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PARENTAL INVESTMENT IN BURYING BEETLES (*NICROPHORUS INVESTIGATOR*): INFLUENCE
OF SEASONAL TIMING AND PRIOR EXPERIENCE
AND
EFFICACY OF MINIMALLY GUIDED INSTRUCTIONAL STRATEGIES IN K-12 AND
UNDERGRADUATE SCIENCE CLASSROOMS: A META-ANALYSIS

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ABSTRACT

This dissertation presents two independent research projects, a field study of an invertebrate species and an educational research meta-analysis. The underlying connection is science.

My biological research focuses on reproductive behavioral strategies in an insect known to demonstrate extensive biparental care, an especially uncommon characteristic among insects. My study expands upon life history and ecology studies of *Nicrophorus investigator* (burying beetles) in a montane ecosystem at Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. A number of physiological cues such as age and condition influence reproductive performance in *Nicrophorus investigator*, however environmental cues and their effects have received much less attention. Additionally, investigating physiological and seasonal influences on reproductive behavior under natural conditions has been less common than laboratory studies. I used lab and field studies to investigate the reproductive strategies of *Nicrophorus investigator*. Breeding beetles in the laboratory identified age related effects independent of environment. Breeding beetles exposed to natural environmental conditions added a realistic complexity not obtained in the lab and revealed environmental influences on reproductive investment.

My results suggest that age alone did not significantly reduce the brood success or parental investment behaviors of montane burying beetles, whereas investing in a brood did. In all cases, second broods were not as productive as first broods, even after a period of rest and feeding between reproductive events. I successfully delayed eclosion of adult beetles by manipulating the overwintering environment of pre-pupae by reducing the

temperature. Though limited, there is evidence of terminal investment behaviors, with adults that emerge late in the breeding season producing a larger brood. In conclusion, unmanipulated adult *N. investigator* can produce a comparable first brood at any time during the breeding season and retain the ability to produce a second brood in the event that the first fails or a second, appropriate breeding resource is discovered.

Environmental conditions limit *N. investigator*'s ability to breed toward the end of the constrained breeding season, but further studies are necessary to reveal responses to this environmental cue.

Educational initiatives in science, technology, engineering, and mathematics (STEM) have resulted in a large body of literature evaluating learning in STEM disciplines. The meta-analysis presented here focuses on science education in the United States and addresses the efficacy of minimally guided instructional strategies. It presents an updated synthesis of educational research across all science disciplines, utilizing a broad range of alternative teaching techniques, and includes K-12 and post-secondary settings. Meta-analysis of qualifying studies conducted in K-12 and undergraduate classrooms shows that minimally guided instructional strategies result in a positive increase in student achievement levels (cumulative effect sizes, respectively, $d = 0.31$, undergraduate $d = 0.44$). A rank-ordered list of study characteristics provides insight into the essential features of the most successful programs. A discussion of implications and areas for further consideration is included as well.

Chapter 1: Literature Review:

Life history theory, reproductive and parental investment strategies

ABSTRACT

My research investigates parental investment into offspring during reproduction in an invertebrate species that exhibits a unique combination of two life history traits, iteroparity (multiple reproductive bouts) and extended biparental care. I present a brief introduction to components of life history theory that affect the trade-offs organisms face when reproducing and caring for their offspring. I provide a historical account of the development of general life history theory as well as a description of specific reproductive strategies that influence an organism's ability to maximize its fitness. I analyze the costs, benefits, and implications of the trade-offs faced within these strategies, synthesizing empirical data from a wide range of species. I conclude with a review of the reproductive behavior and natural history of my research organism, *Nicrophorus investigator*, a montane species of burying beetle, and discuss its suitability for studies of life history evolution.

INTRODUCTION

A fundamental concept in biology is the efficient and effective partitioning of finite resources to maximize fitness. Life history theory seeks to explain variations among organisms in key behavioral, physical, and demographic characteristics that produce optimal strategies. Reproductive behaviors and life history patterns are coupled in that selection favors the behaviors and strategies that maximize fitness, under the constraints imposed by trade-offs in the partitioning of finite resources (Brommer 2000, Roff 2002, Kaplan & Gangestad 2004). Some of the characteristics involved in the study of life history theory are size at birth, growth rate, mortality rate, age-specific fecundity rates, and age and size at maturity (Stearns 2000, Roff 2002). These demographic relationships result in an integrated suite of physical and behavioral traits, observed throughout an organism's life (MacDonald 1997). Understanding life history patterns significantly contributes to predictions of organisms' responses to environmental perturbations or selective pressures (Brommer 2000, Kaplan & Gangestad 2004).

My research focuses on seasonal timing of life cycle stages, number of reproductive attempts and age-specific reproductive investments, including number and size of offspring and parental care, for a species of burying beetle, *Nicrophorus investigator*. Specifically, I investigate the possibility that *Nicrophorus investigator* exhibits adaptive plasticity in the duration of parental care in response to previous reproductive experience or seasonally induced time constraints. Together, life history theory supported by empirical evidence offer a strong approach to understanding how reproductive and parental care behaviors might differ under varying environmental conditions (Harris & Uller 2009).

TIMING OF LIFE CYCLE STAGES

Daily and seasonal cycles are important to most terrestrial animals (Bradshaw *et al.* 2006). A self-sustained circadian clock typically aids in regulating daily activities in eukaryotes (Dunlap *et al.* 2004); a number of plants and animals utilize the length of day to regulate seasonal activities (Garner & Allard 1920, Truman 1971). Organisms have evolved adaptive responses to daily and seasonal environmental cues to exploit favorable conditions (Tauber & Tauber 1976, Saunders 1980, Saulich & Musolin 1996, Bradshaw & Holzapfel 2007).

Photoperiod (Garner & Allard 1920) strongly influences plant reproductive processes as well as most aspects of insects' lives (Marcovitch 1924). As a result, the functions of clock mechanisms resulting in responses to circadian and seasonal rhythms have received considerable research. A landmark reference in the field of insect seasonality was published by Danilevskii in 1965 and was the first to formally address geographical implications in the study of photoperiodism (Takeda & Skopik 1997). Photoperiodism affects insect life histories and results in evolutionary responses to rhythms of the biosphere, including recognizing optimal seasonal reproductive periods (Gotthard *et al.* 1999, De Block & Stoks 2003, Homeny & Juliano 2007). Species that are subjected to seasonal time constraints and respond to photoperiodism are expected to exhibit marked developmental and behavioral plasticity (Johansson & Rowe 1999).

Holometabolous insects undergo complete metamorphosis, dramatic changes in different developmental stages. The most common stages of insect metamorphosis include eggs, larvae, pupae, and adult. During metamorphosis, wings are developed

internally and adult insects emerge (eclose) from the pupal stage (imaginal ecdysis) according to species specific circadian rhythms (Beck 1980). One benefit of two distinct developmental stages is to separate adults from the immatures is to reduce intraspecific competition between mature and immature stages and weaken predation and parasitism pressures (Grimaldi & Engel 2006, Gotthard 2008). Environmental stimuli influence the process of metamorphosis as well as other life history traits, including times of activity, growth, reproduction, and diapause (Tauber & Tauber 1976, 1978).

To synchronize growth and reproductive activities with suitable environmental conditions, a number of insects and acarines undergo a state of relative dormancy, called diapause (Tauber & Tauber 1976). Insects may undergo diapause in any of their life-cycle stages cued by daily or seasonal changes. Diapause can be induced with a combination of environmental cues, including photoperiod and temperature, and can occur in organisms avoiding extremely cold temperatures [overwintering] or avoiding extreme heat [estival diapause] (Beck 1980). Diapause involves physiological changes such as: 1) a decrease in metabolic rate, 2) suppression of growth, 3) a decrease in body water content, and 4) an increase in accumulation of body fat reserves (Beck 1980). Some insects enter diapause as mature, larval instars, overwinter as pre-pupae, and immediately pupate upon the termination of diapause, eclosing in the spring as adults (Beck 1980, Wolda 1988). Although the events that initiate the cessation of diapause are still unknown, there are a number of factors that contribute to its termination. In some species, a cue for diapause to terminate is long day-length coupled with exposure to cold temperatures for an extended period of time (Špicarová 1972, Tauber & Tauber 1976, Wolda 1988). For a given species, the timing and duration of diapause is dependent of

the life history characteristics as well as environmental stimuli that induce and terminate the process.

One consideration for organisms that undergo complete metamorphosis is the degree to which the larval development influences the adult phase (Rowe & Ludwig 1991, Blake *et al.* 1996, Pechenik 2006, Gotthard 2008). Studies have shown that in most holometabolous insects, there is a significant correlation between the two life stages for a species' physiologic state (Pechenik & Eyster 1989, Pechenik 2006). However, molecular markers have shown that no link is expressed between larval and adult biological clocks. Essentially, the process of metamorphosis in these species, including *Drosophila melanogaster*, resets the physiological clock and upon eclosion, the adult is 'newborn' (Blake *et al.* 1996).

NUMBER OF REPRODUCTIVE ATTEMPTS

The critical component in maximizing fitness is reproduction (Stearns 1976). The spatial and temporal spread of reproductive effort can be described as a continuum with semelparity and iteroparity as the alternate strategies (Verberk *et al.* 2008). Semelparity is reproducing only once in life (Cole 1954, Fritz *et al.* 1982). This strategy is favored when juvenile survival probability is high compared to adult survival (Murphy 1968). Iteroparity is repeated reproduction and is a strategy to distribute reproductive effort over time and space (Cole 1954, Fritz *et al.* 1982). This strategy is favored in variable environments where the probability of adult survival is greater compared to juvenile survival (Stearns 1976) and is common among insects (Fritz *et al.* 1982, Tallamy & Brown 1999). Field observations and studies have revealed unexpected combinations

and strategies exhibited along the semelparity-iteroparity continuum in plants, insects, and fish (Tallamy & Brown 1999, Hautekèete *et al.* 2001, Lesica & Young 2005, Christiansen *et al.* 2008).

Among plants, several species are known to possess plasticity in their reproductive strategy and depending upon environmental conditions, can exhibit either semelparity or iteroparity (Lesica & Young 2005, Hautekèete *et al.* 2001, Young & Augspurger 1991). Facultative iteroparity (or facultative semelparity) describes some insects (Tallamy & Brown 1999) and fish (Christiansen *et al.* 2008). These organisms, while usually semelparous, are iteroparous under exceptionally favorable conditions. Burying beetles (*Nicrophorus investigator*) have been classified as facultatively iteroparous (Tallamy & Brown 1999).

REPRODUCTIVE INVESTMENTS

Life history theory provides a robust framework for understanding specific species characteristics and behaviors. Partitioning resources in order to maximize fitness within an organism's lifespan can include complex life cycle stages, seasonal time constraints and diverse reproductive strategies. Flexibility within life history traits is documented to be adaptive and while this plasticity complicates the study of life history theory, it illustrates the diversity of the natural world (Rowe & Ludwig 1991, Gotthard *et al.* 1999, Homeny & Juliano 2007). Insects, in particular, display a diverse range of unique reproductive tactics that are adaptive responses to environmental or seasonal time constraints (Johansson & Rowe 1999, Homeny & Juliano 2007). Some of the early ideas that contributed to the overall development of life-history theory have also proven to be

cornerstones in the study of specific reproductive investments. According to Stearns (1992) over forty-five of these reproductive investments and resulting trade-offs have been described through a number of theoretical models and empirical studies. This review focuses on the traits applicable to my research project and while they are addressed somewhat independently for organizational purposes, they are complicated components of life-histories interwoven with one another.

AGE AT REPRODUCTION

Historical perspective

Maturity is the pivotal point at which an organism moves from a life of preparation to a life of reproduction (Stearns 1992). For animals, age at maturity is defined as age at first reproduction and according to senescence theory (Williams 1957), this is also the age at which mortality rates begin to increase. Since Edney and Gill's (1968) demonstration that altering the age at which organisms are allowed to breed influences lifespan, studies of reproductive maturity have moved in two directions (Promislow & Bugbee 2000).

One approach has been to study sexual maturity, the age at which an organism is physically capable of reproduction, and its effects on lifespan (Promislow & Bugbee 2000, Koons *et al.* 2008). Early sexual maturation typically leads to shorter lifespan (Gadgil & Bossert 1970) and increased survival to maturity, due to the short amount of time spent as a juvenile (Bell 1980). Later maturity typically leads to longer life span, with lower juvenile mortality rates (Bell 1980, Stearns 1992). Later maturation would be selected for in constant environments when the result is greater reproductive output.

However, constant environments are rare (Koons *et al.* 2008). In fact, uncertain environments favor late maturation (or delayed reproduction) in semelparous organisms and earlier maturation in iteroparous species (Koons *et al.* 2008).

In addition to age at maturity, research has also focused on the influence of demographic delays in reproduction, or the age at which an organism actually breeds, on life history traits (Promislow & Bugbee 2000). Williams (1957) predicted that selection for decreased ‘vigour’ in youth should lead to increased longevity. Investigating demographic delays in breeding addresses this prediction. Individuals that are unable to breed until later in the reproductive season are expected to not only live longer (Williams 1957), but are most likely to exhibit adaptive phenotypic responses resulting from the seasonal time constraints (Carrière *et al.* 1996, Gotthard 1998, Johansson & Rowe 1999, Homeny & Juliano 2007). For example, damselflies, *Lestes viridis*, that hatch into adults while exposed to a photoperiod that resembles late in their breeding season decrease their development time and increase their growth rate (De Block & Stoks 2004).

Empirical data

Delayed reproduction has been shown to influence several life history traits, although the patterns are not consistent among species. In some cases, longer life span is genetically correlated with forced delays in reproduction in *Drosophila melanogaster* (Rose 1984, Partridge & Fowler 1992, Partridge *et al.* 1999). Willow-carrot aphids, *Cavariella aegopogii*, and vetch aphids, *Megoura viciae*, also exhibit a positive effect from delayed reproduction, an increase in size and fitness of offspring (Dixon *et al.* 1993). Moreover, seed beetles, *Acanthoscelides obtectus*, utilize a better developed reproductive system when forced to breed later and therefore also gain increased fitness

(Maklakov *et al.* 2007). However, there are a number of instances wherein the delay results in decreased fitness. The beet armyworm, *Spodoptera exigua* (Hübner), exhibited decreased longevity as well as decreased fitness the longer that reproduction was delayed (Rogers & Marti Jr. 1997). The longevity of the European corn borer, *Ostrinia nubilalis*, is not affected by delayed reproduction. However, fitness declines significantly as older females are unable to access spermatophore nutrients to the same extent as younger females (Fadamiro & Baker 1999). Cockroaches, *Nauphoeta cinerea*, also exhibit an age-related decline in fecundity due to delayed reproduction (Moore & Moore 2001). Older females take extended time to produce their first brood and the numbers of offspring in each brood throughout life are significantly reduced compared to females that reproduce early in life. A follow-up study revealed that older females experience this reduction in fitness because they lose oocytes via apoptosis (Vaux & Strasser 1996) while early reproduction allows oocytes to be protected in younger females (Moore & Sharma 2005). Univoltine crickets that are subjected to strict seasonal time constraints experience significantly reduced fitness when forced to eclose later in the reproductive season (Carrière *et al.* 1996); in spite of their physiological youth relative to their early-emerging conspecifics (Blake *et al.* 1996).

Central to all life history traits, including age at reproduction, is the phenomena of trade-offs in allocation of resources. Other than supporting Williams' (1957) general prediction that decreased vigor in youth leads to increased longevity, the evidence does not seem to indicate a clear trend in the influence of demographic delays in reproduction (Clutton-Brock 1984). Constraints of empirical studies often lead to the use of a relatively small number of state variables as indicators of fitness (Ericsson *et al.* 2001).

The differential allocation of resources according to species specific life history traits means that there can be any number of fitness outcomes due to delays in reproduction. As a result, age-specific variation in reproduction is difficult to predict at large taxonomic levels and much more complex than has been appreciated (Reid *et al.* 2003, Lock *et al.* 2007). As indicated by the varying fitness outcomes presented above, strong consideration must be given to the species specific level of parity, an individual's state of development, experience, and senescence, as well as the influence of population density (Clutton-Brock 1984, Reid *et al.* 2003). Empirical studies from natural populations is needed to develop and explore life history theories (Reid *et al.* 2003, Lock *et al.* 2007). In particular, the study of life history theory must include adaptively flexible reproductive strategies, as well as effects of seasonal time constraints, and constant versus variable environments (Gotthard 1998, De Block & Stoks 2003, 2004).

NUMBER AND SIZE OF OFFSPRING

Historical Perspective

Smith and Fretwell (1974) provided the first formal graphic and mathematical model calculating the size and number of offspring a female must produce in order to maximize fitness (Messina & Fox 2001). However, studies regarding optimal offspring number began much earlier such as with Lack's work on avian clutch size (1947). Lack (1947) observed an inverse relationship between numbers of nestlings and nestling survival and hypothesized that there would be an intermediate number of offspring that would produce the most fledglings thereby maximizing parental fitness. However, since then, several factors have been identified that result in a clutch smaller than the optimal

“Lack size” (Messina & Fox 2001). Charnov and Skinner (1984) pioneered the use of foraging theory, the marginal value theorem, to describe the optimal number of eggs in a clutch of parasitoid wasps. They established that for organisms that distribute offspring across several resource areas, the optimum number of eggs at one host site is a function of the costs of finding and handling additional hosts (Messina & Fox 2001).

Physiological constraints on production of eggs may also contribute to smaller optimal clutch numbers (Roff 1992). Finally, bet-hedging can be selected for in heterogeneous habitats, reducing the variance of success and reducing an organism’s risk of reproductive failure in harsh conditions (Messina & Fox 2001).

The seminal Smith-Fretwell (1974) paper relating offspring size and number is based upon two assumptions: (1) there is a trade-off between offspring number and size, and (2) there is a direct relationship between offspring fitness and increased parental investment per offspring (Winkler & Wallin 1987). Smith and Fretwell (1976) also assume that the total quantity of resources allocated to reproduction is fixed, although it is actually quite variable in most situations. For example, variation in parental care behaviors (see below) or acquisition of resources as an adult would alter the amount of resources allocated to reproduction (Messina & Fox 2001). Although the Smith-Fretwell model applies to a wide range of reproductive behaviors, across many taxa, there are some instances when it does not provide accurate predictions about the investment relationship. Such cases include individuals experiencing postpartum estrus or partitioning of energy by other than the parents in addition to instances when selection actually favors decreased fecundity or there are physiological constraints to producing

offspring at either end of the size spectrum (Smith & Fretwell 1974, Messina & Fox 2001).

The Smith-Fretwell (1974) model provided the springboard for further exploration into the evolution of the offspring size and number trade-off and its premise remains at the core of current, more complex models. Winkler and Wallin (1987) distinguished between the parents' total reproductive effort and the effort for each individual offspring. They developed a more precise method of predicting optimal total effort expended by considering: (1) effort per offspring, (2) the ratio of realized juvenile survival rate to the maximal adult survival rate, and (3) the threshold at which adult survival rates dramatically suffer from increasing effort (Winkler & Wallin 1987). At the same time, McGinley *et al.* (1987) was investigating offspring investment in variable environments. Optimal offspring size in a spatially heterogeneous environment (without regard for offspring density) is the mean of the optimum in each separate habitat (McGinley *et al.* 1987). When considering offspring density, the model indicates that offspring of variable sizes are only more favorable in a very limiting set of circumstances, like the parent being able to guide offspring dispersal according to offspring size. If there is a strong likelihood that dispersal will be random, an intermediate offspring size is optimal (McGinley *et al.* 1987). The one instance in which McGinley *et al.* (1987) suggested that variation in offspring size would be advantageous is in an environment that experiences temporal variability, both within- and between-year.

Empirical data

Seed beetles (*Stator limbatus*) exhibit egg size plasticity, and in turn, a trade-off between egg size and egg number. Female seed beetles can facultatively adjust egg size based on the host plant upon which they are ovipositing, large eggs on the blue paloverde and small eggs on the catclaw acacia (Fox *et al.* 1997). The interaction between egg size and residual reproductive value means that females laying eggs on paloverde exhibit decreased fecundity (Parker & Begon 1986). In the parent bug (*Elasmucha grisea* L.), female size and clutch size are positively correlated (Mappes & Kaitala 1994). The optimal clutch size is a function of the female's size and her ability to ward off predators. For carabid beetles (*Notiophilus biguttatus* F.), phenotypic variation in reproductive effort could mask trade-offs between egg size and number, but some notable trends have been detected (Ernsting & Isaaks 2000). Increases in environmental temperatures increased the number of total eggs laid, the total egg mass, and these eggs were significantly smaller compared to eggs laid at lower temperatures. The connection between environmental variability and brood size has also been established in dung beetles and bees. Brood mass is directly related to resource quality; which, because it is unpredictable can lead to seasonally unpredictably large broods late in the breeding season for dung beetles, *Onthophagus ater* (Kishi & Nishida 2009). In a leaf-cutter bee, *Megachile apicalis*, brood size is also directly related to resource quality which is also directly related to seasonal progression (Kim & Thorp 2001). One explanation for the seasonal shift in offspring size and number exhibited in the side-blotched lizard, *Uta stansburiana* Baird and Girard, is the 'bet-hedging hypothesis' (Nussbaum 1991). Smaller clutches of relatively larger offspring can be selected for in uncertain

environments because they are less likely to fail, resulting in the observed shifts during a lifespan (Stearns 1976, 1992).

Offspring size and number relationships take on particularly interesting dimensions for holometabolous insects, whose fitness outcomes are influenced by both larval survival and adult eclosion. For example, the costs and benefits associated with size at metamorphosis can vary within a season and there can be time constraints on size at emergence (Rowe & Ludwig 1991). Decreased size at emergence of individuals reared under high time stress (imposed seasonal time constraints) is evident in numerous insect families (Satyrine butterflies: Nylin et al. 1996, Gotthard 1998; damselflies: Johansson & Rowe 1999, De Block & Stoks 2004, Strobbe & Stoks 2004, Śniegula & Johansson 2010; caddisfly: Shama & Robinson 2009; horned beetles: Plaistow *et al.* 2005; crickets: Carrière *et al.* 1996). For *Gryllus pennsylvanicus*, significant fitness costs are incurred by early-emerging individuals exposed to low temperatures as well as late-hatching individuals experiencing decreased growth resulting from the shortened favorable season (Carrière *et al.* 1996). Conversely, increased resource availability or decreased predation pressures is known to increase fitness for some early-emerging individuals (Leimar 1996, Johansson et al. 2001, Orizaola et al. 2010).

The Smith-Fretwell (1974) model provides a general representation of the trade-offs predicted for number and size of offspring. It provides the broad underpinning upon which empirical studies are based and the intricate nuances of specific species life history strategies can be investigated. Size and number of offspring plays an important role in maximizing fitness and is predicted to be directly related to levels of parental care (Clutton-Brock 1991, Roff 2002).

PARENTAL CARE

Historical Perspective

Williams (1966), Trivers (1972), and Maynard Smith (1977) were the early life history pioneers who focused on reproductive strategies specifically related to parental care. Although Lack (1947) acknowledged partitioning of resources as a limiting factor in clutch size, he was initially looking for trends in the number of eggs laid and it was not until later that data was extrapolated to include adult survival and associated parental care investment (Roff 1992, 2002). Of these possible investments, parental care and the degree to which it changes under variable conditions most informs my research project.

Williams illustrated the first formal appreciation for the costs involved in parental care (Clutton-Brock 1991). Historically, Williams was the first to represent an organism's behaviors in terms of a cost/benefit ratio, and it was upon this foundational idea that Trivers' built the structural framework for current theories in parental care (Clutton-Brock 1991, Roff 2002). Trivers (1972) coined the term *parental investment* and provided seminal definitions (Clutton-Brock 1991, Roff 2002, Houston *et al.* 2005). According to Clutton-Brock (1991), in addition to arguing for the intricate link between parental investment and sexual selection, Trivers' notably emphasized that there are both pre-zygotic and post-zygotic ways in which parents contribute to their offspring and that invariably, investment in one offspring precludes investment in another offspring at the same time. At the same time, E.O. Wilson (1971) dispelled the myth that insects were too primitive to exhibit parental care (Tallamy & Schaefer 1997).

Maynard Smith's (1977) analysis of parental investment contributed a new perspective on animal behaviors being shaped according to future expectations and not solely on past expenditures (Houston *et al.* 2005). He developed models of sexual strategies in relation to parental care and the likelihood of desertion combined with the chances of the deserting mate finding additional breeding opportunities. In short, his analysis indicated when, in a constant habitat, males and females might be most likely to cease parental investment behaviors in favor of desertion and seeking additional copulations (Maynard Smith 1977). He concluded his paper with a brief review of sexual strategies in major vertebrate groups reinforcing the intricate complexities within life history traits, providing a foreshadowing of the diverse reproductive strategies among invertebrates.

Parental investment is known to include diverse parental care behaviors (Clutton-Brock 1991). These behaviors might be indicative of differential allocation or reproductive compensation exhibited by some species when presented with variable mate quality, for example, increased egg production in relation to an attractive mate (de Lope & Møller 1993, Weddell 1996, Cunningham & Russell 2000, Galeotti *et al.* 2006, Harris & Uller 2009). Additionally, investment might range from pre-copulatory behaviors, such as preparation of nests, to post-zygotic care, such as social assistance provided to mature offspring (Clutton-Brock 1991). In addition to broadening the range of parental care behaviors, Clutton-Brock identified further costs involved with parental care such as the energy expenditure of breeding, investment in propagule size, viviparity and incubation, gestation, and lactation and how these may reduce the parent's residual reproductive value. Finally, Clutton-Brock formally recognized the sheer complexity and

how a considerable number of factors, including predictability of resources, environmental stochasticity, and the relatedness among competitors, impact the cost/benefit ratio associated with parental care.

As a result of preliminary work in the area of offspring size and number and parental care, Roff (2002) and others have devised very specific life history models to calculate individual reproductive investments and related fitness gains. Parental care behaviors will evolve when there is a need to limit reproduction to specific times and locations and when the benefit of such behaviors outweighs the costs involved (Tallamy 1984). For example, increased clutch sizes tend to cause a decrease in adult survival and residual reproductive value and as a result, actual clutch sizes may be smaller than predicted according to Lack's calculations (Tallamy 1984, McGinley *et al.* 1987, Stearns 1992, Roff 2002). Clearly, it is paramount to include the complex and sometimes hidden costs of parental care behaviors when analyzing care strategies and trends.

Empirical data

Recognizing parental care as a major component of life history theory provides the framework, theoretical modeling, observations, and manipulative experiments, for examining specific reproductive behaviors in a variety of species. These behaviors include thresholds of brood abandonment, environmental induced changes in parental care, and differential offspring provisioning. Predictions from models can provide a basis for study, and as the empirical data demonstrates, these models must be expanded to include a diverse range of environmental and physiological conditions affecting organisms.

A trade-off between investing in a current reproductive bout versus deferring the investment to some future opportunity is at the heart of an abandonment threshold for iteroparous species (Williams 1957). Peterson (2007), studying solitary bees, *Megachile rotundata*, and Steinhart (2008), studying small mouth bass, *Micropterus dolomieu*, confirmed that the risks and costs of parental care vary enormously, even within a species. They found: [1] parents in environments with high predation risks, to themselves, provide less care to their offspring, settling for a relatively smaller fitness payoff, than parents exposed to less predation risk (Peterson 2007, Steinhart 2008), [2] when faced with uncertain relatedness to even a portion of the offspring in the current brood, parents invest much less care, even to the point of abandonment or infanticide (Steinhart 2008), and [3] when food availability decreases or the energy expenditure of parental care becomes too great, parents are likely to abandon or commit infanticide (Peterson 2007, Steinhart 2008). In order to optimally allocate resources to maximize fitness, natural selection should favor flexible (plastic) life history strategies, including parental care (Drummond & Rodríguez 2009). Organisms exhibiting semelparity are not constrained by trade-offs between a current clutch and the opportunity for future reproduction and will typically exhibit some level of offspring care. Conversely, an iteroparous strategy is considered incompatible with offspring care because of the investment in seeking additional reproduction opportunities (Tallamy & Brown 1999).

The trade-off between investing in current reproduction and the possibility of future reproduction takes on an intricate complexity when considering the results of age- and/or seasonally- related pressures organisms face (Roff 2002, Dearborn 2008). In long-lived, univoltine birds, such as albatross, *Phoebastria immutabilis*, the trend for older

parents to out-perform younger parents in overall reproductive success is evident, but this might not be directly related to investing more in reproduction later in life, but just not as efficiently (Dearborn 2008). Older birds may well be better foragers and can offer better care while younger birds are actually investing heavily into their offspring (Dearborn 2008). In only one breeding season, when the age difference between reproductive bouts is much reduced, blue-footed boobies, *Sula nebouxii*, show a significant decline in the relative mass of eggs laid in a clutch as the breeding season drew to a close (D'Alba 2007). This adjustment amounted to a heavier second egg early in the season and a heavier first egg later. The level of parental investment differed for these females not only because of the possibility of future reproduction later in the season, but also in response to the environmental demands of worsening conditions affecting her survival until the following season (D'Alba 2007). King Penguins, *Aptenodytes patagonicus*, also exhibit differential levels of parental investment when low body mass thresholds are attained early in the breeding season compared to later in the season. Breeders that reached a critical energy depletion stage early in the reproductive season abandoned their egg and attempted additional breeding after re-feeding and returning to the breeding location (Robin *et al.* 2001). However, breeders that reached the critical body mass threshold later in the season, upon returning to the breeding location from re-feeding, did not engage in additional reproductive attempts. Although the late breeders spent more time re-feeding, food availability was not related to the length of feeding or the lack of breeding upon return. Rather Robin *et al.* (2001) hypothesize that the trade-off between survival and reproduction for King Penguins is based upon energy status and an internal clock regulating reproductive investments. When brood sizes were experimentally

enlarged for young and middle-aged collared flycatchers, *Ficedula albicollis*, all females had to greatly increase their parental care efforts but the younger females produced significantly less fit offspring compared to the older females (Sendecka *et al.* 2007). In fact, in subsequent years, the young females whose broods were artificially enlarged continued to lay smaller clutches compared to all other females. Young females were unable to cope with increased parental demands and reproductive costs compared to older females (Sendecka *et al.* 2007). Parental care experience is also an influential factor in the reproductive success of female Columbian, *Spermophilus columbianus*, and Cape, *Xerus inauris*, Ground squirrels (Broussard *et al.* 2008, Pettitt *et al.* 2008). Multiparous (experienced) female Columbian Ground squirrels exhibit significantly greater reproductive investments and success compared to primiparous (inexperienced) females, despite statistically controlling for age and body condition (Broussard *et al.* 2008). Similarly, multiparous female Cape Ground squirrels weaned more juveniles to successful emergence compared to primiparous females (Pettitt *et al.* 2008).

Organisms that exhibit behaviors contrary to life history theory predictions are especially useful in illustrating the complexities of the natural world. A unique set of parental care behaviors are exhibited by sand gobies, *Pomatoschistus minutus*; they breed for only one season, produce multiple broods, demonstrate extensive parental care, in this instance paternal care, and practice filial cannibalism (Lissåker 2007). Life-history models would predict that early in the season, males would sacrifice more eggs to maintain vigor for later reproduction and toward the end of the season cannibalism would decline as males' residual reproductive value also declines. However, studies of sand gobies do not support these predictions. Increased level of filial cannibalism later in the

season results from unexpected complexities sand gobies face, such as water temperature conditions and a decrease in the number of fertile females available (Lissåker 2007).

In comparison, burrower bugs, *Sehirus cinctus*, rely on an ephemeral reproductive resource and exhibit brood defense and provisioning of young. The duration of maternal care and the temporal limitation of host-plant fruits allows for a maximum of two reproductive cycles in a season. Life history theory predicts an increase in reproductive effort as residual reproductive value declines; however, burrower bugs do not follow this trend (Clutton-Brock 1991, Kight 1997). Prior to offspring independence, females caring for broods late in the season (females were in three treatments, producing and caring for a first brood, producing a first brood and not caring for it, and not producing the first brood until late), consistently terminated care substantially reducing reproductive success (Kight 1997). Physiological constraints from senescence and energy depletion are predicted to more strongly influence behavior compared to a classic life history trade-off.

Heterogeneous or adverse environments potentially offer parents the greatest challenge in allocating available resources optimally and offering adequate parental care. Differences in parental care strategies can be observed when two populations of the same species live in nearby, but very contrasting sites. For example, the level of parental care of male mountain bluebirds, *Sialia currucoides*, has been analyzed at two nesting sites that differed by approximately one thousand meters of mean altitude. Males at higher altitudes exhibited increased feeding of offspring, more often and later into development, than males at the lower altitudes (Johnson 2007). This trend of increased parental care in harsh conditions has been observed numerous times across several taxa (Stearns 1992, Roff 2002, Johnson 2007).

Parental care behaviors are relatively rare among invertebrates, particularly arthropods; however there have been a few recorded instances of unique and extensive parental care (Clutton-Brock 1991, Mappes & Kaitala 1994). Both dung beetles, *Onthophagus atripennis*, and soil mites, *Sancassania berlesei*, are known to differentially provision offspring according to resource quality (Kishi 2006, Plaistow 2007). This tactic is common in species that encounter a lottery-style reproductive system based on the variability of their reproductive environment (Hanski & Cambefort 1991). As would be expected, smaller quantities of a high quality resource is necessary, compared to a lower quality resource, to produce more numerous and larger offspring (Kishi 2006, Plaistow 2007). Additionally, both species highlight a dynamic shift in reproductive strategy. Younger mothers produce more numerous eggs and older mothers produce larger eggs (Clutton-Brock 1991, Stearns 1992, Kishi 2006, Plaistow 2007).

Parental care behaviors evolve even in the presence of costs such as increasing predation risk. For example, to ensure offspring survival, female leeches, *Helobdella stagnalis*, attach their eggs and developing larvae on their ventral surface for several weeks, although the added mass makes them more susceptible to predation (Mann 1957). Similarly, although they become more vulnerable to predation, female intertidal beetles, *Bledius spectabilis*, will remain with their young to prevent suffocation with incoming tides (Wyatt 1986, cited in Clutton-Brock 1991). Subsocial shield bugs, *Parastrachia japonensis*, incur huge costs in provisioning their larvae by repeatedly leaving their nests and foraging on the ground, sometimes a great distance from protection. However, this increased risk is countered by the benefit of an ephemeral resource that must be monopolized within a limited breeding season (Filippi *et al.* 2002). The maternal

behavior of the lace bug, *Gargaphia solani* Heidemann, exemplifies the physiological compromises that dictate parental care strategies. Without predators, nymph survival exceeds 80%, even without maternal protection; and with predators present, maternal protection increases survival over seven times (Tallamy 1984). Maternal parental care, however, is very costly in so far as protecting each clutch accounts for nearly half the female's life span and egg production is suppressed throughout, considerably reducing fecundity.

While extensive parental care does not typically occur in invertebrates exhibiting strict iteroparity, facultative iteroparity has been proposed as a mechanism to explain the occurrence of parental care tactics in species that are physiologically capable of reproducing more than once (Tallamy & Brown 1999, Stegmann & Linsenmair 2002, Futami & Akimoto 2005, Nagano & Suzuki 2008). In some crab spider species, (Thomisidae family), the extent of the strategy is limited to a second, compensatory ovipositing immediately after separation from their brood or early total brood failure (Futami & Akimoto 2005). In this case, if the brood is lost in the early stages of egg-guarding, the females are able to resume feeding and produce a replacement clutch of eggs. However, females allocate, on average, 31% of their weight to egg-guarding the first clutch of eggs, suggesting that the costs of parental care prohibit additional reproduction attempts (Futami & Akimoto 2005). In spiders, semelparity is the base strategy, and avoiding reproductive failure appears to be the condition driving facultative iteroparity.

Tallamy and Brown (1999) suggest that facultative iteroparity (also referred to as functional semelparity) is selected for in species that exhibit parental care and are capable

of producing more than one clutch when environmental and nutritional conditions are favorable. After rearing one brood, *Sehirus*, burrower bugs, and *Nicrophorus*, burying beetles, among others, retain the capability to reproduce again; however, the likelihood of locating reproductive resources due to availability or length of reproductive season is minimal, rendering most of these insects incapable of producing a second brood (Kight 1997). The fecundity costs of parental care are significantly reduced as the probability of finding ephemeral resources a second time decreases, while the parents gain considerable benefits from investing in their already successful first brood (Tallamy & Brown 1999).

Considering the numerous complex components of individual life history strategies, species that exhibit tactics that paradoxically appear to be at odds are particularly interesting. The inclusion of adaptive phenotypic plasticity broadens the study of life history traits by adding complexity that can account for otherwise previously unexplained phenotypic trade-offs for certain species.

NICROPHORUS INVESTIGATOR AS A STUDY ORGANISM

The genus *Nicrophorus* (Coleoptera: Silphidae), burying beetles, is well-known for being obligate carrion breeders, exhibiting extensive bi-parental care, and having limited breeding seasons. Species constrained by strict environmental conditions are expected to demonstrate phenotypic plasticity (Johansson & Rowe 1999, De Block & Stoks 2004). Burying beetles, with their interesting and uncommon combination of life history traits coupled with strict seasonal constraints, provide an outstanding model system to investigate the influence of environmental cues and aging on reproductive success. They are maintained and manipulated within a laboratory setting in which the

entire reproductive cycle can be monitored. The flexibility of their reproductive behavior can be thoroughly investigated in a field setting that replicates their natural habitat much more closely than laboratory trials.

Discovery of a potential breeding carcass prompts a pair of beetles to begin preparation of the carcass for burial and breeding, the first of many parental investment behaviors. Although unpredictable in the field, vertebrate carcasses ranging in size from 7-300 g (depending on beetle species) are utilized as breeding resources (Pukowski 1933, Milne & Milne 1944, 1976, Trumbo & Fernandez 1995, Scott 1998). Body size of burying beetles is under direct selection (Smith & Heese 1995). As a result, if several beetles of both sexes discover a carcass, which occurs often, aggressive intrasexual interactions typically lead to the exclusion of beetles of inferior size (Pukowski 1933, Eggert & Sakaluk 2000). By virtue of gaining access to larger carcasses, larger adult beetles are capable of producing greater numbers of offspring compared to smaller beetles and larger larvae exhibit decreased overwintering mortality compared to smaller larvae (Müller *et al.* 1990, Eggert & Müller 1992, Eggert & Müller 1997, Smith 2002). Although individual reproductive success may be compromised in instances of communal breeding, several species, including *N. tomentosus*, *N. vespilloides*, and *N. defodiens* are known to co-breed on very large carcasses (Scott 1997, Eggert & Sakaluk 2000). In the Pacific Northwest, *N. investigator* has been found communally breeding on large salmon carcasses (Hocking *et al.* 2006).

Females can bury and successfully utilize carcasses as a breeding resource and raise a clutch without male assistance. Females that mate prior to arriving at a carcass can store viable sperm for several weeks and produce healthy diploid offspring without

the assistance of a male (Müller & Eggert 1989, Scott 1998). Males that arrive at a carcass will attempt to attract a female and may even begin carcass burial, but if a female does not arrive, the males abandon the resource (Pukowski 1933). If a female abandons a clutch, or dies immediately after laying eggs, the male beetle can successfully maintain the carrion ball and raise the brood to pupation (Trumbo 1991, Scott & Gladstein 1993, Trumbo & Fernandez 1995, Müller *et al.* 1998).

The breeding adult pair excavates soil from under the carcass and as the interring process continues, the beetles remove fur or feathers from the remains (Milne & Milne 1976, Scott 1998). Once the skin is cleaned, the carrion is balled up and the beetles apply antimicrobial oral and anal secretions to the surface of the ball. These secretions slow the decomposition of the carcass allowing the larvae to feed on the carcass until fully developed (Pukowski 1933, Milne & Milne 1976, Scott 1998, Rozen *et al.* 2008). The length of time beetles spend burying a carcass is inversely related to the ambient temperature (Scott 1990, Smith & Heese 1995, Eggert & Sakaluk 2000). Additionally, as a reproductive season progresses and soil becomes drier and more difficult to excavate, carcasses are buried more superficially compared to carcasses buried early in the season (Eggert & Sakaluk 2000, Bishop *et al.* 2002). The breeding season for this holarctic genus can span from very early spring to late fall, but for *Nicrophorus investigator* in montane regions of Western Colorado, it is strictly limited to the time between emergence, typically early July, and the first frost in early fall, typically late August (Smith 2002).

During the carcass interring process, the female oviposits a clutch of eggs a short distance from the burial chamber which will hatch within a few days. The young will

make their way through the soil to the carcass. The clutch is often larger than can be supported on the carcass and some young are culled by both adults upon hatching (Trumbo 1990, Eggert & Müller 1997, Eggert *et al.* 1998, Steiger *et al.* 2007). In preparation of the larvae arriving, the beetles continue investing in the carcass, placing an opening at the top of the ball and forming a conical depression with a suspension of regurgitated carrion (Pukowski 1933, Milne & Milne 1976, Scott 1998). Upon arrival at the carcass, the larvae demonstrate begging behaviors and they are fed regurgitated carrion by both parents (Pukowski 1933, Milne & Milne 1976, Trumbo & Fernandez 1995, Rauter & Moore 1999). Larvae in the smaller species, *N. vespilloides* and *N. defodiens*, can survive without parental feeding after the first instar, while the larvae of the larger species require longer parental care (Scott 1998). Species such as *N. orbicollis* and *N. sayi*, develop mandibles after the second instar and are able to feed themselves. Development is complete in approximately two weeks and the larvae disperse from the consumed carcass to pupate (Scott 1998, Meierhofer *et al.* 1999, Smith *et al.* 2000, Rauter & Moore 2002). In addition to being inversely related to the length of time it takes adult beetles to bury a carcass, temperature also impacts larval development. As temperatures cool or if the carcass is buried in the shade, larval development is slowed and dispersal will occur later compared to clutches raised in warm, open areas (Scott 1990, Smith & Heese 1995, Meierhofer *et al.* 1999). Typically, adult females remain with their brood while males desert prior to complete larval development. Throughout the process of larval development, any feeding on the carcass by the adults is thought to indicate the possibility of a second reproductive attempt by the parents (Creighton *et al.*

2009). Mass gained by adults is the result of part of the reproductive resource being made unavailable to the current brood or possibly from the ingestion of culled larvae.

The number of larvae raised on a carcass is controlled by the adult beetles. During the burial process, the adult beetles have assessed the volume or mass of the carcass, and in response the brood is culled as the larvae arrive (Müller *et al.* 1990, Trumbo 1990, Smith & Heese 1995, Trumbo & Fernandez 1995). *Nicrophorus investigator*'s fitness is maximized when, given the size of the breeding resource, the optimal size and number of larvae is produced (Milne & Milne 1944, Scott 1998, Smith 2002). Larval size is directly and positively correlated to timing of eclosion and adult size. Large larvae are more likely to survive the overwintering period and will emerge later compared to smaller larvae (Pukowski 1933, Smith 2002). There is a direct and positive correlation between adult size and likelihood of securing a carcass as a reproductive resource (Pukowski 1933). However, the smallest larvae, eclosing into the smallest adults, tend to emerge earliest in the season, allowing them the most time to seek reproductive opportunities (Smith unpublished data). The larger larvae take longer to emerge and may experience greater success because of their size; yet, that is offset by the increased possibility of losing access to quality early-season carcasses, which could lead to a decreased probability of successfully reproducing twice in one season (Milne & Milne 1976, Rowe & Ludwig 1991).

Depending upon species, the development of larvae through pupation and concluding with imaginal ecdysis (the emergence of an adult beetle) can be as short as a few weeks, or as long as 11 months (Smith 2002). Species that pupate quickly (3-4 weeks) enter diapause as adults and spend the winter in this stage (Rauter & Moore 2002,

Schnell *et al.* 2007). Species that follow this pattern include: *N. germanicus*, *N. humator*, *N. vespilloides* (Pukowski 1933), *N. vespillo* (Pukowski 1933, Meierhofer *et al.* 1999), *N. pustulatus* (Rauter & Moore 2002), *N. marginatus* (Bedick *et al.* 2006), and the endangered *N. americanus* (Bedick *et al.* 2006, Schnell *et al.* 2007). Other species spend the winter in a prepupal stage, emerging the following spring as adults. These species include: *N. investigator*, *N. forsor* (Pukowski 1933), and *N. tomentosus* (Wilson *et al.* 1984). Eclosion of adults is strongly influenced by soil temperature and secondarily, day length (Špicarová 1972). In the montane habitat of *Nicrophorus investigator*, the first adults begin to emerge approximately 21 days after the first bare-ground day in the spring (Smith unpublished data).

Studies on *Nicrophorus* species have revealed diverse reproductive patterns. Several species are capable of producing more than one clutch in a breeding season, particularly under laboratory conditions (Trumbo 1991, Smith & Heese 1995, Eggert & Müller 1997, Scott 1998). When provided with 100 g carcasses and maintained in a laboratory, *N. quadripunctatus* can produce more than six clutches, with little reduction in brood size (Nagano & Suzuki 2007). Under similar conditions, *N. vespillo* and *N. orbicollis* both have significantly smaller second consecutive broods (Müller 1987, Scott & Traniello 1990) and *N. vespilloides* demonstrates a substantial decline in the size of the third brood (Eggert & Müller 1997). Meanwhile, *N. tomentosus* is unable to produce a third brood at all (Trumbo 1990). *N. orbicollis* produces considerably larger first broods early in the reproductive season when compared with first broods late during the season (Scott & Traniello 1990) while early and late season initial broods for *N. vespillo* are similar in size (Meierhofer *et al.* 1999).

Additionally, there has been both theoretical and empirical work on the duration of parental care and its impacts on reproductive success within the genus, producing mixed results. Termination of parental care is experimentally determined when adult beetles emerge from the brood chamber and attempt to fly away or do not return to the chamber immediately after a slight physical jostle (Scott & Traniello 1990). In field studies, the mean duration of care for *N. orbicollis* adults was significantly longer than for *N. defodiens* or *N. tomentosus* and, on the average, females of all species studied thus far remain with the brood longer than males (Scott & Traniello 1990, Scott & Gladstein 1993, Scott 1998b, Meierhofer *et al.* 1999). Carcass weight and mean offspring weight are directly and positively correlated to female length of care in *N. orbicollis* (Scott & Traniello 1990). The early study of Scott and Traniello (1990) concluded that the seasonal timing of reproduction does not influence duration of care in male or female *N. orbicollis*. When considered independently, a follow-up theoretical model predicted that duration of paternal care is also not influenced by the probability of finding a subsequent carcass and producing another brood or by the level of competition for carcasses (Scott & Gladstein 1993). However, a later study on the same species contradicted these results (Scott 1998b). Adults reared in isolation (presumed perception of limited competition for carcasses) provided significantly less care than adults reared in either groups or wild-caught (Scott 1998b). Males cared for their first and second broods equally and remained with their third brood significantly longer while females remained with their second brood longer and did not survive to care for a third brood. When males produced their first broods late in the season, the length of care was significantly longer when compared to initial broods early in the season and females exhibited no difference in care with

regard to the timing of the brood (Scott 1998b). Meierhofer's (1999) work with *N. vespillo* indicates that parental pairs remained with their developing broods longer when reproduction took place in May as compared with pairs that reproduced in June or August. This trend in the duration of care was inversely related to the temperatures to which the beetles were exposed.

Special consideration for the effects of multiple components of reproductive success, like senescence, has been included in laboratory studies of reproductive patterns including, early versus late brood sizes and parental care strategies for two *Nicrophorus* species. In *N. vespilloides*, females that experienced delayed reproduction produced significantly heavier larvae compared to early-reproducing females (Lock *et al.* 2007). However, larvae raised by early-reproducing females were not significantly different in weight compared to larvae raised by females that delayed reproduction. Age-related parental care tactics ameliorated effects of the female's age on initial larval size (Lock *et al.* 2007).

Studies of *N. orbicollis* investigate the effects of age on the investment ratio of the current brood value and the possibility of future reproduction (Creighton *et al.* 2009, Trumbo 2009). Creighton *et al.* (2009) focused on specifically measuring the amount of resource allocated by a female for herself and her brood and the impacts of overinvesting in a current clutch or age of the female. Experimental female beetles buried a 30 g mouse carcass and laid eggs based on the size of the mouse initially provided. As larvae appeared on the carcass after hatching, the 30 g mouse was replaced with a 20 g, previously prepared mouse carcass. In the meantime, control beetles buried either a 30 or a 20 g mouse, laid eggs accordingly and raised larvae on the same carcass. Females that

produced an appropriate number of eggs to be raised on a 30 g carcass and instead raised the larvae on a 20 g carcass exhibited significantly reduced reproductive success compared to either of the control groups. Although these females utilized their carcasses most efficiently, they had the fewest reproductive attempts, produced the fewest offspring, and had the most rapidly declining clutch size over consecutive broods (Creighton *et al.* 2009). Moreover, the females that overproduced eggs based on carcass size invested the least into the possibility of their future reproduction; they gained the least weight compared to the control groups. The control females that reproduced on 20 g carcasses invested the most into body mass for future reproduction, produced relatively constant sized larvae, made the most reproductive attempts and lived the longest. In a follow-up manipulation, females either bred early or late on either a 20 or a 30 g carcass. The initial number of larvae of early and late breeders was similar; however, by the time development was complete, the late breeders had significantly more larvae. Early breeding females exhibited a greater investment in future reproduction and gained more mass compared to the late breeders. Larval mass did not differ between early and late breeders on smaller carcasses, but, on larger carcasses, early breeders raised fewer, larger larvae compared to late breeders (Creighton *et al.* 2009).

Trumbo (2009) also investigated parental investments based on the value of a current brood versus the possibility of future reproduction. His focus was on the reproductive success of relatively young and old *N. orbicollis* during brood initiation, defense and development. Younger beetles took a longer time to oviposit and were more likely to experience failure in brood initiation, and, contrary to Creighton *et al.* (2009), there was no effect of age on number and size of larvae produced (carcasses were 24 to

28 g, Trumbo 2009). No effect of age was detected in a resident female's ability to ward off an intruder. However, when allowed the opportunity to usurp a carcass, older females were significantly more successful compared to younger females.

The studies of Lock *et al.* (2007), Creighton *et al.* (2009), and Trumbo (2009) do not provide unambiguous evidence indicating the effects of aging on reproductive success in a genus that utilizes ephemeral breeding resources and provides elaborate parental care. However, they do illustrate that incorporating multiple components of resource allocation and reproductive performance is critical in assessing the effects of a state variable, such as age, on intricate life history strategies. Species that offer extensive parental care may exhibit phenotypic plasticity that allows for behavioral adjustments in response to declining residual reproductive value (Cameron *et al.* 2000, Lock *et al.* 2007). The studies suggest that differential allocation of resources for current and future reproduction is not purely an age-related function, but also a response to the female's declining residual reproductive value later in life. This terminal investment behavior could be in response not only to natural aging, but also to environmental cues (Creighton *et al.* 2009). Although these recent studies have addressed more complicated elements to the changes in reproductive success across a constrained breeding season, the influence of environmental cues independent of age within the *Nicrophorus* genus is as yet, untested.

The physiological clock in holometabolous species is 'reset' upon eclosion and whether the adults appear early or late in the season, they are considered to be the same physiological age the day they emerge (Blake *et al.* 1996, Creighton *et al.* 2009). Obviously, beetles that emerge early in the reproductive season will be exposed to

different environmental signals compared to beetles emerging later in the season. Beetles of the same age, exposed to different environmental cues may well exhibit plastic reproductive strategies, which is the focus of my study. Although the entire genus is ideal for study, there is a paucity of Nicrophorine investigations done under natural conditions (Meierhofer *et al.* 1999). Among those studied in the field, *Nicrophorus investigator* is one of the least studied species in western montane habitats (Smith & Heese 1995). It demonstrates some plasticity with regard to reproductive behaviors at various altitudes (Smith *et al.* 2000, Pontius 2007). An innovative manipulation of burying beetle adult eclosion timing can provide a unique way to evaluate reproductive behaviors can be evaluated throughout the breeding season while eliminating the added confoundedness of age. By allowing some beetles to emerge naturally and forcing some to postpone emergence until mid-way through the breeding season, experiments can be conducted with newly emerged adults of the same age but exposed to different seasonal cues. Such an approach can incorporate the unique components of reproductive and parental care behaviors of the burying beetle system into further understanding of age-related fitness outcomes and further evaluate evolutionary life history theory.

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Chapter 2: Parental investment in burying beetles (*Nicrophorus investigator*):

Influence of seasonal timing and prior experience

ABSTRACT

Species specific reproductive strategies can result from trade-offs incurred while attempting to maximize fitness within the constraints of finite resources. Members of the genus *Nicrophorus* (Coleoptera: Silphidae) makes repeated reproduction attempts and employs bi-parental care, two reproductive strategies not commonly exhibited in tandem in invertebrates. I investigated reproductive strategies of *Nicrophorus investigator* (burying beetles) in a montane ecosystem at Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. Breeding success for *N. investigator* can be influenced by small mammal carcass availability as well as intraspecific and intrageneric competition for carcasses. Some beetles breed multiple times in a season, while others may have only one opportunity to breed later in the season. To test the hypothesis that parental investment behaviors are influenced by 1) the previous reproductive experience of the adult beetles and 2) the timing of reproduction during the course of the season, I manipulated breeding opportunities for beetles in the laboratory and in the field. I hypothesized that beetles that were breeding for a second time and beetles that delayed breeding until late in the season would exhibit significantly more parental investment behaviors resulting in greater brood success, compared to beetles breeding for the first time early in the season. Under natural conditions, approximately three weeks after snowmelt the temperature warms enough such that adult burying beetles emerge from overwinter pupation. I relocated groups of beetles into shaded, cooler areas in order to

delay their emergence from pupation until mid-way through the breeding season. Parental investment behaviors were compared between beetles that emerged early and began reproduction immediately versus beetles that emerged early and were forced to wait to reproduce versus beetles that emerged late and had only one opportunity to breed. Delaying reproduction, alone, did not influence brood success or parent investment levels. Second broods were not as productive as first broods, even after adults were afforded rest and feeding in between reproductive opportunities. Moreover, eclosion can successfully be delayed by changing the temperature of overwintering habitat. However, more experimentation with this technique is necessary to determine the latest that beetles can emerge and still produce offspring. This additional work is recommended because this study provides limited evidence of terminal investment behaviors for beetles exposed to environmental cues of the impending end to the breeding season.

INTRODUCTION

Theoretical predictions of reproductive investments assert that an individual's residual reproductive value (RRV) declines as age progresses (Williams 1957, Creighton *et al.* 2009, Trumbo 2009). The premise is that reproductive effort varies according to the relationship between the value of the current brood to lifetime fitness and the RRV contribution to future fitness (Curio 1983). However values of RRV may be artificially inflated for three reasons: 1) organisms that rely on ephemeral, yet highly rewarding, breeding resources may not find additional resources (Hanski & Cambefort 1991, Trumbo 2009), 2) organisms may increase their reproductive efficiency with age and experience (Cameron *et al.* 2000, Creighton *et al.* 2009, Trumbo 2009), or 3) organisms may replace lost nutrients and return to good condition between reproductive bouts. In addition, relationships between reproductive investment and benefit may not be linear, such that, increases in effort may lead to diminishing returns per unit effort (Williams 1966, Trivers 1972, Maynard Smith 1977, Trumbo 2009). Finally, differential survival of above-average reproducers may interact with senescence to influence breeding strategies (Williams 1957, Reid *et al.* 2003, Creighton *et al.* 2009). Successfully predicting reproductive investments requires simultaneously including considerations of age, environmental cues, constraints, and resources, and behavioral plasticity (Johansson *et al.* 2001, Lock *et al.* 2007, Creighton *et al.* 2009).

Circadian clocks and environmental stimuli are known to influence daily and seasonal activities of virtually all known eukaryotic organisms (Garner & Allard 1920, Truman 1971, Dunlap *et al.* 2004). In response to environmental cues and in order to exploit suitable conditions, insects enter and break a state of dormancy known as

diapause (Tauber & Tauber 1976). During diapause, the larval and adult physiological states are closely correlated among holometabolous insects, however, there is no such link exhibited in their biological clocks (Pechenik & Eyster 1989, Blake *et al.* 1996, Pechenik 2006). Adults that eclose early and those that eclose late are the same physiological age on the day they emerge, despite differences in chronological age, or calendar date (Blake *et al.* 1996, Creighton *et al.* 2009). Thus, adults of the same physiological age may coexist at the same time within a population. Crickets, *Gryllus pennsylvanicus*, experience significantly reduced reproductive success when forced to eclose late in the breeding season (Carrière *et al.* 1996).

Facultatively iteroparous insects exhibit plasticity in reproductive strategies based upon environmental conditions (Tallamy & Brown 1999). Iteroparous species with parental care may be able to respond to environmental indicators of the impending end of the breeding season and adjust their behaviors (Lock *et al.* 2007). Life history models predict that reproductive effort should peak in an act of terminal investments when a female recognizes that she has no future opportunity for reproduction (Williams 1957, Clutton-Brock 1991, McNamara & Houston 1996). Outdoor experimentation is critical for investigating the influence of environmental stimuli on insects with ages that are physiologically or chronologically manipulated. Comparisons of reproductive investments in adult beetles that emerge naturally and then age and reproduce throughout the summer to a cohort of pre-pupae individuals manipulated to postpone emergence until mid-way through the breeding season would clarify the influence of environmental cues (seasonality) versus age and maturity on reproduction.

My study objectives were to investigate the influence of previous reproductive experience and the timing of reproduction during the breeding season in a facultatively iteroparous insect. Both indoor and outdoor quasi-field experiments were conducted to more fully describe the reproductive behaviors of a montane species of burying beetle, *Nicrophorus investigator*. In many *Nicrophorus* species, the influence of age on reproductive success has been studied, while the impacts of environmental cues have been widely ignored. Investigating the potential for physiology and seasonal cues to influence reproductive behavior under natural conditions has been an uncommon practice (Meierhofer et al. 1999); however, a two-step empirical design is a reasonable method to adopt to study those behavioral responses under natural conditions. Initially, breeding under laboratory conditions can help to identify age related effects independent of environment (Lock et al. 2007, Trumbo 2009). Breeding beetles that are exposed to natural environmental conditions adds a realistic complexity not obtained in a lab (Meierhofer et al. 1999) and can reveal environmental influence on reproductive investments (Creighton et al. 2009).

Members of the genus *Nicrophorus* (Coleoptera: Silphidae), burying beetles, are well-known for being obligate carrion breeders, having seasonally defined breeding seasons, and exhibiting extensive bi-parental care (Milne & Milne 1976, Eggert & Müller 1997, Scott 1998). In the majority of species, adult beetles emerge in the spring, fly in search of small animal carrion and engage in competition to secure a carcass as a breeding resource (Pukowski 1933). Hair or feathers are removed from the carcass during the process of interment and the male and female beetles coat the carcass with oral and anal anti-bacterial secretions that act to slow decomposition of the resource. The

prepared carcass serves as nutrition to developing larvae and brood-tending adults (Rozen *et al.* 2008). Typically, both male and female care for the brood. The male leaves the brood first and the female departs upon the completion of larval development. However, adult males and adult females are capable of rearing a brood uniparentally if one member of the adult pair departs from the breeding site following copulation or egg-laying (Müller & Eggert 1989). Feeding on the carcass by the adult beetles may occur during larval development. An adult gaining mass is indicative of an investment in future, rather than current reproduction, as that portion of the breeding resource is unavailable to the current brood (Creighton *et al.* 2009).

Several species produce more than one clutch in a breeding season, particularly in laboratory conditions (Trumbo 1991, Smith & Heese 1995, Eggert & Müller 1997, Scott 1998, Tallamy & Brown 1999, Pennell personal observation). Reproductive success declines for *N. orbicollis*, *N. vespilloides*, and *N. vespillo*, resulting in reduced clutch sizes in consecutive successful breeding attempts (Müller 1987, Scott & Traniello 1990, Eggert & Müller 1997). However, *N. quadripunctatus* can produce more than six relatively similar sized clutches (Nagano & Suzuki 2007). Parental care also varies among reproductive attempts. *Nicrophorus orbicollis* females increase the duration of care for their second brood produced (Scott & Gladstein 1993).

The effects of delays in reproduction have been studied in some nicrophorine species. Initial broods for *N. vespillo* are similar in size regardless of when, early or late, in the breeding season they are produced (Meierhofer *et al.* 1999) while *N. orbicollis* produces larger first broods early in the season compared to first broods produced late in the season (Scott & Traniello 1990). *Nicrophorus vespilloides* females with delayed

reproduction produced significantly heavier larvae compared to early-reproducing females (Lock *et al.* 2007). Two recent studies with *N. orbicollis* focused on the complex tradeoffs between current broods, RRV, and parental care. Creighton *et al.* (2009) reported no difference in larval mass between early and late breeders on smaller carcasses (20 g). However on larger carcasses (30 g), early breeders raised fewer, larger larvae. When compared to females that reproduce at an early age, *N. orbicollis* females that delay reproduction are more successful at establishing and defending a brood and raising larvae through development to dispersal (Creighton *et al.* 2009). Trumbo (2009) reported no effect of age on size of larvae produced on carcasses between 24 – 28 g.

Nicrophorus investigator, a species of burying beetle with holarctic distribution (Anderson & Peck 1985, Peck & Kaulbars 1987), is found in montane habitats of the western United States (Smith & Heese 1995, Smith *et al.* 2000a, Smith *et al.* 200b, Smith 2002). Unlike the majority of nicrophorine species (Peck & Anderson 1985), *N. investigator* overwinters in a pre-pupae state and emerges as breeding adults the following year. The process of metamorphosis resets physiological clocks and upon eclosion, sexually mature adults are essentially newborn (Blake *et al.* 1996). Soil temperature and day length influence the timing of eclosion of adult burying beetles (Špicarová 1972). In Colorado at higher elevations, breeding season for *N. investigator* is limited to the period from late June to the first frost in early fall, typically late August (Smith 2002).

Although some reproductive strategies have been studied for this species, particularly brood size, carcass selection, and effects of altitude (Smith & Heese 1995, Smith *et al.* 2000, Smith 2002, Pontius 2008), the influence of multiple reproductive

attempts and environmental cues on parental investment behaviors and reproductive success are unknown. In addition, the influence of internal cues, such as demographic delays in breeding, isolated from external cues, or environmental effects late in the breeding season, has not been measured for *N. investigator*.

To investigate behavioral outcomes and reproductive success my study had three objectives:

- 1) to decouple the combined effects of prior reproduction and physiological age by comparing reproductive investment and success in first and second breeding attempts of early and late breeders.
- 2) to determine whether the extent of decline in reproductive investment and success between first versus second broods could be reversed through rest and feeding of beetles.
- 3) to determine whether females exposed to natural cues of the end of the breeding season would engage in a “terminal investment”, independent of their physiological age.

During my study, I measured a variety of characteristics related to reproductive investment and success to compare between adult beetles reproducing at different ages and/or at different times during the breeding season. Measures of reproductive investment included: time to accomplish carcass burial, duration of brood care by males and females, and changes in adult beetle body mass. Attributes of reproductive success included: total brood mass and average larval size upon larval dispersion from the carcass and size and number of larvae.

MATERIALS AND METHODS

Nicrophorus investigator adults were collected at the Rocky Mountain Biological Lab (RMBL) in Gothic, Gunnison County, Colorado (N 38° 57', W -106° 00'; 2,900 m elevation). The region consists of areas forested with aspen or Engelmann spruce, *Picea engelmannii*, and willow, *Salix* genus, bogs scattered among alluvial grasslands and meadows (Langenheim 1962). Snowmelt typically occurs in early- to mid-May, with the average 'bare-ground' day occurring around May 21 (Smith unpublished data).

Nicrophorus investigator adults emerge in late June. Low and high temperatures in the summer months are, respectively: May -2° and 13.3°C; June 0.5° and 20°C; July 3.3° and 23.4°C; August 3.3° and 22.8°C (source EPA weather station, RMBL, 1981 to 2009).

Adult collection methods, both years

Wild beetles were captured in baited traps. Metal #10 cans filled with approximately 2,100 cm³ of damp soil and baited with raw chicken were covered with an inverted cone of screen attached to the top to allow the beetles entry and prevent their escape. The cans were hung about 1.5 meters off the ground attached to aspen trees, *Populus tremuloides*, approximately 10 m apart and were placed at the edges of meadow openings around the townsite of Gothic. Traps were put up for 14 days during the last week in June and the first week in July to capture wild beetles just after eclosion at the beginning of the reproductive season. The timing of the capture, almost immediately after wild beetles are known to emerge (Scott 1997, Smith *et al.* 2000, Smith 2002), and the use of bait that beetles do not attempt to utilize as a reproductive resource (Eggert & Sakaluk 2000, Rauter & Moore 2002) made the capture of reproductively experienced beetles highly unlikely. Traps were checked daily and all captured beetles were brought

to the lab, separated by sex, and maintained in ventilated plastic tubs with moistened paper towels and raw chicken *ad libitum*.

Additionally, recently eclosed beetles were collected as they emerged from the soil from overwintering containers which had been buried in outdoor exclosures (Smith 2002). The previous year, 25-30 larvae from captive breeding experiments were placed in each container, allowed to position themselves at any depth in the soil, and the containers were placed outside. The exclosure was a pit dug into soil measuring about 1.5 meters by 1.5 meters and 20 cm deep. Overwintering containers were steel #10 cans with wire mesh covering both ends filled with soil to approximately 1 cm of the top of the can. Soil was used to fill the spaces between the cans and was slightly mounded upon hardware cloth that had been placed over the cans. The dirt and hardware cloth were removed approximately two weeks prior to expected emergence and the cans were checked several times daily for newly emerged adults on top of the soil. These adults were maintained as described for the wild caught beetles.

Breeding methods, year one

Commercially available (frozenrodent.com™) whole white mice (*Mus musculus*) were used as the carcass resource for the breeding experiments and kept frozen until used in the experiments. Mouse carcasses ranged in size from 20 - 30 g. Each reproducing pair of beetles received a randomly selected mouse carcass within that range. To examine whether seasonal timing or prior reproductive experience influences parental investment within the breeding season, the carcass sizes were held constant for beetle pairs breeding twice (Meierhofer *et al.* 1999). This reduced variation between broods due to variation in carcass size (Rauter & Moore 2002, Nagano & Suzuki 2007).

For all breeding experiments, small plastic terraria (length 18 cm, width 14 cm, height 9.5 cm) were filled with approximately 1,600 cm³ damp soil and kept in a ventilated room and exposed to natural lighting. I established three treatments of 30 replicated pairs of beetles. Each pair of beetles was placed in a container and provided a mouse carcass on top of the soil. The adult beetles were matched for mass to minimize complications of alternative reproductive strategies due to size difference between sexes (Eggert & Sakaluk 2000, Smith pers. comm.). Females were selected randomly from the ventilated plastic tub in which they were held and measured. Males were also selected randomly and as they were measured, they were placed with a female of similar mass. Some males were rejected and returned to the tubs if a female of appropriate mass was not in use for experimentation. Paired males and females were within 0.08 grams of one another, which was approximately 23-24% of total adult mass in year one and approximately 19-20% of total adult mass in year two. Observations throughout the reproductive cycle included: adult beetle mass prior to and post reproduction (after adults leave brood chamber), number of days to bury the carcass, and the number and mass of living larvae produced.

Completion of brood development was determined when the larvae consumed the carcass and abandoned the brood chamber (Scott 1998, Meierhofer *et al.* 1999, Smith *et al.* 2000, Rauter & Moore 2002). At that time, the larvae were counted, weighed, and placed in cans filled with soil to overwinter in the ground at RMBL (Smith 2002). Adult beetle mass was measured and males were switched to pair with a different female (again matched for mass). The switch of males was designed to simulate natural events. It is highly unlikely that any pair of beetles would raise a second brood together (Jenkins *et al.*

2000). Instead, in nature, breeding pairs separate upon brood development and individuals seek to become a member of a new pair competing for a new carcass. All pairs reproducing for a second time were provided a mouse with mass within 1 g of the female's first mouse.

Reproductive investments can be costly and influence future reproductive attempts. The second objective of my study was to isolate the influence of physiological costs, such as maternal body mass loss and reduced nutritional condition, from the influence of the experience of prior reproduction. To accomplish this task, two treatments were designed to allow for reversal of the physiological decline that might occur due to the investment in the first brood (Trumbo 1991, Steiger *et al.* 2007).

Experimental treatments, year one

Experiments were conducted indoors, under natural light and regulated temperature. The three treatments were as follows (Table 2.1):

- 1) Consecutive broods without a resting period. Beetle pairs were bred early in the season. Immediately upon complete larval development of the first brood, individuals were re-matched as describe above to a new adult beetle that had also just completed a first brood. The pair received a new mouse in the female's breeding container. The soil remained unchanged but had been sifted through while collecting the previous brood's larvae. The former brood chamber was not available for burial. Thirty replicates, each breeding twice, N = 30.

- 2) Consecutive broods with a resting period. Beetle pairs were bred early in the season. Upon complete larval development of the first brood, individuals were re-matched as above to new adult beetles that had also just completed a first brood and the

pair was rested and fed raw chicken *ad libitum* for three days. After rest, the pair received a new mouse for breeding. The soil remained unchanged but had been sifted through while collecting the previous brood's larvae. The former brood chamber was not available for burial. Thirty replicates, each breeding twice, N = 30.

3) Seasonally “late” breeders (early emerging beetles, reproducing for the first time as an older beetle). Adult beetles were held captive, and separated by sex until the early season larvae from the first broods of Treatments 1 and 2 (above) completed development. Individuals were matched according to mass, placed in brood chambers that had been prepared as for the other treatments, and provided a mouse for their first breeding opportunity (as above). Thirty replicates, breeding once, N = 30.

Breeding methods, year two

Commercially available (rodentpro.com™) whole white mice (*Mus musculus*) were used for the carcass resource for the breeding experiments and kept frozen until used in the experiments. Mouse carcasses ranged in size from 23 - 26 g. Just as in the first year, each reproducing pair of beetles received a randomly selected mouse carcass within that range and carcass sizes were held constant for pairs breeding twice.

Breeding experiments were conducted outdoors in #10 cans filled with approximately 2,100 cm³ of damp soil and covered with wire screening secured with multiple large rubber bands. The bottoms of the cans had been removed and a wire screen was secured with wire to ensure drainage of the soil and still prevent escape (Smith 2002). Several large boxes (1.25 m x 2.5 m x .5 m) with secure mesh lids were built outdoors to house 40 cans each to prevent scavengers (foxes, *Vulpes vulpes*,

weasels, *Mustela erminea*, martens, *Martes americana*, bears, *Ursus americanus*) from disturbing the experiments. The boxes were filled with soil and the breeding containers were buried in the soil up to approximately 5 centimeters from the top of the can. Adult beetles were paired according to mass as in year one.

The same treatment designs were followed as in year 1, however, each treatment consisted of 20 replicates, and was conducted outdoors under natural conditions. Observations throughout the reproductive cycle included the same measures as in year 1. Added in year 2, were parental investment behavioral observations. The completion of larvae development was determined in the same fashion, and at that time, larvae were counted, weighed and buried in overwinter cans. Adult beetles were weighed again and the males were switched to a new, equally sized female. The second mouse for beetle pairs reproducing twice was within 1 g of the female's first mouse.

Behavioral observations and parental investment

Parental investment was recorded as the days to bury the carcass, the duration of parental presence in the brood chamber, and the difference in parental mass after reproduction (Müller 1987, Eggert & Müller 1997, Scott 1998, Meierhofer 1999, Futami & Akimoto 2005). The reproductive output included overall count and mass of brood (Müller 1987, Scott & Traniello 1990, Smith & Heese 1995, Eggert & Müller 1997, Scott 1998, Meierhofer 1999). Parental care was noted as the number of days each parent was tending the brood chamber. During the course of larval care, parents would be seen inside or near the brood chamber or on the surrounding soil. Although beetles might be found on top of the soil or appearing to wander around the can, beetles that were still actively participating in the rearing of offspring would move back to the brood chamber

when slightly jostled. The day on which each parent was found out of the brood chamber and did not return with some prompting was considered the termination of parental care for that brood (Trumbo 1991, Smith pers. comm.). The mass of both parents was measured when care had terminated. Only replicates in which both parents survived were included in statistical analysis because death of one mate influences the duration of parental care for the remaining parent (Meierhofer 1999).

Experimental treatments, year two

Experiments were conducted both outdoors under natural light and temperature and indoors under natural light with regulated temperature. The five treatments were as follows (Table 2.1):

1) Consecutive broods without a resting period. Pairs consisted of beetles that emerged naturally and were bred early in the season. Immediately upon complete larval development of the first brood, individuals were re-matched according to mass with new adult beetles (who had also just completed a first brood), the pair received a new mouse and clean, sifted local soil. Twenty replicates, each breeding twice, N = 20.

2) Consecutive broods with a resting period. Pairs consisted of beetles that emerged naturally and were bred early in the season. Upon complete larval development of the first brood, individuals were re-matched according to mass with new adult beetles (who had also just completed a first brood) and the pair was rested and fed raw chicken *ad libitum* for three days before receiving a new mouse for breeding. Twenty replicates, each breeding twice, N = 20.

3) “Late breeders, early emergers” (reproducing for the first time as an older beetle). Adult beetles were held captive, and separated according to sex,

until the early season larvae, from the first broods of Treatments 1 and 2, completed development. Individuals were matched according to mass, placed in brood chambers that had been prepared as for the other treatments, and provided a mouse for their first breeding opportunity (as above). Twenty replicates, breeding once, N = 20.

4) “Late breeders, late emergers” (reproducing for the first time as a younger beetle). Pairs consisted of beetles that emerged late and bred late in the season. To delay eclosion, containers of overwintering beetles were moved to a cooler location before emergence. On May 28, 2009, after the snow melted off the overwinter cans and prior to beetles emerging, seven cans of larvae that had been buried the previous year were removed from the overwinter plots. The cans were moved into the shade in an aspen grove. On July 27, 2009, the cans were returned to the overwintering pit area that experiences warmer temperatures than the aspen grove. These beetles emerged later in the season (mid-August) and were “younger” than beetles that emerged earlier, either wild-caught or from the overwinter containers (Blake *et al.* 1996). Pairs of beetles (matched for mass as above) were placed in breeding containers with sifted soil and a mouse. This group of reproducing beetle pairs did not successfully bury any mice or produce any offspring. The adult mass change was recorded for pairs that survived three weeks (the typical length of brood development). Twenty replicates, breeding once, N = 20.

5) Late emerging, late breeders, indoor controlled conditions. To validate that beetles emerging late in the season could still reproduce (because of the failures of beetle pairs to bury carcasses in treatment described above), five pairs of late emerging beetles

were placed on soil in terraria used in year 1 and provided a mouse. These were the only late emerging beetles that produced larvae and raised them to complete development. $N = 5$.

Statistical analysis, both years

All statistical analyses were performed using SPSS version 16.0. A significant difference in experimental design between the two years of research led to independent analysis of each year's data. Year, treatment, and carcass size were maintained as independent variables and the dependent variables included adult beetle weights prior to and post reproduction (after adults leave brood chamber), number of days to bury the carcass, the number and mass of living larvae produced, and the duration of parental care (year 2 only). These measures were first tested for normality using Shapiro-Wilks W before proceeding with analyses. One-way ANOVAs were used to produce F -statistics on normally distributed data and Kruskal-Wallis tests were used to produce Chi-Square statistics on non-normal data. When appropriate, all analyses, parametric or nonparametric, included Bonferroni corrections for multiple comparisons. Two dependent variables, adult beetle masses and duration of adult care, had two within subjects factors (gender and reproductive attempt) and one between subjects factor (treatment level). The ANOVAs used to analyze these variables tested for main effects of gender, reproductive attempt and treatment as well as all possible interactions. The Kruskal-Wallis test was also utilized in analyzing the distribution of initial adult masses within each year.

In order to isolate the effect of age, data collected from the beetles forced to delay reproduction was compared to data collected from the first broods of the un-rested, unfed

and rested, fed treatment groups. The replicates in these groups did not experience any treatment changes until after their first brood completed development. In order to increase sample size, both groups were combined to analyze the first brood. To identify effects of prior reproduction, the same measures from delayed breeders were compared to the second broods of the un-rested, unfed and rested, fed treatment groups. Repeated measures ANOVAs were used to determine if between brood decline can be reversed with rest and feeding. Brood production and adult body conditions were compared between the first treatment that bred consecutively without rest and the second treatment that rested between reproductive opportunities. To detect evidence of terminal investment behaviors, data collected on delayed emergers (young beetles reproducing for the first time late in the season) was compared to the second broods and delayed broods of naturally emerging (older) adults. Finally, Pearson's simple correlation tests were run within treatment results to compare trends in brood success and investment behaviors.

RESULTS

Average mouse mass in year one was 24.092 g (± 0.19 SE) and mean mouse mass in year two was 24.008 g (± 0.04 SE). Mouse carcasses were not significantly different within or between years ($F_{1,273} = 0.285$, $p = 0.594$). Furthermore, carcass mass among treatments for year one and two were not significantly different, respectively: $F_{2,147} = 0.254$ ($p = 0.776$) and $F_{4,120} = 1.699$ ($p = 0.155$). The average initial (prior to reproductions) beetle mass in year one was 0.328 g (± 0.009 SE) and was 0.369 g (± 0.006 SE) in year two. The distribution of pairs of beetles, based upon initial body mass, was significantly different between years (Kruskal-Wallis test, $X^2_{15} = 85.530$, $p < 0.001$). The

distribution was not significantly different within the first year 1, $X^2_5 = 4.626$ ($p = 0.463$). In the second year, when the five late emerging beetle pairs that breed indoors are included in the analysis, there is a significant difference in the distribution, $X^2_9 = 16.747$ ($p = 0.032$). However when these replicates are removed from analysis, there is no difference in distribution of pairs across treatments, $X^2_7 = 3.270$ ($p = 0.355$). The mean female and male mass in the indoor breeding group was, respectively, 120% and 95% of the mean mass of the other beetles reproducing in the second year (Table 2.2). In year one and year two, respectively, the average daily temperature for the early reproductive attempt was 13.2 °C and 12.8 °C and the average daily temperature for the late reproductive attempt was 11.3 °C and 11.2 °C.

Analysis for Objectives 1 and 3

To decouple the effects of age and prior reproductive experience, reproductive measures were compared between young and old reproducing beetle pairs and between beetle pairs of the same age, but with differing reproductive experience. Additionally, comparing reproductive measures between experienced and inexperienced beetle pairs during the second year will provide evidence to address the possibility of terminal investment behaviors independent of age. The three measures of reproductive success were: 1) total number of larvae in a brood, 2) total mass of the brood, and 3) mean larval size. In year 1, when the reproductive success of young and old adult beetles was compared there was no statistical difference in the number of larvae ($F_{1,82} = 1.582$, $p = 0.212$; Figure 2.1, graph a), the total mass of the brood ($F_{1,82} = 0.357$, $p = 0.552$; Figure 2.2, graph a), or the mean larval mass ($F_{1,82} = 1.411$, $p = 0.238$; Figure 2.3, graph a). However, when the same measures were compared for first time broods and second

broods late in the season, fewer larvae were produced in second broods (Kruskal-Wallis test, $X^2_2 = 26.315$, $p < 0.001$; Figure 2.1, graph b) and the total mass of the second broods was lower ($F_{2,48} = 32.070$, $p < 0.001$; Figure 2.2, graph b). Although the adults that were rested and fed between broods produced larvae that were, on average, similar to those larvae produced by older beetles forced to delay reproduction, the unrested and unfed adults produced significantly smaller larvae, and overall differences were not significant ($F_{2,48} = 2.834$, $p = 0.069$; Figure 2.3, graph b).

In year 2, when treatment 5 (indoors) is included in the analysis, younger beetles produced significantly fewer larvae than beetles that were forced to delay breeding (Kruskal-Wallis test, $X^2_2 = 6.102$, $p = 0.047$; Figure 2.4, graph a). When treatment 5 is excluded, there is no such difference ($F_{1,40} = 0.865$, $p = 0.429$). The number of larvae produced by previously reproducing beetles was significantly less than the number produced by inexperienced beetles at the same time during the breeding season. When the indoor replicates are included in the analysis ($F_{2,20} = 5.508$, $p = 0.012$; Figure 2.4, graph b). Without treatment 5, there is no such difference ($F_{1,16} = 0.326$, $p = 0.727$). The overall mass of larvae produced in broods of younger beetles was significantly less than the brood mass of beetles forced to delay reproduction (including indoor replicates, $F_{2,44} = 9.834$, $p < 0.001$; Figure 2.5, graph a). Excluding treatment 5, there is no such difference ($F_{1,40} = 1.484$, $p = 0.267$). The mass of broods produced by experienced beetles was significantly less than the brood mass of inexperienced beetles reproducing at the same time in the breeding season (including indoor replicates, $F_{2,20} = 19.637$, $p < 0.001$; Figure 2.5, graph b). Without these replicates, there is no such difference ($F_{1,16} = 0.096$, $p = 0.909$). This trend also describes the average size of larvae produced in the

second year (Figure 2.6). Including treatment 5, the larvae produced by younger beetles were significantly smaller than the larvae produced by beetles forced to delay reproduction ($F_{2,44} = 4.095$, $p = 0.023$; Figure 2.6, graph a). However, without the indoor replicates, there is no difference ($F_{1,40} = 1.042$, $p = 0.362$). Experienced beetles also produced smaller larvae compared to inexperienced beetles, if indoor replicates are included ($F_{2,20} = 10.798$, $p < 0.001$; Figure 2.6, graph b). Without these replicates, again, there is no difference ($F_{1,16} = 2.747$, $p = 0.096$).

Measures of reproductive investment, days for the adult pair to bury the carcass, adult body mass lost, and, the duration of adult care of the brood, in year 2 only, were compared for young and old beetles as well as experienced and inexperienced beetles. With regard to the number of days for the adults to bury the carcass in year 1, there was no significant treatment effect between early and late first reproduction events (Kruskal-Wallis test, $X^2_2 = 0.740$, $p = 0.691$; Figure 2.7, graph a), however, there was a significant treatment effect between late first reproduction and second reproduction (Kruskal-Wallis test, $X^2_2 = 36.910$, $p < 0.001$; Figure 2.7, graph b). Carcasses were buried more rapidly for the second reproduction attempt compared to the first reproduction attempt occurring at the same time in the breeding season.

When younger and older adults in year 1 are compared, females that were forced to delay breeding lost significantly more of their initial body mass than did females that bred when they were younger (Student's t : $t = 2.36$, $df = 80$, $p = 0.023$; Figure 2.8, graph a). While younger and older males lost relatively the same amount of body mass (Student's t : $t = 0.81$, $df = 80$, $p = 0.423$). When body mass lost of inexperienced breeders is compared to experienced breeders, there is a significant treatment effect for

females ($F_{2,49} = 3.717$, $p = 0.031$; Figure 2.8, graph b). The females breeding for a second time exhibited significantly less body mass loss than the females forced to delay reproduction until late in the season.

In the second year, the younger beetle pairs took significantly longer to bury the carcass compared to older beetle pairs that were forced to delay reproduction ($F_{2,79} = 5.475$, $p = 0.002$; Figure 2.9, graph a). Within that comparison, the beetles that emerged late in the season took significantly fewer days to bury carcasses compared to beetles that emerged naturally at the start of the season (post-hoc comparison, after Bonferroni corrections, $p < 0.001$). Without the late emerging replicates that bred indoors, there is still a difference between late first attempts and early first attempts (Student's t : $t = 2.681$, $df = 59$, $p = 0.008$). Experienced beetles buried carcasses late in the season faster than inexperienced beetles ($F_{3,78} = 4.431$, $p = 0.006$; Figure 2.9, graph b). The experienced group that was rested and fed in between broods buried the second carcass fastest of all groups (post-hoc comparisons, Figure 2.9). Without the indoor, late emerging replicates, there is still a significant difference between late first carcass burial and second carcass burial times ($F_{2,74} = 5.399$, $p = 0.006$).

With regard to the percent of adult mass lost during reproduction in year 2, when primiparous adults breeding late in the season are compared to primiparous adults breeding early in the season, there is a significant difference in mass loss between treatments, ($F_{3,73} = 94.232$, $p < 0.001$; Figure 2.10), but no interaction ($F_{3,73} = 2.096$, $p = 0.106$) or main effect of gender ($F_{1,75} = 0.031$, $p = 0.861$). Post-hoc comparisons reveal that the naturally early emerging adults, reproducing both early and late, lost significantly more mass (as a percent of initial mass) than the beetles forced to emerge and then

reproduce late in the season (comparisons indicated in Figure 2.10). When adults producing late first broods are compared to adults producing second broods there is a significant interaction effect of gender and treatment ($F_{4,72} = 13.488$, $p < 0.001$; Figure 2.11). Within the treatment where adults were fed and rested between reproductive opportunities (treatment 2), the females lost significantly more mass than the males (post-hoc comparison, after Bonferroni corrections, $p < 0.001$). The late emerging females that did not produce any offspring outside (treatment 4) and the late emerging females that produced offspring inside (treatment 5) all lost significantly less body mass than either the rested and fed females producing a second brood or the naturally emerging females forced to delay reproduction (Table 2.3). While the adult males forced to delay breeding lost significantly more mass than either of the late emerging and breeding groups of males (Table 2.3).

The second year, one additional measure of reproductive investment was recorded for reproducing adult beetle pairs. When care of late first broods is compared to care of early first broods, there is a within subject effect of sex, females remained longer, ($F_{1,80} = 79.047$, $p < 0.001$; Figure 2.12) and a between subject effect of treatment, delayed breeders remained longer ($F_{2,79} = 6597.821$, $p < 0.001$; Figure 2.12). Statistical analysis without late emerging beetles breeding indoors reveals no significant gender and treatment interaction ($F_{2,74} = 1.082$, $p = 0.344$) but a within subjects effect of gender ($F_{1,74} = 68.755$, $p < 0.001$) and a between subjects effect of treatment ($F_{1,74} = 5523.105$, $p < 0.001$), when late first broods are compared to early first broods. When late first broods are compared to second broods, the same trend is revealed, females remained longer than males ($F_{1,69} = 41.870$, $p < 0.001$), and delayed breeders cared for the brood longer than

repeat breeders ($F_{3,67} = 6126.283$, $p < 0.001$; Figure 2.13). Again, statistical analysis without late emerging beetles breeding indoors reveals no significant gender and treatment interaction ($F_{2,63} = 1.760$, $p = 0.180$) but a within subjects effect of gender ($F_{1,63} = 41.073$, $p < 0.001$) and a between subjects effect of treatment ($F_{2,63} = 5885.381$, $p < 0.001$).

Analysis for Objective 2

To determine if between brood decline in reproductive success or investment could be reversed reproductive measures were compared between a group of adult beetles that received *ad libitum* food and rest for prior to the second reproductive attempt and a group of adult beetles that immediately started the second attempt upon complete larval development of the first brood. Brood success measures were compared between consecutively produced broods in year 1 (only two breeding pairs successfully produced consecutive broods in year 2). Both the un-rested and rested groups showed a significant decline in the number of larvae produced in the second brood ($F_{1,21} = 53.735$, $p < 0.001$; Figure 2.14). However, the decline was similar across treatments ($F_{1,21} = 1.116$, $p = 0.303$). Similarly, both groups showed a significant difference in the total mass of the first and second broods ($F_{1,21} = 85.847$, $p < 0.001$; Figure 2.15), and the decline was comparable across treatments ($F_{1,21} = 0.111$, $p = 0.743$). There was a significant decline in the average size of larvae produced in the second brood by the adults that did not rest and feed between broods while the larvae produced by the adults that did rest and feed were similar size in both broods ($F_{1,21} = 5.370$, $p = 0.031$; Figure 2.16).

Measures of reproductive investments were compared between consecutive breeding attempts in year 1 and year 2. In the first year, both un-rested and rested groups

had a significant decline in the number of days to bury the second carcass, $F_{1,54} = 178.920$, $p < 0.001$, however, the decline was similar across treatments, $F_{1,54} = .0892$, $p = 0.349$ (Figure 2.17, graph a). In the second year, there was a significant interaction between treatment and reproductive attempt, $F_{1,37} = 3.948$, $p = 0.054$ (Figure 2.17, graph b). Both treatments showed a significant decline in the number of days to bury the second carcass, and the number of days to bury the first carcass for the first broods are significantly different between the un-rested and rested groups (Bonferroni corrected p-value for multiple comparisons, $p = 0.053$). Due to the small number ($n = 2$) of beetles that produced larvae in consecutive broods during year 2, only year 1 beetles were analyzed for percent of initial mass lost. Across the treatments, there is a significant difference in percentage of mass lost, with the rested and fed treatment losing less mass ($F_{1,22} = 164.926$, $p < 0.001$; Figure 2.18, graph a). Within the treatments there is a significant difference between the mass lost in males and females, with the male losing more mass ($F_{1,22} = 5.453$, $p = 0.029$; Figure 2.18, graph b) and there is a significant difference between reproductive attempts, with the most mass lost during production of the first brood ($F_{1,22} = 22.612$, $p < 0.001$; Figure 2.18, graph c).

To determine the level of relatedness between the percent of initial adult mass lost and the number of days it took breeding pairs of beetles to bury the carcass, or the duration of care offered by adults in the second year, a Pearson's simple correlation was conducted for both sexes in both years. The correlation coefficient (R) indicates a slightly positive relationship for the percent of initial adult mass lost and the number of days to bury a carcass in all four cases (Table 2.4). The relatedness between female mass lost in year one and male mass lost in year two and the number of days to bury the

carcass significant, respectively: $t = 2.531$, $df = 104$, $p = 0.013$ and $t = 1.952$, $df = 94$, $p = 0.054$. The relationship between mass lost and the duration of parental care in the second year is negative for both sexes (male $R = -0.165$, female $R = -0.236$). The correlation coefficient for females is significant, $t = 2.115$, $df = 80$, $p = 0.038$.

DISCUSSION

Although the data from the two years were analyzed separately, there are some clear trends that reveal general characteristics of reproductive investments and success for *Nicrophorus investigator* in montane ecosystems. The first objective of my study was to decouple the effects of prior reproduction and age by comparing brood success and reproductive investments in first and second broods of early and late breeders. Age alone does not appear to significantly impact brood success, in both the indoor and outdoor trials (analyses of year 2 that indicated differences were based on extremely small sample sizes). Thus far beetles that naturally emerge at the beginning of the breeding season, the number of larvae, the total mass of the brood or the average larval size will be comparable if reproduction takes place early in the season or late in the season. This result is inconsistent with previous results from other Nicrophorine species that exhibit greater brood success with early first broods (Scott & Traniello 1990, Lock *et al.* 2007). One possibility is that the naturally short breeding season in montane habitats leads to little difference, temporally or environmentally, between early and late broods. Additionally, the previous studies were conducted in habitats that support four or more species of burying beetles, making competition a strong selective force on reproductive behaviors. In Western Colorado, *N. investigator* competes with most often only one (but at most, two) other Nicrophorine species and would be equally as likely to experience

brood failure from flies and mites or environmental extremes (Smith unpublished data, Pennell personal observation). This possibly relaxed selective pressure from other burying beetle species may contribute to the comparable success of broods either early or late in the season. Additionally, because reproductive resources are ephemeral and competition can lead to intruders usurping a carcass or total brood failure, *Nicrophorus investigator* is capable of raising a second brood. This study shows that, consistent with many species in the genus, the second brood is smaller than the first. My data suggests maintaining this facultative iteroparity led a higher level of fitness success compared to a strictly semelparous reproductive approach. Furthermore, being able to produce equally successful first broods early or late in the season allows any carcass that is suitable for reproduction to be utilized to its full potential.

Reproductive investments, as measured by days to bury a carcass, mass lost and duration of care are not as straight forward in their response to age and experience. Without exposure to natural environmental conditions, age did not affect burial rate, but previous reproductive experience did shorten the length of time required to inter a carcass. Under natural conditions younger breeding pairs of beetles took longer to bury a carcass than did older, inexperienced beetles. This is consistent with Creighton *et al.*'s (2009) recent work on *N. orbicollis* that showed that older females (this study describes a breeding pair) are more successful at establishing a brood compared to younger females. In contrast, under controlled indoor conditions, *N. orbicollis* takes longer to bury a carcass as temperatures decrease (Scott 1990). However, environmental cues indicating the impending end of the reproductive season may contribute to the speed of burial by older beetles in Colorado. In this study, the statistical correlation between days to bury a

carcass and mass loss is a positive one; suggesting that, based on the previous result, younger beetles would lose greater mass compared to older beetles. However, the beetles that delayed breeding (and buried carcasses the fastest) lost the greatest percentage of their initial body mass. This indicates a number of components contribute to mass loss during reproduction, not simply carcass burial rate.

The second objective of my study was to determine whether the extent of decline in reproductive success and investment between consecutive broods could be reversed through rest and feeding. There were too few beetle pairs that consecutively reproduced in the second year, and the majority of analyses are from the first year. Two measures of reproductive success, total number and total mass of larvae produced, were not influenced by rest and feeding between broods. However, the rest and feeding paid off for the adults in treatment 2 because the mean larval size produced did not exhibit the same decline as the other two measures. While the overall brood produced was smaller in the second attempt for both treatments, the larvae of the rested and fed adults gained the advantage of increased size for overwintering, compared to the other second brood larvae. One of the measures of reproductive investment, the number of days to bury a carcass, was unaffected by rest and feeding, while the percentage of initial adult mass lost positively responded to the treatment. The rested and fed adults lost less mass compared to the unrested, unfed adults. This decrease in lost mass didn't affect the overall number or weight of the second brood that was produced, but it may have contributed to the average larval size. The larger larvae produced by the rested and fed beetles can directly result in increased fitness due to increased overwintering success and emergence the following spring as a larger adult (Smith 2002).

The duration of adult care of the brood was measured in the second year of field work, providing more insight into the mass that adult beetles lose during breeding. The duration of care increased as the breeding season progressed, supporting trends already noted in other *Nicrophorus* species. As the temperature began to decrease, the Pearson's correlation coefficient is slightly negative for both genders which is surprising. A parent that remains with a brood for an extended period of time is expending energy caring for the brood; however, they are also in the presence of a food resource. The ability to feed off the breeding resource may offer enough nutrition to offset the mass lost to care. In addition, culled larvae can not be accounted for in this study and may have been utilized as a source of nutrition for adult beetles as well. Parental care and carcass preparation are certainly only a portion of the investments committed by breeding adults. Further experimentation, under natural conditions, is necessary to discern the additional components contributing to mass loss during reproduction.

This study provided a general measure of two reproductive investments that contribute to the loss of mass during reproduction. Additional experiments could elucidate some of the more detailed components and mechanisms of mass loss in burying beetles. These alternative explanations went untested in my study, but would be worth future investigation. A substantial amount of antimicrobial anal and oral secretions are utilized by adult beetles while interring a carcass, certainly resulting in considerable water mass loss. Additionally, a pair of beetles that buried a carcass can be replaced by an intruding pair that can raise larvae through development without having invested in brood chamber construction and resource preparation. Three treatments can explore the degree to which burying a carcass influences mass loss: 1) beetles that fully prepare

carcasses only, 2) beetles that prepare carcasses and raise larvae to full development, and 3) beetles that raise larvae after usurping a previously buried carcass. Furthermore, lipids constitute approximately 30-40% of the dry weight of oocytes and the process of ovipositing would lead to considerable loss of mass in females; at the same time, bred males will have lost substantial glycogen reserves in both spermatophores and non-spermatophore secretions (Boggs 1995, Arrese & Soulages 2010). Using a combination of dehydration and differential centrifuging processes, the nutritional costs of copulation, ovipositing, and resource preparation can be examined. Mass loss can also differ between wild populations that fly to detect and secure reproductive resources and captive populations that are given a mouse for breeding. Presumably, wild beetles will lose more mass to the fueling of their flight muscles compared to beetles that are put on a mouse in captivity. Differences in mass loss due to flight requirements can be considered by contrasting beetles that naturally arrive at and bury reproductive resources placed in the open to beetles that are placed on a mouse in a closed environment, such as a breeding container.

The third objective of my study was to determine if females exposed to natural cues of the end of breeding season would engage in ‘terminal investment’ behaviors by forcing the late emergence of some beetles. Eclosion of adult montane burying beetles can be delayed by altering the temperature of the pre-pupae’s overwintering habitat. The beetles that emerged late as a result of this manipulation did not effectively bury a carcass or produce offspring under natural conditions. However, late emerging adults retain the ability to rear offspring, as evidenced by the five replicates placed indoors to breed. The success of these broods was so substantial that a small number of replicates forced

significant statistical shifts in results. Although, the statistical analyses that include these beetle pairs breeding indoors must be regarded with caution, the results do support the hypothesis that environmental cues, independent to age, lead to terminal investment behaviors. Reproductive success was significantly affected by late emergence, the total brood mass was higher, the number of larvae was increased and the average larval size was greater. Additionally, in spite of being young, when the beetles that emerged late were placed indoors, they buried carcasses significantly faster than any previous group, losing the least percentage of their initial mass compared to naturally emerging beetles. Such substantial increases in brood success and decreases in reproductive investments by chronologically young beetles indicate terminal investment behaviors could play strongly into late season breeding for *Nicrophorus investigator*. More experimentation with delaying eclosion is necessary to determine when the critical time for emergence occurs, such that the beetles can still reproduce under natural field conditions. In all measures of reproductive success and investment, *Nicrophorus investigator* exhibits the plasticity in reproductive strategies to be considered facultatively iteroparous, in the truest sense (Tallamy & Brown 1999). Moreover, the strict seasonal constraints and lack of strong competition from other burying beetles species offer unique opportunities to further investigate the possibility of detecting terminal investment strategies toward the end of the breeding season. Continued comparisons between early and delayed emerging beetles can elucidate the details of seasonal cues that ultimately influence brood success and levels of reproductive investments for the montane bury beetle.

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Table 2.1. Field season timeline and experimental design.

Field Timeline	Year 1				Year 2		
	TRT	Condition	N	# broods	Condition	N	# broods
Early May: move cans (yr 2)	1 ^a	Indoor	30	60	Outdoor	20	40
Mid-May: bare ground	2 ^b	Indoor	30	60	Outdoor	20	40
	3 ^c	Indoor	30	30	Outdoor	20	40
Late June: first emergers	4 ^d	-	-	-	Outdoor	20	20
	5 ^e	-	-	-	Indoor	5	5
Early July: Start 1 st broods for trt 1 & 2 ~21 days to mature	a Consecutive early/late breeding, no rest between broods b Consecutive early/late breeding, rest between broods c Late breeding only d Late emerging, late breeding only e Late emerging, late breeding only (to ensure beetle viability)						
Late July: 1 st broods of trt 1 & 2 mature, second or late broods started							
Late July, early August: late emergers (yr 2)							
Season length: ~45 days							

Table 2.2. Descriptive statistics for late emerging and breeding adults (treatment 5) indoors, n = 5, year 2.

Measure	Mean	Standard error
Female weight pre-reproduction	0.444 g	0.024
Female weight post-reproduction	0.430 g	0.028
Male weight pre-reproduction	0.347 g	0.011
Male weight post-reproduction	0.336 g	0.010
Days to bury carcass	1.8 days	0.200
Total weight of larvae produced	3.433 g	0.293
Number of larvae produced	9.8	0.663
Average size larvae produced	0.352 g/larvae	0.049
Duration female care	27.8 days	0.663
Duration male care	24.6 days	0.678

Table 2.3. Bonferroni corrected p-values of pairwise comparisons of mean percentage of initial mass lost in adult beetles, late broods, year 2. When late first broods are compared to second broods there is a significant interaction effect of gender and treatment ($F_{4,72} = 13.488$, $p < 0.001$).

Across treatments	Female p-values	Male p-values	Within treatment	p-values
1 & 5	0.475	21.050	1 unrested, unfed n = 2	1.567
2 & 5	8.253E-05	13.858	2 rested, fed n = 17	8.068E-08
3 & 5	6.261E-05	1.474E-07	3 late broods n = 33	0.218
4 & 5	2.326	22.699	4 late emerger outside n = 20	0.235
1 & 4	3.084	22.079	5 late emerger inside n = 5	23.408
2 & 4	4.532E-05	11.538		
3 & 4	7.286E-06	1.23E-15		
1 & 3	13.532	0.0025		
2 & 3	17.761	20.111		
1 & 2	11.440	21.434		

Table 2.4. Pearson Correlation results. Year 1 & year 2, Pearson correlation of the percent of initial body mass lost (female and male beetles) and the number of days beetle pairs took to bury the carcass. Year 2, Pearson correlation of the percent of initial body mass lost and the duration of adult care (fewer beetles survived through care than buried carcasses). R indicates Pearson correlation coefficient, t-statistic is the test statistic used to measure the relationship between the two mean values, and the p-value provides a measure of the significance of the relationship. Correlation coefficients for both genders, both years for days to bury the carcass are positive. Correlation coefficients for both genders for duration of care are negative.

Year 1 (n = 106)	Mean	SE	R	t-statistic	p-value
Female % initial mass lost	0.098	0.007	0.241	2.531	0.013
Male % initial mass lost	0.111	0.007	0.055	0.562	0.575
Days to bury carcass	3.349	0.167	-	-	-
Year 2 (n = 96)					
Female % initial mass lost	0.125	0.008	0.084	0.834	0.406
Male % initial mass lost	0.122	0.01	0.193	1.952	0.054
Days to bury carcass	3.653	0.209	-	-	-
Year 2 (n = 82)					
Female % initial mass lost	0.125	0.008	-0.236	2.115	0.038
Female duration of care	22.577	0.380	-	-	-
Male % initial mass lost	0.145	0.012	-0.165	1.456	0.150
Male duration of care	19.372	0.431	-	-	-

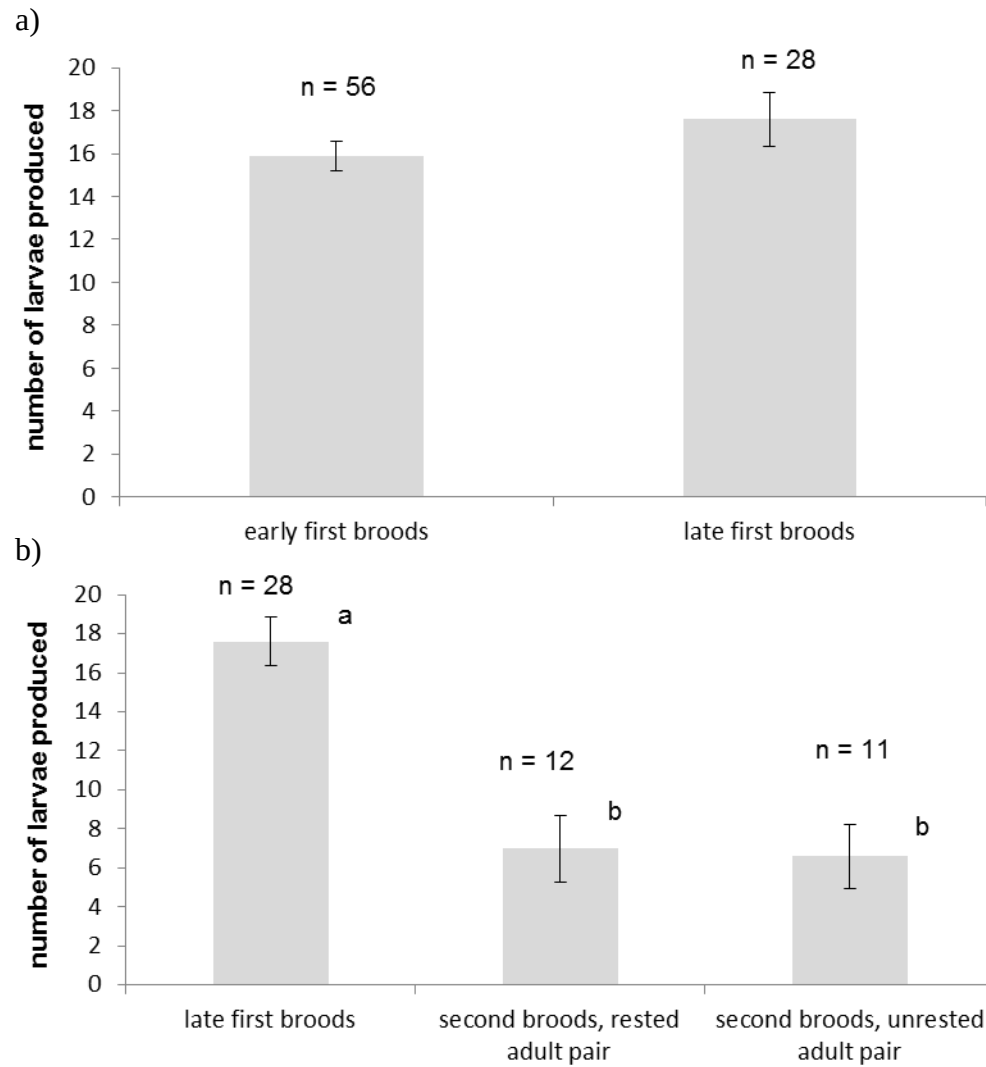


Figure 2.1. Mean number larvae ($\pm$ SE) produced, year 1. There is no significant difference in the number of larvae produced in early and late first broods, $F_{1,82} = 1.582$, $p = 0.212$ (graph a). However, there is a significant treatment effect between late first broods and second broods, (Kruskal-Wallis test), $X^2_2 = 26.315$, $p < 0.001$ (graph b). Treatments not sharing letters (a, b) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

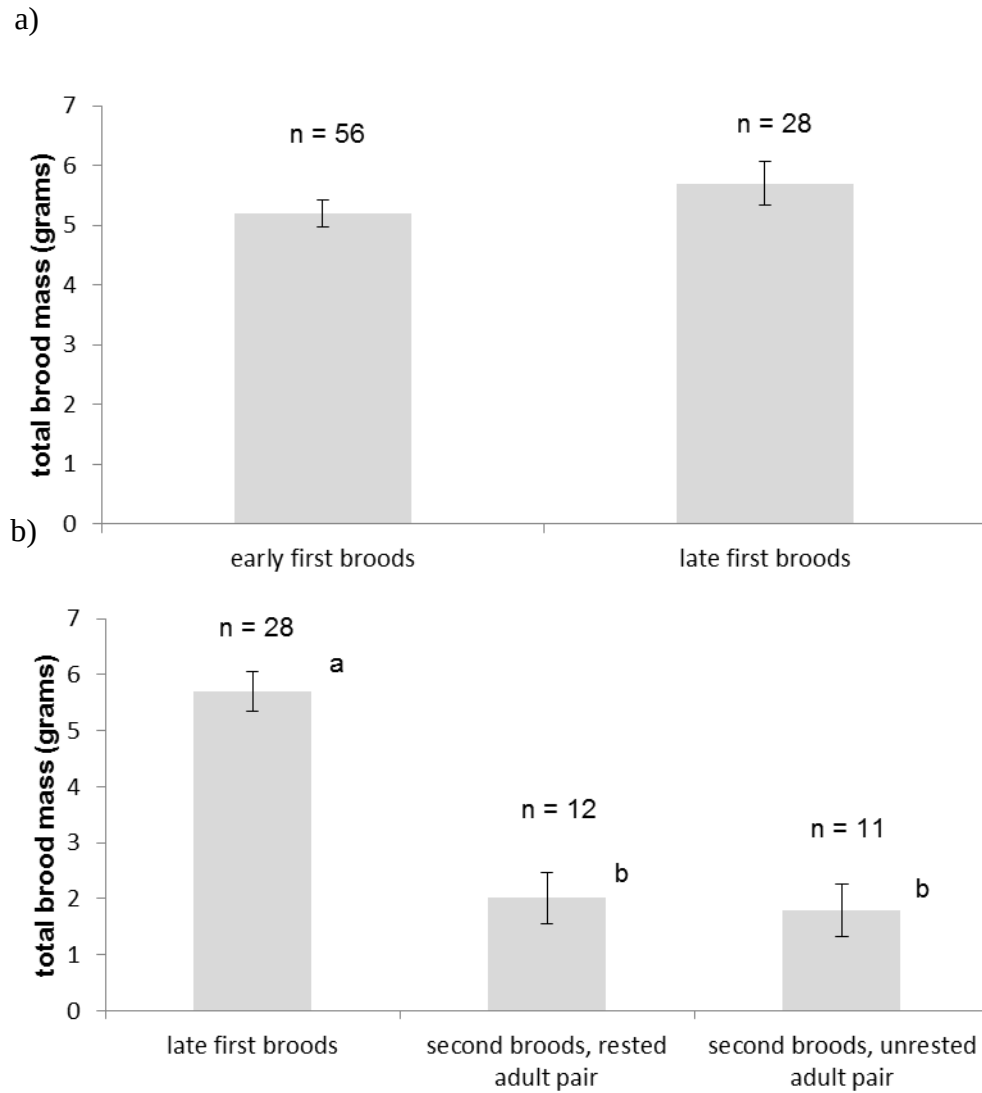


Figure 2.2. Mean mass ($\pm$ SE) of broods produced, year 1. There is no significant difference in the total brood mass of early and late first broods, $F_{1,82} = 0.357$, $p = 0.552$ (graph a). However, there is a significant treatment effect between late first broods and second broods, $F_{2,48} = 32.070$, $p < 0.001$ (graph b). Treatments not sharing letters (a, b) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

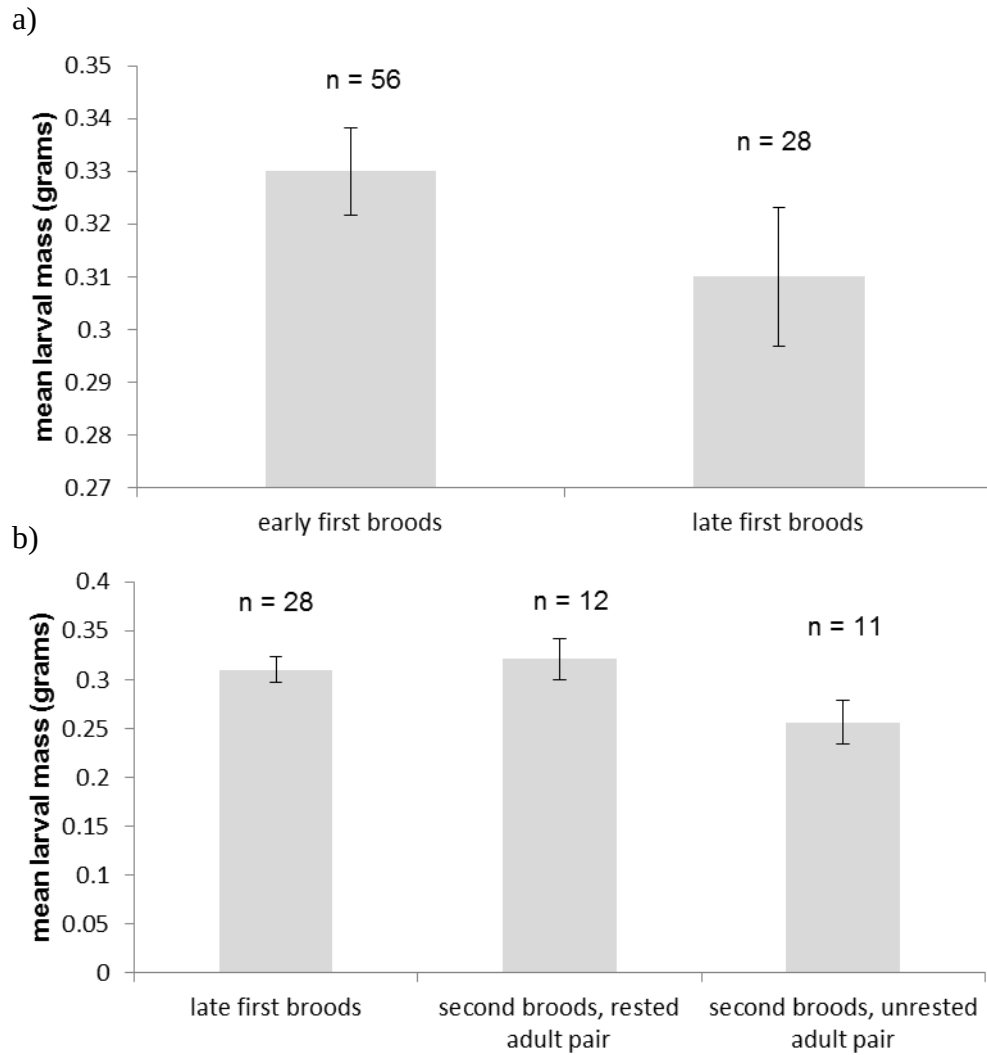


Figure 2.3. Mean mass ($\pm$ SE) of larvae produced, year 1. There is no significant difference in the size of larvae produced in early and late first broods, $F_{1,82} = 1.411$, $p = 0.238$ (graph a). There is no significant difference in the size of larvae produced in late first broods and second broods, $F_{2,48} = 2.834$, $p = 0.069$ (graph b).

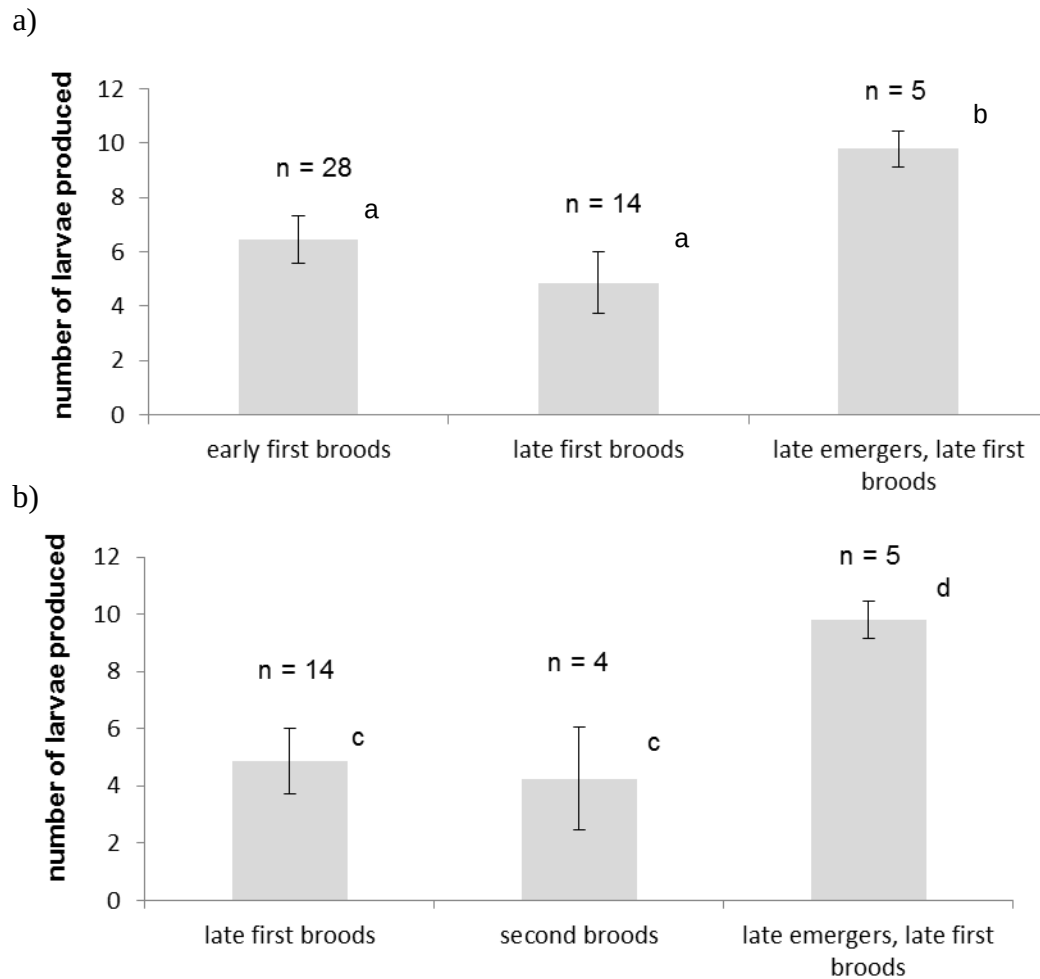


Figure 2.4. Mean number larvae ($\pm$ SE) produced, year 2. There is a significant treatment effect when late first broods are compared to early first broods (Kruskal-Wallis test $X^2_2 = 6.102$, $p = 0.047$, graph a), or second broods ($F_{2,20} = 5.508$, $p = 0.012$, graph b). Treatments not sharing letters (a, b, or c, d) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

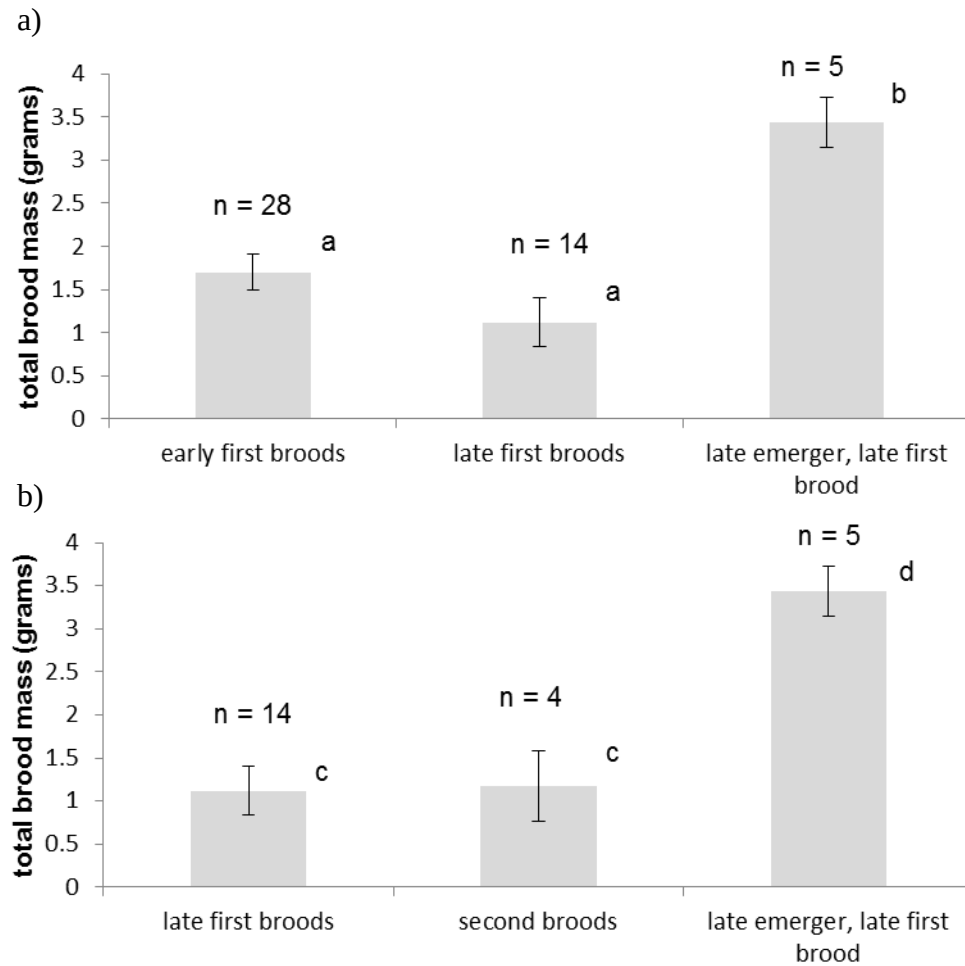


Figure 2.5. Mean mass ($\pm$ SE) of broods produced, year 2. There is a significant treatment effect when late first broods are compared to early first broods ($F_{2,44} = 9.834$, $p < 0.001$, graph a) or second broods ($F_{2,20} = 19.637$, $p < 0.001$, graph b). Treatments not sharing letters (a, b, or c, d) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

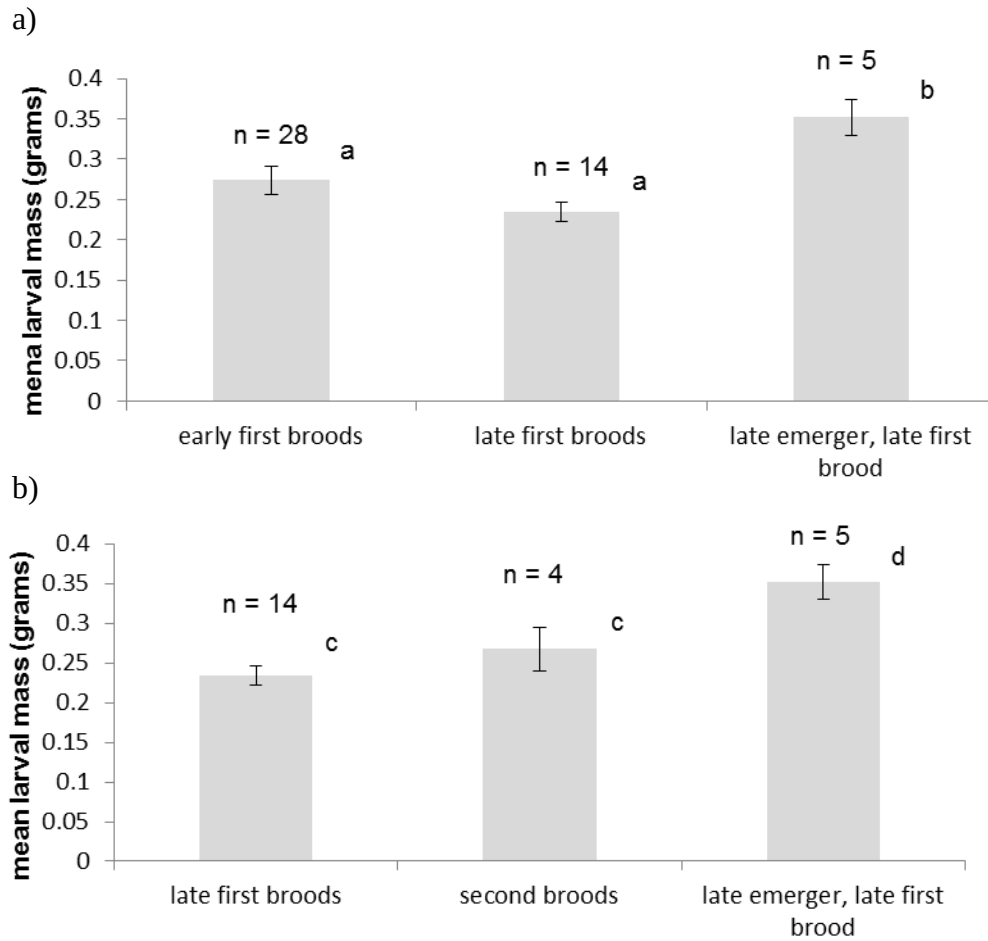


Figure 2.6. Mean mass ($\pm$ SE) of larvae produced, year 2. There is a significant difference in the size of larvae produced in late first broods compared to early first broods ($F_{2,44} = 4.095$, $p = 0.023$, graph a) or second broods ($F_{2,20} = 10.798$, $p < 0.001$, graph b). Treatments not sharing letters (a, b, or c, d) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

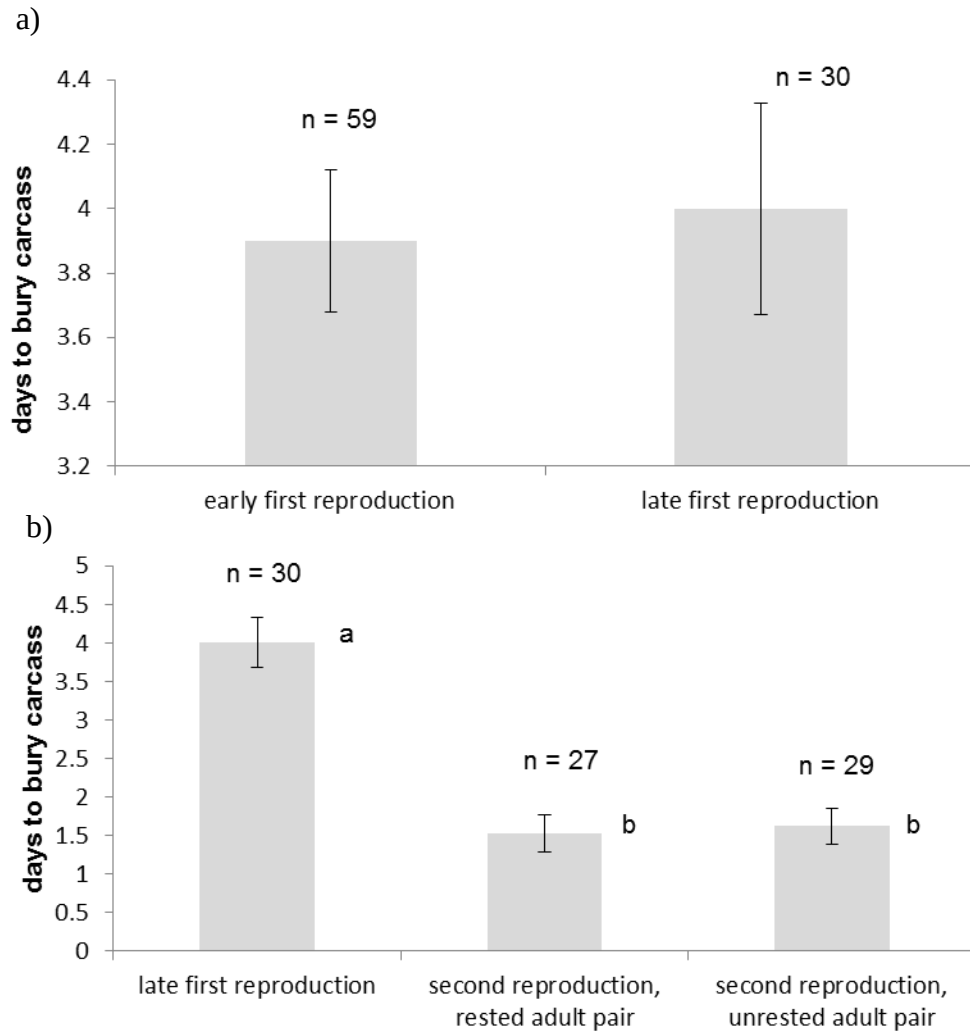


Figure 2.7. Mean days ($\pm$ SE) for adult beetle pairs to bury carcass, year 1. There is no significant difference between the number of days to bury a carcass in the early and late first breeding events (Kruskal-Wallis test), $X^2_2 = 0.740$, $p = 0.691$, graph a. However, there is a significant treatment effect between late first breeding events and second breeding events (Kruskal-Wallis test), $X^2_2 = 36.910$, $p < 0.001$, graph b. Treatments not sharing letters (a, b) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

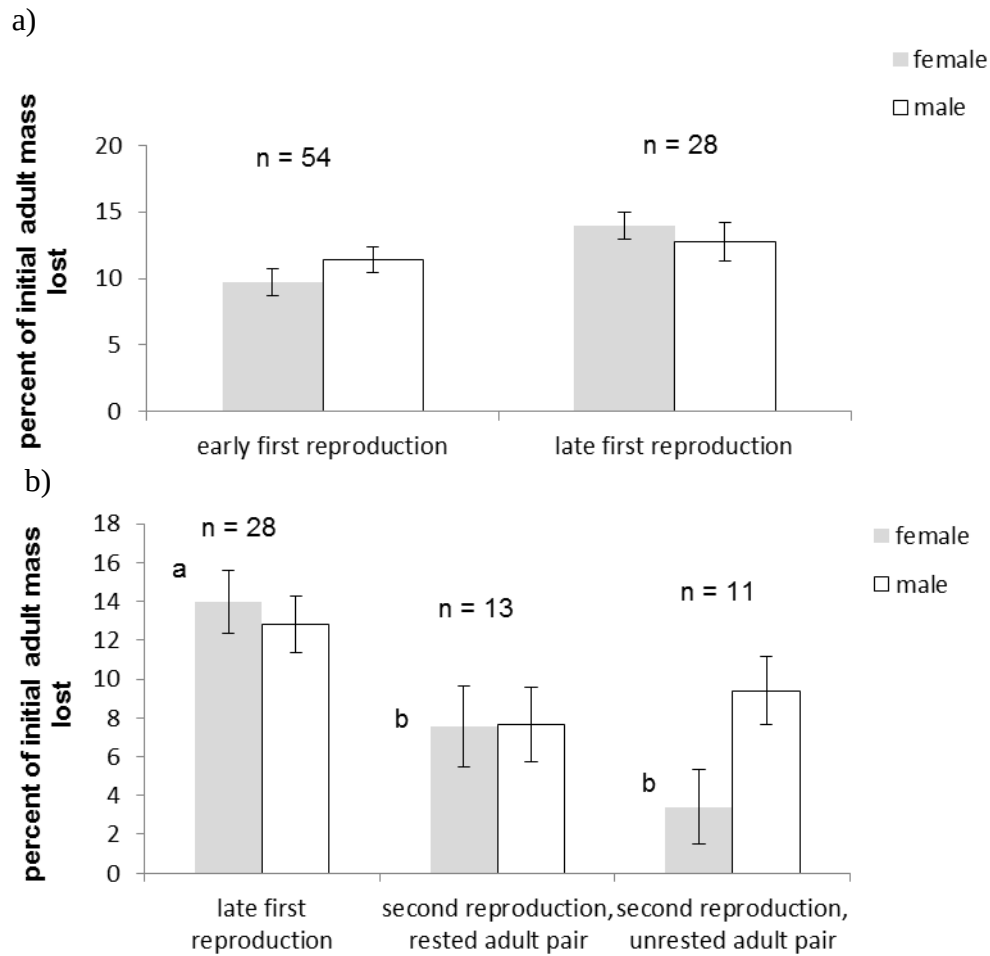


Figure 2.8. Mean percentage ($\pm$ SE) of initial mass lost in adult beetles, year 1. When late first breeders are compared to early first breeders, there is a significant difference in the percent of body mass lost for females (Student's t : $t = 2.36$, $df = 80$, $p = 0.023$), but not for males, (Student's t : $t = 0.81$, $df = 80$, $p = 0.423$) graph a. When late first breeders are compared to second time breeders, there is a significant interaction of gender and treatment, ($F_{2,49} = 3.717$, $p = 0.031$) graph b. Treatments not sharing letters (a, b; females only) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

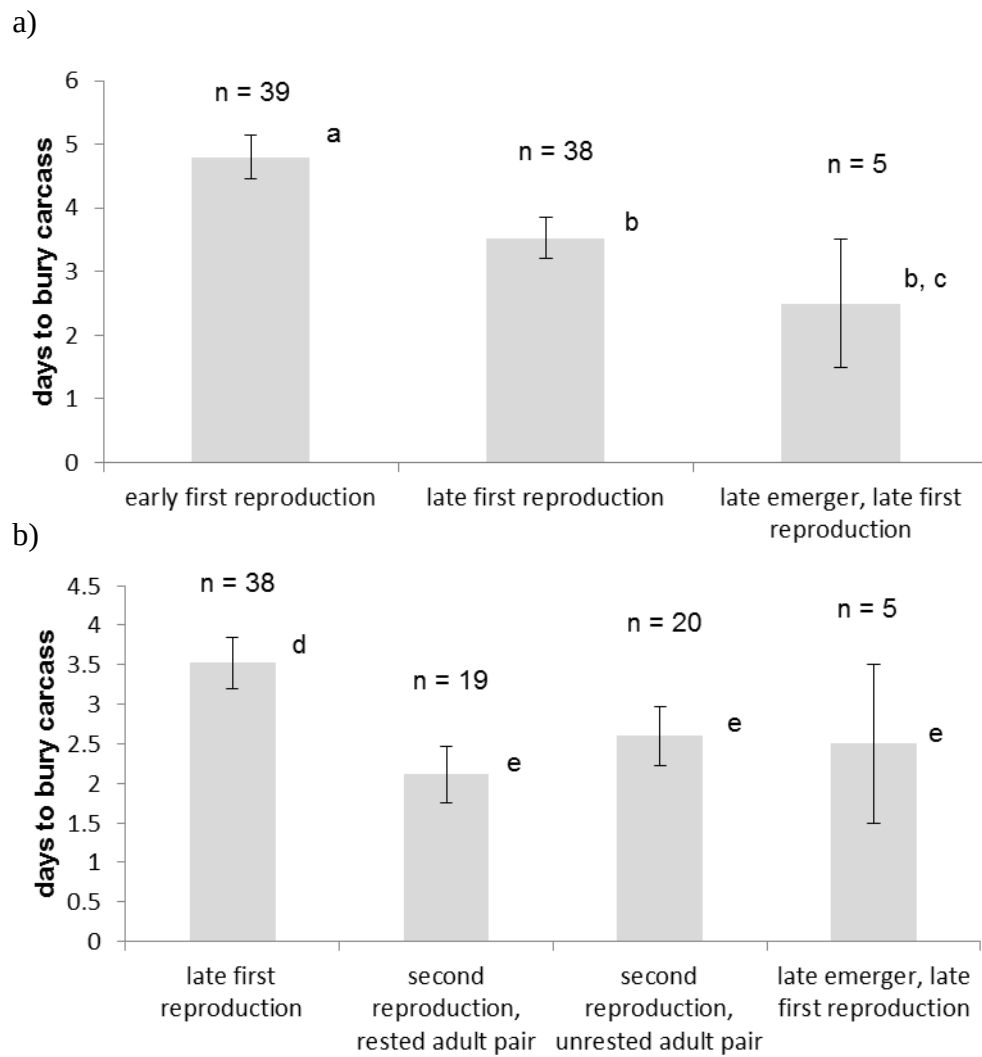


Figure 2.9. Mean days ($\pm$ SE) for adult beetles to bury carcass, year 2. When late first breeding events are compared to early breeding events, there is a significant treatment effect, $F_{2,79} = 5.475$, $p = 0.002$ (graph a). When late first breeding events are compared to second breeding events, there is a significant treatment effect, $F_{3,78} = 4.431$, $p = 0.006$ (graph b). Treatments not sharing letters (a-c, or d, e) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

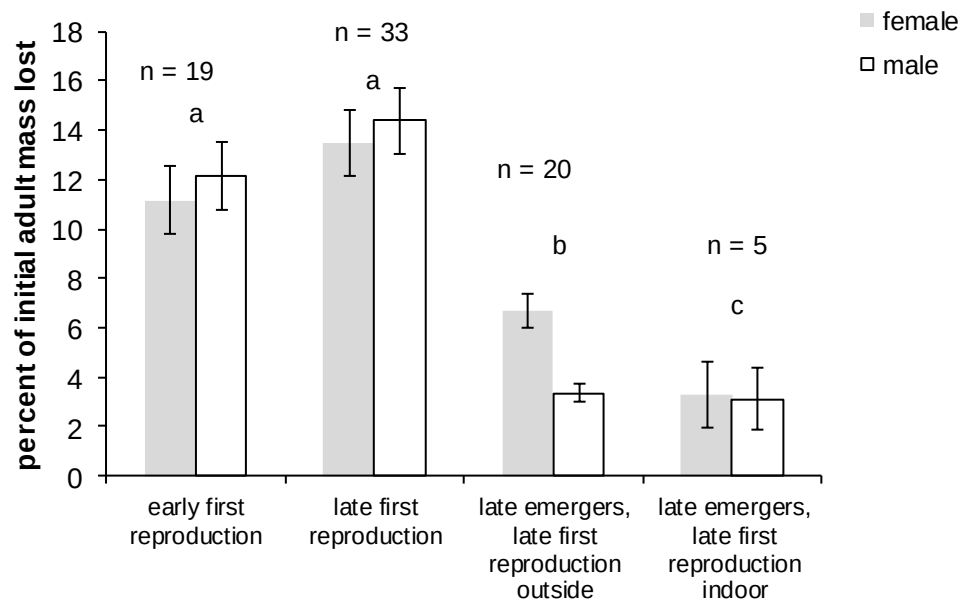


Figure 2.10. Mean percentage ($\pm$ SE) of initial mass lost in adult beetles, first reproductive attempt, year 2. There is no significant interaction ($F_{3,73} = 2.096$, $p = 0.106$) or main effect of gender ($F_{1,73} = 0.031$, $p = 0.861$). There is a significant effect of treatment ($F_{3,73} = 94.232$, $p < 0.001$). Treatments not sharing letters (a, b, c) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

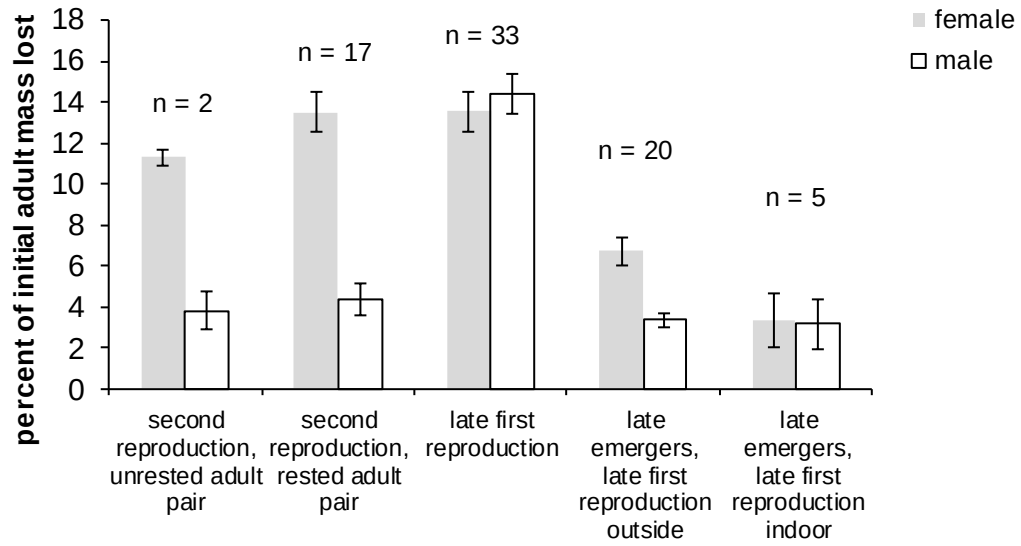


Figure 2.11. Mean percentage ($\pm$ SE) of initial mass lost in adult beetles, late broods, year 2. When adults producing late first broods are compared to adults producing second broods there is a significant interaction effect of gender and treatment ($F_{4,72} = 13.488$, $p < 0.001$). Refer to Table 2.2 for post-hoc comparisons.

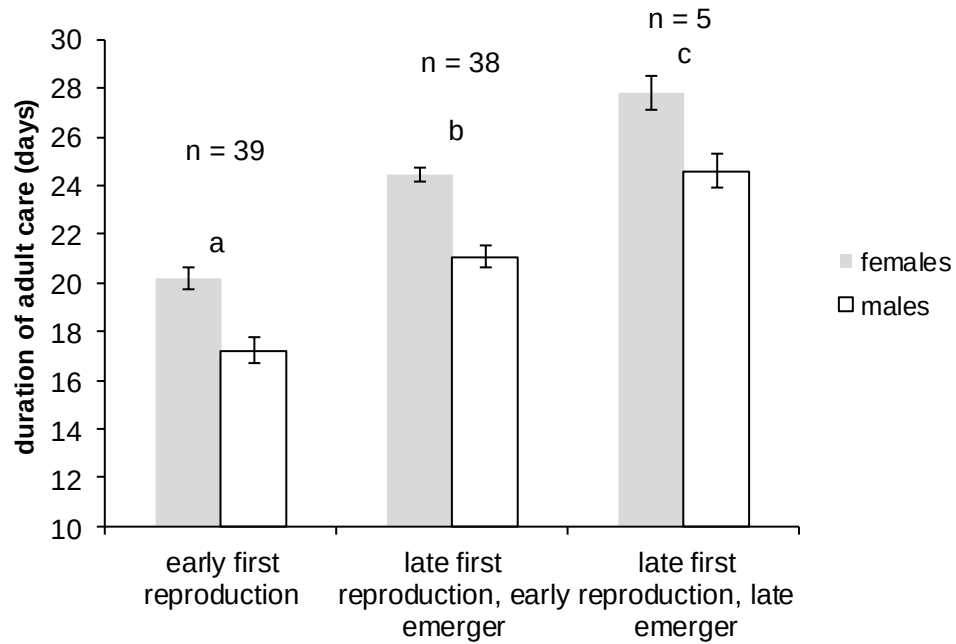


Figure 2.12. Mean duration of adult care ($\pm$ SE) of brood, year 2, early and late first reproduction. There is a within subject effect of sex, $F_{1,80} = 79.047$, $p < 0.001$, females remained longer, and a between subject effect of treatment, $F_{2,79} = 6597.821$, $p < 0.001$, delayed breeders remained longer. Treatments not sharing letters (a,b,c) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

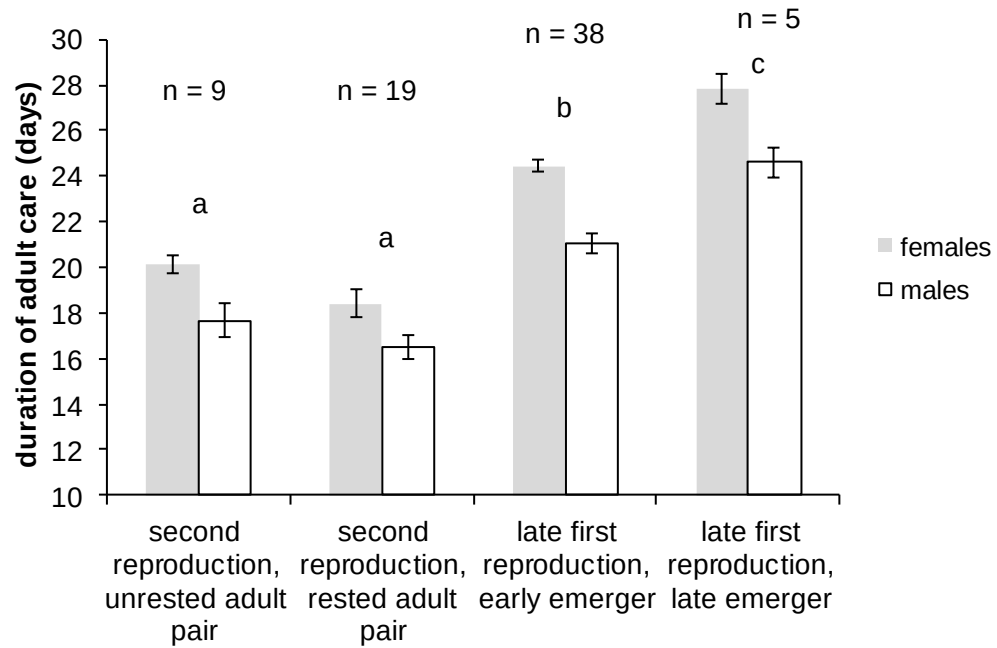


Figure 2.13. Mean duration of adult care ($\pm$ SE) of brood, year 2, second and late reproduction. There is a within subject effect of sex, $F_{1,69} = 41.870$, $p < 0.001$, females remained longer, and a between subject effect of treatment, $F_{3,67} = 6126.283$, $p < 0.001$, delayed breeders remained longer. Treatments not sharing letters (a,b,c) were significantly different in post-hoc comparisons (Bonferroni corrected for multiple comparisons, $p \leq 0.05$).

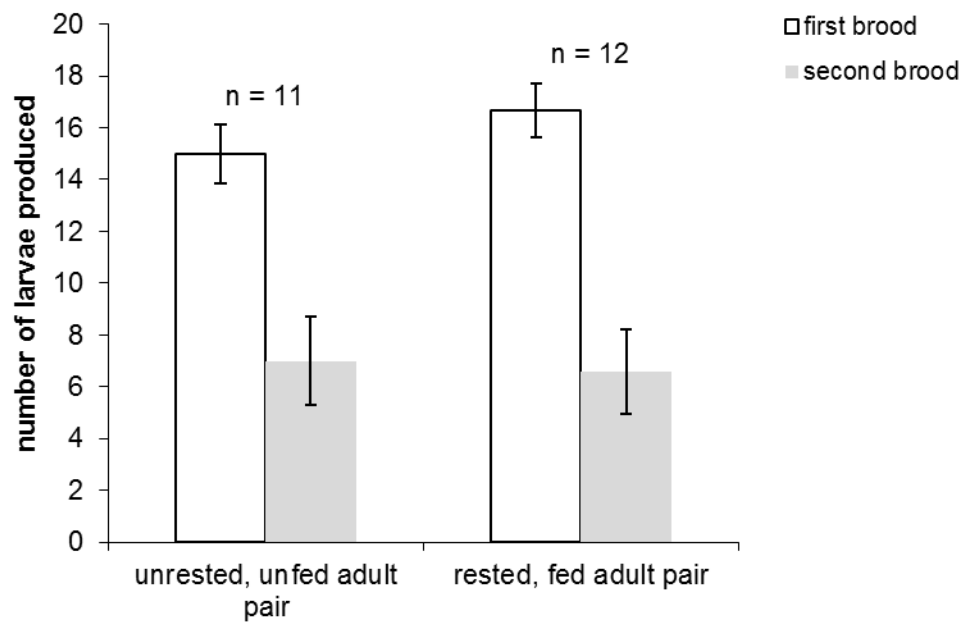


Figure 2.14. Mean number larvae ($\pm$ SE) produced, consecutive broods, year 1. While both treatments showed a significant decline in the number of larvae produced in the second brood, $F_{1,21} = 53.735$, $p < 0.001$, the decline was similar across treatments, $F_{1,21} = 1.116$, $p = 0.303$.

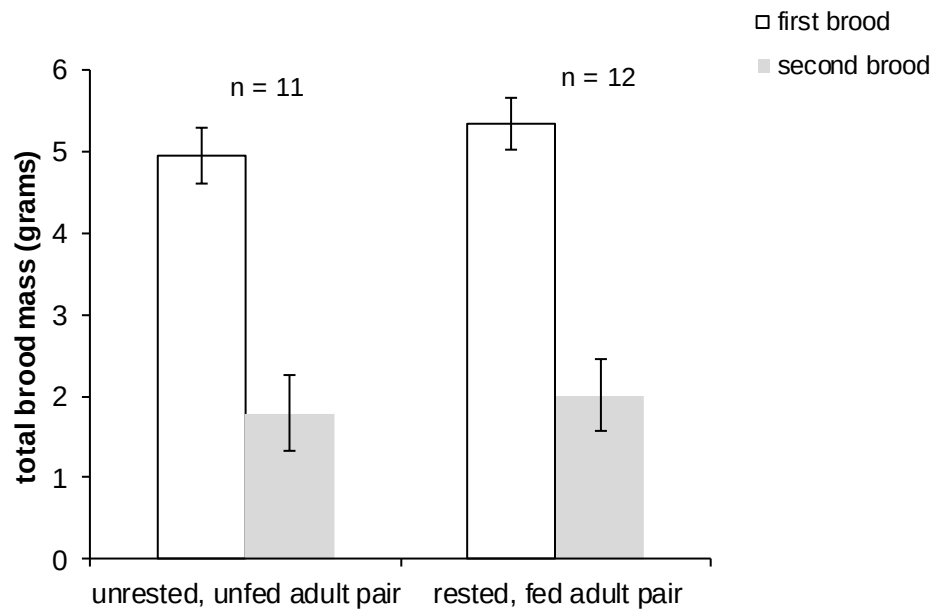


Figure 2.15. Mean mass ($\pm$ SE) of consecutive broods, year 1. While both treatments showed a significant decline in the total mass of the larvae produced in the second brood, $F_{1,21} = 85.847$, $p < 0.001$, the decline was similar across treatments, $F_{1,21} = 0.111$, $p = 0.743$.

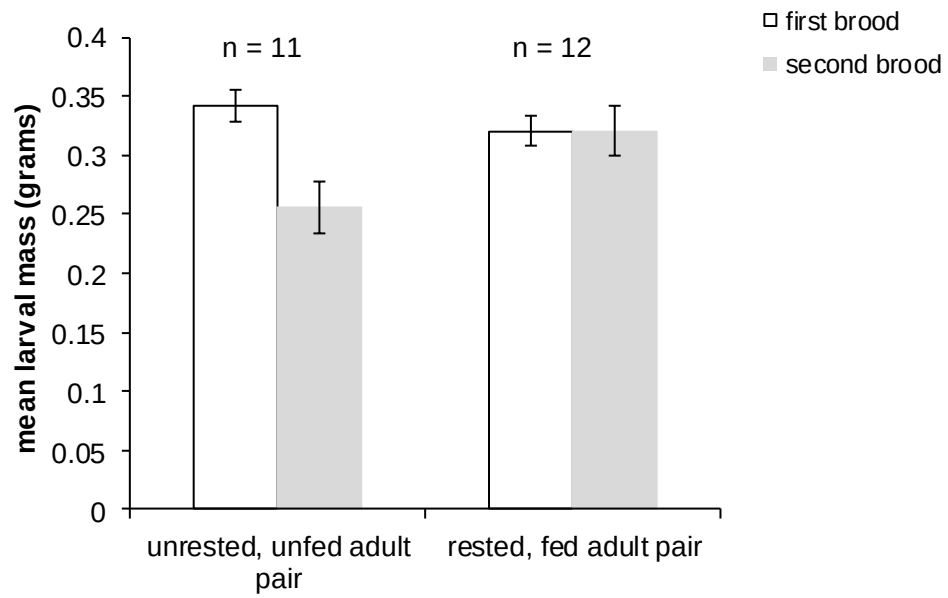


Figure 2.16. Mean mass ($\pm$ SE) of larvae produced consecutively, year 1. There is a significant treatment and reproductive attempt interaction ($F_{1,21} = 5.370$, $p = 0.031$).

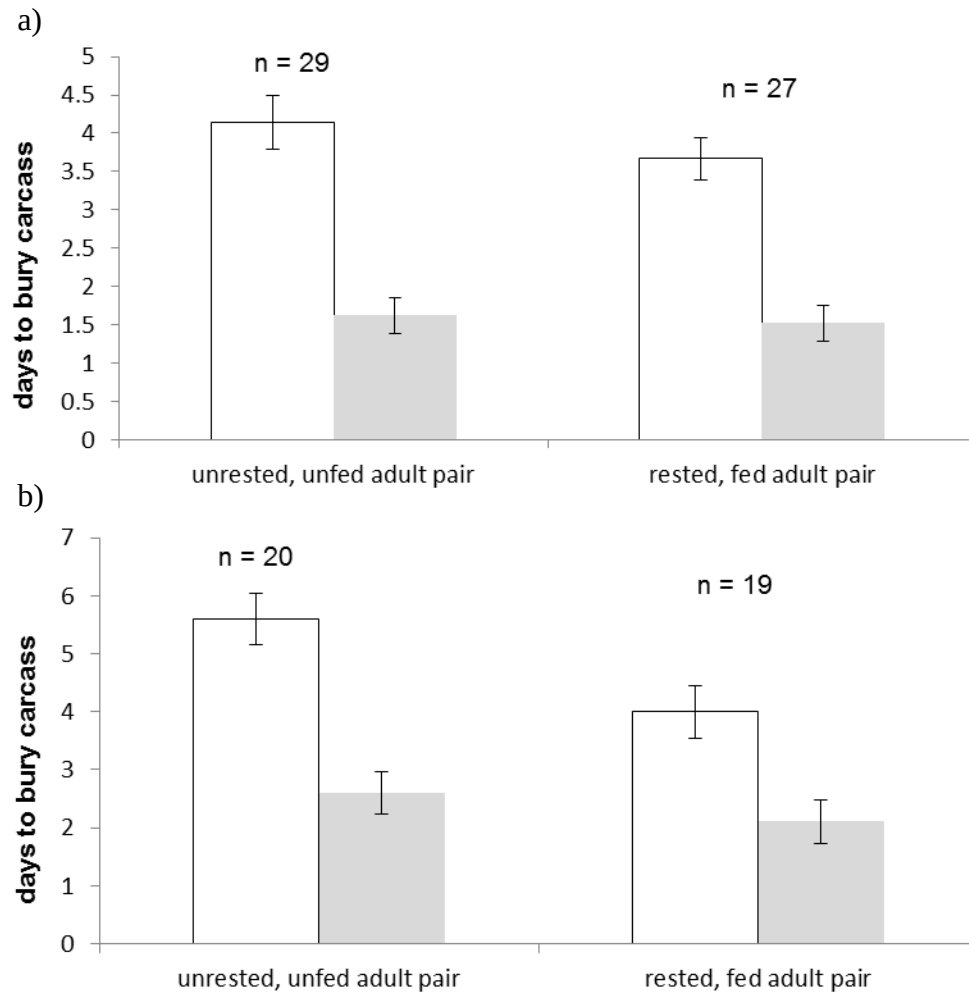


Figure 2.17. Mean days ($\pm$ SE) for adult beetle pairs to bury carcass, consecutive broods, both years. In year 1, both treatments showed a significant decline in the number of days to bury the second carcass, $F_{1,54} = 178.920$, $p < 0.001$, however, the decline was similar across treatments, $F_{1,54} = .0892$, $p = 0.349$, graph a. In year 2, there was a significant interaction between treatment and reproductive attempt, $F_{1,37} = 3.948$, $p = 0.054$, graph b. Both treatments show a significant decline in the number of days to bury the second carcass, and the number of days to bury the first carcass for the first broods are significantly different between the un-rested and rested groups (Bonferroni corrected p-value for multiple comparisons, $p = 0.053$).

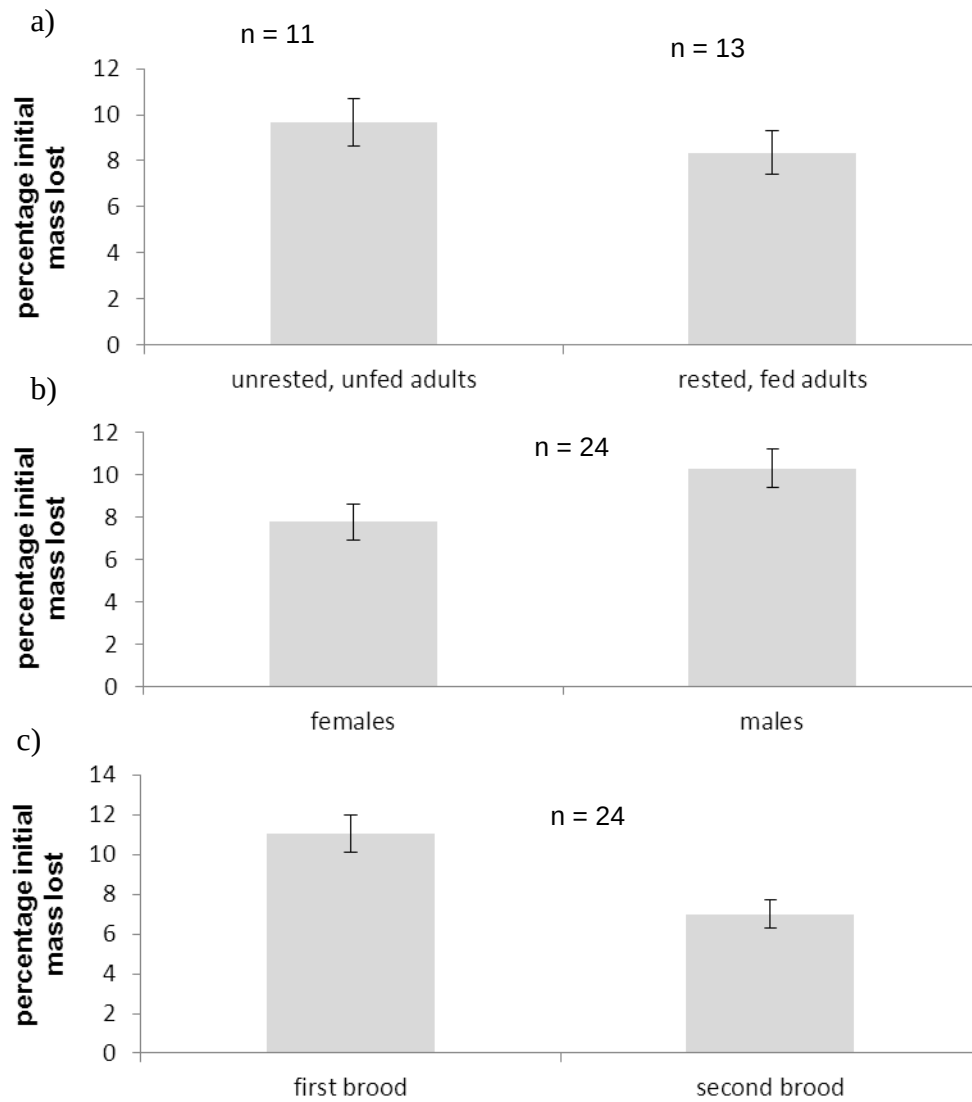


Figure 2.18. Mean percentage ($\pm$ SE) of initial mass lost in adult beetles, consecutive broods, year 1. There is a main effect of treatment, $F_{1,22} = 164.926$, $P < 0.001$, graph a. There is a within subject effect of gender, $F_{1,22} = 5.453$, $p = 0.029$, graph b. And there is a within subject effect of reproductive attempt, $F_{1,22} = 22.612$, $p < 0.001$, graph c.

CHAPTER 3: Efficacy of minimally guided instructional strategies in K-12 and undergraduate science classrooms: A meta-analysis

ABSTRACT

International assessments, research, and a rapidly advancing technologically based global market have all contributed to the call for education reform in the United States, particularly in the disciplines of science, technology, engineering, and mathematics (STEM). Research-based educational reform strategies, including those based on the National Research Council's National Science Education Standards, advocate for student-centered teaching with minimally guided instructional techniques at the core. A number of small, independent programs have published invaluable reports of program characteristics and student achievement scores as a result of attempted reforms. A meta-analysis can synthesize results from multiple individual research studies into a quantitative summary of the effects, in this case the effects of alternative instructional methods. A meta-analysis was conducted to present an updated summary of science education studies at both K-12 and post-secondary levels. The cumulative effect sizes at both levels (K-12 $d = 0.31$, undergraduate $d = 0.44$) confirms the success of minimally guided instructional methods. Rank ordering of effect sizes provided a list of the most effective strategies in each group. Among the K-12 studies, the greatest student increases were observed in studies that implemented curricula that had been endorsed by NSF and offered extensive professional development to teachers. In undergraduate classes, the greatest increases were observed when instructors utilized alternative lecture formats, such as small group and problem based learning settings, and added graduate and

undergraduate learning assistants to assist in the implementation of these strategies.

Continuing research into implementation strategies and curriculum design will help to grow the effectiveness of educational reform movements such as minimally guided instruction.

INTRODUCTION

Scores of contemporary suggestions for focus and reform in the American school system have been made in recent decades, resulting in numerous studies, commissions, and councils aimed at increasing student achievement. The core of the policies and practices implemented, stemming from the development of the National Research Council's (NRC) National Science Education Standards (NSES, 1996) focus on moving towards inquiry and away from the traditional didactic methods of lecturing and assigning homework, where students are expected to passively receive information from the instructor, a dispenser of knowledge (Crawford 2000, Dochy *et al.* 2003, Prince 2004, Smith *et al.* 2007).

The Standards advocate teachers create active learning environments and implicitly state that teachers “focus and support inquiries while interacting with students” and that “inquiry into authentic questions generated from student experience is the central strategy for teaching science” (NRC 1996, Crawford 2000, Schroeder *et al.* 2007). Although there have been numerous ways in which this directive has been implemented, many teachers have adopted minimally guided instruction (Gijbels *et al.* 2005, Hofstein *et al.* 2005, Smith *et al.* 2007). The expression ‘minimally guided instruction’ is an umbrella phrase encompassing instructional strategies associated with constructivism and dynamic learning environments (Phillips 1998, Mayer 2004, Prince 2004, Kirschner *et al.* 2006, Smith *et al.* 2007). Other names for essentially equivalent pedagogical approaches to learning include: discovery, problem-based, project-based, inquiry, experiential, concrete, active, and student-centered (Saunders & Shepardson 1984, Prince 2004, Kirschner *et al.* 2006, Anderson 2002).

Since No Child Left Behind (2001), the preponderance of educational policy reform has been grounded in research based curricular and behavioral studies and focused on primary and secondary educational levels (Wise 1996, NSF 1998, Tretter & Jones 2003, Schroeder *et al.* 2007). Increased pressure for reform in science education, the numerous alternative instructional programs that have been implemented, and the availability of federal funding for plans with positive supporting evidence all beg the question of the relative effectiveness of programs and reforms (Anderson 2002, Schroeder *et al.* 2007).

The most recent aggregation of national research informing science teaching strategies and results was a meta-analysis published in 2007, including K-12 science education studies in the United States from 1980-2004 (Schroeder *et al.* 2007). The calculated overall effect size of the 62 studies was 0.67, indicating a significant increase in student achievement from “alternative teaching strategies”. Previous meta-analyses have provided narrow and quantitatively accurate overviews of science education reform literature, many of them concentrating on one specifically defined aspect of minimally guided instruction (Anderson 2002, Shavelson *et al.* 2003, Collins *et al.* 2004, Schroeder *et al.* 2007). For example, meta-analyses of undergraduate science instruction have targeted studies in only one discipline or those implementing a single technique (Springer *et al.* 1999, Colliver *et al.* 2003, Shavelson *et al.* 2003, Collins *et al.* 2004). No meta-analysis to date has broadly summarized research completed on several minimally guided instructional methods implemented in undergraduate classrooms (Shavelson *et al.* 2003, Collins *et al.* 2004).

The meta-analysis described here fulfills two goals: 1) to update the current body of knowledge on K-12 education reform initiatives since 2004, and 2) to expand a meta-analysis of undergraduate studies to investigate comparative efficacy of several minimally guided instructional methods across all sciences. The scope of the research included in this analysis is intentionally large to avoid issues surrounding interpreting a particular researcher's implementation or instructional strategy focus and to broaden the applicability of the included research (Shavelson *et al.* 2003, Collins *et al.* 2004).

SYNOPSIS OF SCIENCE EDUCATION REFORM

Early works of Dewey (1910, 1938, 1944), Piaget (1954, 1959), and Bruner (1959, 1961) are often cited as foundational theories of learning that should be interwoven within nationwide initiatives for education reform (Mayer 2004, Gijbels *et al.* 2005, Schroeder *et al.* 2007). Some current educational reforms can even trace their roots back to ancient Greece, where Menon explains to Socrates that understanding depends on prior learning (Prawat 1999). The link between the NSE Standards and implementing minimally guided instructional techniques stems from Piaget's learning theory, specifically targeting constructivism (Phillips 1998, Mayer 2004, Schroeder *et al.* 2007). The application of constructivist learning theory can take many forms and it has made broad impacts in the areas of teaching and learning (Mayer 2004). The core premise of the theory is directly linked to the Standards: the design of educational standards and benchmarks that allow students to develop coherent and organized knowledge by participating in an active learning process (Phillips 1998, Mayer 2004, Pine *et al.* 2006). Just prior to the turn of the century, the NSE Standards began increasing awareness

among post-secondary faculty for the need to change from traditional didactic strategies. Although the call for reform was not as immediate as compared to K-12 education, there is a recognizable paradigm shift toward implementing more engaging and student-centered instructional techniques in post-secondary mathematics and science classes (Keefer 1999, Pine *et al.* 2006, Silverthorn *et al.* 2006, Quitadamo *et al.* 2008).

Technological advances and national reliance on industrial sciences drives in current reform efforts that are directly intended to increase student achievement and literacy levels in STEM content areas, particularly at primary and secondary educational levels (Keefer 1999, Siebert & McIntosh 2001, Geier *et al.* 2008). Although not exhaustive, a recent literature search produced a list of recent and current educational reform initiatives:

- Nation at Risk, report by the National Commission on Excellence in Education, 1983 (Richardson & Liang 2008)
- Project 2061, American Association for the Advancement of Science, 1985 (Zoller 2000)
- Benchmarks for science literacy, American Association for the Advancement of Science, 1989, 1993 (Crawford 2000)
- Goals 2000: Educate America Act, PL 103-227, established the National Educational Standards & Improvement Council, 1994 (Richardson & Liang 2008)
- Principles and Standards for School Mathematics, National Council of Teachers of Mathematics, 1995 (Zoller 2000)
- National Science Education Standards & Benchmarks, National Research Council, 1996, 2000 (Zoller 2000, Ingram *et al.* 2004, Smith *et al.* 2007)

- No Child Left Behind, PL 107-110, reauthorization of 1965 Elementary and Secondary Education Act, 2001 (US Department of Education)
- Science Framework for the 2009 National Assessment of Educational Progress (NAEP), report by the United States Department of Education, 2007 (Smith *et al.* 2007)

The sheer number of reform campaigns, coupled with intense political and social pressures for significant improvements, helps to characterize the unprecedented challenges facing science educators today (Silverthorn *et al.* 2006, Quitadamo *et al.* 2008). The current standards-based reform movement at K-12 levels is based upon national standards that have been developed by communities of professional mathematicians, scientists, education researchers, policy makers and teachers (Ingram *et al.* 2004, Smith *et al.* 2007). The National Research Council's (NRC) National Science Education Standards (NSES, 1996) include descriptions of content, teaching strategies, professional development, and assessment techniques (Burrill & Kennedy 1997). Coinciding with the publication of these standards, a strategic framework for K-12 standards-based reform has been implemented to assist and inform groups from National Councils down to individual School Curriculum Committees (Table 3.1, Burrill & Kennedy 1997, Bybee *et al.* 1997). This framework is paramount in establishing lasting and meaningful changes to increase student achievement nationally, as it describes the iterative process of identifying areas of weakness, developing, implementing, evaluating, and revising instructional policies, programs, and strategies (Bybee *et al.* 1997, Smith *et al.* 2007). Some would argue that the United States, as a whole, is still in the dissemination or interpretation stage of this framework (Crawford 2000, Anderson 2002,

Kirschner et al. 2006). However, among education researchers, there is general agreement that the instructional policies and practices that have been developed as a result of the framework are in the throws of Evaluation and Revision (Silverthorn *et al.* 2006, Smith *et al.* 2007, Schroeder *et al.* 2007).

META-ANALYSIS: A RESEARCH TOOL IN SCIENCE EDUCATION

The diverse nature of relatively small scale, independent studies that have been implemented lends itself to a meta-analysis, or compilation, of current studies to evaluate the impacts of minimally guided instructional strategies. Modern meta-analytic methods are common statistical tools that accurately and quantitatively synthesize independent studies in areas such as social sciences, medicine, ecology, and evolution (Rosenberg *et al.* 2000). This method assesses independent quantitative evidence to understand the extent to which a particular hypothesis is supported or rejected.

Precursors to modern meta-analyses include comparing similarly structured studies by overly simplistic vote counting technique as well as combined probability methods. The vote counting technique utilizes three categories into which studies can be classified. With regard to a particular hypothesis, studies are counted as being statistically significant in support, statistically significant in opposition, or not statistically significant. The category with the most tallies is asserted to be the statistical trend summarizing primary studies either to support or refute a hypothesis. This method completely overlooks several key aspects of individual studies. The degree to which the results either support or refute a hypothesis is not considered, nor is the sample sizes of the independent studies counted (Rosenberg *et al.* 2000). Also, variance, due to sample

sizes, study design or strength of results, is disregarded (Hedges *et al.* 1989). Combined probability methods utilize exact probabilities, taking into account sample sizes, from each independent study and can be referred to as *omnibus* tests (Rosenberg *et al.* 2000). However, this method also lacks the ability to quantify the magnitude of the statistically significant finding and cannot assess overall agreement (or lack thereof) among studies. Meta-analysis combined with modern statistical tools concisely synthesizes trends from numerous independent primary research studies.

META-ANALYSIS: MODELS AND THE META-WIN PROGRAM

The general model of a meta-analysis is to calculate and then compare effect sizes (Hedges *et al.* 1989). An effect size is a standardized measure of the degree to which a given treatment is represented, or present, in a given sample. Calculation of effect sizes from similar experimental outcomes, or dependent variables, allows results from numerous independent studies to be compared. One component of standardization is to consider sample sizes of studies, which are incorporated into the associated variances that are calculated along with effect sizes (Hedges *et al.* 1989). The sampling variances of studies provides a mechanism with which individual studies can be weighted in order to compute an overall effect size of a treatment. Weighted statistical models provide the framework for a meta-analysis that can predict the overall, statistically quantifiable, effect present in the data. A fixed-effects model does not allow for generalizations about a population because between study variance is ignored in the analysis. With a random effects model estimates between study variance and population descriptions are possible (Hedges *et al.* 1989, McNamara *et al.* 1998).

For example, the heterogeneity among the studies can be estimated to determine if the studies likely come from one or more populations (Thompson 1994, Rosenberg *et al.* 2000). Cochran's Q , the measure of heterogeneity, is a weighted sum of the pooled effect across studies and the squared differences between individual study effects. Cochran's Q is distributed as a Chi-Square distribution and its probability is used to detect imprecision in the estimate of the effect size for each study (Thompson 1994). If this measure is statistically significant, there is more differentiation between studies than chance can account for and moderator variables may be evident among the studies (McNamara *et al.* 1998). Effect sizes are directly influenced by parameters of the study design or population of subjects, which are broadly labeled moderator variables (Thompson 1994, McNamara *et al.* 1998). If the test of heterogeneity is met, moderator variables can not be ruled out entirely, but rather the combinability of the studies is strengthened.

The MetaWin program calculates an additional statistical component relevant to meta-analysis, fail-safe numbers (N_{fs}). Fail safe numbers provide insight into the level of possible publication bias present within a group of studies. Results of a fail safe calculation provide the number of non-significant or unpublished studies, added to the meta-analysis, that are required to drive the effect size to an insignificant level (McNamara *et al.* 1998, Rosenberg *et al.* 2000). An accepted conservative value against which to test a fail-safe calculation is $5n + 10$, where n is the number of studies in the original meta-analysis (Rosenthal 1979). If the Rosenthal fail-safe number is even conservatively larger than the number of included studies, the overall effect size is a reliable estimate of true effect (Rosenberg *et al.* 2000). For example, if the number of studies in an analysis is 10 (n) and the Rosenthal fail-safe number is $5n + 10$ (60), or

greater, the calculated cumulative effect size is considered to be a dependable measure of the effect of the instructional strategy studied. High fail-safe numbers indicate that a researcher can safely ignore more complicated methodologies within the meta-analysis that address publication bias (Rosenberg 2005).

General rules for understanding the implications of effect sizes can be illustrated by using an alternative curricular treatment as an example. The sign on the effect size is an indicator of the gains in knowledge that can be associated with the new treatment under study. A positive effect size indicates a gain in knowledge that can be attributed to the new curriculum, while a negative sign indicates that no gain in knowledge can be accordingly attributed to the curriculum (McNamara *et al.* 1998). The process of rank ordering effect sizes from lowest to highest results in a list of programs, or curricular treatments increasing in their effectiveness. If the criterion measured across all studies approximates a normal distribution, achievement implications can be easily understood. For an effect size of 1.0, student improvements will be one standard deviation above their previous achievement levels; for example, a student that under the old curriculum scores in the 50th percentile will score in the 84th percentile under the new curriculum. In educational research, effect sizes greater than 1.0 may suggest defects in the design or execution of a study (Hedges *et al.* 1989, McNamara *et al.* 1998, Rosenberg *et al.* 2000).

A random effects meta-analysis model- incorporating weighted statistical analyses- used Hedges' *d* and its accompanying variance to calculate the overall effect present in the data as well as its variance. The overall magnitude of the effect present in the studies is represented in the cumulative effect size and is significantly different from zero if its confidence limits do not bracket zero (Rosenberg *et al.* 2000). In educational

studies, effect sizes greater than 1.0 may suggest improper study design or strongly heterogeneous population structure of study groups and excluding those studies will result in greater preciseness of the cumulative effect size estimation (Hedges *et al.* 1989) - a markedly more precise meta-analysis with greater general applicability (McNamara *et al.* 1998, Rosenberg *et al.* 2000). Weighted histograms identify possible bimodal distribution of the data that is indicative of a small number of studies, or components of studies, with effect sizes greater than 1.0.

METHODS FOR CURRENT STUDY: LITERATURE SEARCH AND INCLUSION CRITERIA

Searches of electronic databases such as Academic Search Complete, Blackwell/Synergy, EBSCO Host, ERIC, ISI Web of Science, and ProQuest yielded hundreds of books, conference papers, journal articles, and the like. Searches included terms such as “science education”, “science teaching”, “inquiry learning”, and “inquiry teaching”. General search strings, “student achievement” and “active learning”, for example, were used with a qualifying and limiting term like “science”. Citation lists were also examined for additional references.

To narrow the scope of the literature appropriate for this meta-analysis, more stringent criteria were imposed upon the studies than were used in initial searches. In order to be included, studies had to:

- address either K-12 or undergraduate science education in the United States
- be published after 2004 for K-12 studies
- measure student achievement or performance as a dependent variable

- use science teaching strategies, other than didactic approaches, as independent variables
- be experimental (incorporate control and treatment groups)
- focus on general populations, not special needs students
- provide statistics necessary to use MetaWin 2.1 to calculate effect size

Two categories of studies were included in the current meta-analysis:

investigations conducted in K-12 classrooms (n = 6 independent studies, yielding 12 component effect sizes, Table 3.2) and investigations conducted in undergraduate settings (n = 12 independent studies, yielding 18 component effect sizes, Table 3.3). All studies utilized a minimally guided instructional strategy with an experimental group of students and traditional, didactic techniques with a control group. Students were randomly assigned to treatment groups and in all studies measures of student achievement were collected using a pre- and post-test format, except one K-12 study, Shymansky 2004, which utilized a post-test only collection format.

In each category, there were two studies that yielded multiple component effect sizes (K-12 studies, Table 3.2; Undergraduate studies, Table 3.3). For example, Lee *et al.* (2010) described the impacts of technology-enhanced inquiry instruction in several science content areas at both Middle and High Schools, resulting in six component effect sizes and one overall effect size for the study. For this study and others like it that produced component and overall effect sizes, only the component effect sizes were included in the calculation of the cumulative effect size for the meta-analysis, to avoid duplication.

ANALYSIS: METAWIN CALCULATIONS

Data, including means, sample sizes and standard deviations for both the control and treatment groups, were input into MetaWin (version 2) for effect size calculation. Hedges' d was selected as the measure of effect size because it is not affected by unequal sampling variances in the experimental and control groups and, if necessary, accounts for effects of small sampling sizes (Rosenberg *et al.* 2000). Two additional output measures were produced from the basic effect size calculation. Cochran's Q provided a measure of heterogeneity to each groups of studies. Rosenthal's fail-safe number provided a reliable estimate of the true effect of minimally guided instructional strategies. Rank ordering of overall effect sizes within each group of studies (K-12 and undergraduate education) allowed for analysis of characteristics of most effective minimally guided science teaching strategies at different educational levels (Rosenberg *et al.* 2000).

RESULTS

K-12 classrooms

The cumulative effect size of the studies in K-12 classrooms indicated that minimally guided instructional strategies results in increased student achievement (ES = 0.31, 95% CI = 0.20 to 0.43, Table 3.2). The test for heterogeneity suggests that, in spite of relatively broad inclusion criteria, the variance in studies is likely due to chance and not to study design or population demographics ($Q_{\text{total}} = 9.46$, $p = 0.58$). Rosenthal's fail-safe number was 76. A rank-ordered cumulative meta-analysis plot helped to examine the general distribution of the data and determine if there was any structure not previously identified in Cochran's Q statistic (Figure 3.1).

After eliminating the outlying study [Lee 2010, high school chemistry study, #10 on Figure 3.1] and reanalyzing the data the effect size remained the same ($ES = 0.31$, $95\% CI = 0.22$ to 0.41), still indicating that minimally guided instructional techniques results in increased student achievement. The second analysis produced a more precise assessment of the treatment as evidenced by the measure of heterogeneity ($Q_{total} = 9.15$, $p = 0.64$). The fail-safe number after reanalysis was 49.

Rank ordering studies according to effect size places the strategies used in each study into descending order of effectiveness (Table 3.4). Among the K-12 studies, the top three overall effect sizes were from studies that implemented research-based and highly rated curricula (according to the National Science Foundation, NSF) and whose instructors, at one time, had been provided intense professional development support (cited in order of decreasing effect size: Wilson *et al.* 2010, Lynch *et al.* 2005, Lee *et al.* 2010). The study with the lowest overall effect size, but still indicated increases in student achievement, was a study designed to implement minimally guided instructional strategies developed in the 1990's (received mediocre ratings from NSF 1998, NSTA) without professional development support for teachers (Pine 2006).

Undergraduate classrooms

The cumulative effect size indicated that an increase in student achievement after implementing minimally guided instructional strategies is exhibited at undergraduate levels as well ($ES = 0.56$, $95\% CI = 0.30$ to 0.82). Additionally, the test for heterogeneity on the undergraduate studies indicated any variances are likely due to chance ($Q_{total} = 11.15$, $p = 0.89$). Rosenthal's fail-safe was 190.

Although the rank-ordered cumulative meta-analysis plot (Figure 3.2) did not indicate any bimodal distribution of the data, examination of a weighted histogram (Figure 3.3) of the effect sizes indicated such a distribution and the analysis was re-run. Studies with outlying effect sizes were eliminated and the effect size decreased slightly ($ES = 0.44$, 95% CI 0.33 to 0.56) and preciseness of the cumulative effect size was similarly effected ($Q_{total} = 11.64$, $p = 0.77$). Rosenthal's fail-safe number was 112.

After eliminating effect sizes greater than one, the undergraduate studies with the top three overall effect sizes were studies that implemented curricular materials, including quizzes, laboratories, technological demonstrations, problem sets, and assessments based upon classroom participation in unique fashions (cited in order of decreasing effect size: Pereira *et al.* 2007, Cibik & Diken 2008, and Knight & Wood 2005). The study with the lowest overall effect size, but still indicated increases in student achievement, incorporated peer-led small group inquiry sessions in a large introductory chemistry course (Lewis & Lewis 2008).

DISCUSSION

This meta-analysis confirms that K-12 teachers and college faculty continue to implement minimally guided instructional strategies and these indeed result in increased student achievement, a result similar to that found in meta-analyses completed prior to 2004 (Schroeder *et al.* 2007) and other more recent, isolated studies (Silverthorn *et al.* 2006, Sirum & Madigan 2010). The effect size in this study is somewhat lower than the earlier meta-analysis (0.67, Schroeder *et al.* 2007), which encompassed studies over almost 25 years. In addition, this analysis showed that the strategies implemented within undergraduate settings results in greater increases in student achievement compared to K-

12 classrooms (ES undergraduate = 0.44 versus ES K-12 = 0.31), even when outlying large effect sizes are removed from the analysis.

The considerable difference between the number of studies (K-12 $n = 6$, undergrad $n = 12$) and the fail-safe calculations from the analysis (K-12 $N_{fs} = 76$, undergrad $N_{fs} = 190$) far exceeds even conservative estimates and is a reliable indication of the true effect of minimally guided instructional strategies (Rosenthal 1979, Rosenberg *et al.* 2000, Rosenberg 2005). This serves as strong supporting evidence that minimally guided instructional strategies will likely boost student achievement levels significantly across a variety of science classrooms.

This analysis targets IF minimally guided instruction increases student achievement and can only begin to examine WHY minimally guided instruction increases student achievement or HOW to structure its implementation. The analysis and rank ordering studies indicate areas for future research that would further address the latter questions of “why” and “how” (Schroeder *et al.* 2007, Sirum & Madigan 2010). Identifying effective specific instructional components or institutional infrastructure necessary for widespread change supports a reform framework that calls for iterations of implementation, evaluation, and revisions (Bybee *et al.* 1997). In fact, a closer examination of the studies’ moderator variables can determine the infrastructure and support required to establish and maintain desired widespread student achievement gains in science (Hedges *et al.* 1989, Rosenberg *et al.* 2000). Moderator variables can include program implementation parameters of: teacher experience or preparedness, professional development opportunities, curriculum materials used, or financial support (Schroeder *et al.* 2007).

According to the rank order of the K-12 studies, the most effective strategies included implementation of NSF endorsed curricula by instructors that were supported by extensive professional development programs. Additionally, adequate financial support from planning grants or private curriculum development companies allowed the instructors in these studies to administer technologically enhanced laboratories and activities. Teachers were intimately familiar with the delivered curriculum in Lee *et al.*'s (2010) study as they had actually participated in the creation of the instructional units. The study that demonstrated the smallest positive effect size was a study that offered minimally guided instructional strategies to teachers, but provided no professional support (Pine 2006).

According to the rank order of the undergraduate studies the most effective strategies included unique implementations of somewhat traditional curricular materials. These studies utilized supplemental materials several times a week and in particular, focused on alternative lecture formats including small group and problem based learning settings. The concentration of the studies was to develop deeper cognitive understanding by presenting information and asking students to demonstrate their knowledge using as many learning modes and styles as possible (Knight & Wood 2005, Pereira *et al.* 2007, Cibik & Diken 2008). Additionally, the design of Knight and Wood's study included adding 8 learning assistants (4 graduate students, 4 undergraduates) while implementing the alternative instructional strategies, which, to no surprise, greatly increased efficacy. The least effective strategy was peer-led small group inquiry sessions (Lewis & Lewis 2008). While the instructional techniques in this study were different from classic

lectures, the summative assessment strategies remained unchanged for all sections of the course, including those that retained traditional didactic approaches.

Although national calls for reform have advanced awareness of the need to increase student achievement at all stages of science education, more extensive studies have been conducted at the K-12 than the post-secondary level (Smith *et al.* 1993, NSF 1998, NRC 2003, Silverthorn *et al.* 2006). A decline in US student performance relative to other nations combined with mandatory attendance laws results in political pressure to increase student achievement (TIMSS 2007, National Center for School Engagement). Additionally, the use of norm-referenced achievement tests and comparable age classes of students leads to the trend of reform movements being initiated at K-12 more often than post-secondary level (Hofstein *et al.* 2005, NRC 2007, Taitelbaum *et al.* 2008). The focus on change at the level of K-12 education stems from oversight of the U.S. Department of Education and the compulsory attendance laws enforced by every state in the Nation (Committee on Undergraduate Science Education 1997, 1999; NSF 1998; Lewis & Lewis 2008). The U.S. Department of Education and individual state legislatures establish, monitor, and ensure adherence to strict criteria for certification of K-12 instructors (Burrill & Kennedy 1997, Richardson & Liang 2008, US Department of Education). Such criteria are implied for post-secondary instructors, however, there is no regulatory body or standardized certification criteria as evidenced at primary and secondary levels (Committee on Undergraduate Science Education 1997, 1999, Keefer 1999, Silverthorn *et al.* 2006).

Although there is a much greater top-down push for reform in K-12, a long-term commitment is necessary to observe sustained changes in student achievement resulting

from the implementation of alternative instructional techniques (Prince 2004, Silverthorn *et al.* 2006). Professional development programs for primary and secondary teachers currently in classrooms continue to improve and evolve and despite not having experienced the techniques as students or being formally trained in minimally guided instruction, there are a number of teachers enthusiastic about changing the way they teach (Yeany & Padilla 1986, Crawford 2000, Crawford 2007, McNeill & Krajcik 2008). For some teachers, however, the experience of having been a student of didactic techniques and the pressure to implement minimally guided instructional strategies without extensive professional development support leads to a decline in the effectiveness of new strategies being implemented (Jones & Eick 2007, Smith *et al.* 2007, Marshall *et al.* 2009). Student achievement often does not increase in tandem with a teacher's comfort with and confidence in the instructional techniques and unsupported teachers are more likely to abandon changes compared to teachers receiving professional support (Smith & Anderson 1999, Anderson 2002, Songer *et al.* 2002, Zion *et al.* 2007). For all of these reasons, minimally guided instructional strategies must continue to be implemented in primary and secondary classrooms, professional development opportunities should be strongly encouraged and easily accessible for teachers, and resulting changes in student achievement must continued to be researched (Silverthorn *et al.* 2006). Moreover, an evaluation of the reform movement's focus upon alternative teaching techniques will be in order after the U.S. Department of Education releases the results from the 2009 NAEP Assessment in Science and the Trends in International Mathematics and Science Study given in 2011. In addition to reform in instructional methods, basic educational infrastructure, such as class size, length of class periods, and length of school year are

suitable and timely areas recommended for further research (Mayer 2004, Schroeder *et al.* 2007).

Implementing minimally guided instruction at the undergraduate level may be driven by faculty and be slower to become widespread, but the results may be more dramatic. A number of studies suggest that older students make superior gains in academic achievement when encountering alternative teaching strategies compared to younger students (Lee *et al.* 2006, Howes 2008, Lee *et al.* 2010, this meta-analysis). Because teachers currently in primary or secondary classrooms are likely to have been taught with traditional didactic methods and in turn, teach they way they were taught, focusing on undergraduate students will result in teachers with greater content knowledge and the confidence to implement minimally guided practices by virtue of having been extensively exposed as students and practiced as pre-service teachers (Darling-Hammond & Youngs 2002, Crawford 2007, Jones & Eick 2007, McDonald & Songer 2008, Quitadamo *et al.* 2008, Sirum & Madigan 2010).

The Doctor of Arts (DA) degree is a graduate program specifically designed to develop content area expertise as well as pedagogical preparation in candidates seeking to become undergraduate faculty members (White & McBeth 2003, ISU 2011). While many doctoral programs are beginning to incorporate pedagogical components, the DA degree includes rigorous studies of learning theories and practical experiences such as collaborative teaching under the guidance of mentoring faculty (White & McBeth 2003). Doctor of Arts graduates that go on to become faculty in teacher preparation programs, insure that students gain knowledge through minimally guided instructional strategies and gain experience in implementing those strategies. Inquiry experiences both as students

and pre-service teachers results in teachers entering the K-12 field better prepared to instruct in like fashion (Yeany & Padilla 1986, Heywood & Heywood 1992, Settlage 2007). Moreover, as new teachers, enter the educational system, students that continue on to undergraduate experiences might possibly encounter additive, synergistic effects after having been exposed to minimally guided instructional strategies in primary, secondary, and post-secondary settings.

In light of historically declining U.S. student performance in STEM disciplines and calls for wide-spread instructional modification, a strategic framework for standards-based educational reform has been implemented nationwide (Burrill & Kennedy 1997, Bybee *et al.* 1997). The reform movement and the evaluation and revision stages of the framework have long focused on the implementation of minimally guided instructional strategies at the K-12 level, techniques that are known to increase student achievement particularly when the teachers delivering the curriculum receive professional development and support (Krajcik *et al.* 2007, Schroeder *et al.* 2007, Taitelbaum *et al.* 2008, this meta-analysis). However, as this meta-analysis shows, the difference in student achievement after implementing these strategies at the K-12 and undergraduate levels warrants further research. This nation's interest in sustaining increased student achievement in STEM disciplines might be best served by concentrating on the content knowledge and the inquiry teaching skills of the next generations of teachers. The same strategic framework that has proven useful at the K-12 level could be effectively implemented more widely at the undergraduate level to affect this new focus.

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Table 3.1. Strategic framework for standards-based reform. From Bybee *et al.* 1997.

Stage	Description	Goal
Dissemination	Getting the word out	Develop awareness
Interpretation	Getting the idea	Increase understanding & support
Implementation	Getting the job done	Change policies, programs & practices
Evaluation	Getting it right	Monitor & adjust policies, programs & practices
Revision	Doing it all again	Improve efficacy & influence of Standards

Table 3.2. Effect sizes and variance of K-12 studies included in meta-analysis (n= 6). Calculations are based on Hedge's d and were calculated using MetaWin, 2.1.

Year	Author: subcategory	Effect size (d)	Variance of ES (Var d)	Study focus
	Lee (overall)	0.34	0.001	
	Lee Middle School:			
	Physical science	0.13	0.004	
2010	Life science	0.09	0.004	technology-enhanced
	Earth science	0.63	0.003	inquiry
	Lee High School:			instruction
	Physics	0.35	0.014	
	Chemistry	1.35	0.023	
	Biology	0.16	0.006	
2005	Lynch	0.38	0.002	highly rated curriculum,
2006	Pine	0.14	0.004	cognitive ability
	Shymansky (overall)	0.25	0.020	inquiry for
2004	Shymansky: multiple choice	0.62	0.020	achievement
	Shymansky: constructed response	-0.11	0.020	tests
2010	Wilson	0.42	0.071	BSCS instruction
2008	Wolf	0.26	0.025	inquiry for achievement

Table 3.3. Effect sizes and variance of Undergraduate studies included in meta-analysis (n= 12). Calculations based on Hedge's d and were calculated using MetaWin, 2.1.

Year	Author: subcategory	Effect size (d)	Variance of ES (var d)	Study focus
2009	Carmichael (overall)	0.23	0.019	team-based learning
	Carmichael: test 1	0.32	0.019	
	Carmichael: test 2	0.35	0.019	
	Carmichael: test 3	0.21	0.019	
	Carmichael: test 4	0.02	0.019	
2008	Cibik	0.74	0.055	inquiry, botany
2008	Fernández-Santander	1.22	0.043	cooperative learning, biochemistry
2005	Knight	0.71	0.029	lecturing less
2008	Lewis	0.13	0.004	peer-led inquiry, chemistry
	Lord (overall)	0.46	0.020	traditional vs. constructivist
1997	Lord: ecology	0.43	0.023	
	Lord: cytology	0.39	0.023	
	Lord: genetics	0.60	0.024	
	Lord: evolution	0.41	0.023	inquiry labs
2006	Lord	0.34	0.042	
2004	Luckie	0.47	0.016	collaborative inquiry, general biology
2007	Pereira	0.89	0.034	inquiry in human anatomy
2008	Quitadamo	0.39	0.018	inquiry & critical thinking, general biology
2001	Sneddon	1.70	0.025	interactive instruction, botany
2002	Tien	0.63	0.002	peer-led inquiry, organic chemistry

Table 3.4. Rank ordered effect sizes of K-12 studies included in meta-analysis (n = 6). Studies in bold indicate independent studies and/or overall effect sizes, studies in italics are component effect sizes of particular content areas within studies that contributed to the overall effect size of the entire study. Matrix number refers to the number assigned to the study within the MetaWin program. These numbers are used in the cumulative meta-analysis plot (Figure 3.1) to identify studies.

Author: subcategory	Effect size	Matrix number
<i>Lee HS Chemistry</i>	1.35	10
<i>Lee MS Earth science</i>	0.63	8
<i>Shymansky: multiple choice</i>	0.62	4
Wilson	0.42	1
Lynch	0.38	12
<i>Lee HS Physics</i>	0.35	9
Lee (overall)	0.34	
Wolf	0.26	2
Shymansky (overall)	0.25	
<i>Lee HS Biology</i>	0.16	11
Pine	0.14	3
<i>Lee MS Physical science</i>	0.13	6
<i>Lee MS Life science</i>	0.09	7
<i>Shymansky: constructed response</i>	-0.11	5

Table 3.5. Rank ordered effect sizes of Undergraduate studies included in meta-analysis (n = 12). Studies in bold indicate independent studies and/or overall effect sizes, studies in italics are component effect sizes of particular content areas within studies that contributed to the overall effect size of the entire study. Matrix number refers to the number assigned to the study within the MetaWin program. These numbers are used in the cumulative meta-analysis plot (Figure 3.2) to identify studies.

Author: subcategory	Effect size	Matrix number
Sneddon	1.70	18
Fernández -Santander	1.22	17
Pereira	0.89	13
Cibik	0.74	2
Knight	0.71	8
Tien	0.63	14
<i>Lord: genetics</i>	<i>0.60</i>	5
Luckie	0.47	15
Lord (overall)	0.46	
<i>Lord: ecology</i>	<i>0.43</i>	3
<i>Lord: evolution</i>	<i>0.41</i>	6
Quitadamo	0.39	4
<i>Lord: cytology</i>	<i>0.39</i>	16
<i>Carmichael: test 2</i>	<i>0.35</i>	10
Lord	0.34	7
<i>Carmichael: test 1</i>	<i>0.32</i>	9
Carmichael (overall)	0.23	
<i>Carmichael: test 3</i>	<i>0.21</i>	11
Lewis	0.13	1
<i>Carmichael: test 4</i>	<i>0.02</i>	12

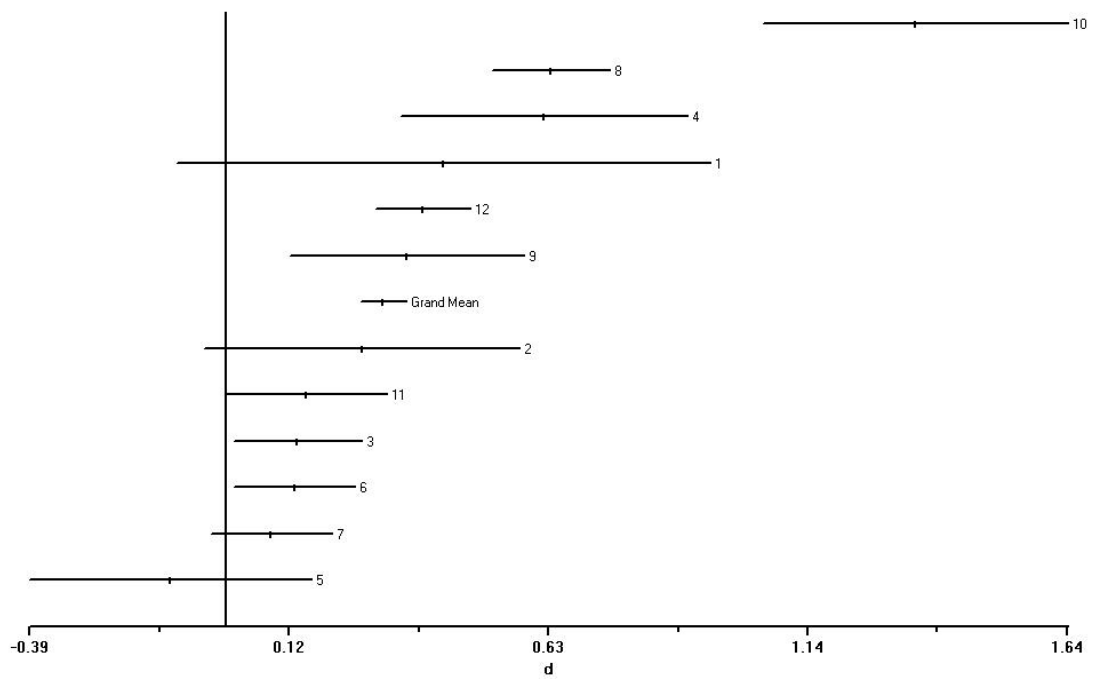


Figure 3.1. Rank-ordered cumulative meta-analysis plot of individual study effect size (95% CI) and cumulative effect size (Grand Mean), K-12 studies ($n = 6$). Vertical axis is located at $d = 0$.

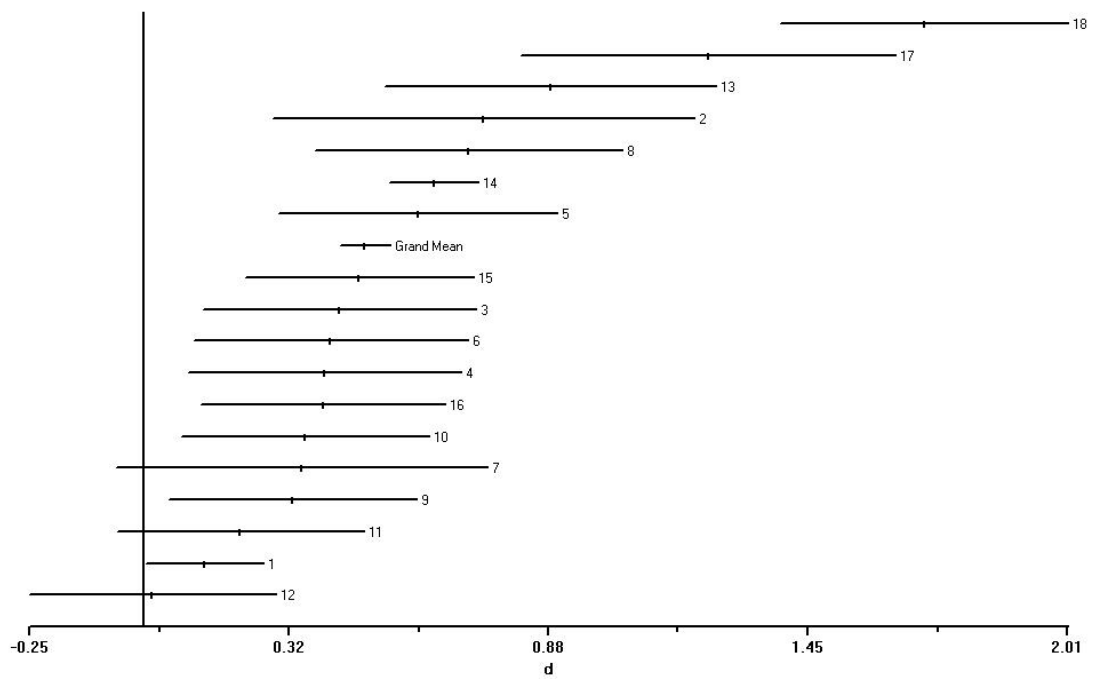


Figure 3.2. Rank-ordered cumulative meta-analysis plot of individual study effect size (95% CI) and cumulative effect size (Grand Mean), Undergraduate studies ($n = 12$). Vertical axis is located at $d = 0$.

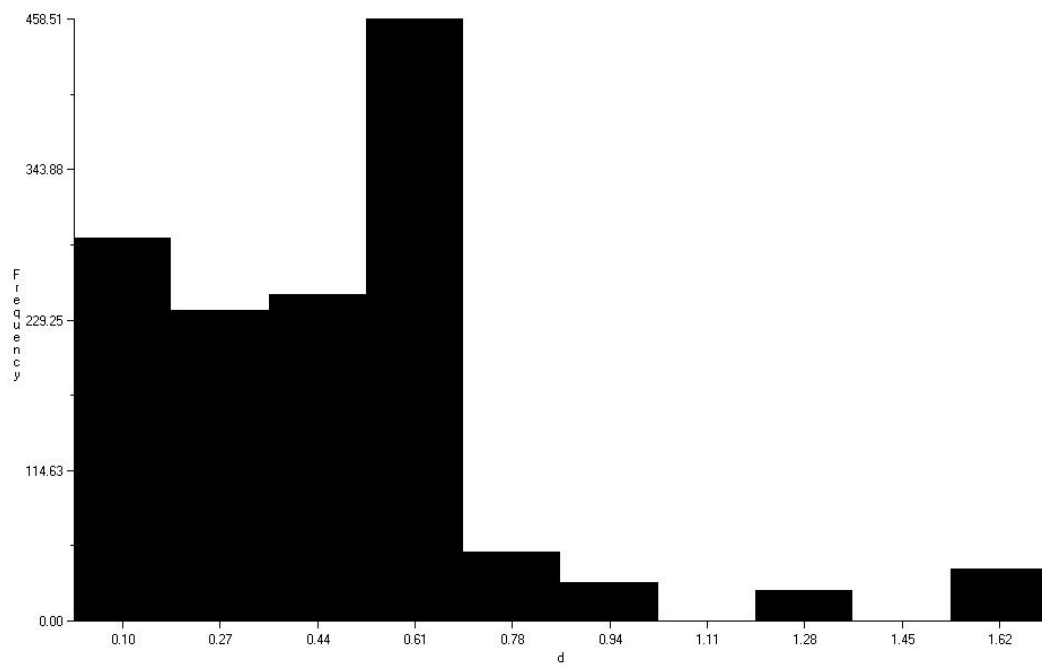


Figure 3.3. Weighted histogram of effect sizes (d) of Undergraduate studies (n = 12) of minimally guided instructional strategies. Height of bars represents combined weight of studies that fall within the class.