SEASONAL METABOLISM AND BIOCHEMICAL COMPOSITION OF TWO UNIONID MUSSELS, *ACTINONAIAS LIGAMENTINA* AND *AMBLEMA PLICATA*.

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ABSTRACT

We examined the seasonal patterns of physiology and biochemical composition in two unionid bivalves, *Actinonaias ligamentina* (subfamily Lampsilinae) and *Amblema plicata* (subfamily Ambleminae). We found that (i) *A. ligamentina* and *A. plicata* displayed different seasonal changes in physiology and biochemical composition, (ii) larval brooding affected the physiology but not the biochemical composition of *A. ligamentina*, and (iii) *A. plicata* had a greater carbohydrate content and condition index than *A. ligamentina*. *A. ligamentina* and *A. plicata* had different patterns of ammonia excretion rate, O:N ratio, and clearance rate, while patterns of oxygen uptake rate were similar between the two species. Overall, weight specific metabolic rates were higher in *A. ligamentina* than in *A. plicata*. Both species had low protein and high carbohydrate content in early summer. Brooding specimens of *A. ligamentina* had lower oxygen uptake and ammonia excretion rates and higher O:N ratios than non-brooding specimens. Differences in condition and carbohydrate content between the two species could explain some of the species-specific mortalities observed since the introduction of zebra mussels.

INTRODUCTION

Freshwater mussels (Order Unionoida) are distributed worldwide in lotic and lentic habitats. As filter feeders, freshwater mussels are ecologically important; they control seston, recycle nutrients, and provide a trophic link between primary producers and predators (Lewandowski & Stanczykowska, 1975; Kasprzak, 1986; Nalepa, Gardner & Malczyk, 1991). Unionid mussels reached their greatest diversity in North America (Williams, Warren, Cummings, Harris & Neves, 1993). However, their abundance and diversity have declined in the last 30 years and they are now among the most imperiled groups of animals in the world. Habitat destruction, including increased siltation, pollution, and river modification, loss of fish hosts, commercial exploitation, and introduced species, are among the causes of their decline (Bogan, 1993).

Despite the importance of freshwater mussels to riverine systems, relatively little is known regarding their physiological ecology. This is especially surprising since marine bivalves have been examined extensively in this regard. In marine bivalves, seasonal variations in physiology and biochemical composition have been linked to annual cycles of food availability, temperature, salinity, oxygen tension and reproduction (Bayne, Thompson & Widdows, 1976; Gabbott, 1976; Pieters, Kluytmans, Zurburg & Zandee, 1979).

We chose to study two species of unionid mussel that are common to the Mississippi River system, *Actinonaias ligamentina* (Lamarck) (subfamily Lampsilinae) and *Amblema plicata* (Say) (subfamily Ambleminae). These mussels belong to different subfamilies and have distinct reproductive periods; *A. ligamentina* is a long-term brooder (bradytictic) while *A. plicata* is a short-term brooder (tachytictic) (Parmalee & Bogan, 1998). These species also respond differently to infestation by zebra mussels (*Dreissena polymorpha*) (Pallas) (Haag, Berg & Garton, 1993; Baker & Hornbach, 1997). Based on their differences in taxonomic status, reproductive strategy, and response to zebra mussel infestation, we hypothesized that *A. ligamentina* and *A. plicata* have different seasonal patterns of physiology and biochemical composition. Annual physiological data for unionid mussels may be useful in scheduling recovery and management efforts, understanding species-specific habitat requirements, and in predicting their response to long-term changes in the environment. We examined (i) condition index, a commonly used measure of nutritive status in marine bivalves, (ii) metabolism, as measured by oxygen uptake and ammonia excretion, (iii) O:N ratio, used as a measure of physiological stress (Bayne & Widdows, 1978), (iv) food clearance rates, and (v) biochemical tissue composition (carbohydrate, protein, lipid, and inorganic contents).

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MATERIALS AND METHODS

Mussel collection and maintenance

Specimens of *Actinonaias ligamentina* were collected by SCUBA from the St. Croix River at Wild River State Park, Minnesota (USA), in June, August, and October 1994 and April and June 1995. At the time of collection, water temperatures were 25, 20, 11, 9, and 24°C, respectively. Specimens of *Amblema plicata* were collected from the St. Croix River at Lakeland, Minnesota in May, July, September, and November 1995. River temperatures were 11, 25, 20, and 5°C, respectively. Mussels were transported to the laboratory in buckets of aerated river water. Physiological measurements were completed within three days of collection. Mussels were maintained until use in 20-gallon aquaria, where they were inserted into fine gravel in their natural infaunal position. The aquaria were maintained at collection temperature (± 1°C) in environmental chambers. On a daily basis, half of the water was removed from the aquaria and replaced with a combination of river water and aged, dechlorinated tap water. Mussels were fed either dried *Chlorella* (Algae-Feast, The Earthrise Co., Callpatria, CA) or a preserved diatom paste (Diet C, Coast Seafoods, Co., Quilcene, WA) at a rate of approximately 2% of their dry tissue mass per day. On each sampling date, 10 to 19 specimens were used. Specimens of *A. ligamentina* used in the experiments ranged from 54 to 159 g total wet mass; specimens of *A. plicata* ranged from 78 to 259 g total wet mass.

Physiological measurements

Measures of oxygen uptake, ammonia excretion, O:N ratio, and clearance rates were performed as in Baker & Hornbach (1997). Individual mussels were placed in filtered water (0.45 µm) in acrylic chambers (1400 mL). Temperature was maintained the same as when collected by immersing the chambers in a controlled water bath containing submersible magnetic stirrers. Individual rates of oxygen uptake were measured as the oxygen depletion in the chambers. Oxygen concentrations were measured every 30 min for two to four h with YSI oxygen probes (5331) and monitors (5300). Oxygen uptake rates were corrected for the volume of water displaced by the mussels, barometric pressure, and any changes in oxygen concentration in control chambers (see Baker & Hornbach, 1997). Nitrogen excretion, measured as the accumulation of ammonia and urea during the oxygen uptake experiments, was measured using an Orion ammonia electrode (model 95-12) and meter (model 920A). Ammonia excretion rates were calculated using standard curves and corrected for the ammonia concentrations in control chambers (see Baker & Hornbach, 1997). Atomic ratios of O:N were calculated from the oxygen uptake and nitrogen excretion rates.

Clearance rates

Clearance rates of individual mussels were measured as the decrease in light absorbance of an algal suspension using a Brinkman probe colorimeter (PC 800) equipped with a 450 nm filter (see Hornbach, Wilcox, Powers, Layne & Davis, 1991). Individual mussels were placed in the chambers in 1 L of water and a mixture of preserved diatoms (Diet C, Coast Seafoods, Co., Quilcene, WA), was added to the chambers to an initial concentration of 12.3 mg dry mass l^{-1} (6.8 mg ash-free dry mass). This concentration approximates the mean total suspended solids observed in the St. Croix River (Hornbach, unpublished data). Light absorbance of each chamber was recorded every 15 min for 90 min. Clearance rates were calculated according to Coughlan (1969) and Sprung (1984) using a standard curve and corrected for any absorbance changes in a control chamber (see Baker & Hornbach, 1997).

Biochemical composition

Intact mussels were weighed wet and measured for height, length and width. Upon completion of the physiological measurements, the tissues were removed from the shells and the presence or absence of brooded glochidia in the gills was noted. Tissue dry mass of each mussel was determined by freeze-drying to constant mass. Tissues were stored at −70°C until use in the biochemical analyses. Mussel shells were dried for several days at room temperature and weighed.

Condition index is a commonly used measure of gross nutrient reserves or 'fatness' in marine bivalves and is essentially a ratio of tissue to shell mass. Condition index was calculated according to Crosby & Gale (1990):

Condition Index = [dry tissue mass (g) · 1000] / internal shell cavity capacity (g) where, internal shell cavity capacity (g) = total animal wet mass (g) − dry shell mass (g).

Energy stores were determined as in Baker & Hornbach (2000). Freeze-dried tissues were ground to a fine powder and stored overnight in a desiccator. Total lipid was extracted from a preweighed amount of tissue (~50 mg) using a chloroform-methanol method and measured gravimetrically (Barnes & Blackstock, 1973; Barber, Ford & Haskin, 1988). Carbohydrate was measured colorimetrically using a phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956; Barber *et al.*, 1988). Absorbance was read at 490 nm, calibrated against a blank solution. A standard curve of glycogen (Type II, from oyster, Sigma Chemical Co., St. Louis, MO) was run concurrently. Protein was measured colorimetrically using the Folin-phenol method (Lowry, Rosebrough, Farr & Randall, 1951; Barber *et al.*, 1988). Absorbance was read at 750 nm, calibrated against a blank solution. A standard curve of bovine albumin (A-2153, Sigma Chemical Co.) was run concurrently. Inorganic ash content of the tissues was determined by placing a weighed amount of tissue in a tared, pre-combusted aluminum pan. The pan and tissue were combusted for 1 h at 500°C. A blank pan was combusted concurrently.

Statistical analyses

For statistical analyses, O:N ratios were arcsine transformed while oxygen uptake rates, ammonia excretion rates, and clearance rates were natural log transformed (Zar, 1984). Biochemical variables were initially expressed as proportions, and then converted to milligrams · g^{-1} dry mussel tissue. Total calorific values were calculated from the biochemical contents, using the carbohydrate, protein, and lipid energy conversion factors of 4.1, 4.3, and 8.4 kcal · g^{-1},

respectively (Beukema & De Bruin, 1979). Analyses of variance were performed to test for an effect of collection date on physiological and biochemical measures. If significant, Tukey-Kramer multiple comparison tests were used to identify differences between specific dates. Brooding females were excluded from these analyses.

For months in which sufficient brooding mussels were found, *t* tests were conducted to test for effects of reproductive state on physiological and biochemical measures. All statistical analyses were conducted using JMP Version 3.1.6 Software (SAS Institute Inc., 1994).

RESULTS

Actinonaias ligamentina

All physiological variables of *A. ligamentina* varied as a function of collection date (Table 1; Fig. 1). Although analysis of variance indicated that condition index varied with collection date (Table 1), multiple comparisons did not reveal differences between specific months. June 1994 and June 1995 oxygen uptake rates were not significantly different from each other and both were greater than those measured in October. June 1995 oxygen uptake rates were also greater than those in April or August; August and October oxygen uptake rates were significantly different from each other (Table 1; Fig. 1C). Ammonia excretion rates in all months, except April and October, were significantly different from each other (Table 1; Fig. 1D). April O:N ratios were greater than those measured in any other month. June 1995 and October O:N ratios also differed from each other (Table 1; Fig. 1E). October clearance rates were significantly lower than those measured in June 1994, June 1995, or August. August clearance rates were significantly greater than those in April or October, and April and June 1994 clearance rates were significantly different from each other (Table 1; Fig. 1F).

All chemical constituents and total calorific value varied as a function of collection month (Table 1; Fig. 2A). Protein level ranged from 40 to 53% of the total tissue dry mass, carbohydrate from 20 to 36%, inorganic ash from 15 to 18%, and lipid from 5 to 7%. Multiple comparisons revealed that protein contents were significantly greater in October than those measured in June 1994 or June 1995; all other pairs of months were not significantly different (Table 1; Fig. 2A). June 1994 and June 1995 carbohydrate contents were not significantly different from each other and both were greater than those measured in October. June 1994 carbohydrate contents were also greater than those measured in April and August (Table 1; Fig. 2A). Only June 1994 and October ash contents were significantly different from each other; all other pairs of months were not significantly different (Table 1; Fig. 2A). April lipid contents were significantly greater than those measured in June 1994, June 1995, and August. All other pairs of months were not significantly different from each other (Table 1; Fig. 2A). Total calorific values were significantly greater in June 1994 than in August, while all other pairs of months were not significantly different (Table 1; Fig. 2A).

Two of 12 specimens of *A. ligamentina* were brooding glochidia in April 1995, and six of 15 were brooding

Table 1 *Actinonaias ligamentina.* ANOVA: effect of collection date on physiology and biochemical composition.

Variable	df	F	P<	Pairs of significantly different months
Condition index	59	3.4	0.0138	NS
Oxygen uptake	58	11.6	0.0001	Jun '94 vs. Oct Jun '95 vs. Apr, Aug, and Oct Aug vs. Oct
Ammonia excretion	58	55.1	0.0001	Jun '94 vs. Apr, Aug, and Oct Jun '95 vs. Apr, Jun '94, Aug, and Oct Aug vs. Apr and Oct
O:N ratio	56	16.5	0.0001	Apr vs. Jun '94, Jun '95, Aug, and Oct Jun '95 vs. Oct
Clearance rate	59	12.3	0.0001	Jun '94 vs. Apr, and Oct Jun '95 vs. Oct Aug vs. Apr and Oct
Protein	58	4.9	0.0018	Oct vs. Jun '94 and Jun '95
Carbohydrate	58	14.0	0.0001	Jun '94 vs. Apr, Aug, and Oct Jun '95 vs. Oct
Inorganic ash	58	3.3	0.0156	Jun '94 vs. Oct
Lipid	59	4.8	0.0020	Apr vs. Jun '94, Jun '95, and Aug
kcal	57	3.1	0.0215	Jun '94 vs. Aug

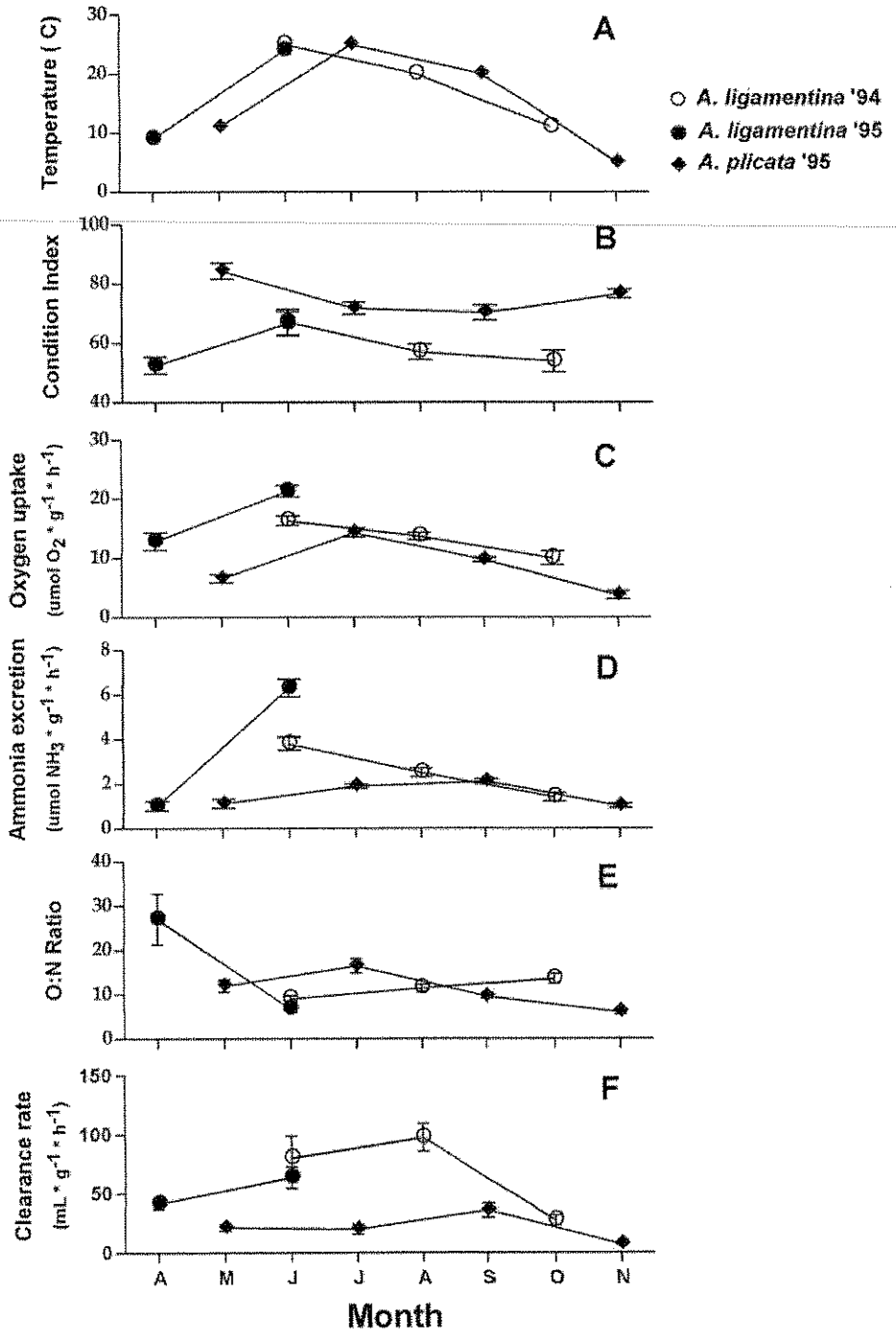

Figure 1. *Actinonaias ligamentina* and *Amblema plicata*. Seasonal changes in **A.** Collection temperature. **B.** Condition Index. **C.** Oxygen uptake. **D.** Ammonia excretion. **E.** O:N ratio. **F.** Clearance rate. (Means ± s.e., n = 10–19).

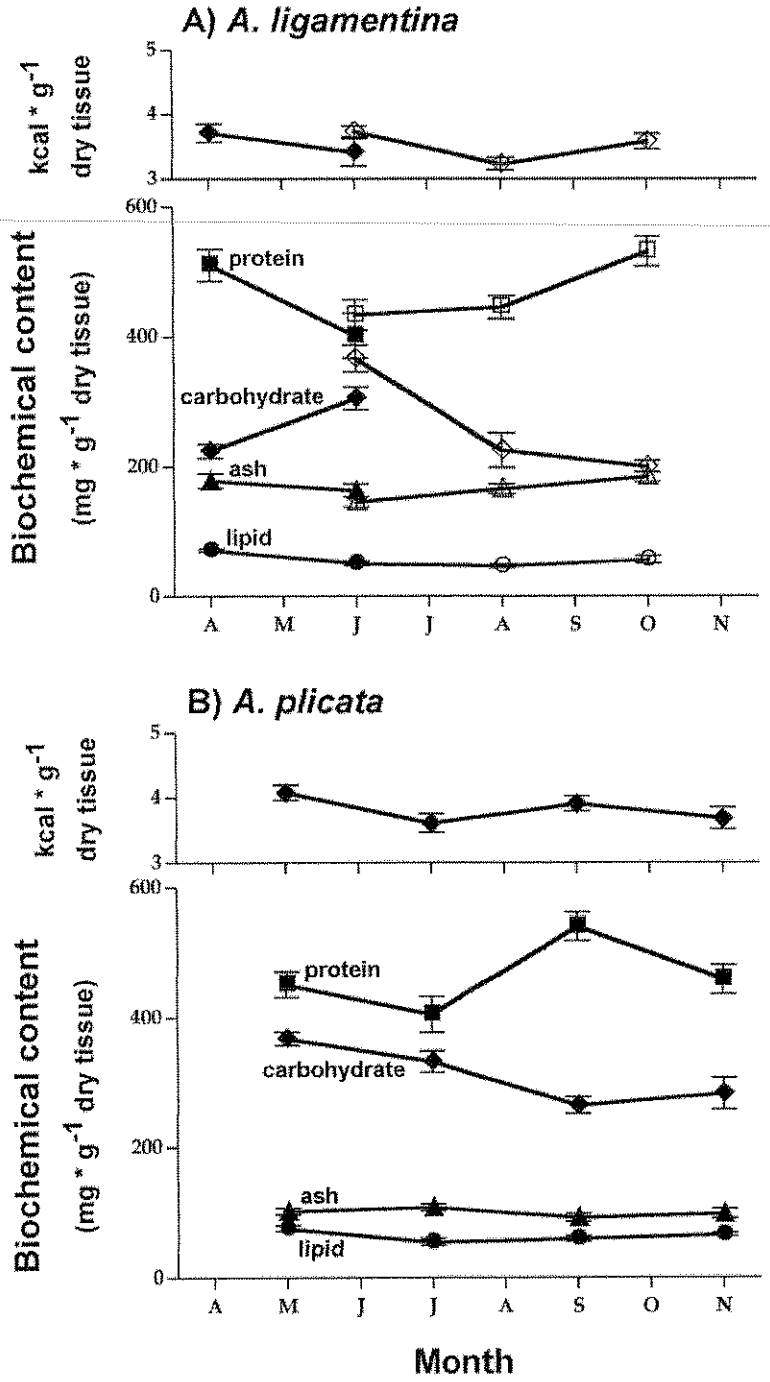

Figure 2. *Actinonaias ligamentina* and *Amblema plicata*. Seasonal variation in the total calorific content (kcal) and contents of protein, carbohydrate, inorganic ash and lipid. **A.** *A. ligamentina*. **B.** *A. plicata*. (Means ± s.e., n = 10–19).

in June 1995. The number brooding in June 1995 was sufficient to test the effects of brooding on physiology and biochemical composition. Oxygen uptake, ammonia excretion, and O:N ratio varied as a function of brooding (Fig. 3). Brooding individuals had lower oxygen uptake rates ($t = 2.2$, $P = 0.0482$), lower ammonia excretion rates ($t = 3.0$, $P = 0.0105$), and greater O:N ratios ($t = -2.7$, P = 0.0187) than non-brooding mussels. Brooding had no effect on biochemical contents or total calorific value.

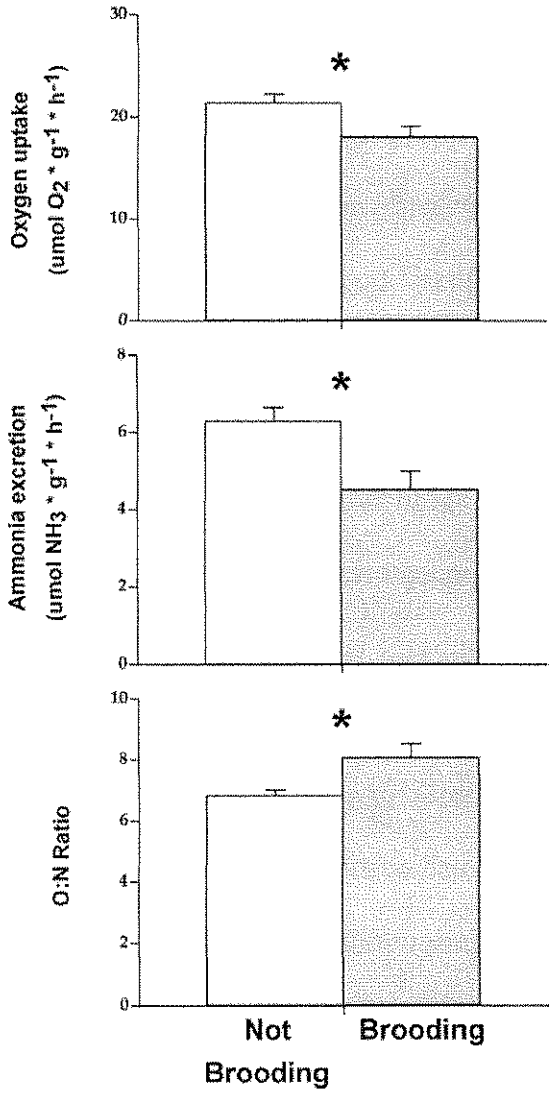

Figure 3. *Actinonaias ligamentina*. Effects of brooding on oxygen uptake rates, ammonia excretion rates and O:N ratios in June 1995. Asterisks indicate significant differences. (Means ± s.e., non-brooding n = 9, brooding n = 6).

Amblema plicata

No brooding specimens of *A. plicata* were collected. All physiological variables of *A. plicata* varied as a function of collection month (Table 2; Fig. 1). Multiple comparisons revealed that condition indices were significantly greater in May than those measured in July or September; all other pairs of months were not significantly different (Table 2; Fig. 1B). Oxygen uptake rates were significantly different between all pairs of collection dates (Table 2; Fig. 1C). July and September ammonia excretion rates were not significantly different from each other and both were greater than those measured in May or November (Table 2; Fig. 1D). July O:N ratios were significantly lower than those measured in any other month. May and November O:N ratios also differed from each other (Table 2; Fig. 1E). November clearance rates were significantly lower than those measured in any other month. July and September clearance rates also differed (Table 2; Fig. 1F).

All biochemical constituents except inorganic ash varied as a function of collection month (Table 2; Fig. 2B). Protein level ranged from 40 to 54% of the total tissue dry mass, carbohydrate from 26 to 37%, inorganic ash from 9 to 11%, and lipid from 5 to 8%. Multiple comparisons revealed that protein contents were significantly greater in September than those measured in May or July; all other pairs of months were not significantly different (Table 2; Fig. 2B). May carbohydrate contents were significantly greater than those measured in September or November. July and September carbohydrate contents also differed from each other (Table 2; Fig. 2B). May lipid contents and total calorific value were significantly greater than those measured in July; all other pairs of months were not significantly different (Table 2; Fig. 2B).

DISCUSSION

In our seasonal study of *A. ligamentina* and *A. plicata*, we found that (i) *A. ligamentina* and *A. plicata* displayed different seasonal changes in physiology and biochemical composition, (ii) larval brooding affected the physiology but not the biochemical composition of *A. ligamentina*, and (iii) *A. plicata* had a greater carbohydrate content and condition index than *A. ligamentina*.

A. ligamentina and *A. plicata* had differing seasonal patterns of ammonia excretion rate, O:N ratio, and clearance rate, while patterns of oxygen uptake rate were similar between the two species. Overall, weight specific metabolic rates were higher in *A. ligamentina* than in *A. plicata*. In both species, oxygen uptake rates

Table 2. *Amblema plicata.* ANOVA: effects of collection date on physiology and biochemical composition.

Variable	df	F	P<	Pairs of significantly different months
Condition index	53	7.3	0.0003	May vs. Jul and Sep
Oxygen uptake	55	33.6	0.0001	May vs. Nov
				Sep vs. May and Nov
				Jul vs. May, Sep, and Nov
Ammonia excretion	54	14.0	0.0001	Jul vs. May and Nov
				Sep vs. May and Nov
				Aug vs. Apr and Oct
O:N ratio	51	12.0	0.0001	May vs. Nov
				Jul vs. May, Sep, and NOv
Clearance rate	54	13.8	0.0001	May vs. Nov
				Jul vs. Nov
				Sep vs. Jul and Nov
Protein	55	5.9	0.0014	Sep vs. May and Jul
Carbohydrate	54	10.8	0.0001	May vs. Sep and Nov
				Jul vs. Sep
Inorganic ash	49	1.5	0.2329	NS
Lipid	55	4.4	0.0072	May vs. Jul
kcal	54	2.9	0.0453	May vs. Jul

were positively correlated with temperature, as is typical of ectothermic animals such as bivalves (Bayne & Widdows, 1978; Hornbach, Wissing & Burky, 1983). It is interesting, however, that the oxygen uptake rate of *A. ligamentina* was higher in April than might be expected at a temperature of only 9°C. Summer oxygen uptake rates (25°C) were 2.5 and 2.9 times greater than those at the lowest temperatures (9 and 5°C) for *A. ligamentina* and *A. plicata*, respectively. This is similar to the ratio of 2.3 measured for *Pisidium walkeri* (1–26°C) (Burky & Burky, 1976).

The atomic oxygen to nitrogen ratio (O:N) is a measure of the balance between the breakdown of proteins and the catabolism of carbohydrates and lipids. Low O:N ratios (< 30) in marine bivalves indicate a reliance on protein catabolism and are usually associated with starvation (Widdows, 1978). Freshwater mussels, however, appear to have low O:N ratios even under favorable conditions, with previously published values ranging from 13 to 78 (Fujikura, Segawa & Okutani, 1988; Baker & Hornbach, 1997, 2000). Low O:N ratios in freshwater mussels are attributed to the breakdown of dietary rather than body protein and may be the result of low rates of growth and protein deposition. In this study, O:N ratios were lower than previously published for freshwater mussels; O:N ratios of *A. ligamentina* ranged from 7 to 27, while those of *A. plicata* ranged from 6 to 17.

Seasonal patterns of O:N ratio differed between *A. ligamentina* and *A. plicata*. O:N ratios of *A. ligamentina* were highest in April, as a result of relatively high oxygen uptake rates and low ammonia excretion rates. This comparatively high O:N ratio suggests an increased reliance on carbohydrate and lipid catabolism. Compared to those of *A. ligamentina*, *A. plicata* had a smaller range of O:N ratios throughout the year. The highest O:N ratio of *A. plicata* occurred in July, as a result of a high oxygen uptake rate but a relatively low ammonia excretion rate. Again, this combination suggests a relatively greater use of carbohydrates and lipids than during the rest of the year.

A. ligamentina and *A. plicata* also had different seasonal patterns of clearance rate. In *A. ligamentina*, clearance rates were positively correlated with temperature, as is typical of many freshwater bivalves (Hornbach, Way, Wissing & Burky, 1984; Burky, Benjamin, Conover & Detrick, 1985). In many other bivalves, there is no consistent seasonal pattern of clearance rate because temperature acclimation occurs (Bayne & Widdows, 1978; Bayne, Moore, Widdows, Livingstone & Salkeld, 1979). Such thermal acclimation of clearance rate appears to have occurred in *A. plicata,* although the low clearance rate in July (25°C) may indicate thermal stress (Widdows, 1978).

A. ligamentina and *A. plicata* had differing patterns of seasonal biochemical composition. However, both species had low protein and high carbohydrate content in early summer (June for *A. ligamentina*, May for *A. plicata*). High carbohydrate content at his time may have been associated with gonad development (Jadhov & Lomte, 1982). Protein content within each species changed more dramatically over the year than did the

other biochemical constituents. Although glycogen is regarded as the major form of energy reserve in bivalves (de Zwaan & Zandee, 1972), protein reserve may be used simultaneously with carbohydrate, or even as the primary energy source (Ansell & Sivadas, 1973; Riley, 1976). Cycles of nutrient storage and use reflect complex interactions between food supply, temperature, growth, and reproduction (Gabbott, 1976).

We found that there were differences in physiology between brooding and non-brooding *A. ligamentina*. Brooding specimens had lower oxygen uptake, lower ammonia excretion rates, and higher O:N ratios than did non-brooding individuals. This relationship between larval brooding and metabolism has also been observed in other freshwater mussels (Tudorancea & Florescu, 1969; Burky & Burky, 1976; Tankersley & Dimock, 1993). In some freshwater mussels, however, oxygen uptake increases during the reproductive period (Way, Hornbach & Burky, 1981). Rates of ammonia excretion and O:N ratios can change during reproduction depending on the protein source (Kreeger, Hawkins, Bayne & Lowe, 1995).

The introduction of zebra mussels (*Dreissena polymorpha*) to North American freshwater systems has caused a dramatic decline in the abundance of unionid mussels (Gillis & Mackie, 1994; Nalepa, 1994). Mortality rates due to infestation are species-specific; for example, there has been a greater reduction in the number of unionid mussels belonging to the subfamily Lampsilinae than in those belonging to the subfamily Ambleminae (Haag *et al.*, 1993; Ricciardi, Whoriskey & Rasmussen, 1996). In addition, *A. ligamentina* appears to be more physiologically sensitive to infestation than is *A. plicata* (Baker & Hornbach, 1997). In this study, we found that *A. ligamentina* had a lower carbohydrate content and condition index than did *A. plicata*. If this is also true of other species in the subfamilies Lampsilinae and Ambleminae, differences in carbohydrate content and condition, combined with greater weight-specific metabolic rates, could help explain species-specific mortality rates due to zebra mussel infestation. Based on our observations, we predict that freshwater mussel species with high metabolic rates and low energy reserves will starve more quickly when infested by zebra mussels than will species that have greater energy reserves and lower weight-specific metabolic rates.

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