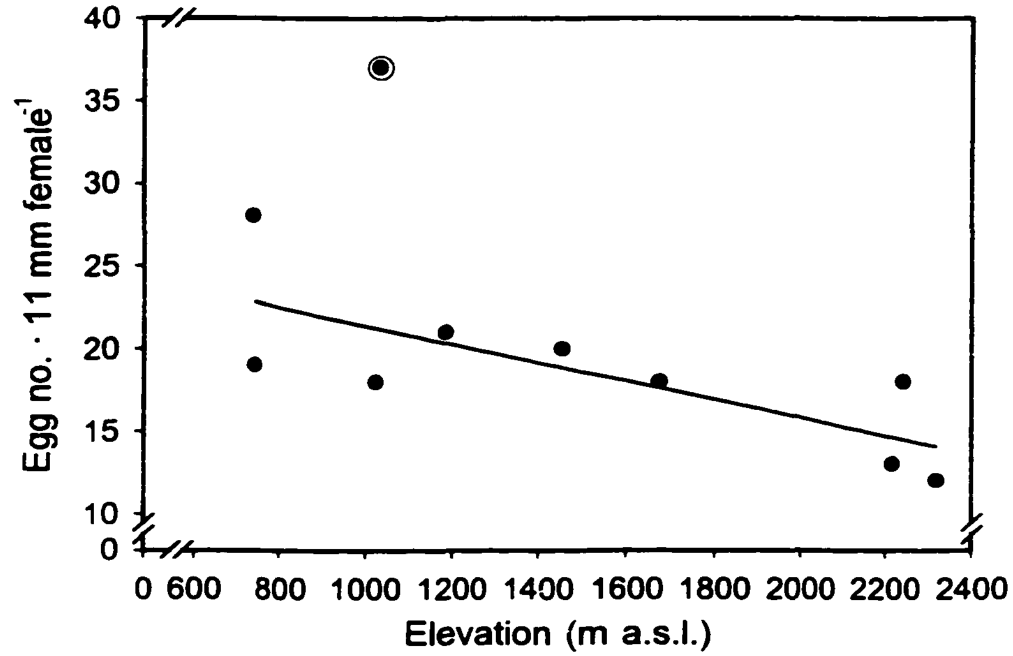

Figure 2-14. The relationship between fecundity for a standardized 11 mm female and elevation for ten populations of *Gammarus lacustris*. Fecundity was positively related to female size in each population, except Mildred Lake (circled) which was not included in regression analysis because fecundity was not significantly related to female size. Linear regression equation is in the form $y = mx$ (±95% CI) + b(±95% CI): $y = -0.006x$ (0.005) + 26.9 (6.88), $R^2 = 0.58$.

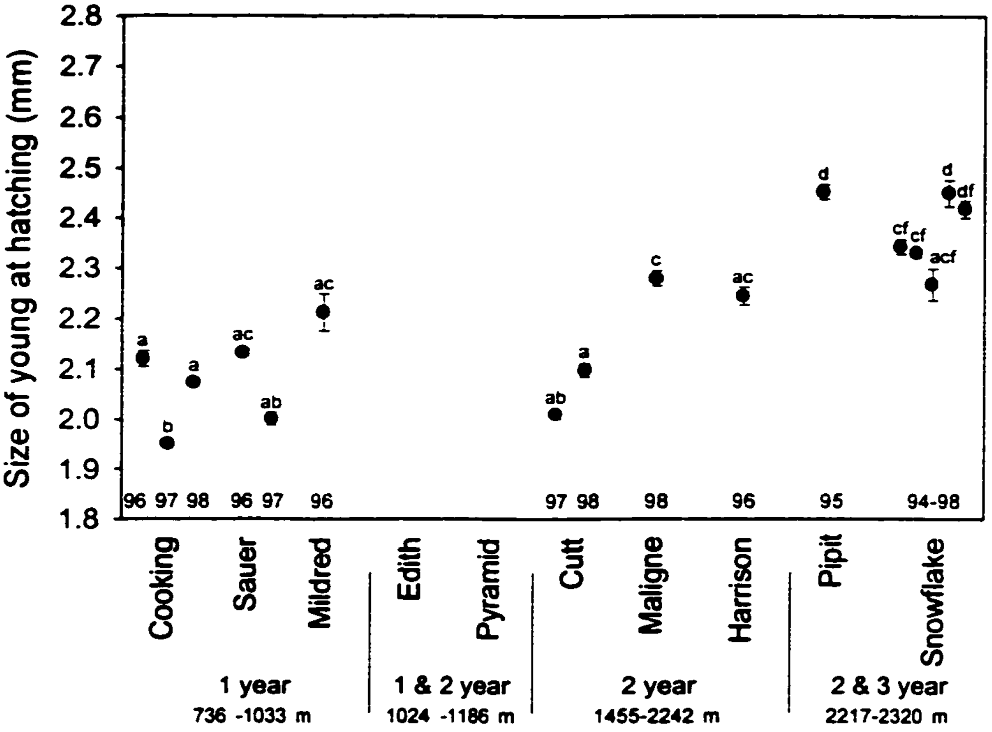

Figure 2-15. The size of *Gammarus lacustris* young at hatching from eight lakes in western Canada. Means±SE are presented and letters indicate similar pairs of means determined with a Tukey post hoc test.

populations were examined in these studies, so that factors which contribute to the variation in age at maturity were not compared among populations. The variation in both of these traits can be explained by the variation in temperature along the altitudinal gradient. Metabolic rates are usually positively related to temperature (e.g. Sandeman and Lasenby 1980), and organisms with a low metabolic rate tend to have a younger physiological age than those with high metabolic rates. Thus, organisms at cold temperatures with low metabolic rates can achieve older chronological ages. This has been shown experimentally for houseflies in which the accumulation of metabolic end products was positively correlated with temperature and negatively with longevity (Sohal et al. 1981; Sohal et al. 1985). A slow metabolic rate and short growing season can also explain the older age at maturity of *G. lacustris* in alpine lakes compared to prairie lakes.

Age at maturity can also be influenced by food availability; a poorly nourished population may take longer to reproduce than a well-fed one (Stearns 1992). Lake productivity (Chl *a* and TP) was negatively related to elevation and positively to temperature in the study lakes (Table 2-1). This may have influenced the food available to *G. lacustris* between lakes. However, the growth rate of individuals from Snowflake Lake was higher than that of individuals from Cooking Lake indicating that food was not limited in the less productive alpine lake (Figure 2-12). In addition, *G. lacustris* occupied similar substrates high in organic detritus in each lake.

The high between-year fluctuations in water temperature in Snowflake Lake likely contributed to the switch in the age at maturity of *G. lacustris* between two and three years. The El Nino year of 1994 was characterized by early ice-out, high water temperature during the ice-free season and a long warm fall. This allowed individuals from all cohorts to grow to a large size before freeze-up (Figure 2-11C). Individuals released in 1993 grew 2 to 4 mm between August and the end of September, allowing them to reproduce in 1995 at age two. Young released in 1994 grew to 6 mm in size by the end of September. In contrast, 1995 was characterized by cold air temperatures, frequently overcast skies and high precipitation including snow in late July and early August. In addition, ice-out did not occur until July 6. Young released in 1995 were less than 6 mm in size one year after they were born (Figure 2-11C). Because of the low water temperature, females of cohort 93 grew only 1 mm in size before they reproduced for a second time at age three in 1996 (Figure 2-11C). A few large individuals of cohort 94 reproduced at age two in 1996, while the majority delayed maturity until 1997 at age three. This suggests that if individuals experience above average water temperatures in the year they are born followed by average water temperatures the next year, they can reach maturity by age two. However, if young experience below average or average temperatures during the ice-free season in the year they are born, then are likely to require three years to mature.

The change in age at maturity and the longevity of individuals ensures the exchange of genetic material between cohorts. For example, females that mature at age two in Snowflake Lake can breed with males that are three years old. Similarly, young that fail to mature in one year in Edith and Pyramid Lakes can breed at age two with individuals that are one year old. Genetic exchange between cohorts in Harrison Lake may be limited because cohorts can interbreed only if females survive to reproduce a second time at age three. If interbreeding among cohorts did not occur, then cohorts would be reproductively isolated and could be considered as separate populations in lakes where the age at maturity is longer than one year. In addition, individuals that survive to reproduce in a second year add further insurance against the consequences of recruitment failure. This is especially advantageous at higher elevations where environmental extremes may make recruitment failure more likely.

The size at maturity was highly variable among lakes and between years and was not related to age or elevation (Figure 2-13A). Reproduction in *G. lacustris* is controlled by day length rather than temperature which helps ensure that young are released in spring at the beginning of the ice-free season (de March 1982). In addition, fecundity is positively related to female size (Chapter 3). Thus, the simplest strategy is for individuals to grow as large as possible before diverting energy to reproduction. This could explain the large size of females in lakes with a one year life cycle where individuals generally experience the longest growing season. The difference in size at maturity in lakes with one year life cycles (e.g. Cooking, Sauer and Mildred Lakes) could also indicate the influence of other factors such as predation by fish and waterfowl and remains to be examined. The between-year variation in size of mature individuals in other lakes (e.g. Mildred, Maligne, and Snowflake Lakes) would indicate that size at maturity is not under genetic control, but that size at maturity is population-specific.

Gammarus lacustris does not seem to follow the body size-temperature pattern generally observed among ecotherms. Generally, small adult sizes occur at warm temperatures (e.g. Atkinson 1994, 1995). However, mature females of *G. lacustris* in Cooking Lake (warm prairie) were similar in size to mature females in the Snowflake Lake (cold alpine) (Figure 2-11). The seemingly divergence of G. lacustris from the general ectotherm pattern for which no single overriding physiological constraint or adaptive explanation has yet been found to account for the general size reduction observed in the majority of ectotherms (Atkinson 1995), may be an artefact influenced by local population-specific conditions. For example, the large size of females and hence fecundity may be necessary in prairie lakes to overcome high rates of juvenile mortality, while in alpine lakes, female size is probably related to the short growing season, and the need to accumulate adequate reserves for overwinter survival and the production of large eggs. Thus, the general pattern may be obscured by prevailing local selection.

Predation by fish can also explain some of the variation in size at maturity between lakes. The size-selective nature of fish predators is widely recognized (e.g. Brooks and Dodson 1965; Galbraith 1967; Vonder Brink and Vanni 1993). In Harrison Lake, bull trout preyed selectively on the largest individuals of *G. lacustris* (Wilhelm et al. in press b; Chapter 4). Similar selectivity by predatory fish which are not gape-limited may also occur in the other lakes that contain fish (Table 2-1). Thus, predation represents a strong selective pressure against large size, because the probability of successfully producing young decreases with increasing size. Although fecundity decreases with size, it is better to have fewer offspring than none at all. The larger size of female *G. lacustris* in Maligne Lake than in Harrison Lake (Figure 2-13A) suggests that the selective pressure against large amphipods was weaker. This is probably related to the physical size of Maligne Lake. The large size of the lake would reduce the encounter rate between fish predators and amphipod prey compared to smaller lakes.

The variation in size at maturity within lakes may be related to the inter-annual variation in the length of the ice-free season and summer temperatures. Although these variations were large in Snowflake Lake (Table 2-2), the small size at maturity in 1997 when temperatures were high is difficult to explain. It was similar to that in lakes with fish predators. This could be related to physical changes in Snowflake Lake between 1995 and 1996. Two beavers that had occupied Snowflake Lake and maintained a dam on the outflow disappeared from the lake in 1995. This led to a ~30 cm decrease in the water level in the lake in the spring of 1996. As a result, large dark peaty areas that *G. lacustris* had occupied previously became inaccessible. It is likely that water in these sheltered areas warmed earlier in spring and remained warm longer in fall than the main basin of the lake. Therefore, it is possible that the loss of this habitat, especially for young, has resulted in an overall reduction of the growing season and further destabilisation of environmental conditions. In response, *G. lacustris* may have adopted a reproductive strategy to produce young as soon as possible. Confirmation of this hypothesis will require further monitoring of the population and environmental parameters.

Reproductive investment by females was highly variable and not related to female size or elevation (Figure 2-13B). This suggests it is not under genetic control. For example, in Sauer Lake the reproductive investment varied from 16.2% in 1996 to 26.3% in 1997 even though female size at maturity remained similar (Figure 2-13A). In Maligne and Snowflake Lakes, reproductive investment decreased as female size at maturity decreased. Comparisons with other amphipod species or other freshwater invertebrates is precluded by the lack of suitable data, because egg weights are rarely measured by other researchers. In fish, reproductive investment within species is also highly variable, e.g. 4 to 9% in pumpkinseeds, *Lepomis gibbosus* (Danylchuk and Fox 1994); 12 to 26% in guppies, *Poecilia reticulata* (Reznick et al. 1990, and 11 to 20% in roach, *Rutilus rutilus* (Vøllestad and L'Abée-Lund 1990). This suggests that the variation observed for *G. lacustris* is comparable to other aquatic species.

Gammarus lacustris appears to display "countergradient variation" in growth rates (Levins 1969). Countergradient variation is the evolution of traits or processes such as the capacity for growth in a direction opposite to that of the phenotypic pattern, to partially counteract the negative influence of the physical environment (Conover and Present 1990). For example, metabolic processes in poikilotherms are temperature dependent and would be expected to be slower in organisms at cold temperatures than in those at warmer temperatures. However, the usual observation is that the metabolic rate of populations or species from cold temperatures is higher at a given temperature than those at warm temperatures (Schmidt-Nielsen 1983). Thus, temperature compensation partially counteracts the negative influence of the physical environment. Although our field-determined growth rates of *G. lacustris* were quite variable, the growth rate for a given temperature was higher in Snowflake Lake than in Cooking Lake (Figure 2-12). The similar slope among the relationships indicates that the temperature-growth rate response curve has shifted to a lower range of temperatures in high-altitude populations. This is somewhat different from the countergradient variation in growth rates of Atlantic silversides, *Menidia menidia* (Conover and Present 1990) in which the growth rate of high- and low-latitude populations is similar at low temperatures but at high temperatures, the growth rate of high-latitude populations is much greater than that of low-latitude populations. Although growth rates of *G. lacustris* over the natural temperature range observed in all three lakes were best described by a linear relationship, we expect the growth rates to decline at high temperatures. This shift in the temperature-growth rate response with elevation also means that a population-specific temperature must be used to calculate physiological age or time. The two exceptionally high growth rates in Snowflake Lake are difficult to explain, but could indicate considerable plasticity in the population. To determine the extent to which genetic variation versus phenotypic plasticity accounts for the variation in the response of growth rate to temperature requires that stocks of *G. lacustris* from populations at different elevations be reared under controlled temperatures in the laboratory (e.g. Conover and Present 1990), or transplanted into a natural environment with a different temperature regime.

The positive relationship between size of young and elevation and the negative relationship between fecundity and elevation hints at an underlying size-number trade-off (Figure 2-13 and 2-14). Indeed, high-altitude females produce larger, but fewer eggs than females from lakes at low altitudes (Wilhelm and Schindler in press; Chapter 3). This trade-off is examined in detail in the next chapter.

In addition, large young hatch from large eggs while small young hatch from small eggs. Thus, not only do young in alpine lakes have a higher capacity for growth at lower temperatures but they are also born at a larger size than in prairie lakes. These traits are adaptive in environments with cold temperatures and growing seasons of variable lengths, if there is an advantage to being large, for example, if over-winter mortality was size-dependent. Large young survived longer under starvation conditions at cold temperatures than small young (Chapter 3), showing that large young are better suited to survive the long ice-covered periods in alpine lakes than small young.

Another possible explanation for large offspring size in alpine lakes is the threat of cannibalism. Adult cohorts overlap with young in alpine lakes (Figure 2-11) and adults have been observed to cannibalize young (F. M. Wilhelm pers. obs.). Adults appear to be size-limited in the prey they can successfully subdue (Chapter 5). Thus, a large size would protect against cannibalism by size-limited adults. On the other hand, if the newly born young were vulnerable to cannibalism, the time required by large young to outgrow the vulnerable size range would be shorter than for small young. In addition, large young can be expected to have a lower metabolic rate than small young and would need to forage less, thereby reducing their activity level and potential exposure to adults (e.g. Johansson and Rowe 1999). It appears that the investment in fewer but larger young at higher elevations is advantageous from several perspectives.

In conclusion, life history characteristics of *G. lacustris* varied along the elevation gradient from prairie to alpine. The age of maturity, growth rate, egg size and size of young at hatching increased with elevation, while fecundity decreased. The correlation between the life history characteristics of *G. lacustris* and the range of temperatures in lakes at different elevations may represent an evolutionary shift in life history strategy or a simple phenotypic response to differing temperatures. From the between-year comparison of *G. lacustris* within lakes, some differences were clearly a phenotypic response. To distinguish potential genetic differentiation between populations requires that individuals are raised under similar environmental conditions (e.g. common garden experiments) and should be a future initiative.

The life history strategies of *G. lacustris* appear well suited to the temperature and predation regimes of their habitats. If the life history strategies represent phenotypic plasticity, then we would expect populations in currently cold montane and alpine lakes to adopt strategies presently found in warmer lakes, if lakes warm as a result of global climatic warming. However, the likelihood of extreme weather conditions (e.g. Francis and Hengeveld 1998) could destabilize alpine populations if individuals are unable to match appropriate life history strategies to frequently and sometimes drastically changing environmental conditions.

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3. REPRODUCTIVE STRATEGIES OF *Gammarus lacustris* (CRUSTACEA: AMPHIPODA) ALONG AN ELEVATION GRADIENT[1]

Introduction

Fecundity and egg size are key life history traits which directly influence fitness. Because not all life history characteristics that determine reproductive rates can be maximized simultaneously, a trade-off exists between the production of a few large or many small young (Smith and Fretwell 1974; Elgar 1990; Roff 1992; Stearns 1992). Such a trade-off has been found in a wide variety of plants and animals (e.g. Roff 1992 p.357). Interspecific comparisons for evaluating trade-offs are informative with regard to life history theory in general. However, it is intraspecific trade-offs that provide insight to reaction norms (the variation of a phenotype as a continuous function of an environmental signal) (Stearns 1989, 1992) and/or the genetic control of reproductive traits among populations (e.g. Willows 1987; Baur and Baur 1997; Hancock et al. 1998). Factors that affect reproductive trade-offs can have important effects on a species' ecology. For example, Willows (1987) found that early breeding females of the rock slater (*Ligia oceanica*) produced large young in spring when diatoms, its major food source, were just beginning to increase in abundance. In contrast, smaller young were produced by females breeding later in summer when food availability was at a maximum. Wellborn (1994) found that in a population of *Hyalella azteca* in which the mortality of adults was high, size at maturity was reduced and females produced many small eggs. In a second population where juvenile mortality was high, adults were large, and produced fewer but larger eggs (Wellborn 1994). Clines of increasing egg size with increasing latitude in polar marine regions (and hence decreasing temperature) have also been reported for marine invertebrates (Wägele 1987; Clarke et al. 1991; Clarke and Gore 1992).

In the future, global climatic warming is expected to increase the temperature of aquatic environments (Schindler et al. 1996; Schindler 1997). As a consequence, those organisms able to tolerate the warmer temperatures of their habitat will experience an increase in metabolic rates. This may influence how income energy is allocated between maintenance, growth and reproduction, and will ultimately determine the success of local populations. Schindler (1997) suggested that investigations of organisms along natural temperature gradients such as those occurring with changes in latitude or altitude should provide a reasonable basis for speculations about the effects of long-term climate warming. Here we use the negative relationship between elevation and water temperature (e.g. Lamontagne et al. 1994) to examine life history characteristics and trade-offs in the freshwater amphipod *Gammarus lacustris*.

We examined fecundity, egg size and weight relationships with elevation in four populations of *Gammarus lacustris* along an elevation gradient ranging from 736 to 2320 m above sea level in western Canada. Given the variation in reproductive strategies observed at different latitudes, we hypothesized that fecundity would decrease with elevation

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[1] *A version of this chapter has been accepted for publication.* Frank M. Wilhelm, and David W. Schindler. *Functional Ecology.*

and that egg size and weight would increase with elevation. Because of the inverse correlation between size and metabolic rate/unit of size, we hypothesized that large eggs would require a longer incubation period than small eggs. Using the same argument, we further hypothesized that young from large eggs would be more resistant to starvation than young produced from small eggs.

Gammarus lacustris is an ideal subject in which to examine variations in fecundity and egg size within a species. It has a wide latitudinal and altitudinal distribution in the northern hemisphere (Bousfield 1958; Wilhelm and Schindler 1996). Females carry developing young in a ventral brood pouch which allows fecundity, egg size and weight to be determined accurately. While the species is omnivorous, it can complete its life cycle on a diet of detritus or algae (Anderson and Raasveldt 1974; Moore 1977), so that its development does not depend on lake productivity alone. Furthermore, individuals from high elevation lakes had higher growth rates than individuals from a low elevation lake (Wilhelm unpub. data) indicating that *G. lacustris* was not food limited in the less productive high elevation lakes.

Materials and Methods

Study lakes

Cooking Lake is a large (36 km^2), shallow (max. depth 4.6 m) hyper-eutrophic prairie (736 m a.s.l.) lake located ~25 km east of Edmonton, in Alberta, Canada (53°25'N 113°03'W) (Mitchell and Prepas 1990). The lake is isothermal and warms to a maximum temperature of 20 to 25°C in mid- to late July. The ice-free season usually lasts from the end of April to the middle of November. After freeze-up, the water becomes anoxic until break-up. In August 1983, phosphorus and chlorophyll *a* concentrations reached 310 $\mu g \cdot L^{-1}$ and 120 $\mu g \cdot L^{-1}$, respectively (Mitchell and Prepas 1990). The lake is slightly saline with a specific conductance near 1400 $\mu S \cdot cm^{-1}$.

Cooking Lake does not contain any sportfish, but some brook stickleback, *Culaea inconstans*, survive in some years. Chironomid larvae dominate the biotic community on a biomass basis, followed by amphipods (*Gammarus lacustris* and *Hyalella azteca*) (Mitchell and Prepas 1990). Large blooms of *Daphnia* occur in some years shortly after ice-out (Wilhelm pers. obs.), but only last for a brief period of one to two weeks. Large numbers of waterfowl use the lake as a breeding and staging area.

Cutt Lake is a small (15.5 ha, max. depth 7.5 m) oligotrophic montane (1455 m a.s.l.) lake in Jasper National Park, Alberta, Canada (52°53'N, 118°20'W). The lake stratifies during the open-water season with maximum surface temperatures near 17°C in August, while bottom temperatures remain near 9°C (Anderson and Donald 1980). The ice-free season lasts from the middle of May to late October. Total phosphorus concentrations of 7.3 $\mu g \cdot L^{-1}$ and 6.9 $\mu g \cdot L^{-1}$ were measured in the fall and spring of 1996 and 1997, respectively. Chlorophyll *a* concentrations on the same sampling dates were 1.32 $\mu g \cdot L^{-1}$ and 0.67 $\mu g \cdot L^{-1}$, respectively (Schindler unpub. data).

The lake was originally fishless, but was stocked with cutthroat trout, *Oncorhynchus clarki*, for 6 years starting in 1960. On average 6 833 trout were stocked per year. The maximum stocked in any one year was 18 000 (Anderson and Donald 1980).

The trout did not reproduce in the lake due to the lack of suitable spawning habitat and the lake is considered to be fishless again (D. Donald, Environment Canada, Regina, Canada, pers. comm.; angling in 1996 and 1997 failed to yield any fish). In the area where *Gammarus lacustris* was collected, it was the most abundant macroinvertebrate. Zygoptera and chironomid larvae also occurred with *G. lacustris*. Anderson and Donald (1980) reported Zygoptera and chironomid larvae as the most abundant macroinvertebrates when they sampled the deepest spot in the lake in 1978. The low abundance of *G. lacustris* at that time may have been related to the presence of fish which preyed on the amphipods.

Maligne Lake is a large (2 066 ha, max. depth 96 m) oligotrophic sub-alpine (1675 m a.s.l.) lake in Jasper National Park, Alberta, Canada (52°40'N, 117°33'W). The lake stratifies weakly during the open water season, which lasts from mid-June to early November. Surface water temperatures reach a maximum of 10 to 13°C in summer (Donald and Anderson 1978), while bottom waters remain near 4°C. Total phosphorus concentrations of 6.8 $\mu g \cdot L^{-1}$ and 3.5 $\mu g \cdot L^{-1}$ were measured in the fall and spring of 1996 and 1997, respectively. Chlorophyll *a* concentrations on the same sampling dates were 0.53 $\mu g \cdot L^{-1}$ and 0.88 $\mu g \cdot L^{-1}$, respectively (Schindler unpub. data).

Fish were not present in the Maligne River watershed before 1928. Brook trout, *Salvelinus fontinalis*, were stocked into Maligne Lake for 13 years starting in 1928 (Donald and Anderson 1978). An average of 75 329 fish were stocked per year. Rainbow trout, *Onchorhynchus mykiss*, were stocked at an average of 29 728 per year for three years starting in 1971. Chironomid larvae were numerically the most abundant (>1000 $ind. \cdot m^{-2}$) macroinvertebrates sampled in 1976 and 1977 (Donald and Anderson 1978), while *Gammarus lacustris* occurred at only 3 $ind. \cdot m^{-2}$. However, amphipods are locally abundant, because we sampled areas with >30 $ind. \cdot m^{-2}$ in 1996-98 (Wilhelm unpub. data). Both species of trout are still present in Maligne Lake and are known to prey on *G. lacustris*. However, given the size of the lake, we considered this predation pressure to be too weak to influence *G. lacustris* size at maturity (cf. Wilhelm et al. in press). In addition, our samples contained large amphipods similar in size to those collected from the other study lakes, which also suggests that fish predation has had little influence on amphipod size in Maligne Lake. Furthermore, Donald and Anderson (1978) found that between 1939-40 and 1976-77 the bottom fauna increased from 805 $ind. \cdot m^{-2}$ and 1.65 $g \cdot m^{-2}$ to 2445 $ind. \cdot m^{-2}$ and 4.06 $g \cdot m^{-2}$. No major change in taxonomic composition was found and Anderson and Donald (1980) concluded that this long-term increase was related to natural processes such as increased sediment loading from the watershed, and not the presence of fish.

Snowflake Lake is a small (7.13 ha, max. depth 12.5 m) oligotrophic alpine (2320 m a.s.l.) lake located in the eastern front ranges of the Canadian Rocky Mountains in Banff National Park (51°35'N, 115°50'W), Alberta, Canada. Ice-out usually occurs between the end of June and mid-July. The lake stratifies weakly in late summer when surface temperatures reach a maximum of 9-12°C. Freeze-up occurs between late September and early October (Anderson 1970). Total phosphorus and chlorophyll *a* concentrations during the open water season range from 3 $\mu g \cdot L^{-1}$ to 8 $\mu g \cdot L^{-1}$ and 0.1$\mu g \cdot L^{-1}$ to 2 $\mu g \cdot L^{-1}$, respectively (McNaught et al. 1999; Schindler unpub. data).

The biotic community of Snowflake Lake has been affected several times by fish and zooplankton introductions. The lake was originally fishless, but was stocked with 15 000 brook trout, 6 000 cutthroat trout and 7 500 rainbow trout between 1960 and 1966. The presence of fish altered the invertebrate community. *Gammarus lacustris* became rare, and *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* were replaced by *Diacyclops* and rotifers (Anderson 1972; McNaught et al. 1999). Fish did not reproduce in the lake and declined gradually, with the last fish caught in 1984. Once the lake was devoid of fish, *G. lacustris* and *D. middendorffiana* populations recovered (McNaught et al. 1999), but *H. arcticus* remained absent. *Hesperodiaptomus arcticus* was reintroduced on July 30, 1992, but did not become abundant in the plankton until late in 1996. *Hesperodiaptomus arcticus* reintroduction has caused the decline in abundance or elimination of all cyclopoid copepod and rotifer species (Schindler unpub. data).

Field collections

Ovigerous *G. lacustris* were collected from Cooking and Snowflake Lakes after ice-out each year from 1996 to 1998. Few females were collected from Snowflake Lake in 1998 and all were used in the incubation experiment (see below). Thus, they were not included in the fecundity analysis for Snowflake Lake. Cutt and Maligne Lakes were sampled in 1997 and 1998. Amphipods were obtained by scraping a 500-µm-mesh dip net over the bottom substrate in water 0.5 m to 1.3 m deep. Ovigerous females were sorted from debris using the method of Wilhelm and Hiebert (1996) and were placed into clean lake water at ambient temperature, in which they were held alive until processed.

Fecundity, egg size and weight

Females were sacrificed in batches of five to ten in dilute (4%) formalin usually within one to two days after collection. Once movement ceased (~10 min), amphipods were transferred back to native lake water for measuring under a Wild M5 dissecting microscope equipped with an ocular micrometer. Total length of females was measured from the tip of the rostrum to the tip of the telson after straightening the body with fine forceps.

Eggs were removed from females by bending the oostegites back and using a gentle jet of water from a Pasteur pipette to displace eggs from the brood pouch. Eggs were counted and classified to development stage (1 to 6) following the description of Sheader and Chia (1970). Egg development per brood was synchronous, so that all eggs from a female could be assigned to a single stage. To obtain the mean size of an egg in each brood, 12 randomly selected eggs from each gravid female were measured. Length was measured (±0.020 mm) as the longest axis of each egg, while the axis perpendicular to it was measured as the width. Volume was calculated using the equation for a prolate spheroid (Beyer 1987):

$$V = 4/3\ \pi\ r_1\ r_2\ r_3$$

where V = volume (mm^3); r_1= length/2 (mm); r_2 and r_3 = width/2 (mm). Depth was not measured separately but was assumed to be equivalent to width. To determine the mean dry weight of an egg in each brood, all eggs from a female were transferred to a pre-weighed aluminum foil drying boat and dried at 55°C for 24 h. After cooling in a desiccator for at least 24 h, they were weighed on a Cahn C-31 Electrobalance to the nearest 0.1 µg. Mean dry weight per egg was calculated by dividing the weight of all eggs per brood by the number of eggs in the brood. Between 5 to 96 females were examined per egg stage from each population. Not all stages were sampled each year.

Development time in relation to egg size

To determine if large eggs required a longer development time than small eggs, females that had recently deposited eggs in their brood pouch were incubated at different temperatures. In 1996 we undertook preliminary experiments with animals from Cooking Lake in an attempt to culture eggs *in vitro* using methods similar to those described by Morritt and Spicer (1996). However, we were unable to successfully maintain eggs until they hatched. Instead, we obtained females with broods for which birth dates were known and let the females incubate the eggs. Precopulatory pairs were collected from the lakes in the spring of 1998 immediately after ice-out, or in the case of the high elevation lakes, while the lakes were still ice covered. Animals were brought into the laboratory and allowed to acclimate to 15°C over two days. Females molted within one to five days and deposited eggs in the brood pouch where they were fertilized by males. After the pairs separated, we removed females to separate aquaria. Between 10 to 15 females were maintained in each 2 L aquarium filled with 1.5 L of 64-µm filtered and aerated lake water. Three aquaria with females from each lake were maintained at each of four temperatures (4, 10, 15 and 20°C) in controlled environmental chambers until young hatched. All aquaria were maintained on a 12 h:12 h light dark cycle. All aquaria were supplied with ad libitum food to eliminate the possibility that the availability of food would skew our results. Each day, 200 mg of crushed trout food pellets were added to each aquarium after siphoning off any food remaining from the previous day. The volume of water was maintained at 1.5 L by adding filtered lake water when needed. Any dead females or females with lost broods were also removed daily. The date when 50% or more of all females in each aquarium had released their young was taken as the hatching date. Generally, hatching times were highly synchronous among females from each population.

Newborn young hatched at 10 °C were measured (length) and dried to constant weight at 55 °C for 24 h to determine if newborn young from the four populations (different sized eggs) differed in weight or size. We chose to focus on young hatched at 10 °C because it was incubation temperature closest to the temperature likely experienced by each population in the field. Furthermore, because young hatched at close intervals it was impossible to measure and weigh young from each lake for each incubation temperature.

Starvation times

To determine if young from large eggs survived longer than young from small eggs, we investigated starvation times of newly emerged young. Young released from females kept at 10°C were removed from the 2 L aquaria immediately after they emerged from the brood pouch and placed into individual pre-washed vials filled with 40 mL of 0.22-µm filtered lake water. Fifteen vials from each population, except Snowflake Lake, were incubated at each of the four temperatures. Each day, young were checked for survival and half of the water was exchanged with newly filtered lake water. Any exuviae present were also removed daily so that young could not ingest them. A mean starvation time was calculated for each temperature and population. Snowflake Lake females experienced high mortality at all incubation temperatures, resulting in too few young to run the starvation experiment.

Analyses

We first examined regression relationships between egg volume and number of eggs per brood and female size using the method of least squares. We then assessed the relationship between fecundity and female size in each population for all years by regressing the number of eggs in the brood pouch on female body size. We limited the comparisons to stage 1 and 2 eggs because brood mortality or egg loss from the marsupium during development would result in an underestimation of brood size and egg production if later stages were considered (Sheader 1983). To determine whether populations differed in size-specific fecundity, we compared populations using analysis of covariance (ANCOVA), with egg number as the response variable, female body size as the covariate, and population as the grouping variable. We examined both linear and log-log relationships, but used the linear regressions because they provided the highest correlation coefficients.

We compared population differences in dry weight and volume of eggs at each development stage using analysis of variance (ANOVA).

Development time was related to temperature through the allometric function $D=aT^{-b}$; where D = hatching time (days); T = temperature (°C); and a and b are fitted constants. This was one of three models favoured by Pöckl and Timischl (1990) who compared 11 mathematical relationships commonly used to describe the relationship between development time and temperature. To determine whether large eggs had a longer development time than small eggs, we compared populations using ANCOVA, with development time as the response variable, temperature as the covariate, and population as the grouping variable. Data from Maligne Lake were not included in the analysis, because all females at 20°C died and no young hatched at 4°C. The weight and size of newly released young were compared using ANOVA.

Starvation time was related to temperature through the allometric function $S=aT^{-b}$; where S = starvation time (days); T = temperature (°C); and a and b are fitted constants. To determine whether young from large eggs survived longer than young from small eggs, we compared starvation times between populations using ANCOVA, with starvation time as the response variable, temperature as the covariate, and population as the grouping variable. No data were available for Snowflake Lake because too few young were born.

In all analyses the data and residuals were checked for normality and homogeneity of variance to ensure that regression and ANCOVA assumptions were met. Significant ANOVA and ANCOVA tests were followed by Tukey tests (Sokal and Rohlf 1981; Zar 1996) to detect means which differed.

Results

Fecundity, egg size and weight

Regressions of egg volume versus egg number did not differ (P = 0.099 to 0.977) from zero, meaning that egg volume was independent of fecundity. Four of ten regressions of egg volume versus female size differed (P = 0.009 to 0.027), of which three were positive and one was negative. However, the amount of variation in egg volume explained by female size was low (R^2 = 0.23 to 0.30) for these regressions. Therefore, we considered egg volume to be poorly predicted by female size in all populations. Fecundity was positively related to female size in all populations (Figure 3-1). Slopes of lines were similar (ANCOVA, $F_{3,227} = 2.61$, $P = 0.052$), but elevations of the lines differed (ANCOVA, $F_{3,230} = 82.47$, $P < 0.001$). Post hoc comparisons showed that elevations differed ($P < 0.001$) among all populations except for Cutt and Maligne Lakes ($P = 0.907$; Figure 3-1).

Egg volume increased with elevation (Figure 3-2A). Females in Cooking Lake (prairie) produced the smallest eggs at 0.119 mm^3 (stage 2) while females in Cutt and Maligne Lakes (montane and sub-alpine) produced intermediate sized eggs at 0.145 mm^3 and 0.141 mm^3 (stage 2), respectively. The largest eggs were produced by females in Snowflake Lake (alpine) where stage 2 eggs had a volume of 0.176 mm^3 (Figure 3-2A). Egg volumes for all stages of development differed between populations (Table 3-1; Figure 3-2A). Egg volume also increased with development stage (Figure 3-2A). The increase in egg volume with development stage was highest in Snowflake Lake, and decreased progressively as elevation of lakes decreased (Maligne, Cutt, and Cooking Lake). The mean volume of Maligne Lake eggs was similar to Cutt Lake eggs at stage 2 and Snowflake Lake eggs at stage 3 (Figure 3-2A), indicating that increases in egg volume between development stages varied among populations.

Egg weights also increased with elevation in a manner similar to egg volume. Females in Snowflake Lake produced the heaviest eggs at 0.077 mg (stage 2) while females in Cutt and Cooking Lakes produced progressively lighter eggs weighing 0.055 mg and 0.045 mg (stage 2), respectively (Figure 3-2B). Egg weights at each stage of development differed (Table 3-1) among populations, except for stage 3 and 4 for Snowflake and Maligne Lakes (Figure 3-2B). The weight of Cutt Lake eggs remained constant throughout the stages sampled. An initial increase in egg weight to stage 3, followed by a graduate decline was found for Cooking and Maligne Lakes, while a slight decline was observed between stages 2 to 5 in Snowflake Lake (Figure 3-2B).

Development time in relation to egg size

The development time of embryos decreased as incubation temperature increased (Figure 3-3). No young were produced at 4°C and embryos from all populations only developed to late stage 4 before disintegrating. Beyond stage 3, lipid droplets were less numerous in embryos at 4°C compared to embryos at warmer temperatures. Prior to their disintegration, stage 4 embryos at 4°C were nearly transparent, indicating that all energy reserves were depleted. We used the last day of observation before embryos disintegrated as the endpoint for the development time - temperature curves. Regression slopes of development time versus temperature were similar (ANCOVA; $F_{3,32} = 0.547$, $P = 0.654$) among populations, but elevations of the regression lines differed (ANCOVA; $F_{3,35} = 7.498$, $P < 0.001$). Development times were similar for Cutt and Snowflake Lakes (Tukey post hoc test; $P = 0.899$), which were longer ($P < 0.001$) than for Cooking Lake, indicating that development time for large eggs was longer than for small eggs (Figure 3-3).

Length and weight of newborn young

The size of newly hatched young differed among populations (ANOVA; $F_{3,70} = 127.82$, $P < 0.001$; Figure 3-4A). The size of young from Cooking and Cutt Lakes was similar at 2.07 mm and 2.10 mm, respectively. Young from Maligne and Snowflake Lakes were progressively larger at 2.28 mm and 2.42 mm, respectively (Figure 3-4A).

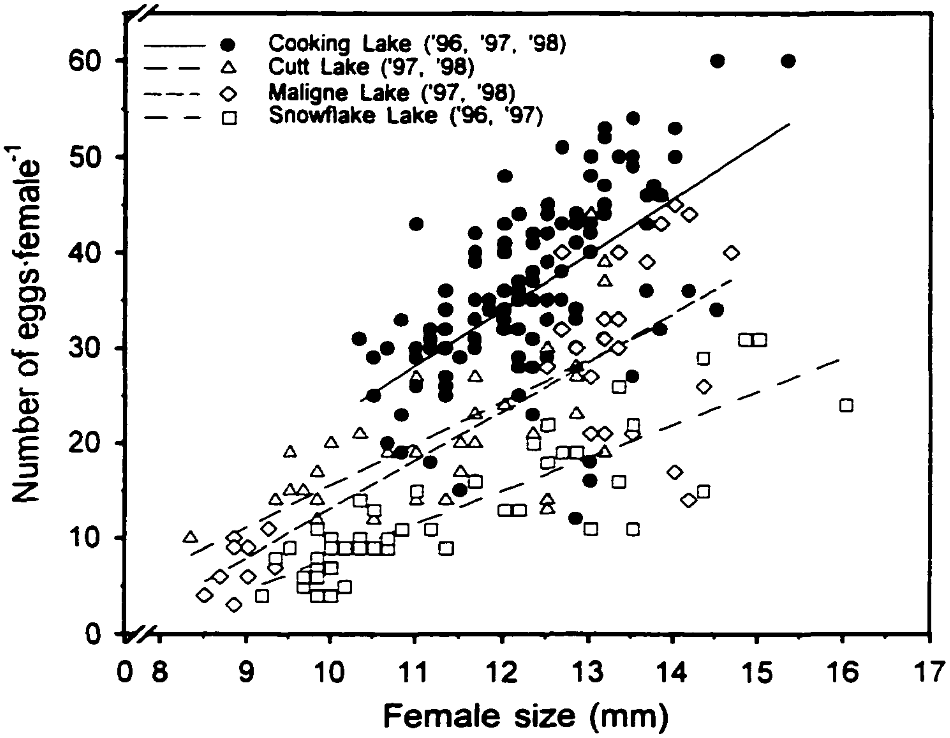

Figure 3-1. The relationship between fecundity and female body size for four populations of *Gammarus lacustris* from lakes along an elevation gradient in western Canada. Elevations (m) above sea level: Cooking Lake = 736 (prairie); Cutt Lake = 1455 (montane); Maligne Lake = 1675 (sub-alpine); Snowflake Lake = 2320 (alpine). Regression equations in the form $y = mx$ (±95% C.I.) + b (±95% C.I.) are: Cooking Lake $y = 5.8x$ (1.46) - 35.7 (18.1), $R^2 = 0.34$; Cutt Lake $y = 4.4x$ (1.59) - 28.5 (18.1), $R^2 = 0.49$; Maligne Lake $y = 5.13x$ (1.31) - 38.2 (16.1), $R^2 = 0.67$; Snowflake Lake $y = 3.5x$ (0.62) - 26.8 (7.1), $R^2 = 0.74$.

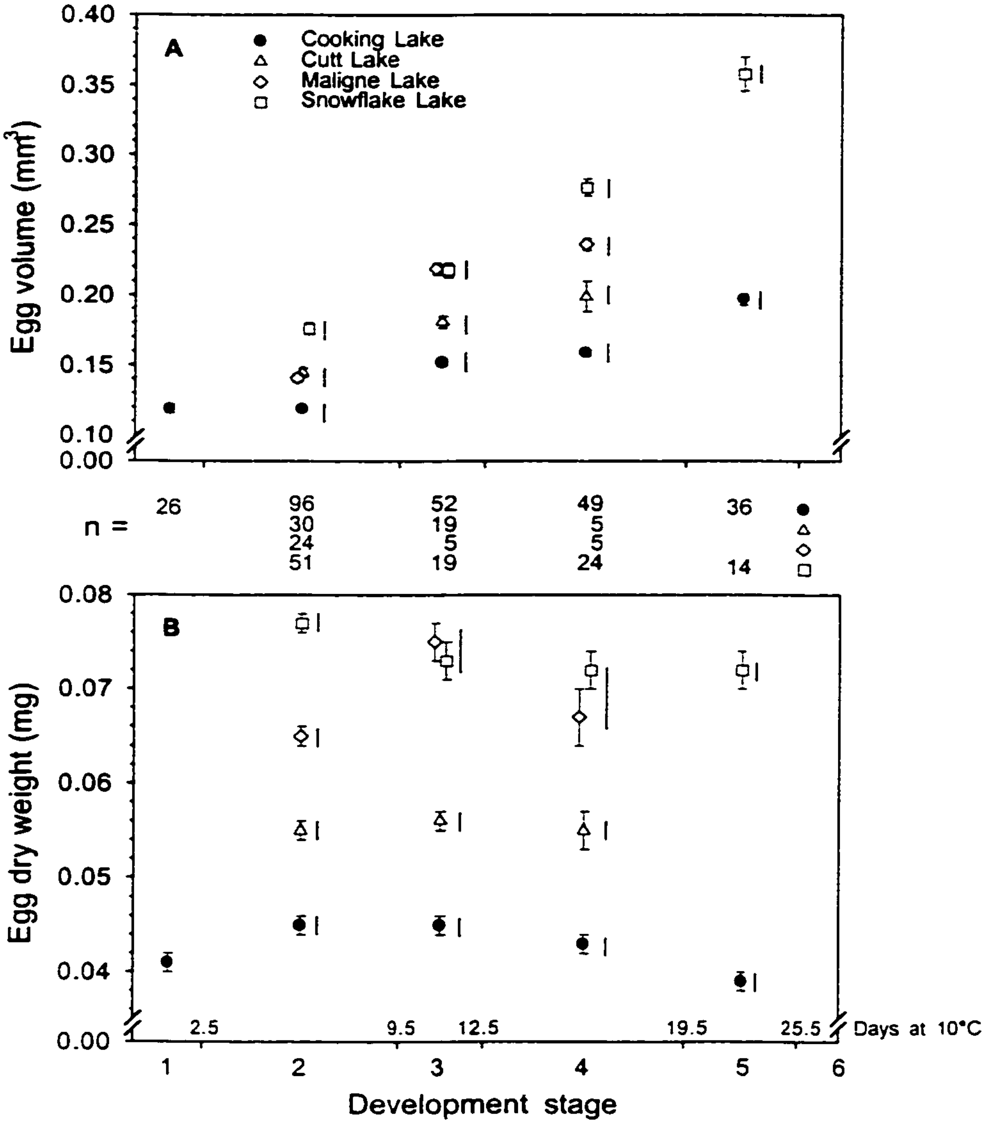

Figure 3-2. The change in mean volume (A) and weight (B) of *Gammarus lacustris* eggs from four populations during development. The duration of each development stage shown on the abscissa was determined from Cooking Lake eggs incubated at 10°C in 1996. Overall means±SE of eggs are plotted for samples collected from Cooking Lake in 1996-98; Cutt Lake 1997-98; Maligne Lake 1997-98 and Snowflake Lake 1996-97. The number of eggs sampled from each population for each stage is indicated between panels. Vertical bars join similar means (ANOVA Tukey post hoc test).

Table 3-1. Summary of ANOVA results for the comparisons of egg volumes among four populations of *Gammarus lacustris*. Differences among individual means detected with Tukey post hoc tests are indicated on Figure 3-2.

	Stage	*F*	D.F.	*P*
Egg volume	2	260.9	3, 197	<0.001
	3	145.8	3, 91	<0.001
	4	145.9	3, 79	<0.001
	5	317.5	1, 48	<0.001
Egg weight	2	119.6	3, 197	<0.001
	3	80.8	3, 91	<0.001
	4	176.7	3, 79	<0.001
	5	260.4	1, 48	<0.001

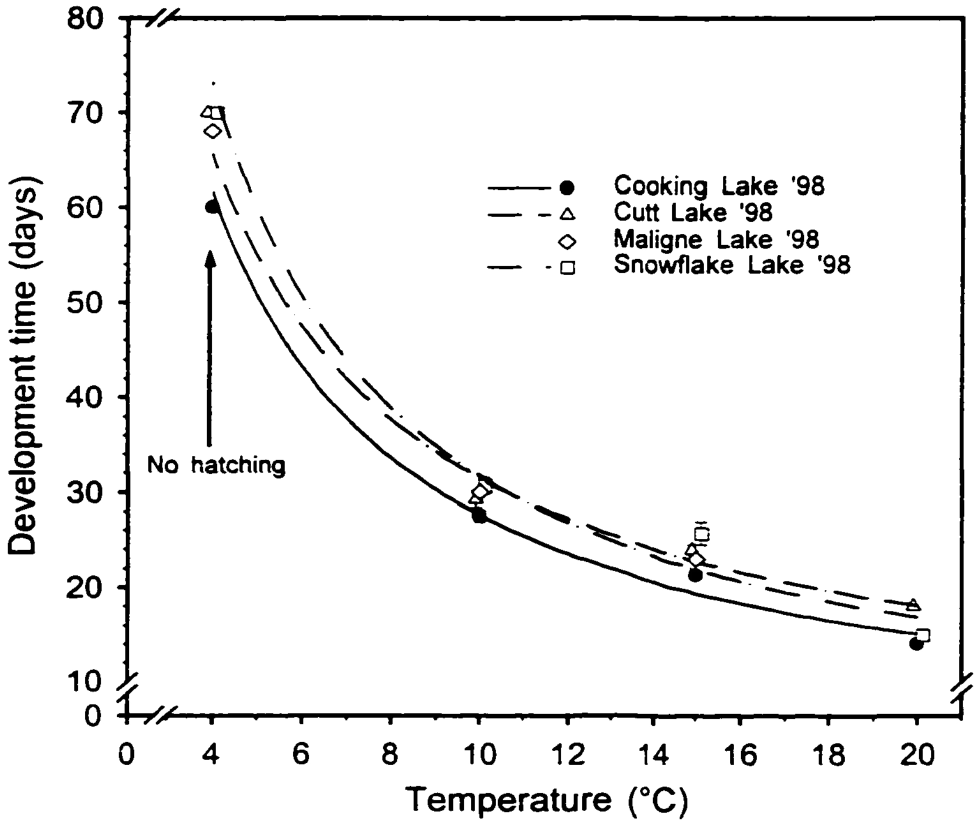

Figure 3-3. The relationship between development time and temperature for four populations of *Gammarus lacustris* from lakes along an elevation gradient in western Canada. No young hatched at 4°C; means ±SE are plotted. Regression equations in the form $\mathrm{Log}_{10}\, y = bx$ (± 95% C.I.) + Log_{10} a (± 95% C.I.) are: Cooking Lake $y = -0.88x$ (0.12) + 2.32 (0.13), $R^2 = 0.97$; Cutt Lake $y = -0.802x$ (0.11) + 2.3 (0.12), $R^2 = 0.98$; Snowflake Lake $y = -0.91x$ (0.20) + 2.42 (0.23), $R^2 = 0.93$.

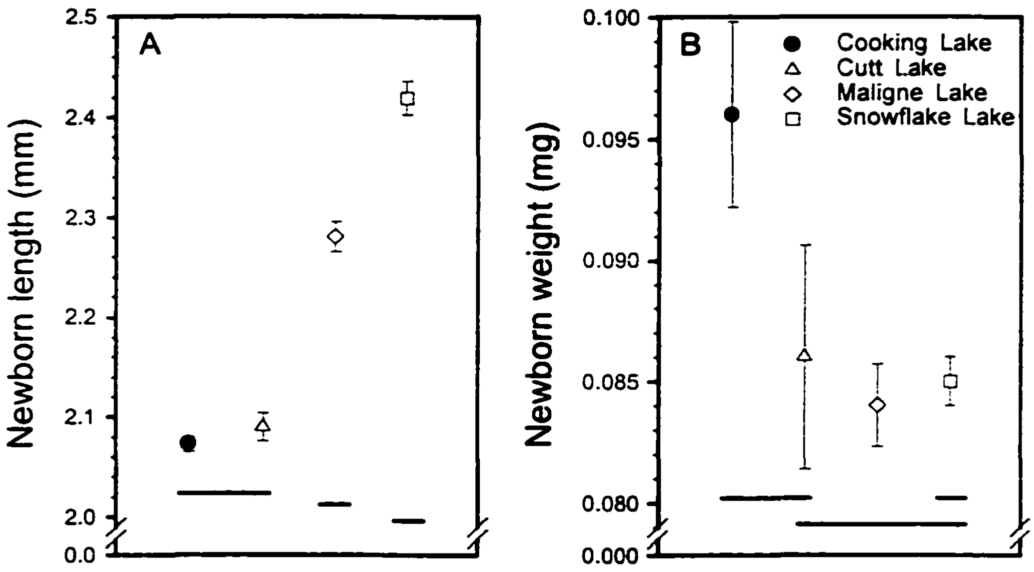

Figure 3-4. Mean lengths±SE (A) and weights±SE (B) of newly hatched *Gammarus lacustris* from four populations. Young were measured from females incubated at 10°C in the laboratory. Horizontal lines indicate similar means as determined by Tukey post hoc tests.

The weight of newly hatched young also differed among lakes (ANOVA; $F_{3,70}$ = 3.779, P = 0.014, Figure 3-4B). However, weights were highly variable, and young from Cooking Lake were the heaviest at 0.096 mg. The weight of young from Cutt, Maligne and Snowflake Lakes was similar at 0.85 mg, 0.84 mg, and 0.84 mg, respectively. Newly hatched young from Cooking and Snowflake Lakes (Figure 3-4B) were 2.5 and 1.2 times heavier than stage 5 embryos (Figure 3-2B).

Starvation times

The time to starvation of newly hatched young decreased with increasing environmental temperature (Figure 3-5). Regression slopes of starvation time versus temperature were different (ANCOVA; $F_{2,174}$ = 9.291, P < 0.001), precluding comparisons of differences in adjusted means between populations. Survival of young from all populations was similar at 10°C and 20°C, but differed at 4°C and 15°C. At 15°C, the starvation time of young from Cooking and Cutt Lakes was longer than at 10°C (Figure 3-5). At 4°C, young from Cutt and Maligne Lakes survived for an average of 31 and 25 days, respectively, while young from Cooking Lake only survived for an average of 13 days (Figure 3-5).

Discussion

Fecundity, egg size and weight

Although the fecundity of *G. lacustris* is known to be highly variable across a wide geographic range (Menon 1966, Biette 1969; de March 1981; Sarviro 1983), this variation has not previously been examined with respect to environmental gradients. Our study shows that a large fraction of the variation in fecundity among populations of *G. lacustris* can be explained by the trade-off between many small or few large eggs. Females in Cooking Lake (736 m) produced more, smaller eggs than in higher elevation lakes where females produced fewer but larger eggs. Although such a trade-off is known for many groups of invertebrates (e.g. Lawlor 1976; Allan 1984; Willows 1987; Wägele 1987; Clarke et al. 1991), few studies have focussed on it across latitudinal or altitudinal gradients in freshwaters. Sarviro (1983) investigated the effect of temperature on the growth of *G. lacustris*. His data indicate the potential for a fecundity/egg size trade-off similar to that presented here; populations from cold lakes had lower fecundity than those from warm lakes. However, egg size was not reported.

Underlying factors that may bring about such a trade-off include physiological constraints, food conditions for females and young, size-specific mortality regimes, and environmental temperature. Complications may arise through their interaction. Egg size in some species may be constrained physiologically by body size, but our data show that female size was always a poor predictor of egg size in *G. lacustris*. Food availability may affect the amount of energy allocated to reproduction in general and also how it is partitioned during vitellogenesis (Skadsheim 1984; Clarke et al. 1985). It is unlikely that food availability for either females or young influenced reproductive traits in our study lakes. *Gammarus lacustris* is described as an opportunistic species, eating whatever is available (de March 1981). It can survive on a diet of algae (Moore 1977), or complete its life cycle on sediment

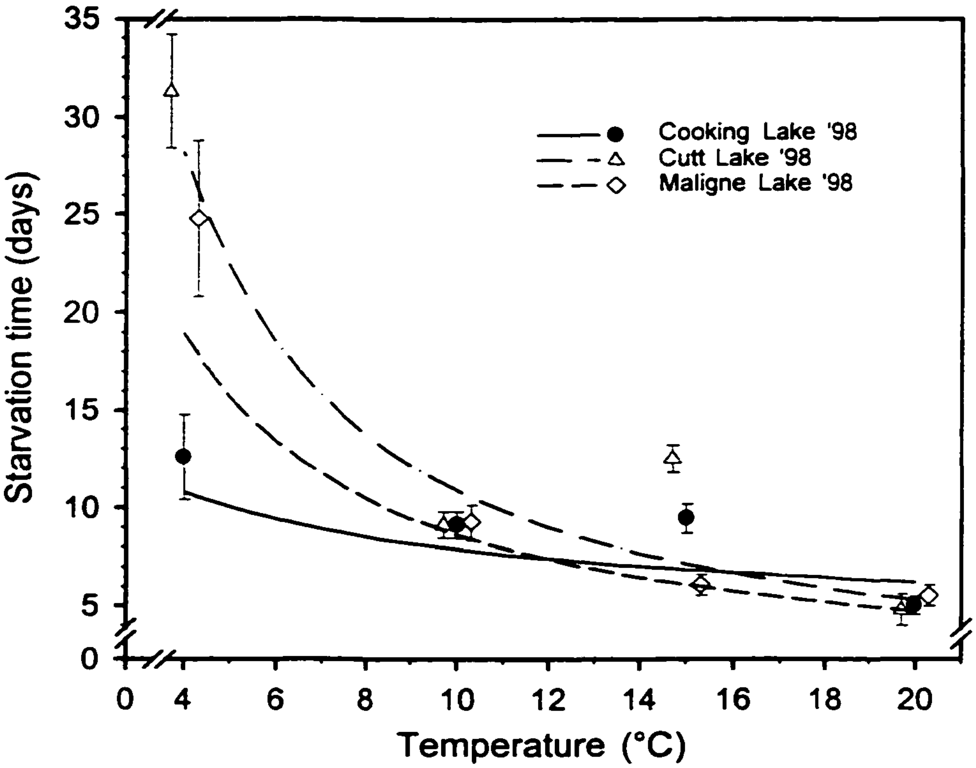

Figure 3-5. The relationship between starvation time and temperature for three populations of *Gammarus lacustris*. Young were obtained from females incubated at 10°C in the laboratory. Snowflake Lake young were not included in the experiment because too few young hatched from females; means±SE are plotted. Regression equations in the form $\text{Log}_{10}\, y = bx$ (± 95% C.I.) + Log_{10} a (± 95% C.I.) are: Cooking Lake $y = -0.35x$ (0.23) + 1.2 (0.24), $R^2 = 0.14$; Cutt Lake $y = -1.0x$ (0.25) + 2.1 (0.26), $R^2 = 0.55$; Maligne Lake $y = -0.86x$ (0.23) + 1.8 (0.25), $R^2 = 0.48$.

and detritus (Anderson and Raasveldt 1974). In fishless lakes and ponds it also preys on zooplankton (Anderson and Raasveldt 1974; Kortelainen 1990; Wilhelm and Schindler in press). In all our study lakes, amphipods were always associated with substrates covered with an organic layer and we often observed individuals in the water column, presumably preying on zooplankton. Furthermore, the growth rate of individuals from Snowflake Lake was higher than that of individuals from Cooking Lake, indicating that food was not limiting in the less productive alpine lake (Wilhelm and Schindler unpub. data).

Differences in adult survival may contribute to the fecundity/egg size trade-off in our study lakes. Females in prairie lakes reach sexual maturity within one year (Menon 1966; de March 1981; Wilhelm and Schindler 1996). Most females die after reproducing, but some produce a second brood in the same year (Menon 1966). In mid- and high elevation lakes, females require two and three years, respectively, to reach sexual maturity. After reproducing, up to 30% of females survive until the following year in Snowflake Lake (Wilhelm unpub. data), and reproduce for a second time. Therefore, the size-specific survival of adults increases with elevation. Because future reproduction is determined by expected rates of survival, growth and fecundity, the effect of high adult mortality is to lower expected future reproduction (Wellborn 1994). Thus, fitness can be maximized by reproducing earlier (younger age, smaller size) and producing more offspring. High adult mortality has not influenced the size at maturity in Cooking Lake because females in all study lakes span a similar size range (Figure 3-1). However, the production of many small eggs is consistent with expectations from life history theory (Stearns 1992).

The differences in adult mortality among the *G. lacustris* populations is most likely related to environmental temperature. *G. lacustris* in Cooking Lake may be living close to its upper maximum temperature. Smith (1973) reported that although *G. lacustris* could survive for brief periods at temperatures of 21 to 26°C, he doubted that populations could maintain themselves above 18°C. The temperature in Cooking Lake regularly exceeds 18°C in summer. However, Smith used *G. lacustris* from Minnesota in his experiments, and some differences in temperature tolerance can be expected between geographic regions. Nevertheless, the metabolic cost of carrying developing young at a high water temperature probably results in an energy deficit from which females in Cooking Lake can not recover.

Temperature and survival of young

Different mortality regimes of young between the study lakes may also contribute to the fecundity/egg size trade-off. Due to the multi-year life cycle in mid- and high elevation lakes, *G. lacustris* generations overlap. Large immatures and adults are cannibalistic (F. M. Wilhelm pers. obs.); thus newly released young in montane, sub-alpine and alpine lakes are exposed to a higher mortality risk than young in prairie lakes. However, adult *G. lacustris* have difficulty handling large prey (e.g. *Daphnia* larger than 3 mm, F. M. Wilhelm et al. unpub. data). Therefore, the mortality of young can be reduced by increasing the investment per offspring (Roff 1992; Stearns 1992; Wellborn 1994). This ensures that either large young are produced or that young hatch with sufficient reserves to grow rapidly through the vulnerable size range. The production of large eggs and young by females in mid- and high elevation lakes (Figure 3-2, and 3-4) is qualitatively consistent with this prediction from life history theory. However, it is difficult to explain the production of intermediate sized eggs in mid elevation lakes.

The cline in egg size along the elevation gradient can be best explained by the decline in water temperature and ice-free season with increasing elevation. The ice-free season in Snowflake Lake is ~90-120 days compared to ~230 days in Cooking Lake, while the mid-summer surface water temperatures are approximately 7.9°C and 22.5°C, respectively (Calculated from the relationship of Lamontagne et al. 1995). Therefore, young at high elevations experience a shorter growing season and lower average temperature than young in prairie lakes. In addition, the development time of eggs is longer at cold temperatures (Figure 3-3). If we assume that density independent mortality dominates in juvenile stages, a longer time spent as small juveniles will result in a higher mortality (Kolding and Fenchel 1981). This explanation is supported by our data, which show that large young hatch from large eggs (Figure 3-2 and 3-4A) and survive longer than small young at a low water temperature (Figure 3-5).

A high starvation tolerance of large young has also been noted for other aquatic species including cladocerans (Tessier et al. 1983; Goulden et al. 1987), prawns (Mashiko 1985), and fish (Miller et al. 1988). The decline in metabolic rate per unit body mass in large individuals is broadly known in animals (Peters 1983). Therefore, the production of progressively larger eggs at higher elevations ehances the survival of young released into environments with cold temperatures and short growing seasons.

Although the metabolic argument is consistent for the size (length) of young and weight of eggs between our study populations, the weight of hatched young from our incubations does not agree with this argument (Figure 3-4B). We believe that newly hatched young gained weight by feeding in the brood pouch before emerging, because newly hatched young were 2.5 and 1.2 times heavier than stage 5 eggs from Cooking and Snowflake Lakes, respectively (Figure 3-2, and 3-4B). Young with full digestive tracts have been found in the brood pouch of females collected from the field (Wilhelm pers. obs.), indicating that feeding occurs before leaving the brood pouch. In addition, young are known to re-enter the brood pouch in some amphipod species (Sheader and Chia 1970). Therefore, to obtain accurate weights of young at hatching, females should be examined individually after eggs have reached development stage 5. However, in preliminary experiments we found that manipulating females with a large brood resulted in the premature release of eggs. Thus, some method to examine the brood without disturbing the female must be devised.

The increase in egg weight between the early development stages (Figure 3-2) is difficult to explain, because it is not possible for females to apportion further resources to eggs once they are extruded from the ovary. Sheader (1983) also observed an increase in egg weight between development stages of the amphipod *Gammarus duebeni*. It has been suggested that the weight gain of eggs in the brood pouch may be related to the absorption of minerals. Morritt and Spicer (1996) have demonstrated that females of some amphipod species are able to osmoregulate the embryonic environment of the brood pouch. Although this has not been investigated in *G. lacustris*, such a mechanism could explain the weight increase of eggs.

Our results show that *Gammarus lacustris* displays a high variability in reproductive traits, such as egg size and number. This variation appears to be a key factor in the maintenance of successful populations in a wide range of aquatic habitats. Our data suggest that as aquatic habitats warm, *G. lacustris* reproduction should shift along a continuum from few large eggs to many small eggs. Therefore, we would predict that the warming of currently cold montane and alpine lakes should result in a shorter life cycle duration and an increase in population density as a result of increased fecundity. However, our study does not provide insights into whether these traits are under environmental or genetic control. For example, using a transplant experiment Hancock et al. (1998) found that egg size of the Australian atyid shrimp, *Paratya australiensis*, was under strong genetic control, but fecundity was influenced by environmental conditions. Similar transplant experiments should be carried out with populations of *G. lacustris* in ecoregions subjected to global warming to predict the fate of populations to altered temperature regimes.

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4. SEASONAL FOOD HABITS OF BULL TROUT FROM A SMALL ALPINE LAKE IN THE CANADIAN ROCKY MOUNTAINS[1]

Introduction

Past surveys of mountain lakes in western Canada have shown that plankton communities in lakes with native cutthroat, *Oncorhyncus clarki*, and bull trout, *Salvelinus confluentus*, populations were more similar to those of fishless lakes than lakes stocked with non-native salmonids (Anderson 1980). Plankton communities in mountain lakes stocked with non-native salmonids such as brook trout, *Salvelinus fontinalis*, were severely impoverished in terms of abundance and species diversity (Reimers 1958; Anderson 1980; Amann 1980; Parker and Schindler 1995; McNaught et al.1999). Anderson (1980) suggested that the lack of feeding by cutthroat trout during their spring reproduction coincided with the critical reproductive period of many invertebrates, allowing their populations to coexist. However, no explanation was offered for the coexistence of plankton and fall spawning bull trout. These findings suggest that: i) stocking programs should be focussed on native species to preserve and maintain plankton diversity; and, ii) that it may be possible to increase the number of bull trout populations in mountain lakes without severely impacting native plankton communities.

The decline and local extirpation of bull trout populations in northwestern North America as a result of overharvesting, habitat destruction, and the introduction of other *Salvelinus* species (Roberts 1987; Buktenica 1997; Colpitts 1997; Fitch 1997; Rhude and Stelfox 1997), has led to concern among fisheries managers and prompted the implementation of recovery plans (Berry 1994). For such plans to be effective, the ecology of the species must be understood. However, the past unfavourable status of bull trout among fishermen and managers (Colpitts 1997) and the difficulty of access to remote mountain lakes have meant that few detailed studies of the ecology of bull trout in high elevation lakes were conducted. A few of these lakes were spared introductions of other fish species during the past stocking era, and today contain bull trout populations in a pristine state. It is estimated that only 6-7 lakes with native adfluvial bull trout populations (lake based populations that spawn in rivers or streams) remain in the Canadian Rocky Mountains (Donald and Alger 1993; Donald and Stelfox 1997). These populations are valued as important gene pools and have been considered as possible donor stocks for introduction to other lakes (Carl et al. 1989). They also represent a valuable resource from which to collect baseline data and examine the mechanism by which bull trout coexist with a diverse invertebrate prey fauna.

To our knowledge, no thorough investigation of the seasonal diet of adfluvial bull trout populations in high elevation lakes has been undertaken. Information on diet and feeding strategy is necessary to assess the suitability of potential lakes for stocking or restoration. Knowledge of the feeding habits is also important to evaluate potential impacts

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[1] *A version of this chapter has been accepted for publication.* Frank M. Wilhelm, Brian R. Parker, David W. Schindler and David B. Donald. *Transactions of the American Fisheries Society.*

of bull trout on lake food webs in lakes identified for potential stocking and could indicate possible mechanisms whereby bull trout coexist with invertebrate populations. Here we describe the seasonal food habits of an unmanipulated adfluvial bull trout population from a small alpine lake in relation to the availability of prey species in the lake.

Materials and Methods

Study lake

Harrison Lake (Figure 4-1) is a small oligotrophic alpine (2,243 m a.s.l.) lake located in Banff National Park, Alberta, Canada (51°32'W 115°48'N). It has a surface area of 8.4 ha and a maximum depth of 10.7 m. The ice-free season lasts approximately 100 days from early July until late September to mid-October. Usually, maximum surface water temperatures reach approximately 12°C in August (Anderson and Donald 1978, D. W. Schindler unpub. data). The lake stratifies weakly; temperature differences between the surface and bottom are usually less than 3°C.

Three small streams; one on the west side and two on the southwest side direct snowmelt runoff to the lake. Inflow volume decreases as the catchment snowpack diminishes throughout the summer. A single shallow outflow is located on the north shore of the lake. A small waterfall approximately 20 m downstream of the outlet prevents immigration of fish from the stream. Bull trout is the only fish species present in Harrison Lake, determined from fishing records earlier this century, gill net surveys in 1977, and continued monitoring in 1996, 1997 and 1998.

Harrison Lake is approximately bowl shaped with a shallow ice-scoured shelf to a depth of approximately 1 m which extends 1 to 5 m from shore (Figure 4-1). Macrophytes are absent and chironomids, cyclopoid copepods (mostly *Diacyclops bicuspidatus thomasi*), *Daphnia pulex var.*, and the amphipod *Gammarus lacustris* are the most abundant invertebrates. The bull trout population is considered unexploited because of the lake's remote location; it is approximately 36 km from the nearest motorized vehicle access.

Bull trout sampling.

On August 17, 1977, bull trout were collected with monofilament gill nets of mixed mesh sizes (9 m each of 25, 38, 51, 76, and 102 mm stretched-mesh). Of the 18 fish, ranging in size from 350 to 440 mm fork length (FL), all contained food and were examined. Although no effort was made to capture young fish, they were observed in shallow (<15 cm deep) water close to shore along the east and north side of the lake. In 1996, bull trout were caught by angling on August 2, 7, and 9, using artificial lures with barbless hooks. Captured bull trout ranged from 170 to 450 mm FL, but only fish >250 mm FL were examined for stomach contents. Of the 31 examined, six had empty stomachs.

In 1997, sampling occurred on July 1, 13, 14, and 24; August 10, 22 and 24; and September 23, and 24. Adult bull trout were angled as in 1996, while juveniles were obtained with coarse mesh (approx. aperture = 1 mm) dipnets. The 323 captured fish, ranged in size from 29 to 540 mm FL, with most individuals in the 120 to 280 mm FL size range, reflecting our increased effort to capture juvenile fish. Of the 126 individuals

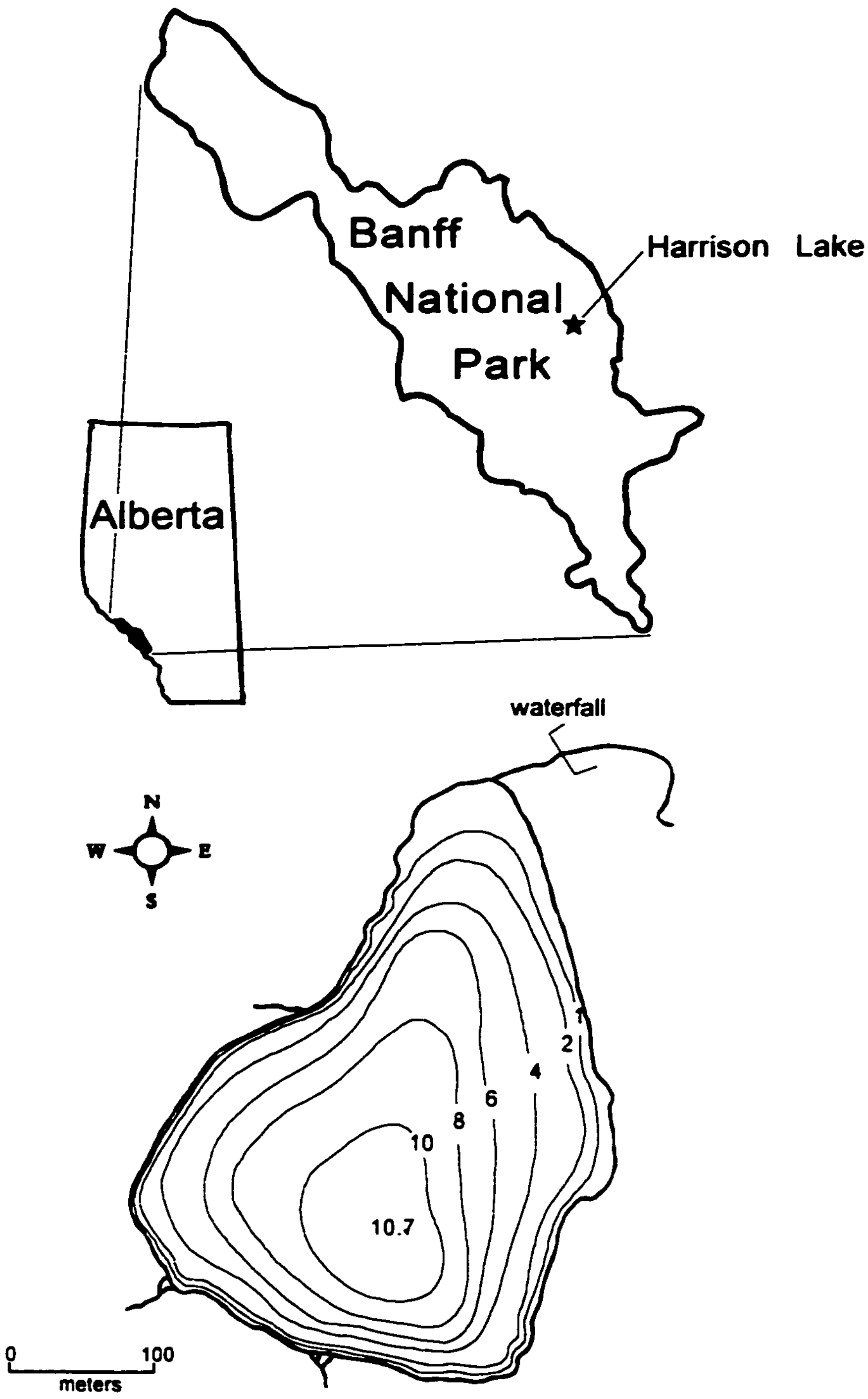


Figure 4-1. Bathymetric map of Harrison Lake showing location of inflows and outflow. Map adapted from Anderson and Donald (1978).

examined for stomach contents, 32 had empty stomachs. Of the 94 with gut contents, 45 were small (≤250 mm FL), and 49 were large (>250 mm FL).

Fork lengths were recorded to the nearest mm in 1996. In 1997, both weight and fork lengths were recorded. For data analysis, bull trout were grouped into small (≤250 mm FL) and large (>250 mm FL) size classes corresponding to their spatial segregation in the lake. Small fish were caught from beneath two cutbanks close to the lake outflow and were observed mainly near shore in shallow (<1 m deep) water, while large fish were caught from deep (>1 m) water and were rarely seen in shallow water. Sampling times were combined by month.

Diet analysis.

To analyse diet, entire stomachs were removed and preserved in dilute formaldehyde in 1977. In 1996 and 1997, we anaesthetized fish with MS222 (tricaine methane sulphonate) and used a flushing technique similar to that employed by Meehan and Miller (1978) to retrieve the stomach contents. Fish were held in a recovery tank until fully recovered from anaesthesia and then released back into the lake. Stomach contents were transferred to labelled plastic bags and fixed with 4% sugared formalin for transport back to the lab where samples were transferred to glass jars and transferred to 70% ethanol for storage until analysis. We used a dissecting microscope to sort and count prey items. The majority of sampled stomachs yielded intact prey items that were easily identified and counted. For broken-up or partially digested food items, we used head capsules of chironomids and *Gammarus* or carapaces of *Daphnia* to identify and count prey items.

We used measures of frequency of occurrence, mean contribution (volume %) to the stomach contents, and prey-specific volume to describe diet. Frequency of occurrence indicates the percentage of fish that had eaten the prey. The mean contribution to the stomach contents expresses the average degree of fullness for each prey taxon as a percentage of the average degree of total fullness (based on points estimates, see below) in the sample (Wallace 1981). Prey-specific volume is the volumetric percentage a prey taxon comprises of all taxa in only those predators in which the prey occurs (Amundsen et al. 1996). Limitations of these methods have been discussed by Hyslop (1980), Wallace (1981) and Amundsen et al. (1996).

Volume was subjectively estimated by a "points" method (Donald and Alger 1993). The stomach contents of each fish were assigned a total of 10 points. Then, each of the major food groups in a stomach was assigned from 1 to 10 points, depending on its contribution to the total volume of food, regardless of stomach size or total content volume. Empty stomachs were not included in the calculations. The points for each food category in a sample of N fish were then summed and expressed as a percentage of the total points (N×10) for the sample. In 1996, we measured the volume of intact invertebrates by water displacement in a graduated cylinder to compare displacement mean volume with estimates of the points method. We placed as many individuals as possible in the cylinder and calculated the volume displaced per individual. Mean prey volumes estimated with the points method compared well to volumes from displacement measurements (Table 4-1), indicating that prey volumes estimated via the points method yielded reliable volume estimates. Further, because data were analyzed only with the points method in 1977, we could compare it with our current data.

Table 4-1. Comparison of bull trout diet mean volume (%) obtained from displacement volume calculations and the points method. Blank cells indicate that not enough items occurred in stomachs to obtain accurate volume measurements, while a plus sign in the points column indicates prey presence, but an insignificant contribution to total stomach volume.

Prey Species	Mean volume (%) (Displacement)	Mean volume (%) (Points)
Daphnia pulex var.	45.1	49.6
Gammarus (adults and immature)	33.4	25.2
Chironomidae (pupae)	19.7	20.8
Pisidium		0.8
Conifer Needles	1.8	2.0
Trichoptera		+
Plecoptera		+
Corixidae		0.8
Adult Diptera		0.8

Prey-specific volume (defined above; Amundsen et al. 1996) was calculated from points volumes as:

$$P_i = \frac{\Sigma S_i}{\Sigma S_{ti}} \times 100$$

where: P_i is the prey-specific volume of prey *i*;

S_i the stomach content (volume %) comprised of prey *i* in individual fish;

S_{ti} the total stomach content in only those predators with prey *i* in their stomach.

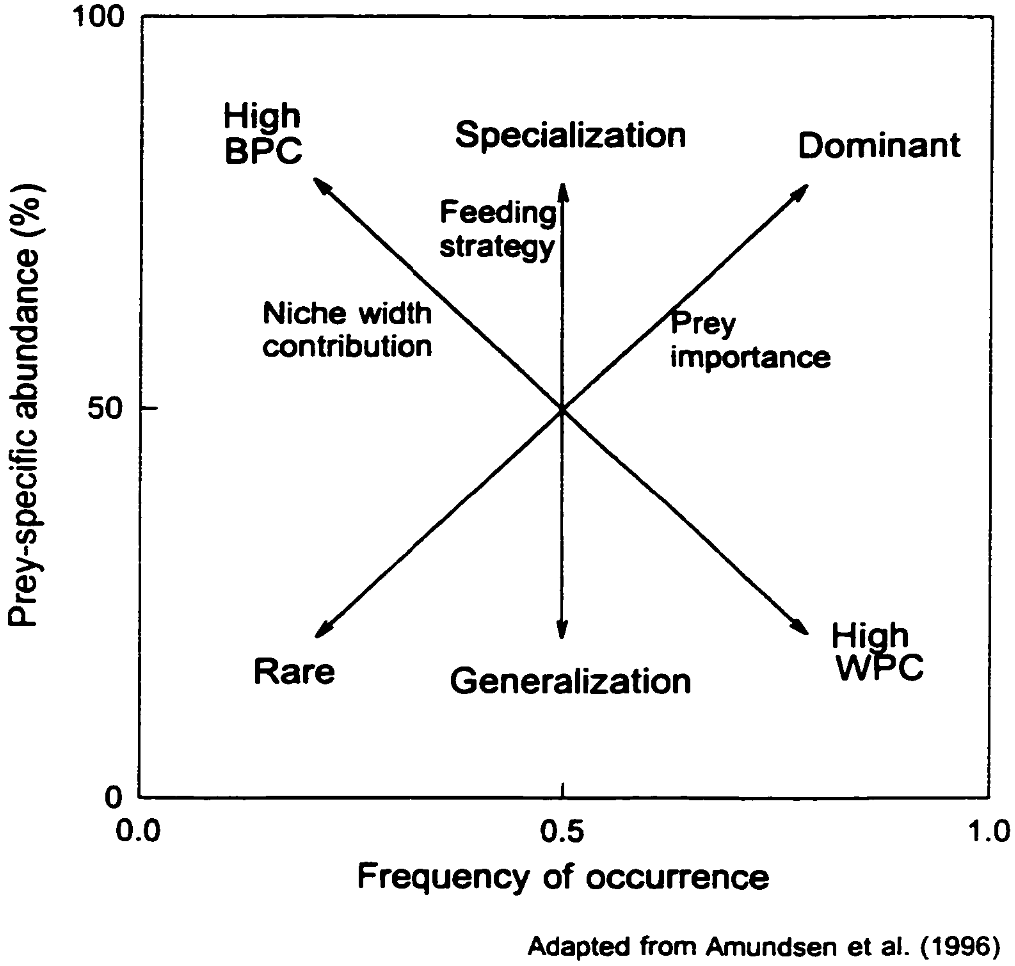
Prey-specific volume was plotted versus frequency of occurrence to compare the among-year and seasonal among-size class feeding strategies of the bull trout population.

We use Amundsen et al.'s (1996) graphical method to present our diet data because it offers advantages over traditional tabular methods. This method allows prey importance, feeding strategy and the inter- and intra-individual components of diet niche width to be easily interpreted (see Amundsen et al. 1996 for a detailed description of the method). Briefly, prey importance and predator feeding strategy information are given by the distribution of points along the diagonals and axes of the plot (Figure 4-2). A measure of prey importance is provided by the percent abundance, increasing along the diagonal from the lower left to the upper right corner. Dominant prey will be clustered in the upper right, while rare and unimportant prey are represented in the lower left corner. The feeding strategy in terms of specialization or generalization is represented on the vertical axis (Figure 4-2). Prey points in the upper part of the graph indicate predators with specialized feeding. If prey points are positioned in the lower part, generalized feeding is inferred. Prey points in the upper left corner indicate specialization of individual predators, while points in the upper right represent specialization of the predator population as a whole and indicate a narrow niche width. A broad niche width for the population is indicated by the location of prey points along or below the diagonal from the upper left to the lower right and a lack of points in the upper right of the plot.

Prey items clustered in the upper left or the lower right corner represent prey types that make the same overall contributions to the population diet, but are indicative of different feeding strategies of individual predators. Prey with high specific abundance and low occurrence will have been consumed by few individuals (specialization), whereas prey with a low specific abundance and a high occurrence will have been eaten occasionally by most individuals. Thus the distribution of prey points along this diagonal are indicative of the contributions of between- and within-phenotype components to the diet niche width (BPC and WPC Figure 4-2). This graphical method allows the rapid interpretation of predator feeding strategies in terms of specialization and generalization, both at the individual and population level. As well, the contributions of between- and within-phenotype components to niche width can be inferred.

Zooplankton and benthos sampling.

Zooplankton were sampled with a 30-cm.-diam., 64-μm-mesh, Wisconsin-style



Adapted from Amundsen et al. (1996)

Figure 4-2. Amundsen et al. 1996 feeding strategy plot. See text for details.

plankton net. Five replicate hauls from 9 m were taken on Aug. 9, 1996, Jul.1, 13, 24, Aug. 10, 22 and Sep. 23, 1997 and preserved with 4% sugared formalin. *Daphnia* density was estimated in 1997 for the Jul. 13, Aug. 10, and Sep. 23 samples by bringing the preserved volume to 500 mL, thoroughly mixing the sample and withdrawing three 10 mL subsamples. All *Daphnia* were counted under a Wild M5 dissecting microscope and density was calculated as individuals per liter.

Gammarus and other benthic invertebrates were sampled from the benthos on August 9, 1996, Jul. 13, Aug. 11 and Sep. 23, 1997 by scraping the substrate with a dipnet (mesh aperture = 500 μm) along the ice-scoured shelf at a water depth of approximately 1 m. This area was chosen because bull trout were repeatedly observed feeding there (Wilhelm 1998). The dipnet was scraped over one square meter of bottom to disturb the top layer of sediment and organisms. Organisms were then collected from the water column with the dipnet. Contents of the dipnet were emptied into a sieve and *Gammarus* were separated from the sediment and debris by trapping them in the surface tension of water (Wilhelm and Hiebert 1996). Other invertebrates were counted and released, or preserved. We calculated relative *Gammarus* and 'other' (all remaining invertebrates) density for the 1997 sampling dates.

Dietary overlap.

Dietary overlap among fish size-classes was calculated using Schoener's (1970) similarity index (a):

$$a = 1\text{-}0.5(\sum|P_{xi} - P_{yi}|)$$

where P_{xi} and P_{yi} are the proportions by points of food category i in the diet of size classes x, and y. Values for a range from 0, representing no overlap, to 1, for complete overlap. Overlap values greater than 0.60 are generally considered to indicate significant overlap in the diet (prey items) of size class x and y (Wallace 1981; Brodeur and Pearcy 1990).

Prey size-selection

The lengths of *Daphnia* and *Gammarus* in the stomach contents from 1996 and 1997 were measured and compared to size distributions present in the lake to test for size-selective predation by bull trout. *Daphnia* size was measured from the top of the head to the inflection of the tailspine (cf. Dodson 1981) with the aid of an ocular micrometer. *Daphnia* were measured from all fish stomachs in which they occurred. All, or a minimum of 50 *Daphnia* were counted from stomachs containing ≤1000 individuals, while a minimum of 100 *Daphnia* were measured when >1000 individuals were present. To estimate the *Daphnia* size distribution in the lake, the lengths of the first 200 to 420 *Daphnia* encountered from the zooplankton samples were measured. The number counted was dependent on the density of *Daphnia* in the samples.

We also measured 30 of the largest *Diacyclops bicuspidatus thomasi* copepods from the August 22, 1997 sample to examine maximum copepod size in relation to *Daphnia* size ingested by the bull trout. We were interested to determine if copepods reached a size vulnerable to bull trout predation, because we only found one copepod in one bull trout stomach.

Gammarus from benthic samples were transported live to our field camp for measuring under a dissecting microscope. Body length of all individuals was measured from the tip of the rostrum to the tip of the telson after straightening the body with fine forceps. The body size of *Gammarus* from fish stomachs was estimated from head capsule lengths (cf. Wilhelm and Lasenby 1998) based on a regression established from fresh monthly samples collected in the summers of 1995 through 1997. All *Gammarus* occurring in fish stomachs were measured. Young-of-the-year (YOY) *Gammarus* from fish stomachs were not included in the analyses because it was impossible to determine if YOY were consumed by the fish or had been released from ingested ovigerous females (cf. Wilhelm 1996). The 1996 stomach samples were taken at the time of peak YOY release (Wilhelm unpub. data). Lack of YOY in 1997 stomach samples suggested that the presence of YOY in stomachs in 1996 likely resulted from the ingestion of ovigerous females.

We used non-parametric Kolmogorov-Smirnov two-sample tests (Sokal and Rohlf 1981) to successively test the similarity of *Daphnia* and *Gammarus* size-frequency distributions in the lake versus in small and large fish. Between-sampling date comparisons were also conducted. The level of significance was Bonferroni-corrected ($P = 0.003$) to maintain an overall significance level of $\alpha = 0.05$.

Seasonal food volume

To examine seasonal patterns in the volume of food ingested by each fish size-class, we calculated the average total food volume present in small and large fish for each month. Total volume (mL) in individual fish was determined from the number of prey items present and their displacement volumes (Table 4-1). Volume for each fish size-class was obtained by averaging over the sampling month. Large fish with less than 1 mL of food were considered to have empty stomachs for this analysis and were not included. We used a one-way ANOVA for each fish size-class to test the hypothesis that food volumes were similar between sampling dates. Significant results were further examined with a Tukey post hoc test to determine which means differed. Data were log transformed to meet ANOVA assumptions of homogeneity of variance and normality. We plotted log back-transformed means and 95 % confidence intervals (Sokal and Rohlf 1981).

Results

Bull trout diet

Overall, the Harrison Lake bull trout population displayed a narrow feeding niche throughout the open-water season. After ice-out, the population mainly fed on chironomid pupae, switching to *Daphnia* and *Gammarus* in mid-August and September (Table 4-2). Only two cases of piscivory were observed, one successful, and one resulting in the death of both the predator and prey fish (Wilhelm 1998). Only one large copepod was found in one bull trout, suggesting copepods (mean *D. bicuspidatus thomasi* length 0.49 mm ± 0.04 SD, n = 30) were either too small to be retained by bull trout gill rakers or were not selected. Sixty-four and 53% of all fish stomachs in 1996 and 1997, respectively, contained *Gammarus*, conifer needles, *Pisidium* and/or stones, indicating the bull trout were

Table 4-2. Mean percentage points (i.e. relative volume) of the various prey taxa found in non-empty stomachs of small (≤250 mm FL) and large (>250 mm FL) bull trout, *Salvelinus confluentus*, from Harrison Lake. A plus sign indicates the prey item was present in the stomach but contributed insignificantly to the overall stomach content volume compared to other prey items. Blank cells indicate the food item was absent.

	Fish size	≤250 mm FL			>250 mm FL				
	Month	Jul.	Aug.	Sep.	Jul.	Aug.			Sep.
Prey taxa	Year	'97	'97	'97	'97	'77	'96	'97	'97
Chironomidae	larvae	1.4	+	0.6	0.3			+	+
	pupae	43.4	+		82.4	0.8	20.8	0.2	
	adults	13.2	1.3		8.3			0.7	
Daphnia		8.3	60.9	65.5	6.0	58.5	49.6	72.3	44.0
Gammarus		21.2	28.4	25.1	1.2	24.0	25.2	12.3	44.8
Fish								4.5	
Pisidium		+	+	+	0.7	11.4	0.8	+	+
Oligochaeta						4.4			
Trichoptera		1.8	1.3	5.6	+	0.3	+	4.5	10.0
Ephemaroptera		0.9	6.8	+	0.3			0.7	
Corixidae			0.8		+		0.8		
Adult Diptera		0.3	+	0.6	+		0.8	0.7	0.4
Plecoptera				1.0	+	0.6	+		0.8

Table 4-2. Continued.

Fish size		≤ 250 mm FL			> 250 mm FL				
	Month	Jul.	Aug.	Sep.	Jul.	Aug.			Sep.
Prey taxa	Year	'97	'97	'97	'97	'77	'96	'97	'97
Conifer Needles		0.3	0.5	+	0.8		2.0	0.5	+
Stones		+	+	+	+		+	+	+
Simuliidae		9.2			+				
Copepoda					+				
Coleoptera								3.6	
Lepidoptera				1.6					

benthivorous when feeding on *Gammarus*.

Between-year diet comparison of large bull trout

The between-year diet of large fish in August was remarkably similar and included *Daphnia* and *Gammarus* as the major prey items (Figure 4-3A, and 4-3B, 4-4D), indicated by their high placement along the prey importance diagonal. In August 1996, chironomid pupae were present in the diet in a large proportion of the population, but were less important than either *Daphnia* and *Gammarus* (Figure 4-3B). The chironomid hatch was delayed in 1996 because a high snowpack slowed the thaw and prevented early warming of the water. In 1997, chironomids hatched during a short time interval during July, explaining their absence in the diet in August and September (Figure 4-4).

Diet seasonality in small bull trout

In July 1997, after ice-out, small bull trout fed on a wide variety of invertebrates, but their diet was dominated by chironomid pupae (Figure 4-4A). A small proportion also fed on *Gammarus* (Figure 4-4A). In August 1997, small bull trout switched prey and fed primarily on *Daphnia* and *Gammarus* (Figure 4-4C). Other prey taxa such as Ephemeroptera, Trichoptera, corixids and chironomids were also consumed, but they contributed little to the diet of the small fish. In September 1997, small bull trout continued to specialize on *Daphnia* and *Gammarus* (Figure 4-4E). Similar to August, other prey taxa were consumed but they did not contribute significantly to the overall diet (Figure 4-4E).

Diet seasonality in large bull trout

In July, large bull trout fed almost exclusively on chironomid pupae (Figure 4-4B). Although chironomid adults were present in some bull trout stomachs, these individuals still had their pupal case attached to their abdomen. Only one large bull troutfed primarily on *Daphnia* at the end of July (Figure 4-4b). Large bull trout switched prey species in August. with the majority of the population feeding on *Daphnia*. *Gammarus* were the second most important prey (Figure 4-4D), but were less important in the diet of the large fish compared to small fish (Figure 4-4C). Some individual fish specialized on Coleoptera, and in one case. on small (~40 mm FL) bull trout (Figure 4-4D). In September 1997, large bull trout continued to specialize on *Daphnia* and *Gammarus* (Figure 4-4F). At this time, *Gammarus* and *Daphnia* co-dominated the diet of large trout (Figure 4-4F). Several large bull trout specialized on Trichoptera larvae, which had large (~ 35-40 mm long ×6-8 mm dia.) cases built of dark stones making them highly visible on the silty lake bottom and susceptible to predation. The reliance among large trout on three prey species coupled with the decline in food volume in the stomachs over the open-water season (see below) suggests a high potential for intra-size class competition.

Prey availability

Prey availability changed over the course of the open-water season in 1997. Emerging chironomids were abundant after ice-out until the end of July. The large proportion of chironomid pupae in the diet of all bull trout in July, followed by their near absence from the diet in August (Figure 4-4), mirrors our personal observations of the abundance of emerging adults and is probably a reasonable, if coarse, index of their

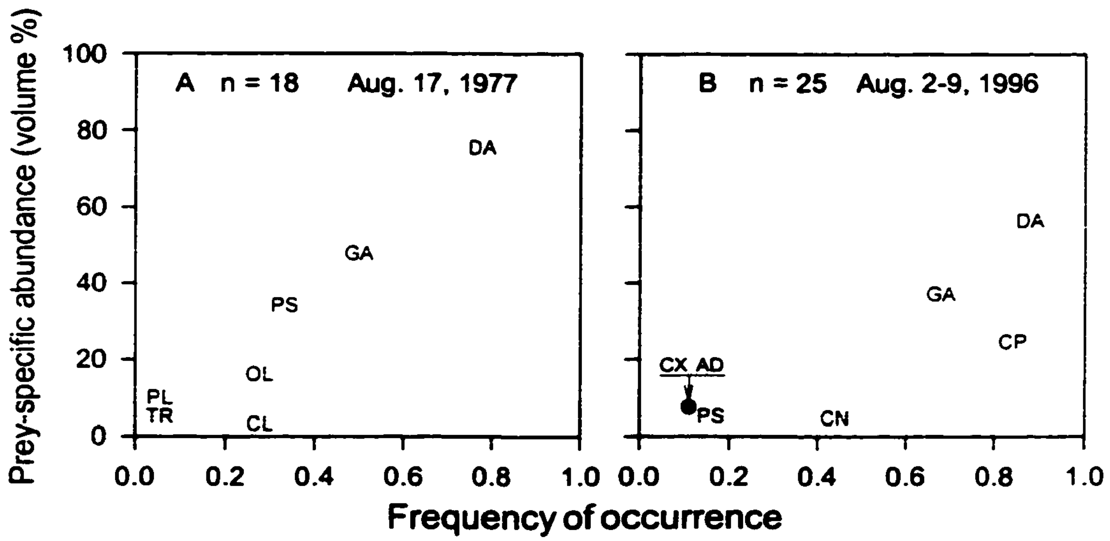


Figure 4-3. Feeding strategy plots for large (>250 mm) bull trout from Harrison Lake on **A**) August 17, 1977, and **B**) August 2, 7, and 9, 1996. Prey items identified as follows: CP - chironomid pupae, CA - chironomid adults, CL - Chironomid larvae, DA - *Daphnia*, GA - *Gammarus*, SI - Simuliidae larvae, TR - Trichoptera nymphs, EP - Ephemeroptera nymphs, PL - Plecoptera nymphs, PS - *Pisidium*, CX - Corixidae, AD - adult diptera, LE - Lepidoptera adults, CO - Coleoptera, FI - fish, OL - oligochaeta, CN - conifer needles.

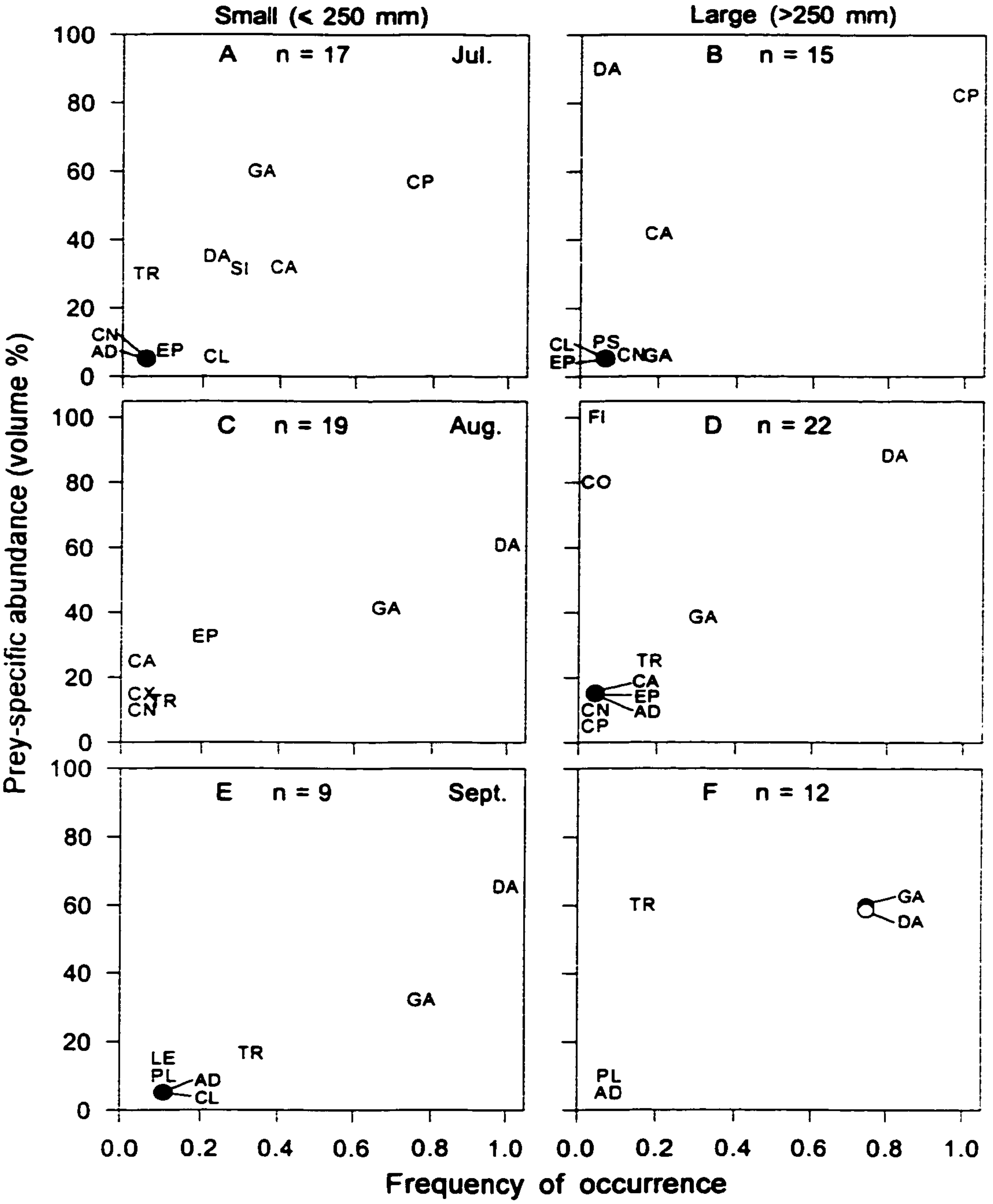


Figure 4-4. Seasonal feeding strategy plots for small (≤250 mm FL) and large (>250 mm FL) bull trout from Harrison Lake in 1997. N - number of fish with food in their stomachs examined for each size class on each date. Prey points abbreviated as in Figure 4-3.

availability in the lake.

Although *Gammarus* abundance increased from July to September, this increase was due to the release of young by mature females in August (Figure 4-5). These YOY were too small to be susceptible to bull trout predation; therefore, the abundance of *Gammarus* available to bull trout declined from approximately 129 to 50 $ind.{\cdot}m^{-2}$ (Figure 4-5). Adult *Gammarus* increased in size with age, making them increasingly susceptible to bull trout predation as the season progressed (see below). Other benthic invertebrates such as Trichoptera and Ephemeroptera nymphs, corixids, Coleoptera and fingernail clams collected with *Gammarus* in benthic sweeps were not abundant, and together averaged between 18 and 20 $ind.{\cdot}m^{-2}$ between July and September (Figure 4-5).

Daphnia density peaked in August at 9.9 $ind.{\cdot}L^{-1}$, increasing from 2.7 $ind.{\cdot}L^{-1}$ in July and decreasing to 3.5 $ind.{\cdot}L^{-1}$ at the end of September (Figure 4-5). Most gravid females were observed after ephippial egg production in early August. Ephippia were produced by females ranging between 1.5 to 2 mm in size.

Dietary overlap

Diets of both bull trout size-classes overlapped considerably each month (July a = 0.60, August a = 0.76, September a = 0.76). Both size-classes fed largely on chironomid pupae in July, and *Daphnia* and *Gammarus* in August and September. However, each bull trout size-class concentrated on a different size of *Daphnia* (see below). The predominance of one or two prey items in the diet of the bull trout population (Figure 4-3, and 4-4) also indicates a strong dietary overlap among individuals in each fish size-class.

Prey size-selection

Bull trout selectively preyed on large *Daphnia*. The mean size of *Daphnia* from bull trout stomachs was significantly ($P < 0.003$) larger than those in the lake for all months (Figure 4-6A, and 4-7). Although large *Daphnia* were present in the water column in 1997 shortly after ice-out (Figure 4-7A), few were consumed by bull trout until early August (Figure 4-7A, and 4-7B, 4-4B, and 4-4D). Widespread ephippial egg production was noted during the first week of August, before *Daphnia* occurred frequently in many bull trout stomachs. The average size of *Daphnia* collected from the lake increased ($P < 0.001$) between July and September from 1.2 mm to 1.6 mm (Figure 4-7C to 4-7D). This increase in mean size could be attributed to the late-season decline in the number of young (Figure 4-5).

Large bull trout preyed on the largest *Daphnia* in 1996 and 1997, consistently focusing on the 2.5 mm size class (Figure 4-6A, and 4-7). The size-distributions of consumed *Daphnia* in August between-years were similar. Between-month comparisons in 1997 showed that large bull trout selected similar-sized *Daphnia* in July and August (mean size = 2.5 mm). However, in September, significantly ($P = 0.001$) smaller (mean size = 2.3 mm) *Daphnia* were selected (Figure 4-7).

Small bull trout preyed on intermediate sized *Daphnia* (mean length = 1.9 to 2.0 mm) during the open water season in 1997. The mean size of consumed *Daphnia* was larger ($P < 0.001$) than the mean size in the lake (Figure 4-7B to 4-7D). Between-month comparisons showed that the mean size of *Daphnia* selected by small bull trout decreased

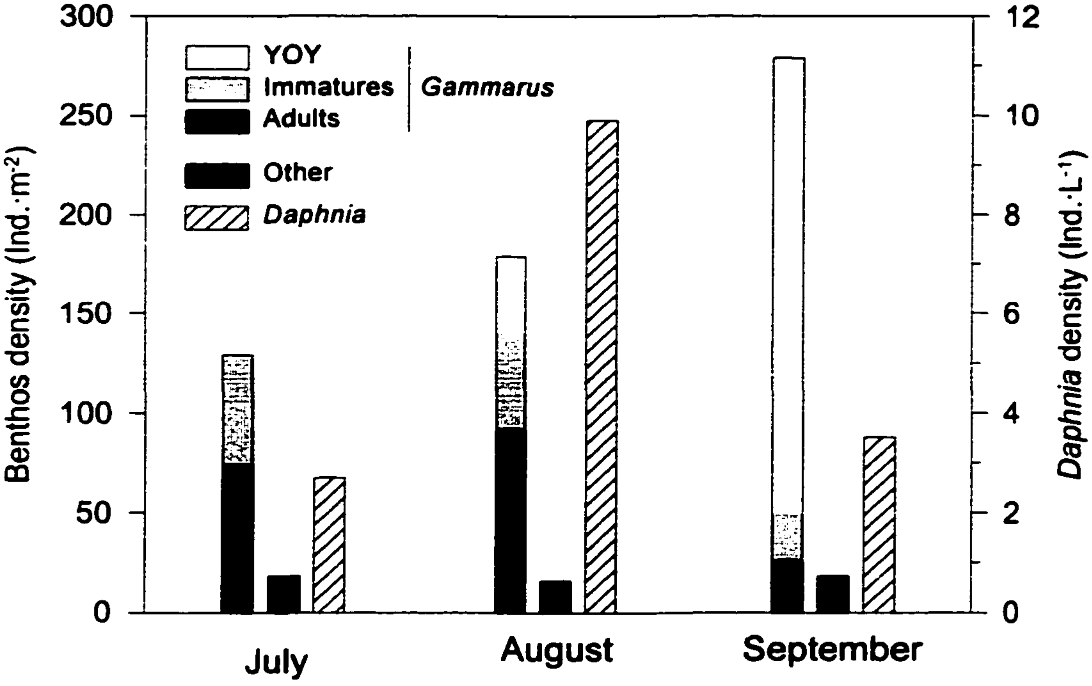


Figure 4-5. Seasonal abundance of prey organisms in Harrison Lake in 1997. *Gammarus* and 'other' benthic invertebrate densities are approximate densities per square meter. (see methods) on left y-axis, while *Daphnia* densities are given on right y-axis.

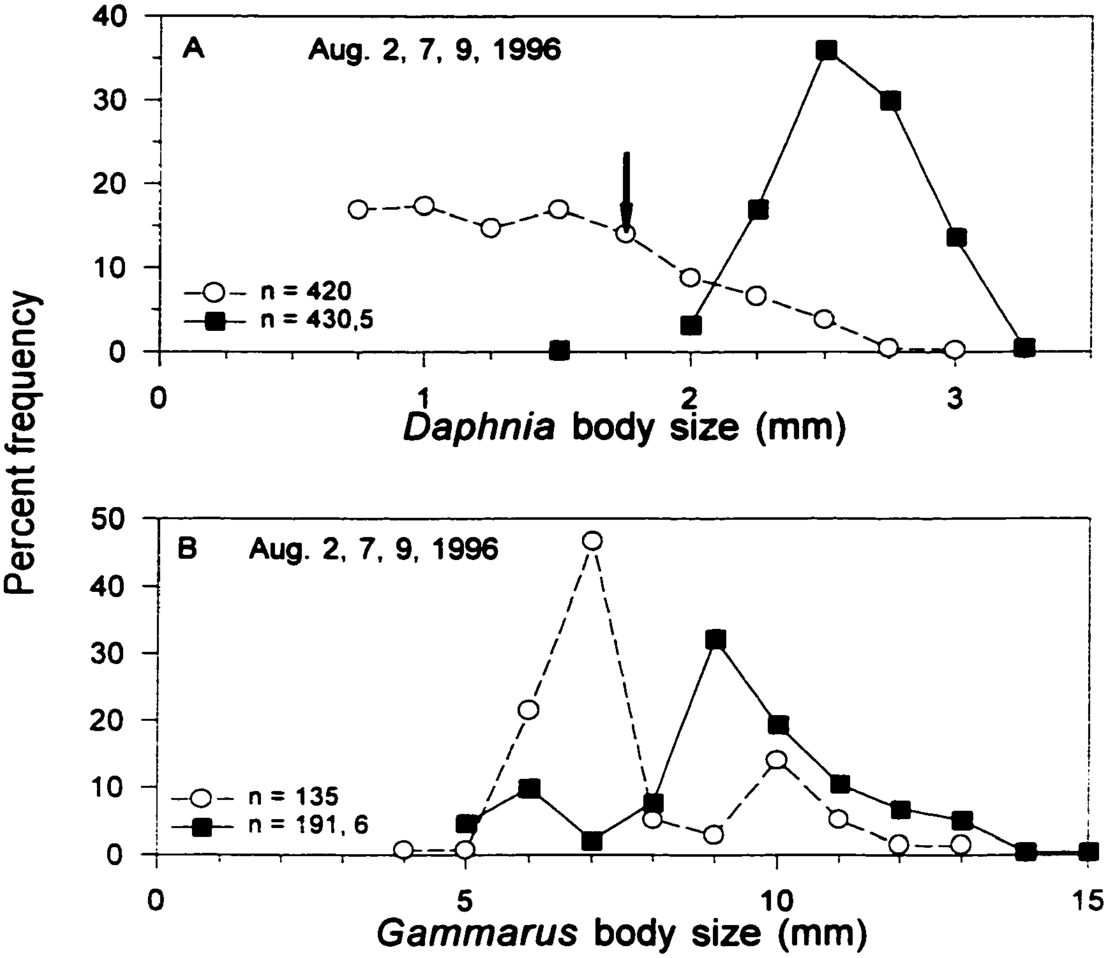


Figure 4-6. Invertebrate prey size distributions for individuals in stomachs and those in the lake showing size-selective predation for A) *Daphnia* and B) *Gammarus lacustris* in 1996. Arrow indicates the smallest *Daphnia* size class with eggs; n, n - indicates the number of prey measured and the number of fish with that prey in their stomach; symbols: ○ lake and ■ large (>250 mm) fish.

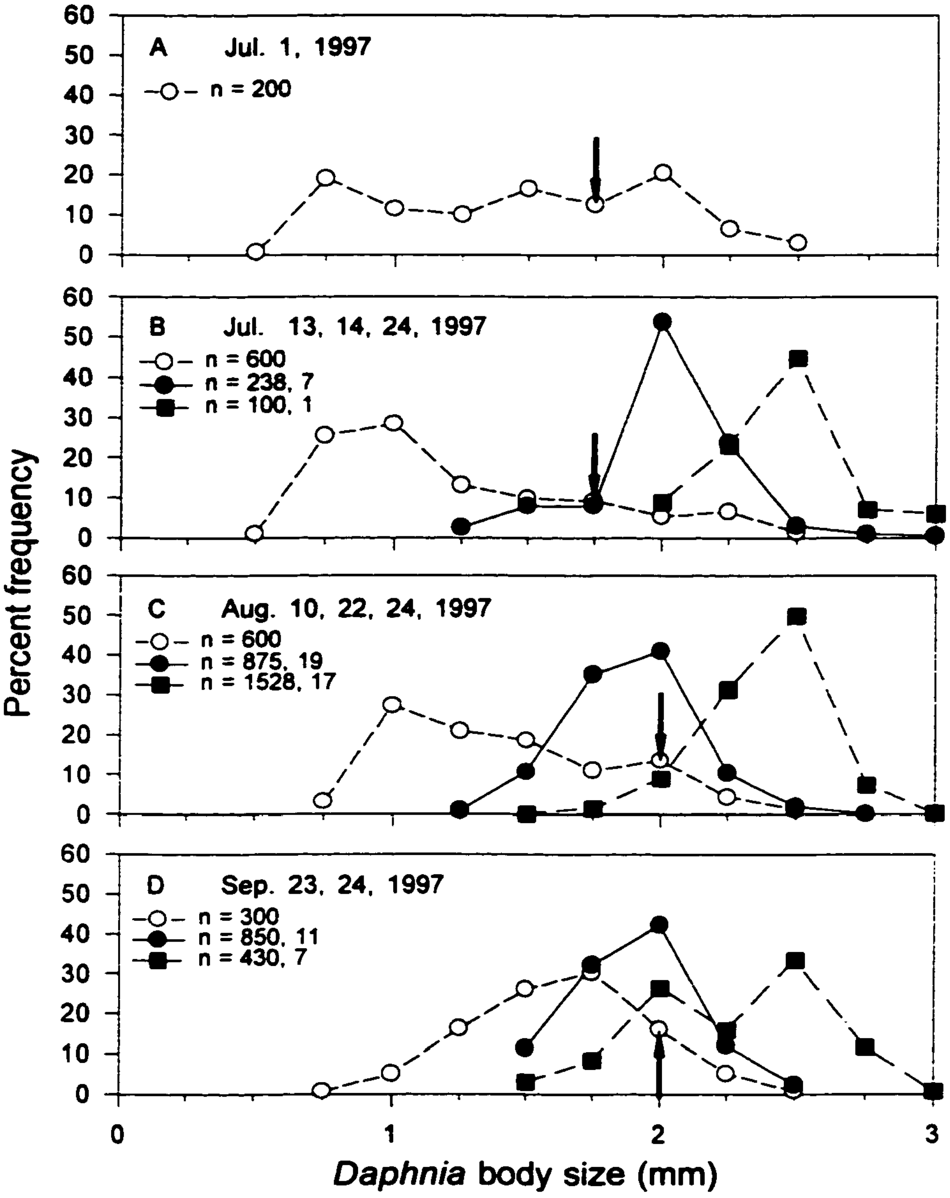


Figure 4-7. A comparison of seasonal size distribution of *Daphnia* in fish stomachs with those in Harrison Lake in 1997, showing size-selective predation on large *Daphnia* by bull trout. Arrows indicate smallest *Daphnia* size class with eggs. Ephippia were only observed in the August 10 sample. No predation on *Daphnia* was observed on July 1. N, n - indicates number of *Daphnia* measured and number of fish with *Daphnia* respectively. Symbols: ○ lake; ● small (≤250 mm) fish; and ■ large (>250 mm) fish.

significantly ($P < 0.001$) between July and August, but remained constant ($P = 1.00$) between August and September.

Bull trout selectively preyed on large *Gammarus* (Figure 4-6B, and 4-8). In 1996, the mean size of *Gammarus* preyed on by large bull trout was larger ($P < 0.001$) than the mean size in the lake (Figure 4-6B). In July 1997, small and large bull trout selected similar ($P = 0.42$) sized *Gammarus* (mean size = 8.7 and 8.8 mm respectively) that were larger ($P < 0.001$) than the mean size (7.5 mm) present in the lake (Figure 4-7B). In August 1997, small and large bull trout selected similar ($P = 0.113$) sized *Gammarus* (mean size = 9.7 and 9.1 mm respectively). Although these were larger than the mean size (7.5 mm) in the lake, we could only conclude that *Gammarus* in the small fish were larger ($P = 0.001$) than in the lake given our Bonferroni-corrected significance level. In September, small and large bull trout preyed on similar ($P = 0.081$) sized *Gammarus* (mean size = 9.9 and 9.4 mm respectively) which were significantly smaller ($P < 0.001$) than the mean size (10.6 mm) in the lake (Figure 4-7C). Bull trout predation probably explains the absence of large *Gammarus* in Harrison Lake compared to nearby fishless Snowflake Lake where adult *Gammarus* reach sizes of 15 to 20 mm and densities of 400 ind.$\cdot$m^{-2} (Parker et al. 1996).

Seasonal food volume

Average total volume of food in small fish increased over the season (ANOVA, $F_{2,42} = 7.18$, $P = 0.002$). Food volume was constant ($P = 0.904$) in July and August at 1.1 and 1.3 mL, respectively, but increased ($P = 0.006$) to 3.0 mL in September. The higher food volume in small bull trout during September resulted from a larger volume of *Daphnia* (Figure 4-9). In large fish, food volume declined significantly from July to August and September (ANOVA, $F_{2,34} = 5.75$, $P = 0.007$), due to the loss of chironomid pupae from the diet (Figure 4-9). Food volume in July (7.0 mL) was greater ($P = 0.022$) than in August (3.2 mL) and September (2.6 mL), when food volume was constant ($P = 0.748$) (Figure 4-9).

Length-Weight Relationship

The 1997 length-weight relationship for bull trout was best described by the allometric function:

$$\text{Weight (g)} = 4.8 \cdot 10^{-6} \cdot \text{Fork Length (mm)}^{3.15}$$
$$R^2 = 0.995,\ n = 142,\ \text{(Figure 4-10).}$$

The data were normally distributed and residual variance was homogeneous. However, a large proportion of adults over 425 mm FL were in poor condition (Figure 4-10). These emaciated individuals had disproportionately large mouths, heads, and slender bodies. These individuals were predicted to be 14 to 41 % underweight based on the above length-weight relationship.

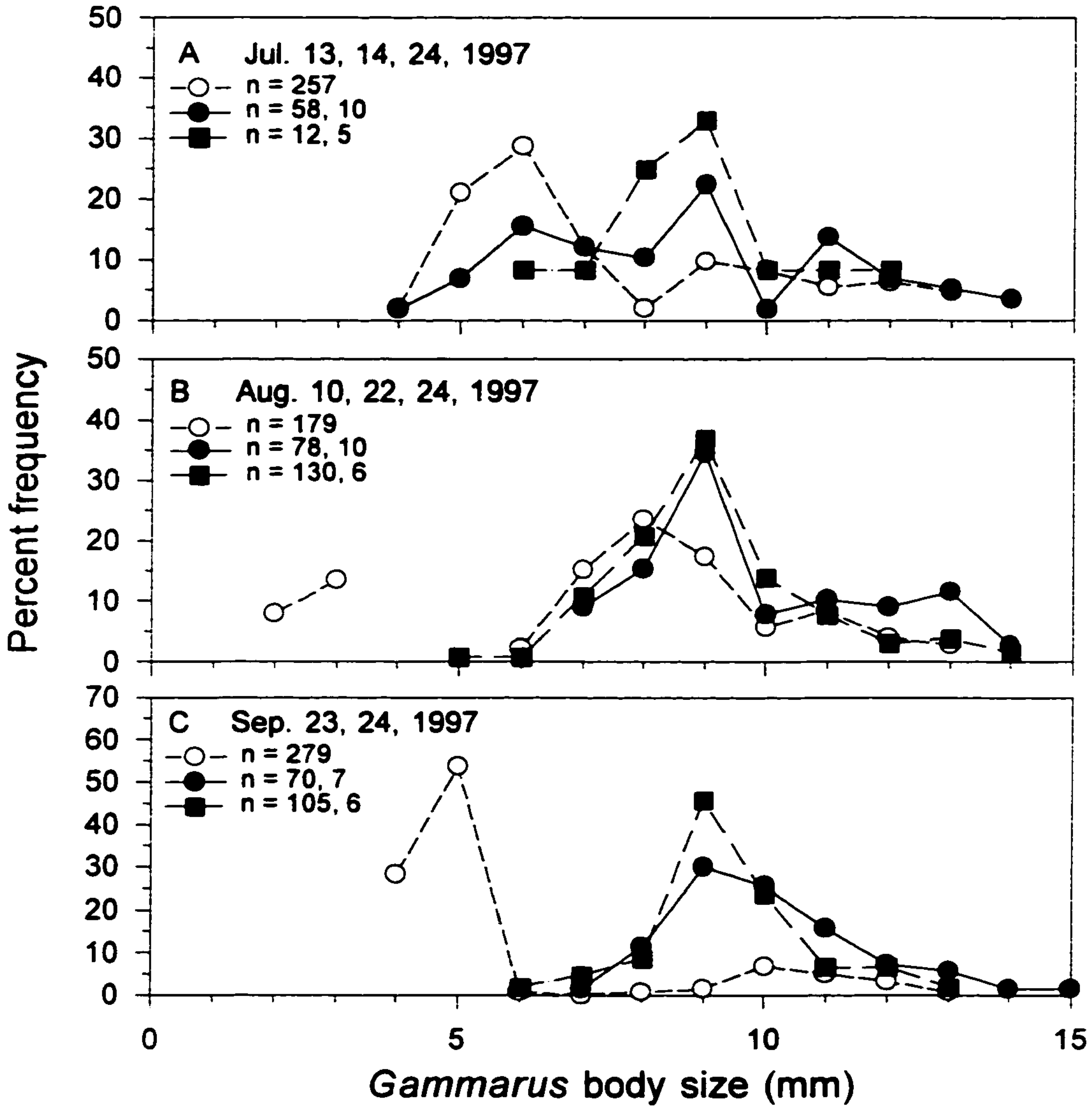


Figure 4-8. A comparison of seasonal size distribution of *Gammarus* in fish stomachs with those in Harrison Lake in 1997. N, n - indicates number of *Gammarus* measured and number of fish with *Gammarus* respectively. Symbols: ○ lake; ● small (≤250 mm) fish; and ■ large (>250 mm FL) fish. YOY were not included in analysis, but are shown for completeness.

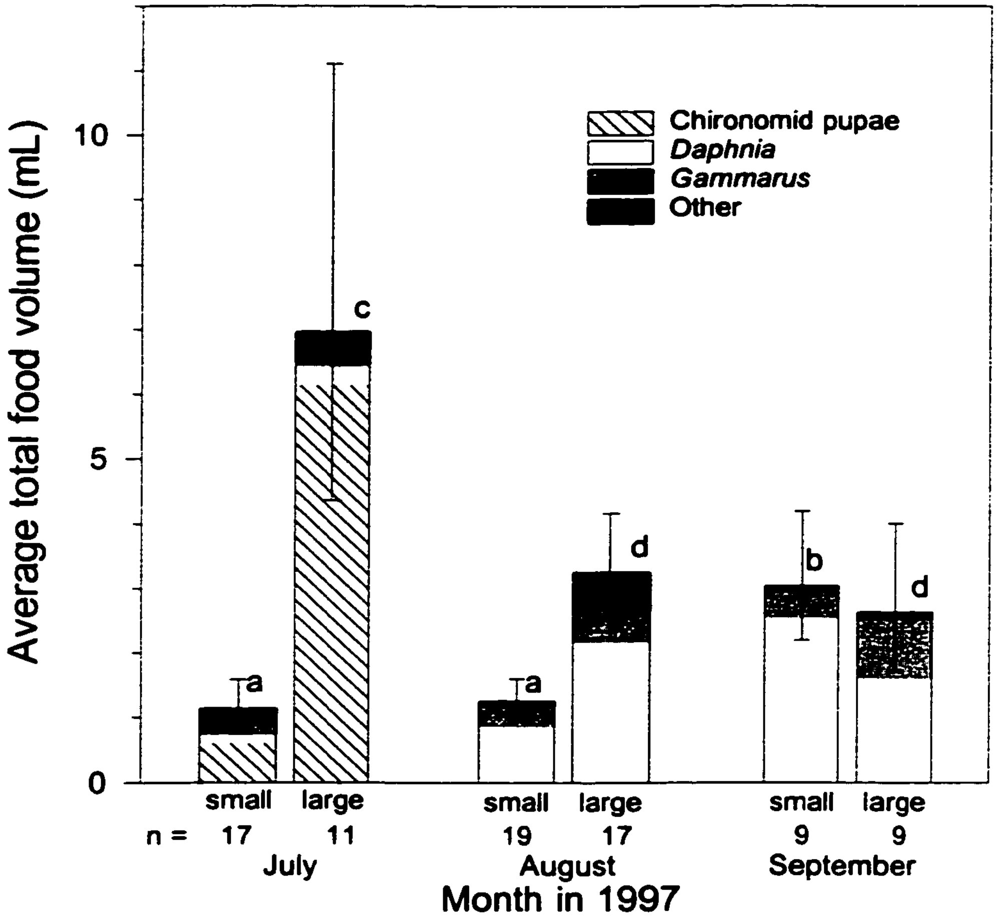


Figure 4-9. Average total food volume (mL) in the stomach of small (≤250 mm FL) and large (>250 mm FL) bull trout on each sampling date in 1997. Bars are log back-transformed means, and error bars represent 95 % confidence intervals. N is the number of fish. Letters indicate similar mean volumes; size classes analyzed separately.

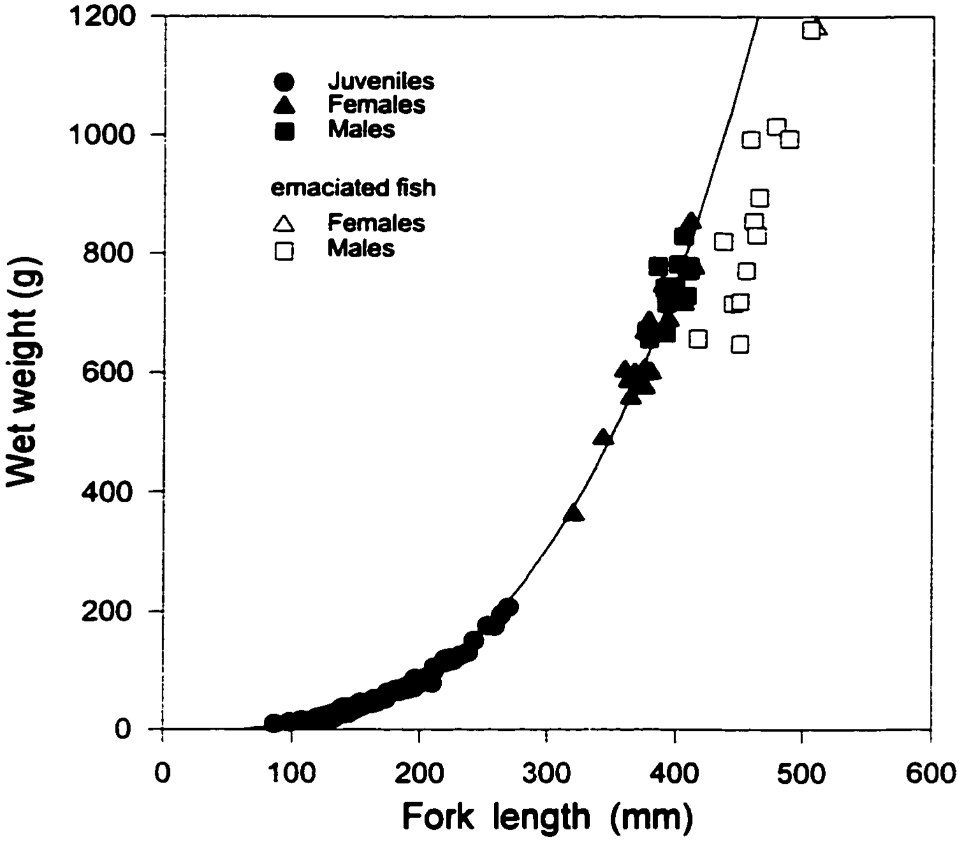


Figure 4-10. Bull trout length-weight regression for individuals captured in Harrison Lake in 1997. Regression in the form Y= aL^b: Weight (g) = 4.8·10^{-6}·L(mm)$^{3.15}$, R^2 = 0.995, n = 142. Emaciated fish were not included in the regression. Sex classification of the emaciated fish is unreliable.

Discussion

Factors allowing survival of bull trout and prey species

Size-selective predation by bull trout in Harrison Lake is similar to that reported for other salmonid and zooplanktivorous species (Brooks and Dodson 1965; Galbraith 1967; Wellborn 1994). However, bull trout did not eliminate *Daphnia* as stocked, non-native salmonids did in nearby Snowflake and Pipit Lakes (Anderson and Donald 1978; McNaught et al. 1999), and in Sporely Lake, Michigan (Galbraith 1967). The size distribution of *Daphnia* in Harrison Lake has remained constant among years. Although individual *Daphnia* attain a large size (3.0 to 3.25 mm), they are smaller than the 4-mm *Daphnia* present in nearby fishless Pipit Lake (Wilhelm et al. 1998). Bull trout predation may have resulted in a smaller maximum adult size in the *Daphnia* population as predicted from life history theory (Roff 1992; Stearns 1992).

The intense feeding by small and large bull trout on *Daphnia* in late August and September appears to be the main reason for the reduction in abundance of the largest *Daphnia*. Generally, *Daphnia* populations in most north temperate lakes decline after the late-summer chlorophyll *a* maximum and production of ephippia (Hall 1964). However, this is not the case in alpine lakes, where adult *Daphnia* overwinter (Wilhelm et al. 1998; McNaught et al. 1999). The overwinter survival of *Daphnia* adults is probably a function of a low metabolic rate resulting from the cold summer lake temperatures. The low predation on *Daphnia*, coupled with ephippia production early in the open-water season while bull trout feed on chironomid pupae, promotes the year-to-year persistence of the *Daphnia* population. The intense predation on *Daphnia* late in the open-water season therefore probably has little influence on population dynamics the following year.

The spatial segregation of bull trout by size in Harrison Lake, with large fish in the pelagic and small fish in the nearshore littoral zone (Wilhelm 1998), also benefits the *Daphnia* population. Large bull trout either cannot retain *Daphnia* under 2 mm or elect not to consume them (Figure 4-6A, and 4-7C to 4-7F). *Daphnia* commence reproduction and ephippial production between 1.5 - 2.0 mm. Therefore, many *Daphnia* can reproduce before they become vulnerable to size-selective fish predation in the pelagic zone. Although small *Daphnia* were consumed by small fish, the latter were excluded from the pelagic zone due to the risk of cannibalism by large fish (See Wilhelm 1998).

The low predation on *Gammarus* in early summer also coincided with their critical reproductive period. Ovigerous females were present from ice-out until approximately the first week in August, when they released their young. Because bull trout predation was focused on chironomid pupae and adults during this time, many female *Gammarus* successfully released their young. The timing of the switch by bull trout from chironomids to amphipods likely varies between years and is probably determined by the rate of warming after ice-out, because this would determine the duration of chironomid emergence (cf. Figure 4-3B). The effect of a change in timing of the prey switch on the *Gammarus* population is unknown.

The presence of chironomid pupae in Harrison Lake and their selection by bull trout in July is a key feature allowing *Daphnia* and *Gammarus* to survive in Harrison Lake. Chironomid pupae in Harrison Lake are either entirely black or opaque white and approximately 10 to 15 mm in length, making them highly visible and easy targets for the bull trout. Their high abundance in bull trout stomachs suggests they are energetically rewarding. In comparison, the smaller, unpigmented *Daphnia* are present at low densities in early July which may make them less appealing and beneficial as prey than the larger pupae. Although *Gammarus* are present in the lake year round, they must be selected from bottom depressions filled with organic debris (Wilhelm 1998). This makes them more difficult to capture than large chironomid pupae.

Dietary overlap

The diet similarity between large and small bull trout in Harrison Lake was striking, because in general, different size-classes of charr in the same lake utilize different prey (Rawson 1961; Martin 1966; Power 1980; Johnson 1980; Gerstmeier 1985; Fraley and Shepard 1989). However, our prey-size analyses indicate that the overlap may be less than suggested by the similarity index, because each bull trout size-class concentrated on a different size of *Daphnia*. This finding suggests that significant diet overlap values from indices, such as the Schoener's similarity index, generated by the reliance of predators on one or two prey species, should be further examined to determine if apparent overlaps can be separated based on prey size.

The predominance of a few prey species in the diet of each bull trout size class suggests a high potential for intra-size class competition. The lack of widespread specialized feeding beyond the common food items may have been the result of the absence of other abundant prey types. However, the specialization of several large bull trout in August (Figure 4-3D) indicates that, similar to other salmonids, bull trout may concentrate on specific prey species (Bryan and Larkin 1972). This may be the result of a local concentration of prey items that a particular fish has discovered by chance. Bull trout diet studies from lower elevation lakes suggest that they are generalists and prey opportunistically on abundant prey items (Leathe and Graham 1982; Donald and Alger 1993; Mushens and Post 1997). The lack of other abundant prey items in Harrison Lake combined with the decline in the amount of food in the stomachs of large bull trout over the course of the open water-season (Figure 4-9) suggests that large fish were food limited and supports the argument of high intra-size class competition for the food that is available. Furthermore, the upper size limit reached by bull trout in this lake, compared to lakes where prey fish are available also suggests that the population is food limited (see below).

Seasonal food volume

In contrast to small fish, food intake in large bull trout declined in August and September in 1997 compared to July. We do not have caloric values for the consumed food, but considering that the prey species and volumes were nearly identical to those of the small fish in September, we suggest that large bull trout may be food limited. This decrease in food quantity may explain the presence of many large emaciated individuals. Large bull trout probably obtain adequate food during the chironomid hatch early in the summer, allowing them to grow or maintain weight. But they appear to be incapable of capturing the smaller prey that are available in late August and September. Similar feeding maxima occurring shortly after ice-out or early in the open-water season have been reported for salmonids in arctic and alpine environments (Johnson 1980; Dawidowicz and Gliwicz 1983;

Hofer and Medgyesy 1997).

Cannibalism on small bull trout could alleviate the food limitation faced by large fish. However, the low rate of cannibalism that we observed from stomach analyses in Harrison Lake suggests it is either infrequent or occurs under-ice in winter. Vulnerability to cannibalism may increase after freeze-up, when over 1 m of ice would displace small fish from their shallow water (<15 cm deep) lake refuges if they did not emigrate to the stream outflow. We do not know if large bull trout switch to cannibalism or even feed during the ice-covered period, when water temperatures fall below 1.5°C throughout the water column. Winter diet data for lake trout suggest little under-ice feeding, with yearly minimum gut fullness occurring just before ice break-up (Martin 1954). A similar pattern may also occur in bull trout.

The poor condition of some large bull trout could also be caused by spawning the previous fall. Weight loss following spawning by adfluvial bull trout in Lower Kananaskis Lake, Alberta, ranges between 8-17% (Mushens and Post 1997); while Johnson (1980) reported a weight loss of up to 26% after spawning in arctic charr populations. If we assume a similar weight loss due to spawning, it would account for only six of the 14 emaciated adults we captured. The remaining eight individuals, which were 30 to 40% underweight, may represent a post-reproductive senescing portion of this unexploited population.

Bull trout size and diet

Salmonids generally change diet as they grow, switching from planktivory to piscivory if forage fish are available (Martin 1966; Rawson 1961; Nilsson and Pejler 1973; Langeland 1978; Leathe and Graham 1982; Boag 1987). Thus, larger bull trout would be expected in lakes with forage fish than in lakes where bull trout subsist on invertebrates. This was the basis for Donald and Alger's (1993) model of increasing maximum trout size with increasing lake food-web complexity. Bull trout generally reach 440 mm FL in Harrison Lake where forage fish are absent and the occurrence of cannibalism is presumed to be low. Similarly, in Pinto Lake, another bull trout-only lake, maximum size was approximately 500 mm FL (Carl et al. 1989). In contrast, in Flathead Lake, Montana, and Lower Kananaskis Lake, Alberta, where prey fish are present, bull trout exceed 600 mm FL (Leathe and Graham 1982; Mushens and Post 1997; Stelfox 1997). Preliminary diet data from Lower Kananaskis Lake show that bull trout under 450 mm FL fed mainly on large invertebrates, including the opossum shrimp, *Mysis relicta* (Mushen and Post 1997). Therefore, the absence of forage fish suggests a 'trophic bottleneck' as defined by Heath and Roff (1996) and indicates that a maximum size of 400-500 mm FL can be expected for bull trout restricted to an invertebrate diet.

Unlike non-native salmonids, which frequently decimate the native invertebrate fauna in lakes where they are stocked (Gliwicz 1980; Pechlaner 1984; Parker and Schindler 1995; Parker et al. 1996; McNaught et al. 1999), native bull trout coexist with their invertebrate prey, including large crustacean species. We have shown that a self-sustaining bull trout population can exist in an alpine lake with only three major invertebrate prey species. The fact that these three prey species are common in other high elevation lakes, bodes well for potentially establishing bull trout populations in other mountain lakes.

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5. EFFECTS OF *Gammarus lacustris* (CRUSTACEA: AMPHIPODA) ON PLANKTON COMMUNITY STRUCTURE IN AN ALPINE LAKE[1]

Introduction

In this paper we present evidence that predation by *Gammarus lacustris*, a common freshwater amphipod of northern North America, Europe, and Siberia (Bousfield 1958; Ökland 1969; Segerstråle 1954; Zhadin and Gerd 1963), can alter the plankton community structure of fishless alpine lakes. The impact of size-selective predation on zooplankton communities by vertebrate and invertebrate predators such as *Alosa*, *Chaoborus*, *Leptodora*, and some diaptomid and cyclopoid copepods, is well established (Brooks and Dodson 1965; Neill 1981; Paul et al. 1995). Some benthic invertebrates, such as *Mysis relicta* and *Chaoborus spp.* also affect pelagic zooplankton communities (Neill 1981; Lasenby 1991; Langeland et al. 1991). Maly et al. (1980) reported that flatworms prey on *Daphnia*, influencing zooplankton composition and dynamics in high elevation ponds. Investigations by Dick (1992) and Dick et al. (1993) indicate that cannibalism and mutual predation among some gammarid species are important in structuring their communities. Amphipods are conspicuous members of benthic communities in freshwaters and are generally classified as detritus feeders and/or scavengers (Barnes 1980), not as predators. Among these, *Gammarus lacustris* is commonly considered as an herbivorous scavenger (Bagge 1968) and detritivore (Moore 1977). However, predatory feeding by *G. lacustris* on diaptomid copepods, daphnids and *Chaoborus* has been noted (Forbes 1893; Anderson and Raasveldt 1974 and references therein; Kortelainen 1990).

Anderson and Raasveldt (1974) and Anderson (1980) concluded that zooplankton community composition in some prairie ponds and alpine lakes was consistent with *G. lacustris* predation patterns observed by Anderson and Raasveldt (1974), suggesting that *G. lacustris* may be important in structuring zooplankton communities in fishless lakes and ponds. These conclusions, however, were based on extrapolations from *G. lacustris* predation experiments in 100 mL experimental vessels. Cautions about extrapolating from inappropriately scaled experimental chambers and enclosures to whole lake ecosystems are frequent in the literature (e. g. de Lafontaine and Leggett 1987; Piercey and Maly 1998; Schindler 1998). The use of small containers usually requires higher prey densities than present *in situ*. As well, small containers generally yield higher predation rates compared to large containers because encounter frequency between prey and predators is enhanced and prey tend to be more active (de Lafontaine and Leggett 1987; Piercey and Maly 1998). In addition, small containers prevent spatial separation which may be important to vertically migrating species. Ideally, manipulative experiments should be executed at a scale closely reflecting field conditions. We used large (2800 L) *in situ* enclosures to experimentally control *G. lacustris* density to test the hypothesis that *G. lacustris* predation alters the zooplankton community structure in fishless alpine lakes.

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Materials and Methods

Study Lake

Snowflake Lake is a small (7.13 ha, max. depth 12.5 m) ultra-oligotrophic alpine (2320 m a.s.l.) lake located in the Front Ranges of the Canadian Rocky Mountains in Banff National Park (51°35'N, 115°50'W), Alberta, Canada. A map and further chemical and physical descriptions are provided in Anderson (1970a) and McNaught et al. (1999). Ice-out usually occurs between the end of June and mid-July and the lake stratifies weakly in late summer when surface temperatures reach a maximum of 9-12°C. Freeze-up occurs between late September and early October (Anderson 1970a).

The biotic community of Snowflake Lake has been manipulated several times through fish and zooplankton introductions. The lake was originally fishless, but was stocked with salmonids for 5 years between 1960 and 1966. The presence of fish altered the zooplankton community from one dominated by *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* to one dominated by *Diacyclops* and rotifers (Anderson 1972; McNaught et al. 1999). Fish did not reproduce in the lake and declined gradually, with the last fish caught in 1984. Once the lake was devoid of fish, *Gammarus lacustris* and *D. middendorffiana* returned (McNaught et al. 1999), but the large copepod, *H. arcticus*, remained absent and small cyclopoid copepod species continued to dominate the zooplankton. *Hesperodiaptomus arcticus* was reintroduced on 30 July 1992, but did not become abundant in the plankton until late in 1996. *Hesperodiaptomus arcticus* reintroduction has caused the decline in abundance or elimination of all cyclopoid copepod and rotifer species (Schindler unpub. data).

Mesocosm Sampling

We used 12 enclosures in Snowflake Lake to test *G. lacustris* predation at different densities on the zooplankton community in 1996. The enclosures were 1.0 × 1.0 × 3.0 m deep and constructed of transparent woven polyethylene. They were closed at the bottom. with a volume of 2800 L when filled. Enclosures were suspended from floating frames constructed of wood and closed cell styrofoam anchored at a water depth of 8 to 10 m. They were filled on July 11, approximately 1 week after ice-out, by submersing each enclosure to 6 m, opening the top and pulling it up through the water column. After suspending all enclosures, *G. lacustris* and large zooplankton captured during filling were removed by hauling a square (0.95 × 0.95 m) 500-µm-mesh screen 4 times from the bottom to the top of each enclosure. We then performed 4 additional vertical hauls with an 64-µm-meshed 30-cm-diam. Wisconsin plankton net to remove as much as possible of the smaller zooplankton. Each enclosure was then stocked with zooplankton collected from 11 m vertical hauls taken with the 30-cm-diam. plankton net from the deepest spot in the lake. Eight hauls, from which all *G. lacustris* were immediately removed, were used to stock each enclosure. *Gammarus lacustris* were collected with a sweep net from the east shore of the lake and added at densities of 40 (Low), 200 (Medium), and 400 (High) individuals per m^{-2} to triplicate enclosures on July 15 (= Day 0). Three enclosures did not receive any *G. lacustris* and served as controls. Only adult *G. lacustris*, at a male/female ratio of 1:1, were stocked because this cohort dominates the diurnal migrations into the pelagic zone (Wilhelm unpub. data). *Gammarus lacustris* density in lakes in the vicinity ranges between ~40 to > 400 ind.·m^{-2} (Parker et al. 1996)

Water samples were collected from the enclosures and the lake (near the enclosures) using a clear polyvinyl chloride tube 2.75 m in length and 0.05 m inner diameter. DeVries and Stein (1991) reported that such a tube sampler yielded zooplankton densities comparable to those from a vertical net tow and a Schindler-Patalas trap, even for highly mobile calanoids. The tube was dropped vertically into each enclosure or the lake, plugged at the top, removed and emptied into a bucket. The procedure was repeated three times, removing approximately 16.2 L of water, or about 0.6% of the enclosure volume. Aliquots were removed for algal cell counts (0.25 L), chlorophyll *a* (1 L) and water chemistry (total phosphorus (TP) and total dissolved nitrogen (0.5 L)) analyses. The remaining 14.45 L were filtered through a 10-µm-mesh net and preserved with sugared formalin for zooplankton counts. The minimum detectable limit for plankton using the tube sampler (assuming a Poisson distribution and a 95% probability of detection) was 0.2 ind.·L^{-1}. To minimize depletion effects on enclosure populations, larger samples were not taken. At the end of the experiment, the contents of each enclosure were retained in a top-net with 64-µm-mesh panels and a cod end. This top-net was attached to close the top of each enclosure after it was loosened from the frame. After concentrating, the contents were preserved with 4% formalin. Amphipods remaining at the end of the experiments were counted to obtain a survival estimate.

Zooplankton in the lake was enumerated from five replicate 11-m hauls, preserved together, taken at the deepest spot of the lake with the 30-cm-diam. 64-µm-mesh plankton net. We used an efficiency correction of 50 % based on Anderson's (1970b) findings of efficiencies of up to 40 % for a similar net towed in a more productive montane lake over a vertical distance exceeding 30 m. Keys from Edmondson (1959), Chengalath et al. (1971), Torke (1974), Grothe and Grothe (1977), and Czaika (1982) were used to identify zooplankton species. Zooplankton were enumerated by allowing the entire sample to settle for 24 h, and then counting all individuals using both inverted and dissecting microscopes. Lake samples were subsampled (10 to 25 mL) from a final sample volume of 250 to 500 mL. Rotifer density was based on *Keratella hiemalis* abundance, as it was the dominant rotifer. Other rotifers (*Keratella cochlearis*, *Polyarthra dolichoptera*, *Notholca spp.* and *Synchaeta spp.*) were collected infrequently and contributed little to overall rotifer abundance. Chlorophyll *a*, used as an indicator of phytoplankton biomass, was determined fluorometrically with a Shimadzu Model RF-1501 spectrofluorometer (Mandel Scientific, Guelph, ON, Canada) following the method of Welschmeyer (1994). Phytoplankton biomass, water chemistry and zooplankton densities were compared using a repeated measures ANOVA with one between-subjects (*Gammarus* density) and one within-subject factor (Date) (Von Ende 1993). Treatment (*Gammarus* density) × time (Date) interactions were also considered for each test. Only samples from Days 7, 20 and 35 were included in the repeated measures analysis, as initial stocking densities (Day 0) for all enclosures were estimated from 2 samples (4 × 11 m hauls preserved together) collected at the same time as zooplankton samples for stocking the enclosures. Calanoid nauplii, cyclopoid copepodites and cyclopoid adult results were not included in the analysis because densities often were below the detection limit of the sampler. However, we present them graphically to show the trends.

Results

Gammarus lacustris in the enclosures migrated similarly to individuals in the lake (Wilhelm pers. obs.), remaining on or near the bottom of the enclosures during the day and swimming throughout the water column to within 0.1 m of the surface at night. *G. lacustris* survival decreased slightly with increasing density over the course of the experiment from 93±7, 89±5 to 81±3 percent (mean±SE) of initial densities in the low, medium and high density treatments respectively.

Daphnia middendorffiana density increased 13, 24 and 12 times in the lake, the control and low density *Gammarus* enclosures, respectively, over the course of the experiment (Figure 5-1A). In the medium density *Gammarus* enclosures, a small (3.5 times) increase occurred during the last sampling interval (Figure 5-1A), while in the high density *Gammarus* enclosures, *D. middendorffiana* density remained constant until Day 20 and then declined to zero (Figure 5-1A). We examined the treatment trend on the last sampling date by regressing *Daphnia* density versus Log *Gammarus* density. This showed that *D. middendorffiana* density declined strongly with increasing *G. lacustris* density (Figure 5-2A; $R^2 = 0.60$, DF = 11, $P = 0.003$).

Rotifer abundance declined ($P < 0.001$) in all enclosures over the course of the experiment (Table 5-1, Figure 5-1B). The treatment effect was weak ($P = 0.347$, Table 5-1) indicating that the decline in rotifer abundance may have been related to factors other than *Gammarus* density. Although rotifer abundance was 2.5 times higher in the lake than in the enclosures on Day 0, it rapidly declined to zero by Day 30 (Figure 5-1B).

Calanoid copepods were almost exclusively *Hesperodiaptomus arcticus*. Only 2 other diaptomid (*Diaptomus tyrrelli*) specimens were encountered in all samples. Nauplii declined in all enclosures and in the lake over the course of the experiment (Figure 5-1C). The decline was most rapid in the medium and high density *Gammarus* enclosures, but the density was at or below our detection limit for most of the samples. Calanoid copepodite density was variable in the enclosures after stocking, but declined in all enclosures by the end of the experiment (Figure 5-1D). Treatment and the interaction effect were weak (P = 0.066, 0.055 respectively, Table 5-1). Copepodite density in the lake, however, increased with time (Figure 5-1D). *Hesperodiaptomus arcticus* adult density increased ($P < 0.001$) in all enclosures and the lake (Table 5-1, Figure 5-1E). The treatment effect was insignificant ($P = 0.120$, Table 5-1), as was a least squares regression analysis of adult *H. arcticus* density versus Log *Gammarus* density for the final date (Figure 5-2B; $R^2 = 0.27$, DF = 11, $P = 0.083$). *Hesperodiaptomus arcticus* adult density in the lake did not increase until after Day 20 (Figure 5-1E).

Cyclopoid nauplii density decreased ($P < 0.001$) from a high of 1 ind.$\cdot$L^{-1} to near zero in all enclosures without a treatment or interaction effect (P = 0.638, 0.876 respectively; Table 5-1, Figure 5-1F)). Nauplii density in the lake also declined sharply over the 35 day experimental period. Although cyclopoid copepodites were present on Day 0 at densities of 0.59 and 0.88 ind.$\cdot$L^{-1} in the enclosures and the lake respectively, they declined to near zero by the first sampling date (July 19) and remained near undetectable levels for the remainder of the experiment (Figure 5-1G). The higher nauplii density in the lake compared to the enclosures on Day 0 may reflect a depletion effect of nauplii from the

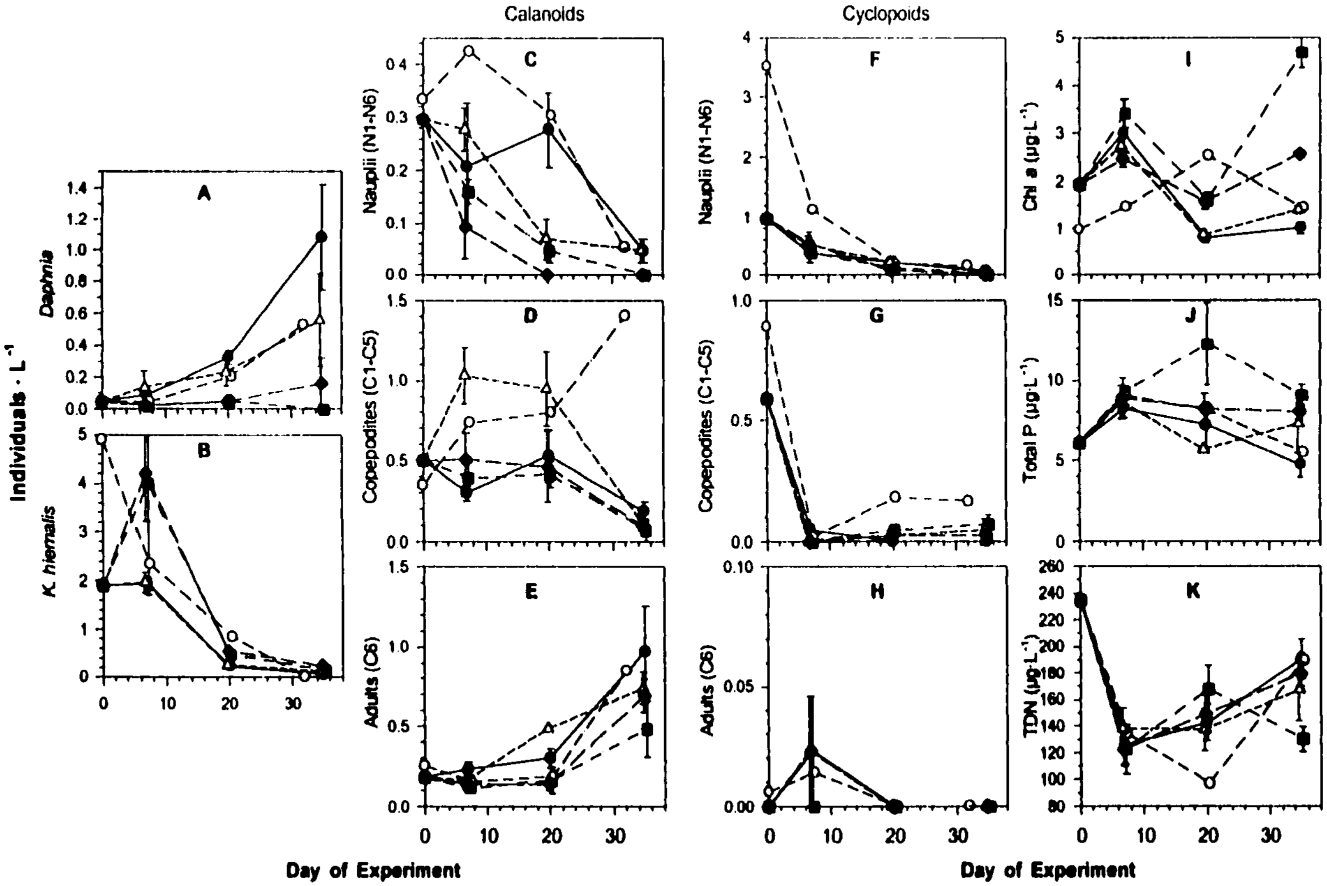

Figure 5-1. Mean densities of Snowflake Lake zooplankton and selected water chemistry parameters in enclosures with varying densities of the predator *Gammarus lacustris* for 1996. Treatment *Gammarus* density: (●) - Control (0 ind.·m^{-2}), (△) - Low (40 ind.·m^{-2}), (♦) Medium (200 ind.·m^{-2}), (■) High (400 ind.m^{-2}), (○) Lake (200-400 ind.·m^{-2}). Error bars represent ±1 SE. Lake zooplankton samples represent a single collection and therefore do not have errors bars associated with them. Error bars less than the size of the symbol in other samples are not shown.

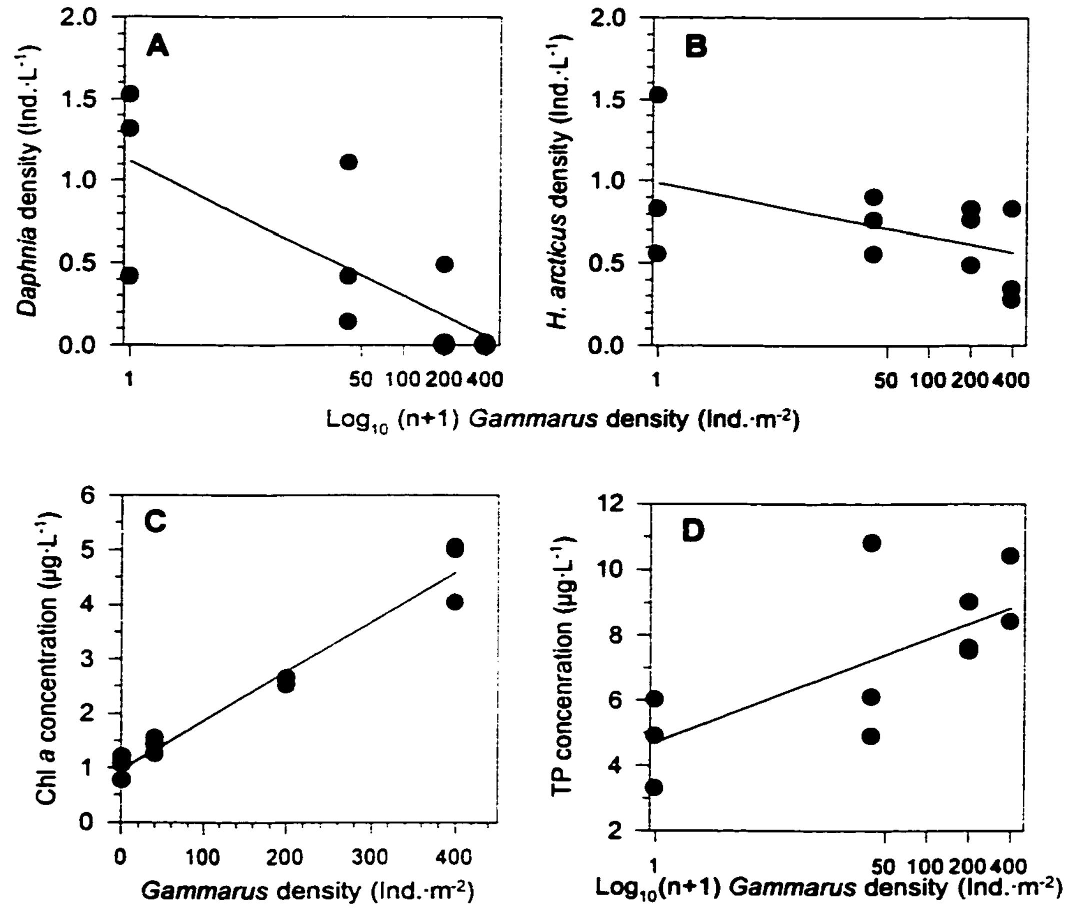

Figure 5-2. Relationships showing the effect of *Gammarus lacustris* density on zooplankton abundance, chlorophyll *a* and total phosphorus concentration at the end (Day 35) of the enclosure experiment. **A** - *Daphnia middendorffiana* abundance versus $\text{Log}_{10}(n+1)$ *G. lacustris* density ($Y = -0.411\ X + 1.12$, $R^2 = 0.60$, DF = 11, $P = 0.003$); **B** - *Hesperodiaptomus arcticus* abundance versus $\text{Log}_{10}(n+1)$ *G. lacustris* density ($Y = -0.16\ X + 0.98$, $R^2 = 0.27$, DF = 11, $P = 0.083$); **C** - Chlorophyll *a* concentration versus *G. lacustris* density ($Y = 0.01\ X + 0.96$, $R^2 = 0.96$, DF = 11, $P < 0.001$); **D** - Total phosphorus (TP) concentration versus $\text{Log}_{10}(n+1)$ *G. lacustris* density ($Y = 1.58\ X + 4.71$, $R^2 = 0.52$, DF = 11, $P = 0.008$).

Table 5-1. Repeated measures analysis of variance for zooplankton abundance and water chemistry from Snowflake Lake enclosures in 1996 using different densities of the predator *Gammarus lacustris*.

Test	Source	DF	MS	*P*
Daphnia				
Between subjects	Treatment	3	0.435	0.039
	Error	8	0.096	
Within subject	Date	2	0.473	0.001
	Interaction	6	0.165	0.018
	Error	16	0.045	
K. hiemalis				
Between subjects	Treatment	3	2.113	0.347
	Error	8	1.659	
Within subject	Date	2	30.883	<0.001
	Interaction	6	1.298	0.553
	Error	16	1.534	
Calanoids C1-C5				
Between subjects	Treatment	3	0.317	0.066
	Error	8	0.088	
Within subject	Date	2	0.843	<0.001
	Interaction	6	0.097	0.055
	Error	16	0.037	
Calanoid Adults C6				
Between subjects	Treatment	3	0.120	0.120
	Error	8	0.045	
Within subject	Date	2	1.055	<0.001
	Interaction	6	0.041	0.369
	Error	16	0.035	
Cyclopoid nauplii				
Between subject	Treatment	3	0.013	0.638
	Error	8	0.022	
Within subject	Date	2	0.547	<0.001
	Interaction	6	0.014	0.876
	Error	16	0.036	
Chl *a*				
Between subject	Treatment	3	5.226	<0.001
	Error	8	0.085	
Within subject	Date	2	9.106	<0.001
	Interaction	6	2.061	<0.001
	Error	16	0.089	
TP				
Between subject	Treatment	3	21.780	0.069
	Error	8	6.195	
Within subject	Date	2	7.066	0.145
	Interaction	6	6.477	0.125
	Error	16	3.229	
TDN				
Between subject	Treatment	3	259.303	0.687
	Error	8	509.488	
Within subject	Date	2	4783.470	0.012
	Interaction	6	1250.188	0.228
	Error	16	810.668	

lake during hauls taken to stock the enclosures. Cyclopoid adults were rare in Snowflake Lake in 1996. The few that were present in the lake and the enclosures on Day 0 rapidly declined to undetectable levels (Figure 5-1H). Even though we used five vertical hauls to sample zooplankton in the lake on each date, cyclopoid adults were not encountered after Day 20 (Figure 5-1H).

Phytoplankton biomass in the enclosures was almost twice as high as in the lake on Day 0 (Figure 5-1I), and may be related to the selective retention of large species such as diatoms and *Dinobryon* in the net during enclosure stocking. Phytoplankton biomass increased in the lake and the enclosures until the first sampling date, after which phytoplankton in the lake increased until Day 20, while it declined in all enclosures (Figure 5-1I). After Day 20, phytoplankton biomass in the lake declined while it increased ($P < 0.001$) in all enclosures. After Day 20, phytoplankton biomass increased faster in the presence of higher amphipod densities ($P < 0.001$ treatment × date interaction, Figure 5-1I). Phytoplankton biomass was also positively ($P < 0.001$) related to *Gammarus* density (Table 5-1). A regression analysis for the last sampling date showed that 96 % of the variation in Chl *a* concentration was explained by *Gammarus* density (Figure 5-2C; $R^2 = 0.96$, DF = 11, $P < 0.001$).

The total phosphorus (TP) concentration increased by approximately 3 $\mu g \cdot L^{-1}$ in the enclosures and the lake between Day 0 and the first sampling date, after which it was variable without trends with either time ($P = 0.145$) or treatment ($P = 0.069$) (Table 5-1, Figure 5-1J). However, concentrations in the medium and high density *Gammarus* enclosures were slightly higher than in the control and low density *Gammarus* enclosures (Figure 5-1J). A least squares linear regression of TP concentration versus *Gammarus* density on the final sampling date showed that TP was positively related to *Gammarus* density (Figure 5-2D; $R^2 = 0.68$, DF = 11, $P < 0.001$).

Total dissolved nitrogen (TDN) was highest in the enclosures and the lake at the beginning of the experiment, after which it declined to a minimum and then increased again (Figure 5-1K). The minimums in the enclosures occurred around Day 7 while the lake did not reach its minimum until near Day 20. This change with time is reflected in the date effect ($P = 0.012$, Table 5-1). *Gammarus* density did not ($P = 0.687$) influence TDN concentrations (Table 5-1).

Discussion

Our enclosure results show that *Gammarus lacustris* significantly influenced the abundance of pelagic zooplankters and phytoplankton biomass. The abundance of *D. middendorffiana* was reduced in the medium and high density *Gammarus* enclosures compared to the control and low density *Gammarus* enclosures (Figure 5-1A, and 5-1E). *Hesperodiaptomus arcticus* density was not reduced significantly in our experiment. Although *G. lacustris* density in Snowflake Lake was between 200-400 ind.$\cdot m^{-2}$, as determined from night vertical plankton net and daytime bottom closing net hauls (Wilhelm unpub. data) (corresponding to the medium and high enclosures), *D. middendorffiana* density in the lake increased similar to the low *Gammarus* density enclosures (Figure 5-1A), indicating a discrepancy between the enclosures and the lake. The larger vertical separation between *D. middendorffiana* and *G. lacustris* in the lake compared to the enclosures may represent a pelagic refugium where *D. middendorffiana* encounters are reduced. *Gammarus lacustris* gut content analyses show that predation on zooplankton is limited to the time they spend in the water column at night (Wilhelm unpub. data). Therefore, the pelagic zone over deep water may serve as a refugium for zooplankton from *G. lacustris* predation. Similarly, fish and large diaptomid copepods typically coexist in mountain lakes only if they are deeper than 16 m and larger than 16 ha (Donald et al. 1994).

The decline in rotifer abundance in all enclosures may be attributable to predation by *H. arcticus*. Rotifer regulation by *H. arcticus* adults and copepodites in Snowflake Lake has been demonstrated previously (Paul et al. 1995). Our results show that the regulation of rotifers is possible at a low *H. arcticus* density. Rotifer density in all enclosures declined to less than 1 ind.$\cdot$L^{-1} by Day 20 while *H. arcticus* density remained near 0.5 and 0.2 ind.$\cdot$L^{-1} for copepodites and adults, respectively (Figure 5-1D, and 5-1E). Regulation of rotifers by copepod predation in alpine lakes is possible because low food availability and cold water temperatures slow rotifer reproduction (McNaught et al. 1999). All of our enclosures had approximately 2 times higher chl *a* concentration than the lake at the start of the experiment, which should have ameliorated low rotifer food conditions. Either this phytoplankton was unavailable to the rotifers, or, more likely, copepod predation diminished any advantages from increased food levels. Similar conclusions from fertilized mesocosms were reached by Paul et al. (1995). The simultaneous decline of rotifer abundance in the lake represents the decline and near elimination of rotifers in the lake after *H. arcticus* became the dominant zooplankter in late 1996.

The decrease in calanoid nauplii and copepodite abundance in the enclosures (Figure 5-1C, and 5-1D) over the course of the experiment is expected because of recruitment to the adult cohort. Nauplii declined from a density of 0.3 ind.$\cdot$L^{-1} to near zero, while copepodites declined from approximately 0.5 ind.$\cdot$L^{-1} to as low as 0.18 ind.$\cdot$L^{-1}. The continued copepodite increase in the lake may be related to the high nauplii density in the lake on Day 7 (Figure 5-1C) which indicates that peak nauplii recruitment from resting eggs deposited in lake sediment occurred after we had started the experiment. If we assume zero mortality during development, adult *H. arcticus* density should have increased by 0.62 ind.$\cdot$L^{-1} to 0.82 ind.$\cdot$L^{-1} at the end of the experiment. Our assumption of no mortality during development is probably incorrect; however, we currently do not have any *H. arcticus* mortality rates for Snowflake Lake. Rigler et al. (1974) reported an average mortality rate of 59% for *Limnocalanus macrurus* from naupliar to adult stages in Char Lake in the high Canadian arctic. *Hesperodiaptomus arcticus* mortalities in our experiment must be less than 59% because only the mortality rate in the high-density treatment could have been that high (Figure 5-1E). Lower mortalities are necessary to explain the density of adults in the other enclosures (Figure 5-1E).

A longer experiment may have resulted in a significant reduction of adult *H. arcticus*. The ability of *G. lacustris* to capture and consume diaptomid copepods has been reported by Forbes (1893) and Anderson and Raasveldt (1974). In single prey feeding experiments with *H. arcticus* that we conducted in the field in 1995, *G. lacustris* also captured and consumed *H. arcticus* adults (Wilhelm unpub. data.). However, when presented with a choice of *D. middendorffiana* or *H. arcticus*, *D. middendorffiana* were always selected. This may indicate that *G. lacustris* does not switch to prey on *H. arcticus* until after *D. middendorffiana* density declines below some threshold, which may have occurred in a longer experiment. However, the duration of our experiment was constrained by the open-water season and poor late-season access to Snowflake Lake. In the lake, such a switch by *G. lacustris* may not occur until late fall or after the lake is ice-covered. Predation of adult *H. arcticus* by *G. lacustris* could explain the slow the re-establishment of *H. arcticus* as the dominant copepod in Snowflake Lake after its reintroduction in 1992 as well as the continued density below that characteristic of other lakes in the area (Anderson 1980; Schindler unpub. data), as the *G. lacustris* density in Snowflake Lake is higher than in other nearby Lakes (Wilhelm unpub. data). A seasonal examination of *G. lacustris* diet in Snowflake Lake would show if and when predation on *H. arcticus* occurred.

The disappearance of cyclopoids from the enclosures was consistent with previous research showing that codominance of zooplankton by predaceous diaptomid and cyclopoid species is unlikely (Anderson 1970c), and that pristine mountain lakes are likely to be dominated by species of *Hesperodiaptomus* (Anderson 1980). Anderson (1970c) reported *H. arcticus* to consume between 6 to 20 cyclopoids per day and predation experiments carried out at Snowflake Lake in 1995 showed that *H. arcticus* selectively preyed on cyclopoid nauplii and copepodites compared to its own young (A. S. McNaught, Dept. Biol., Central Michigan University unpub. data). Although cyclopoids can prey on diaptomids and exclude them from lakes (Anderson 1970c), the role of predator or prey is determined by the relative size or instar of the two groups. Adult *H. arcticus*, because of their larger size compared to cyclopoid adults typically present in mountain lakes, are not vulnerable to cyclopoid predation, whereas cyclopoids remain vulnerable to predation by *H. arcticus*. The decline of cyclopoids in Snowflake Lake in 1996 (Figure 5-1F to 5-1H) marked their collapse after dominating the zooplankton assemblage for nearly 20 years while *H. arcticus* was absent from the lake.

Phytoplankton biomass at the end of the experiment was highest in the high-density *Gammarus* enclosures where zooplankton abundance and hence grazing pressure was lowest (Figure 5-1I, and 5-2C). We can not explain the decline observed in all enclosures between Days 7 and 15, but it may be related to a change in algal species composition, settling of cells, or UV effects. The increase of phytoplankton in the high-density *Gammarus* enclosure was mediated directly and indirectly by *G. lacustris*. Predation by *G. lacustris* on *Daphnia* reduced grazing pressure on phytoplankton, while *G. lacustris* excretion and egestion regenerated nutrients that stimulated phytoplankton growth (Figure 5-1I).

The significant positive relationship of TP with *Gammarus* density on the final sampling date (Figure 5-2D) may be related to the altered plankton communities present among treatments. *Daphnia* have a high dietary P requirement and are known to sequester P relative to other zooplankton (Anderson and Hessen 1991). High *Daphnia* densities in the control and low density *Gammarus* treatments may have contained much of the TP, while in the medium and high density *Gammarus* treatments, predation on *Daphnia* by *G. lacustris* released the phosphorus, increasing the TP concentration.

Our large enclosure experiment in Snowflake Lake supports the hypothesis that predation by *Gammarus lacustris* influences the plankton community structure in fishless alpine lakes. *Daphnia middendorffiana* abundance decreased with increasing *G. lacustris* density. This large zooplankter would be expected to be reduced or eliminated in the presence of planktivorous fish, as seen earlier in the century when Snowflake Lake was stocked with fish (McNaught et al. 1999).

Although in Snowflake Lake *G. lacustris* was present at densities similar to the medium and high enclosures, predation in the lake was weaker, possibly because a pelagic deepwater refugium reduces predator-prey encounters. If so, the impact of *G. lacustris* on plankton community structure would be expected to be most intense in small lakes and ponds whereas in large deep lakes, weaker influences on prey population dynamics and life history structure, similar to other invertebrate predators, would be expected.

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6. THE INFLUENCE OF EXPERIMENTAL SCALE ON ESTIMATING THE PREDATION RATE OF *Gammarus lacustris* (CRUSTACEA: AMPHIPODA) IN AN ALPINE LAKE[1]

Introduction

Predator-prey interactions and selective predation are strong determinants of plankton community structure in lakes (e.g. Brooks and Dodson 1965; Neill 1981). Although most freshwater research has been directed at pelagic predators (e.g. Hall 1964; Carpenter and Kitchell 1988; Paul et al. 1995), the ability of benthic invertebrate predators such as *Mysis relicta*, *Chaoborus spp.* and flatworms to modify plankton communities has been noted (Maly et al. 1980; Lasenby 1991; Langeland et al. 1991). Recently, Wilhelm and Schindler (in press) showed that the amphipod, *Gammarus lacustris*, generally considered a benthic scavenger and detritivore, was also an important predator of *Daphnia* in an alpine lake.

In reviewing the literature, we noted that a wide variety of experimental scales and methods have been used to estimate predation rates and effects on prey. These ranged from small controlled laboratory chambers to estimates based on field surveys (e.g. Anderson and Raasveldt 1974; Vonder Brink and Vanni 1993; Elser et al. 1987). However, rarely is the same manipulation applied at different experimental scales (e.g. Ives et al. 1993; Carignan and Planas 1994; Sarnelle 1997). For invertebrate predators, researchers have generally favoured feeding experiments under controlled laboratory conditions where predation rates are estimated from the consumption of a known number of prey at the start of a trial (e.g. Dodson 1984). Although gut-content enumerations have also been used (Matsakis and Conover 1991), they are time-consuming and the identification of prey species may be difficult. In contrast, in mesocosm studies and whole-lake surveys, predatory impacts are surmised from predator abundance and correlated negative changes in prey populations (e.g. Vanni 1988). Actual predation rates are seldom computed and reconciled with those obtained from laboratory experiments.

Discrepancies between experimental findings obtained from laboratory studies, mesocosms and whole-lake manipulations are widely recognized by aquatic ecologists (Levine and Schindler 1992, 1999; Carpenter 1996; Schindler 1998; Lodge et al. in press; Pace in press). Micro- and mesocosms fail to represent whole-lake systems for a number of reasons, such as: lack of inclusion of potential keystone species, species turnover potential, physical refugia, and restriction or exclusion of air-water and water-sediment interfaces. Aquatic ecologists continue to use micro-/mesocosms because they offer the advantages of speed, replication, statistical power, affordability, and insights into mechanistic processes. However, researchers need to be aware of the scale to which their mesocosm results can be extrapolated with confidence (Carpenter 1996; Schindler 1998; Pace in press; Lodge et al. in press). Schindler (1998) suggested that an important role of whole-lake studies is to calibrate and verify that mesocosm results properly represent the interplay of ecosystem-

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scale processes to an extent that results can be extrapolated with confidence. Thus, researchers should avail themselves of opportunities which permit the critical evaluation of predictions obtained from a range of experimental scales.

We examined the effect of experimental scale on the predation rate of *Gammarus lacustris* feeding on *Daphnia middendorffiana* in an alpine lake. Predation rates were compared from small (20-L) cubitainers, large (2800-L) mesocosms and three diurnal whole-lake surveys. For the diurnal lake surveys, we also examined the effect of zooplankton stratification on estimated predation rates of *G. lacustris*, and investigated prey size selection by *G. lacustris*. We also investigated the species and size of prey selected by *G. lacustris* in multiple-prey and prey-size preference experiments.

Materials and Methods

Study lake

Snowflake Lake is a small (7.13 ha, max. depth 12.5 m) oligotrophic alpine (2320 m a.s.l.) lake located in the eastern Front Ranges of the Canadian Rocky Mountains in Banff National Park (51°35'N, 115°50'W), Alberta, Canada. A map and further chemical and physical descriptions are provided in Anderson (1970) and McNaught et al. (1999). Ice-out usually occurs between the end of June and mid-July. The lake stratifies weakly in late summer when surface temperatures reach a maximum of 9-12°C. Freeze-up occurs between late September and early October (Anderson 1970).

The biotic community of Snowflake Lake has been affected several times by fish and zooplankton introductions. The lake was originally fishless, but was stocked with salmonids for five years between 1960 and 1966. The presence of fish altered the zooplankton community from one dominated by *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* to one dominated by *Diacyclops* and rotifers (Anderson 1972; McNaught et al. 1999). Fish did not reproduce in the lake and declined gradually, with the last fish caught in 1984. Once the lake was devoid of fish, *G. lacustris* and *D. middendorffiana* returned (McNaught et al. 1999), but the large copepod, *H. arcticus*, remained absent and small cyclopoid copepod species continued to dominate the zooplankton. *Hesperodiaptomus arcticus* was reintroduced on July 30, 1992, but did not become abundant in the plankton until late in 1996. *Hesperodiaptomus arcticus* reintroduction has caused the decline in abundance or elimination of all cyclopoid copepod and rotifer species (Schindler unpub. data).

Small-scale prey selection and predation experiments

Two sets of predation experiments were conducted: selection experiments with mixed prey, and predation experiments with single prey. These small-scale (20-L) experiments were conducted in the lake outflow stream at our base camp at an elevation of 1800 m a.s.l., approximately 8 km from the lake. Experiments at this location could be readily monitored, whereas access to the lake after the start of an experiment was unpredictable due to inclement weather or the presence of grizzly bears.

In prey selection experiments conducted between Aug. 2-8, 1996, one adult *G. lacustris* predator was offered either adult *H. arcticus* and *D. middendorffiana* or medium and large *D. middendorffiana* prey. Experiments were run in 20-L clear polyethylene cubitainers at a prey density of 1.5 ind.·L^{-1}. We used this density to approximate the density of each zooplankton species (<1 to ~3.2 ind.·L^{-1} for *H. arcticus* and <1 to >10 ind.·L^{-1} for *D. middendorffiana*) common in lakes in the vicinity (Anderson and Donald 1978; Paul and Schindler 1994; McNaught et al 1999). *Gammarus lacustris* were collected with a sweepnet from the east shore of the lake. *Daphnia middendorffiana* and *H. arcticus* were collected with a 30-cm-diam. 64-µm-mesh Wisconsin-style plankton net from the centre of the lake. Although predators and prey are separated by day, *G. lacustris* migrate into the pelagic zone at night. Predators and prey were transported in separate one litre insulated containers to base camp, where they were emptied into 6 liter holding tanks set in the stream until the cubitainers were prepared (~1 h). Each cubitainer was filled with stream water filtered through a 64-µm-mesh screen and prey were added before a single adult *G. lacustris* predator was added. Experiments with both male and female predators were run. *Hesperodiaptomus arcticus* selected for the experiments included stages CV and adults, but no females with egg sacs, while *Daphnia* spanned the entire size range present in the lake. For selection experiments with medium and large *Daphnia* as prey, the mean size (±SE, range) of *Daphnia* selected was 2.10 mm (±0.10, 1.00 - 2.40 mm) and 3.50 mm (±0.08, 3.10 - 4.15 mm) for the medium and large treatments, respectively. This size was determined from individuals in control chambers after the completion of the experiment. Control chambers contained only prey species and were run with each trial to account for mortality not related to predation. All experimental densities and control chambers were run in triplicate. Experiments were run for 48 h because of the slow predation rates expected at the prevailing lake and stream temperatures (6-8 °C). Experiments were terminated by filtering the predators and prey remaining in each cubitainer through an 64-µm-mesh screen and preserving them with 4% sugared formalin. Animals were counted and measured with the aid of a stereomicroscope equipped with an ocular micrometer. *Gammarus lacustris* selection was calculated with Manly's alpha (Manly 1974):

$$\alpha_i = \frac{\log p_i}{\Sigma_{j=1}^{m} \log p_j}$$

as given in Krebs (1989) where:

α - Manly's alpha (preference index) for prey type i
p_i, p_j - proportion of prey i or j remaining at end of experiment = e_i/n_i
e_i - number of prey type i remaining uneaten at end of experiment
n_i - initial number of prey type i in experiment
m - number of prey types

We used this formula to account for the declining prey density over the course of the experiments (Manly 1974). When selective feeding does not occur, $\alpha_i = m^{-1}$. If $\alpha_i > m^{-1}$, prey species i is preferred in the diet. Conversely, $\alpha_i < m^{-1}$ indicates avoidance of prey species i.

Predation experiments with single prey were run in 20-L cubitainers between August

29-31, 1995. Single adult *G. lacustris* predators (males only) were offered *D. middendorffiana* prey at initial densities of 1-, 1.5- and 2 ind.·L^{-1}. The rationale for these densities was the same as for the mixed prey selection experiments. Identical procedures as those described above were followed for collections, handling and set up. All experimental densities and control chambers (1 ind.·L^{-1}) were run in triplicate for 48 h.

Mesocosm experiments

Large (2800-L) mesocosms were suspended in Snowflake Lake in 1996 to examine the predatory effect of *Gammarus lacustris* on zooplankton populations. Details of the mesocosm setup are given in Wilhelm and Schindler (1999). Briefly, the mesocosms were 1.0 × 1.0 × 3.0 m deep, constructed of translucent woven polyethylene, and closed at the bottom. They were filled on July 11, approximately one week after ice-out. After filling, all *G. lacustris* and large zooplankton were removed with a square (0.95 × 0.95 m) 500-µm-mesh screen hauled vertically through each mesocosm. Additional hauls with a 30-cm-diam. 64-µm-meshed Wisconsin-style plankton net were also taken to remove as much as possible of the smaller zooplankton. Mesocosms were stocked with zooplankton collected from the lake on July 11, and densities of 40-, 200-, and 400 ind.·m^{-2} adult *G. lacustris* at a 1:1 male:female ratio on July 15. Three mesocosms did not receive any *G. lacustris* and served as controls. All treatments were run in triplicate, for a total of 12 mesocosms.

Mesocosms were sampled on Days 7, 20, and 35 by dropping a clear polyvinyl chloride tube 2.75 m in length and 0.05 m inner diameter vertically into each mesocosm, plugging the top and removing it to a bucket. The procedure was repeated three times, removing approximately 16.2 L of water, or about 0.6% of the mesocosm volume. Larger samples were not taken to minimize depletion of mesocosm populations. Of the sample removed, 14.45 L were filtered through a 10-µm-mesh net and preserved with sugared formalin for *D. middendorffiana* enumeration. Counting was completed with the aid of a stereomicroscope after allowing the entire sample to settle for 24 h.

Here we only calculated *G. lacustris* predation rates from the low and medium (40 and 200 *G. lacustris*·m^{-2}) treatments, because the number of *G. lacustris* per liter (0.014 and 0.071, respectively) was in the range of densities (0.003 to 0.052 ind.·L^{-1}) observed in the lake between 1995 and 1997 (F. M. Wilhelm unpub. data). Furthermore, Daphnia were eliminated from the high density treatment during the experiment, precluding the calculation of predation rates. *Daphnia* density (ind.·L^{-1}) was calculated as the average density between sampling days for each enclosure treatment. The number of *Daphnia* lost to predation in treatment mesocosms between sampling days was calculated as the difference from increases in control enclosures divided by the number of *Gammarus* and the sampling interval (days). It may be argued that this calculation method caused us to overestimate the predation rate during the course of the experiment because we failed to account for increases in control mesocosm density resulting from multiple generations. However, we are confident that this is not the case in our experiment. In Snowflake Lake, recruitment of young occurs from either resting eggs or large overwintering females. Overwintering females are generally very large 3-4 mm individuals (Wilhelm et al. 1998) which are not very vulnerable to *Gammarus* predation (see below). Thus, we would expect these individuals to persist in both the control and *Gammarus* mesocosms. Young recruited from resting eggs or overwintering females, however, would be vulnerable to *Gammarus*. The average generation time in the cold alpine lake varies between 14-21 d (Clare 1998). Therefore, only one cohort would have been produced during the experiment and differences in *Daphnia* densities between the control and treatment mesocosms can be attributed directly to *Gammarus* predation.

Vertical migration and gut content analysis of G. lacustris *in Snowflake Lake*

The diurnal vertical migration of *Gammarus lacustris* in Snowflake Lake was studied on July 9, 31, and Aug. 27, 1995 at the deepest point of the lake. This was done by taking stage hauls using a 30-cm-diam. 64-µm-mesh Wisconsin-style plankton net over a 24 h period at ~6 h intervals, starting between 12:00 h and 14:00 h on each date. Three hauls were pooled from each of the 0-3 m, 3-6 m, 6-8 m, and 8-11 m strata and preserved in 4% sugared formalin. By sampling the lake on three dates, we made use of the naturally increasing *Daphnia* density during the open-water season to examine the whole-lake predation rates of *G. lacustris* at different prey densities.

Gut contents of adult *G. lacustris* were analyzed by dissecting the fore-, mid- and hindgut from individuals and mounting them in glycerol gelatin. In samples with <15 *G. lacustris*, all individuals were examined. At least half of all individuals were examined in samples with >15 individuals. Prior to covering slides with a coverslip, the gut lining and peritrophic membrane were carefully removed and the contents spread with a dissecting needle. Slides were examined at 100-200× with a Leica Dialux 22 EB compound microscope equipped with an ocular micrometer. The number of *D. middendorffiana* consumed was calculated from either the number of post abdominal claws or the number of mandibles divided by two. The greatest average number of *D. middendorffiana* ingested by adult *G. lacustris* during each 24 h sampling period was taken as the daily predation rate. Gut passage time was determined to be in excess of ten hours because most individuals caught in the early morning still contained a small amount of sediment in the hindgut from the previous day's feeding, while the remainder of the gut was filled with zooplankton parts. Similarly, individuals caught in the evening generally still had some zooplankton remains in the hindgut, while the rest of the gut was filled with sediment.

Daphnia middendorffiana density in Snowflake Lake was calculated in two ways. *Daphnia* density was calculated based on traditional whole-water column plankton samples taken on or within two days of our sampling dates (McNaught et al. 1999). Five replicate net hauls, using the net described above, from a depth of 11 m to the surface were combined and preserved with sugared formalin. In the laboratory, preserved samples were brought up to a volume of 800 mL and 5-mL subsamples were enumerated at ×50 using a stereomicroscope. *Daphnia* density was then back-calculated from the number counted in the subsamples, the volume of subsamples and the volume filtered with the net. This method assumes an even distribution of *Daphnia* in the water column, and is commonly use to estimate zooplankton density (e.g. McNaught et al. 1999). We also calculated *Daphnia* density from each stratum sampled with the stage hauls. This allowed us to examine if *Daphnia* were depth stratified. If the *Daphnia* distribution was depth-stratified, then migrating *G. lacustris* could encounter a higher *Daphnia* density than that calculated from the whole-water column hauls.

Size-selective predation by G. lacustris

To determine if *G. lacustris* preyed size-selectively on *D. middendorffiana* in Snowflake Lake, we estimated the size of *D. middendorffiana* ingested by *G. lacustris* from a *Daphnia* mandible length - body length regression established from 24 *D. middendorffiana* collected concurrently in the net hauls with *G. lacustris*. Mandible length was measured linearly along the length of the raised molar ridge, while body length was measured from the top of the head to the inflection of the tailspine. The size distribution of *Daphnia* present in the lake was estimated by randomly measuring 200 *Daphnia* from the whole-water column samples.

Analyses

For each experimental scale, the number of prey consumed per *G. lacustris* predator per day was calculated and plotted versus prey density at the beginning of the experiment. For the lake survey, the number of prey consumed was plotted versus prey density on each sampling date. Rates were analyzed using least squares linear regression analysis through the origin (Zar 1996), which assumed no prey consumption in the absence of prey *a priori*. We used a linear model because we found it was the simplest model that best described the data at each scale. Regression slopes were compared using analysis of covariance, followed by pairwise comparisons to detect differences (Zar 1996). The predation rate in the lake determined from *Daphnia* densities from the whole-water column hauls was not included in the overall analysis because the *Daphnia* density in the lake was grossly underestimated from these samples (see results below). The size distribution of ingested *D. middendorffiana* was compared to the size distribution in the lake with a two-sample Kolmogorov-Smirnov test (Sokal and Rohlf 1981). We limited our statistical comparison to the Aug. 27-28 sample because of either the low number of *G. lacustris* or ingested *Daphnia* on the other sampling dates.

Results

Prey selection by G. lacustris

When offered either *D. middendorffiana* and *H. arcticus* prey, male and female *G. lacustris* strongly selected *D. middendorffiana* (Table 6-1). This strong preference for *Daphnia* indicates that predation rates from single-prey experiments should be reflective of *G. lacustris* selection in mixed-prey assemblages (i.e. mesocosms and the lake). Prey depletion in these trials ranged from 17 to 44% in the cubitainers with female and male *G. lacustris*, respectively. When offered medium and large *D. middendorffiana*, male and female *G. lacustris* selectively preyed on medium (2.1 mm) *Daphnia,* although some large *Daphnia* were consumed as well (Table 6-1). Prey depletion ranged from 35 to 73% in the female ana male treatments, respectively, reflecting the strong preference of *G. lacustris* for *Daphnia*.

Gammarus lacustris *predation on* Daphnia middendorffiana

At all experimental scales, consumption of *D. middendorffiana* by *G. lacustris*

Table 6-1. *Gammarus* prey preference calculated using Manly's (1974) alpha preference index. Prey choices offered were *Daphnia middendorffiana* versus *Hesperodiaptomus arcticus* and medium versus large *D. middendorffiana*. Means±SE of three replicates completed for each predator-prey trial are given; * denotes selection for a prey item.

Gammarus Predator	α *Daphnia*	α *H. arcticus*
Males	0.98 ± 0.02*	0.02 ± 0.02
Females	0.92 ± 0.04*	0.08 ± 0.04
	α Medium *Daphnia*[a]	α Large *Daphnia*[a]
Males	0.73 ± 0.04*	0.27 ± 0.04
Females	0.81 ± 0.10*	0.19 ± 0.10

[a] Average body sizes were 2.08 mm (± 0.10 SE, range 1.00 - 2.41 mm) and 3.54 mm (± 0.08 SE, range 3.10 - 4.15 mm) for the medium and large *Daphnia* respectively. *Daphnia* body size was measured on individuals from the control chambers after the experiment.

increased linearly with prey density (Figure 6-1), possibly indicating a Type I (Holling 1959) response over a wide range of prey densities. Predation rate (slope) differed (ANCOVA, $F_{3,8} = 68.71$, $P < 0.001$) among experimental scales. It was highest in the 20-L cubitainers, intermediate in the mesocosms and lowest in the lake (Table 6-2; Figure 6-1). Although predation on *Daphnia* by *G. lacustris* was high in the 20-L cubitainers, *Daphnia* abundance was not reduced to zero in any replicate. Pairwise comparisons indicated that predation rates in the 20-L cubitainers and in low and medium *Gammarus* density mesocosm treatments were similar ($P > 0.05$), but each differed ($P < 0.001$) from the whole-lake rate.

Daphnia densities calculated from the whole-water column samples were much lower than those calculated from the stratified samples (Figure 6-1) because it is assumed that *Daphnia* are evenly distributed throughout the water column. However, the stratified samples showed that the highest *Daphnia* density (70 to 95% of all daphnids in the water column) always occurred in the 0-3 m stratum without any diurnal changes (A. S. McNaught unpub. data). Thus, *G. lacustris* that migrate vertically in Snowflake Lake only encounter a high *Daphnia* density only near the surface. As a result, amphipods that are not in the top 3 m of the water column encounter few *Daphnia*, resulting in the lower predation rate when consumption is plotted versus the high densities from the stratified hauls (Figure 6-1).

Gammarus lacustris migrated into the water column at night with peak abundances occurring near midnight (Figure 6-2). Amphipod swimming in the water column oscillated; pulses of upward swimming intermixed with periods of sinking, especially after striking prey. This resulted in the distribution of *G. lacustris* throughout the water column versus their concentration in the stratum containing the prey. Therefore, *Daphnia* densities encountered by active *G. lacustris* in surface waters were probably higher than indicated by the passive densities from the stratified samples. The increase of *G. lacustris* density over the open-water season can be accounted for by the recruitment of maturing individuals into the adult cohort.

Size-selective predation by G. lacustris

Daphnia middendorffiana body size was directly related to mandible length by the relationship Body length (mm) = 0.102 + 26.1×Mandible length (mm); ($R^2 = 0.94$, $P < 0.001$, n = 24). The difference (measurement error and actual differences) between pairs of dissected mandibles was 0.003 mm, meaning that larger differences distinguished different *Daphnia* in *G. lacustris* gut contents.

Daphnia mandibles in *G. lacustris* guts were usually intact. On rare occasions one, but never both mandibles in a set was crushed. In such situations, only the intact mandible was measured. On Aug. 27, 1995, 62 different *Daphnia* were identified from mandible measurements in 27 adult *G. lacustris*. Adult *G. lacustris* selectively ($P < 0.001$) preyed on medium and large *Daphnia* (Figure 6-3). The medium (2.0 mm) size-class preyed on most frequently by *G. lacustris* in the lake was analogous to the medium sized (2.1 mm) *Daphnia* selected in the selection experiments (Table 6-1).

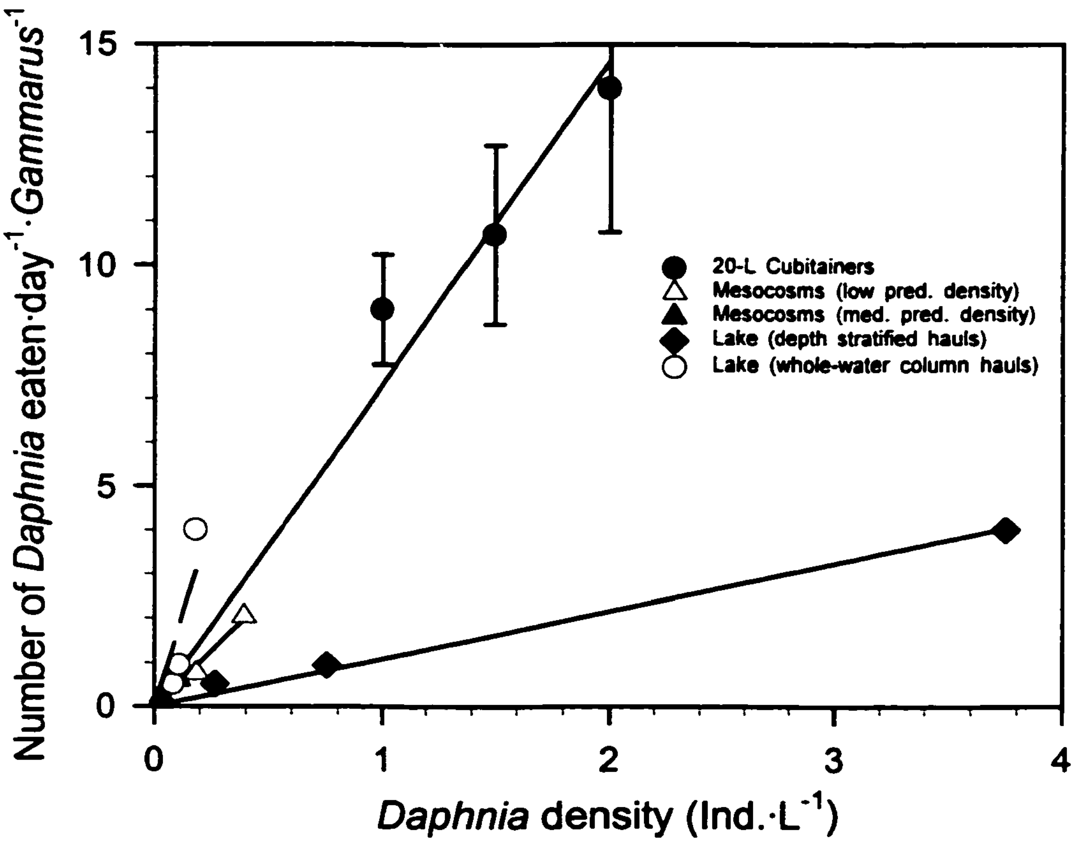

Figure 6-1. Relationships of predation rates of *Gammarus lacustris* in relation to *Daphnia middendorffiana* density at three experimental scales in Snowflake Lake. Differences in whole-lake predation rate stem from the calculation of *Daphnia* densities based on whole-water column hauls and depth stratified samples. Error bars represent ±SE; lack of error bars indicates samples for which error could not be calculated.

Table 6-2. Least squares estimate for predation rates of *Gammarus lacustris* preying on *Daphnia middendorffiana* at three experimental scales. The regression equation is in the form Number of *Daphnia* eaten· $\text{day}^{-1}\cdot \textit{Gammarus}^{-1} = b \times \textit{Daphnia}$ density (±95% confidence interval); n - number of samples; *R* - correlation coefficient; *P* - probability value.

Relationship	*b*	n	*R*	*P*
20-L Cubitainers	7.3 (±1.68)	3	0.984	<0.001
2800-L mesocosms (Low)	5.0 (±1.38)	3	0.991	0.004
2800-L mesocosms (Med.)	5.7 (±1.36)	3	0.99	<0.001
Lake (stratified hauls)	1.1 (±0.16)	3	0.998	<0.001
Lake (whole-water column)	16.7 (±17.38)	3	0.859	0.026

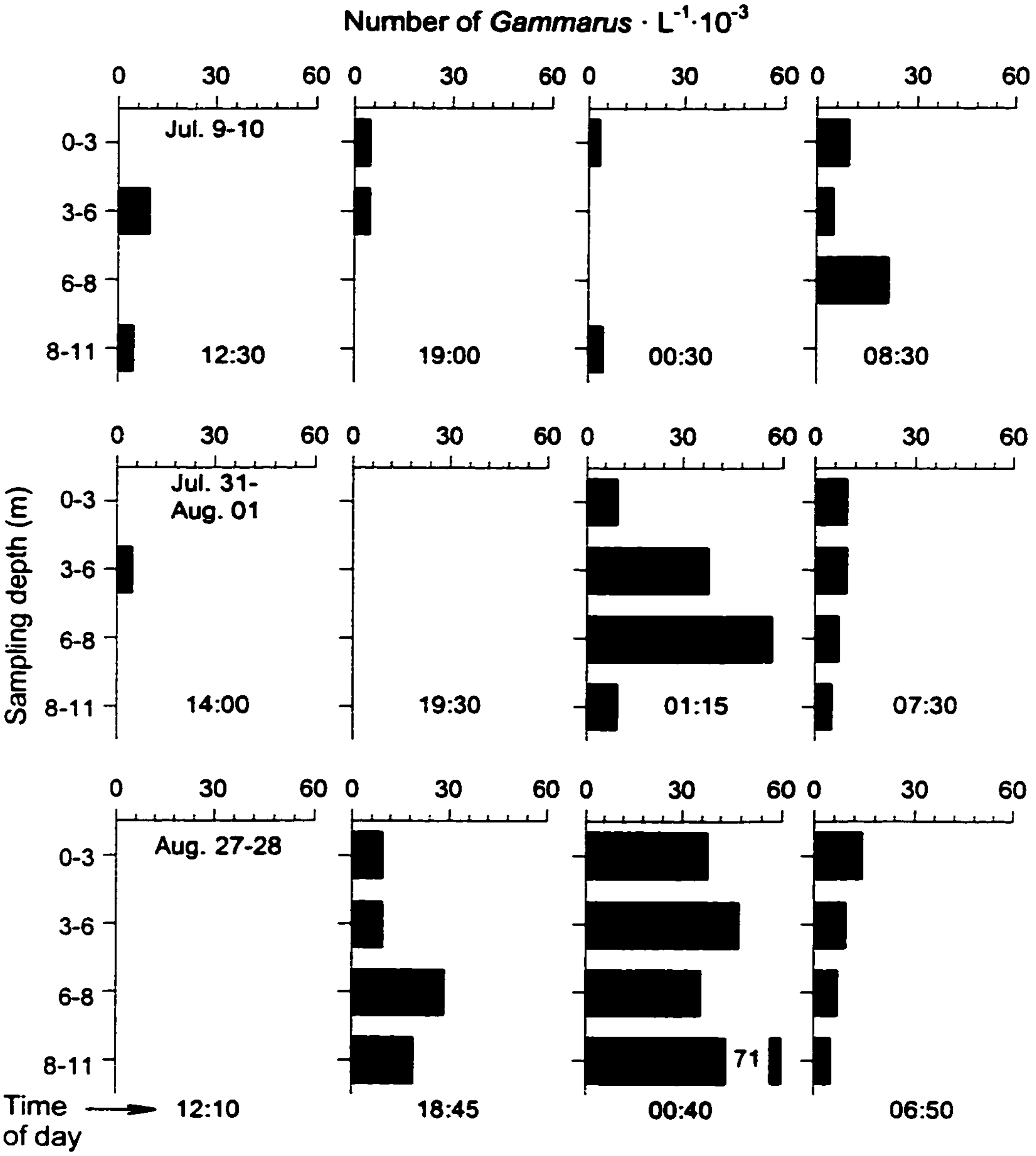

Figure 6-2. Depth-stratified *Gammarus lacustris* abundance in the Snowflake Lake water column over a 24 h period for three dates in 1995. Error bars were not calculated because for each series of hauls, animals were preserved together.

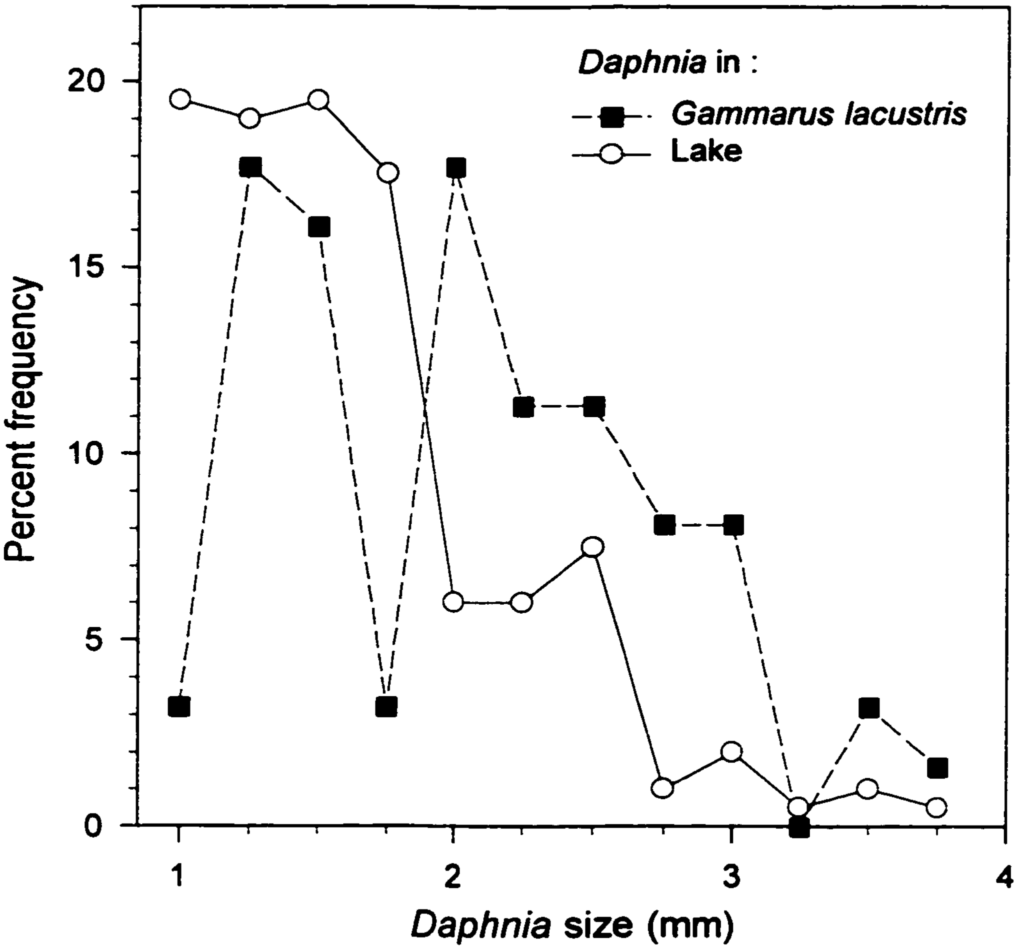

Figure 6-3. A comparison of the *Daphnia middendorffiana* size distribution in *Gammarus lacustris* gut contents with those in Snowflake Lake for the August 1995 sampling date, showing size-selective predation on large *Daphnia* by adult *G. lacustris*.

Discussion

Our results show a strong inverse relationship between experimental scale and *G. lacustris* predation rate. Even in the large mesocosms, predation rates were approximately five times higher than in the lake (Figure 6-1). Although we initially thought that the 3 m deep mesocosms would be large enough to eliminate the effect of container size for the relatively small zooplankton and *G. lacustris*, we were mistaken. Higher predation rates in the cubitainers and mesocosms probably arose from several factors, including a higher encounter frequency between prey and predators, higher activity of prey and predators due to encounters with container sides, lack of prey refugia, and restricted spatial separation preventing migratory behaviours (de Lafontaine and Leggett 1987; Piercey and Maly 1998). *Gammarus lacustris* migration patterns in the mesocosms were similar to those in the lake (Wilhelm and Schindler 1999); however, the vertical separation in the mesocosms was much less than in the lake. This likely reduced the time between prey encounters. The high predation rate in the mesocosms helps explains why Wilhelm and Schindler (1999) observed lower *Daphnia* densities in mesocosm treatments corresponding to the predator-prey density in Snowflake Lake than in the lake.

Inter-annual differences in lake temperature can not explain the difference in predation rates between the lake survey in 1995 and the mesocosm experiment in 1996. Average lake temperatures on the 1995 survey dates were 5.0°C, 7.3°C, and 6.7°C on July 9, 31, and Aug. 28 respectively. On the 1996 mesocosm sampling dates, temperatures were 7.2°C, 7.6°C, and 8.7°C, on July 22, Aug. 4 and 19, respectively. Therefore, temperatures in the mesocosm were up to 2°C higher than during the lake survey. Even if we assume that predation rates doubled with a 2°C increase in temperature, our conclusions would not change. The relationship between predation rate and ambient temperature would be useful to predict predatory impacts in lakes with different thermal regimes. We would expect the predation rates of *G. lacustris* to be related to temperature in a manner similar to the filtration rates of zooplankton (e.g. Schindler 1968; Burns 1969; Chisholm et al. 1975).

Predation rates from either the 20-liter cubitainers or 2800-L mesocosms would overestimate the effect of *G. lacustris* predation in lakes. Anderson and Raasveldt (1974) and Anderson (1980) concluded that the zooplankton community composition in some prairie ponds and alpine lakes was consistent with *G. lacustris* predation patterns observed by Anderson and Raasveldt (1974). However, these conclusions were based on extrapolations from predation experiments with *G. lacustris* carried out in 100-mL containers. These patterns of community composition should be re-evaluated in light of our findings. We believe that *G. lacustris* predation influences zooplankton community structure. However, the exact relationship between lake size and depth, *G. lacustris* density, and zooplankton community composition remains unknown.

Our results also show the need to accurately determine the spatial distribution of prey organisms when assessing predation rates. The congregation of *D. middendorffiana* in the top 3 m of the water column and their lack of migration greatly altered our calculated predation rates (Figure 6-1). Determining zooplankton densities from whole-water column samples is commonly used (e.g. McNaught et al. 1999), for it is much less time consuming and expensive than the separate analysis of individual strata. However, prey densities and encounter frequencies obtained in this manner may be inaccurate leading to spurious results and inappropriate conclusions regarding the impact of predators on prey populations. If we had assumed a uniform *Daphnia* density throughout the water column, we would have overestimated the predation rate of *G. lacustris* by more than a factor of 10. Thus, prey behaviour and distribution under natural conditions must be taken into account when designing large-scale experiments or whole-lake surveys.

The linear increase in predation rate with food availability suggests that *G. lacustris* follows a Type I feeding response (Holling 1959). Generally, predators display a Type II feeding response, where consumption initially increases with food density, but approaches an asymptote at high food density as handling time becomes limiting. Type I responses are rare in natural systems, but have been reported for *Daphnia* feeding on yeast cells (Rigler 1961) and brown lemmings foraging on arctic grasses (Batzli et al. 1981). The apparent Type I response observed for *G. lacustris* may be related to the low *Daphnia* densities in our experiments and Snowflake Lake. Although we used *Daphnia* densities representative of alpine lakes in general, these may be too low to induce predator saturation typical of Type II responses. It also suggests that *G. lacustris* may be food limited when preying on zooplankton in alpine lakes in which prey densities are low.

The strong selection by *G. lacustris* for *D. middendorffiana* versus *H. arcticus* probably reflects the slower swimming and weaker escape of the former. Copepods have a strong catapulting reaction to tactile stimuli. However, *G. lacustris* confined in vessels with high densities of *H. arcticus* will readily consume them (Anderson and Raasveldt 1974, F. M. Wilhelm pers. obs.). In addition, *D. middendorffiana* density was higher than *H. arcticus* density in the lake (McNaught et al. 1999), which would also have contributed to their high occurrence in the diet of *G. lacustris*.

The *Daphnia* selection experiments and the gut content analyses of *G. lacustris* indicate that predation by *G. lacustris* may be size-limited. *Gammarus lacustris* attempting to feed on very large *D. middendorffiana* in small beakers were unable to seize or subdue their prey (F. M. Wilhelm pers. obs.). This size-limited *Daphnia* predation may explain the occurrence of the unusually large *Daphnia* in fishless alpine lakes where *G. lacustris* is the only predator (e.g. Wilhelm et al. 1998). Reproduction by these large overwintering *Daphnia* probably contributes to rapidly increasing population density in spring. A rapid increase in numbers and the non-migration may serve to ensure the survival of the *Daphnia* population in lakes with *G. lacustris* predators. However, at high predator densities, no or few young *Daphnia* would be expected to survive and the population would be eliminated (Wilhelm and Schindler 1999). The frequency of co-occurrence of large *Daphnia* and *G. lacustris* in permanent alpine lakes and ponds remains to be investigated.

Diurnal vertical migrations by zooplankton are common phenomena in many aquatic environments. A number of reasons, such as predator avoidance (e.g. Gliwicz 1986), thermal advantages (e.g. McLaren 1963), and higher food availability in sub-surface water (Williams et al. 1996) have been proposed to explain migrations. The non-migration of *D. middendorffiana* in Snowflake Lake could be explained as a *G. lacustris* avoidance strategy. By staying at the top, *Daphnia* achieve the maximum possible vertical separation between themselves and their predators, allowing individuals to grow under low predation pressure. However, by remaining near the surface during the day, *Daphnia* would be exposed to high levels of potentially damaging UV radiation, because alpine lakes are very clear and contain little dissolved organic carbon (DOC), a major attenuator of UV radiation in freshwaters (Scully and Lean 1994; Schindler et al. 1996). Similar to other *Daphnia* in alpine and arctic lakes, *D. middendorffiana* in Snowflake Lake have darkly pigmented carapaces. Melanization of the carapace is thought to confer high tolerance of, and resistance to damage by short wavelength light including UV radiation (Hessen 1996; Siebeck and Böhm 1994; Luecke and O'Brien 1983). However, Hessen (1996) found that melanic morphs had slower growth rates than non-melanic morphs resulting from the metabolic cost of melanin synthesis. Although a slower growth rate would mean that *Daphnia* remain vulnerable to predation by *G. lacustris* for a longer period of time, our findings from Snowflake Lake suggest that the trade-off between reduced growth and melanin synthesis is outweighed by the ability to coexist with *G. lacustris* predators.

In conclusion, we have shown that *G. lacustris* predation rates were significantly influenced by experimental scale. Even in large mesocosms, predation rates were significantly higher than whole-lake rates. This implies that extrapolations of predation rates determined experimentally will exaggerate the influence of *G. lacustris* in natural systems. It may be argued that our results are specific to *G. lacustris*, or benthic-pelagic predators. Predation rates by other invertebrate predators need to be compared across experimental scales. In addition, the gut content analysis of *G. lacustris* from the lake surveys supports our argument that *G. lacustris* is an active pelagic predator of zooplankton and that ingestion of *Daphnia* is not a result of their confinement in the cubitainers or mesocosms. Given the wide geographic distribution of *G. lacustris* and its occurrence in small fishless alpine and arctic lakes and ponds where the structure of the zooplankton community has been related primarily to pelagic predators, our results should serve to stimulate further research into the possible structuring of pelagic communities from predation by *G. lacustris*.

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7. THE CONTRIBUTION OF *Gammarus lacustris* TO PHOSPHORUS RECYCLING IN A FISHLESS ALPINE LAKE[1]

Introduction

Phosphorus is typically the limiting nutrient for primary production in temperate fresh waters (Schindler 1978). Much effort has been expended to estimate the contribution of various parts of the biotic community to the cycling of P within lakes. For example, past attention focussed on the role of zooplankton as a major source of P in the epilimnion of lakes (e.g. Peters and Rigler 1973; Lehman 1984), although it is now known that protists and bacteria dominate nutrient regeneration in the plankton (Dodds et al. 1991; Hudson and Taylor 1996). Recently, bioenergetic modelling and fish manipulations have been used in an attempt to quantify the importance of fish and the effect of food web structure on P cycling (e.g. Schindler et al. 1993; Schaus et al. 1997). However, some researchers have suggested that regeneration of P by fish is insignificant (Nakashima and Leggett 1980; Mazumder et al. 1992). The release of P from sediments by benthivorous fish and its contribution to eutrophic conditions in shallow lakes is well known (e.g. Lougheed et al. 1998). The importance of P regenerated by fish has been assessed in relation to a variety of P sources such as that supplied by tributaries (Brabrand et al. 1990), but not in relation to the total amount of P regenerated (e.g. Hudson and Taylor 1996). We propose that the role of organisms in the cycling of P is put in context by comparing it to the planktonic P regeneration rate.

Benthic invertebrates also release P from sediments (Granéli 1979; Gallep 1979; Gardner et al. 1981). Of particular interest are species such as *Mysis* and *Chaoborus* which migrate between the benthic and pelagic zones. Nutrients may be removed from sediment and carried into the pelagic zone as the invertebrates migrate vertically. Phosphorus regenerated in this manner represents 'new' P for pelagic consumers because it originated in the sediment. In fishless lakes of the Rocky Mountains, the amphipod *Gammarus lacustris* undergoes similar diurnal migrations (Anderson and Raasveldt; Wilhelm and Schindler in press). During the day *G. lacustris* is epibenthic and feeds on sediment. At night it migrates into the pelagic zone where it preys on zooplankton. Adult *G. lacustris* are large (15-18 mm long, 9-16 mg dry weight) and abundant (400 ind.$\cdot$m^{-2}), sometimes exceeding several thousand ind.$\cdot$m^{-2} (Anderson and Raasveldt 1974). Therefore, *G. lacustris* may represent an important benthic-pelagic link in oligotrophic alpine lakes by providing pelagic consumers with P recycled from sediments.

We estimated the net P regeneration of *G. lacustris* during its nocturnal migration into the pelagic zone in Snowflake Lake, an alpine lake in the Canadian Rocky Mountains. We measured the P released by *G. lacustris* experimentally. Phosphorus released in- and removed from the water column due to predation on zooplankton was estimated from gut content analyses and published P values for zooplankton. We evaluated the regeneration of P by *G. lacustris* by comparing it to the P regeneration rate of the plankton community.

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[1] *A version of this chapter has been accepted for publication.* Frank M. Wilhelm, Jeff J. Hudson and David W. Schindler. *Canadian Journal of Fisheries and Aquatic Sciences 56: 1679-1686*

The planktonic regeneration rate was measured using the ^{33}P technique of Hudson and Taylor (1996). We conclude by comparing the contribution of the P regenerated by *G. lacustris* in Snowflake Lake to that of *Mysis relicta* in Lake Michigan.

Materials and Methods

Study lake

Snowflake Lake is a small fishless alpine lake (7.13 ha, mean depth 6.1 m, maximum depth 12.5 m, 2320 m a.s.l.) located in the eastern Front Ranges of the Canadian Rocky Mountains in Banff National Park (51°35'N, 115°50'W), Alberta, Canada. The lake is oligotrophic, with TP of 3-8 $\mu g \cdot L^{-1}$ and chlorophyll *a* of 0.1-2 $\mu g \cdot L^{-1}$. The waters are well buffered (pH values of 7.9-8.4; alkalinities of 900-1350 $\mu eq. \cdot L^{-1}$). The lake stratifies weakly for short periods during the open-water season. High winds and snow during July and August can easily destratify the lake. Other chemical conditions and a map with detailed basin description can be found in McNaught et al. (1999).

The biotic community of Snowflake Lake has been manipulated several times through fish and zooplankton introductions. The lake was originally fishless, but was stocked with salmonids for 5 years between 1960 and 1966. The presence of fish altered the zooplankton community from one dominated by *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* to one dominated by *Diacyclops* and rotifers (Anderson 1972; McNaught et al. 1999). Fish did not reproduce in the lake and declined gradually, with the last fish caught in 1984. Once the lake was devoid of fish, *Gammarus lacustris* and *D. middendorffiana* returned (McNaught et al. 1999), but the large copepod, *H. arcticus*, remained absent and small cyclopoid copepod species continued to dominate the zooplankton. *Hesperodiaptomus arcticus* was reintroduced on July 30, 1992, but did not become abundant in the plankton until late in 1996. *Hesperodiaptomus arcticus* reintroduction has caused the decline in abundance or elimination of all cyclopoid copepod and rotifer species (Wilhelm and Schindler 1999).

The benthic fauna is dominated by the amphipod *Gammarus lacustris*, which is typically benthic by day and pelagic by night. While migrating, material ingested from benthic feeding on sediment is excreted and egested in the pelagic zone.

Gammarus lacustris *density*

Gammarus lacustris density in Snowflake Lake was determined by vertical net hauls of a 30-cm-diam., 64-µm-mesh size Wisconsin-style plankton net and a bottom closing net (BCN, 0.168 m^2 opening and 500-µm-mesh). Vertical hauls with the plankton net were taken on three dates in 1995 over the deepest point in the lake. On each date, three stage hauls from each 0-3 m, 3-6 m 6-8 m and 8-11 m stratum were taken over a 24-h period at ~6 hour intervals, starting between 12:00 and 14:00 h. Two additional whole-water column tows (0-11 m) were taken at each sampling time on two of those dates. In 1997, we took triplicate hauls at depths of 3, 7 and 11 m on 5 dates with the BCN during daylight hours only. We used the density of *G. lacustris* to estimate the regeneration rates of P by the amphipod population in the lake.

Movement of phosphorus from the benthic to the pelagic zone by G. lacustris

Gammarus lacustris release of P derived from the sediment was measured experimentally in the field on July 17 and August 7, 1997. The July 17 run was a preliminary experiment to determine the amphipod density at which P release could be detected in a 2 h experiment. We collected *G. lacustris* with a dipnet from a boat just after dusk, when individuals had migrated into surface waters with guts filled with sediment from daytime benthic feeding (Wilhelm pers. obs). On shore, we rapidly transferred 10, 40 and 75 amphipods to duplicate polycarbonate Nalgene chambers containing 500 mL lake water. All experimental chambers were filled with water from a common sample that was collected from the surface at an offshore location. Experimental chambers and syringes had previously been washed (0.1% Contrad-70), rinsed (ethanol), and leached (0.1 N HCl). A density of 75 amphipods per chamber was selected as necessary to document P release. This experimental density was greater than ambient densities in Snowflake Lake. For the August 7, 1997 experiment, we transferred 75 amphipods to each of eight experimental chambers. Four chambers were filled with adults only and another four were filled with a mixture of adults and immatures. Two control chambers contained lake water only. All chambers were incubated partially submerged in a water bath filled with lake water. The water in the water bath was changed once during each experiment to avoid possible temperature fluctuations.

To determine P release rates, 35-mL water samples were removed with a plastic syringe before, immediately after (time zero), and at 5, 15, 45, and 120 min after introducing the amphipods. We were careful to only withdraw surface water to avoid faecal pellets released by the amphipods. The time and volume of water removed from each chamber were recorded. At the end of each experiment, the remaining water was decanted and the amphipods dried to a constant mass. TP in each water sample was analyzed using the method of Parsons et al. (1984). Our TP values do not include large particles that were generated by the amphipods, and we view the change in TP over time comparable to planktonic regeneration (see below). The change in TP in a chamber over the course of an incubation was used to calculate the net release rate from *G. lacustris*. The release of TP over time was modelled with a quadratic function and the release rate was determined by taking the derivative of this function at time zero. We used the rate at time zero because we expected TP release to decline over time, as we did not provide the amphipods with food. Results were combined into two groups; adults only and adults plus immatures.

Gammarus lacustris *phosphorus regeneration from preying on zooplankton*

While *G. lacustris* are in the water column at night they prey on zooplankton (Wilhelm and Schindler 1999). Thus, predation on zooplankton and subsequent migration of *G. lacustris* to the sediment represents the removal of P from the pelagic zone. This would decrease the effect of new P released during the upward migration. We did not measure removal from the pelagic zone experimentally due to logistic constraints, but estimated it as follows. From nighttime *G. lacustris* collections and gut content analyses in 1995, it was found that *Daphnia* made up 96% (by number) of all zooplankton consumed by *G. lacustris*. Over the 10-h period that *G. lacustris* spent in the water column, adults consumed an average of four, 2-mm *Daphnia middendorffiana* (F. M. Wilhelm unpub. data). We used these data to estimate P dynamics as a result of zooplankton predation. *Daphnia middendorffiana* length was converted to dry weight using Edmondson's (1955)

D. middendorffiana length-weight regression. *Daphnia* P content was estimated using Andersen and Hessen's (1991) value of 1.43 % P of dry weight. Because *G. lacustris* does not ingest zooplankton whole, but rather in bits by shredding it, some food (and P) is lost before ingestion (sloppy feeding). Although food loss due to sloppy feeding has not been examined for *G. lacustris*, we conservatively estimated this value at 40% based on loss rates for *Bythotrephes* and *Mysis relicta*, two raptorial zooplankton predators that also exhibit sloppy feeding (Lehman 1993; Smokorowski et al. 1998). Lehman (1993) reported that P ingestion efficiency by *Bythotrephes* was 58% (42% loss) when feeding on ^{33}P-labelled *Daphnia rosea*. Smokorowski et al. (1998) reported that mysids only ingested 31.7% of ^{113}Cd and 13.8% ^{65}Cu when fed labelled *Daphnia magna*, indicating an approximate loss rate of 70 to 85%. This is considerably higher than the loss estimated by Lehman (1993) for P, and may indicate differences between P and metals and (or) differences in the degree of sloppy feeding between the predators. Thus, we consider a loss rate of 40% as conservative.

The ingested material is then assimilated to some degree, while the remainder is egested and excreted. Amphipod assimilation rates vary with food type, ranging from 0 to 40% for leaves, and up to 87% for algae and bacteria (Nilsson 1974; Lopez and Elmgren 1989). However, none of these include zooplankton food. Lehman (1993) argued that the selection of soft prey tissues (the apparent sloppy feeding) by *Bythotrephes* led to a highly assimilable diet (85% for C). Smokorowski et al. (1998) reported *Mysis* assimilation efficiencies of 72% and 21% for ^{113}Cd and ^{65}Cu, respectively. To be conservative, we used an assimilation efficiency of 85% for *G. lacustris*; a lower assimilation efficiency would increase P release to the water column as a result of zooplankton feeding. We assumed that half of the nonassimilated portion of dietary P was released in an available form via excretion to the water, while the other half was sequestered in a temporarily nonavailable form e.g. faecal pellets.

Planktonic phosphorus regeneration in the water column

Detailed methods for measuring total planktonic P regeneration are outlined in Hudson and Taylor (1996). Briefly, on August 13, 1997, lake water (20 L) was removed from the surface waters from the middle of Snowflake Lake and placed in a clear polyethylene container that had been pre-cleaned as described above. Water was then transported to the laboratory (~8 h by helicopter and car). In the laboratory, lake water (4 L) was placed in a clear pre-cleaned polyethylene container. This water was incubated at ambient temperature (11 °C) with carrier-free radiophosphate ($^{33}PO_4$, ~1000 $Bq \cdot mL^{-1}$, ICN Biomedicals) for approximately 35 h to label the planktonic community which did not include any *G. lacustris*. Incubations were terminated with the injection of competitive inhibitor ($^{31}PO_4$, final concentration 1 mg $P \cdot L^{-1}$). This prevented re-incorporation of ^{33}P that was released from the plankton. The increase of ^{33}P in the dissolved pool over time was then measured. The slope of this line provided an estimate of the release rate of dissolved ^{33}P. The lake water remaining in the 20 L field container was analyzed for TP (Parsons et al. 1984). The release rate of dissolved ^{31}P from the planktonic community was calculated from the relationship:

$$\frac{{}^{31}\mathrm{P\ released}}{{}^{33}\mathrm{P\ released}} = \frac{{}^{31}\mathrm{TP}}{{}^{33}\mathrm{TP}}$$

with the following formula:

$$^{31}\mathrm{P\ release\ rate} = {}^{33}\mathrm{P\ release\ rate} \times {}^{31}\mathrm{TP} \div {}^{33}\mathrm{TP},$$

because at equilibrium $^{31}\mathrm{TP}/^{33}\mathrm{TP} = {}^{31}\mathrm{TPP}/^{33}\mathrm{TPP} = {}^{31}\mathrm{TDP}/^{33}\mathrm{TDP}$

where TPP is total particulate P and TDP is total dissolved P. Therefore, our definition for phosphorus regeneration was the transfer of phosphorus from the particulate pool (>0.2 µm) to the dissolved pool (<0.2 µm) over time. Egestion, excretion, exudation, decay, cell lysis and sloppy feeding all contribute to this process. Activity was measured with a liquid scintillation counter and corrected for background counts. Quenching of samples was not present.

Net phosphorus regeneration by G. lacustris

We obtained the net P regeneration by *G. lacustris* from the pelagic zone as follows:

$\mathrm{ng\ P{\cdot}L^{-1}{\cdot}d^{-1}} = ((\mathrm{ng\ P{\cdot}mg^{-1}{\cdot}h^{-1}} \times \mathrm{mg{\cdot}ind.^{-1}} \times \mathrm{ind.{\cdot}L^{-1}}) + (\mathrm{ng\ P{\cdot}L^{-1}{\cdot}h^{-1}}$ release from predation on zooplankton) - ($\mathrm{ng\ P{\cdot}L^{-1}{\cdot}h^{-1}}$ assimilation + $\mathrm{ng\ P{\cdot}L^{-1}{\cdot}h^{-1}}$ faecal pellet production)) × 10 $\mathrm{h{\cdot}d^{-1}}$.

The first term accounts for the population regeneration of P from excretion and egestion of sediment derived from the benthic zone. The density term ($\mathrm{ind.{\cdot}L^{-1}}$) is the density of *G. lacustris* in the lake. The last two terms apportion the amount of P released in- and exported from the pelagic zone due to predation on zooplankton. We then multiply by 10 h, the time spent per 24 h period in the water column. Gut passage time was determined to be about 10 h, based on findings from 24 h surveys (F. M. Wilhelm unpub. data.).

Results

Gammarus lacustris *density*

In August 1995, the average nighttime *G. lacustris* density in the water column was 0.02 $\mathrm{ind.{\cdot}L^{-1}}$, not including juveniles, of which only 3 were caught in all hauls. Juveniles rarely migrate in Snowflake Lake (F. M. Wilhelm unpub. data). Densities estimated from the daytime BCN hauls in 1997 ranged from 0.003 to 0.052 $\mathrm{ind.{\cdot}L^{-1}}$, and encompass the density estimate obtained from nighttime vertical hauls in 1995. Because we did not perform nighttime hauls in 1997, we used the 1995 densities to estimate the regeneration of P by the amphipod population.

Phosphorus regeneration by G. lacustris *from sediment*

The amount of phosphorus released by *G. lacustris* decreased over time (Figure 7-1). Mean weight-specific P release rates for the July 17 preliminary experiment ranged from 34.8 to 83.1 ng P·mg dry weight^{-1}·h^{-1} (Table 7-1). On August 7, 1997, we measured mean weight-specific P release rates of 45.6 and 209.1 ng P·mg dry weight^{-1}·h^{-1} for adults-only and adults plus immatures, respectively (Table 7-1). No mortality occurred during either experiment.

The mean dry weight of adult amphipods in the July 17 experiment ranged from 6.8 to 7.7 mg (Table 7-1). Mean dry weight of adults on August 7 was slightly lower at 6.2 mg, while the mean dry weight of individuals in the adult plus immature chambers was 4.5 mg (Table 7-1).

Phosphorus regeneration by G. lacustris *preying on zooplankton*

The mass of four 2.0-mm *Daphnia middendorffiana* was estimated to be 146.6 µg, which resulted in an hourly ingestion rate of 14.66 µg of *Daphnia*·*Gammarus*$^{-1}$·h^{-1}. This represented a P ingestion rate of 209.6 ng P·*Gammarus*$^{-1}$·h^{-1}, assuming that 1.43 % of dry weight in *Daphnia* was phosphorus (Andersen and Hessen 1991). Of this, 93.2 ng P·*Gammarus*$^{-1}$·h^{-1} (40% loss due to sloppy feeding plus half of the unassimilated portion) was released to the water column, 106.9 ng P·*Gammarus*$^{-1}$·h^{-1} was assimilated, and 9.4 ng P·*Gammarus*$^{-1}$·h^{-1} was sequestered in faecal material.

Planktonic regeneration of phosphorus

The planktonic P regeneration rate (without amphipods) in Snowflake Lake was 23 ng P·L^{-1}·h^{-1} on August 13, 1997. A second rate measured on July 23, 1998, but not included in our calculations, was 28.2 ng P·L^{-1}·h^{-1}.

Net phosphorus regeneration by G. lacustris

The net P regeneration rate by the *G. lacustris* population in Snowflake Lake was 52.2 ng P·L^{-1}·d^{-1} (7.8 to 137.2, range) and 181.4 ng P·L^{-1}·d^{-1} (27.3 to 449.5, range) for adults only and adults plus immatures, respectively. This represented between 9.5 and 32.9 % of the daily planktonic regeneration rate, assuming a constant planktonic regeneration rate over 24 h.

Discussion

Our measured release rates of P for *G. lacustris* fell within the range of P release rates reported for other invertebrates (Table 7-2). However, direct comparisons between our measured P release rates and other studies is difficult because P release rates are highly variable and often reflect methodological and environmental differences. *Gammarus lacustris* release rates of P were up to 15 times higher than P release rates of fed fish (Table 7-2). Our planktonic regeneration rate is similar to the mean regeneration rates of P (36.0 and 40.0 ng P·L^{-1}·h^{-1}) reported for two small oligotrophic lakes (TP 3.2 to 13 µg·L^{-1}) in southcentral Ontario (Hudson and Taylor 1996). Our rate is also similar to a planktonic regeneration rate of 28.9 ng P·L^{-1}·h^{-1} reported for Flathead Lake, a large oligotrophic (TP

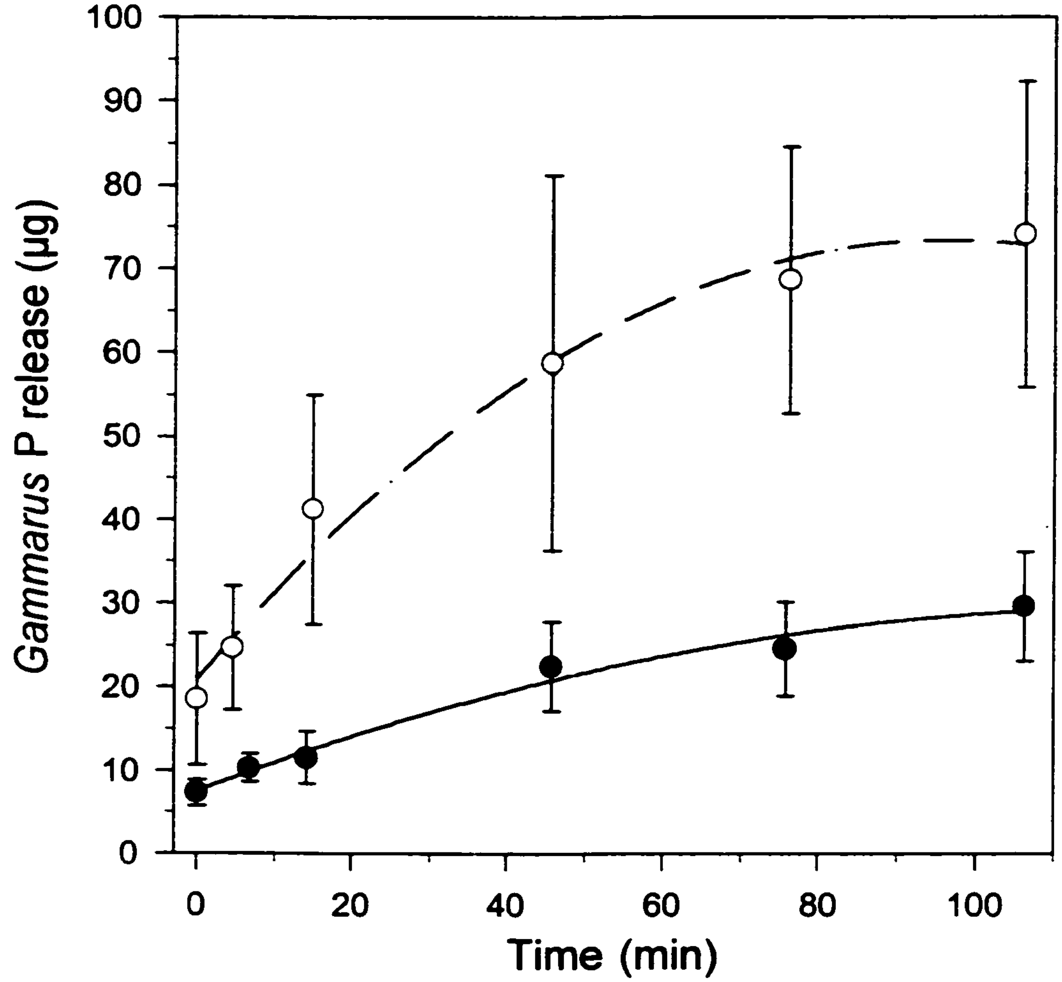

Figure 7-1. Sediment derived phosphorus release by *Gammarus lacustris* in Snowflake Lake. Seventy-five amphipods were incubated *in situ* in 500 mL chambers. A quadratic regression was fitted to P release. Then the derivative of the regression was taken and the P release rate was determined at time zero. (●) adults-only μg $P \cdot min^{-1} = -1.47 \cdot 10^{-3} \cdot min^2 + 0.358 \cdot min + 7.49$, $R^2 = 0.98$; (○) adults plus immatures μg $P \cdot min^{-1} = -5.68 \cdot 10^{-3} \cdot min^2 + 1.09 \cdot min + 20.97$, $R^2 = 0.98$. Means±SE of four replicates for each treatment are presented.

Table 7-1. Mean weight-specific phosphorus release rates for *Gammarus lacustris* from Snowflake Lake at 10°C. Amphipods were collected at dusk after migrating to the lake surface. ($\bar{X}$ - mean, SE - standard error, n - number of replicates).

G. lacustris (No. of ind.)	Date in 1997	Dry weight (mg·ind.$^{-1}$)		TP regeneration rate (ng P·mg^{-1}·h^{-1})		
		$\bar{X}$	SE	$\bar{X}$	SE	n
Adults (10)	Jul 17	7.20	0.83	34.8	24.0	2
Adults (40)	Jul 17	7.70	0.59	22.8	1.9	2
Adults (75)	Jul 17	6.80	0.17	83.1	33.7	2
Adults (75)	Aug 7	6.23	0.03	45.6	13.0	4
Adults + imm. (75)	Aug 7	4.48	0.27	209.1	91.5	4

Table 7-2. Phosphorus release rates of other amphipods, benthic invertebrates and fish.

Species	Phosphorus release rate (ng P·mg^{-1}·h^{-1})	Temp. °C	Source
Amphipods			
G. lacustris - adults	45.6 ± 13.0 (S.E.) (18.6 - 72.5 range)	10	This study
G. lacustris -adults plus immatures	209.1 ± 91.5 (S.E.) (55.6 - 474.7 range)	10	This study
Pontoporeia hoyi	2.5 - 2.8	5	Nalepa et al. 1983
Lembos intermedius	1009.2 0 h post feeding	-	Johannes 1964
	489.6 2 h post feeding		
	161.8 18 h post feeding		
Other invertebrates			
Mysis relicta	29.0 - 409.0	4-7	Madeira et al. 1982
Chironomids[a]	29.7 full gut	20	Gardner et al. 1981
	27.6 cleared gut	20	
	13.0 full gut	5	
	8.4 cleared gut	5	
Chironomids[a]	16.4 - 18.6	16	Nalepa et al. 1983
Tubificids[a]	10.5 full gut	20	Gardner et al. 1981
	9.6 cleared gut	20	
	5.6 full gut	5	
	7.2 cleared gut	5	
	214.7 (0-30 min.)[b]	5	
	56.0 (30-60 min.)[b]	5	
Tubificids[a]	4.0 - 5.6	16	Nalepa et al. 1983
Fish			
Lepomis macrochirus	1.0 - 2.3 unfed	18 - 22	Mather et al. 1995
	1.2 - 5.7 fed	18 - 22	
Dorosoma cepedianum	1.0 - 1.3 unfed	19 - 21	
	2.0 - 13.7 fed	19 - 21	Mather et al. 1995

[a] Rate per mg ash free dry weight (AFDW), 24 h experimental duration.

[b] Time after removal from culture aquaria, short-term experiments.

Table 7-3. *Gammarus lacustris* P regeneration when feeding on sediment and zooplankton in Snowflake Lake in relation to planktonic TP regeneration. Range in brackets is based on range of population density; all P release rates are in ng $P \cdot L^{-1} \cdot h^{-1}$; ± indicates P addition or removal.

	Amphipod density (ind.·L^{-1})	+TP regen. from sediment	+TP regen. from zoop. pred.	-zoop. TP assimilated	±zoop. TP excreta	Total TP regenerated	Total TP lost from zoop. predation	Net *G. lacustris* TP regen. as fraction of hourly planktonic regeneration	Net *G. lacustris* TP regen. as fraction of daily planktonic regeneration[b]
Adults (75)	0.02 (0.003-0.052)	5.68 (0.85-14.77)				7.55 (1.13-19.77)	2.33 (0.35-6.05)	22.7% (3.4-59.7)	9.5% (1.4-24.9)
			1.68 (0.25-4.51)	2.14 (0.32-5.56)	0.19 (0.03-0.49)				
Adults + imm (75)	0.02 (0.003-0.052)	18.6 (2.8-46.0)				20.47 (3.08-51.0)	2.33 (0.35-6.05)	78.9% (11.9-195.4)	32.9 (4.9-81.4)
Planktonic rate[a]		23.0							

[a] Without *G. lacustris*.

[b] Calculated assuming a constant planktonic rate throughout day and a 10 h interval of *G. lacustris* migration into water column in August.

≈5.5 $\mu g \cdot L^{-1}$) lake in north-western Montana, U.S.A. (Dodds et al. 1991). The slightly higher rates of P regeneration in the other lakes compared to our alpine lake may be due to warmer water temperatures, or methodological differences.

On a daily basis, P regeneration by *G. lacustris* was 9.5% to 32.9% (range 1.4% to 81.4%) of the planktonic regeneration rate (Table 7-3). This indicates that *G. lacustris* can be an important source of new P to the pelagic zone in Snowflake Lake. The areal density of *G. lacustris* in Snowflake Lake is intermediate for alpine lakes at 400 $ind. \cdot m^{-2}$ (Parker et al. 1996). Densities of several thousand per square metre occur in some lakes (Anderson and Raasveldt 1974). At such high densities, the amount of P transported and released in the epilimnion could exceed planktonic regeneration rates.

Most of the phosphorus released by *G. lacustris* in the pelagic zone originated in bottom sediments, so that it represents the addition of new P to the pelagic zone. Caraco et al. (1992) estimated that about 35% of the summer algal biomass in Mirror Lake (New Hampshire) required a source of new P, in addition to external sources of P and that recycled within the pelagic zone. The authors measured sources of P that are rarely measured, such as P input from ground water, and bathers, in an attempt to balance their P budget. They hypothesized that vertically migrating invertebrates such as *Chaoborus* may be an important source of P, but did not quantify it. Our results suggest that migrating benthic invertebrates are key factors in benthic-pelagic interactions and provide a mechanism by which nutrients from the benthos are made available to pelagic consumers. Our findings underscore the need of aquatic ecologists to expand pelagic food web studies to include benthic-pelagic linkages.

Several possible errors are involved in our method of measuring P regeneration by *G. lacustris*. These include: the removal of organisms from food, the evacuation of gut contents over time, and crowding (Peters and Rigler 1973; Mullin et al. 1975; Lehman 1984). The removal from food and evacuation of gut contents are problematic in studies with small bodied species of zooplankton because of their high metabolic rates and short gut clearance times. As a result, nutrient release rates quickly decline (Korstad 1983). Our estimate of P release is taken at the beginning of the incubation (t=0) to minimize this bias. We collected the amphipods and started the experiments shortly after they had migrated to the surface, when their guts were still full. *Gammarus lacustris* gut evacuation over the 2 h experimental period was not complete, so our rates should closely approximate ambient release rates.

Crowding of organisms can either increase or decrease the estimated release rates (Peters and Rigler 1973; Mullin et al. 1975). Peters and Rigler (1973) and Korstad (1983) observed decreased release rates at high zooplankton density and attributed this to physiological stress from overcrowding. However, Mullin et al. (1975) attributed large differences in nutrient excretion by crowded compared to individual zooplankters to nutrient leaching due to crowding. We did not directly address artifacts resulting from overcrowding in this study. Our results indicate a slight, but weak positive relationship between P release and *Gammarus* density (Table 7-1). The different rates between the two treatments on August 7, 1997, may also stem from crowding. However, the higher release rate in the adult plus immature treatment chambers could be due to a higher weight-specific excretion rate of the smaller immatures. A more likely explanation is the release of P by injured organisms. *Gammarus lacustris* adults usually prey on the smallest conspecifics (young-of-the-year), but aggression towards immatures has also been observed (Wilhelm pers. obs.). Therefore, antagonistic interactions between adults and immatures may have resulted in the increased release of P.

Alternatively, body P may have been released by immatures when they were accidentally drawn into the syringe and injured as a sample was being withdrawn. When this occurred, the water and amphipod were returned to the chamber and a new sample was withdrawn. Although no mortalities occurred during the experiment, nonlethal injuries may have resulted in the release of body P. However, replicates where individuals were drawn into the syringe did not consistently demonstrate higher release rates than replicates where individuals were not drawn into the syringe. Thus, we think that the antagonistic activity among amphipods was the more important release process.

Due to these interactions, release rates from chambers containing immatures may be overestimates. However, P release from injured animals may also occur under natural conditions in Snowflake Lake. Dense amphipod aggregations that include all sizes of individuals occur in Snowflake Lake. Many individuals from these aggregations have damaged or missing limbs, an indication of aggressive behaviour. Therefore, we view our measured release rate with immatures as a maximum release rate. We consider our adult-only release rate as more typical of lake conditions.

Sloppy feeding by raptorial invertebrate predators results in a large proportion of uningested food and P. Nutrients in this uningested food may not be immediately available, e.g. that associated with the carapace, in which case we have overestimated the sloppy feeding loss. However, Smokorowski et al. (1998) found that 32% of the metal ^{113}Cd in labelled *Daphnia* was released to soluble form after *Mysis relicta* had fed on them for 4 h. *Gammarus lacustris* feeding on *Daphnia* releases finely shredded material (Wilhelm pers. obs.). Therefore, much of the P in the uningested food may be released. In addition, the small particles would have a slow sinking rate and thus be exposed to further breakdown and P release during their transition through the water column, especially since the mixed water column is quite deep. Even if all P from predation on *Daphnia* was exported to the benthos, *G. lacustris* would still represent a net source of P (1.48 to 14.4 ng $P\cdot L^{-1}\cdot d^{-1}$) to the water column (Table 7-3).

Comparison of P regeneration by vertically migrating invertebrates

The paucity of published phosphorus release rates for migrating invertebrates and a lack of adequate planktonic regeneration rates preclude a rigorous comparison of P regeneration by *G. lacustris* to that of vertical migrants in other lakes. However, Hudson et al. (1999) estimated planktonic regeneration rates when lake water TP is known from:

$$\text{Log}_{10}\text{ planktonic regeneration rate (ng P}\cdot\text{L}^{-1}\cdot\text{h}^{-1}) = 1.0077 \times \text{Log}_{10}\text{TP}(\mu\text{g}\cdot\text{L}^{-1}) + 0.7206.$$

We used this relationship to calculate the planktonic regeneration rate for Lake Michigan to compare the contribution of P recycled by *Mysis relicta*, for which release rates are available (Madeira et al. 1982). We calculated the TP in the epilimnion of Lake Michigan in 1977, when Madeira et al. (1982) measured *Mysis* release rates, by interpolating Rousar's 1970-1971 average of 8.9 $\mu\cdot g\cdot L^{-1}$ for station 2 (Rousar 1973) and Shafer and Armstrong's 1990 July average of 5 $\mu g\cdot L^{-1}$ for the epilimnion (Shafer and Armstrong 1994) to arrive at a TP value of 7.5 $\mu g\cdot L^{-1}$. This decline of TP between these studies is consistent with that reported for Lake Michigan between 1976 and 1984 as a result of P management (Scavia et al. 1986). Using the above equation, we estimated the Lake Michigan planktonic regeneration rate at 960.8 ng $P\cdot L^{-1}\cdot d^{-1}$. We calculated *M. relicta* release rates from Madeira et al. (1982; Table 5) in ng $P\cdot L^{-1}\cdot d^{-1}$ (their rates are corrected for time spent in water column). Phosphorus regeneration rates by *M. relicta* ranged from 9.9 to 105.6 ng $P\cdot L^{-1}\cdot d^{-1}$. Thus, P regeneration by *M. relicta* accounted for 1.0 to 10.9% of the planktonic regeneration rate; lower than our rates for *G. lacustris*. Therefore, we conclude that P regeneration by *G. lacustris* in Snowflake Lake is more important than that of *M. relicta* in Lake Michigan. These differences may arise from a number of factors including: population density, sediment P content, tissue stoichiometry, water temperature and differences in methodology. It should also be noted that the rate calculated above for *M. relicta* does not account for the removal of P from predation on zooplankton. However, P removal from *M. relicta* predation on zooplankton should be similar to that for *G. lacustris*, given their analogous predatory behaviour.

By using the planktonic regeneration rate as a basis for comparison, we were able to determine that *Gammarus lacustris* is an important benthic-pelagic link in Snowflake Lake. Through its vertical migrations, it provides pelagic consumers with P recycled from lake sediments. The importance of other organisms in the regeneration of P in lakes could be evaluated similarly by comparing their P regeneration rates with the planktonic regeneration rate.

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8. LIPID LEVELS IN *Gammarus lacustris* FROM LAKES ALONG AN ELEVATION GRADIENT

Introduction

In this chapter I examine differences in lipid reserves between *Gammarus lacustris* populations from lakes at different elevations (730 m a.sl. to >2300 m a.s.l; Table 8-1). Lake water temperature decreases with increasing altitude while the length of the open-water season is reduced (Chapter 2). This gradient is similar to a latitudinal gradient from temperate latitudes to polar latitudes along which the seasonal storage of energy reserves by organisms increase (Pinto-Coelho et al. 1997; Vanderploeg et al. 1992; Cavaletto et al. 1996; Clarke 1977). To my knowledge the seasonal variation in lipid reserves has not been examined in freshwater invertebrates along an altitudinal gradient. Such an investigation should complement life history studies by indicating how energy is apportioned at various life stages under different environmental conditions. Specifically, I hypothesized that lipid levels in *G. lacustris* would increase with elevation to meet the higher metabolic requirement at colder temperatures. In addition, male gammarids are generally larger than females, and large males gain access to the largest and most fecund females (Zielinski 1998; Chapter 2). Because less energy is required to produce sperm than eggs, males should allocate most of their energy to growth in size, whereas females need to retain adequate energy reserves to produce viable offspring. Therefore, I hypothesized that at all elevations, lipid reserves in females would be higher than in males. I also quantified lipid reserves in juvenile, immature male and female *G. lacustris* in Snowflake Lake to examine the seasonal lipid dynamics. Finally, I quantified the reproductive investment (lipid reserves) used by females to carry developing eggs from fertilization to hatching.

A review of the ecological functions of lipids

Lipids are an important class of biomolecules with key functions in organisms such as energy storage (fatty acids, wax esters), membrane integrity (phospholipids), and building blocks for a variety of steroids (Zumdahl 1986). Their high energy density (39.35 $J \cdot mg^{-1}$) compared to proteins (23.63 $J \cdot mg^{-1}$) and carbohydrates (17.18 $J \cdot mg^{-1}$) (Cavaletto and Gardner 1998) make them ideal energy-storing compounds in organisms. Of the various lipid classes, ecologists have been most interested in the groups related to energy storage, which are the most variable of the lipid pools (Arts 1998) directly affecting growth, survival, and reproduction.

Organisms from environments with constant food quality and quantity tend to have small seasonal fluctuations in lipid reserves, except during reproduction (Clarke 1977; Napolitano and Ackman 1989; Pinto-Coelho et al. 1997). In contrast, organisms in environments with marked seasonal variations in food quality and quantity display large fluctuations in lipid reserves (Vanderploeg et al. 1992; Cavaletto et al. 1996; Hagen et al. 1996) because organisms store excess energy during high food abundance which is then used during periods of low food availability. For example, tropical copepods have small seasonal lipid fluctuations (Pinto-Coelho et al. 1997) compared to polar and temperate copepods (Comita et al. 1966; Vanderploeg et al. 1992; Hagen et al. 1993). Lipid reserves in temperate species are highest just after maximum food availability and then decline because the reserves are used to fuel metabolism and reproduction (Vanderploeg et al. 1992). Analogous lipid patterns should persist in aquatic species from high altitude environments because seasonal changes are similar to those in polar regions.

High variation in food quality and quantity usually occurs in environments with large fluctuations in ambient temperature. Generally, low food availability coincides with low environmental temperatures. Metabolic rates of poikilotherms are reduced at low temperatures, reducing energy expenditure. However, at cold temperatures, a high proportion of polyunsaturated fatty acids (PUFA) is required in cell membranes to maintain membrane fluidity, intracellular transport processes and overall cell metabolism (Stanley and Colby 1971; Adams 1998; Olsen 1998). Therefore, in cold environments, a higher proportion of lipid reserves must be allocated to metabolic maintenance than in warm environments. Consequently, we expect lipid levels in species from high altitudes or latitudes to be higher than in populations from warmer environments. In addition, we expect a delay in other processes that compete for lipid reserves, such as growth and reproduction, if energy intake or reserves are limited.

The importance of lipids in the function of cell membranes and enzymes also suggests that a minimum lipid threshold should exist below which survival is threatened. Minimum lipid levels of 1 to 4% of wet wt. have been recorded for the survival of certain fish species (Adams 1998; and references therein). Adare and Lasenby (1994) reported a minimum lipid level near 7% of dry wt. for the freshwater opossum shrimp, *Mysis relicta*. However, lipid levels required for survival have not been well studied in other invertebrates.

Lipid reserves also play a key role in reproduction. The production of eggs or offspring is energetically demanding, usually involving a trade-off between maturation and increased egg production associated with future growth (Stearns 1992). This is especially apparent in poikilothermic species where fecundity generally increases with body size (Bell 1980). Lipid reserves can influence if, when, and to what degree reproduction occurs (Tessier et al. 1983; Rowe et al. 1991). For example, female *Daphnia* with poor energy reserves allocated less lipid per egg than females with higher lipid reserves. Consequently, young from eggs with low lipid levels had lower survival compared to young from eggs with high lipid levels (Tessier et al. 1983). In Atlantic salmon (*Salmo salar*), the maturation of male parr was suppressed when mesenteric fat failed to reach a critical level in May (Rowe et al. 1991). Reabsorption of oocytes is known to occur in some fish under low food conditions and may also occur in amphipods (Sheader 1983; Wilhelm 1994). Hill et al. (1992) suggested that the amphipods *Pontoporeia femorata* and *Monoporeia affinis* need to store lipids to a level of ~20% of dry wt. in order to reproduce. The Great Lakes amphipod *Diporeia* requires lipid reserves of 23 % of dry wt. before it starts reproduction (Quigley et al. 1989). If organisms have a lipid threshold below which reproduction is not initiated, then individuals from highly seasonal environments may take longer to acquire the necessary lipid reserves than those in constant environments. This mechanism has been proposed for the increase in life cycle length with depth for the Great Lakes amphipod *Diporeia* (Winnell and White 1984; Siegfried 1985; Cavaletto and Gardner 1998), and is likely the reason for the progressive increase in *G. lacustris* life-cycle length with altitude (Chapter 2).

Lipids also play an important role in the accumulation of organic contaminants

(Schindler et al. 1995; Landrum and Fisher 1998; and references therein). The accumulation of contaminants in organisms depends on several factors including extent and mode of interaction with contaminated media, lipophilicity of contaminant, and lipid content of the organism. The non-polarity of lipids means they can act as solvents and storage sites for hydrophobic contaminants. By sequestering contaminants in lipid depots, organisms can remove contaminants from sites of toxic action. However, contaminants associated with lipid depots can be passed to offspring when females use lipid reserves for egg production. In addition, contaminants can be remobilized and their toxicity changed, if lipid pools are depleted, as occurs when lipids are used during periods of low food availability (Geyer et al. 1994). Therefore, species with high lipid levels exposed to organic contaminants would be expected to have high contaminant burdens. Campbell (1997) found that concentrations of organic contaminants in fish from Bow Lake in Banff National Park were best explained by lipid concentrations.

Recently it has been recognized that the incorporation of contaminants into lipid pools can modify fatty acid molecules (Landrum and Fisher 1998). The full implications of this are not yet known, but it is hypothesized that such changes could alter membrane functions and provide less energy when the lipids are catabolized. Thus species that require high lipid levels to maintain membrane functions and fuel metabolism in cold environments may not be able to accumulate adequate lipid reserves to survive long periods of cold temperatures and low food.

Materials and Methods

Study lakes and collection of animals

Gammarus lacustris were collected from Cooking, Sauer, Patricia, Mildred, Snowflake, and Harrison Lakes, Alberta in 1996 (see Table 8-1 for collection dates and Chapter 2 for lake characteristics). The lakes range in altitude from 730 m a.s.l. to 2320 m above sea level. At each lake, *G. lacustris* were collected from shallow water (≤ 1.3 m) with a coarse mesh (~500-µm aperture) sweep net. Amphipods were separated from detritus using the method of Wilhelm and Hiebert (1996) and placed into a 12 L pail filled with 64-µm-filtered lake water for transport to the laboratory. Samples from the prairie and montane lakes usually arrived at the laboratory for processing within several hours of collection. Samples from Snowflake and Harrison Lakes were kept cold in 6 L buckets in the outflow stream of Snowflake Lake after collection. Insulated 1L containers were used to transport them from the field to the laboratory. They usually arrived at the laboratory within 2-3 days of collection.

For lipid extraction, amphipods were sacrificed in dilute (~ 0.1%) formaldehyde solution, sorted by sex and reproductive condition (with or without eggs) and measured as described in Wilhelm and Lasenby (1998). Individual amphipods were placed in small pre-weighed test tubes (6 mm diam.×50 mm length) held in an ice-cooled tray. I obtained five individuals of each stage and sex for each lake and sampling date, however, some of these were lost during processing. For young-of-the-year, ten individuals were pooled in each tube to obtain adequate mass for extraction. For eggs, the entire egg mass from each gravid female was placed in a separate tube. Samples were dried for 24 h at 50°C under a constant

Table 8-1. Summary characteristics of lakes from which *Gammarus lacustris* were sampled for lipid analysis in 1996, including details of samples collected from each lake.

Lake	Elevation (m above sea level)	Date sampled	Life stages Sampled	Number collected
Cooking Lake	736 (prairie)	May 26	Mature males	5
			Mature females	5
			eggs (Stage 4)	5
Sauer Lake	740 (prairie)	May 22	Mature males	5
			Mature females	5
			eggs (Stage 4)	5
Mildred Lake	1033 (montane)	June 14	Mature males	5
			Mature females	4* (1)
			eggs (Stage 1)	4* (1)
Patricia Lake	1180 (montane)	June 14	Mature males	5
			Mature females	5
			eggs (Stage 4)	5
Harrison Lake	2243 (alpine)	June 25	Mature males	5
			Mature females	5
			eggs (Stage 2)	5
		August 9	Spent females	5
Snowflake Lake	2320 (alpine)	June 28	Juveniles	5
			Immature males	5
			Immature females	6
			Mature males	5* (5)
			Mature females	5* (5)
			eggs (Stage 2)	5* (3)
		July 15	Juveniles	5
			Immature males	6
			Immature females	4
		August 11	Immature females	9
			Immature males	1
		August 23	Juveniles	5
			Immature males	5

* (#) - indicates samples and number lost during analysis (mainly during centrifugation).

flow of nitrogen gas. The tubes were then placed into individual preserving jars, purged with nitrogen and stored in the dark at -20°C to minimize lipid oxidation.

Total lipids were extracted from dried amphipod tissue samples following modification (Gardner et al. 1985) of the gravimetric technique originally developed by Folch et al. (1957). Briefly, tubes with amphipods were thawed and processed 10 at a time with the addition of 2 blanks and 3 standards for each run. To extract lipids, 200 µL of chloroform:methanol (2:1) was added to each tube and the contents ground with a rotary glass pestle for 1 min. Contents were then transferred to capillary tubes and centrifuged for 1.5 min at >3000 rpm. The supernatant was then drawn off, washed with 0.9% NaCl solution, vortexed and centrifuged again for 1.5 min. at >3000 rpm. This separated the water and chloroform:methanol solution. The tube was then cut just below the water-solvent interface and the chloroform:methanol solution containing the lipids withdrawn and placed into a pre-weighed plastic weighing boat to determine the amount of lipid gravimetrically, after solvent evaporation. Blanks contained only solvent and were used to check for contamination. Freeze-dried and pulverized clam tissue was used as the standard to check for consistency among runs. Clams were obtained from the Battle River in Ponoka, Alberta. The use of a natural tissue standard is preferred to a pure lipid standard, because the extraction method is used to analyze lipids from a tissue matrix rather than pure lipids (M. Arts, NHRI, Saskatoon, pers. comm.).

Statistical analyses

Between-lake lipid levels in mature *G. lacustris* males and females at similar reproductive stages were compared using one-way analysis of variance (ANOVA). Lipid levels between males and females within-lakes were compared using t-tests. Egg lipid levels were compared with two one-way ANOVAs after grouping populations with eggs at similar development stages. Seasonal variation in lipid level in Snowflake Lake juveniles, immature males and females was compared using ANOVA. All ANOVA comparisons were followed by Tukey post hoc tests to detect means which differed (Sokal and Rohlf 1981). A t-test was used to compare the lipid level in Harrison Lake females with stage 2 eggs to lipid levels in spent females to determine the amount of energy spent by females during incubation of young. Data were log-transformed to stabilize variances.

Results

Lipid levels in mature male and female Gammarus lacustris

Lipid levels were highest in the alpine lake population (Harrison Lake) at 13.7% ± 0.97 (mean±SE) and 18.2% ± 1.63 for males and females, respectively (Figure 8-1). Lipid levels in the other populations, all of which had one year life cycles, ranged from 2.5% ± 0.62 to 5.4 ± 0.89 and 6.6% ± 0.56 to 10.6% ± 0.34 for males and females, respectively. Mature male and female lipid levels differed (males ANOVA, $F_{4,20} = 30.55$, $P < 0.001$; females ANOVA $F_{4,19} = 11.58$, $P < 0.001$) among lakes (Figure 8-1), and in all populations males had lower (t-tests, $P < 0.05$) lipid levels than females. Lipid levels of females from Harrison Lake were higher ($P = 0.034$) than in the other lakes where the lipid level of

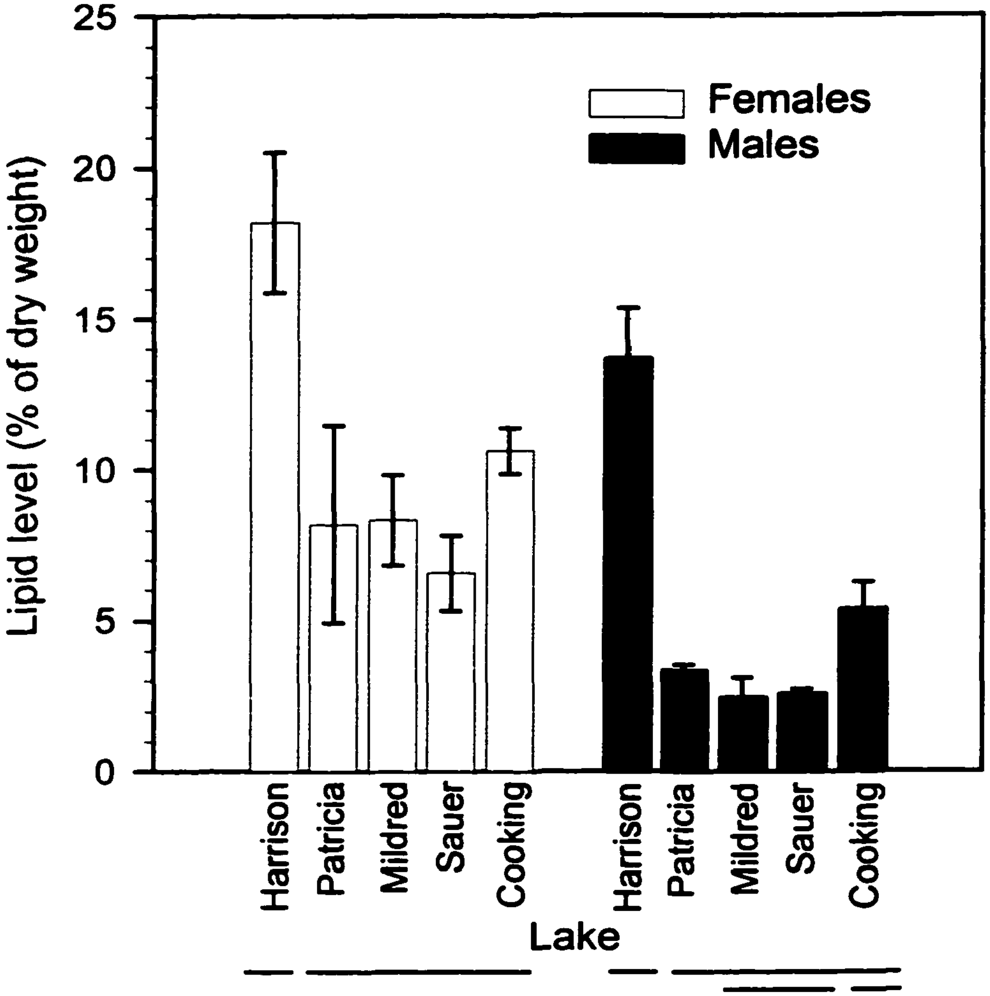

Figure 8-1. Lipid levels of mature male and female *Gammarus lacustris* in 1996 from one- and two-year life cycle lakes along an elevation gradient in western Canada. Similar means detected with a Tukey post hoc test are underlined. Error bars represent ± 1 SE.

females was similar ($P>0.05$) (Figure 8-1; Tukey post hoc test). Similarly, the lipid level of males from Harrison Lake was higher ($P = <0.001$) than in the other lakes. The lipid level of males in Cooking Lake was higher ($P = 0.011$) than in Mildred and Sauer Lakes (Figure 8-1; Tukey post hoc test).

Lipid levels in Gammarus lacustris *eggs*

Egg lipid levels ranged from 21.2% ± 2.23 (mean±SE) in Sauer Lake to 42.7% ± 0.44 in Snowflake Lake (Figure 8-2). The lipid level of stage 1 or 2 eggs from Snowflake, Harrison and Mildred Lakes were similar (ANOVA, $F_{2,8} = 3.04$, $P = 0.104$). The lipid level of stage 4 eggs differed (ANOVA, $F_{2,12} = 7.51$, $P = 0.008$) among Sauer, Patricia and Cooking Lake . In this group of lakes, egg lipid levels were lowest in Sauer Lake (21.2%), intermediate in Patricia Lake (25.4%) and highest in Cooking Lake (29.2%) (Figure 8-2). Lipid levels in these populations may have been higher at earlier developmental stages.

Seasonal variation in juvenile and immature Gammarus lacustris *from Snowflake Lake*

Lipid levels in juvenile *G. lacustris* from Snowflake Lake in 1996 decreased (ANOVA, $F_{2,12} = 7.15$ $P = 0.009$) from 13.2% ± 1.53 (mean±SE) on 28 June (Julian day [JD] 179) to 5.4% ± 0.54 on 23 August (JD 236) (Figure 8-3A). During the same period, dry weight increased (ANOVA, $F_{2,12} = 13.33$, $P = 0.001$) from 0.35 mg ± 0.04 to 0.91 mg ± 0.12 (Figure 8-3A). Lipid levels in immature females increased (ANOVA $F_{2,17} = 14.5$, $P < 0.001$) from 14.8% ± 1.44 to 20.6% ± 0.67 between 28 June (JD 179) and 11 August in 1996 (JD 224)(Figure 8-3B). Weight change over the period was minimal (ANOVA, $F_{2,17} = 2.47$, $P = 0.114$), rising slightly from 3.4 mg ± 0.40 to 4.0 mg ± 0.26 (Figure 8-3B). Meanwhile, lipid levels in immature males did not change (ANOVA, $F_{2,12} = 2.44$, $P = 0.129$) between 28 June (JD 179) and 23 August 1996 (JD236) (Figure 8-3C), although their weight more than doubled (ANOVA, $F_{2,12} = 17.37$, $P < 0.001$) from 2.71 mg ± 0.21 to 5.02 mg ± 0.36 during the same time (Figure 8-3C).

Female reproductive investment associated with brooding

Samples to estimate female investment during brooding were obtained from Harrison Lake. Females with stage 2 eggs were sampled on 25 June, 1996 while the lake was still 3/4 ice-covered. Young were released in the lake during the first 10 days of August. Of the females sampled on 9 August, one had fully hatched young in the brood pouch while others had empty brood pouches but still had setae on their oostegites, indicating the recent release of young. Between 25 June (JD 176) and 9 August 1996 (JD223), female lipid level decreased (t-test, $P < 0.001$) from 18.2% ± 1.04 to 8.4% ± 1.23, while dry weight remained unchanged (t-test, $P = 0.073$) (Figure 8-4). Therefore, the minimum female energy expenditure in carrying the developing young was 19.85 J per female over the 45 day period ($0.44\ J \cdot d^{-1}$), assuming a mean dry weight of 5.15 mg. This does not include additional energy that may have been obtained from food ingested during the incubation period.

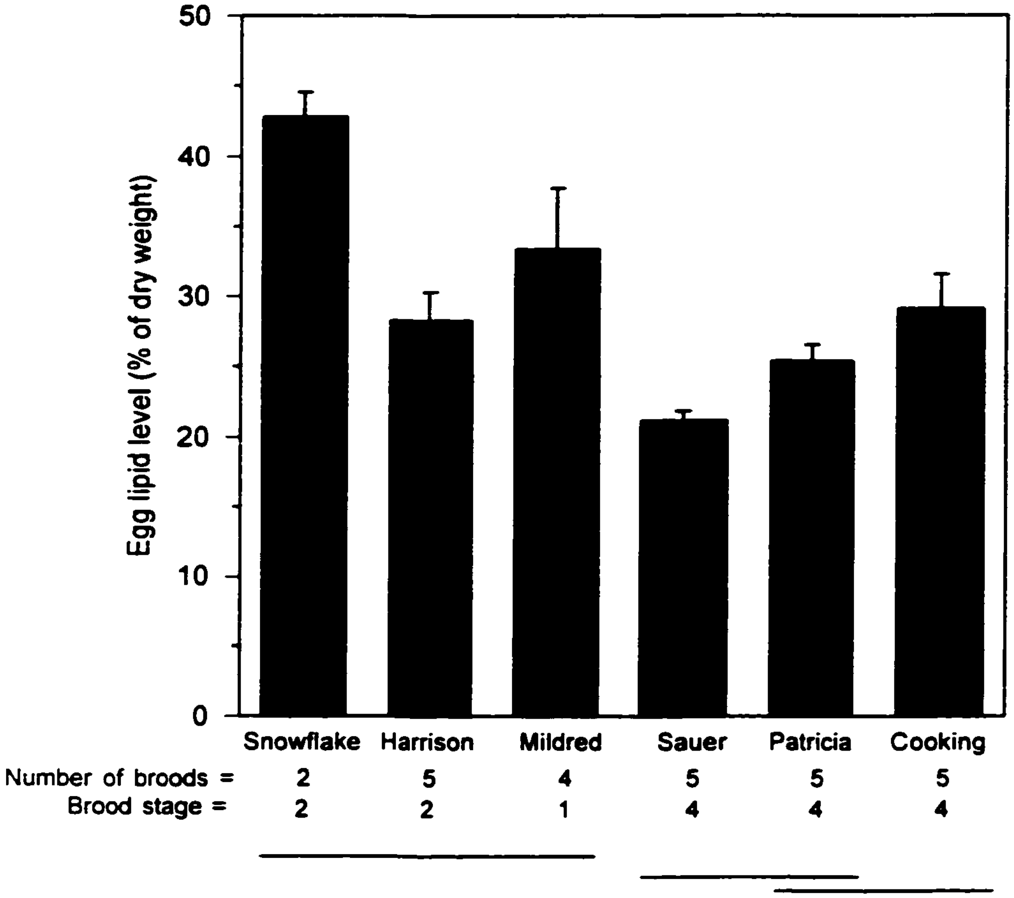

Figure 8-2. Egg lipid levels as percent of dry weight of eggs removed from mature female *Gammarus lacustris* in 1996. Number of broods - indicates the number of egg masses examined from different females; Brood stage - indicates the development stage of the eggs from each lake. Similar means detected with a Tukey post hoc test are indicated by line on the same level; note that Snowflake, Harrison and Mildred Lakes were analyzed separately from Sauer, Patricia and Cooking Lakes because of differences in reproductive stage. Error bars represent ±SE.

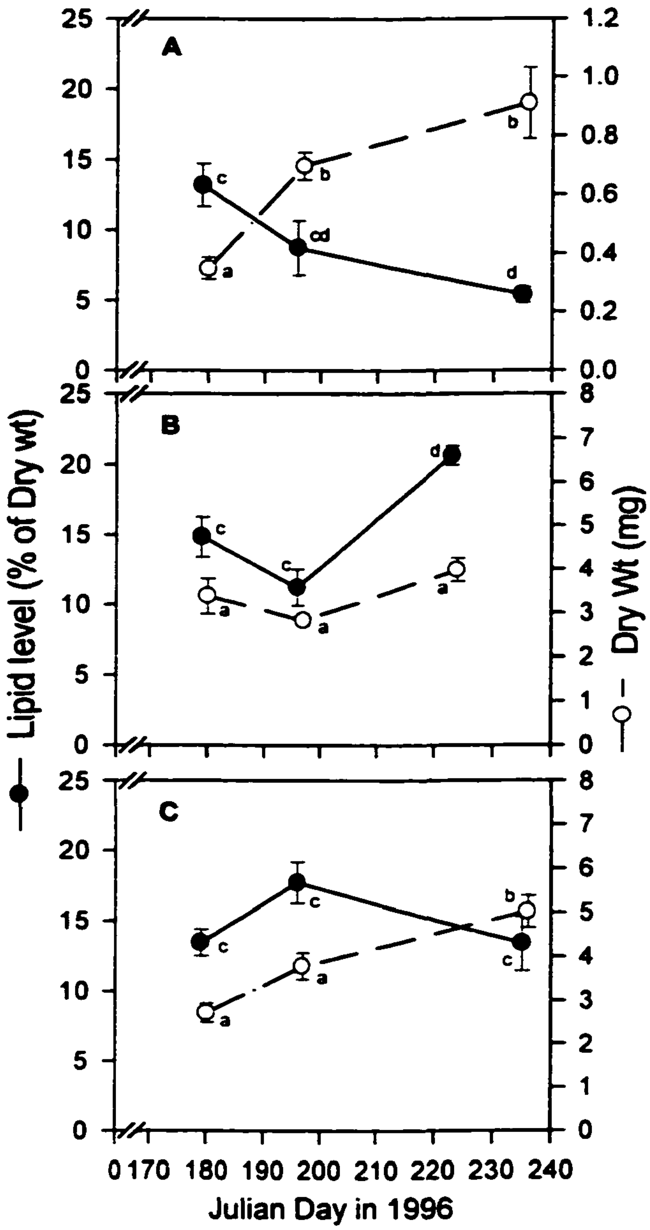

Figure 8-3. Seasonal variation in lipid level and dry weight of A - juvenile, B - immature female and C - immature male *Gammarus lacustris* in 1996 in Snowflake Lake. Letters indicate similar means detected with a Tukey post hoc test; lipid level and dry weight were analyzed separately. Error bars represent ±SE.

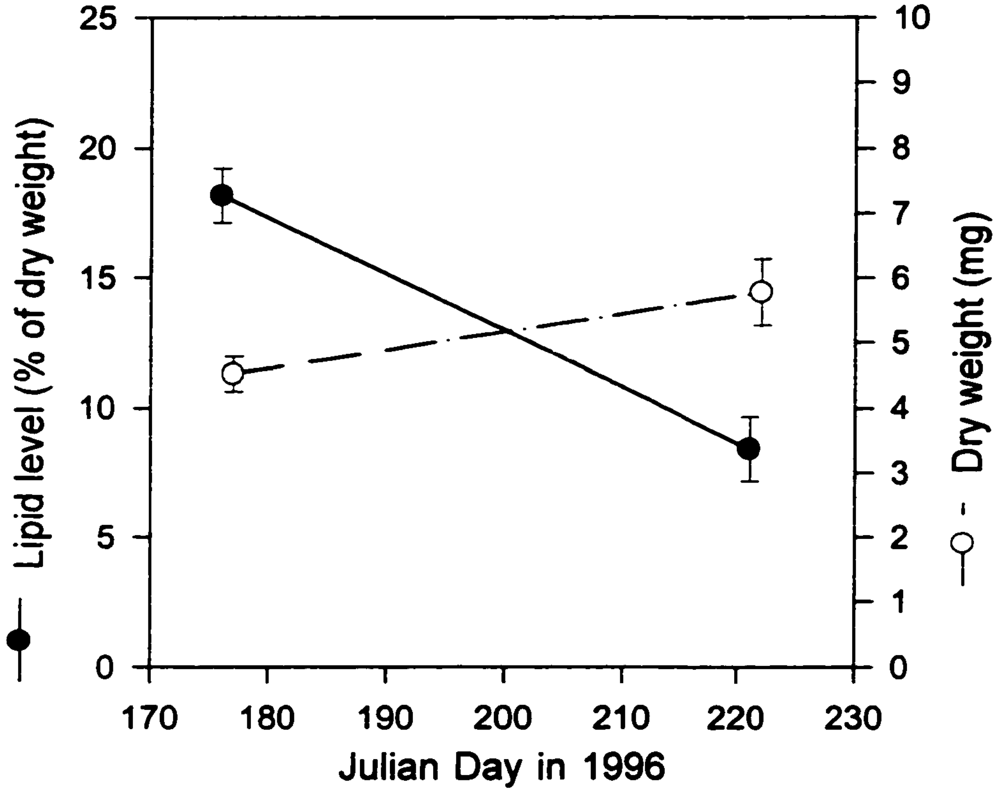

Figure 8-4. Lipid level and dry weight of ovigerous female *Gammarus lacustris* in Harrison Lake before and after hatching of young. Error bars represent ± 1 SE.

Discussion

Gammarus lacustris that had one year life cycles had similar lipid levels, regardless of elevation, while *G. lacustris* from Harrison Lake, which had a two year life cycle, had much higher lipid levels. The montane populations included here have one year life cycles (Chapter 2) which probably explains why the lipid levels are similar to those from the prairie populations (Figure 8-1). I collected the samples from the montane lakes at the beginning of 1996 when I did not know the specific life cycle length in each of the lakes. It was not until the fall of 1996 that I determined that lakes with *G. lacustris* populations that had a two year life cycle were located above ~1400 m a.s.l. in the montane ecoregion. Although Mildred and Patricia Lakes, near the Jasper townsite along the Athabasca River, are generally considered montane lakes and are 300-400 m higher in elevation than the prairie lakes, they have ice-free seasons that are similar to prairie lakes (Anderson and Donald 1980, Mitchell and Prepas 1990). Therefore, they are best regarded as transitional lakes between the prairie and montane ecoregions, with *G. lacustris* in them displaying a predominantly prairie lake life cycle. The formulation of a clear relationship between altitude and lipid levels must wait until further lipid data are analyzed for *G. lacustris* populations from lakes at other elevations, especially from lakes where two and three year life cycles predominate.

The lipid levels that I measured for *G. lacustris* with a one year life cycle are similar to those reported by Arts et al. (1995) for Gursky Pond and Humboldt Lake in Saskatchewan where *G. lacustris* also has a one year life cycle. They found that lipid levels varied from 2.4 to 15.7% during the open-water season. In their study, mean lipid levels of gravid females (including eggs) in spring were ~11% and 15.7% in Gursky Pond and Humboldt Lake, respectively. If I include the egg mass and lipid level with female weight and lipid level, the lipid levels for gravid females for Patricia, Mildred, Sauer and Cooking Lakes are 8.6%, 8.4%, 6.5%, and 10.7%, respectively. Similar to my findings, Arts et al. (1995) reported lower lipid levels in males than females. Lipid levels in mature males from Gursky Pond and Humboldt Lake were 6% and 9%, respectively.

The lipid level I measured for *G. lacustris* from Harrison Lake is the highest recorded for this species so far. The lipid level of mature males was between 2.5 and 5.5× higher than in the other lakes (Figure 8-1). The lipid level of mature females (including eggs) was 21.8%. This value is similar to those reported for the Great Lakes amphipod *Diporeia* (~23%) (Quigley et al. 1989) and the Baltic species, *Pontoporeia femorata* and *Monoporeia affinis* (~20%) (Hill et al. 1992). This correspondence in lipid levels among gravid females of different amphipod species that inhabit cold (2-12°C) water suggests that a lipid level between 20-25% is required for reproduction.

The reason for higher lipid levels in colder environments remains undetermined. Lipid levels in Harrison Lake may be higher than in the other study lakes to satisfy metabolic requirements related to membrane and enzyme function at cold temperatures (e.g. Olsen 1998). Transplant experiments amd /or laboratory studies in which amphipods from the various populations are reared under different temperature regimes will be necessary to fully address lipid - environmental temperature relationships.

The high lipid levels in Harrison Lake may also be associated with iteroparity (i.e. reproduction in subsequent years after reaching maturity; Chapter 2). Adults in Harrison

Lake may maintain high energy reserves to ensure their overwinter survival, growth and reproduction in subsequent years. Such a strategy is advantageous if the survival of young is related to post hatching conditions in the lake. Spring ice-out and summer water temperatures are highly variable in the alpine lakes. Young have low survival in years with late ice-out and cold summer temperatures (Chapter 2). Therefore, females that reproduce in more than one year are likely to leave more successful offspring than if reproduction was dependent on a single reproductive event and year. In populations of American shad for example, the number of repeat spawners increases with latitude and variability in the thermal structure of the home river (Leggett and Carscadden 1978). Because the survival of shad eggs and young is influenced by water temperature, females that reproduce in more than one year increase their chance of reproducing in a year when conditions for survival of young are good.

The lipid level of eggs does not appear to be related to either elevation or life cycle length. The similarity of lipid levels in Snowflake, Harrison and Mildred Lakes, for eggs at closely related development stages (Figure 8-2) but from different altitudes suggests that the lipid level of eggs is fixed. Furthermore, the lipid level of stage 4 eggs from the prairie lakes was similar to those at an initial development stage from Harrison Lake (Figure 8-2). This indicates that either very low amounts of lipid are metabolized during development, or that the lipid level in eggs from prairie populations was even higher at earlier development stages. Because the lipid level of eggs does not vary systematically with elevation or life cycle length, the between-population differences in egg weight (Chapter 3) must be the result of other compounds or elements. Differences in elemental composition of eggs in marine isopods from different latitudes (Clarke and Gore 1992) and in eggs of land snails from different elevations (Baur and Baur 1997) have been noted. Therefore, the large and heavy eggs produced by females in alpine lakes may contain more protein precursors which are used to produce the large young. Few studies of lipid levels in amphipods include data suitable for comparison with my data precluding a within-taxon comparison. However, Chess and Stanford (1998) recently reported lipid levels near 70% for newly deposited eggs of *Mysis relicta* in Flathead Lake, Montana. This level is almost twice that of eggs from *G. lacustris* in Snowflake Lake.

In 1996, juvenile *G. lacustris* in Snowflake Lake depleted their lipid reserves and gained weight during the open-water season (Figure 8-3A). This pattern of lipid depletion and growth is similar to that seen in other invertebrate species (Clarke 1977; Vanderploeg et al. 1992; Hill et al. 1992; Cavaletto et al. 1996). Rapid growth is advantageous for juveniles, especially if they cease to be vulnerable to cannibalism by adults above a certain size. Although it is questionable if juvenile *G. lacustris* can survive the winter with a lipid level of just 5.3% (Figure 8-3A), this lipid level was measured at the end of August, and lipid accumulation may have occurred after this date with a slowing of the growth rate. Thus, I hypothesize that young accumulate lipids towards the end of the open-water season before cold water temperatures reduce metabolic rates to a minimum. However, it is difficult to follow the pattern of lipid accumulation and growth outside of the summer open-water season in Snowflake Lake because the late season working conditions at the remote alpine site are very difficult.

Immature females in Snowflake Lake allocated energy to storage during the open-water season in 1996 rather than growth (Figure 8-3B). At the end of August, lipid levels were just above 20% and probably continued to increase throughout the fall. This level is similar to the 21% measured in mature females from Harrison Lake and further supports the suggestion that females of amphipod species living in cold water need to accumulate lipid levels between 20 to 25% before reproduction (Quigley et al. 1989; Hill et al. 1992) . The lipid level in immature males remained constant near 15%, while their weight almost doubled (Figure 8-3C). Thus, males allocated energy to growth rather than energy storage. This is consistent with the prediction that males should allocate the minimum energy necessary for survival but maximize the energy allocated to growth. Because of size assortative mating, large males have access to large and more fecund females. Although the production of sperm is less energetically demanding than the production of eggs, male amphipods invest energy in carrying the female in precopula before mating. Thus the minimum lipid level stored by males must also include sufficient energy for the precopulation period.

Lipid levels in Harrison Lake females decreased by more than 50% over the 45 day incubation period (Figure 8-4), corresponding to an investment of 19.9 J. In contrast, a maximum of only 2.3 J was invested directly in eggs (assuming eggs to be 100% lipid). This indicates that the major reproductive investment was in carrying developing young, and not the production of eggs. Energy expended by females during brooding or caring for eggs is almost never included in measures of reproductive investment in small invertebrates because of the difficulty in measuring it (Clarke 1987). However, lipid analysis appears to be a suitable method with which to assess reproductive investment by females apart from the production of eggs. Lipid analysis would be most suitable and accurate for females which do not feed during the incubation period, but rely entirely on stored energy reserves. For females that feed during the incubation period, lipid analysis would only provide a minimum estimate of expended energy, provided females used stored lipids, for it would be impossible to account for energy gained from ingested food. I frequently collected gravid *G. lacustris* from Harrison Lake with sediment in their guts, indicating that they fed during the incubation period. Therefore, the 19.9 J expended by females in Harrison Lake must be regarded as the minimum energy expended during brooding.

The large amount of energy expended by females during brooding suggests that female survival may be influenced by temperature and food during the incubation period. Lipid levels of females in Harrison Lake dropped to 8% by the time young were released from the brood pouch. This is near the 7% starvation level observed by Adare and Lasenby (1994) for the opossum shrimp *Mysis relicta*. If *G. lacustris* has a similar starvation level, females that release their brood above the starvation level should be able to recover and continue to live. The presence of large females after ice-out in the spring of 1996 and 1997 (Chapter 2) indicates that females do survive. However, because the bull trout in Harrison Lake prey size-selectively on the largest individuals (Wilhelm et al. in press) it is difficult to know if the low abundance of large females in the population is the result of starvation from inadequate lipid reserves or fish predation. Post-reproductive survival could be addressed by examining lipid levels in females from Snowflake Lake where *G. lacustris* predators are absent.

I believe that lipid analysis is a useful tool in aquatic ecology. With modifications that allow the analysis of individuals, seasonal patterns of energy allocation in different cohorts within a population can be followed. Lipid analysis can also be used to assess the reproductive investment of females other than that directly associated with the production of eggs. Lipid analyses will also complement studies of organic contaminants in organisms. One of the current shortfalls of organic contaminant analyses is that many of the extraction solvents are contaminant specific, and few have ideal lipid extraction efficiencies. In some cases lipid extractions are only 50% efficient (Landrum and Fisher 1998). This complicates cross-study comparisons which are based on lipid normalized data, assuming constant lipid extraction efficiencies among solvents. It may be best to base cross-system/contaminant comparisons on lipid data obtained from lipid analyses, such as those performed here.

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9. GENERAL DISCUSSION

My study includes several features that will be useful in predicting the effect of climate-induced warming on invertebrate communities in aquatic ecosystems. I examined the life history strategy and role of *Gammarus lacustris* in ten lakes along an elevation gradient ranging from prairie (~730 m a.s.l.) to alpine (>2300 m a.s.l.). I used the negative relationship between temperature and elevation to predict the possible response of populations of *G. lacustris* and other invertebrates to global warming.

The life history strategy of *G. lacustris* varied along the elevation and temperature gradient. The age of maturity was negatively correlated with temperature and positively with elevation, ranging from one year in prairie lakes to three years in alpine lakes (Chapter 2). In alpine Snowflake Lake, *G. lacustris* normally required three years to reach maturity. However, females reached maturity at age two and a smaller size in years with early spring thaws that were preceded by warm summers and long, warm autumns (Chapter 2). This suggests that the age of maturity may be a phenotypic response to water temperature.

In lakes where warm temperatures caused a reduction in the age of maturity, size at maturity was also reduced. This resulted in high between-year variability in size at maturity. However, in lakes with one-year life cycles, the size at maturity also varied between years and was difficult to explain. This suggests that reproduction was not under genetic control, but that it commenced once individuals exceeded a minimum size and energy threshold (Chapter 2 and 8).

The size at maturity among lakes did not increase with decreasing temperature as expected for ectotherms (e.g. Atkinson 1994). The expected pattern may have been obscured by populations-specific selective pressures such as predation. For example, the large size of mature females in Cooking Lake was probably influenced by the presence of gape-limited predators such as brook stickleback and odonate larvae (e.g. Wellborn 1994). In contrast, the small size of females in alpine Harrison Lake was influenced by the selective predation of bull trout on large individuals (Chapter 4).

The proportion of energy invested in reproduction by females was not correlated with elevation and differed within lakes between years (Chapter 2). Although difficult to explain, it may have been related to the timing of the spring thaw. However, egg size and egg weight were positively correlated with elevation, while fecundity was negatively correlated with elevation (Chapter 3). Large eggs required a longer incubation time than small eggs at similar temperatures, but young from large eggs were larger than young from small eggs (Chapter 4). Large young also survived longer than small young at cold temperatures when starved. Thus, the production of fewer, larger young is adaptive (e.g. Kolding and Fenchel 1981) at progressively higher elevations where the growing season is shorter.

Gammarus lacustris is also an important link among multiple trophic levels. In Harrison Lake, it is an important food item in the diet of native bull trout (Chapter 4). The availability of abundant alternative prey for fish after ice-out was important for the survival of the amphipod population (Chapter 4). Fish fed on emerging chironomid pupae while female amphipods incubated and released young. In contrast, in lakes where non-native species were present, *G. lacustris* populations were typically reduced to undetectable levels (Anderson and Donald 1978, Anderson 1980).

Predation on zooplankton by *G. lacustris* is important in structuring plankton communities. In Snowflake Lake, *G. lacustris* reduced the abundance of the herbivore, *Daphnia middendorffiana*, in mesocosms with medium and high densities of *G. lacustris*. The combined removal of grazers and the regeneration of nutrients contained in them stimulated an increase in phytoplankton biomass (Chapter 5). However, predation by *G. lacustris* was higher in the mesocosms than in Snowflake Lake (Chapter 6). I hypothesize that the offshore pelagial over deep water, where the spatial separation between prey and predators is large, serves as a refugium for zooplankton (e.g. Donald et al. 1994). Thus, the influence of *G. lacustris* on plankton communities should be greatest in shallow lakes and ponds.

In addition to releasing nutrients contained in herbivorous zooplankton, *G. lacustris* regenerates sediment-derived nutrients in the pelagic zone during its nocturnal vertical migrations in Snowflake Lake (Chapter 7). This sediment-derived P represents the addition of new P to the pelagic community. On average, the net P recycled by *G. lacustris* from both predation and benthic-pelagic coupling represents between 10 to 33% of that recycled by the planktonic community. This finding highlights the need for aquatic ecologists to consider the contribution of often trivialized components of the biotic community in lakes as potentially important sources of nutrients.

The change in the basic ecology of *G. lacustris* and its interaction in aquatic communities in lakes with different temperatures can be used to predict the response of this species and possibly other aquatic invertebrates to changes in water temperature, as expected under various climate change scenarios. In prairie regions, summer increases in air temperature of up to 9°C are predicted with a doubling of atmospheric CO_2 (Manabe and Wetherald 1986). Water temperatures are expected to rise because they closely mimic air temperatures (e.g. Schindler 1997). Predictions for alpine regions are uncertain because changes in temperature will depend on the moisture content of the airmass moving into the mountain regions. Warmer lakes are expected under drought conditions because low snowfall would lead to an earlier thaw and a longer open-water season. Conversely, the advection of moist air would result in higher snowfall and a deeper snowpack which would lengthen the spring melt and delay ice-out. This would reduce the open-water season and water temperatures. Long-term climate records for the town of Banff, ~50 km distant from my study lakes in Banff National Park, show an increase in the mean annual air temperature for the period 1960 to 1994 (Luckman and Seed 1995). Annual precipitation was highly variable from 1960 to 1970, followed by a drought in the late 1970's to the early 1980's. Since then to 1994, annual precipitation increased, but as rainfall in summer, not snowfall in winter (Luckman and Seed 1995). This suggests that a warmer climate will result in a longer ice-free season and warmer water temperatures in alpine lakes in the south central Rockies of Alberta. In contrast, annual air temperatures have decreased and winter precipitation increased since 1950 at Niwot Ridge (3730 m a.s.l.) in the Front Ranges of the Rocky Mountains in Colorado, USA (Williams et al. 1996). For this site, colder water temperatures are predicted under climate change scenarios. Thus, it appears that climate-related temperature changes in alpine areas will be region-specific.

Further increases in water temperature in prairie lakes should cause them to approach or exceed the upper thermal tolerance of 22 to 24°C for *G. lacustris* (Smith 1973) resulting in higher mortalities or the loss of entire populations. Large declines in the abundance of *G. lacustris* were observed in Cooking Lake in 1994 and 1998, years in which summer temperatures were above average (F. M. Wilhelm pers. obs). This suggests that high temperatures in warm years may already exceed the upper thermal limit of the species. Beisner et al. (1996) have shown that the population dynamics of freshwater invertebrates become unstable at high water temperatures, resulting in the collapse of populations. Although lethal water temperatures during the summer may extirpate populations of *G. lacustris* from prairie lakes, it may be difficult to assess such extirpations along major migration routes of waterfowl. New populations may be readily established by *G. lacustris* arriving in the feathers of migrating birds at times when water temperatures are colder. For example, *G. lacustris* are very abundant in small pothole lakes in Manitoba. Thus, large fluctuations in- and the extirpation of populations of *G. lacustris* can be expected in prairie lakes if temperatures become warmer.

The warming of mid- and high-elevation lakes should cause populations of *G. lacustris* to shift their life history strategies to resemble those now found in warmer lakes at lower elevations. Thus, a decrease in the age at maturity, egg size, and weight, and an increase in fecundity would be expected as climate warms if these traits are under phenotypic control or can evolve rapidly. Increases in fecundity would lead to higher population densities, which should shift the regulation of populations in predator-free lakes from temperature-dependent survival of eggs and young to density-dependent resource limitation. Of concern should be the increased likelihood of extreme variations (e.g. Francis and Hengeveld 1998) between years. This could result in the loss of populations because of the inability of individuals to match appropriate life history strategies to rapid and large fluctuations in environmental conditions. For example, the accumulation of lipid reserves by maturing females in warm years may be inadequate for their survival or reproduction the following spring if ice-out is delayed by cold temperatures. Alternatively, females may fail to allocate the appropriate energy reserves per egg, resulting in young that are unable to survive after hatching. The replacement of populations extirpated from mountain regions would probably be low because of the low frequency of animals and waterfowl moving among bodies of water in those areas.

Temperature-dependent processes such as metabolism would increase in warmer waters. Thus, the consumption of food to meet basic metabolic requirements would increase and place a greater demand on food resources, and on prey populations of zooplankton. Although the loss of zooplankton to predation would increase, populations may be able to overcome the loss through faster reproductive rates at warmer temperatures (e.g. Neill 1984). The release of nutrients from ingested zooplankton and sediment would also increase at higher temperatures. Although the temperature-dependence of physiological processes is common among poikilotherms, specific relationships for *G. lacustris* will be necessary to quantify its responses at warmer temperatures.

Future Research

To predict the response of populations to changes in their local environment, it is important to know whether life history traits are under genetic control or if they are phenotypic responses to local conditions. The comparative approach that I used is adequate to identify differences among populations. However, to distinguish potential genetic differentiation between populations requires that individuals from different environments be raised under similar environmental conditions, or a 'common garden' experiment (cf. Smith et al. 1994; Rohr 1997; Tracy 1999). Life history characteristics from such an experiment should ideally be measured on individuals of the F2 generation to ensure that the results are not influenced by maternal carry-over effects (Roff 1992; Blanckenhorn 1997). This would require an experimental duration of greater than six years for temperatures at which *G. lacustris* typically requires three years to reach maturity. It may be difficult to keep a large number of *G. lacustris* alive under artificial conditions for such an extended period of time. An alternative experiment might be to transplant amphipods into separated areas of a natural water body at different temperatures, permitting interactions with natural communities. The simplest outcome would be that the range in life history traits displayed by *G. lacustris* is the result of phenotypic plasticity. However, initial evidence from allozyme and DNA analysis (J. Witt, Department of Zoology, University of Guelph) indicates that some genetic differences exist among the ten populations of *G. lacustris* that I studied. Therefore, populations may be locally adapted. If so, extirpation would represent a loss of biodiversity.

To establish a mortality schedule over the course of an organism's life-span requires a reliable estimate of the number of individuals present in specific age- or size-classes in a population at successive time intervals. Of the wide variety of methods available to census benthic invertebrates, none was adequate to quantitatively sample the peaty or steep rocky shorelines that *G. lacustris* inhabits in the alpine lakes. My density estimates were for mature and maturing individuals which are difficult to age accurately. Thus, reliable mortality estimates for all age classes requires a new method to quantitatively sample all age classes of *G. lacustris*.

Accurate information on growth is fundamental to understanding the population dynamics of *G. lacustris*. Currently, it is difficult to accurately determine the exact age of large individuals, especially reproductive adults. The ages of wild lobsters and crayfish have been determined using relationships between age and the accumulation of lipofuscin in the brain of animals of known age (Belchier et al. 1998). Similarly, adult Diptera have been aged using the accumulation of pteridine in the compound eye (McIntyre and Gooding 1995). Similar methods should be pursued and adapted to determine the age of large, reproductively mature amphipods collected from the field. The determination of accurate ages for *G. lacustris* in alpine lakes would be especially useful, because it would provide detailed information on the age structure of reproducing adults. Such information is necessary to construct accurate life tables and predict population dynamics.

Dynamic energy budget models relate how the energy acquisition of individuals affects demographic processes at the population level (Kooijman 1993, Gurney et al. 1996). Because temperature affects the physiological processes that ultimately determine population growth rates, it is necessary to understand the energetic response of individuals at different temperatures. For example, Beisner et al. (1996) and Beisner et al. (1997a, b) showed that at high temperatures, predator-prey interactions between the flatworm predator *Mesostoma ehrenbergii* and the crustacean herbivore, *Daphnia pulex,* were highly unstable, resulting in the extinction of the herbivore followed by extinction of the predator. These responses were related to the direct effect of temperature on the rate of feeding, energy use, birth and death for both the predator and prey populations. Incorporating such information into a dynamic energy budget allows the population dynamics of predators and prey to be examined in relation to altered environmental conditions such as increased temperatures. Given the importance of *G. lacustris* in aquatic ecosystems, such a model would be a useful tool to predict population dynamics in lakes expected to be subjected to global warming.

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