# Experimental Studies of Interactions between Two Unionids, *Elliptio waccamawensis* and Leptodea ochracea: Variation in Negative and Positive Effects

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## ABSTRACT

We conducted a field experiment in Lake Waccamaw, North Carolina, to elucidate the responses of two unionids (*Elliptio waccamawensis* and *Leptodea ochracea*) to varying population densities. Field enclosures at two depths maintained each species in low, mixed culture, and high densities; intra- and interspecific interactions were inferred from changes in growth, tissue mass, and shell mass. The experiment lasted one year (May, 1995-1996), with interim data collected in September, 1995. *E. waccamawensis* did not respond to density manipulations over the annual experimental period; however, its growth was significantly reduced in the presence of *L. ochracea* during summer at the deeper site. This negative effect on *E. waccamawensis* is consistent with competition for resources, and a laboratory experiment estimating mussel grazing rates provided supporting evidence. For *L. ochracea*, density had no effect on annual growth at the deeper site; at the shallow site, however, high density significantly increased growth. Winter data revealed both negative and positive density-dependence. *L. ochracea* in high density treatments grew significantly less at the deep site but demonstrated increased growth at the shallow location. Thus, both negative and positive interactions occur in this mussel assemblage and vary with season and depth.

## INTRODUCTION

Despite historical records of dense and diverse assemblages, the potential structuring role of density-dependent interactions in freshwater bivalve (Bivalvia: Unionidae) communities has been inadequately examined. A few studies have given equivocal results. Kat (1982) showed that *Elliptio complanata* responded to elevated local density with decreased growth and increased migratory behavior; he inferred the importance of local density and suggested that intraspecific competition may influence unionid assemblages. On the other hand, Hanson et al. (1988) found no evidence for density-dependence in *Anodonta grandis simpsoniana*. The lack of research on interactions in native mussel communities represents a significant void in our ecological understanding of this conspicuous and important fauna. Adult unionids are present in a variety of habitats and manipulable for experimental purposes. They are analogous to marine soft-sediment bivalves where extensive study has demonstrated competitive interactions (Peterson 1977, 1982, Peterson and Andre 1980).

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Lake Waccamaw, a Carolina bay lake (Frey 1949) in North Carolina, contains over 20 gastropod and bivalve species (Porter 1985) and is ideal for evaluating the potential role of intra- and interspecific interactions in structuring relatively undisturbed unionid assemblages. The lake is very shallow ($z_{max}$ = 3.3 m) and has a surface area of 3618 ha (Casterlin et al. 1984). *Elliptio waccamawensis* and *Leptodea ochracea* dominate the unionid community in the southern part of Lake Waccamaw in terms of abundance and biomass (Porter 1985, DiDonato 1998). These species are equally abundant at the lake's shallow periphery, but the endemic *E. waccamawensis* becomes relatively more common in deeper water (DiDonato 1998). That change in community composition prompted the question of whether interactions are present and change along the depth gradient. To test this, mussel densities were manipulated in field enclosures at two depths; intra- and interspecific interactions were inferred from evidence of density-dependence (e.g., negative density-dependence implied competition).

Interactions between marine benthic organisms have received extensive study. While competition for space determines epifaunal community composition (Connell 1961), resource competition also influences benthic communities (Okamura 1988, 1992), including infaunal suspension-feeding bivalves (Peterson 1991). Most freshwater mussels collect food (phytoplankton, other seston; McMahon 1991) like their marine counterparts, and freshwater research using methods borrowed from marine studies will provide comprehensive views of bivalve interactions. Many researchers have assumed that species with higher filtration rates will be superior competitors. While this is not always the case (Strayer 1999), this rate provides a convenient metric for interaction strength and direction. We used a laboratory experiment to test the hypothesis that *E. waccamawensis* and *L. ochracea* consume food resources at different rates.

## METHODS AND MATERIALS

Field Experiment

Manipulations of unionid density were used to explore potential interactions along the depth gradient. Circular enclosures (0.25 m$^2$) were placed at two depths in the southern region of Lake Waccamaw. Enclosures were buried 10 cm, leaving 2 cm above the substrate. Enclosure size mimicked natural clump sizes for these unionids (DiDonato 1998). In total, 15 enclosures were placed at both a shallow location ($\bar{x}$ = 0.60 m) and a deeper site ($\bar{x}$ = 1.25 m); the two sites were >300 m apart. At the deep location, gastropods and *Corbicula fluminea* were abundant (22.0 and 3.5 indiv./m$^2$, respectively); neither was present at the shallow site.

*E. waccamawensis* and *L. ochracea* (17-37 mm shell length) were hand-collected from the substrate over eight days (22-30 May 1995). Shell length, the longest antero-posterior dimension (Johnson and Brown 1998), was measured with vernier calipers to the nearest 0.1 mm. Animals in this size range were used because they are abundant and demonstrate measurable growth rates over short time periods. Mussels were segregated into five treatments groups (Table 1) with similar size distributions. Individuals were marked with paint dots to facilitate identification. Treatment groups were randomly assigned to enclosures and replicated three times at each depth.

Experimental densities were based on prior sampling data. Porter (1985) reported patch densities of *E. waccamawensis* and *L. ochracea* reaching 132 and 26 indiv./m$^2$, respectively. From a more limited survey, DiDonato (1998) reported that *E. waccamawensis* and *L. ochracea* averaged 12 and 4 indiv./m$^2$, respectively, at the deepest areas sampled (>1 m). Patch densities were significantly higher, however, with *E. waccamawensis* and *L. ochracea* found at 32 and 10 indiv./m$^2$, respectively. Treatments reflected these upper densities. Biomass levels were within the natural range (DiDonato, unpubl. data).

Treatments were maintained until the week of 20 September, when mussels were recollected, remeasured, and then returned to enclosures. Two *E. waccamawensis* and 11 *L. ochracea* (1% and 5.7% of the total) were not recollected at this time, but these "missing" animals were not replaced because it was unclear whether they were gone (i.e., dead) or unrecovered (i.e., burrowed into sediment). The experiment ended on 4-5 May 1996; recovered animals were returned to the laboratory. Shell lengths were measured, and animals were sacrificed. Soft tissues were dried for 48 hours at 60 °C. Shells were dried at 60 °C for four hours. Tissue and shell masses were quantified (± 1 mg).

The experiment was analyzed as three separate periods: annual (May - May), summer (May - September), and winter (October - May). Individual growth and mass within those periods were analyzed as responses to density treatments. Size changes over time are suitable for estimating intra- or interspecific effects because mussel reproduction is often correlated with size (Haukioja and Hakala 1978). For the annual period, shell growth, final tissue weight, and final shell weight were examined collectively. Only shell growth was evaluated for summer and winter.

We used enclosure means in statistical tests to avoid pseudoreplication (Hurlbert 1984); the enclosure was the experimental unit. Final tissue and shell masses were averaged over all individuals of each species within an enclosure. Growth during each period was calculated as $\ln(S_2/S_1)$, where $S_2$ and $S_1$ were shell lengths (mm) at the end and beginning, respectively, of the exposure period (Kaufmann 1981). Growth estimates were averaged across all bivalves of each species within an enclosure and evaluated with respect to four potential models: power, Gompertz, logistic, and Bertalanffy (Kaufmann 1981). These models regress growth (or log-transformed growth) against a measure of initial size. The model accounting for the most variation in growth when averaged across all species and depth combinations was used in analyses for that period.

Statistical analyses followed the "cell means" approach (Searle 1987, Dunson and Travis 1991). Three density combinations (8, 16, or 8 individuals + 8 heterospecifics per enclosure) examined at two depths constituted six treatment "cells" for each species (Table 1). For data from the annual period (annual growth, final tissue weight, final shell weight), treatment cells were subjected to a one-way multivariate analysis of covariance (MANCOVA). Initial size from the growth model served as a covariate to remove the influence of animal size. MANCOVA was used because these response variables are often correlated (Haukioja and Hakala 1978), and Roy's greatest root was the critical test statistic because it leads to *post hoc* procedures (Scheiner 1993). Significant effects (p < 0.05) were followed by a series of preplanned contrasts testing the particular effects of DEPTH, INTRASPECIFIC DENSITY, INTERSPECIFIC DENSITY, and the interactions INTRASPECIFIC DENSITY × DEPTH and INTERSPECIFIC DENSITY × DEPTH. These contrasts are consistent with Underwood's (1986) recommendations for critically evaluating competition experiments. The interaction contrasts tested the effects of density and patch composition across the depth gradient. It is important to stress that contrasts were not done unless the overall model detected significant treatment differences (i.e., these were protected contrasts); however, because of the variability of field experiments and low replication, the significance level for contrasts was maintained at p = 0.05. For significant contrasts, treatment means were compared *post hoc* to low density controls to determine the precise effect. For the summer and winter periods, experimental treatments were analyzed with one-way analysis of covariance (ANCOVA), with shell growth as the dependent variable and the measure of initial animal size from the growth model as the covariate. Data were subjected to contrasts and *post hoc* comparisons using the criteria outlined above. Statistical analyses were completed using PROC GLM (SAS 1989). Data were first evaluated for homogeneity of variances ($F_{max}$ test; Sokal and Rohlf 1995) and homogeneity of slopes (partial F-test; Kleinbaum et al. 1988).

Table 1. Treatments for the competition experiment conducted in Lake Waccamaw, N.C. Densities are given as individuals per 0.25 m² enclosure. 'X' indicates a treatment combination; --- indicates a potential combination not used.

| | L. ochracea | E. waccamawensis 0 | 8 | 16 |
|---|---|---|---|---|
| Shallow | 0 | --- | X | X |
| | 8 | X | X | --- |
| | 16 | X | --- | --- |
| Deep | 0 | --- | X | X |
| | 8 | X | X | --- |
| | 16 | X | --- | --- |

It is important to note that growth data from different experimental periods could be analyzed assuming different growth models. Selecting a growth model does not imply that bivalves grow according to one specific model. Rather, this is a pragmatic selection to increase the power of statistical tests by including a covariate accounting for the highest residual variation in bivalve growth outside treatment effects.

Mussel recovery rates were also evaluated. Survivorship is an important variable in experiments studying intra- and interspecific interactions (Peterson 1982). Unfortunately, we could not unambiguously assign a survival status to missing animals. Instead, proportional recovery was used as a surrogate measure of survivorship and examined for effects consistent with experimental treatments.

Laboratory Filtration Experiment

The laboratory methodology used to estimate *E. waccamawensis* and *L. ochracea* filtration rates followed Cahoon and Owen (1996). *E. waccamawensis* and *L. ochracea* were collected on 20 June 1997 from Lake Waccamaw (<0.5 m water depth). Mussels acclimated for four days in aerated aquaria (23 ± 1 °C). Lake Waccamaw water was fertilized with a modified f/2 medium (Guillard and Ryther 1962) diluted to f/10 strength (176 $\mu$M $NaNO_3$, 7.3 $\mu$M $NaH_2PO_4 \cdot H_2O$, and 22 $\mu$M $Na_2SiO_3 \cdot 9H_2O$) and thoroughly mixed. Fertilization was intended to increase phytoplankton in the water and minimize any artifacts of bivalve excretion during the experimental trial (Cahoon and Owen 1996). Water was aerated in carboys under natural illumination during the acclimation period.

Experiments were done in 10 - 13 L plastic buckets arranged in four blocks of three. Within each block, three treatments were randomly assigned: control bucket (no mussels), *E. w.* bucket (two individuals of *E. waccamawensis*), *L. o.* bucket (two individuals of *L. ochracea*). Lake water (8 L) was added to each bucket, and a glass finger bowl containing 200-250 mL of clean beach sand was carefully sunk to the bottom. A sample (100 mL) was collected from the top $\frac{1}{3}$ of each bucket and stored temporarily on ice. Two individuals of each species ($\bar{x}$ = 33.2 mm for *E. waccamawensis* and $\bar{x}$ = 32.1 mm for *L. ochracea*) were added to the finger bowl. Several times daily, buckets were stirred for 15 seconds to distribute and/or resuspend any settled plankters. Water was periodically aerated.

Samples were collected at two-day intervals for eight days and vacuum-filtered through glass fiber filters within one hour. Filters were frozen until analysis. Chlorophyll pigments were extracted in 90% acetone for 24 hours under refrigeration, and chlorophyll *a* (chl *a*) was read via spectrophotometry (Parsons et al. 1984). Grazing and filtration rates of each species were estimated using the equations of Frost (1972, cited in Cahoon and Owen 1996). Since changes in the concentration of chl *a* arise from both algal growth and bivalve grazing, algal growth rates (k, day⁻¹) were estimated for each

two-day interval in control buckets. Grazing rates (g, day$^{-1}$) were calculated with this series of equations:

$$(k - g) = \ln (C_2 / C_1) / (t_2 - t_1) , \text{ and}$$
$$g = k - (k - g),$$

where $C_2$ = chl $a$ ($\mu$g/L) in one bucket at the end of the interval $t_2$-$t_1$, and $C_1$ = chl $a$ ($\mu$g/L) at the beginning of interval $t_2$-$t_1$. Filtration rate (F, L/day·indiv.) was calculated as:

$$F = Vg / N,$$

where V = volume (L), g = grazing rate (day$^{-1}$), and N = number of individuals.

Chlorophyll $a$ in the controls was analyzed with repeated-measures analysis of variance (rmANOVA, Winer et al. 1991) to examine phytoplankton dynamics in the absence of grazing. TIME served as the repeated factor. Bivalve grazing rates were evaluated with repeated measures techniques using MANOVA (Von Ende 1993), with SPECIES serving as the main effect and TIME serving as the repeated factor. The test statistic for the MANOVA was Roy's greatest root. All analyses were done with PROC GLM or PROC ANOVA (SAS 1989).

Sediment Cores

Ten cores (8 cm length, 5 cm diameter) were taken at each depth to assess sediment particle sizes. Samples were frozen until analysis. Sediments were dried for 48 hours at 60 °C and dry-sieved. Sand was defined as anything unable to pass through a 0.063 mm sieve, while material passing through a 0.063 mm screen was silt/clay. Composition (by weight) of sediments was noted. Additionally, 2-5 g subsamples were baked for two hours at 400 °C to estimate percent organic matter (determined as weight loss upon ignition).

## RESULTS

Field Experiment

Recovery of experimental animals after the summer period was 94% for *L. ochracea* and 99% for *E. waccamawensis*. Of the missing individuals, the remains of only one *L. ochracea* were found. Shell remnants suggested predation. At the experiment's conclusion, 80.7% and 63.0% of all *E. waccamawensis* and *L. ochracea*, respectively, were recollected (Figure 1). *E. waccamawensis* was recovered in higher proportions at the shallow site (92.7%) compared to the deeper site (70.8%). This was a significant difference (ANOVA on arcsine-transformed proportions followed by a DEPTH contrast, p = 0.0079). For *L. ochracea*, 67.7% and 58.3% were recovered at the shallow
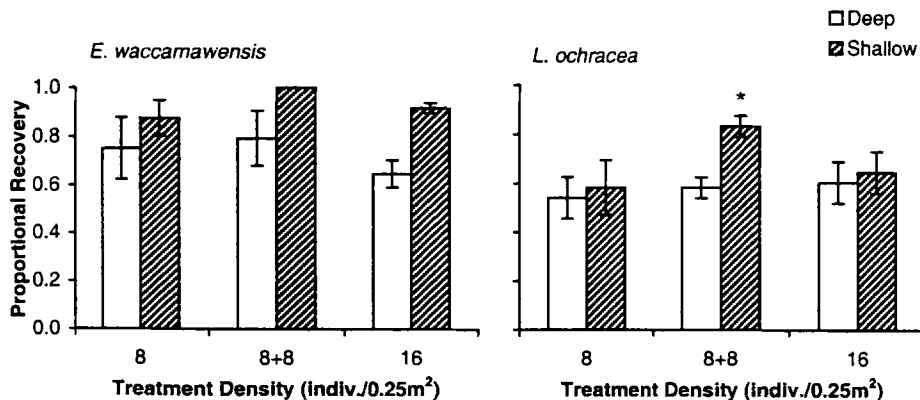


Figure 1. Proportional recovery (± 1 S. E.) of *E. waccamawensis* and *L. ochracea* following the field experiment. The asterisk indicates a higher recovery of *L. ochracea* in mixed treatments than in low density controls at the shallow site.

and deep sites, respectively. At the shallow site, there was higher recovery of *L. ochracea* from the mixed treatment than the low density treatment (p = 0.0313).

*Annual Period*. The growth model selected for analyzing the annual period was the logistic model, for which initial size $S_1$ explained 54% of the variation. The assumptions of homoscedasticity and homogeneity of slopes for all three variables for both species were satisfied; these were assumed to hold for the multivariate analysis (Scheiner 1993).

MANCOVA indicated a significant overall effect on *E. waccamawensis* ($F_{5,11}$ = 49.80, p = 0.0001). Contrasts revealed an effect of DEPTH (p = 0.0001), with growth, tissue weight, and shell weight increased at the shallow location relative to the deeper site (Table 2). The variables were not significantly correlated (p>0.05), and univariate tests were done to confirm the multivariate analysis. These tests (results not shown) verified that only DEPTH had a significant effect on *E. waccamawensis* over this period.

There were differences among treatment cells for *L. ochracea* ($F_{5,11}$ = 5.13, p = 0.0113); contrasts revealed a significant DEPTH effect (p = 0.0279) on the three variables (Table 2). Again, responses were not significantly correlated, and univariate ANCOVAs demonstrated both a significant DEPTH effect (p = 0.0069) and an INTRASPECIFIC DENSITY × DEPTH interaction (p = 0.0219) on shell growth (Table 3, Figure 2). *Post hoc* comparisons indicated that density did not influence growth at the deep site. However, at the shallow location, *L. ochracea* at high density grew significantly more than those at low density (p = 0.0204), suggesting a positive effect of density on growth. Contrasts indicated only a DEPTH effect for tissue and shell weight.

*Summer Period*. The Gompertz model explained 47% of the variation in summer growth, and ln $S_1$ served as the covariate for analyses. Tests revealed homoscedasticity and homogeneity of slopes. ANCOVA demonstrated a significant effect ($F_{5,11}$ = 10.79, p = 0.0005) on *E. waccamawensis* summer growth. Contrasts revealed the effects of DEPTH and INTERSPECIFIC DENSITY (Table 4). *E. waccamawensis* grew more at the shallow than the deep site (Figure 3). The endemic mussel also showed reduced growth in the presence of *L. ochracea* at the deep site when compared to low density treatments (p = 0.0378) but was unaffected by the heterospecific at the shallow site.

ANCOVA revealed treatment effects on *L. ochracea* ($F_{5,11}$ = 8.99, p = 0.001), with DEPTH affecting growth (Table 4). Contrary to the endemic, *L. ochracea* demonstrated higher shell growth at the deeper site (Figure 3). No other contrasts were significant.

*Winter Period*. The Bertalanffy model accounted for 13% of the total variation in winter growth, and reciprocal size ($1/S_1$) served as the covariate in subsequent analyses. Data demonstrated homoscedasticity and satisfied the assumption of nonsignificant interaction for the covariance analysis.

Table 2. Contrast F-statistics following multivariate analysis of covariance on growth, tissue weight, and shell weight of *E. waccamawensis* and *L. ochracea*.

| Contrast[a] | *E. waccamawensis* | *L. ochracea* |
|---|---|---|
| LOCATION | 67.41***[b] | 4.88* |
| INTRASPECIFIC DENSITY | 1.32ns | 0.93ns |
| INTRASPECIFIC DENSITY × DEPTH | 0.59ns | 1.97ns |
| INTERSPECIFIC DENSITY | 2.82ns | 1.34ns |
| INTERSPECIFIC DENSITY × DEPTH | 1.56ns | 1.19ns |

[a]Degrees of freedom for all multivariate contrasts are 3,9.
[b]ns (not significant) p>0.05, *p<0.05, ***p<0.001

Table 3. Contrast F-statistics following analysis of covariance of *L. ochracea* growth over the annual period.

| Contrast[a] | F |
|---|---|
| DEPTH | 10.98**[b] |
| INTRASPECIFIC DENSITY | 0.02ns |
| INTRASPECIFIC DENSITY × DEPTH | 7.12* |
| INTERSPECIFIC DENSITY | 2.77ns |
| INTERSPECIFIC DENSITY × DEPTH | 2.52ns |

[a]Degrees of freedom for each contrast are 1, 11.
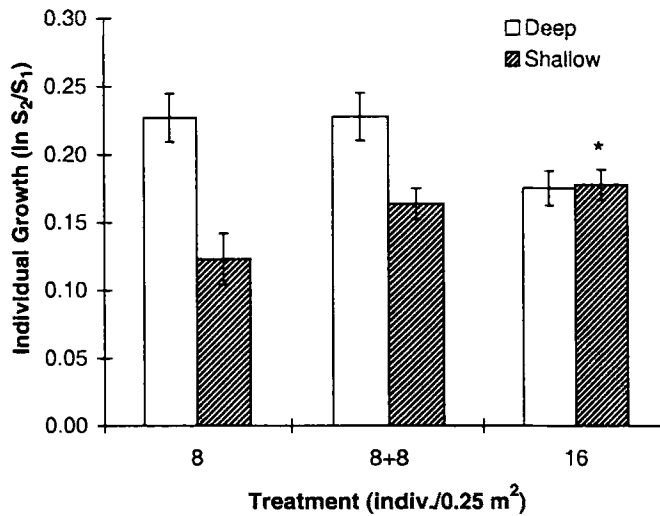[b]ns (not significant) p>0.05, * p<0.05, **p<0.01



Figure 2. Mean adjusted individual growth of *L. ochracea* (± 1 S. E.) over the annual period. The asterisk indicates a significant difference between that treatment and the corresponding low density control.

Table 4. Contrast F-statistics following analysis of covariance of *E. waccamawensis* (*E. wacc*) and *L. ochracea* (*L. och*) growth during summer and winter.

| Contrast[a] | Summer | | Winter | |
|---|---|---|---|---|
| | *E. wacc* | *L. och* | *E. wacc* | *L. och* |
| DEPTH | 51.28***[b] | 11.45** | 111.5*** | 70.92*** |
| INTRASPECIFIC DENSITY | 0.86ns | 2.52ns | 1.75ns | 0.00ns |
| INTRASPECIFIC DENSITY × DEPTH | 0.19ns | 0.20ns | 0.37ns | 57.23*** |
| INTERSPECIFIC DENSITY | 6.01* | 0.21ns | 0.63ns | 40.89*** |
| INTERSPECIFIC DENSITY × DEPTH | 0.84ns | 0.04ns | 0.49ns | 99.42*** |

[a]Degrees of freedom for each contrast are 1,11.
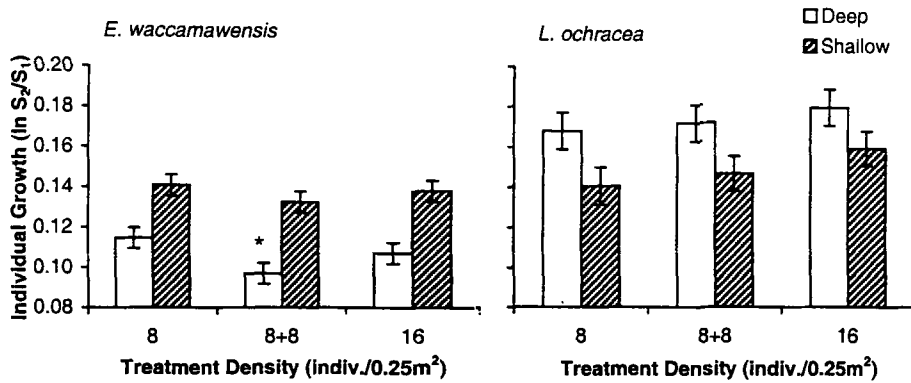[b]ns (not significant), p>0.05, * p<0.05, **p<0.01, ***p<0.001

Figure 3. Mean adjusted individual growth for *E. waccamawensis* and *L. ochracea* (± 1 S. E.) over the summer period. The asterisk indicates a significant difference between that treatment and the corresponding low density control.

The overall model showed a significant effect on *E. waccamawensis* growth ($F_{5,11}$ = 19.73, p = 0.0001), but contrasts highlighted only a DEPTH effect (Table 4). Animals at the shallow site grew more than ones at the deeper location (Figure 4).

There were many treatment effects on *L. ochracea* growth (Table 4). Animals at the shallow location grew more than those at the deeper site (p = 0.0001), which differed from summer. *L. ochracea* growth increased in the presence of the endemic at the shallow site (p = 0.0001) relative to low density controls (Figure 4), but that was reversed at the deeper site (p = 0.0181). The interaction between depth and intraspecific density was also highly significant (p = 0.0001). The presence of *E. waccamawensis* was associated with increased growth of *L. ochracea* in shallow water (p = 0.0001) but diminished growth of *L. ochracea* (p = 0.0003) at the deep location.
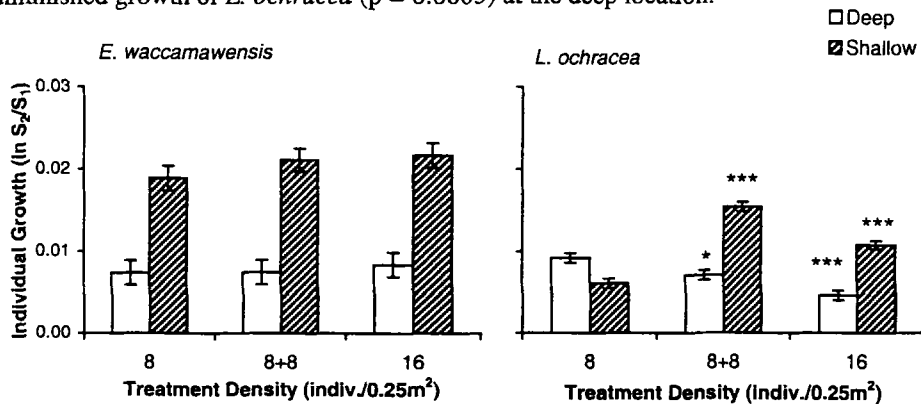


Figure 4. Mean adjusted individual growth for *E. waccamawensis* and *L. ochracea* (± 1 S. E.) over the winter period. Asterisks indicate significant differences between treatments and the corresponding low density control.

Filtration Experiment

In the grazing experiment, the final sample taken from one *L. ochracea* replicate did not contain detectable levels of chlorophyll, and the entire replicate was deleted from the analysis. After grazing rates were calculated, MANOVA revealed significant effects of TIME ($F_{3,3}$ = 23.82, p = 0.0136) and a TIME × SPECIES interaction ($F_{3,3}$ = 79.82, p = 0.0023). There was no SPECIES effect (p > 0.05). The significant TIME × SPECIES interaction indicates that *L. ochracea* removed phytoplankton at a greater rate over the

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first time interval than *E. waccamawensis* (Figure 5). *E. waccamawensis*, on average, grazed the algal growth occurring over the two-day interval, while *L. ochracea* exhibited grazing rates significantly above 0 during that same period. Both species showed similar filtering capacities. *E. waccamawensis* filtration rates ranged from 0 to 2.0 L/day·indiv., while *L. ochracea* filtered slightly larger volumes (0.4 to 2.4 L/day·indiv.).

Sediment Characteristics

The substrate at both locations consisted of >99% sand. The shallow site was dominated by particles between 0.5 and 0.125 mm, while particles at the deeper location were predominantly 1.0 to 0.25 mm. Sediment composition suggest relatively high exposures and benthic turbulence (Bailey 1988). Sediment organic content at each location was similar but generally very low. Organic matter ranged from 0.24 to 0.60% ($\bar{x}$ = 0.31%) and 0 and 0.96% ($\bar{x}$ = 0.46%) at the shallow and deeper site, respectively.
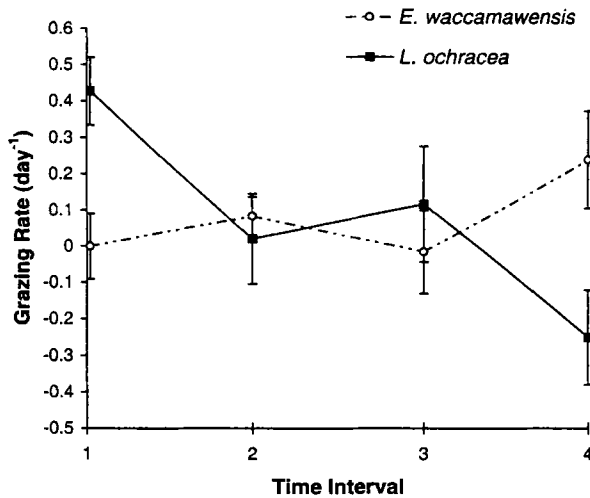


Figure 5. Grazing rates (day$^{-1}$), based on changes in the quantity of chlorophyll *a* ($\mu$g/L) over two-day intervals, for each bivalve species.

## DISCUSSION

Unionid Responses to Density Manipulations: Spatial and Temporal Effects

Experimental data show that water depth was a strong determinant of unionid growth. Over the annual period, *E. waccamawensis* exhibited reduced shell growth, tissue weight, and shell weight at the deep site. This finding is consistent with prior research demonstrating reduced growth of *E. complanata* in deeper water (Strayer et al. 1981). Hanson et al. (1988) also found an effect of depth on *Anodonta grandis simpsoniana* growth and concluded that the lower temperature of deeper water was responsible. Temperatures during summer at the shallow location in Lake Waccamaw were slightly higher (appx. 1 °C), and elevated temperature during the period of highest growth may contribute to *E. waccamawensis'* depth response. Resource levels (chl *a*) are typically higher in shallow water (Porter 1985; pers. obs) and may also enhance *E. waccamawensis* growth. The battery of explanations for a depth effect on the endemic are all plausible, but this work cannot determine the crucial environmental variable(s). Furthermore, we have no explanation for the converse pattern of higher *L. ochracea* growth in deeper water. Beyond the depth effects, density-dependence over the annual period is suggested by the significant INTRASPECIFIC DENSITY × DEPTH interaction. Growth of *L. ochracea* in shallow water increased with elevated density.

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We anticipated that interactions within and between species would be prevalent during summer. Contrary to this expectation, there was no evidence of intraspecific density-dependence during summer for either unionid. In the case of *E. waccamawensis*, this may be an artifact of test densities. Sampling around the experimental site revealed *E. waccamawensis* patch densities up to 32 indiv./m², but densities up to 132 indiv./m² have been recorded in other regions (Porter 1985). Furthermore, using small animals kept biomass low and in turn may have prevented consistent density pressure necessary to detect interactions over short temporal scales (<1 yr). The absence of summer intraspecific effects in *L. ochracea* is also difficult to explain. While low density treatments were three times higher than observed densities, biomass in those treatments was within natural levels. While we anticipated reduced growth as a response to doubling density (and biomass), no intraspecific effects were evident.

There was limited evidence of interspecific interactions during the summer period. The presence of *L. ochracea* diminished *E. waccamawensis* growth at the deeper site, while the effect of *L. ochracea* on the endemic at the shallow site was in the same direction but not significant. The interspecific effect at the deep site without corresponding intraspecific effects was unusual and could imply interference competition, possibly competition for space. However, since animals occupied < 2% of the enclosed area, the excess space makes interference competition unlikely (Peterson 1982). Instead, this result is more consistent with exploitative competition for resources.

When water temperatures are colder and phytoplankton less abundant during winter, resource competition may intensify. These data reveal strong effects of density and depth and the interaction between density and depth. Regardless of patch composition, elevated density diminished growth for *L. ochracea* at the deep site but increased growth at the shallow location. However, since growth increments are so small (< 1 mm), it is difficult to decide whether these statistically significant results have biological significance. We point out only that the positive density-dependence for *L. ochracea* noted in winter is consistent with its expression over the annual period.

This experiment demonstrates that the outcome of unionid density manipulations can vary in space and time. Research from Lake Waccamaw has provided indirect evidence that bivalves are food-limited (Lauritsen 1985 cited by Cahoon et al. 1992, Stiven and Arnold 1995). Given that evidence, we might have anticipated the consistent negative density-dependence associated with competition. Instead, we document variable interactions. *L. ochracea* reduced growth of *E. waccamawensis* at the deeper site during summer. During winter, though, *L. ochracea* exhibited a reduction in growth at the deep site in the presence of *E. waccamawensis*. This may signify a seasonal shift in competition, with *L. ochracea* the competitive dominant during summer and *E. waccamawensis* dominant during winter. The seasonal change in density-dependence over one year underscores the import of selecting appropriate temporal scales for experiments. Significant details would have been missed had our experiment lasted only one summer or one year without periodic measurement.

Mechanisms of unionid interaction

Rocky intertidal animals typically compete via interference tactics (e.g., crushing, overgrowing, or dislodging competitors; Peterson 1991). Soft-sediment infauna partition habitat depth (Lee 1996), nullifying the interference mechanisms prevalent in hard substrate assemblages. Negative interactions instead arise via resource removal. Experiments have produced evidence for local resource competition among infaunal marine bivalves (Peterson 1982) and other suspension-feeding invertebrates (Okamura 1988, 1992). Discerning the mechanism of interaction often requires detailed study.

The competitive ability of a bivalve is a combination of grazing rate, foraging efficiency, and resource requirements (Strayer 1999). We used the laboratory experiment

to measure grazing rates, as this is one aspect of resource removal that might lead to species interactions. That test showed that *L. ochracea* demonstrated higher grazing rates early in the experiment. Furthermore, filtration rates for *L. ochracea* were between 0.4 and 2.4 L/day·indiv. *E. waccamawensis* filtered from 0 to 2.0 L/day·indiv., which compares favorably to the 1.78 L/day·indiv. reported by Cahoon and Owen (1996). While these data do not prove that *L. ochracea* can consistently deplete the local food supply, they do suggest that resource pre-emption may occur and result in the growth differences detected in the summer.

If we are to infer species-specific filtration as one mechanism leading to negative density-dependence, species must show similar resource requirements. Unionids ingest particles ranging in size from 4 to 80 μm (Parker et al. 1998), implying significant resource overlap. Of course, there will be some interspecific variation. Paterson (1986) reported that *E. complanata* removed particles of ≈4 μm diameter with the highest efficiency, while Vanderploeg et al. (1995) demonstrated that *Lampsilis radiata siliquoidea* effectively filtered 1-4 μm particles. Different efficiencies at the extremes of the distribution curve may translate somewhat into resource partitioning, although there appears to be broad overlap in unionid diets.

Anecdotal evidence from our work suggests there may be some differences in resource consumption in our mussels. During the feeding experiment, *E. waccamawensis* were observed with green pseudofeces containing (apparently viable) algal cells around the exhalant siphon. *L. ochracea* did not have similar clouds, suggesting a higher assimilation or filtration efficiency for that species. Alternatively, *L. ochracea* may feed on a larger range of particle sizes (or types) than *E. waccamawensis*. Field observations also indicate that *L. ochracea* reopen their valves sooner after disturbance than *E. waccamawensis*. These behaviors may introduce disparities in access to food as well as the amount of food consumed by *L. ochracea* compared to the endemic.

Positive interactions in unionid communities

One unique result from this study is the positive density-dependence observed for *L. ochracea* over the winter and annual periods. Positive density-dependence in growth and survivorship was unexpected, and our work does not offer much insight into a potential mechanism. Marine research, however, offers a testable hypothesis. Dense assemblages of benthic suspension-feeders can deplete the benthic boundary layer (Fréchette et al. 1989, O'Riordan et al. 1993, Wildish and Kristmanson 1997), but Ertman and Jumars (1988) suggest that filtering by active suspension-feeders may enhance the deposition of particles via the flow convergence towards the incurrent siphons. While higher local density implies lower per capita food intake, the combined filtering of more animals may enhance food consumption. Water depth may modify this effect. In shallow water, surface plankton are closer to benthic unionids; mussel feeding and turbulence may quickly replenish locally-depleted resources. In deeper water, the benthic boundary layer may be depleted for longer periods because of reduced mixing, despite enhanced deposition from mussel pumping. This effect of water depth could account for the observed differences in interaction across depth.

These data from Lake Waccamaw are evidence for positive density-dependence in unionids, akin to Bertness's and Leonard's (1997) concept. The study of positive interactions in ecological communities is returning to the forefront of research. Intertidal research has demonstrated the crucial role that positive interactions can play in recruitment (Bertness and Grosholz 1985) and species distribution (Bertness 1989). Research demonstrates the positive influence of neighbors in procuring food resources. Under some flow regimes, feeding rates of the bryozoan *Electra pilosa* are higher when heterospecifics are adjacent (Okamura 1988). Continued research on positive interactions will further refine our understanding of community structuring forces.

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