Journal of Ecological Research, 3, 34-39 (2001)

ENERGETICS OF BROODING IN THE FRESHWATER AMPHIPOD *GAMMARUS MINUS*

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ABSTRACT

Reproductive strategies among organisms vary greatly, as do the energetic investments associated with producing young. Amphipod crustaceans carry their eggs in a brood pouch and release them as juveniles. However, very little is known about the reproductive energy investments of amphipod females during the period when they are carrying their young. The goal of this research was to determine the energetic costs associated with carrying a brood in the freshwater amphipod, *Gammarus minus*. We hypothesized that there would be no difference in metabolic rate between *G. minus* females carrying a brood and females from which the brood was removed. To test this hypothesis we measured oxygen consumption of female amphipods carrying broods, debrooded females, and their removed eggs. We found no difference in metabolic rate between females carrying broods and debrooded females ($t = 0.38$, df = 44, $P = 0.705$). However, we found that the combined metabolic rate of debrooded females and their removed young was higher than that of females carrying broods ($t = 5.28$, df = 44, $P < 0.001$). These results indicate that there is no additional metabolic cost to carrying a brood, suggesting that conditions within the brood pouch lower the metabolic rate of the embryos.

Keywords: amphipods, brooding, energetics, Gammarus, *metabolism, oxygen consumption*

INTRODUCTION

The act of brooding (carrying eggs in an external pouch) is a form of parental care that offers an increased chance of survivorship for the young (Dick et al. 1998). However, the actual energetic cost to mothers of carrying their broods has not been estimated for any amphipod species. Behavioral research performed on the freshwater amphipod *Crangonyx pseudogracilis* has offered some indication of "active" pre-emergence brood care (Dick et al. 1998). They found that amphipods expand their brood pouches, allowing for pouch ventilation, increased suspension of the eggs and possible cycling of the eggs within the pouch. Females were also found to actively eject non-viable eggs from their pouches, another active mechanism of pre-emergence brood care. However, research performed on the brooding cladoceran *Daphnia magna* revealed no extra energetic investment to carrying their broods as estimated by oxygen consumption rates (Glazier 1991). This finding suggests that mothers are able to ensure increased survivorship of their young with no additional energy costs to the female.

Gammarus minus is a species of freshwater amphipod locally abundant in cold, alkaline springs in central Pennsylvania (Glazier et al. 1992). We tested the hypothesis that there should be no difference in metabolic rate as measured by oxygen (O_2) consumption rate, between *G. minus* females carrying a brood and females from which the brood was removed.

MATERIALS AND METHODS

We collected brooding female amphipods from Petersburg Spring in Petersburg, Pennsylvania, and acclimated them to their native temperature of 10° C (Glazier et al. 1992) in an environmental control chamber for a minimum of 24 hours. Petersburg springwater filtered using GF/C filter paper to remove bacteria, algae, and fungi, was used for all of our experiments. We randomly selected 12 brooding females from a holding tank and placed them in starvation chambers for 24 hours. Starving the females was important so that no feces were produced during respiration measurements because decaying feces consume oxygen, thus affecting the oxygen consumption estimates of the amphipods. Starvation also ensured that all extraneous activities (e.g., digestion) other than those required for basic survival were eliminated to reduce possible sources of variation. A starvation chamber consisted of one specimen cup with the bottom replaced by a screen, placed inside another specimen cup. This screen allowed for feces to fall through making it unreachable to the coprophagic amphipod.

We used a flow-through respirometer housed in a walk-in environmental control chamber kept at 10° C (Fig. 1). This respirometer consisted of an aerated 10-gallon carbuoy as a reservoir, a peristaltic pump, 10 5-ml syringes, and a wastewater collection tank. Water was pumped from the carbuoy and directed into each of the 10 syringes where the water flowed through the syringes at an adjustable rate. After the starvation period was complete, we selected eight amphipods from the twelve and placed them in the respirometer. A piece of fine netting was placed in the syringe to minimize the females' amount of movement by providing a surface to which they could cling. We did not place organisms in 2 of the 10 syringes, which served as controls for the system. We varied the placement of the controls between runs to account for any flow-rate or oxygen concentration variability within the system.

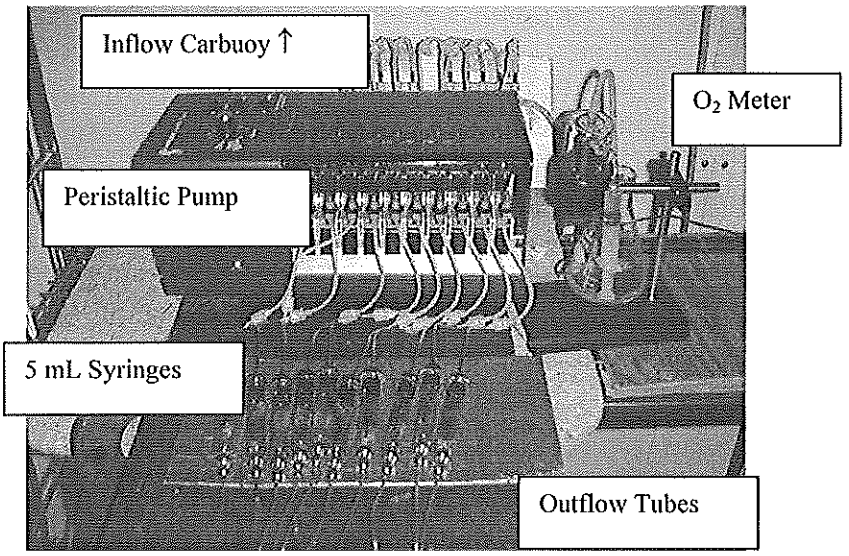


Figure 1. Picture of flow-through respirometry system

After the females were placed in the syringes, we tapped out air bubbles to ensure that no additional oxygen was being added to the system. The amphipods remained in the respirometer for approximately 16 hours. During the 16th hour we connected the outflow tubes from the syringes to a Strathkelvin dissolved oxygen meter. We took readings after allowing the water to flow through the meter for 3 minutes to ensure the passage of any air bubbles introduced into the meter and to allow for equilibration. We repeated this process for all 10 syringes during the 16th and 18th hours. Before each set of readings, we adjusted the fine calibration knob to 160 torr using water saturated with oxygen. Between the first and second readings, we determined the flow rate of each syringe by directing the discharge tubes into 10-ml burets and recording the volume after 30 minutes.

After the readings were taken, we removed the females from the respirometer and briefly anesthetized them in carbonated water. They were then immobilized on a piece of dental wax using insect pins and we flushed their eggs from the brood pouch using filtered Petersburg springwater. We next placed the females back in fresh Petersburg springwater for 24 hours and fed them cultured elm leaves.

We counted and staged the removed eggs according to development (stage I = amorphous eggs, stage II = comma shaped embryos; following Dick et al. 1998) and immediately placed them in a static respirometry system consisting of 10 2-ml syringes kept in a 10°C control chamber. Any eggs lost or ruptured in the debrooding process or in the transfer to and from the respirometer were noted. We removed all air bubbles from the syringes and left exactly 2 ml of water in the syringes. We allowed the eggs to incubate for 6 hours at which time the syringes were connected to the oxygen meter. Water was injected into the meter and allowed to sit for a 2-minute period at which point oxygen concentrations were recorded for each brood. We then placed the broods in foil packs and stored them in a –70° C cooler.

Twenty-four hours prior to placing the debrooded females back into the respirometer, we removed them from their feeding chambers and starved them again for 24 hours. We placed them back in the respirometer for 16 hours and repeated the aforementioned process of taking readings on the 16th and 18th hours. We then removed the mothers from the respirometer, euthanized them, and measured their lengths. They were stored in a –70° C cooler for later retrieval.

We collected the above measurements on 45 different females for a total of over 250 hours worth of observations. Any females that died after the debrooding were removed from the data set and replaced in later runs. After we completed collecting data, we freeze-dried all of the females and their respective broods, and measured their dry masses using a CAHN electrobalance.

To calculate oxygen consumption rates, we used two different equations based on whether a flow-through or static system was used. Total consumption for the females was determined by $\mathbf{R = [(P_E - P_C) SAF]/m}$, where R is the oxygen consumption rate, P_E is the oxygen reading obtained for the organism, P_C is the control reading, S is the solubility coefficient of oxygen at 10° C, A is the volume of 1 mole of oxygen at STP, F is the flow rate of the system (averaging about 3 ml/hr), and m is the dry mass of the female (following Glazier and Sparks 1991). Total oxygen consumption of eggs was calculated using $\mathbf{R = [(P_E - P_C) SAV/t]}$, where V is the total volume of water in the syringe and t is the incubation time (6 hours for this experiment) (following Glazier 1991).

After testing for normality using an Anderson-Darling test and equal variances using both a Levene's and an F-test, we analyzed the data in MiniTab using either a paired t-test, a Welch's t-test, a 2-sample t-test, or a Mann-Whitney U test. We considered differences to be significant if $P \leq 0.05$. Values between $P = 0.05$ and 0.10 were considered marginally significant.

RESULTS

We found no difference in metabolic rate between brooding (M = -0.3053) and debrooded (M = -0.3169) females ($t = 0.38$, df = 43, $P = 0.705$) carrying both stage I ($t = -0.26$, df = 21, $P = 0.794$) and stage II ($t = 0.89$, df = 22, $P = 0.386$) eggs (Fig. 2A). We also found that the combined metabolic rate of debrooded mothers and their removed young (M = -0.4956) was significantly higher than the rates of brood-carrying females ($t = 5.28$, df = 43, $P < 0.001$) for both stage I ($W = 582.0$, df = 21, $P = 0.0423$) and stage II ($t = 5.49$, df = 22, $P < 0.001$) eggs (Fig. 2B). We also found that eggs outside of the brood pouch (M = -0.1786) had a higher metabolic rate than those found within the brood pouch (M = 0.0117) as calculated by the difference between the combined rate of mothers with their removed broods and the brood-carrying mothers ($t = 5.64$, df = 43, $P < 0.001$). This trend was apparent for both stage I ($t = -5.91$, df = 21, $P < 0.001$) and stage II ($t = -10.27$, df = 22, $P < 0.001$) eggs (Fig. 2C). The metabolic rate of stage II eggs outside of the brood pouch (M = -1.303) was marginally significantly greater than that of stage I eggs outside of the pouch (M = -1.050) ($W = 579.0$, df = 43, $P = 0.0997$). However we found no metabolic difference between stage I (M = -0.0126) and stage II (M = 0.0349) eggs within the brood pouch ($t = -0.78$, df = 43, $P = 0.439$) as seen in Fig. 2D.

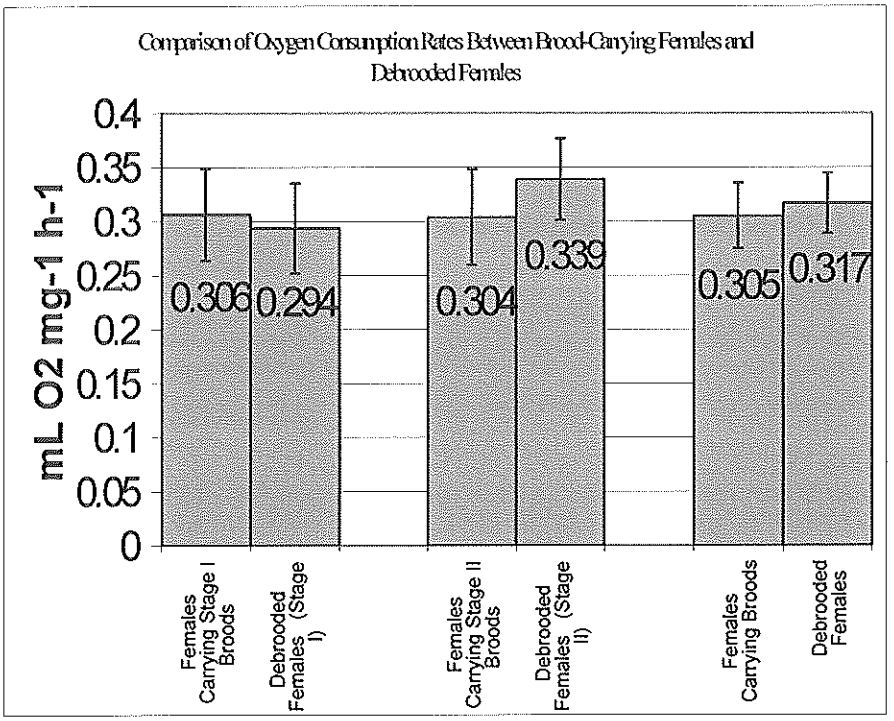
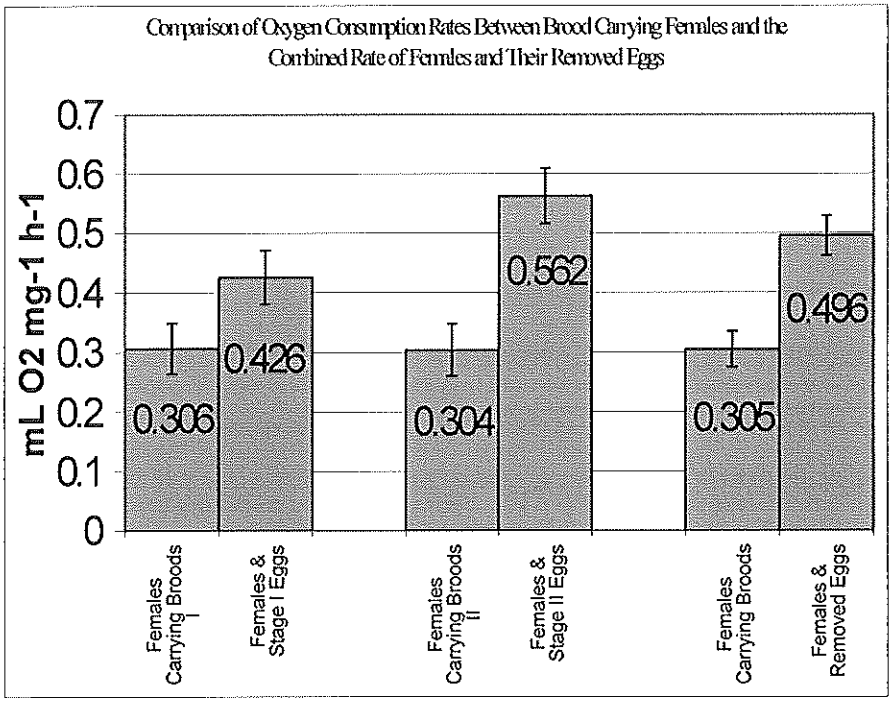
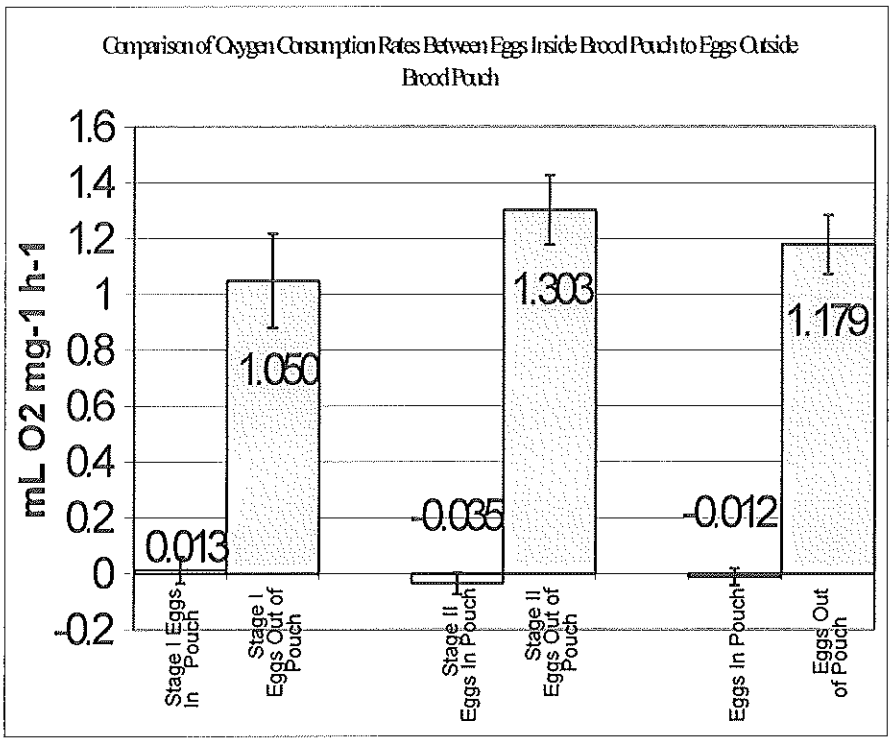

Comparison of Oxygen Consumption Rates Between Brood-Carrying Females and Debrooded Females
mL O2 mg-1 h-1
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.306
0.294
0.304
0.339
0.305
0.317
Females Carrying Stage I Broods
Debrooded Females (Stage I)
Females Carrying Stage II Broods
Debrooded Females (Stage II)
Females Carrying Broods
Debrooded Females

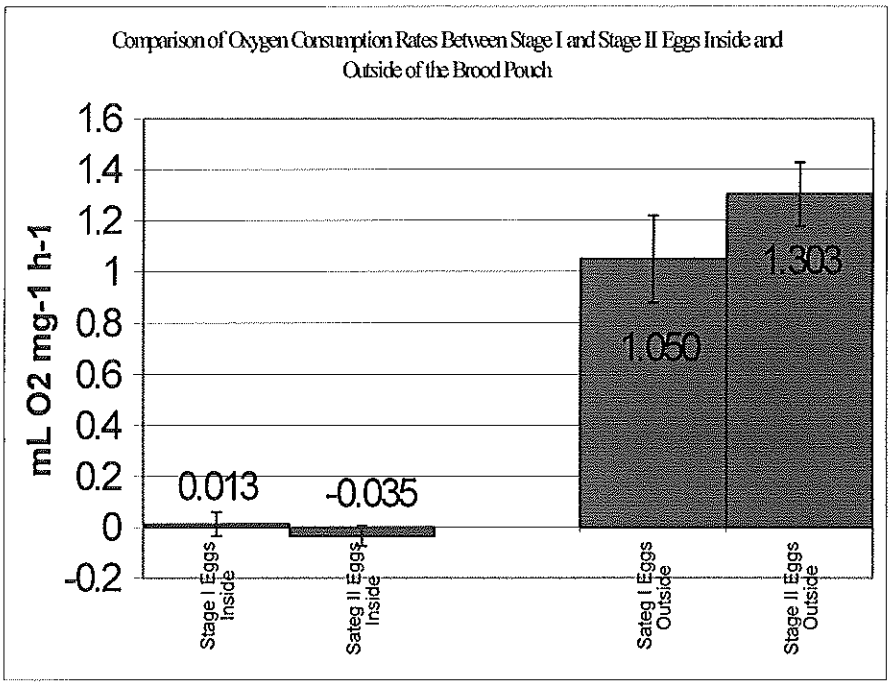
Figure 2A


Comparison of Oxygen Consumption Rates Between Brood Carrying Females and the Combined Rate of Females and Their Removed Eggs
mL O2 mg-1 h-1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.306
0.426
0.304
0.562
0.305
0.496
Females Carrying Broods I
Females & Stage I Eggs
Females Carrying Broods II
Females & Stage II Eggs
Females Carrying Broods
Females & Removed Eggs

B


Comparison of Oxygen Consumption Rates Between Eggs Inside Brood Pouch to Eggs Outside Brood Pouch
mL O2 mg-1 h-1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0.013
1.050
-0.035
1.303
-0.012
1.179
Stage I Eggs In Pouch
Stage I Eggs Out of Pouch
Stage II Eggs In Pouch
Stage II Eggs Out of Pouch
Eggs In Pouch
Eggs Out of Pouch

C


Comparison of Oxygen Consumption Rates Between Stage I and Stage II Eggs Inside and Outside of the Brood Pouch
mL O2 mg-1 h-1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0.013
-0.035
1.050
1.303
Stage I Eggs Inside
Sateg II Eggs Inside
Sateg I Eggs Outside
Stage II Eggs Outside

D

DISCUSSION

We failed to detect any difference in O_2 consumption rates between *G. minus* females carrying a brood and females from which the brood had been removed. This suggests that no additional energetic investment is associated with carrying young in a brood pouch in this species. This finding appears to be a highly adaptive strategy in that females can successfully protect their young without needing to increase their energetic intake. Dick et al. (1998) observed brooding female *C. pseudogracilis* engaging in active care to improve the conditions within the brood pouch for the young. At first glance, these activities appear to contradict the findings of this study in that our data showed no increased metabolic activity in brooding females. However, it is possible that, to allow for such embryonic care, reductions are made by the female in other activities. Energy may be reallocated from active swimming, for example, and put toward brood-care activities.

We initially expected the combined rate of the debrooded females and their young (for both stages I and II) to be equal to that of the female carrying her brood. However, we found that the combined rate of debrooded females and their young was actually higher than that of the brood-carrying female. This could have been caused by an increase in the metabolic rate of the eggs after they were removed from the brood pouch or due to a slight increase in O_2 consumption of debrooded females.

If the eggs actually do consume less O_2 inside of the brood pouch, this implies that conditions inside the pouch limit the metabolic activity of the developing embryos. One possible limiting factor could be anoxic conditions, which may exist within the crowded pouch. Another factor could be the reduced exposed surface area of the eggs in the pouch due to crowding, leading to lower O_2 exchange rates. These results differ from the findings of Glazier (1991) who found that metabolic rates of embryonic *D. magna* were not limited by conditions within the brood pouch. However, this comparison to amphipods may be inappropriate given the phylogenetic differences between the organisms. Our findings suggest that not all brooding organisms conform to the same reproductive strategies.

If the metabolic rate of the female increases after debrooding, this could be linked to recovery stress associated with debrooding, preparation for another reproductive event, or increased activity associated with trying to find their lost young. Increased metabolic activity in both the eggs and the debrooded females may have acted together or independently and more research must be conducted to determine the cause of this difference.

We also found that stage II eggs had a slightly higher O_2 consumption rate outside of the pouch, than did stage I eggs. However, there was no difference between the consumption rates of stage I and stage II eggs inside of the brood pouch. This may further confirm our suggestion that brood conditions are limiting the metabolic rate of the embryos.

The results of our study pertain to only one population of amphipods, and further studies should include additional populations of *G. minus*, as well as other amphipod species. In particular, it would be worthwhile to determine any evolutionary differences in reproductive investment among freshwater, marine and terrestrial populations.

ACKNOWLEDGEMENTS

We thank Dr. Douglas Glazier and Chuck Yohn for all of their help on this project.

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