The Phenology and Inducible Defenses of *Lepidurus lemmoni* (Crustacea: Notostraca)

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Matthew A. Hill

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Major Professor: Frank M. Wilhelm, Ph.D.
Committee Members: James B. Johnson, Ph.D.; Christine M. Moffitt, Ph.D.
Department Administrator: Lisette P. Waits, Ph.D.

Authorization to Submit Thesis

This thesis of Matthew A. Hill, submitted for the degree of Master of Science with a Major in Natural Resources and titled "The Phenology and Inducible Defenses of *Lepidurus lemmoni* (Crustacea: Notostraca)," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: ______________________________ Date: __________________
Frank M. Wilhelm, Ph.D.

Committee
Members: ______________________________ Date: __________________
James B. Johnson, Ph.D.

______________________________ Date: __________________
Christine M. Moffitt, Ph.D.

Department
Administrator: ______________________________ Date: __________________
Lisette P. Waits, Ph.D.

Abstract

Growth, hatching, and anti-predator defenses were studied in *Lepidurus lemmoni* from several lakes throughout its range. Growth of *L. lemmoni* was well described by a sigmoidal curve, with growth slowing after sexual maturity. Carapace sexual dimorphism of *L. lemmoni* developed well before sexual maturity and differed significantly between females, males, and immatures. Average hatching day was inversely correlated with temperature, and not affected by salinity. Cumulative egg hatch was inversely related to temperature in Goose Lake and Middle Alkali Lake, but peaked at 13.5°C in Dowling Lake *L. lemmoni*. Cumulative hatch was uniformly lowest across all temperatures at 0.0ppt, significantly so in Goose Lake *L. lemmoni*. Middle Alkali Lake *L. lemmoni* had significantly higher egg mortality than *L. lemmoni* from Goose or Dowling lakes. Defensive morphology was induced by temperature, and non-lethal injury to a limited extent, but not alarm cues or kairomones.

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Chapter 1: General Introduction

The Great Basin is a large endorheic basin in western North America encompassing 477,664 km^2, comprising almost the entire state of Nevada, as well as portions of Oregon, California, Arizona, Utah, and Idaho. Despite being classified as a cold desert, the Great Basin contains a large number of perennial and seasonally temporary water bodies, especially in the western half (Grayson, 2011). The permanence and reliability of these water bodies is threatened by anthropogenic climate change, which has been implicated in increasing the frequency of extreme events, including flooding from high precipitation, and drying due to persistent drought. High variation in precipitation in the Great basin over the last 1000 years suggests that non-anthropogenic mechanisms can also threaten the availability of these waters to wildlife, and may work synergistically with anthropogenic climate change to magnify the perturbations (Melack *et al*., 1997).

Great Basin waters may be critical to the life history needs of certain North American shorebirds, and are threatened by development, ground- and surface water diversions, hydroelectric projects (Engilis and Reid, 1996), and climate change (Melack *et al.*, 1997). The waters of the western Great Basin are used as breeding areas by nine species of shorebirds, and as migratory stopovers by an additional 24 species (Oring and Reed, 1996). The shallow, saline and alkaline lakes of various sizes are major or critical contributors to the species persistence of American Avocets, Snowy Plovers, Long-billed Dowichers, Red-necked and Wilson’s Phalaropes, and Willets (Oring and Reed, 1996). These birds arrive in great abundance to these habitats during wet years, but either fail to stop or leave immediately after arrival during dry years (Warnock *et al.,* 1997). Increases in the variability in the frequency of inundation of these water bodies, as is predicted with anthropogenic climate change (Melack *et al.*, 1997) could have serious negative impacts on the persistence of these shorebird species.

Shorebirds use the water bodies of the Great Basin for a number of purposes. The shallow, saline lakes provide shelter from predators, water, and an abundant source of food. These lakes are often highly productive, and can contain a large invertebrate biomass (Mahoney and Jehl, 1985). The macroinvertebrate assemblages of these lakes are often dominated by Branchiopod Crustacea, especially members of the orders Anostraca and Notostraca (Rogers, 2001; Mahoney and Jehl, 1985). The most common notostracan in the Great Basin is Lynch's Tadpole Shrimp, *Lepidurus lemmoni* Holmes, 1894, which occurs in many of the lakes frequented by migratory and nesting shorebirds (Oring and Reed, 1996; Rogers, 2001). It is likely that Notostraca, and especially *L. lemmoni,* are an important component in the diets of shorebirds in the western Great Basin. Caspian terns have been observed feeding Notostraca to their chicks at Summer Lake in southeastern Oregon (D. Banks, ODFW, pers. comm.). Summer Lake is surrounded by large playas inhabited by *L. lemmoni* (pers. obs.), sometimes in phenomenal numbers (D. Banks, ODFW, pers. comm.). Thus, it is highly probable that the Caspian Terns of Summer Lake were using *L. lemmoni* to feed their chicks. Because of the potential importance to migratory shorebirds, basic biological and ecological data on *L. lemmoni* is needed to understand populations in different lakes of the western Great Basin as they change in response to climatic changes. For example, extirpation of populations of *L. lemmoni* due to long periods of drought or permanent drying may negatively affect populations of shorebirds. To predict future trajectories of shorebirds requires that we understand the biology of its prey.

Lepidurus lemmoni is a primitive crustacean typical of large, temporary, alkaline lakes in western North America. It has been recorded from Alberta, Montana, Washington, Oregon, California, Wyoming, Nevada, New Mexico, Arizona, and Baja California (Rogers, 2001). It is especially common in the Great Basin and Mojave Desert (Rogers and Hill, 2013). The playas in which they live are filled by winter and spring precipitation, and are shallow, turbid, and very alkaline, with pH values of 8.2-11 (Lynch, 1966).

Little is known about the life history and phenology of *L. lemmoni*. Mature specimens have been collected between April and September, but the growth rate is unknown, though surmised to slow with age (Lynch, 1966). Nothing is known about the timing of hatching, and no laboratory studies examining the relationship between temperature, salinity, intrinsic factors (e.g., egg size) and hatching rates have been completed. Also, there is no explanation for the high degree of morphological variability in *L. lemmoni*, especially in regard to the carapace armature. Management of the migratory birds and the Great Basin habitats that they require for survival will require an understanding of the basic biology of an important prey species, which is explored in this thesis.

In chapter 2, I used in- and outdoor cultures to determine the minimum size at sexual maturity, time to sexual maturity, and the development of sexual dimorphism in *L. lemmoni*. I also tested the hypothesis that the growth rate was related to the latitude of origin of specimens. These data allow estimation of the inundation time required for successful completion of the life cycle, and comparison of the phenology of *L. lemmoni* to other, better-studied Notostraca.

In chapter 3, I examined the effect of temperature and salinity on cumulative egg hatch and average hatching day of several populations of *L. lemmoni* to test the following hypotheses: that the response of hatching (cumulative hatch and duration of incubation) to temperature and salinity was related to the latitude of origin of eggs, that hatching success of eggs and duration of incubation was related to egg diameter and embryo mass, and if the duration of incubation is adaptively driven by short hydroperiod or competition/predation avoidance with *B. gigas*. These data allow comparison of the hatching of *L. lemmoni* to other, better-studied Notostraca. These data also allow determination of the factor(s) that affect hatching, and the long-term survival of *L. lemmoni* populations, based on estimations of how anthropogenic climate change will affect these factors. Furthermore, these data will help establish optimal hatching conditions for *L. lemmoni* for future laboratory studies.

In chapter 4, I address inter-population morphological variability in *L. lemmoni*, which was previously given taxonomic relevance (Linder, 1952), as a response to fish predation. I test the hypothesis that exposure of *L. lemmoni* to alarm cues and/or fish kairomones induces defensive morphology. I also test the hypothesis that non-lethal injury can induce defensive morphology, both in regenerated body parts at the site of the injury, and in the whole organism. The results of these experiments will allow me to determine if the *echinatus* form

(Linder, 1952) in *L. lemmoni* is an inducible defense, and one that occurs in response to fish predation.

In chapter 5, I discuss incidental observations made during other experiments. These observations offer insights into the nature of the *echinatus* form, the environmental cues that induce it, and its possible adaptive significance. In chapter 6, I summarize the findings in the previous data chapters, and suggest future directions in the study of *L. lemmoni*.

Each of these chapters was written as individual manuscripts, and therefore contains separate introductions, methods, results, and discussions. When being considered for peer-reviewed publication, it is likely that some chapters will be merged, and will not appear in the same form as in this thesis. For this reason, individual abstracts and references were not provided for each chapter.

Chapter 2: Growth of *Lepidurus lemmoni* in Laboratory and Outdoor Culture

Introduction

Lepidurus lemmoni Holmes, 1894 is a primitive crustacean typical of large, temporary, alkaline lakes in western North America. It has been recorded from Alberta, Montana, Washington, Oregon, California, Wyoming, Nevada, New Mexico, Arizona, and Baja California (Rogers, 2001). It is especially common in the Great Basin and Mojave Desert (Rogers and Hill, 2013). The playas in which they live are filled by winter and spring precipitation, and are shallow, turbid, and very alkaline with pH values of 8.2-11 (Lynch, 1966).

Little is known about the life history and phenology of *L. lemmoni*. Mature specimens have been collected between April and September, but the growth rate is unknown, though surmised to slow with age (Lynch, 1966). Based on studies of similar Notostraca, individuals likely attain sexual maturity at a carapace length of approximately 10 mm (Ahl, 1991; Fry-O'Brien and Mulla, 1996; Meintjes, 1996). The growth of several other species of Notostraca has been previously described. In *Triops longicaudatus* (LeConte, 1846) *Lepidurus packardi* Simon, 1886, and *Lepidurus apus* (L., 1758), growth is rapid early in life, then slows significantly, roughly concurrent with the onset of sexual maturity (Scott and Grigarick, 1978; Ahl, 1991; Cvetković-Miličić and Petrov, 1999). *Triops granarius* (Lucas, 1864) from South Africa has a growth curve without a distinct decrease in growth after maturity (Seaman *et al.*, 1991).

L. lemmoni, like most other gonochoristic (with separate sexes) Notostraca, displays noticeable sexual dimorphism, especially in regard to the shape of the carapace (Longhurst, 1955; Lynch, 1966) (Fig. 2.1). The carapace of the male is flatter, making it appear broader than that of the female (Lynch, 1966). No data exist on when this dimorphism develops in *L. lemmoni* or any other Notostraca.

Methods

Study Overview

I describe the growth rate of *L. lemmoni*, as well as the development of sexual dimorphism in carapace shape. These data allow estimation of the inundation time required for successful completion of the life cycle, and comparison of the phenology of *L. lemmoni* to other, better-studied Notostraca. An outdoor mesocosm was used to rear individuals of *L. lemmoni* to collect general phenology data under near-natural conditions because there were no nearby natural populations of *L. lemmoni.* Additionally, experience with the outdoor mesocosm was used to inform the laboratory common garden experiments. A laboratory common garden experiment, with different populations side-by-side subjected to identical conditions, was used to examine if the latitude of origin of a population affected its growth rate.

Study Populations

Populations of *L. lemmoni* representing the north (Dowling Lake, 51° 45’ 04”N 112° 04’ 58”W, AB, Canada) and south (Edwards Air Force Base, 34° 50’ 20”N 117° 55’ 01”W, CA, USA) extremes, as well as the center of the latitudinal range (Goose Lake, 41° 46’ 30”N 120° 27’ 44”W, CA, USA; Middle Alkali Lake, 41°31’53”N 120°07’28"W, CA, USA) were selected for study.   Lakebed sediments were collected from these localities during the dry season. The top 2-3 cm of sediments were collected from several locations in each lake, and combined into a composite sample.

Outdoor Mesocosm Study

To examine the growth rate of *L. lemmoni*, a common garden experiment in outdoor mesocosms was undertaken. This experiment was completed by sinking three 120cm diam. × 12 cm deep polyethylene pools into the ground such that the top of the side wall was 2 cm above ground level on the University of Idaho campus (46° 43’ 41.5”N 117° 01’ 32.4”W) Moscow, ID, USA to ensure that all were exposed to similar environmental conditions (wind, sun, etc.) (Fig. 2.2). The pools were installed in December 2012, and each was inoculated with approximately 8L of dry soil, one with Goose Lake and two with Middle Alkali Lake sediments. Only one pool was inoculated with Goose Lake sediments due to limited availability of sediments. After adding the sediments, the pools were allowed to fill with snow and rain water. Once weekly observations of the pools commenced at ice-out (March), and water temperature and conductivity were recorded with a Yellow Spring Instrument Co. (YSI - Yellow Springs , Ohio, USA) model 85 multi-probe.

Laboratory Common Garden Study

For each of two duplicates from each lake, four liters of dry lakebed soil were mixed with 100 L of distilled water in a 120 cm diam. × 12 cm deep polyethylene experimental chamber floated in a 1000 L circular fiberglass flow-through tank (Fig. 2.3) supplied with chilled and ambient water mixed to maintain a fixed temperature. All tanks were started at 5-6°C, and slowly raised to 15-17°C over three weeks, then increased to 20°C eight weeks after inundation to simulate the temperature changes associated with filling and inundation of pools in early spring from snowmelt. Light was provided with overhead fluorescent tubes, timer-controlled to match seasonal light levels at 47°N latitude. Evaporative loss was compensated with 20 L distilled water added to each pool 25, 62, and 77 days into the experiment. Gentle aeration was provided to each pool via compressed air delivered with a 10 cm air stone. Food in the form of 0.1 g sinking tropical fish food (Shrimp Pellets®, HBH Pet Products, Springville, UT) was provided twice weekly. The experiment was started on 31 January 2014, and terminated on 30 May 2014, after the last individual died.

Sampling and Analysis

Sampling of *L. lemmoni* in both experiments took place weekly. To sample hatched *L. lemmoni* in the outdoor mesocosms, a 1 mm mesh aquarium net was gently swept back and forth across the length of the pool ten times in quick succession. Because this method did not reliably yield *L. lemmoni*, the pools in the laboratory common garden were sampled by sweeping a 1 mm mesh aquarium net back and forth through each pool for ten minutes. The contents of the net were examined visually by eye for any *L. lemmoni*. All *L. lemmoni* captured were transferred to a 10 cm diameter glass Petri dish with a thin layer of water from the pool from which they were collected. The Petri dish was placed on a sheet of graph paper with 5 mm grids, after which all specimens were individually photographed. The carapace length and maximum carapace width was recorded for each individual using the graph paper for reference. After measurement, all *L. lemmoni* were returned to the originating pool. The date that hatchlings first appeared, as well as the date on which sexually mature individuals (measured by the presence of brood pouches on the eleventh thoracic limb of females) were present was recorded. The pools were monitored in this fashion until they dried completely in the outdoor mesocosm, and until the last individual died in the laboratory mesocosms. Carapace length was plotted as a function of time for each population. Only four weeks of observations were possible in the outdoor mesocosm, which was insufficient to fit a growth curve, or for analysis of sexual dimorphism. Individuals hatched occasionally during the first two months of observations in the laboratory common garden, so the data were recruitment-adjusted to remove any lag effect these late-hatching individuals would have on the growth curve. Recruitment-adjusted data was produced by excluding all individuals that were smaller than the smallest individual in the previous week's observation. A scatterplot of carapace length as a function of time was produced with the recruitment-adjusted data, and the general shape of the plot was used to select an appropriate curve to fit to the data. To test the hypothesis that growth curves did not differ among populations, I used the method of Kappenman (1981) to compare non-linear growth curves.

Because only males were present in both Dowling Lake pools, only individuals from Goose Lake were used for analysis of sexual dimorphism. To examine sexual dimorphism in carapace shape, the carapace length of immature *L. lemmoni* (carapace length less than 10.5 mm), mature males, and females was plotted as a function of carapace width and examined using least-squares linear regression. The slope of each line (female, male, and immature) was compared to 1 with the 95% confidence interval to determine if the relationship between carapace length and width changed with growth. The average carapace length: width ratio of each group was compared with a one-way ANOVA, with the ratio as the response variable and development stage as the treatment, followed by a Fisher's protected Least Significant Difference test (Ott and Longnecker, 2010) to detect means which differed from each other. All analysis was performed in R (R Core Team, 2013).

Results

Outdoor Mesocosm Study

All pools filled with snow shortly after placement on December 24, 2012 and became ice-free on 21 March 2013. Conductivity in all pools ranged from 1000 to 20,000 µS/cm (Fig. 2.4). The conductivity in both Middle Alkali Lake pools was similar on each observation date and ranged from 5000 µS/cm to 20,000 µS/cm. Middle Alkali pool A dried approximately 30 days before pool B. Just before pool B dried, a decrease in conductivity was observed (Fig. 2.4), which may have been related to the crystallization of salts along the edges of the pool. Interestingly, the conductivity in the Goose Lake pool was lower and remained below 1000 µS/cm until the last week before it dried out completed when the conductivity rose to approximately 5000 µS/cm (Fig. 2.4).

At ice-out (21 March 2013), juveniles and adults of the anostracan *Branchinecta mackini* Dexter, 1956 were present in all pools, which persisted until 6 May 2013. A second cohort of *B. mackini* appeared on 14 May 2013 in Middle Alkali Lake pool B, and persisted until 30 May 2013. *L. lemmoni* was first recorded in the Goose Lake pool on 23 April 2013, after which individuals were present until the pool dried on 14 May 2013, (Fig. 2.5). The *L. lemmoni* were large when first observed, with carapace lengths (CL) of 6.8-16.0 mm (Fig. 2.6). It is unlikely that these individuals hatched and grew to this size in less than one week.

This suggests that the sampling procedures were insufficient to collect smaller *L. lemmoni*, and hatching occurred at an earlier, unknown date. The smallest mature female (determined by presence of ovisacs) had a CL of 11.4 mm. A single *L. lemmoni* with a carapace length of 3.7 mm was observed in Middle Alkali Lake pool B on 14 May 2013. No other *L. lemmoni* were observed in either Middle Alkali Lake pool before or after this date.

Laboratory Common Garden Study

The first *L. lemmoni* were observed in the Goose and Dowling pools 17 days after inundation. No hatching of *L. lemmoni* was observed in pools containing sediments from Edwards AFB. Growth of *L. lemmoni* from Goose Lake and Dowling Lake (Fig. 2.7) were both well described by sigmoidal curves. Based on sum of squares analysis, the growth curves of each lake were best described with separate rather than combined curves (Table 2.1). The smallest gravid female observed was 10.53 mm CL, which roughly corresponded to the boundary between the exponential and transitional stages for both Goose Lake and Dowling Lake growth curves. The growth of Goose Lake *L. lemmoni* lagged substantially behind the growth of Dowling Lake *L. lemmoni*, especially during the exponential growth stage. Assuming earliest sexual maturity at a carapace length of 10.53 mm based on Goose Lake data, Dowling Lake *L. lemmoni* reached maturity much sooner than Goose Lake *L. lemmoni* (58.6 vs 70.6 days post inundation, based on growth curves). Despite this difference, the two populations were nearly identical during the lag and stationary phases of their respective growth curves achieving a carapace length of 18 mm (Fig 2.7).

Sexual dimorphism in carapace shape was readily visible in *L. lemmoni* (Fig. 2.8). The carapace length: width ratio of immature Goose Lake *L. lemmoni* (CL=1.60-10.47 mm) averaged 1.01±0.08 (SD). The smallest individual with clear female carapace morphology was 7.4 mm CL, though individuals as large as 10.5 mm CL with ambiguous carapace morphology occurred. Males and females had an average carapace length: width of 1.06±0.06 and 1.16±0.09, respectively. The carapace length: width ratio differed significantly (ANOVA $F_{1, 2} = 31.13$, $P<0.001$) between immature, male, and female *L. lemmoni*. A Fisher's LSD showed that all three groups differed from each other (Fig. 2.9). The relationship between carapace length and width in females, males, and immatures were all well described with linear regressions (fig 2.10). Based on the 95% confidence intervals of the slopes (Table 2.2), the slope of the immature line differed significantly from 1, so the carapace length: width ratio increased with growth in immature *L. lemmoni*. The slope was not significantly different from 1 in females and males, though the female slope showed a non-significant positive trend (Table 2.2).

Discussion

In the outdoor mesocosm study, Goose Lake *L. lemmoni* was first noted in late April. Because of their large size when they were first noted, hatching must have occurred much earlier, probably sometime in March. The large amount of dead plant material that fell into the pools obscured smaller *L. lemmoni* and likely prevented their detection earlier. The majority of Goose Lake *L. lemmoni* did not reach sexual maturity until the last three weeks of the hydroperiod, greatly limiting their reproductive output. In these mesocosms, the drainage area/lake area ratio was 1.0. A pool with a larger drainage area would have received more water, which in turn would have taken longer to evaporate. While the Goose Lake *L. lemmoni* were barely able to complete their life cycle in the outdoor mesocosm before the pool dried out, natural populations living in lakes with larger drainage areas would have more time to reproduce. However, it does demonstrate that *L. lemmoni* can complete one life cycle in a very compressed period. This is what likely allows them to survive in lakes and pools of varying sizes widely distributed throughout their range.

Brown and Carpelan (1971) found that *B. mackini* eggs stopped hatching above salinities of 1‰, or approximately 1968 µS/cm. Because *B. mackini* was able to hatch in the Middle Alkali Lake pools, and *L. lemmoni* largely was not, it can be inferred that *L. lemmoni* requires lower conductivities than *B. mackini* for hatching (i.e., - less than 1‰ or 1968 µS/cm). The conductivity of the pools increased with time (Fig. 2.4), resulting in an inverse relationship between conductivity and the amount of time that water will remain in the pool. Therefore, conductivity may be a cue for the hatching of the eggs of *B. mackini* and *L. lemmoni* because conductivity changes in relation to the amount of water in a pool, and thus its hydroperiod (Prophet, 1963). In the outdoor mesocosm pools, *B. mackini* had reached sexual maturity by 21 March, while the first gravid *L. lemmoni* was not observed until May 1. Because *L. lemmoni* took longer to reach sexual maturity than *B. mackini*, and a low conductivity forecasts a long hydroperiod, it follows that *L. lemmoni* eggs may require a lower conductivity to hatch than *B. mackini*.

In the Middle Alkali pools, there was a second hatching of *B. mackini* and the single *L. lemmoni* hatching recorded on 14 May 2013. The conductivity was 21140 µS/cm on 14 May 2013, well above the 1‰ threshold discussed above, and therefore hatching of *B. mackini* and *L. lemmoni* should have been inhibited. However, this hatching event followed a light rainfall (0.127 cm) that occurred the day before, which may have formed a thin lens of water with a lower conductivity on top of the saline water allowing eggs to hatch. If this was the case, it would indicate that timing of egg hatching responds quickly to changes in conductivity. This response timing should be explored further in relation to temperature, and location, as it may offer insights into population response in different parts of the animal's range.

In the laboratory common garden study, growth of Goose Lake and Dowling Lake *L. lemmoni* could not be explained with the same growth curve, but both showed the same pattern of fast early growth that slowed after sexual maturity. This pattern is similar to that found in *L. packardi* (Ahl, 1991), *L. apus* (Cvetković-Miličić and Petrov, 1999), and *T. longicaudatus* (Scott and Grigarick, 1978), but not *T. granarius* (Seaman *et al.*, 1991). The size at sexual maturity was also similar to *L. packardi* and *T. longicaudatus*, with minimum sizes at sexual maturity of 10 mm and 10.26 mm carapace length, respectively (Ahl, 1991; Fry-O'Brien and Mulla, 1996). *L. lemmoni* did mature at a much larger size than *T. granarius,* which matures at a CL of 6.5 mm (Seaman *et al.,* 1991). These data suggest that *L. lemmoni* is not an unusual member of the genus with respect to size at maturity or growth patterns.

L. lemmoni grew slowly, reaching sexual maturity 8 and 10 weeks after inundation in Dowling and Goose lakes, respectively. This explains the success of *L. lemmoni* in habitats such as large playas with reliably long hydroperiods (Rogers and Hill, 2013). The growth of Goose Lake *L. lemmoni* lagged substantially behind the growth of Dowling Lake *L. lemmoni*, especially during the exponential growth stage. It may be that the faster growth to maturity in Dowling Lake *L. lemmoni* is an adaptation to the temporary nature of Dowling Lake (Vermeer, 1970), versus the semi-permanent status of Goose Lake, or an adaptation to the shorter growing season in Alberta versus that in Northern California. However, the lack of a population from the extreme southern end of the species' range in this study makes it difficult to draw firm conclusions about the effect of latitude on growth rate. A repeat of this study including a southern population would clarify potential latitudinal patterns. Also, the addition of a central population from a temporary habitat, more similar to northern and southern habitats, would take into consideration any confounding effect the semi-permanent nature of Goose Lake may have had on this study. The observed minimum size at sexual maturity coincides with the change from exponential phase to transitional phase in the growth curves of both Dowling Lake and Goose Lake *L. lemmoni*. This suggests that reproduction incurs a substantial metabolic cost, energy which otherwise could go towards growth.

This study represents the first description of the development of sexual dimorphism in the Notostraca meaning that *L. lemmoni* cannot be compared to other Notostraca in this regard. However, because *L. lemmoni* is so similar to other Notostraca in regard to size at maturity and shape of the growth curve, it is likely that other gonochoristic Notostraca have similar patterns of development in sexual dimorphism. Lynch (1966) found sexual dimorphism in total size, female *L. lemmoni* being 30% larger than males. If this is true, the smaller males may utilize the broader carapace as protection against gape-limited predators, while females are similarly protected by their overall larger size. Alternatively, the narrow carapace of the female may be a modification to allow the male to successfully amplex the female with the modified endites on thoracopod 2. Observations in the outdoor mesocosm study suggested that mating in *L. lemmoni* is performed ventral to ventral, though the brevity of the mating event (< 10 s) and the turbidity of the water made detailed observations difficult. Further study observing the mating of *L. lemmoni* in clear water with visual recording equipment could allow clarification if the role of carapace shape in amplexus and mating.

This study has provided some basic information on the life history of *L. lemmoni*, which can be used to evaluate the potential effect anthropogenic climate change will have on this species. Anthropogenic climate change is predicted to increase the variability in precipitation in the geographic range of *L. lemmoni* (Melack *et al.*, 1997). This, in conjunction with increasing human water use, means that the playas in which *L. lemmoni* occurs may be inundated less frequently, and remain inundated for a shorter period of time. In the short term, *L. lemmoni* may become restricted within its current range to those lakes with the very largest catchment basins, where sufficient water would collect to allow *L. lemmoni* to complete its life cycle. In the long term, anthropogenic climate change may cause the geographic range of *L. lemmoni* to substantially shrink or shift. To ameliorate the effects of climate change on *L. lemmoni*, water managers should manage water withdrawals in the drainage basins of *L. lemmoni* habitat to either allow lakes to hold water for at least 10 weeks, or keep pools dry. A 10-week hydroperiod would allow *L. lemmoni* to complete its life cycle, and resting eggs will remain viable in the dry lakebed in low water years. Thus, *L. lemmoni* should easily survive under these extreme conditions, while repeated years with hydroperiods too short for the completion of *L. lemmoni*'s life cycle would eventually exhaust the egg bank and cause extirpation.

Figures and Tables

Table 2.1 – Parameters of non-linear sigmoidal growth models for *Lepidurus lemmoni* from Dowling and Goose lakes, and a combined model of both lakes. All models follow the form $f=a/(1+\exp(-(x-x_0)/b))$, where f=carapace length; x=days post inundation, and a and b are fitted constants.

Lake	**a**	**b**	**x_0**	**R^2**	**SS**
Dowling	15.94	12.14	50.54	0.92	207.16
Goose	16.09	16.99	59.69	0.90	236.39
Combined Model	15.93	15.07	55.34	0.89	564.66

Table 2.2 – Parameters of linear models of carapace length plotted as a function of carapace width for immature, male, and female *Lepidurus lemmoni*. All models follow the form $f=y_0+ax$, where f=carapace length and x=carapace width, y_0 is the y-intercept, a is the slope coefficient, and R^2 is the coefficient of determination.

Sex	***n***	**y_0 (95%CI)**	***a* (95%CI)**	**R^2**	***P***
Female	20	-0.70 (-4.40, 3.00)	1.22 (0.91, 1.54)	0.89	<0.001
Male	29	1.12 (-0.87, 3.11)	0.97 (0.82, 1.12)	0.87	<0.001
Immature	46	-0.27 (-0.52, -0.02)	1.07 (1.03, 1.11)	0.98	<0.001

Figure 2.1 – Female (left) and male (right) adult *Lepidurus lemmoni* reared from Middle Alkali Lake sediments. Photos by author.

Figure 2.2 – Set up of common garden experiment for rearing of *L. lemmoni* on the University of Idaho Campus in Moscow, Idaho. Each pool was weighed down with a concrete block to prevent it from blowing away in the wind.

Figure 2.3 – Temperature controlled set-up used to culture of *Lepidurus lemmoni* in the laboratory common garden study. A 120 cm plastic wading pool was floated in a 1000 L fiberglass tank through which temperature-controlled water was passed to maintain a constant water temperature. Gentle aeration was provided via an airstone. Turbidity resulted from the suspension of very fine sediment particles when the sediments were wetted, and are reflective of conditions in the native lake habitats which were simulated in this experiment

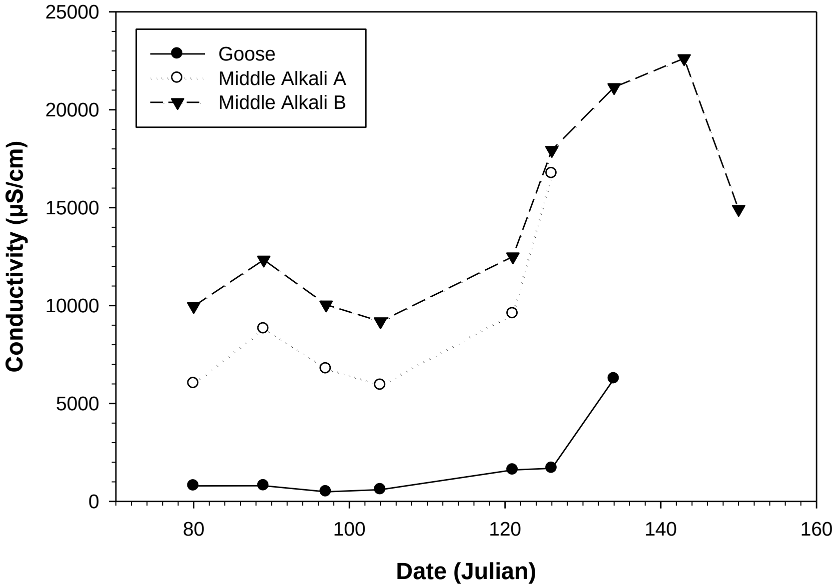

Figure 2.4 – Conductivity as a function of time in three outdoor mesocosms during the 2013 hydroperiod.

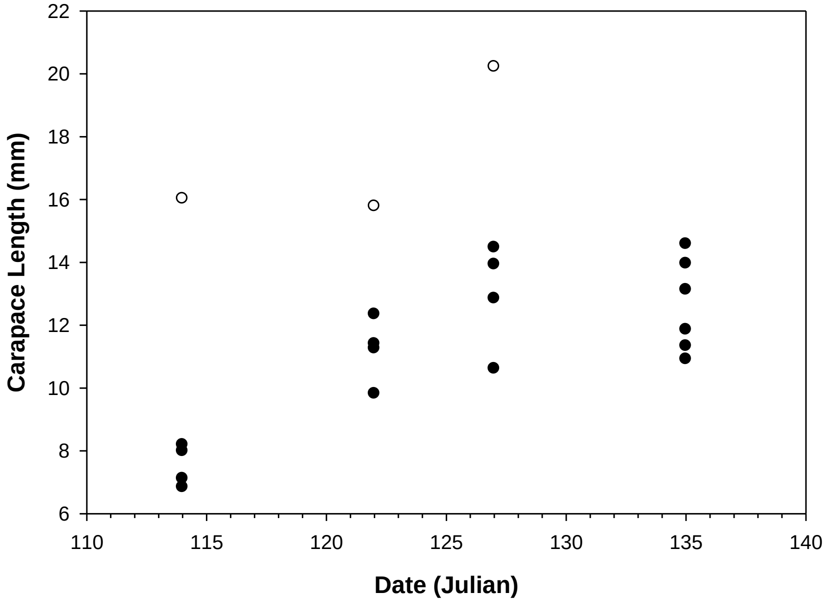

Figure 2.5 – Carapace length as a function of time for *Lepidurus lemmoni* hatched from Goose Lake sediments in the outdoor mesocosms. The empty circles represent a very large specimen that probably hatched several weeks prior to the main cohort.

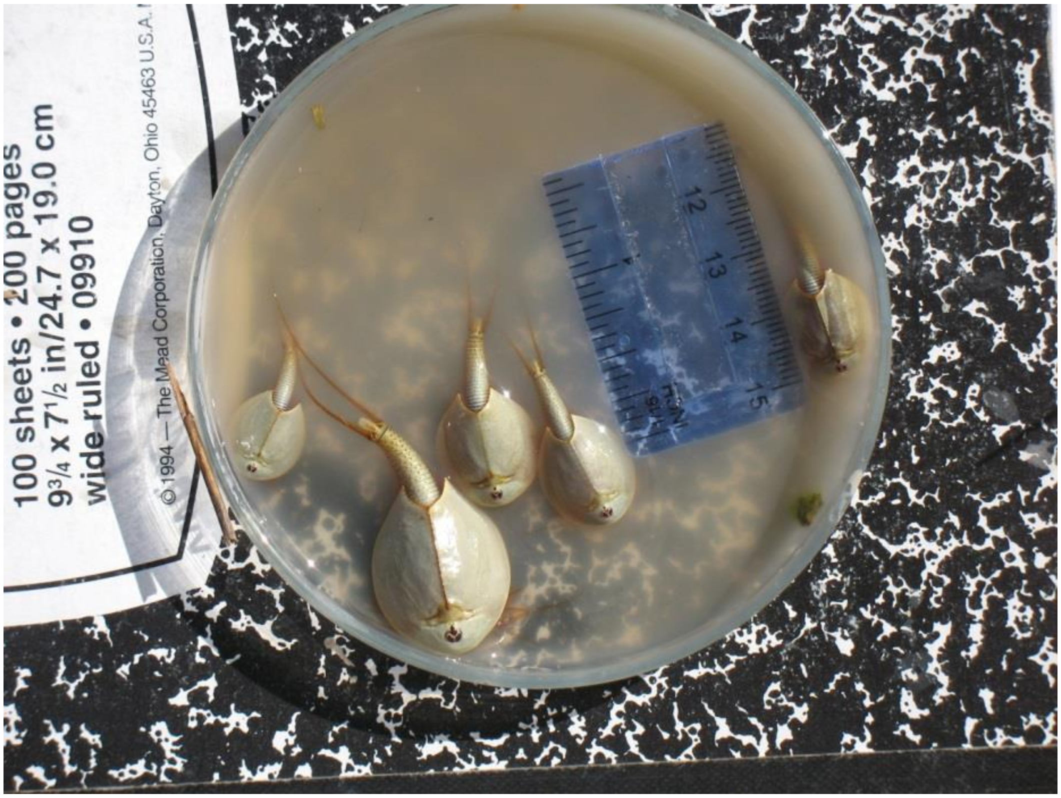

Figure 2.6 – Photograph of individuals of *Lepidurus lemmoni* on 6 May 2013 hatched from Goose Lake sediments during the outdoor mesocosm study. Given the disparity in size of the individual at 7 o'clock relative to the others, it likely hatched several weeks before the other individuals in the dish.

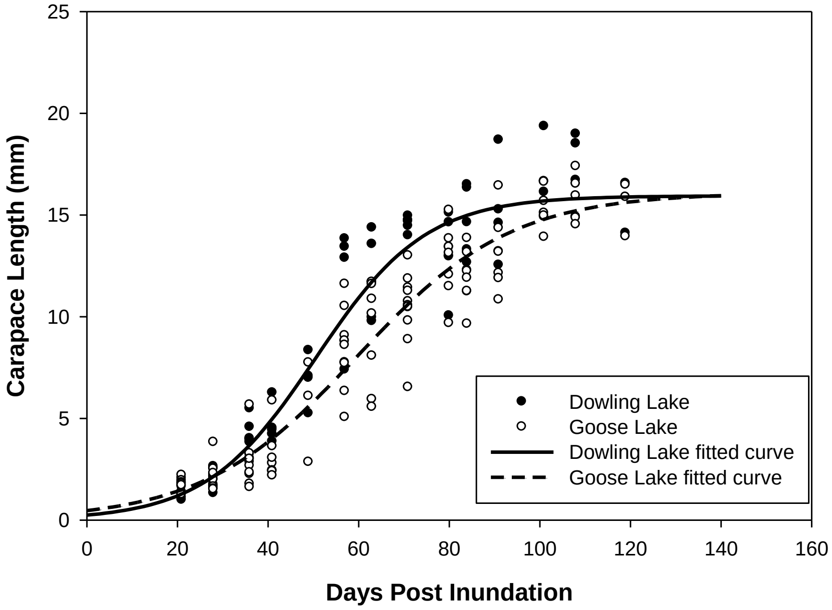

Figure 2.7 – Carapace length as a function of time of *Lepidurus lemmoni* from Dowling and Goose lakes during the laboratory common garden study. All models follow the form $f=a/(1+\exp(-(x-x_0)/b))$. See Table 2.1 for model parameters.

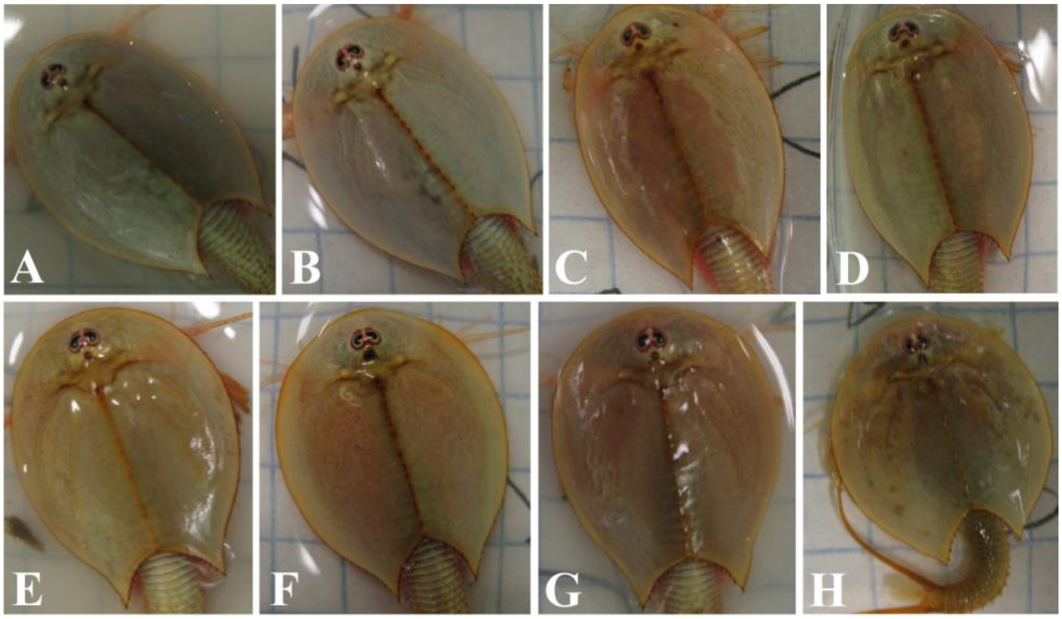

Figure 2.8 - Examples of sexual dimorphism in carapace shape in *Lepidurus lemmoni*. Females (A-D) have a higher carapace length: width ratio than males (E-H). Carapace lengths (CL) and length: width ratios (L:W) are: (A) CL=14.9mm, L:W=1.10;(B) CL=16.5mm, L:W=1.18; (C) CL=16.6mm, L:W=1.25; (D) CL=16.5mm, L:W=1.40; (E) CL=16.6mm, L:W=1.10; (F) CL=16.5mm, L:W=1.04; (G) CL=15.7, L:W=0.95; (H) CL=13.9, L:W= 0.93.

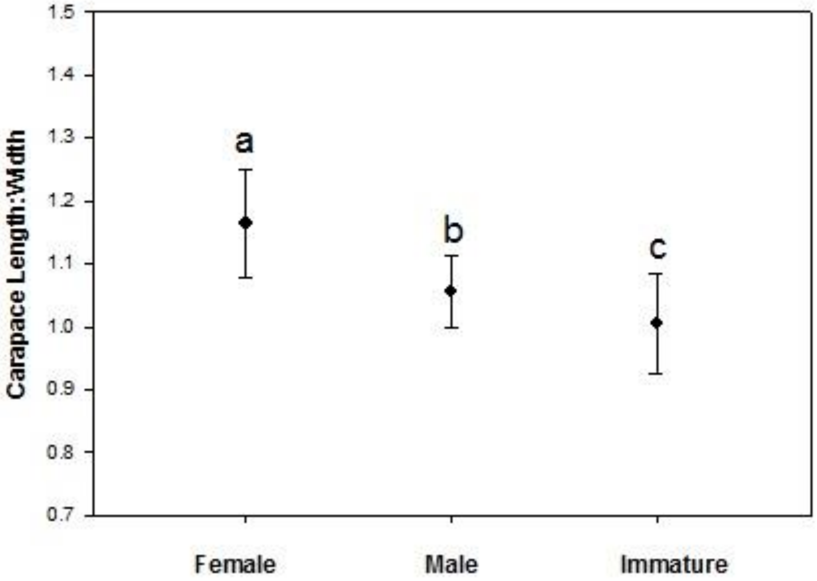

Figure 2.9 – Plots of mean ± SD comparing carapace length: width ratios between immature, male, and female *Lepidurus lemmoni*. Letters indicate life stages that differ from each other in this metric.

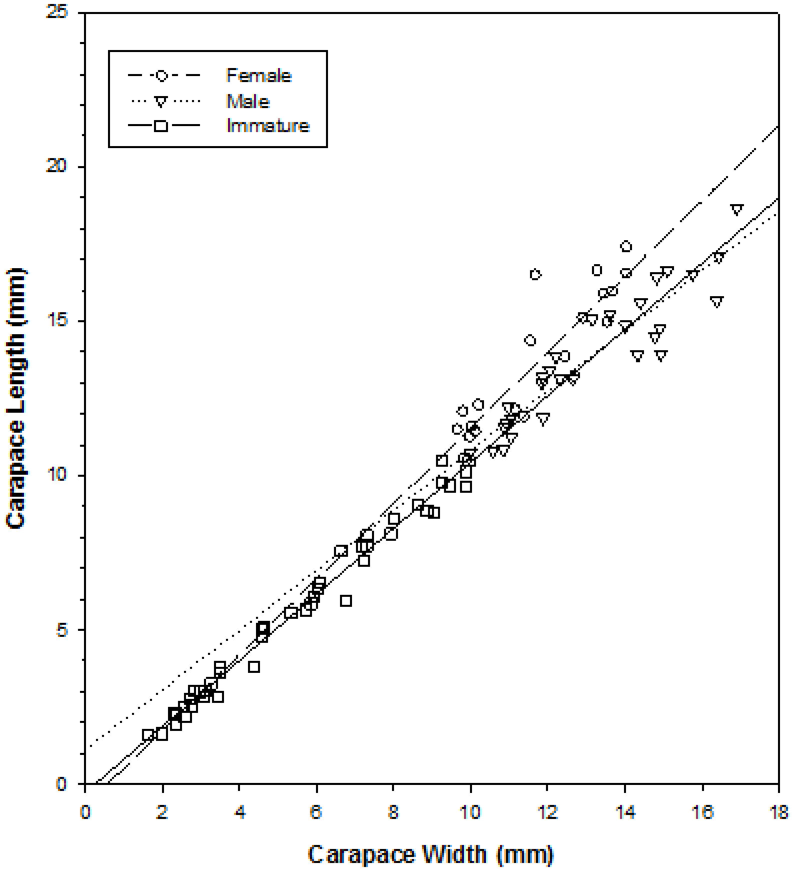

Figure 2.10 - Carapace length plotted as a function of carapace width for immature, male, and female *Lepidurus lemmoni*.

Chapter 3: Intrinsic and Extrinsic Factors Affecting Egg Hatch in *Lepidurus lemmoni*

Introduction

Lepidurus lemmoni Holmes, 1894 is a primitive crustacean typical of large, temporary, alkaline lakes in western North America. It has been recorded from Alberta, Montana, Washington, Oregon, California, Wyoming, Nevada, New Mexico, Arizona, and Baja California (Rogers, 2001). It is especially common in the Great Basin and Mojave Desert (Rogers and Hill, 2013).

The astatic (temporary) waters of the Great Basin and Mojave Desert, like the habitats of most non-cladoceran Branchiopoda, are variable and unpredictable in their occurrence and duration of inundation. This uncertainty is considered the most important factor determining the life history of species that inhabit astatic waters (Brendonck, 1996). To maximize the likelihood of reaching sexual maturity and reproducing, *L. lemmoni* should hatch only if a pool has filled sufficiently to ensure its persistence until the life cycle is completed. Thus, it would be advantageous for the eggs of *L. lemmoni* to hatch in response to low temperatures and salinity, because this combination should forecast a sufficiently long hydroperiod to allow completion of the life cycle in the Great Basin, where the majority of annual precipitation occurs in winter (Grayson, 2011). Two Anostraca, *Branchinecta mackini* Dexter, 1956 and *Branchinecta gigas* Lynch, 1937, that invariably co-occur with *L. lemmoni* follow this pattern by hatching soon after pools fill when temperature and salinity are low (Broch, 1988).

While the unpredictable nature of the habitat is undoubtedly a primary driver of the life history of *L. lemmoni*, competition or predation may also play a role. *Branchinecta gigas*, a giant (up to 160 mm) predatory fairy shrimp that co-occurs with *L. lemmoni,* feeds heavily on *B. mackini* and calanoid copepods (Lynch, 1937; Daborn, 1975), which are important prey items for *L. lemmoni* (Lynch, 1966). *B. gigas* may compete with *L. lemmoni* for food, and therefore it would be advantageous for *L. lemmoni* to minimize temporal co-occurrence with *B. gigas. B. gigas* hatches soon after pools fill with water, and die out relatively early (Broch, 1988). *L. lemmoni* could avoid competition with *B. gigas* by hatching later in the season. If this is the case, *L. lemmoni* eggs would hatch in response to high temperatures and salinity, because this combination should forecast the absence of *B. gigas.* In addition, *B. gigas* has been observed to feed on immature *L. lemmoni* (D.C. Rogers, Kansas Biological Survey, pers. comm.), thus *L. lemmoni* could avoid predation by *B. gigas* by hatching later in the season, again mediated by high temperature and salinity. This may explain why *L. lemmoni* is found mostly in very large, long-lasting pools (Rogers and Hill, 2013), where the hydroperiod may be long enough for *L. lemmoni* to complete its life cycle after *B. gigas* has died out for the year.

Like other Notostraca, *L. lemmoni* inhabits relatively unpredictable habitats strongly influenced by variations in weather and climate, so a temperature- and salinity-driven life cycle as seen in other Notostraca would be advantageous and allow *L. lemmoni* populations to survive variable conditions. This, coupled with their ease of dispersal in the resting egg stage (Fryer, 1996), suggests that life history traits such as hatching time, growth rate, and time to sexual maturity do not vary by latitude, though a certain amount of plasticity in these traits may exist. However, the anostracan *Branchinecta lindahli* Packard, 1883 shows a negative correlation between ideal hatching temperature and latitude (Rogers, 2015), and *L. lemmoni* may show a similar pattern.

For *L. lemmoni,* nothing is known about the timing of hatching, and no laboratory studies examining the relationship between temperature, salinity, or intrinsic factors (egg diameter and embryo mass) and hatching rates have been completed. However, two other species of *Lepidurus* have been studied in this regard. Kuller and Gasith (1996) reported optimal hatching temperatures of 16-20°C for *Lepidurus apus lubbocki* Brauer, 1873 from Israel, with hatching occurring on day 5 at 20°C and day 12 at 10°C. Hann and Lonsberry (1991) reported an optimal temperature of 20°C for the hatching of *Lepidurus couesii* Packard, 1875 eggs from Manitoba, with overall hatching success peaking at 78%. This study also found that hatching began three days after inundation at 20°C, but not until day five at 17°C. However, Braswell (1963) reported an optimal hatching temperature of 10°C for *L. couesii* from northern Utah. The reason for the large difference in optimal hatching temperature between these two populations of *L. couesii* is unknown. Given the control of temperature on the hatching (Ahl, 1991; Braswell, 1963; Hann and Lonsberry, 1991; Scott and Grigarick, 1979) and growth rates (Fry-O'Brien and Mulla, 1996) of other Notostraca, it is reasonable to hypothesize a similar influence on the life history characteristics of *L. lemmoni*.

Variation in egg size is one way that invertebrates adapt to different habitat temperatures (Wilhelm and Schindler, 2000; Mura, 1991), predictability (Mura, 1991), and predation pressures (Belk, 1977). Among the Branchiopoda, egg size varies in the Anostraca in relation to habitat parameters. For example, Mura (1991) found no correlation between female size and egg diameter in *Chirocephalus diaphanus* Prévost, 1803, but strong positive correlation between egg diameter and habitat predictability and altitude. A similar correlation between altitude and both egg diameter and mass were reported for the freshwater amphipod *Gammarus lacustris* Sars, 1863 by Wilhelm and Schindler (2000). Variation of egg size in relation to habitat parameters has not been studied within the Notostraca. With its broad latitudinal range, *L. lemmoni* is a good candidate for such research to explore potential egg size – latitude relationships.

Development time responds inversely to temperature in other aquatic Crustacea (Wilhelm and Schindler, 2000). Longer development times require more energy to maintain the embryo, so eggs require more energy to hatch in cold environments than in warm environments. The northern extreme of *L. lemmoni*'s range (central Canada) is colder than the southern extreme (southern California), so the northern populations likely face longer development times, and therefore require more energy to hatch, and should then have larger and heavier eggs to accommodate the necessary energy stores. Conversely, southern populations of *L. lemmoni* should have small eggs. Warmer temperatures allow faster development, which requires less energy, and therefore successful hatching can be achieved with smaller eggs. It is therefore expected that egg diameter and embryo mass in *L. lemmoni* varies directly with latitude.

If playa hydroperiod length is a critical factor shaping phenology in *L. lemmoni*, then it would be expected that its eggs should ideally hatch at low temperature and salinity.

However, *L. lemmoni* inhabits only the largest pools, so the duration of inundation of a pool may not be the critical factor shaping its life history, and other processes such as interspecific competition with *B. gigas* may be more important. If the phenology of *L. lemmoni* is driven by interspecific competition with *B. gigas*, then it would be expected that its eggs would preferentially hatch at high temperature and salinity.

In this study, I determined the optimum temperature and salinity conditions of *L. lemmoni*, to test the hypothesis that populations from different latitudes differ in optimum hatching temperature and salinity as an adaptation to local conditions. I also tested the hypothesis that egg size and embryo mass varies with latitude as an adaptation to local conditions.

Methods

To test hypotheses related to the influence of temperature, salinity, egg diameter, and embryo mass on hatching success, populations of *L. lemmoni* representing the north (Dowling Lake, 51° 45’ 04”N 112° 04’ 58”W, AB, Canada) and south (Edwards Air Force Base, 34° 50’ 20”N 117° 55’ 01”W, CA, USA) extremes, as well as the center of the latitudinal range (Goose Lake, 41° 46’ 30”N 120° 27’ 44”W, CA, USA and Middle Alkali Lake, 41°31’53”N 120°07’28"W) were selected for experiments. Dry lakebed sediments collected from each location were rinsed through a 180-µm-mesh sieve with tap water, after which the contents were examined with the aid of a dissecting microscope for eggs of *L. lemmoni* which were removed with forceps. The eggs of *L. lemmoni* were distinguished from the eggs of *B. mackini* and *B. gigas* based on characters described in Hill and Shepard (1997) (Fig. 3.1). The sediment was processed such that all eggs picked were wetted for no more than three hours, to prevent premature initiation of the hatching process. Eggs removed from sediments were dried at room temperature for 24 hours, after which the diameter of a subset of 30 randomly selected eggs was measured with the aid of a dissecting microscope equipped with a calibrated ocular micrometer. After drying, eggs from each lake were stored individually in 1 mL glass shell vials stoppered with 1 cm cubes of 100% polyurethane foam at -25°C for two weeks until the beginning of the experiment.

To test the hypothesis that timing of hatching is related to salinity required knowledge of the ionic composition of water in pools with *L. lemmoni*. Because such data were not available, I based the composition on data from an astatic lake in Lower Grand Coulee, Grant County, Washington which contained *B. mackini* and *B. gigas* (Broch, 1988), which often co-occur with *L. lemmoni* (Eriksen and Belk, 1999). A 10 ppt stock solution containing the ions reported from Lower Grand Coulee (Table 3.1) was mixed 48 hours before the beginning of the experiment. The stock solution was diluted with distilled water to create treatments of 0.0, 0.1, 0.4, and 0.8 ppt, triplicates of which were set up by adding 1.0 L of salinity treatment water to 2 L plastic incubation chambers (ZipLoc™, Racine, WI). To allow air flow, while minimizing evaporation, four holes approximately 2 mm in diameter each were made in the lids of the incubation chambers. Incubation chambers were then partially submerged in water baths at 8.0, 13.5, and 19.0°C (Fig. 3.2). Temperatures were allowed to equalize for 24 h before beginning the experiment.

To start the experiment, each vial containing one egg was filled with water from the randomly assigned salinity and temperature treatment with a syringe. Three replicates of five eggs from each lake were placed in polyethylene cups, which were then submerged into the incubation chamber in each of the three temperature baths and weighted with color-coded marbles to identify replicates. Temperature of the water baths was recorded daily with an electronic thermometer, and each vial was individually examined with a 15x hand lens to determine if eggs had hatched. Any hatched individual was removed and preserved in 70% EtOH. The experiment continued for 28 days, or until no eggs hatched from the treatment for five days. At the end of the experiment, all unhatched eggs were examined under a dissecting microscope to assess viability which was tested by applying gentle pressure to each egg with forceps. Viable eggs resisted pressure due to the presence of an intact chorion and embryo, while nonviable eggs collapsed easily. The diameter of each viable egg was measured with the aid of a microscope with a calibrated ocular micrometer. Eggs in which the embryo had partially hatched, but failed to escape the prenaupliar envelope and rotted, were counted as dead.

To determine if egg mass varied between populations, 30 randomly selected eggs from each lake were decapsulated using a method adapted from Sorgeloos *et al.* (1977). The eggs were soaked in deionized water for 1 h, then transferred to a 2% hypochlorite bleach solution until all of the chorion had dissolved, approximately 7-15 minutes. Decapsulated embryos were rinsed thoroughly in deionized water, then placed in aluminum weigh boats and dried to constant mass at 65°C for 24 h. The mass of dried eggs was determined on a Mettler Toledo AT21 Comparator electronic balance to the nearest µg after eggs had cooled in a desiccator for 1 h.

Mean diameter of dry and wet eggs among populations was analyzed using a one-way analysis of variance (ANOVA), in which egg diameter was the response variable and population of origin was the treatment variable. Separate analyses were run for wet or dry eggs. Because the ANOVAs of dry and wet egg diameters among populations indicated no among-population difference, I combined all dry diameter data and used a two-sample t-test with unpooled variances to examine if it differed from the pooled wet diameters. Mean embryo mass among populations was analyzed with a one-way ANOVA, in which embryo mass was the response variable, and population was the treatment variable. Average hatching time was analyzed using a two-way ANOVA, in which average hatching time was the response variable, and temperature and salinity were the treatment variables. Separate analyses were run for each population. Hatching time was not significantly related to salinity, so analysis was focused on the effects of population and temperature on hatching time. Average hatching time was analyzed using a one-way ANOVA, in which average hatching time was the response variable, and temperature and population were the treatment variables. Cumulative hatching success was analyzed using a two-way ANOVA, in which cumulative hatching success was the response variable, and temperature and salinity were the treatment variables. Separate analyses were run for each population. Egg mortality was analyzed using a one-way ANOVA, in which egg mortality was the response variable, and population was the treatment variable. Egg mortality in each population was analyzed using a two-way ANOVA, in which egg mortality was the response variable, and temperature and salinity were the treatment variables.

In all analyses, the data and residuals were checked for normality and homogeneity of variance to ensure that test assumptions were met. Data which failed to meet assumptions of ANOVA were $\log_{10}$-transformed and rechecked. Data that continued to violate assumptions were analyzed with a Kruskal -Wallis rank sum test in place of one-way ANOVA. Significant ANOVA tests were followed by Fisher's protected Least Significant Difference tests to detect means that differed (Ott and Longnecker, 2010). Significant Kruskal-Wallis tests were followed by Mann-Whitney tests with Bonferroni correction and approximate p (Ott and Longnecker, 2010) to detect means that differed. In Dowling Lake egg mortality, the main effects (Fig. 3.3) showed disordinal interaction, so no analysis of Dowling Lake egg mortality was done. Goose Lake egg mortality data violated homogeneity and normality of error assumptions, which $\log_{10}$ and square root transformations did not fix, so no analysis of Goose Lake egg mortality was done.

Results

No *L. lemmoni* eggs were found in 20 kg of sediment collected from four different locations on Edwards Air Force Base, despite the remains of adult *L. lemmoni* being present from samples at three locations. Soil collected from other potential southern *L. lemmoni* habitats (Silurian Lake, Silver Lake, Soda Lake, and an unnamed lake South of Boulder City, NV) yielded no Notostracan eggs (Soda Lake), or only *T. longicaudatus* eggs (Unnamed, Silurian, and Silver lakes).

Egg Diameter and Embryo Mass

The dried eggs of *L. lemmoni* were spherical, orange-brown, and unornamented (Fig. 3.4). However, embryos from Dowling Lake and Middle Alkali Lake *L. lemmoni* were almost uniformly bright orange, while the embryos of Goose Lake *L. lemmoni* were mostly white (Fig. 3.5). Egg diameter of dry eggs ranged from a mean of 370 µm ± 13.4 (mean ± SD) in Middle Alkali Lake to 374.2 µm ± 19.4 in Goose Lake, while the diameter of wet eggs ranged from 425.4 µm ±16.2 in Middle Alkali Lake to 430.6 µm ± 24.5 in Goose Lake. The diameter of dry and wet eggs did not differ among lakes ($\text{ANOVA}_{\text{dry}}$, $F_{2,1}= 0.6$, $p=0.551$; $\text{ANOVA}_{\text{wet}}$, $F_{2,1}= 1.688$, $p=0.187$). However, the average diameter of wet eggs (428.3 µm ± 19.9) was significantly (t-test, $t = -28.0$, df = 200.3, $n = 359$, $p<0.001$) larger than dry eggs (372.4 µm, ±15.0). Embryo mass ranged from a mean of 15.2 µg ± 0.1 in Middle Alkali Lake to 15.5 µg ± 0.7 in Dowling Lake. Embryo mass did not differ among lakes (ANOVA, $F_2= 0.471$, $p=0.646$). Middle Alkali Lake embryos showed greater variation in mass than those from Goose and Dowling lakes (Fig. 3.6).

Average Hatching Day

Eggs from all lakes hatched in all incubation treatments (temperature and salinity) in the hatching experiment, with the average hatching day ranging from 6.6 to 19.3. Average hatching day of eggs of *L. lemmoni* was significantly (ANOVA, $F_2=26.23$, $p<0.001$) affected by population, with average hatching days of 8.9, 14.3, and 13.3 in eggs from Dowling, Goose, and Middle Alkali lakes, respectively. Average hatching day of eggs of *L. lemmoni* was significantly (ANOVA, $F_2=61.56$, $p<0.001$) affected by temperature, with average hatching days of 17.5, 10.5, and 6.9 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively. Average hatching day of eggs of *L. lemmoni* was not significantly (ANOVA, $F_3=1.383$, $p=0.25$) affected by salinity, with average hatching days of 13.9, 11.2, 11.6, and 12.9 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively. No significant (ANOVA, $F_{2, 3}=0.504$, $p=0.805$) interaction between temperature and salinity existed.

Average hatching day of eggs of *L. lemmoni* from Dowling Lake was significantly (ANOVA, $F_{2, 3}=13.679$, $p<0.001$) affected by temperature, with average hatching days of 17.8, 9.1, and 6.6 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively (Fig. 3.7). Average hatching day of eggs of *L. lemmoni* from Dowling Lake was not significantly (ANOVA, $F_{2,3}=1.164$, $p=0.333$) affected by salinity, with average hatching days of 10.4, 7.7, 8.6, and 9.5 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively (Fig. 3.8). No significant (ANOVA, $F_{2, 3}=0.189$, $p=0.965$) interaction between temperature and salinity existed.

Average hatching day of eggs of *L. lemmoni* from Goose Lake was significantly (ANOVA, $F_{2, 3}=18.697$, $p<0.001$) affected by temperature, with average hatching days of 17.8, 12.1, and 7.0 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively (Fig. 3.7). Average hatching day of eggs of *L. lemmoni* from Goose Lake was not significantly (ANOVA, $F_{2,3}=2.556$ $p=0.063$) affected by salinity, with average hatching days of 16.0, 12.5, 13.8, and 15.8 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively (Fig. 3.8). No significant (ANOVA, $F_{2, 3}=2.267$, $p=0.058$) interaction between temperature and salinity existed.

Average hatching day of eggs of *L. lemmoni* from Middle Alkali Lake was significantly (ANOVA, $F_{2,3}$=27.610, $p<0.001$) affected by temperature, with average hatching days of 16.9, 10.0, and 8.7 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively (Fig 3.7). Average hatching day of eggs of *L. lemmoni* from Middle Alkali Lake was significantly (ANOVA, $F_{2,3}$=3.825, $p=0.023$) affected by salinity, (Fig. 3.8), with average hatching days of 19.3, 13.2, 12.1, and 11.9 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively (Fig. 3.8). No significant (ANOVA, $F_{2,3}$=2.508, $p=0.084$) interaction between temperature and salinity existed.

Cumulative Hatch

Cumulative egg hatch ranged from 0.05 to 0.96. Temperature significantly affected cumulative hatch in all lakes, but salinity was significant only in Goose Lake *L. lemmoni.* Cumulative egg hatch of *L. lemmoni* from Dowling Lake was significantly (ANOVA, $F_{2,3}$=22.750, $p<0.001$) affected by temperature, with cumulative egg hatch of 0.08, 0.57, and 0.37 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively (Fig. 3.9). Cumulative egg hatch of *L. lemmoni* from Dowling Lake was not significantly (ANOVA, $F_{2,3}$=1.369, $p=0.276$) affected by salinity, with cumulative egg hatch of 0.24, 0.33, 0.40, and 0.38 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively (Fig. 3.10). No significant (ANOVA, $F_{2,3}$=1.798, $p=0.142$) interaction between temperature and salinity existed.

Cumulative egg hatch of *L. lemmoni* from Goose Lake was significantly (ANOVA, $F_{2,3}$=35.167, $p<0.001$) affected by temperature, and by salinity (ANOVA, $F_{2,3}$=9.111, *p*<0.001). A significant (ANOVA, $F_{2,3}$=4.611, *p*=0.003) interaction between temperature and salinity existed. The graph of main effects (Fig. 3.11) shows that cumulative hatch was uniformly low (0.18 ± 0.16) at all temperatures at 0.0 ppt. At higher salinities, the cumulative hatch was high (0.69 ± 0.20) at 8.0°C and (0.73 ± 0.20) at 13.5°C, but was very low (0.09 ± 0.11) at 19.0°C. Because of the significant interaction of main effects, further analysis was not carried out.

Cumulative egg hatch of *L. lemmoni* from Middle Alkali Lake was significantly (ANOVA, $F_{2,3}$=5.560, *p*=0.010) affected by temperature, with cumulative egg hatch of 0.27, 0.22, and 0.05 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively (Fig. 3.9). Cumulative egg hatch of *L. lemmoni* from Middle Alkali Lake was not significantly (ANOVA, $F_{2,3}$=1.387, *p*=0.271) affected by salinity, with cumulative egg hatch of 0.09, 0.24, 0.18, and 0.20 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively (Fig. 3.10). No significant ANOVA, $F_{2,3}$=1.987, (*p*=0.107) interaction between temperature and salinity existed.

Egg Mortality

Egg mortality occurred in eggs from all populations, with average egg mortality in all incubation treatments (temperature and salinity) ranging from 0.00 to 0.51. Egg mortality differed significantly (Kruskal-Wallis, χ^2=59.49, *p*<0.001) between lakes (Fig. 3.12), with average egg mortality of 0.12, 0.13, and 0.24 in Dowling, Goose, and Middle Alkali lakes, respectively. There were significant differences between Middle Alkali and Goose lakes

(Mann-Whitney, $p<0.001$, $r=0.74$) and Middle Alkali and Dowling lakes (Mann-Whitney, $p<0.001$, $r=0.75$).

Egg mortality of *L. lemmoni* from Middle Alkali Lake was not significantly (ANOVA, $F_{2,3}=0.302$, $p=0.992$) affected by temperature, with average egg mortalities of 0.42, 0.42, and 0.42 at the treatment temperatures of 8.0, 13.5, and 19.0°C, respectively (Fig. 3.13). Egg mortality of *L. lemmoni* from Middle Alkali Lake was not significantly (ANOVA, $F_{2, 3}$=,11.721 $p<0.631$) affected by salinity, with average egg mortalities of 0.36, 0.40, 0.51, and 0.40 at the treatment salinities of 0, 0.1, 0.4, and 0.8 ppt, respectively (Fig. 3.14). No significant (ANOVA, $F_{2, 3}=0.116$, $p=0.299$) interaction between temperature and salinity existed.

Discussion

The external morphology of *L. lemmoni* eggs is similar to the eggs of *Triops cancriformis* (Longhurst, 1955; Thiéry and Gasc, 1991), *T. longicaudatus* (Scott and Grigarick, 1978), *L. couesii* (Braswell, 1963), and *L. apus* (Thiéry and Gasc, 1991). The variation in embryo color observed between populations in this study has been noted in other Notostraca in which the color of the embryo was due to carotenoid pigments reflective of the maternal diet (Longhurst, 1955). Fox (1949) reported that starved *T. cancriformis* females produced white embryos, while those well-fed produced pink embryos. This suggests that females in the *L. lemmoni* population in Goose Lake may have had a more variable diet than females in the other lakes where more reddish colored eggs were produced. Although egg coloration may indicate a variable maternal diet, this variation did not affect egg hatching or mortality in my experiments. It is unknown if a variable diet of females influences the number of eggs produced. This should be examined in future studies.

The diameter of *L. lemmoni* eggs increased by about 15.2% after wetting which has not been studied in other Notostraca, the closest comparison being in *L. apus* from France, which showed an 8-9% difference between undried and dried eggs (Thiéry and Gasc, 1991). The diameter of dried *L. lemmoni* eggs I measured (372±15 µm) also was significantly smaller than the dried eggs of *L. apus* (474±4.07 µm) (Thiéry and Gasc, 1991) and *L. apus lubbocki* (447±16 µm) (Kuller and Gasith, 1996).

The average diameter of wet *L. lemmoni* eggs I measured were smaller than the 470 µm previously recorded by Lynch (1966), and barely within the range (410-520 µm) he gave. Lynch (1966) measured eggs dissected from the ovisacs of the neotype, so the measurements and range represent that of undried eggs. Based on this, it is likely that the diameter of *L. lemmoni* eggs, and probably those of all Notostraca, are largest when freshly laid, smallest when dried, and larger, though not as large as freshly laid eggs, when rewetted. The role of diameter increase in hatching success is unclear, but likely reflects tissue rehydration. It also indicates the chorion has some elasticity associated with it, allowing water to pass through. It would be interesting to know if swelling related to rehydration is predictive of time of hatching. Future studies on egg morphology in the Notostraca should include diameters of fresh, dried, and rewetted eggs. This will allow more complete comparison between species, and allow for more confident identification of unhatched eggs in all states. Most published data on egg diameter reports only one measurement, and it is not always clear the state of hydration for which measurements are reported.

The inverse relationship between the average date of hatching and temperature observed across the three population of *L. lemmoni* was not unexpected given this is a typical pattern among poikilotherms (e.g., Burden and Hart, 1989; Crisp, 1981). The similarity of average hatching day with temperature among populations suggests that this trait is invariant in *L. lemmoni* at mid and northern latitudes of its range. While average hatching day was delayed by low temperatures, it was not to an extent that would suggest an adaptation to escape competition with *B. gigas.* Juvenile *L. lemmoni* co-occurred with juvenile *B. gigas* in all Dowling Lake and Goose Lake laboratory mesocosms (chapter 2), further suggesting that there is no attempt by *L. lemmoni* to temporally segregate with *B. gigas.* Evidence for any competition between *L. lemmoni* and *B. gigas* is circumstantial, and should be investigated directly. It may be that the benthic habit of *L. lemmoni* and the nektonic habit of *B. gigas* allows for sufficient separation to reduce competition. The patterns of cumulative hatch discovered across temperatures among the populations may represent an adaptive response to higher latitudes, where the lake may be more likely to refreeze early in the season, killing any *L. lemmoni* that hatched early in the season at cold temperatures. At lower latitudes, high temperatures may signal that the habitat will dry soon, and it is therefore disadvantageous to hatch at high temperatures.

The lack of effect of salinity on average hatching day and cumulative hatch in *L. lemmoni* is unexpected, as salinity should indicate pool size more accurately than temperature. Salinity should be inversely correlated with time since inundation, low salinities therefore signaling a long-lasting pool, and should produce a maximum hatch (Prophet, 1963). Unexpectedly, all populations had the lowest cumulative hatch at 0.0ppt salinity, though Goose Lake was the only population where this trend was significant. However, a zero salinity habitat may not be suitable for juvenile or adult survival (Longhurst, 1955), and therefore inhibit hatching. This may be because very low salinity could indicate a permanent body of water which may be unsuitable due to the presence of other predators (e.g., fish).

The relationship between egg mortality and population was significant. The higher egg mortality observed in Middle Alkali Lake *L. lemmoni* may be explainable by the large variance in embryo mass in Middle Alkali Lake, compared to the other two lakes. If embryo mass correlates to available energy stores, as it does in *G. lacustris* (Wilhelm and Schindler, 2000), then a large proportion of Middle Alkali Lake *L. lemmoni* embryos had relatively low energy stores. These low energy stores could translate to many of the embryos being unable to successfully complete hatching. If Middle Alkali Lake eggs did indeed have lower energy stores than the other two lakes, this could explain the high mortality and relatively lower hatching success in Middle Alkali Lake *L. lemmoni*. Further studies examining the embryo total energy and lipid content could clarify if a relationship exists between embryo total energy and hatching success. However, the mortality of Middle Alkali Lake *L. lemmoni* eggs did not vary by temperature, as would be expected if variable energy stores were the cause of the high mortality. While the eggs from all three lakes studied were handled in an identical fashion, some unforeseen or unobserved variation in handling may have damaged Middle

Alkali Lake eggs, and this could be the cause of the high mortality. This hypothesis could be tested by repeating the study with special attention paid to handling of the eggs.

Anthropogenic climate change is predicted to increase the variability in precipitation in the geographic range of *L. lemmoni* (Melack *et al.*, 1997). This means that the lakes in which *L. lemmoni* occurs may be inundated less frequently, and remain inundated for a shorter period of time. Increasing human water use may exacerbate this situation. This increasing unpredictability of its habitat, coupled with the long development time (Chapter 2), means that *L. lemmoni* will reproduce less frequently, and may not be able to maintain populations in all of its current range. This could cause shrinking and/or shifting of the geographic range of *L. lemmoni* in the future.

Figures and Tables

Table 3.1 – Types and amounts of salts used to create artificial lake water for the hatching studies.

Salt	**g/L (for 10ppt stock solution)**
K_2CO_3	0.82
Na_2CO_3	6.34
$NaHCO_3$	1.54
NaCl	1.28
$CaCl_2$ (anhydrous)	0.01
$MgSO_4$ (anhydrous)	0.002

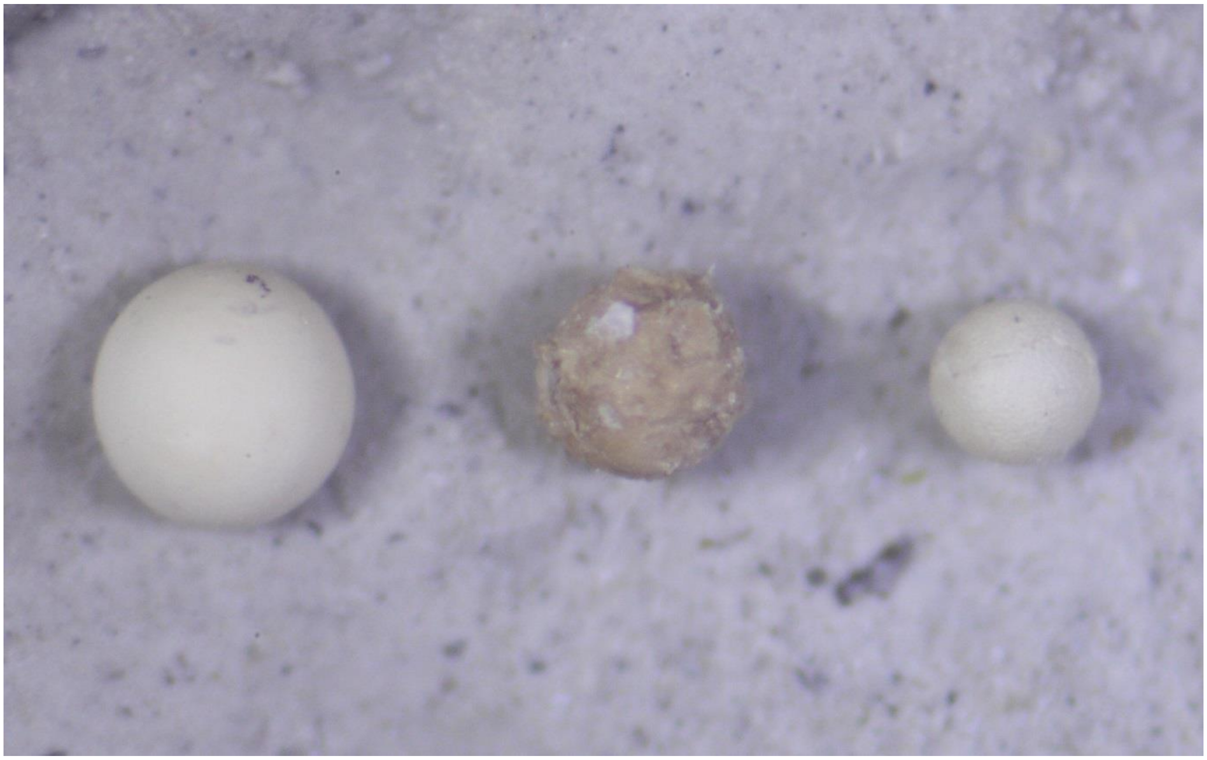

Figure 3.1 – Eggs of *Branchinecta gigas* (left), *Lepidurus lemmoni* (center), and *Branchinecta mackini* (right) in sediment from Middle Alkali Lake. Egg diameters are 488 µm for *B. gigas*, 375 µm for *L. lemmoni*, and 313 µm for *B. mackini*.

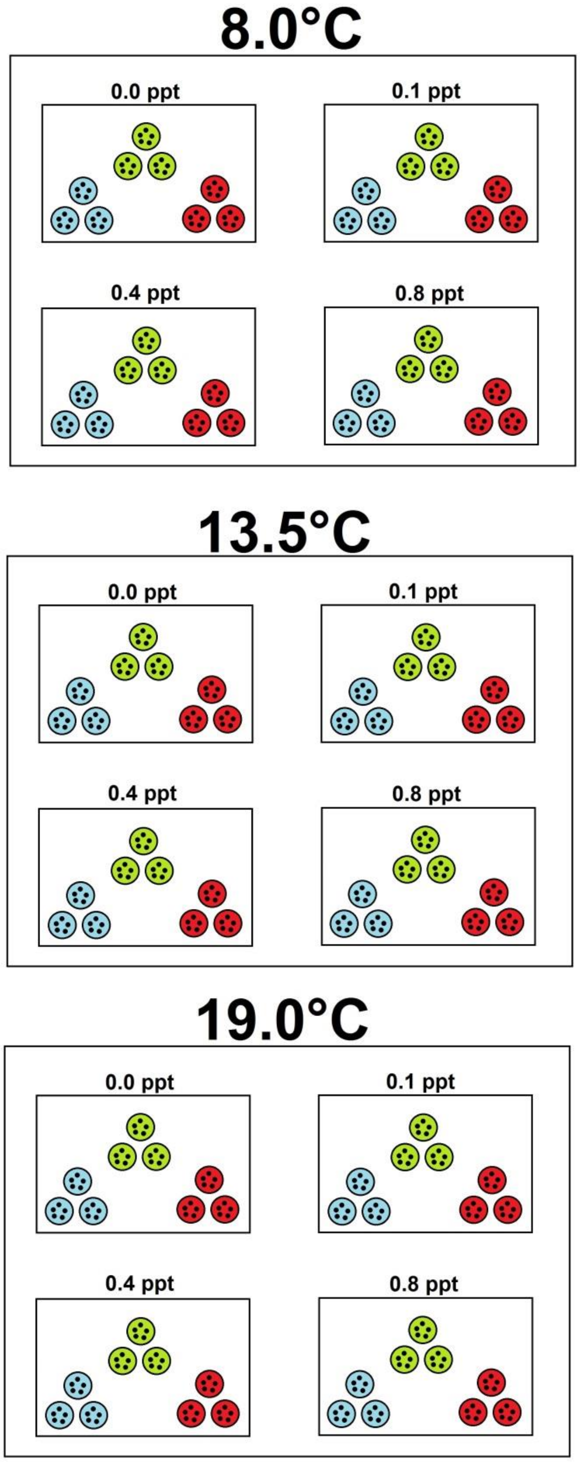

Figure 3.2 – Experimental layout of the hatching study. Colored circles (Dowling Lake – blue, Goose Lake – green, Middle Alkali Lake – red) represent replicates, each containing five eggs each.

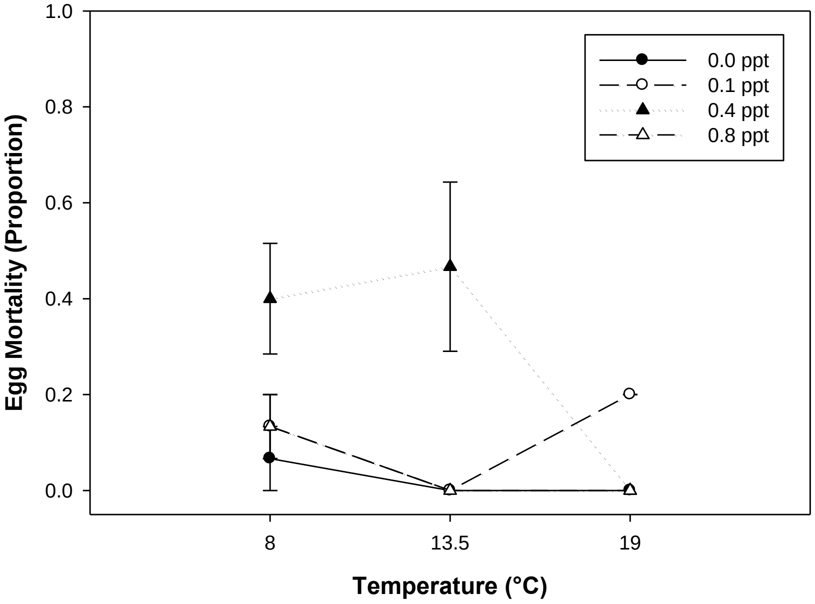

Figure 3.3 – Interaction of main effects (temperature and salinity) on egg mortality in Dowling Lake *Lepidurus lemmoni.*

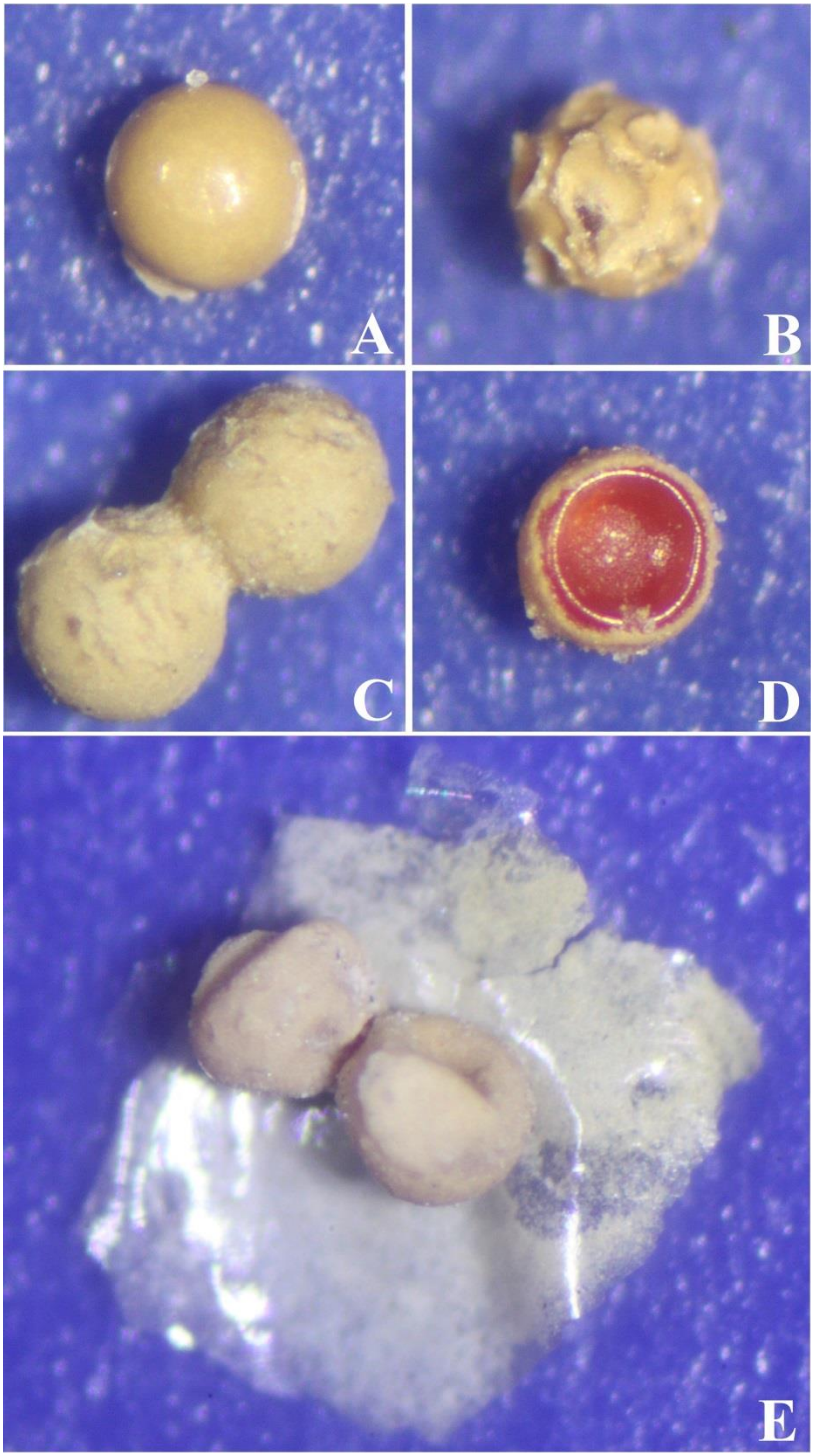

Figure 3.4 – Dry eggs of *Lepidurus lemmoni*: A, egg lacking adhesive coat; B, egg with adhesive coat, with scars from detached sand grains; C, two eggs firmly attached to each other; D, egg with broken chorion, showing bright red embryo; E, two eggs adhered to a fragment of adult exuvium.

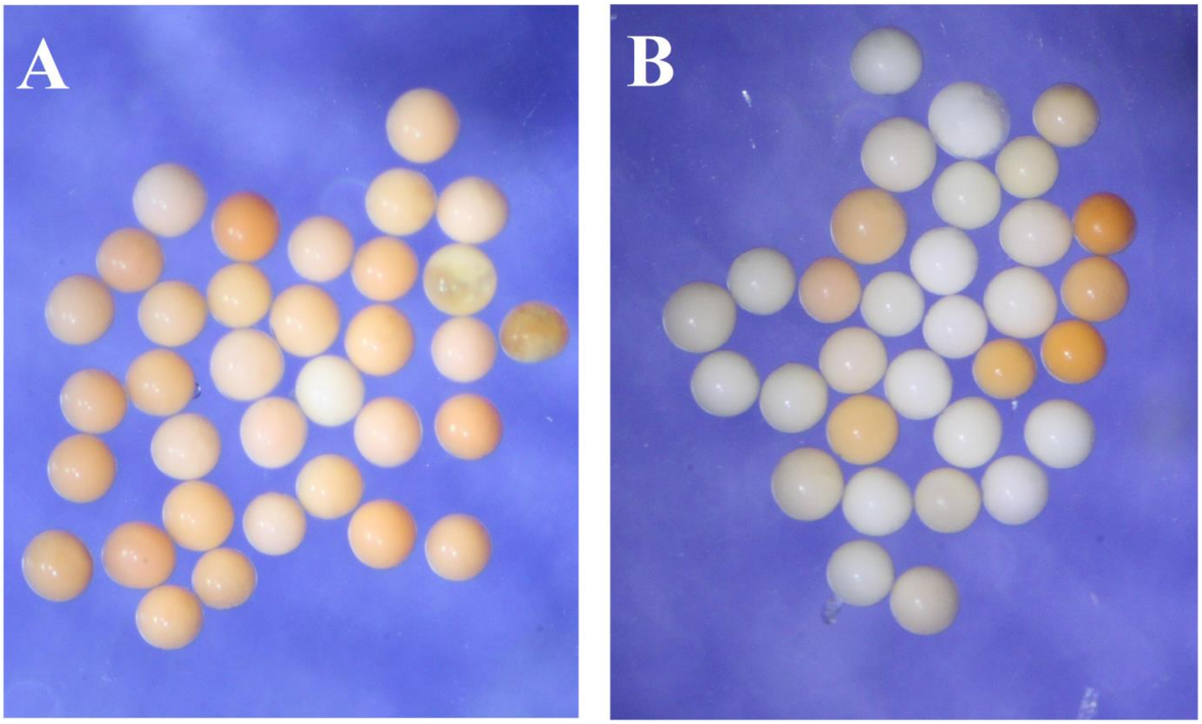

Figure 3.5 – Decapsulated embryos of *Lepidurus lemmoni* from Middle Alkali Lake (A) and Goose Lake (B). Goose Lake embryos were mostly white while Middle Alkali Lake and Dowling Lake (not pictured) were uniformly orange.

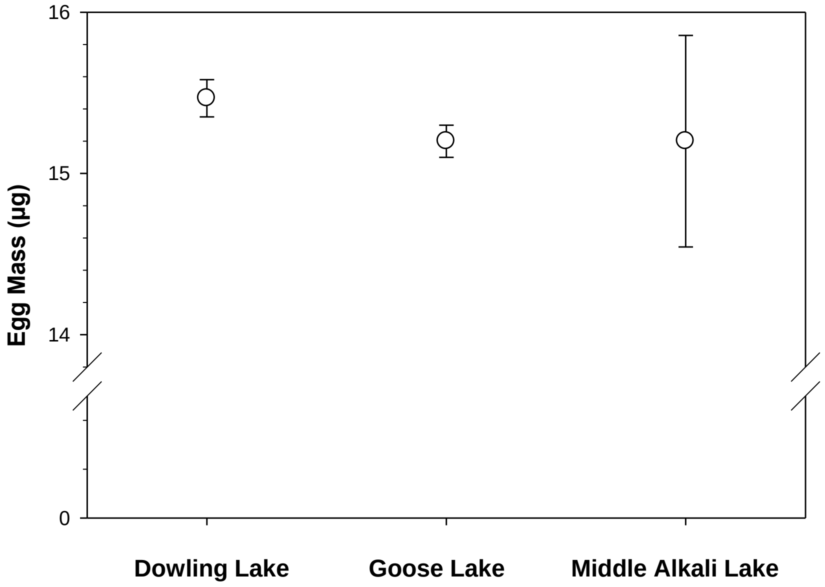

Figure 3.6 – Plot of individual embryo dry mass (mean ±SD) of *Lepidurus lemmoni* from three lakes in its described distribution range.

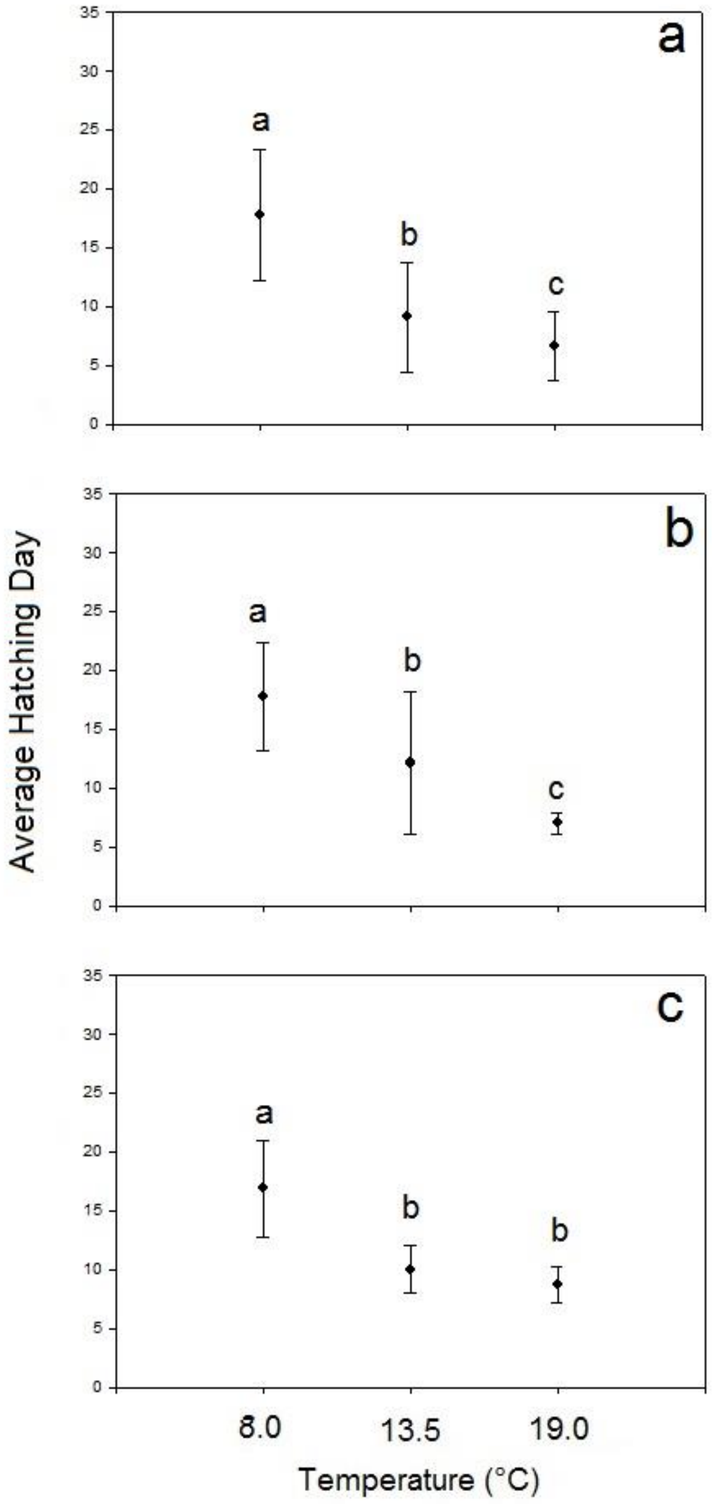

Figure 3.7 – Average hatching day as a function of incubation temperature in *Lepidurus lemmoni* from (A) Dowling, (B) Goose, and (C) Middle Alkali lakes. Letters represent significant differences derived from Fisher's LSD.

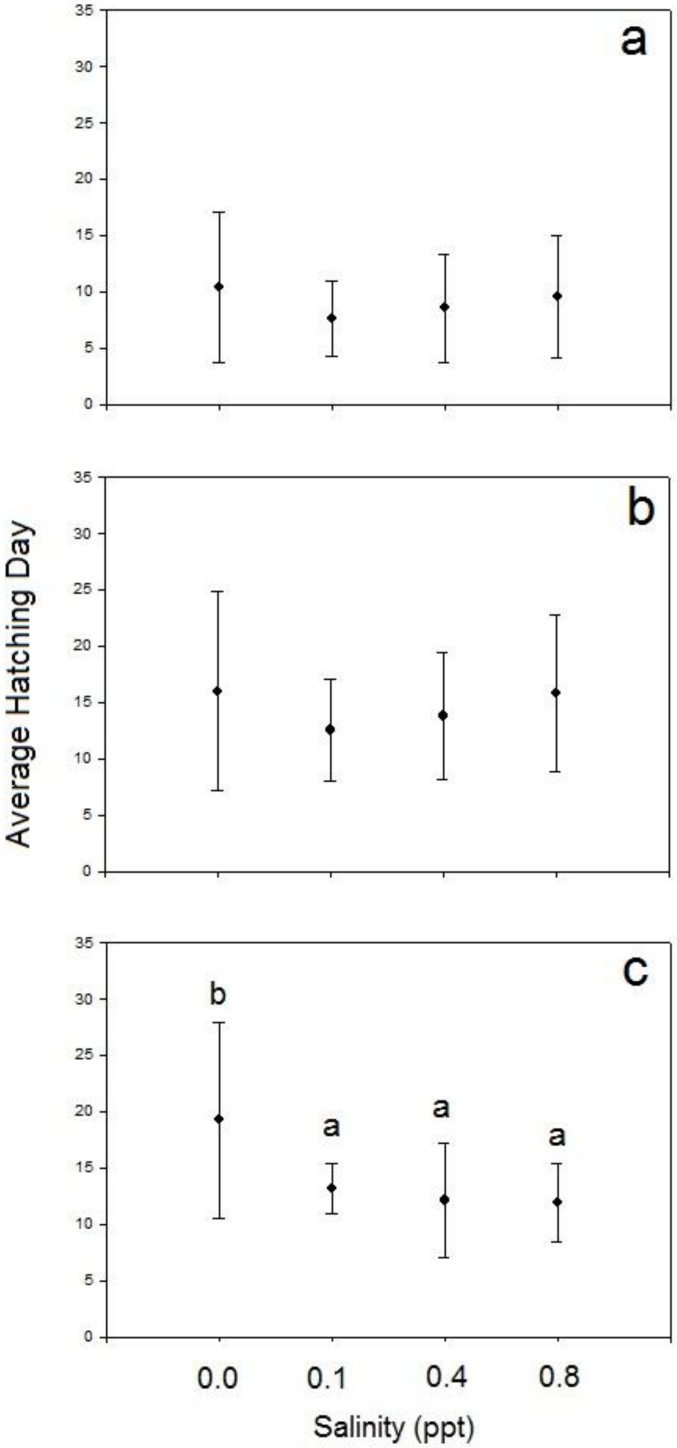

Figure 3.8 – Average hatching day as a function of incubation salinity in *Lepidurus lemmoni* from (A) Dowling, (B) Goose, and (C) Middle Alkali lakes. Letters represent significant differences derived from Fisher's LSD.

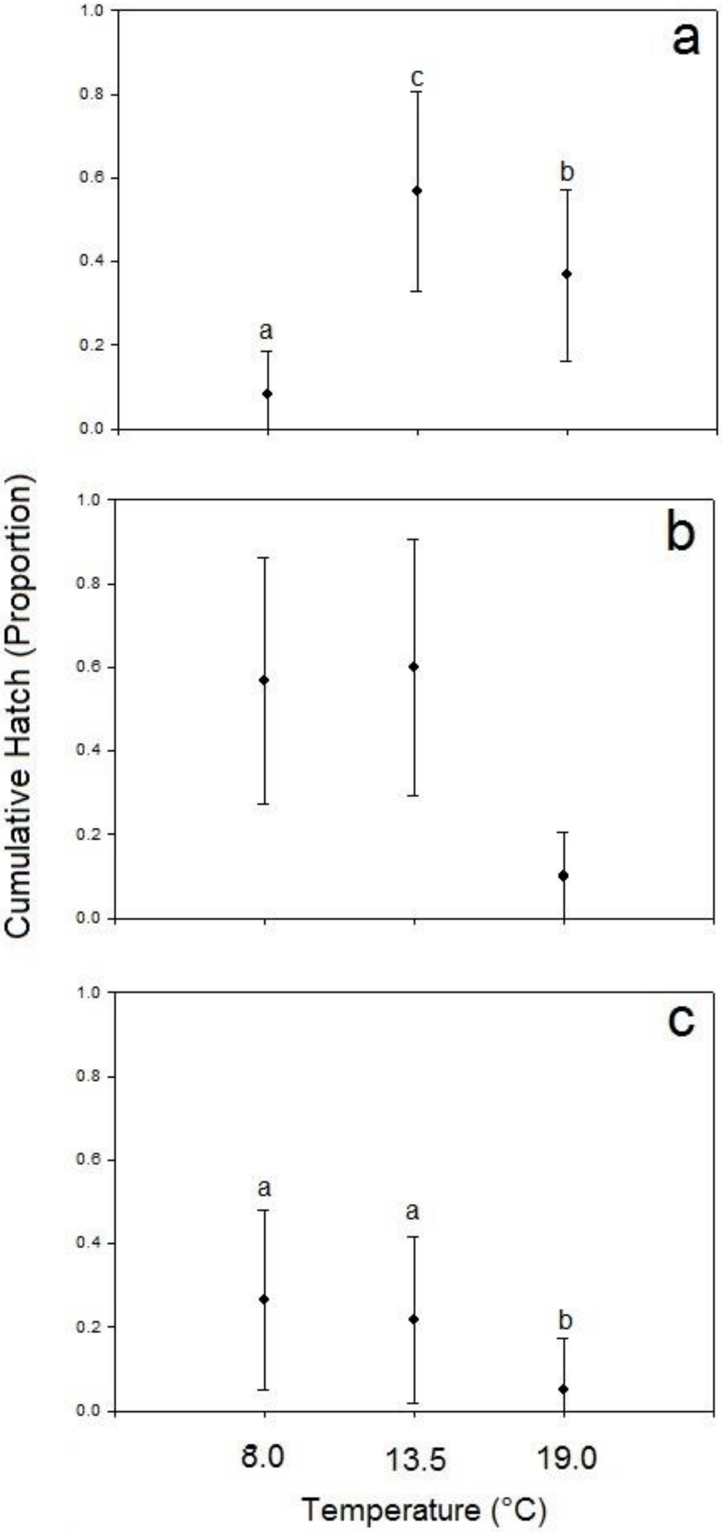

Figure 3.9 – Cumulative hatch as a function of incubation temperature in *Lepidurus lemmoni* from (A) Dowling, (B) Goose, and (C) Middle Alkali lakes. Letters represent significant differences derived from Fisher's LSD. Significant interaction between temperature and salinity in Goose Lake *L. lemmoni* prevented further analysis.

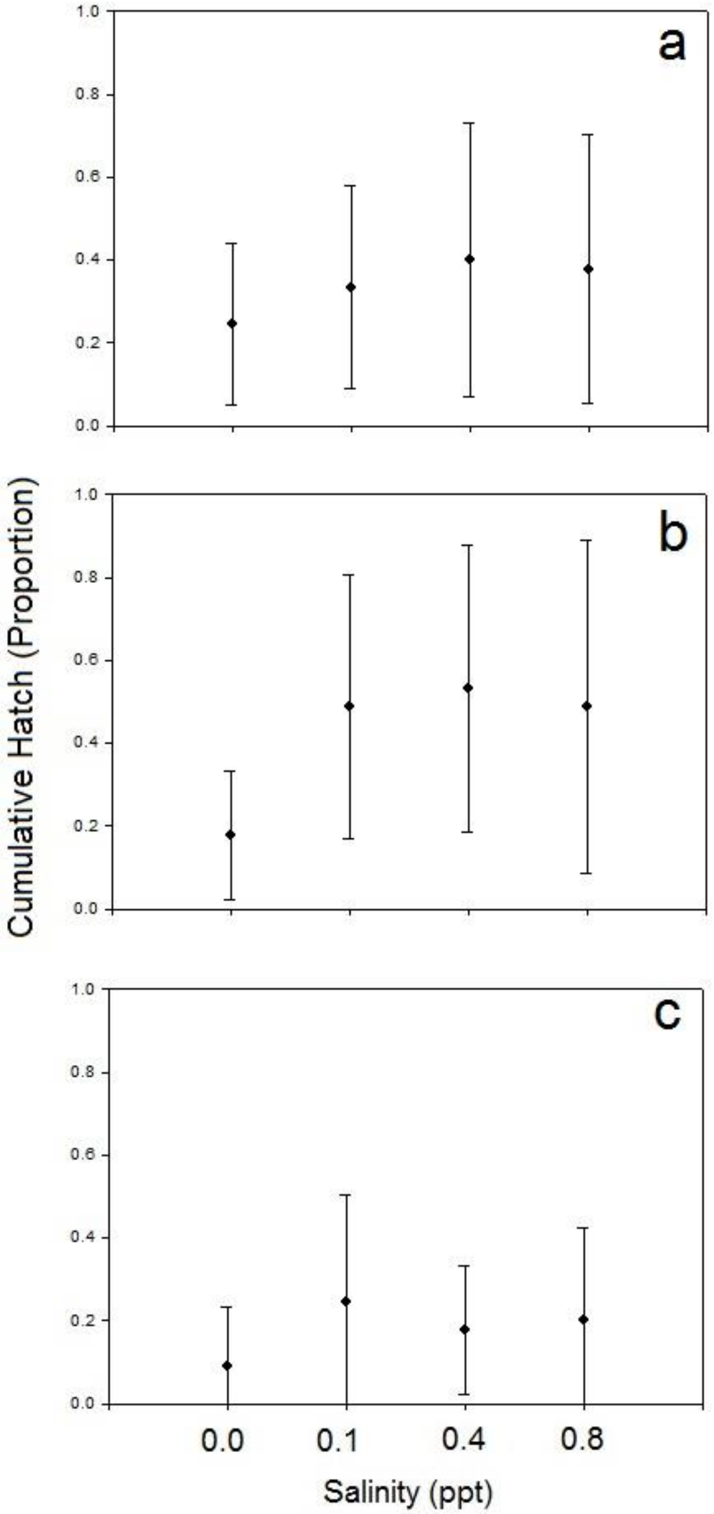

Figure 3.10 – Cumulative hatch as a function of incubation salinity in *Lepidurus lemmoni* from (A) Dowling, (B) Goose, and (C) Middle Alkali lakes.

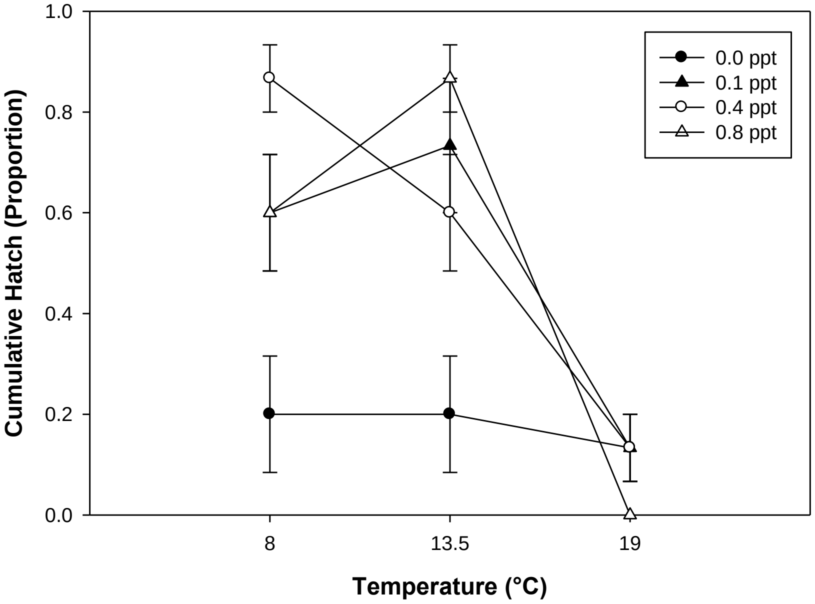

Figure 3.11 – Interaction of main effects (temperature and salinity) on cumulative hatch in Goose Lake *Lepidurus lemmoni.*

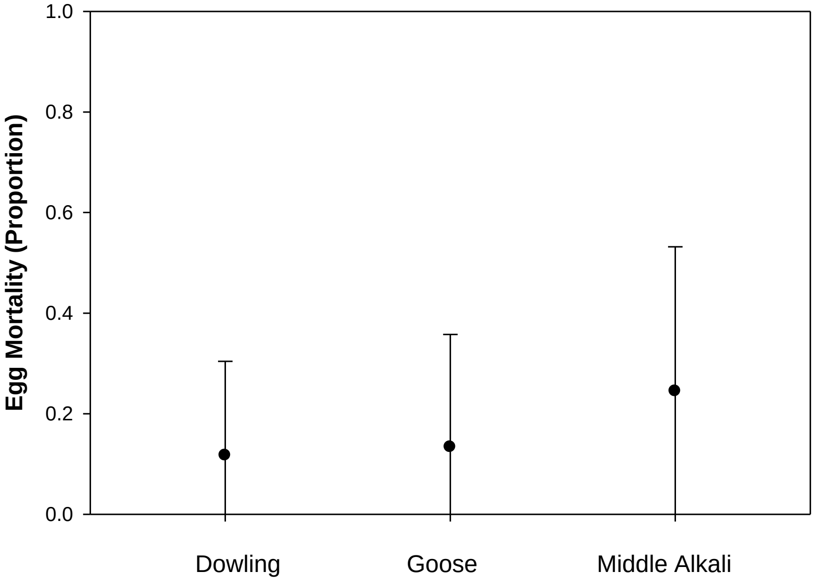

Figure 3.12 – Egg mortality in *Lepidurus lemmoni* from Dowling, Goose, and Middle Alkali lakes across all incubation treatments. Egg mortality in Middle Alkali Lake is significantly different from egg mortality in Dowling and Goose lakes.

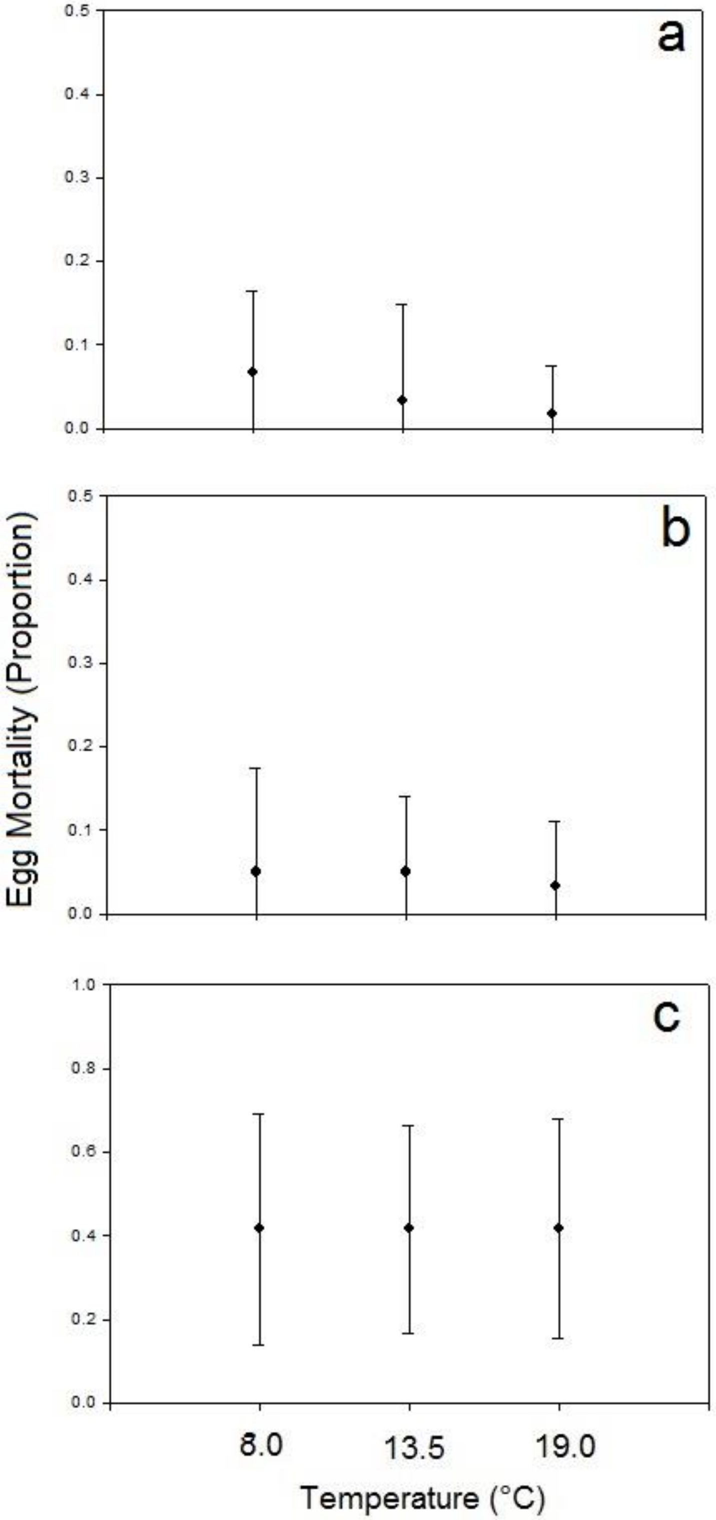

Figure 3.13 – Egg mortality as a function of incubation temperature in *Lepidurus lemmoni* from (A) Dowling, (B) Goose, and (C) Middle Alkali lakes.

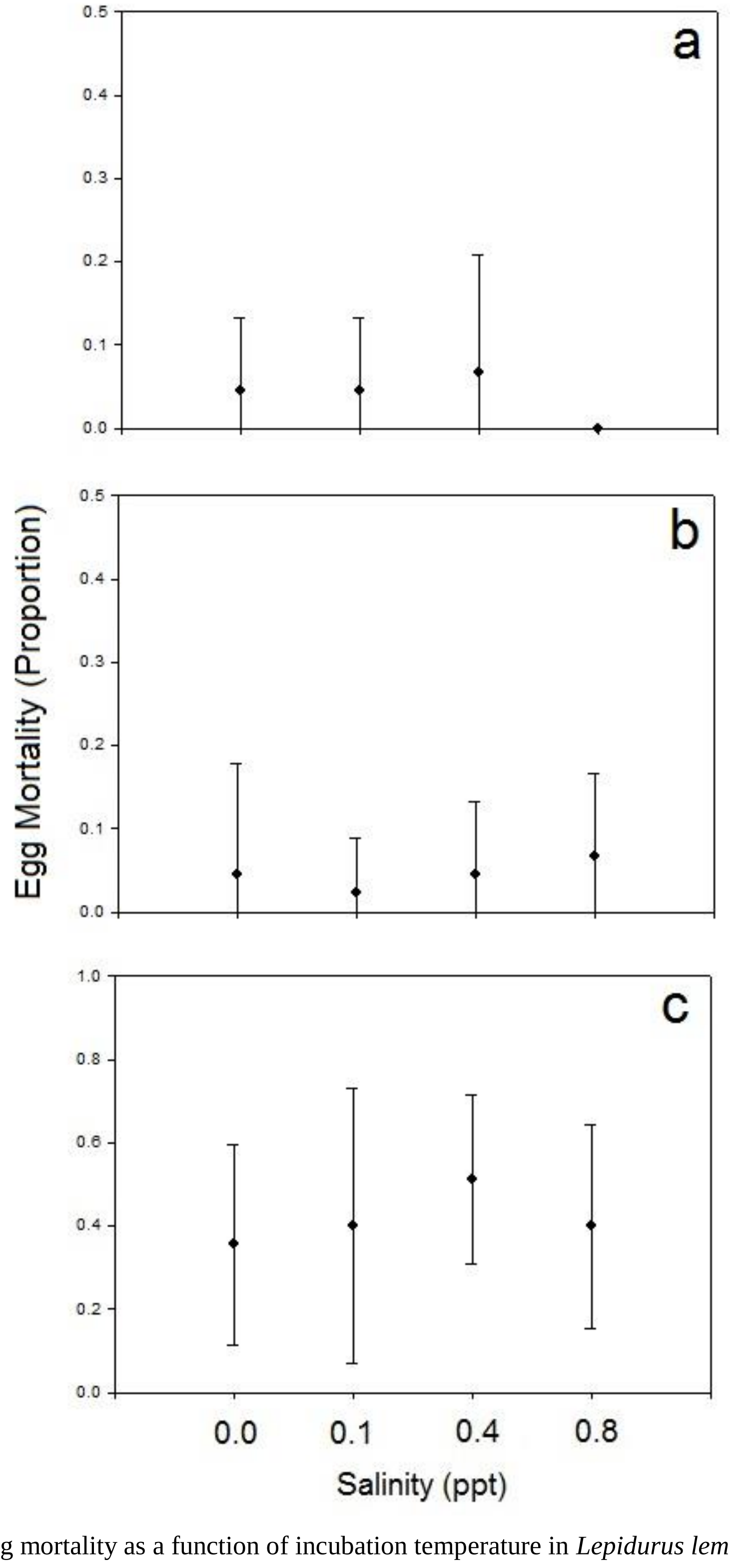

Figure 3.14 – Egg mortality as a function of incubation temperature in *Lepidurus lemmoni* from (A) Dowling, (B) Goose, and (C) Middle Alkali lakes.

Chapter 4: Inducible Defenses in *Lepidurus lemmoni*

Introduction

Lepidurus lemmoni Holmes, 1894 is a primitive crustacean typical of large, temporary, alkaline lakes in western North America. It has been recorded from Alberta, Montana, Washington, Oregon, California, Wyoming, Nevada, New Mexico, Arizona, and Baja California (Rogers, 2001). It is especially common in the Great Basin and Mojave Desert (Rogers and Hill, 2013). Its habitat is typically shallow, turbid, and very alkaline, with pH values of 8.2-11 (Lynch, 1966).

After its initial description, the species fell into obscurity until Linder's (1952) review of the North American Notostraca considered the original description unfit by modern taxonomic standards, and recommended abandoning the name. In the same paper, he described a new species of *Lepidurus* from specimens from eastern Washington and southeastern Oregon as *Lepidurus lynchi* and *L. lynchi* var. *echinatus*, respectively. Linder (1952) described *L. lynchi* var. *echinatus* based on twelve specimens from Goose Lake, Oregon. The variety is much more spinose than the typical form, bearing large, erect spines on the carina, sulcus, and lateral carapace margins. As described, typical *L. lynchi* had few or no small spines on the carina and lateral carapace margins. The sulcus is also shallower in the *echinatus* variety, causing more of the abdomen to be covered by the carapace (Fig.4.1). No ecological explanation for this more spinose from was given. In 1966, *L. lemmoni* was redescribed by Lynch (1966), and *L. lynchi* and *L. lynchi* var. *echinatus* were subsumed into *L. lemmoni*. The taxonomy of *L. lemmoni* has been stable since then.

In his redescription of *L. lemmoni*, Lynch (1966) synonymized both *L. lynchi* and *L. lynchi* var. *echinatus* with *L. lemmoni*. His rationale was that *L. lemmoni* from the type locality, Honey Lake, CA, overlapped morphologically with *L. lynchi* var. *echinatus.* Linder did not have access to type material at the time of his work, so his choice was understandable. He did mention the similarity between *L. lynchi* var. *echinatus* in his collections and the published description of *L. lemmoni* (Linder, 1952). Lynch considered Linder's *L. lynchi* var. *echinatus* to merely represent subspecific variation without taxonomic importance. Neither Lynch nor Linder noted that Goose Lake is home to four species of fish (Scheerer *et al*., 2010), at least two of which (redband trout and tui chub) are potential predators of *L. lemmoni*. Lynch did note that tui chub (as *Siphateles obesus*) co-occurred with *L. lemmoni* in the type locality (Lynch, 1966). It is noteworthy that *L. lemmoni* from Honey Lake possessed more and larger spines than any of the other populations examined in the redescription, excluding those from Goose Lake. Honey and Goose lakes are also the only two published localities of *L. lemmoni* that are relatively permanent, both only drying out every few years or decades, respectively (Lynch, 1966; Scheerer *et al*., 2010). Thus, it may be possible that the more armored *echinatus* form represents a morphology in response to the presence of predators, also known as an inducible defense.

Inducible defenses are common in the animal world and have been observed in most of the Branchiopod groups. The Spinicaudata, especially the suborder Cladocera, are well known for their elegant and sometimes dramatic morphological and behavioral responses to various predation pressures (Tollrian and Dodson, 1999). Recently, limnadiid clam shrimp have been shown to demonstrate morphological changes in response to alarm cues and non-lethal injury (Rogers *et al*., 2012). For example, eggs of cyzicid clam shrimp will not hatch in the presence of kairomones from larval salamanders (Blaustein, 1997). Some fairy shrimp (order Anostraca) modify their behavior in the presence of a predatory turbellarian (Brendonck *et al*., 2002). Fairy shrimp also produce special predation-resistant eggs, possibly in response to the kairomones of predatory Turbellaria (Dumont *et al*., 2002). If the *echinatus* form of *L. lemmoni* represents an inducible defense, it would be the first record of one in the Notostraca. However, the Notostraca display a great deal of intraspecific morphological variation (Longhurst, 1955), which has traditionally been deemed to be "of little significance systematically or, probably, adaptively" (Longhurst, 1958). At least some of this morphological plasticity may be explained in light of inducible defenses.

The only other Notostracan known to regularly co-occur with fish is the circumboreal *Lepidurus arcticus* (Pallas, 1793) (Rogers, 2001). *L. arcticus* is a primary summer food source for brown trout *Salmo trutta* L. and arctic charr *Salvelinus alpinus* (L.) in deep lakes in the arctic and alpine subarctic (Børgstrom, 2001; Lien, 1978; Adalsteinsson, 1979; Larsson *et al*., 1978). No research has been done on *L. arcticus* to determine if it displays any morphological or behavioral responses to the presence of fish predators. Morphological variation occurs between populations of *L. arcticus*, and to a lesser extent, haplotypes (Hessen *et al*., 2004). Populations with exceptionally large body size and well-developed carina spines occur in eastern Siberia, but it is unknown if these populations co-occur with fish or any other predators (Hessen *et al*., 2004). Only very limited conclusions on morphological variability can be drawn from Hessen *et al*. (2004) because they neglected to relax specimens before fixation, likely causing severe contraction of the trunk (Linder, 1952).

Further work should be done with *L. arcticus* to elucidate its defensive adaptations to salmonid predation, as well as the true nature of its morphological variation.

Rogers (Kansas Biological Survey, pers. comm.) has found that *L. lemmoni* can be induced to take the *echinatus* form by exposing them to alarm cues in the form of crushed conspecifics. Considering that both Goose and Honey lakes hold fish, and both contain spiny forms of *L. lemmoni*, it seems plausible that the *echinatus* form represents an inducible defense against fish predation. It is possible that this is a trait specific to these two populations. It may also be that the *echinatus* form is caused solely in nature by fish predation. *L. lemmoni* are exposed to a number of predators throughout their range including *Dytiscus* spp., *Notonecta* spp. (Daborn, 1975), *Branchinecta gigas* (D.C. Rogers, Kansas Biological Survey, pers. comm.), and a number of wading birds (Plissner *et al*., 1999; Plissner *et al*., 2000; pers. obs.). If these predators could induce the *echinatus* form, then it would likely have been found elsewhere.

Most *L. lemmoni* habitat occurs in the pluvial lake basins of the Great Basin and Mojave Desert (Rogers, 2001; Rogers and Hill, 2013). Its more northern localities, the channeled scablands of eastern Washington, and the glacial lakes of northern Montana and Alberta (Lynch, 1966; Hartland-Rowe, 1965), were not available for colonization until the late Pleistocene (Alt, 2001; Gillespie *et al*., 2004). It is therefore likely that *L. lemmoni* evolved in or near the pluvial lakes of the Great Basin and Mojave Desert. These pluvial lakes fluctuated greatly in size and depth during the Pleistocene and Holocene, changing from large, permanent, freshwater lakes with diverse fish assemblages to smaller, usually temporary, fishless, alkaline or saline pools (Grayson, 2011). This means that *L. lemmoni* may have been exposed intermittently to predation by fish over the last 400,000 years. This variable predation pressure is considered one of the key prerequisites for the evolution of inducible defenses (Tollrian and Dodson, 1999), and could be the reason for the observed morphological differences reported for populations from Honey and Goose lakes. The *echinatus* form only occurs in semi-permanent lakes where fish can and do co-occur, so it can be surmised that the *echinatus* form is induced by fish predation, which will be tested in this study.

Chemicals are a good source of information in aquatic habitats, especially in turbid waters, like the habitat of *L. lemmoni* (Lynch, 1966), where visual range is limited. Chemicals can also transmit information over large spatial and temporal scales. Alarm cues and kairomones are two forms of chemical communication used by aquatic organisms to signal predation risk. Alarm cues are here defined as cellular and extracellular fluids released during injury, and probably not released by specific glands (Laforsch *et al*., 2006). Kairomones are chemicals used in interspecific signal transmission which are exclusively advantageous to the receiving species. In short, they are chemicals released by predator species that signal their presence to prey species (Tollrian and Dodson, 1999). Both alarm cues and kairomones have been shown to induce defensive morphologies and behaviors within the Branchiopoda.

Kairomones can induce defensive morphological, behavioral, and life history changes in *Daphnia* (Tollrian and Dodson, 1999). These reactions in *Daphnia* can be induced by kairomones from a number of fish species, including non-planktivorous species (Loose *et al*., 1993). These reactions can also be induced by kairomones from invertebrate predators such as *Chaoborus* and *Notonecta* (Tollrian and Dodson, 1999). *Daphnia magna* Straus, 1820 will respond to kairomones produced by predators that have not been feeding on conspecifics (Loose *et al*., 1993). In *Daphnia cucullata* Sars, 1862, kairomones produced by predators that have fed on conspecifics (specific kairomones) produce a stronger response than non-specific kairomones produced by predators feeding on *Chydorus sphaericus* (Müller, 1785) (Laforsch *et al*., 2006). Reactions to kairomones are concentration-dependent, and at low predator densities there is a threshold for the reaction (Tollrian and Dodson, 1999). Laforsch *et al*. (2006) also found that a dose-response curve, with a plateau after a certain signal concentration, was produced with *D. cucullata*. Kairomones can also act synergistically with abiotic factors such as light intensity to increase the defensive reaction (Brewer *et al*., 1999). *Daphnia* has been shown to have a much stronger response to kairomones than to alarm cues (Laforsch *et al*., 2006), though many studies on kairomone responses in *Daphnia* actually observe responses to a mixture of alarm cues and kairomones (Weber and Declerck, 1997). Certain responses require a mixture of alarm cues and kairomones (Ślusarczyk, 1999). In nature, the mixture of these two signals may be what signals predation pressure to *Daphnia* and induces defensive changes (Laforsch *et al*., 2006). In some, but not all cases, these defensive morphological responses (inducible defenses) are known to have a negative impact on the fecundity of individual *Daphnia* (Tollrian and Dodson, 1999).

There is evidence that juvenile and adult prey species can display different morphological defenses in response to the same predation cues. Rabus and Laforsch (2011) studied the inducible defenses of *D. magna* in relation to one of its predators, the notostracan *Triops cancriformis.* When juvenile *D. magna* are exposed to kairomones from *T. cancriformis,* they develop elongated tail spines. As *D. magna* mature in the presence of *T. cancriformis* kairomones, tail spine length decreases, but general somatic bulkiness increases. Both morphological changes provide effective protection from the gape-limited *T. cancriformis*.

Little is known about the nature of kairomone molecules. Experiments have shown them to be low molecular weight (<500 Dalton), heat stable, non-volatile molecules. They are stable over a broad pH range, and a lack of specific enzymatic degradation suggests that they are not proteins. They remain active after drying and reconstitution, but disappear within 24 hours in septic conditions (Loose *et al*., 1993; Beklioglu *et al*., 2006). If kept at -20°C, kairomones will retain their induction capabilities for at least two years (Ślusarczyk and Rybicka, 2011). Kairomones can be isolated from water, fish mucous, viscera, and feces (Tollrian and Dodson, 1999; Ślusarczyk and Rygielska, 2004). Feces are the primary source of kairomones that induce avoidance diapause in *D. magna* (Ślusarczyk and Rygielska, 2004).

As can be seen above, these defensive reactions are well-studied in *Daphnia*, a highly derived branchiopod crustacean related to *L. lemmoni* (both are in the class Branchiopoda). Because of this relation, information on the inducible defenses of *Daphnia* may be applicable to *L. lemmoni.* I plan to determine whether fish kairomones or alarm cues induce the *echinatus* form, if there is a difference in the response to these separate signals and the signals combined. I will also explore the possibility of differential responses to alarm cues and kairomones in adult and juvenile *L. lemmoni*. Experiments were designed to test three hypotheses; that inducible defenses differ with life stage, that alarm cues, kairomones, and the two combined have different effects on morphology, and that non-lethal injury can induce defensive morphology. These experiments were limited to the Goose Lake population, because the Goose Lake population is one of only two known *L. lemmoni* populations that co-occurs with fish, so it was reasonable to expect that this population would demonstrate the strongest and best-developed inducible defenses of all the populations of *L. lemmoni*.

Methods

Test Animals

All *L. lemmoni* used in these experiments were reared from a stock culture started from lakebed soil collected during the 2012 dry season from Goose Lake, Modoc County, CA. Four liters of dry lakebed soil were mixed with 100L of distilled water in a 120cm diam. × 12 cm deep polyethylene experimental chamber floated in a 1000L circular fiberglass flow-through tank supplied with chilled and ambient water mixed to maintain a fixed temperature. The tank was started at 5-6°C, and slowly raised to 15-17°C over three weeks to simulate the temperature changes associated with early spring. Gentle aeration was provided via compressed air delivered with a 10 cm airstone. Food in the form of 0.1 g sinking tropical fish food (Shrimp Pellets®, HBH Pet Products, Springville, UT) was provided twice weekly. To record the carapace length of all hatched *L. lemmoni* before use in the experiments, they were transferred from the stock culture with a net to a 10cm diameter glass Petri dish with a thin layer of culture water. The dish was placed on top of a sheet of graph paper with a 5 mm grid and all specimens were individually photographed. Carapace length was measured along the midline from the anterior margin of the carapace to the end of the carina at the sulcus using the gridded paper for reference.

Alarm Cue Collection

To collect alarm cues, a procedure was adapted from Mirza and Chivers (2003). Briefly, 12 adult *L. lemmoni* (11.5mm+ carapace length) from the stock culture were homogenized in a mortar, mixed with 25mL distilled water, and filtered through a Büchner funnel with 101 Fast qualitative filter paper (Lab Nerd, St. Augustine, FL). The filtrate was diluted to 0.067 *L. lemmoni*/mL with distilled water and dispensed into 1.6mL Eppendorf tubes in 1.0mL aliquots and stored at -25°C until needed (typically within one year of production).

Kairomone Collection

For the collection of fish kairomones, two anglers from the public harvested rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) which were then donated to the project. All fish were immediately stripped of feces as in Stone *et al.* (2008). Feces were stored on ice for transport to the laboratory (approx. 25 km distant) where they were homogenized in a mortar, mixed with 25mL distilled water, and filtered through a Büchner funnel with 101 Fast qualitative filter paper (Lab Nerd, St. Augustine, FL). The filtrate was diluted to 0.067 trout feces/mL with distilled water and dispensed into 1.6mL Eppendorf tubes in 1.0mL aliquots which were stored at -25°C until needed (within one year of production).

Effects of Alarm Cues and Kairomones

To examine the response of *L lemmoni* to kairomones and alarm cue, 16 juvenile (carapace length 7.23 mm, SD=1.15) and adult (carapace length 14.10 mm, SD=1.43) *L. lemmoni* from the stock culture were used. The *L. lemmoni* were placed into individual 1.87L plastic aquaria 22.5x14.6x14.0cm (L*W*H) (Lee's®, San Marcos, CA) filled with 1.5 L culture water mixed with distilled water with a standardized conductivity of 450 µS/cm, measured with a YSI-85 probe. Each aquarium was provided with gentle aeration via an airstone. Treatments consisted of alarm cue, kairomone, alarm cue+kairomone, and a control (distilled water addition). Treatments were made by mixing three frozen aliquots of alarm cue or kairomone with 5mL distilled water of which 1 mL was added to each assigned aquarium. Alarm cue aquaria received 1.0 mL of alarm cue, kairomone aquaria received 1.0 mL of kairomone, and alarm cue+kairomone aquaria received 1.0 mL each of alarm cue and kairomone. Control aquaria received 1.0 mL distilled water. This treatment produced the equivalent of one predation event per 30 L, one trout per 30 L, and one predation event plus one trout per 30L in the alarm cue, kairomone, and alarm cue+kairomone treatments, respectively. The actual density of fish, and predation events, in Goose Lake is unknown, so this concentration was chosen as a plausible, if high, concentration of these signals. I erred on the high side of the concentration to improve the probability of defenses being induced.

Each treatment was administered on four replicates. Each replicate was randomly placed within each of four blocks. All blocks were kept in a light exclusion chamber made of black plastic to simulate the reduced light availability in the highly turbid natural habitat, and to reduce evaporative loss. Barriers made of 25 cm high strips of plastic drop cloth separated individual aquaria to eliminate any transfer of treatment between aquaria due to splashing or spattering related to the air bubbles from the airstone breaking at the surface. After 24 hours of acclimation, treatments were added daily. Food in the form of commercial sinking fish food pellets (Shrimp Pellets®, HBH Pet Products, Springville, UT), was added twice weekly. Treatments were administered for four weeks, after which the aquaria were left for five days to allow animals to undergo a final molt. After this five-day rest period, each *L. lemmoni* was narcotized in carbonated water and preserved in 70% ethanol until detailed morphological analysis.

For detailed morphological analysis, the number of carina and sulcus spines (Fig. 4.2), as well as the presence/absence of *echinatus* morphology (heavily sclerotized carina with large spines, erect spines present on carapace margins), was recorded for each individual. Morphology was analyzed using one-way analysis of variance (ANOVA), in which carina and sulcus spine count was the response variable and chemical signal (alarm cue, kairomone, alarm cue+kairomone, and distilled water) was the treatment variable. The expected errors of the adult carina spine count did not have a mean of zero, so ANOVA could not be used. Due to the violated assumption, the Kruskal-Wallis rank sum test was used , where carina spine count was the response variable and chemical signal (alarm cue, kairomone, alarm cue+kairomone, and distilled water) was the treatment variable. Separate analyses were run for juveniles and adults. The effect of age (juvenile vs. adult) on carina and sulcus spine counts was tested with t-tests where carina and sulcus spine count was the response variable and age (juvenile or adult) was the treatment variable.

Effects of Non-Lethal Injury

To test the hypothesis that non-lethal injury can induce defensive morphology, 10 adult *L. lemmoni* (carapace length 13.60mm, SD=0.92) were selected randomly from the stock culture and five individuals were placed in a treatment group (cercopods removed with dissecting scissors at the point level with the posterior margin of the caudal lamina) or control group (no manipulation beyond handling). Animals were placed individually in 22.5x14.6x14.0cm 1.87L plastic aquaria (Lee's$^{®}$, San Marcos, CA) filled with 1.5L culture water mixed with distilled water to achieve a standardized conductivity of 450 µS/cm, which was measured with a YSI-85 probe. Each aquarium was provided with gentle aeration via an airstone. Food in the form of commercial sinking fish food pellets (Shrimp Pellets®, HBH Pet Products, Springville, UT), was added twice weekly. At the end of 40 days, each *L. lemmoni* was narcotized in carbonated water and preserved in 70% ethanol until analysis.

The number of spines per cercomere in the dorsal aspect was recorded from five complete cercomeres just beyond the distal margin of the caudal lamina, and the length of each counted spine was measured. The width of the cercopod just beyond the distal margin of the caudal lamina was also measured. The number of carina and sulcus spines was recorded for each individual. Each individual was also checked for the presence/absence of *echinatus* morphology (heavily sclerotized carina with large spines, erect spines present on carapace margins).

The effect of injury and regeneration on mean cercomere spine length was tested with a t-test where mean cercomere spine length was the response variable and injury was the treatment variable. The effect of injury and regeneration on mean cercomere spine count was tested with a t-test where mean cercomere spine count was the response variable and injury was the treatment variable. The effect of injury and regeneration on mean cercopod width was tested with a t-test where mean cercopod width was the response variable and injury was the treatment variable. The effect of injury on carina spine count was tested with a t-test where carina spine count was the response variable and injury was the treatment variable. The effect of injury on sulcus spine count was tested with a t-test where sulcus spine count was the response variable and injury was the treatment variable.

Results

Effects of Kairomones and Alarm Cues

In experiments with kairomones and alarm cues, no juvenile or adult *L. lemmoni* from any treatment group displayed the *echinatus* morphology. Sulcus spine count in adult *L. lemmoni* was not significantly (ANOVA, $F_{3,3}$=0.103, *p*=0.957) affected by treatment, with mean sulcus spine counts of 17.67, 16.75, 17.33, and 13.75 in the treatments of alarm cue, kairomone, alarm cue+kairomone, and distilled water respectively (Fig. 4.3). Carina spine count in adult *L. lemmoni* was not significantly (Kruskal-Wallis, χ^2=7.068, df=3, *p*=0.070) affected by treatment, with mean carina spine counts of 3.67, 11.25, 15.33, and 5.75 in the treatments of alarm cue, kairomone, alarm cue+kairomone, and distilled water, respectively (Fig. 4.4). Sulcus spine count in juvenile *L. lemmoni* was not significantly (ANOVA, $F_{3,3}$=0.694 *p*=0.573) affected by treatment, with mean sulcus spine counts of 19.75, 17.00, 17.00, and 14.75 in the treatments of alarm cue, kairomone, alarm cue+kairomone, and distilled water, respectively (Fig. 4.5). Carina spine count in juvenile *L. lemmoni* was not significantly (ANOVA, $F_{3,\,3}$=1.175, *p*=0.36) affected by treatment, with mean carina spine counts of 2.75, 7.00, 9.00, and 9.00 in the treatments of alarm cue, kairomone, alarm cue+kairomone, and distilled water, respectively (Fig. 4.6). Mean sulcus spine counts of adults (16.93) was not significantly (t-test, *t* = -0.1301, df = 28, *n* = 30, *p*=0.897) different from juveniles (17.13). Mean carina spine counts of adults (8.93) was not significantly (t-test, *t* = 0.9513, df = 28, *n* = 30, *p*=0.350) different from juveniles (6.94).

Effects of Non-Lethal Injury

No *L. lemmoni* from treatment or control groups displayed the *echinatus* morphology. Removed cercopods regenerated to varying degrees, both between and within individuals (Fig. 4.7). Mean sulcus spine counts of injured *L. lemmoni* (15.6) was not significantly (t-test, *t* = -1.059, df = 8, *n* = 10, *p*=0.321) different from uninjured *L. lemmoni* (13.4) (Fig. 4.8). Mean carina spine counts of injured *L. lemmoni* (3.2) was not significantly (t-test, *t* = -1.173, df = 4, *n* = 10, *p*=0.306) different from uninjured *L. lemmoni* (0.0) (Fig. 4.9). Mean cercomere spine counts of injured *L. lemmoni* (4.64) was not significantly (t-test, *t* = 0.475, df = 48, *n* = 50, *p*=0.637) different from uninjured *L. lemmoni* (4.48) (Fig. 4.10). Mean cercomere spine lengths of injured *L. lemmoni* (108.26 µm) was significantly (t-test, *t* = -8.690, df = 226, *n* = 228, *p*<0.001) different from uninjured *L. lemmoni* (84.16 µm) (Figs. 4.11, 4.12). Mean cercopod width of injured *L. lemmoni* (382.5 µm) was not significantly (t-test, *t* = -0.496, df = 4.454 *n* = 10, *p*=0.643) different from uninjured *L. lemmoni* (372.5 µm) (Fig. 4.13).

Discussion

There is no evidence for the *echinatus* form in *L. lemmoni* being an inducible defense. No combination of kairomones or alarm cues induced anything that could be considered *echinatus* morphology. The inability to distinguish difference in treatments from controls was likely hampered by the small sample size and high variance of remaining individuals. The lack of individuals was the result of low recruitment in the laboratory. Any future studies must take this into consideration and focus on recruiting a high abundance of individuals from laboratory cultures, because ideally all individuals should be of the same or known age. The inability to induce the *echinatus* form suggests that this form may represent an ecological race of *L. lemmoni*, specially adapted to coexist with fish. This calls into question anecdotal reports of the *echinatus* form being induced by exposing *L. lemmoni* to crushed conspecifics and the taxonomic classification as it exists currently. An analysis of DNA from both forms should be undertaken to help resolve these questions.

Regenerated cercopods had significantly longer spines than uninjured cercopods. This response to non-lethal injury is similar to that of the limnadiid clam shrimp *Eulimnadia texana* (Packard, 1871), which regenerated antennae with spines instead of setae (Rogers *et al.,* 2012). Unlike the response to injury seen in *E. texana*, the regenerated cercopods in *L. lemmoni* were not wider. While injury did alter the morphology of regenerated tissues, the effect did not extend to other structures in the body. Rogers *et al.* (2012) did not discuss whether this was the case in *E. texana*. This defense may be geared towards larger predators (*e.g.,* fish) or to cannibalistic conspecifics. The biting off of the cercopods of conspecifics was observed in *L. lemmoni* in the stock cultures by the author, as well as in other Notostraca

(*Triops longicaudatus* and *T. granarius*). This suggests that the regeneration of injured cercopods with longer spines may be a defense against cannibalism, and not fish predation, and therefore that cannibalism may be significant threat to *L. lemmoni*. Wild populations of *L. lemmoni* should be studied to determine how common cannibalism is in comparison to predation by other species.

This study represents the first investigation of anti-predator defenses in the Notostraca, and as such *L. lemmoni* cannot be compared to other Notostraca in this regard. However, the Notostraca display high intraspecific morphological variation (Longhurst, 1955). At least some of this morphological variability may be explainable as population-level adaptations to local predation pressures. Further research in this area would increase understanding of the mechanisms related to morphological variation in the Notostraca.

Figures and Tables

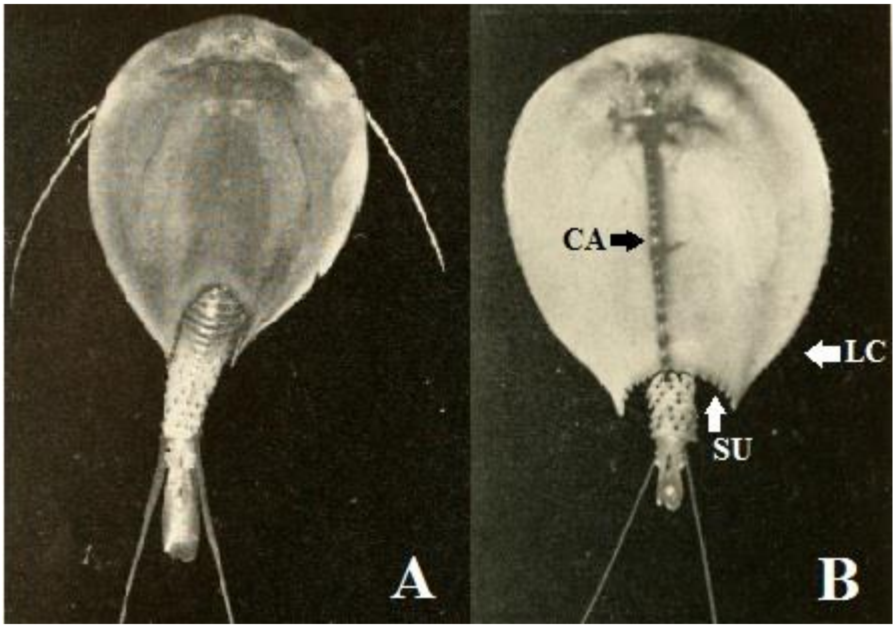

Figure 4.1– Typical form (A) and *echinatus* variety (B) of *Lepidurus lemmoni* from Linder (1952) (as *L. lynchi*). Note the heavily-spined carina (CA) and sulcus (SU) of the carapace, as well as the erect spines on the lateral carapace (LC) margins of the *echinatus* variety.

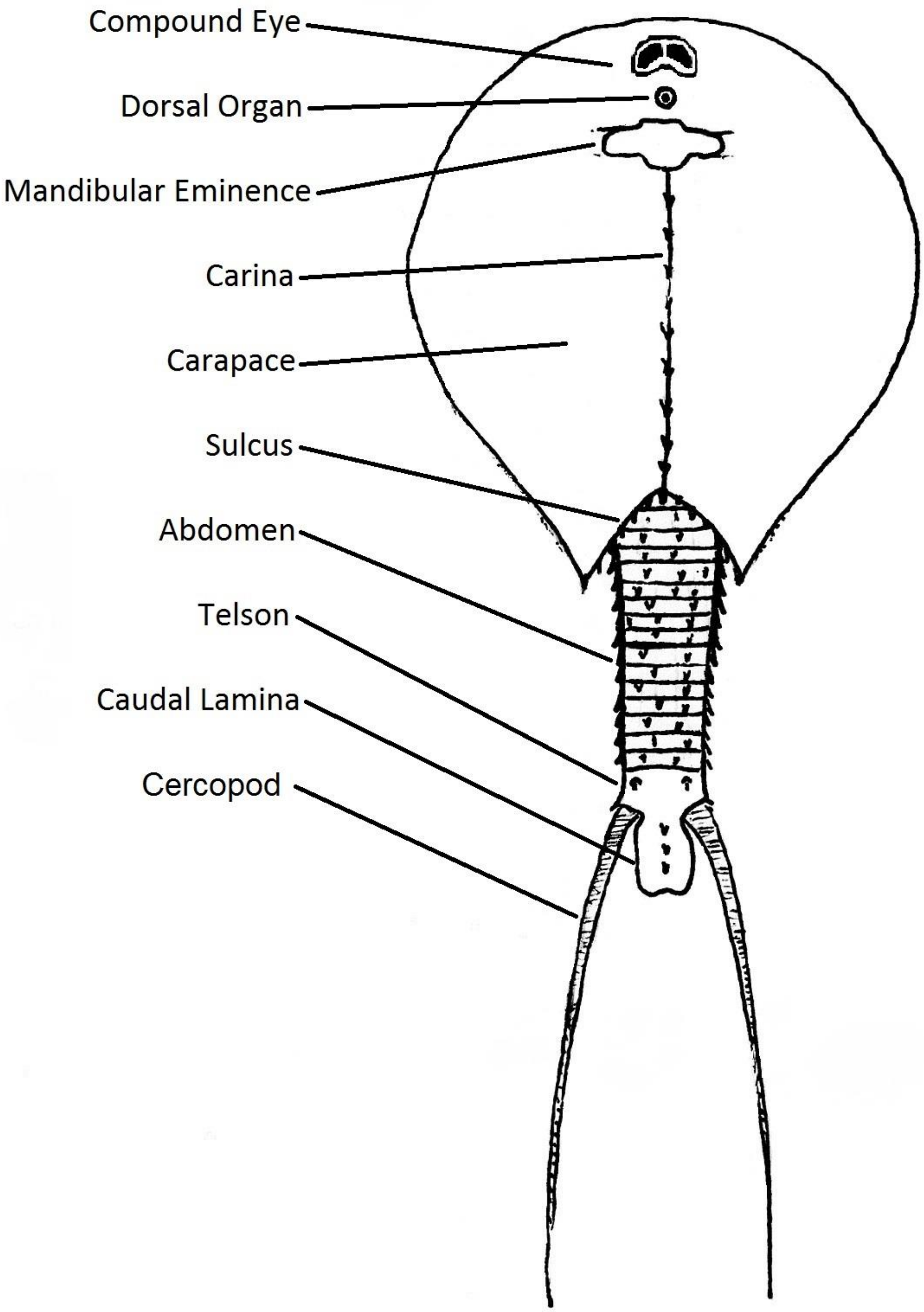

Figure 4.2 – Dorsal view of *Lepidurus lemmoni* with major morphological features labeled. Appendages and shell gland omitted. Figure drawn by the author.

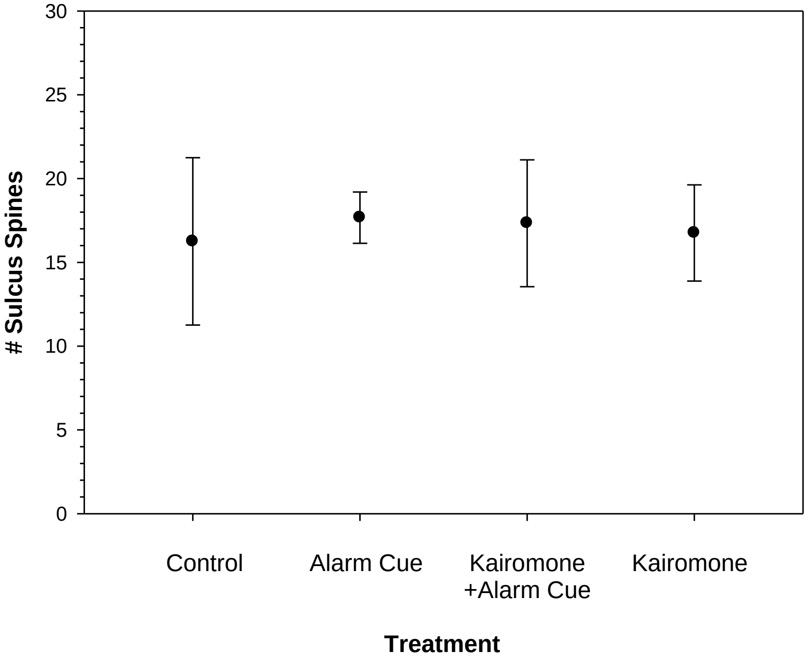

Figure 4.3 – Effect of alarm cues and kairomones on mean (±SD) sulcus spine count in adult *Lepidurus lemmoni* (mean±SD). All means among treatments were similar.

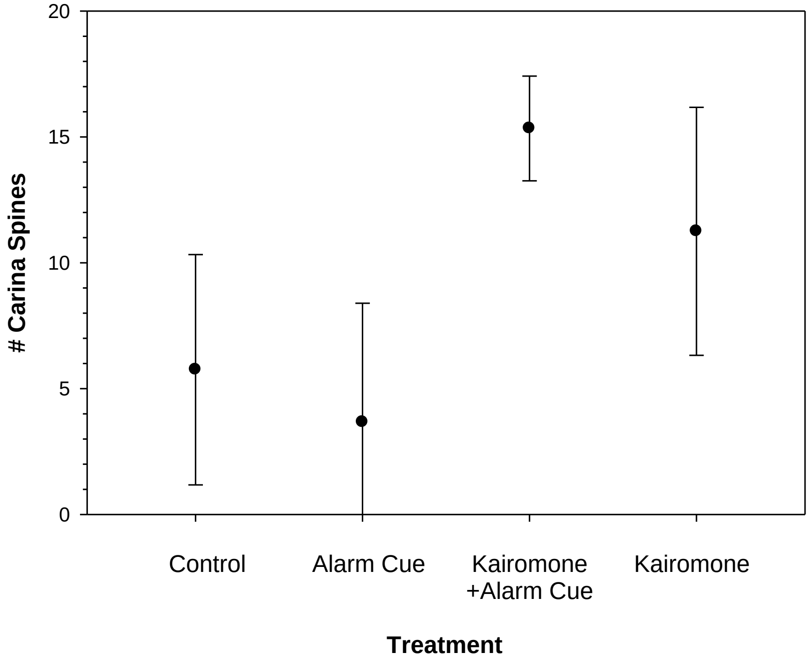

Figure 4.4 – Effect of alarm cues and kairomones on mean (±SD) carina spine count in adult *Lepidurus lemmoni*. All means among treatments were similar.

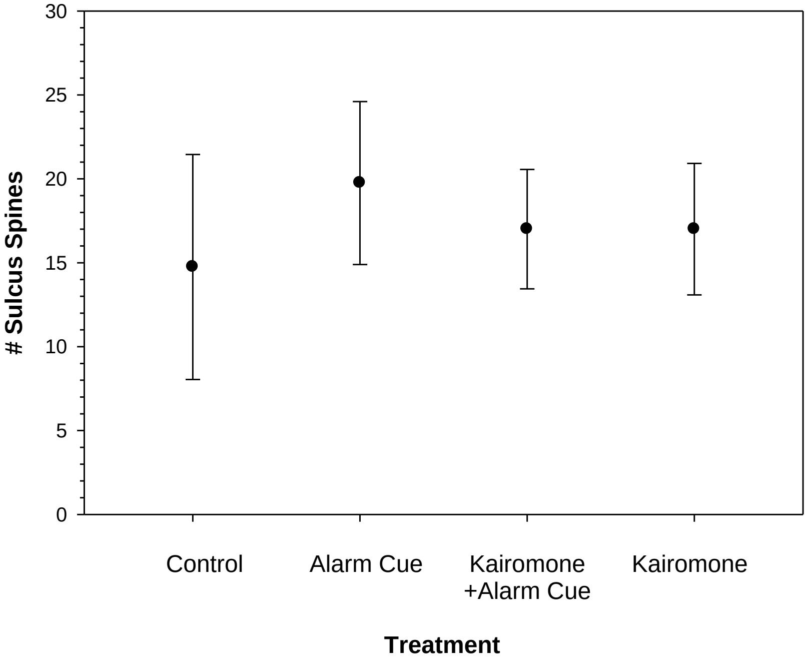

Figure 4.5 – Effect of alarm cues and kairomones on mean (±SD) sulcus spine count in juvenile *Lepidurus lemmoni*. All means among treatments were similar.

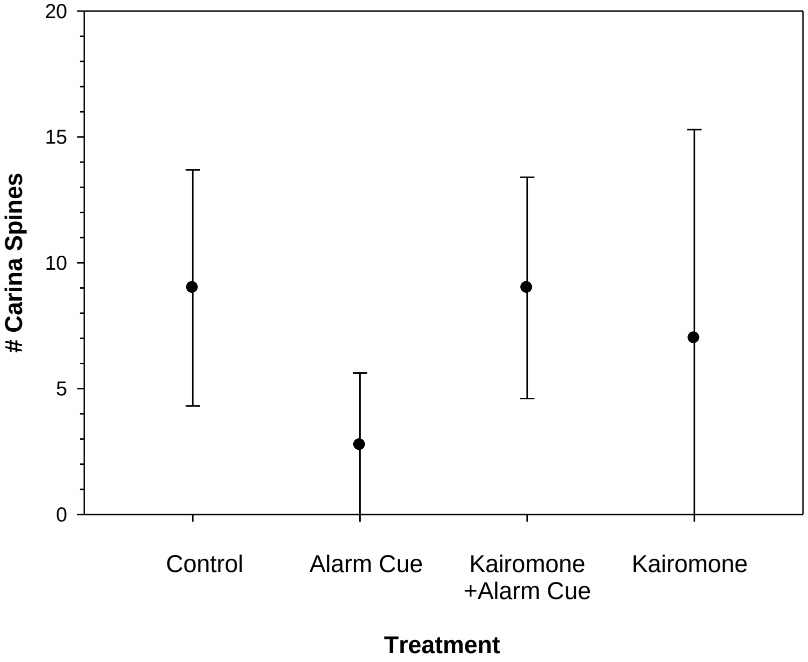

Figure 4.6 – Effect of alarm cues and kairomones on mean (±SD) carina spine count in juvenile *Lepidurus lemmoni*. All means among treatments were similar.

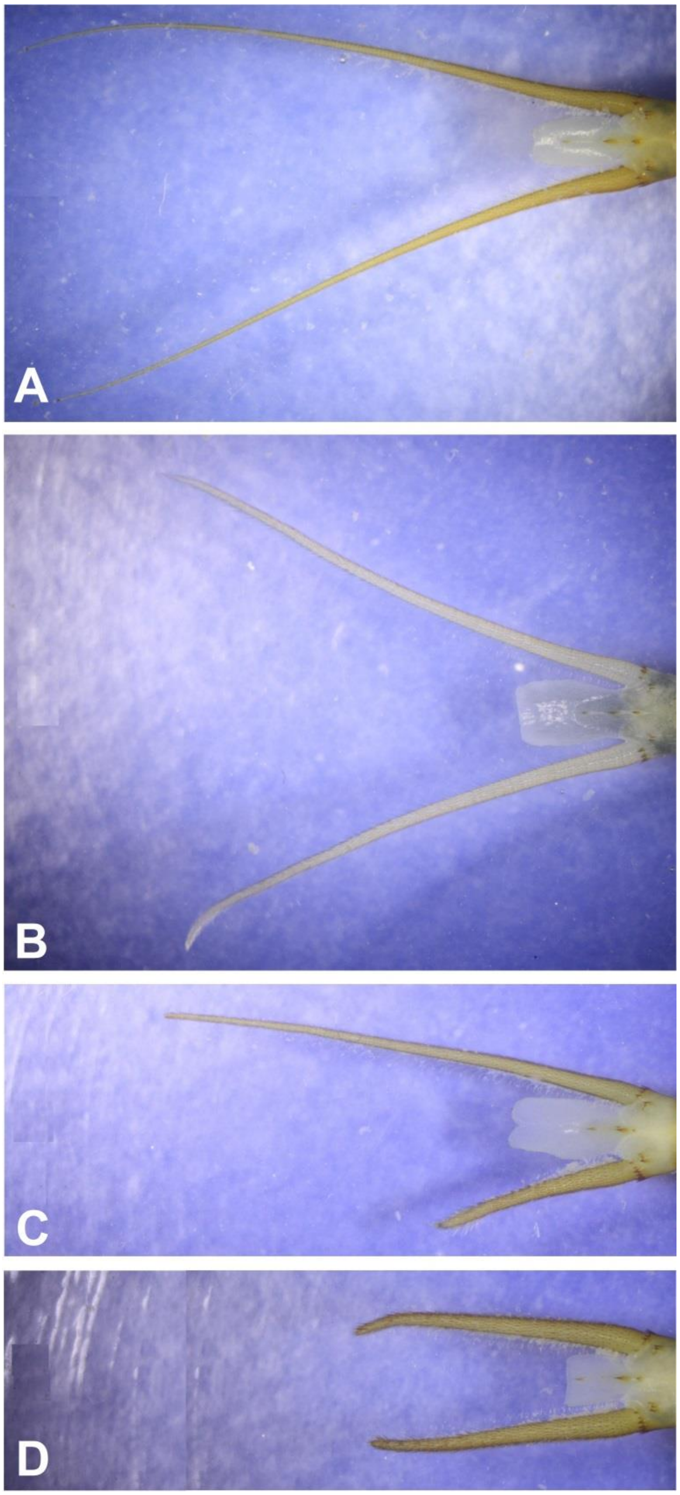

Figure4.7 – Regenerated cercopods of *Lepidurus lemmoni*. A, specimen from the control group with normal cercopods; B, specimen with cercopods that have nearly completely regenerated after removal; C, specimen with cercopods that have regenerated to different extents after removal; D, specimen with cercopods that have regenerated poorly after removal.

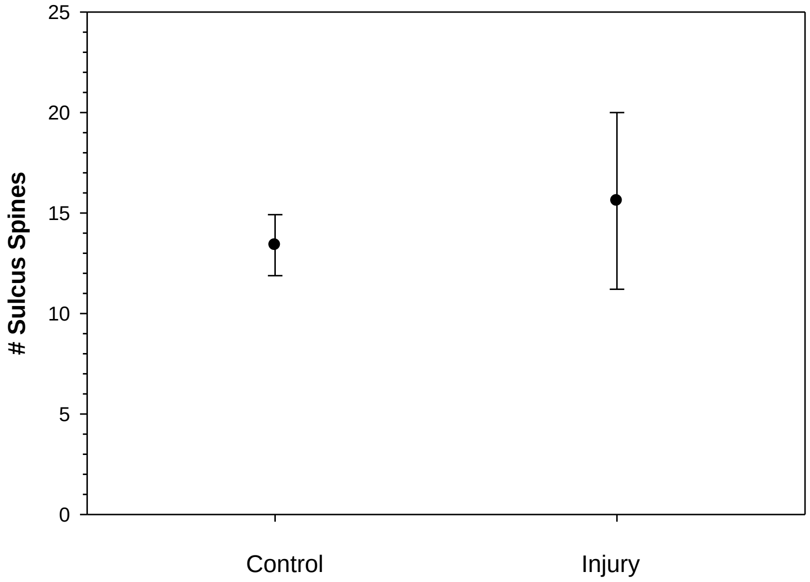

Figure 4.8 – Effect of non-lethal injury on mean (±SD) sulcus spine count in *Lepidurus lemmoni*. All means among treatments were similar.

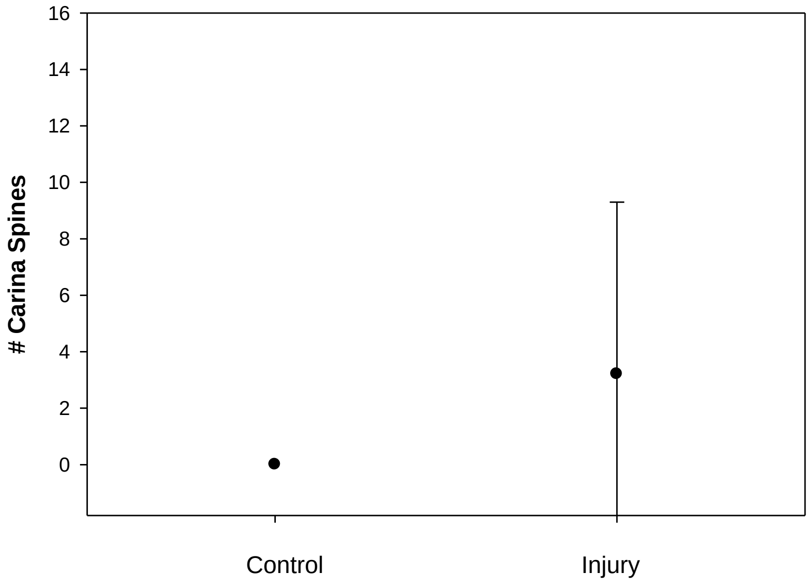

Figure 4.9 – Effect of non-lethal injury on mean (±SD) carina spine count in *Lepidurus lemmoni*. All means among treatments were similar.

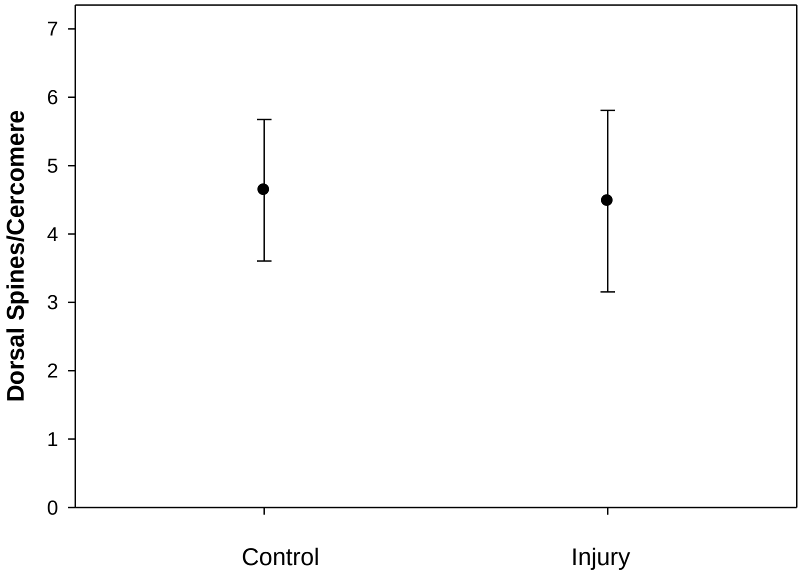

Figure 4.10 – Effect of regeneration on mean (±SD) cercomere dorsal spine counts in control and regenerated cercopods of *Lepidurus lemmoni*. All means among treatments were similar.

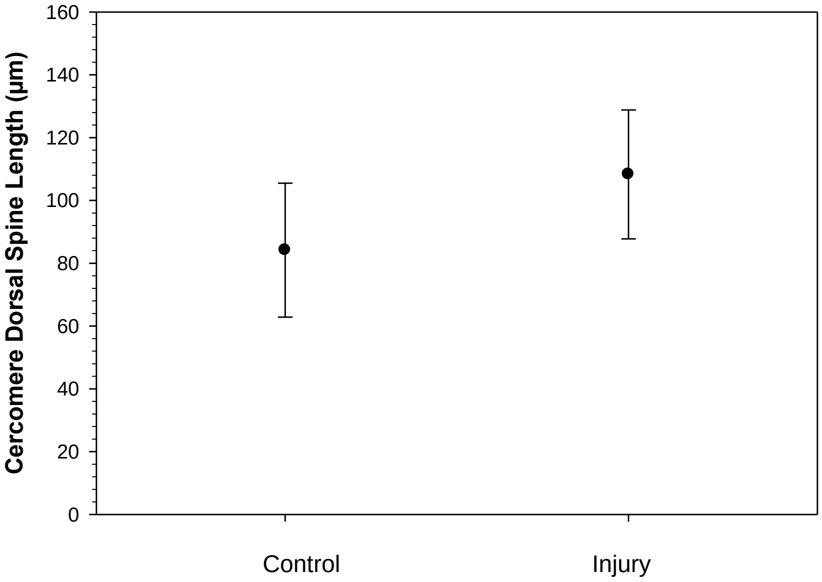

Figure 4.11 – Effect of regeneration on mean (±SD) cercomere dorsal spine lengths in control and regenerated cercopods of *Lepidurus lemmoni*. The treatments differed significantly from each other.

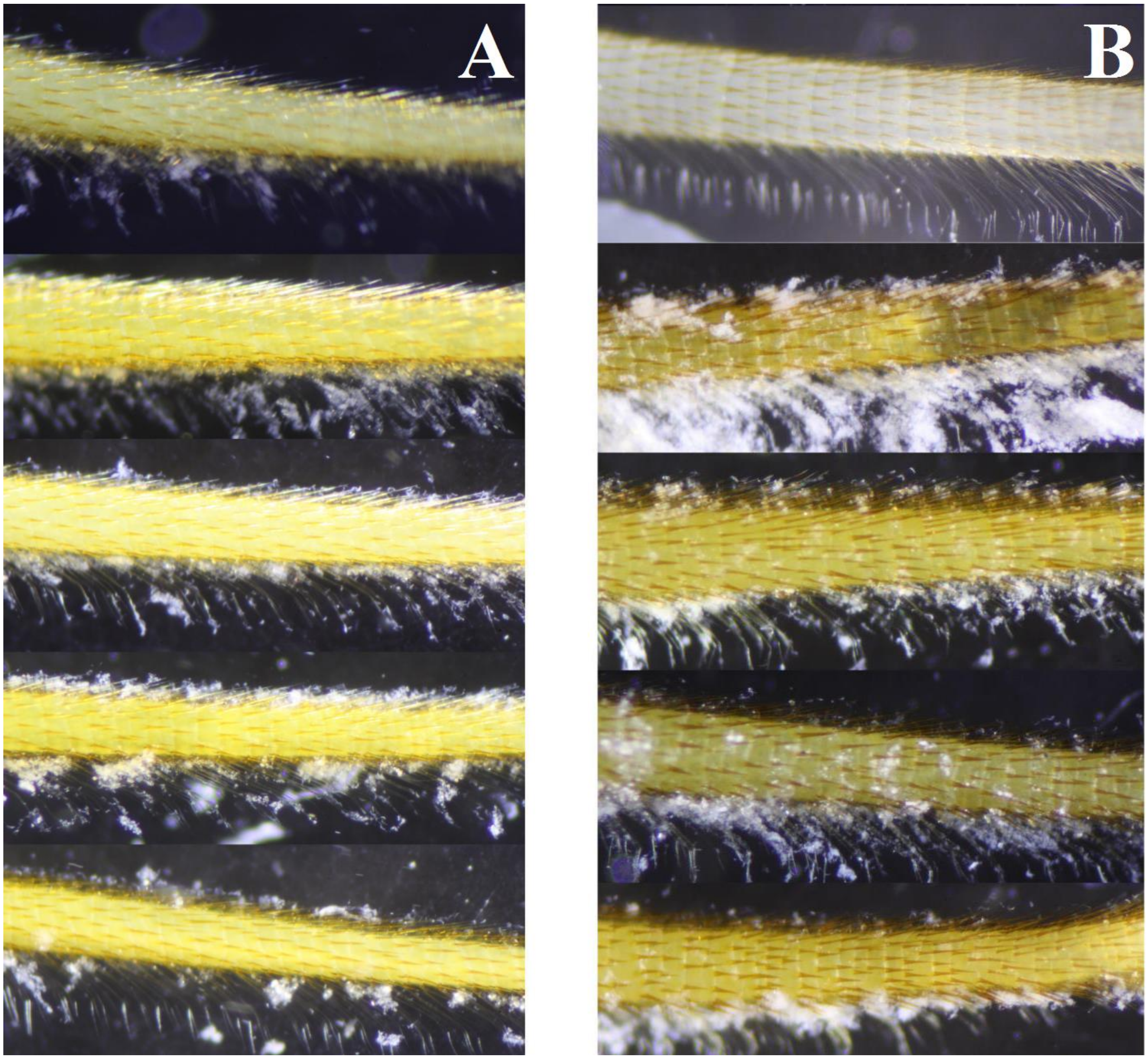

Figure 4.12 – Magnified view of (A) uninjured and (B) regenerated cercopods of *Lepidurus lemmoni*. Regenerated cercopods had longer, but not more dorsal spines on the cercopods.

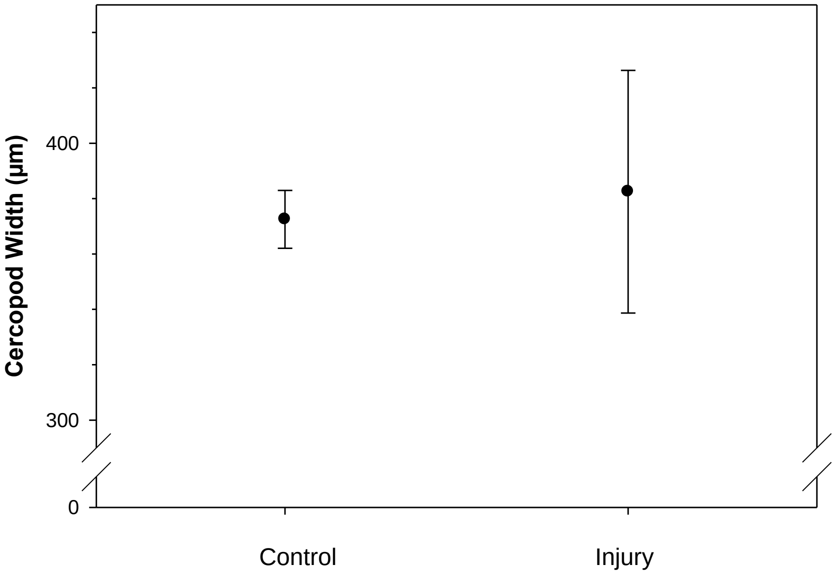

Figure 4.13 – Effect of regeneration on mean (±SD) cercopod width in *Lepidurus lemmoni*. All means among treatments were similar.

Chapter 5: A Note on the Temperature-mediated Body Morphology of the tadpole shrimp, *Lepidurus lemmoni*, from Goose Lake, Oregon, USA

The tadpole shrimp *Lepidurus lemmoni* Holmes, 1894 is a crustacean that lives in temporary, shallow, turbid, alkaline lakes with a pH of 8.2-11 (Lynch 1966) in western North America with a range from Alberta, Canada, south to Baja California, Mexico (Rogers, 2001). It is especially common in the Great Basin and Mojave Desert (Rogers and Hill, 2013). Its taxonomy has undergone several changes (Linder 1952, Lynch 1966) and has included the amalgamation of what appeared to be several morphologically distinct forms which were dismissed as ‘insignificant’ (Lynch 1966). Of particular interest here is the ‘normal’ body morphology and the ‘*echinatus*’ from which was only described from two lakes (Goose and Honey lakes, OR, USA) which are relatively permanent bodies of water, both only drying out every few years or decades, respectively (Lynch, 1966; Scheerer *et al*., 2010). Specifically, the normal body form is characterized by a weak carina with few or no small spines, small, sparse spines on the sulcus, and the weak spination on the carapace margins, while the *echinatus* form is characterized by a sclerotized carina, large carina and sulcus spines, and large, erect spines on the lateral carapace margins.

L. lemmoni is exposed to a number of predators throughout its range including *Dytiscus* spp., *Notonecta* spp. (Daborn, 1975), *Branchinecta gigas* (D.C. Rogers, Kansas Biological Survey, pers. comm.), and a number of wading birds (Plissner *et al*., 1999; Plissner *et al*., 2000; pers. obs.). However, the e*chinatus* form only occurs in lakes with fish, suggesting that it may represent a defense against fish. Although D.C. Rogers (Kansas Biological Survey, pers.

comm.) was able to induce the *echinatus* form by exposing individuals to alarm cues in the form of crushed conspecifics, I was unable to replicate this with alarm cues originating from crushed conspecifics or trout kairomones (Chapter 4). However, in the course of performing temperature-related experiments I observed the induction of the *echinatus* form. Additional analysis of these observations offers a potential explanation for the occurrence of the *echinatus* form.

While observing the growth rate of Goose Lake *L. lemmoni* in a laboratory mesocosm study at 20°C (Chapter 3), the carapaces of adults became increasingly spiny as carapace length increased. The largest specimens (ca. 17mm carapace length) even developed large, erect spines on the carapace margins (Fig. 5.1), and the carapace became sclerotized – all indications of the *echinatus* morphology. In contrast, *L. lemmoni* from Dowling Lake raised in the same mesocosm study remained relatively free of spines and displayed the normal body morphology (Figure 5.1).

At roughly the same time as the mesocosm study, I had 5 *L. lemmoni* from Goose Lake in a separate 127L aquarium left over from a previous experiment, which were maintained at a constant 13.5°C for general observations. These *L. lemmoni* were similar in size to the largest specimens from the laboratory mesocosm study (ca. 18mm carapace length), but had very small spines on the sulcus and carapace margin, and lacked any carina spines (Fig. 5.2), indicating the normal body morphology. Except for temperature, these cultures received identical treatment, such as addition of food, and air via an airstone.

To determine when the *echinatus* morphology developed in the 16 *L. lemmoni* from Goose Lake reared at 20°C, I established three body morphology categories progressing from normal to *echinatus* which included: smooth individuals with minute or lacking carina and sulcus spines, intermediate individuals with carina and sulcus spines, but lacking heavy sclerotization of the carina and erect spines on the carapace margins, and *echinatus* individuals with large carina and sulcus spines, a heavily sclerotized carina, and large, erect spines on the carapace margins. The *echinatus* morphology first appeared in individuals with a carapace length of 11.5 mm, while all individuals with a carapace length >17.5 mm had transitioned to the *echinatus* morphology (Fig. 5.2)

Temperature-induced defensive morphology has been observed in another branchiopod crustacean, the cladoceran *Daphnia lumholtzi* Sars, 1885 (Yurista, 2000). In this species, defensive spines form at 31°C, but not at lower temperatures. These spines disappear when cultures are maintained at 31°C for long periods, or if the temperature is decreased. The spines induced by high temperatures are larger than those induced by kairomones from planktivorous fish. It is hypothesized that temperature is a proximal cue for spine induction, and kairomones are required for maintenance of the defensive morphology.

The basal metabolic rate of fish scales positively with temperature (Clarke and Johnson, 1999), and consequently so does the amount of food required. Thus, fish predation becomes more intense with increasing temperature. It therefore follows that the *echinatus* form would develop at higher temperatures when fish predation would be more intense, and fail to develop at lower temperatures, when fish predation would be less intense. Whether the defensive response to temperature in *L. lemmoni* works synergistically with alarm cues or kairomones, as is the case in *D. lumholtzi*, is unknown and should be tested in future experiments.

The *echinatus* morphology appeared just after individuals reached sexual maturity, suggesting it may specifically protect individuals about to reproduce. Alternatively, the *echinatus* form may only be effective against gape-limited predators at a certain size. In this case, juvenile *L. lemmoni* would be better served using all available energy to increase carapace size and not spine development. Future experiments should test if the *echinatus* form is an effective defense against fish predation.

Figures and Tables

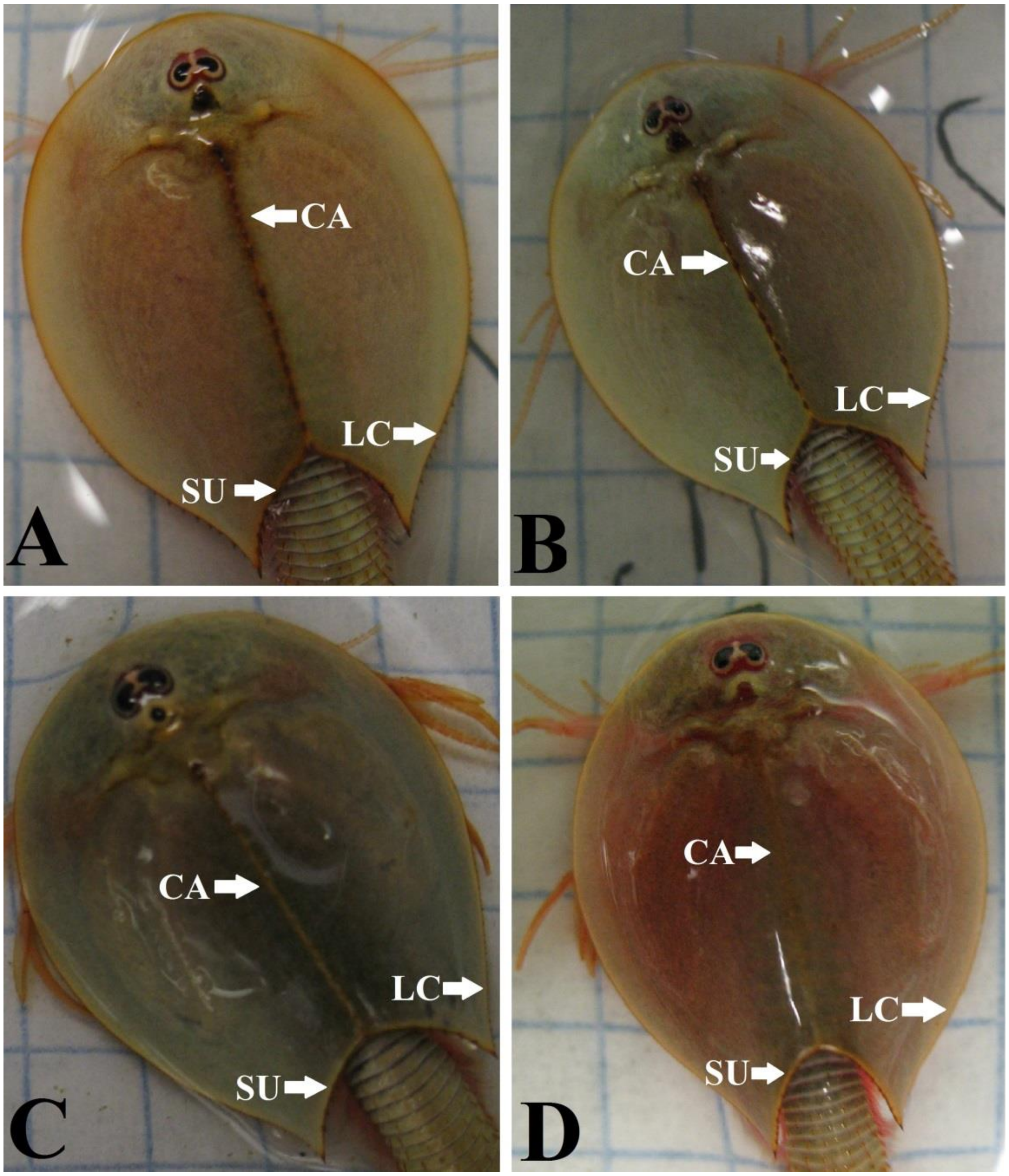

Figure 5.1 – Temperature-mediated morphology in *Lepidurus lemmoni*. A, Goose Lake *L. lemmoni* reared at 20°C, carapace length 16.52 mm; B, Goose Lake *L. lemmoni* reared at 20°C, carapace length 17.11 mm; C, Goose Lake *L. lemmoni* reared at 13.5°C , carapace length 18.82 mm; Dowling Lake *L. lemmoni* reared at 20°C, carapace length 18.49 mm. Note the sclerotized carina (CA), large carina and sulcus spines (SU), and large, erect spines visible on the posteriolateral carapace margins (LC) on specimens A and B displaying *echinatus* morphology, and the lack of spines on the carina (CA), the small, sparse spines on the sulcus (SU), and the weak spination on the carapace margins (LC) on specimens C and D displaying typical morphology.

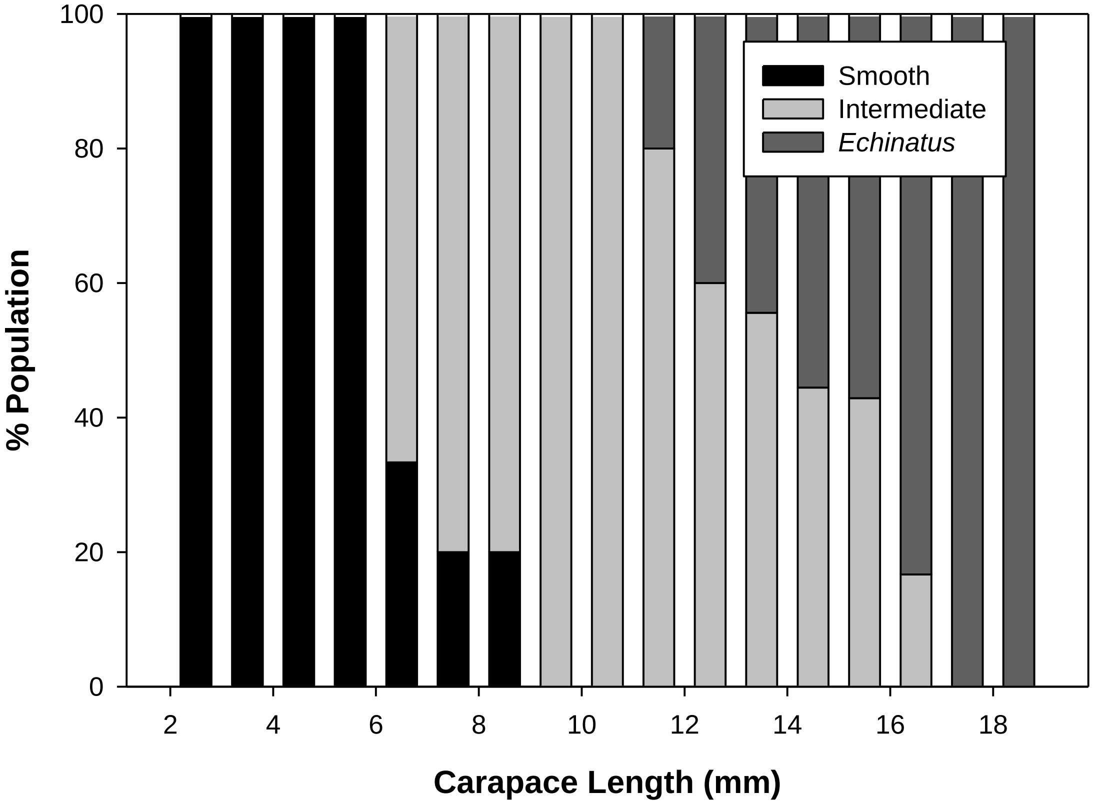

Figure 5.2 – Changes in body morphology in a population of laboratory-reared *Lepidurus lemmoni* from Goose Lake reared at 20 °C.

Chapter 6: General Summary

Lepidurus lemmoni Holmes, 1894 is a widespread, yet little-studied, animal. Before this study, all that was known about the biology of *L. lemmoni* was based on scant incidental observations and inferences from the primary literature based on other species in the genus *Lepidurus*. Nothing was known about its growth rate, incubation requirements, anti-predator defenses, or the variation in phenology in response to latitude. This thesis has provided information in all of these aspects of the biology of *L. lemmoni*.

I examined the phenology of *L. lemmoni* in mesocosms, finding that the northern population had a significantly faster growth rate than the central population, which I interpreted as adaptation to regional climate conditions. Due to the distance to the nearest *L. lemmoni* habitat, repeated observations on organisms in the wild could not be completed. However, the mesocosms were designed to mimic natural conditions as much as possible. The temperature and light was controlled to match the active period of *L. lemmoni*, and the mixing of lake sediments with distilled water should have produced water chemistry very similar to that of the natural habitat. Additionally, the resting eggs of various Anostraca (*Branchinecta gigas*, and *Branchinecta mackini* in Goose Lake or *Branchinecta readingi* in Dowling Lake) and other various microcrustacea (Cladocera, Copepoda, Ostracoda) in the lake sediments should have produced a reasonable analog to natural conditions. However, the laboratory mesocosms did not contain the influx of organic material that occurs in natural lakes from the overland flow of rain and snowmelt into the lake basin. The addition of prepared food may have made up for this lack of organic material, or added a confounding variable. Confounding aspects may have included contamination of the commercial diet that included unknown fish kairomones or alarm cues. Also, the relatively small size of the mesocosms may have allowed nitrogenous wastes to build up to levels not seen in nature, or caused unnaturally crowded conditions. Concurrent field observations would have allowed testing of the validity of the mesocosm experiments, and provided additional data on the phenology of *L. lemmoni*.

I examined the incubation requirements of *L. lemmoni* from northern and central populations, finding that the northern population differed from central populations in ideal incubation temperature. Egg hatch in the northern population was largely inhibited by low temperatures, while the two central populations showed the opposite pattern, with hatching being largely inhibited at high temperatures. These patterns may represent an adaptive response to local conditions. At higher latitudes, lakes may be more likely to refreeze early in the season, killing any *L. lemmoni* that hatched early in the season, so it is therefore disadvantageous to hatch at low temperatures. At lower latitudes, high temperatures may signal that the habitat will dry soon, and it is therefore disadvantageous to hatch at high temperatures. A warming climate may mean that northern lakes do not refreeze and remain inundated for less time. If this is the case, northern populations, which do not hatch at low temperatures, may not have enough time to complete their lifecycle before the lake dries. The northern populations may then be pushed northward or extirpated if appropriate habitat to the north does not exist. Central populations may be able to utilize these conditions to expand their range northward, becoming established in the lakes that once held northern populations.

Representatives from southern populations could not be obtained for the phenology and egg hatch experiments, despite several attempts. The sites at Edwards AFB contained remains of adult *L. lemmoni*, but no eggs. These sites also had flow marks in the sediments, suggesting that water had flowed through the area. In the large, shallow lakes that *L. lemmoni* inhabits, strong winds can push the lake substantially out of its basin (pers. obs.), carrying water and *L. lemmoni* into shoreline areas that are rarely inundated, and stranding the *L. lemmoni*. These flooded sites may remain inundated long enough for observation of adults, but apparently dry out before reproduction and oviposition can occur. All soil collected that did contain abundant *L. lemmoni* eggs came from lakes bisected by causeways with roads, which allowed easy access to the deepest, most reliably filled portions of the lakes. The large size of *L. lemmoni*'s habitat (some lakes exceeding 35km long) makes comprehensive sediment sampling difficult, especially without ready access to deep portions of the lakes via causeways. This may also explain the lack of success in collecting southern populations of *L. lemmoni*. Addition of a southern population, as well as more populations between the latitudinal extremes, to these experiments would elucidate any latitudinal trends in the data.

In chapter 4 I attempted to induce *echinatus* morphology, a putative defense against fish predation, by exposing Goose Lake *L. lemmoni* to alarm cues and fish kairomones. Due to the availability of a limited number of specimens, these experiments were carried out sample groups with few individuals. This, and large variation in morphological features within treatment groups, made separating the effects of treatment vs. control difficult. Repeating these experiments with much larger sample sizes would allow confident rejection of the hypothesis that the *echinatus* form can be induced by perceived predation pressure. Goose

Lake *L. lemmoni* did respond to non-lethal injury (bilateral removal of cercopods) by regenerating cercopods with longer spines on the cercomeres. I concluded that this most likely represented a defense against cannibalism, though testing the hypothesis that it is an effective defense against cannibalism through experimentation would strengthen this conclusion.

In chapter 5, I discussed some incidental observations made during other experiments. I observed that Goose Lake *L. lemmoni* spontaneously expressed the *echinatus* morphology when reared at 20°C, but stayed spineless when reared at 13.5°C. I also found that the *echinatus* morphology first occurred in 11.5mm carapace length specimens, but did not occur in 100% of the population until all individuals were at least 17.5mm carapace length. The *echinatus* morphology first appeared just after individuals reached sexual maturity, suggesting it may specifically protect individuals about to reproduce. Juvenile *L. lemmoni* may direct all available energy to growth and not spine development to reach sexual maturity as fast as possible. After the attainment of sexual maturity, energy may then be directed to spine growth to protect reproductive adults. The *echinatus* form may then only be an effective deterrant to fish predation in conjunction with large body size.

I concluded that the *echinatus* form was induced by high temperatures, and meant as a defense against the increasing feeding rates of fish at those higher temperatures, similar to that found in *Daphnia lumholtzi* Sars, 1885 (Yurista, 2000). While it seems odd that a putative defense against fish predation would be induced by temperature and not chemicals signaling the presence of fish (kairomones) or predation events (alarm cues), it must be remembered that all of these, temperature and chemical signals, are just proxies of the actual threat. In the case of *L. lemmoni*, fish predation may be negligible at lower temperatures, so then temperature would be a better signal for *echinatus* induction than fish kairomones. The hypothesis that alarm cues would induce the *echinatus* form was based on the assumption that Goose lake fish prey upon *L. lemmoni.* There is no direct evidence for this, so it is possible that fish have a non-consumptive, but still negative effect on *L. lemmoni* that requires morphological defense against. Fish may strike at *L. lemmoni*, causing stress and a damaging accumulation of singularly minor injuries. The *echinatus* form may protect *L. lemmoni* from nipping fish, which could otherwise result in declines in health and reproductive output, and potentially death in the long term. These results should be tested under direct experimental examination where the signal threshold can be found, the effectiveness of the *echinatus* form as a deterrent to fish predation can be examined, and where the hypothesis that alarm cues and kairomones can act synergistically with temperature can be tested.

All of the experiments in this thesis were plagued by low sample sizes, hampering analysis throughout. This is the direct result of the difficulty of culturing *L. lemmoni* in large numbers. While a high hatch rate was easily achieved in the laboratory, juvenile and adult *L. lemmoni* are cannibalistic, limiting the density of culture. Future studies should incorporate segregation of individuals, or separation of individuals into narrow size classes to minimize cannibalism and maximize culture production. Also, assumptions about diet were made when commercial tropical fish food was used. It was noted that the anostracan *Branchinecta mackini* Dexter, 1956 disappeared from the laboratory mesocosms following hatching of *L.*

lemmoni. The *B. mackini* may have been eaten by *L. lemmoni*, and a continuous supply of live food is needed for the maintenance of robust, high-density cultures of *L. lemmoni*.

This study has contributed to the information on the basic biology of *L. lemmoni*, including information on growth rate, incubation requirements, sexual dimorphism, and predator defenses. However, the lack of a southern population in these analyses limited my ability to infer patterns and draw conclusions about the biology of *L. lemmoni*, especially in regard to latitudinal gradients in hatching requirements, one of the main goals of my thesis. Further study, on more populations and more individuals, will add to this study, and allow a more complete understanding of the biology of *L. lemmoni*. Additionally, virtually nothing is known about the diet or genetics of *L. lemmoni*. Except for some brief observations on gut contents (Lynch, 1966), the diet of *L. lemmoni* is unknown. Future studies should explore the diet of *L. lemmoni*, and whether it varies by life stage or population. Genetic analysis, in addition to further examination of the phenology, should be performed on more populations of *L. lemmoni* throughout its range. This would also allow for better understanding of the number and sizes of distinct populations, which could guide decisions on the management and protection of distinct populations, and the species as a whole.

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