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TIMING OF METAMORPHOSIS IN A FRESHWATER CRUSTACEAN: COMPARISON WITH ANURAN MODELS[1]

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Abstract. Many crustaceans have complex life cycles characterized by a metamorphosis, yet variation in metamorphic traits, and the causes and consequences of this variation, have rarely been examined. Food concentrations were changed during specific larval stages of the freshwater copepod *Mesocyclops edax* Forbes (Copepoda: Cyclopoida) to examine whether age and size at metamorphosis remain flexible or become fixed during the larval period. Results were compared to predictions of both flexible (the Wilbur–Collins model) and fixed (Leips–Travis model) rate models for the timing of amphibian metamorphosis. Age and size at metamorphosis were variable in all treatments, and age was always more variable than size. Changes in food concentration early in larval development resulted in significant differences in age at metamorphosis among treatments, but changes initiated when 60% of the larval period had passed had no effect on age at metamorphosis. Development appeared to become fixed later in the larval period, before the ultimate larval stage was reached. These results support predictions of the Leips–Travis model. Early changes in food concentrations had significant effects on size at metamorphosis, but changes initiated during the penultimate larval stage (50–60% of larval development) had no effect on metamorph size. Size at metamorphosis in *M. edax* also appeared to be fixed before the ultimate larval stage was reached. Fixation of size at metamorphosis during development is not predicted by either model and may be unique to organisms with rigid exoskeletons that constrain growth within any stage. Patterns of covariation between age and size at metamorphosis suggest that food conditions early in larval development exert a large effect on metamorphic traits, in contrast to patterns observed in several amphibian species.

The Wilbur–Collins model places a fitness premium on delaying metamorphosis to achieve a maximum size, when growth conditions are favorable; it thus may not apply to crustaceans. Selection pressures on the timing of metamorphosis in crustaceans may differ substantially from those identified for amphibians and other organisms. Because of these differences, incorporating crustaceans into studies of metamorphosis will help to clarify the factors affecting this life cycle transition.

Key words: crustacean growth; developmental model; developmental plasticity; Leips–Travis Dynamic Allocation model; life history variation; Mesocyclops edax; *metamorphosis; size constraints; Wilbur–Collins model.*

INTRODUCTION

Metamorphosis is a major life cycle event in diverse organisms, including many crustaceans. It often is accompanied by a shift in habitat or niche (Werner 1988) and is considered an important life history event because size and age at metamorphosis directly affect survival rates, reproductive output, and dispersal ability in many organisms (e.g., Moeur and Istock 1980, Blakley 1981, Semlitsch et al. 1988). Both age and size at metamorphosis are variable in organisms including amphibians (e.g., Wilbur and Collins 1973, Berven 1982, Semlitsch and Gibbons 1985), fishes (e.g., Policansky 1983, Victor 1986 *a, b*; Chambers and Leggett 1992), marine invertebrates (e.g., Jackson and Strathmann 1981, Pechenik 1990), and insects (e.g., Blakley 1981, Palmer 1984, Forrest 1987). Age usually is more variable than size because—or partly because—there often is a critical minimum size below which metamorphosis cannot occur (e.g., Wilbur and Collins 1973, Nijhout 1975, Blakley and Goodner 1978).

The causes and consequences of variation in age or size at metamorphosis have most often been examined in amphibians, from both theoretical and experimental perspectives. Studies of amphibian metamorphosis have produced two general types of models to account for variation in both age and size of metamorphs. These models differ primarily in the degree to which larval development remains flexible throughout the larval period. Wilbur and Collins (1973) proposed that larval development remains flexible throughout development, and that age and size at metamorphosis are functions of recent larval growth history. When recent growth history is poor, both age and size at metamorphosis should vary (Fig. 1A). When growth has been good, individuals should metamorphose at maximum sizes and variable ages (Fig. 1B). In contrast to this flexible development model, several models propose that developmental flexibility is lost ontogenetically either early (Travis 1984) or late (Hensley 1993, Leips and

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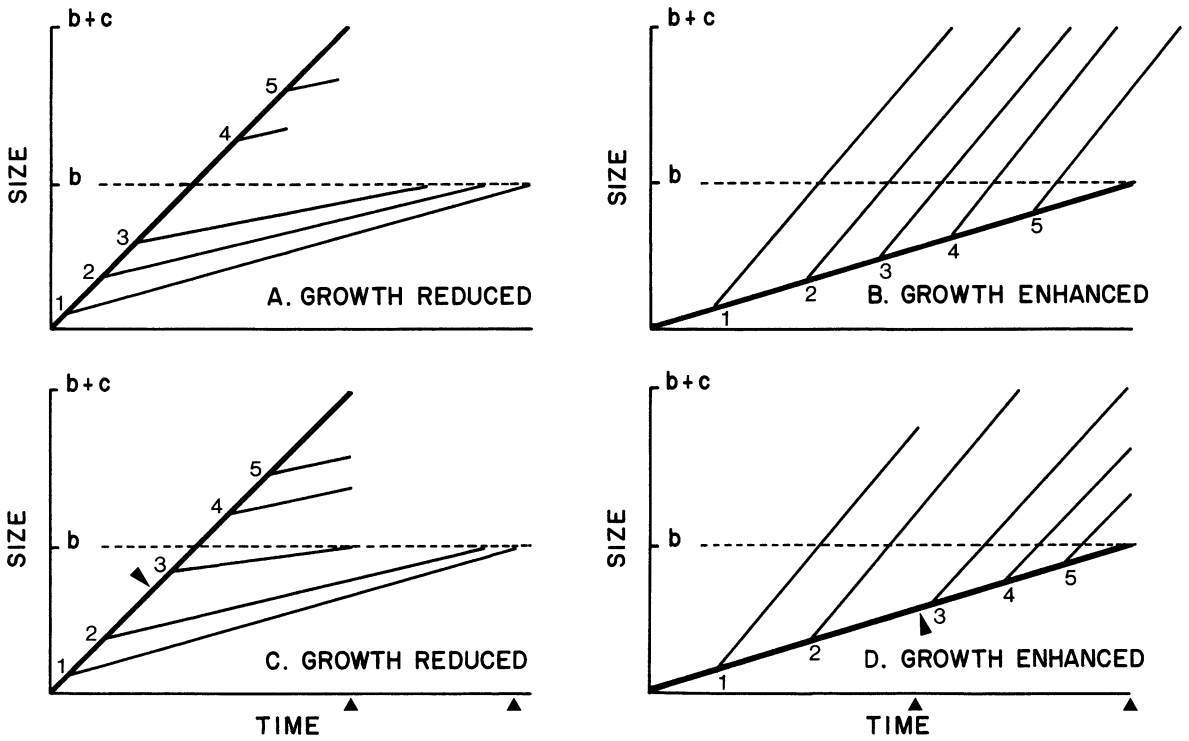

FIG. 1. Hypothetical growth trajectories, sizes at metamorphosis, and lengths of larval periods based on the Wilbur–Collins model (A, B) and the Leips–Travis Dynamic Allocation Model (C, D) (modified from Alford and Harris 1988, Hensley 1993). Limits to the size for metamorphosis predicted by each model: b refers to the minimum, and b+c to the maximum. Heavy lines represent constant growth rates, and numbered branches (thinner lines) off each heavy line represent predicted effects of changes in growth rate at various times during development. Wilbur–Collins model: (A) Decreased growth rate before minimum size is reached (1–3) results in later metamorphosis at smaller sizes compared with constant growth rate. Later decreases (4–5) lead to earlier metamorphosis (end of growth line). (B) Increased growth rate results in larger sizes at metamorphosis and younger ages at metamorphosis (1–4) but may delay metamorphosis (5) relative to individuals with a constant growth rate (note that thin line no. 5 extends beyond the thick line). Leips–Travis model: (C) Early reductions in growth rate (1–2) delay metamorphosis compared to individuals with high constant growth rates. Development is fixed late (arrowhead along the growth trajectory), and subsequent decreases in growth affect size but not age at metamorphosis (3–5). (D) High early growth (1–2) accelerates metamorphosis. Development is fixed late in the larval period (arrowhead pointing to the main growth trajectory), and subsequent increases in growth (3–5) affect only size at metamorphosis. Arrows along the abscissa in C and D indicate minimum and maximum ages at metamorphosis.

Travis 1994) in the larval period. At the point when developmental rate becomes fixed, age at metamorphosis also becomes fixed and can no longer be influenced by environmental conditions. Growth remains flexible over the entire larval period, and size at metamorphosis is most affected by environmental changes that occur late in larval duration after age at metamorphosis has been fixed (Fig. 1C, D; e.g., Hensley 1993, Leips and Travis 1994).

Both flexible and fixed development models can account for the phenotypic variation documented in age and size at metamorphosis, and experimental data gathered on several amphibian species from both temporary and permanent ponds often support predictions of more than one model (e.g., Travis 1984, Alford and Harris 1988, Hensley 1993). The Wilbur–Collins or flexible-development model has most often been tested with organisms other than amphibians. Lacey (1986) found that recent growth history accurately predicted flowering year in *Daucus carota* (Umbelliferae), supporting the Wilbur–Collins model. Development of male guppies (*Poecilia reticulata*) remained flexible in response to differences in food availability and growth rates; these results also support the Wilbur–Collins model (Reznick 1990). Vøllestad (1992) found no correlation between age and size at metamorphosis in the European eel *Anguilla anguilla*. He concluded that developmental pathways are fixed among a number of geographically distinct populations and rejected the Wilbur–Collins model. Tests of both flexible and fixed development models with the pitcher-plant mosquito *Wyeomyia smithii,* an amphibian analogue, support aspects of both models: age at metamorphosis was fixed late in the larval period, but nonzero growth rates during the ultimate larval stage were required for metamorphosis to occur (Bradshaw and Johnson 1995). Neither flexible nor fixed rate models have been tested experimentally for crustaceans, many of which have complex life cy-

cles. Crustaceans have rigid exoskeletons that constrain growth within each instar and which could alter metamorphic responses to environmental conditions.

Freshwater copepods (Crustacea) undergo a metamorphosis between the last larval (or sixth naupliar) stage and the first copepodite (juvenile) stage. Growth occurs in both pre- and post-metamorphic stages, and reproduction is temporally separated from metamorphosis by five subsequent juvenile stages. Little is known about this transition in copepods or about the ecological factors that affect its timing. Among three allopatric species of the calanoid copepod *Boeckella,* Jamieson (1986) found age at metamorphosis more variable than size in one species and size more variable than age in a second; the third species was variable for both age and size. She associated these patterns with specific habitat characteristics of each species; indirectly, these data support some of the predictions of the Wilbur–Collins model. Age and size at metamorphosis vary in at least five other copepod species (Twombly 1993, 1995), and age is more variable than size in each. This repeated pattern suggests that metamorphosis is size-determined in many copepods (see Blakley 1981, Policansky 1983).

In this study, I used laboratory experiments to examine the effects of changes in food concentrations on age and size at metamorphosis in *Mesocyclops edax* Forbes (Copepoda: Cyclopoida) and particularly to determine if larval development remains plastic throughout the larval period. Experiments were designed following the protocol used in many amphibian studies (e.g., Alford and Harris 1988; see also Bradshaw and Johnson 1995): food concentration was increased or decreased at specific larval stages and the effect of these manipulations on age and size at metamorphosis was recorded. The results allowed me to compare predictions from anuran flexible- and fixed-development models with data collected for a freshwater crustacean. If naupliar development remains flexible, I expected to find significant differences in age, but not size, at metamorphosis among all food treatments. Individuals experiencing reduced food concentrations should metamorphose at similar, minimum sizes (Fig. 1A), while individuals experiencing increased food concentrations should delay metamorphosis until they have reached similar maximum sizes (Fig. 1B). If age at metamorphosis becomes fixed during the larval period (Hensley 1993, Leips and Travis 1994), changes in food concentrations after this point should produce significant differences, among treatments, only in size at metamorphosis (Fig. 1C, D). My experiments were not intended to resolve conflicts among existing models but rather to compare specific predictions of these models with results obtained from a common freshwater copepod in order to begin to examine crustacean metamorphosis in a broader ecological context.

Methods

Experiments

Two experiments were conducted to test how naupliar size and age at metamorphosis responded to changes in food concentrations initiated at different times during the larval period. Space and time constraints, together with small clutch sizes in *M. edax,* required that these experiments (which consisted of different treatments) be run consecutively rather than simultaneously. *Mesocyclops edax* produces relatively small clutches of 20–40 eggs each, and I performed all experiments using 15 sibships (a sibship consisted of all eggs produced by a single female at a given time) so that sample sizes for each treatment would be as large as possible and that genetic contributions to metamorphic traits (e.g., Travis 1983) could be separated from environmental (treatment) effects. For all experiments, ovigerous female *M. edax* were collected from Barber Pond, West Kingston, Rhode Island, egg sacs were removed in the laboratory, and eggs were allowed to hatch in ≈15 mL of filtered, autoclaved pond water at 20 ± 1°C and a 14:10 L:D photocycle. Once hatched, nauplii were raised individually in 15 mL of filtered, autoclaved pond water under the same temperature and photocycle conditions, and subjected to one of the treatments described below.

All individuals were observed daily until they metamorphosed. New metamorphs were anaesthetised with carbonated water and traced, at 50×, using a camera lucida attached to a Wild M8 stereomicroscope. Individuals were traced from the anterior end of the cephalothorax to the end of the caudal rami and these traces were transformed to body lengths based on a conversion factor obtained using a stage micrometer. Each individual was measured at least three times to estimate measurement error.

In both experiments, food concentrations were manipulated at specific stages throughout larval development. In the first experiment, individuals raised in high food concentrations were switched to low food to effect a decrease in growth rates. Individuals reared at low food were switched to high food (to effect an increase in growth rates) in the second, complementary experiment. A mixture (1:1 by carbon content) of *Cryptomonas ozolini* and *C. erosa* was used as the food source. The volume (V) of 50 individual algal cells was calculated as an oblate spheroid and converted to mass of cell carbon using the conversion factor $\log_{10}C = 0.866(\log_{10}V) - 0.460$ (Strathmann 1967). Thereafter, cell densities in algal stock cultures (maintained in exponential growth phase in modified MBL medium [Stemberger 1981]) were estimated daily using a hemacytometer, cell densities were transformed to carbon concentration, and the appropriate volume of stock cultures was added to filtered, autoclaved pond water to obtain the food concentrations desired.

Low food concentrations were determined in a pre-

liminary test. Nauplii reached metamorphosis with low mortality at a food concentration of 0.6 μg C/mL but not at 0.2 or 0.4 μg C/mL, and I chose 0.6 μg C/mL as an algal concentration that could limit naupliar growth and development. "High" food concentration was 2.5 times this low concentration (or 1.5 μg C/mL).

In the first experiment (Experiment 1), nauplii reared at high food concentrations were switched to low concentrations at specific larval stages. Ovigerous female *M. edax* were collected from Barber Pond on 7 June 1994, and eggs were hatched as described above. Newborn nauplii from 15 separate sibships were randomly assigned to four treatments: high constant food (HC, 1.5 μg C/mL), high food switched to low food (0.6 μg C/mL) at naupliar stage 3 (HLN3), high food switched to low food at naupliar stage 4 (HLN4), and high food switched to low food at naupliar stage 5 (HLN5). All switches were performed before nauplii had reached the developmental stage at which they were competent to metamorphose. Two nauplii were raised together in small plastic petri dishes and four dishes ($n = 8$ nauplii) were initiated for each treatment, from each of the 15 sibships. This design allowed me to increase sample size in each treatment without doubling the medium or algal food required. Effects of rearing two nauplii together (a dish effect) on age and size at metamorphosis were estimated statistically.

The second experiment was identical to Experiment 1, except that individuals were raised at low food concentrations and switched to high food at specific developmental stages. Ovigerous females were collected from Barber Pond on 30 June 1994, when clutch sizes were slightly smaller than they were on 7 June. Nauplii hatched from 15 sibships were assigned randomly to four treatments (6–8 nauplii/treatment): low constant food (LC), low food switched to high food at naupliar stage 3 (LHN3), naupliar stage 4 (LHN4), and naupliar stage 5 (LHN5). As described for Experiment 1, all switches were completed before nauplii were competent to metamorphose.

Statistics

Experiments were designed for mixed-model, hierarchical analyses of variance, and I evaluated developmental plasticity as variation among food treatments in age and size at metamorphosis. In all ANOVA models, treatment (food regime) was a fixed effect, while sibships, dishes, and individuals were all randomly chosen. Both age and size were $\log_{10}$-transformed to achieve normality and homogeneity of variances among treatments. ANOVA tested for significant effects of treatment, sibship, treatment × sibship interaction, and dish nested within treatment and sibship, on both age and size at metamorphosis; the appropriate error term for each of these effects is shown in Table 1 (Zar 1984: Appendix A). The ANOVA model for size had an additional term, replicate measures of each individual, which provided an estimate of measurement error. Unequal clutch sizes among sibships and mortality resulted in unequal sample sizes, and *F*-ratios were based on Type III sums of squares (Shaw and Mitchell-Olds 1993). Variance components were calculated for all random effects in order to determine how much of the variation observed in age or size at metamorphosis was due to the associated error terms (individual variation for age, measurement error for size).

TABLE 1. Structure of hypothesis testing using a mixed-model analysis of variance for size and age at metamorphosis.

Source of variation	*F* value
Age at metamorphosis	
Treatment	$MS_{treat}/MS_{treat \times sibship}$
Sibship	$MS_{sibship}/MS_{dish(treat\text{-}sibship)}$
Treatment × Sibship	$MS_{treat \times sibship}/MS_{dish\ (treat\text{-}sibship)}$
Dish (Treatment-Sibship)	$MS_{dish(treat\text{-}sibship)}/MS_{error}$
Size at metamorphosis	
Treatment	$MS_{treat}/MS_{treat \times sibship}$
Sibship	$MS_{sibship}/MS_{dish(treat\text{-}sibship)}$
Treatment × Sibship	$MS_{treat\text{-}sibship}/MS_{dish(treat\text{-}sibship)}$
Dish (Treatment-Sibship)	$MS_{dish(treat\text{-}sibship)}/MS_{ind(treat\text{-}sibship\text{-}dish)}$
Individual (Treatment-Sibship-Dish)	$MS_{ind\ (treat\text{-}sibship\text{-}dish)}/MS_{error}$

Because the experiments tested specific predictions of metamorphosis models, planned comparisons were used to discriminate among treatments when ANOVA showed this effect to be significant. First, treatment sums of squares were partitioned into the sums of squares due to differences between controls and treatment groups, and to the sums of squares due to treatments. Dunnett's test (Zar 1984, Day and Quinn 1989) was used to compare all treatments with their appropriate control when the "control vs. treatment" effect was significant. When the "among treatments" effect was significant, I used Tukey's HSD test to discriminate among treatments. Planned comparisons among treatments of age at metamorphosis in Experiment 1 (HL) were invalidated by a significant treatment × sibship interaction term. To examine these treatment differences, I analyzed pairwise treatment-sibship combination means using both Dunnett's (for control-vs.-treatment comparisons) and Tukey's HSD (for comparisons among treatments) tests and looked for general patterns in the resulting treatment-sibship array (J. Travis, *personal communication*). Pearson product–moment correlations were calculated between age and size at metamorphosis within treatments, to examine covariance between these traits. All statistical analyses used SAS procedures (SAS Institute 1985).

RESULTS

The goal of these experiments was to determine whether changes in food concentrations initiated at specific developmental stages during the larval period affected age and size at metamorphosis in *Mesocyclops*

TABLE 2. Sample size (n), mean ($\bar{X}$), standard deviation (SD), and coefficient of variation (CV) for age and size at metamorphosis in all experimental treatments.

Treatment†	n	Age (days) $\bar{X}$	SD	CV (%)	Size (μm) $\bar{X}$	SD	CV (%)
HC	96	11.7	2.7	23.5	492	28.6	5.8
HLN5	104	12.8	3.3	25.7	479	40.2	8.4
HLN4	97	14.7	3.7	25.0	462	28.2	6.1
HLN3	82	16.7	5.1	30.6	468	32.9	7.0
LC	67	19.4	4.6	23.8	450	24.9	5.5
LHN5	76	17.1	2.7	15.9	458	22.6	4.9
LHN4	87	13.0	2.3	17.8	470	20.3	4.3
LHN3	73	14.0	3.5	25.0	464	23.8	5.1

† Treatment codes: HC = high constant food 1.5 μg C/mL); LC = low constant food (0.6 μg C/mL); HLN3 = high food switched to low food at naupliar stage 3: HLN4 = high food switched to low at naupliar stage 4; HLN5 = high food switched to low at naupliar stage 5.

edax. Food concentrations were changed at specific stages before individuals had reached the developmental stage (and perhaps the size) at which they were competent to metamorphose. In Experiment 1, N3 individuals were switched from high to low food after 27% of the HC larval period, and switches for N4 and N5 individuals occurred after 43% and 60% of the HC larval period, respectively. Individuals developed more slowly in Experiment 2 (low food) and were switched from low to high food after 16%, 26%, and 46% of the LC larval period had passed.

Time to metamorphosis

Changing food concentrations at different developmental stages resulted in different mean ages at metamorphosis (Table 2). Age at metamorphosis was variable (coefficients of variation 16–30%) in all treatments. The shortest naupliar period occurred in the highest food concentration (minimum = 9 d; mean = 11.7 d) and the longest period was exhibited by nauplii fed the lowest food concentration (mean = 19.4 d; maximum = 31 d). The latter mean figure is 68% larger than the former. Because different sibships were exposed to high (HC) and low (LC) food concentrations, this increase may be slightly over- or underestimated.

Analysis of variance showed that the timing of the switch in food concentrations (treatment) explained a significant amount of the variance observed in age at metamorphosis (F statistics, Table 3). Increases in food concentrations (Experiment 2) appeared to have a larger effect on age at metamorphosis than did decreases, again based on the associated F statistics. Age at metamorphosis varied significantly among sibships in both experiments, but the treatment × sibship interaction was significant only in Experiment 1 (HL). In both experiments, age at metamorphosis varied among dishes within a sibship and treatment. When variance was partitioned among the random effects in the mixed-model ANOVA, treatment × sibship interactions (Experiment 1), dish effects (Experiment 2), and the error terms (individual variation) accounted for the largest portions of the observed variation.

In Experiment 2, subdividing the treatment sums of squares and treatment degrees of freedom into separate analyses showed significant differences between control and treatment groups as well as among treatments (Table 4). Dunnett's test showed that mean age in each treatment differed significantly from control (LC) values. Thus, switching from low to high food concentrations at three different stages during larval development accelerated metamorphosis relative to controls, which were raised in low constant food concentrations. Among treatments, increases in food at either the earliest (N3) or middle (N4) stage resulted in similar ages at metamorphosis (Tukey's HSD test, Table 4), so that there was no clear cumulative effect when food concentration was increased during the first 25% of the larval period. Increased food later in larval development (N5 stage, 46% of larval period) produced individuals that were significantly older at metamorphosis than were those exposed to LHN3 or LHN4 treatments), although not as old as those raised in constant

TABLE 3. Mixed-model analysis of variance for age at metamorphosis, including estimates of variance components (expressed as percentage of total variance) among all random effects.

Source	df	Type III ss	MS	F	P	Variance components (%)
Experiment 1: HL						
Treatment (T)	3	1.166	0.389	12.93	0.0001	
Sibship (S)	14	0.411	0.029	3.92	0.0001	0.0
Treatment × Sibship	42	1.263	0.030	4.02	0.0001	36.4
Dish (Treatment-Sibship)	153	1.145	0.008	1.42	0.01	12.4
Error	166	0.874	0.005			51.2
Experiment 2: LH						
Treatment (T)	3	1.174	0.39	36.2	0.0001	
Sibship (S)	14	0.286	0.020	2.5	0.0001	8.0
Treatment × Sibship	42	0.453	0.011	1.3	0.137	6.0
Dish (Treatment-Sibship)	102	0.841	0.008	1.9	0.0001	29.8
Error	141	0.589	0.004			56.0

TABLE 4. Partitioning of treatment sums of squares (SS) for age at metamorphosis (Experiment 2 only) into orthogonal comparisons between control and treatment groups and among treatments, followed by Dunnett's test to compare each treatment with its appropriate control and Tukey's HSD test to discriminate among treatment groups.

Source	df	SS	MS	F	P
Control vs. Treatment	1	0.757	0.757	70.16	<0.001
Among treatments	2	0.63	0.315	29.2	<0.001

Dunnett's test:	LHN4	LHN3	LHN5	LC	
Tukey's HSD test:	LHN4	LHN3	LHN5		

Notes: For Dunnett's and Tukey's HSD tests, treatments connected by the same underscore are not significantly different at $P = 0.05$. Treatment codes are arranged in ascending (youngest-oldest) order.

low food (LC controls). These results indicate that the timing of metamorphosis was not fixed during the first 46% of larval development.

Because of the significant treatment × sibship interaction in Experiment 1 (HL), treatment-sibship combination means were analyzed (using Dunnett's or Tukey's HSD multiple comparison tests) to examine general trends in age at metamorphosis among treatments. In 73% of the 60 combination means analyzed, late (N5 stage) decreases in food concentrations produced ages at metamorphosis similar to those of the HC control group, suggesting that larval duration had become fixed by the time 60% of the larval period had passed. Earlier decreases in food concentration (HLN4, HLN3) usually (60% of the combination means) delayed metamorphosis significantly compared with the control or with the HLN5 treatment. Nauplii switched from high to low food concentrations at the N3 and N4 stages (27% and 43% of the HC larval period) were similar in age at metamorphosis, and there appeared to be no cumulative effect of decreasing food density early in larval development.

There was some congruence in results between the two experiments. Early (N3 or N4 stage) switches (either HL or LH) delayed or accelerated metamorphosis, respectively, relative to the control groups, but there were no significant differences in age at metamorphosis between the N3 and N4 treatments in either experiment. These results suggest a threshold, rather than a cumulative, effect of food: individuals experiencing an increase in food (for example) early in the larval period accelerated development, but exposure to high food for 84% of the larval period (LHN3) had no significant effect on the timing of metamorphosis over exposure to high food for only 75% of the larval period (LHN4). These results also suggest that the window of responsiveness of larval development to changes in food concentration encompasses the first 50% of the larval period but closes around 60% of the larval period, after which time age at metamorphosis is fixed. Treatment differences in Experiment 1 (HL) that support this interpretation can only be interpreted as general trends because of the analysis used.

Size at metamorphosis

New metamorphs ranged in size (length) from 400 μm (LC) to 532 μm (HC). Nauplii raised at high food concentrations were always the largest (Table 2), but changes in body size with food regime were much smaller (mean size in LC treatments was 8.6% smaller than mean size in HC treatments) than those achieved for age at metamorphosis (mean age in LC treatments was 68% larger than mean age in HC treatments).

Analysis of variance showed that the stage at which food concentrations were switched had a significant effect on size at metamorphosis (F statistics, Table 5). Differences among sibships also were significant in both experiments, but there was no significant treatment × sibship interaction for size at metamorphosis. Within a treatment and sibship, differences in size at metamorphosis among experimental dishes were nonsignificant, but individual variation in size was highly significant in both experiments. Variance components showed that measurement error accounted for slightly >10% of the observed variation in size. As this analysis excludes variation due to fixed treatment effects, measurement error is overestimated and probably was <10% in both experiments.

Contrasts between controls and treatment groups made up a significant portion of treatment sums of squares for size at metamorphosis in both experiments (Table 6). Changes in food conditions during larval development resulted in smaller (Experiment 1) or larger (Experiment 2) individuals at metamorphosis than those reared in control conditions. Dunnett's test revealed an interesting pattern in both experiments: early (N3 or N4 stage) switches in food conditions produced individuals significantly larger (increased food) or smaller (decreased food) than the control organisms, but changes made at the late (N5) stage produced no changes in size relative to controls even though food was switched before the ultimate larval stage was attained. Contrasts among treatments also contributed significantly to the treatment sum of squares (Table 6), and comparisons among treatment groups showed a consistent ranking. In both experiments, individuals switched at the earliest (N3) stage were statistically similar in size to those switched at the latest (N5) stage, and those switched at an intermediate (N4) stage were either the smallest (HLN4) or the largest (LHN4) of all treatments. Thus, individuals in the N4 stage appeared to be the most sensitive to changed food conditions, exhibiting the largest differences (over con-

TABLE 5. Mixed-model analysis of variance for size at metamorphosis, including estimates of variance components (expressed as a percentage of total variance) for all random effects.

Source	df	Type III SS	MS	*F*	*P*	Variance components (%)
Experiment 1: HL						
Treatment	3	0.111	0.037	13.1	0.0001	
Sibship	14	0.255	0.018	8.8	0.0001	22.7
Treatment × Sibship	42	0.118	0.003	1.4	0.09	5.1
Dish (Treatment-Sibship)	147	0.303	0.002	0.8	0.94	7.5
Ind (Treatment-Sibship-Dish)	155	0.262	0.002	17.2	0.0001	54.3
Error	760	0.075	0.000098			10.3
Experiment 2: LH						
Treatment	3	0.036	0.013	10.4	0.0001	
Sibship	14	0.041	0.003	2.7	0.002	5.8
Treatment × Sibship	42	0.053	0.001	1.2	0.26	3.1
Dish (Treatment-Sibship)	101	0.109	0.001	0.8	0.94	0.0
Ind (Treatment-Sibship-Dish)	141	0.204	0.001	19.8	0.0001	77.5
Error	698	0.051	0.000073			13.5

trols) in sizes at metamorphosis. A similar ranking was observed for age at metamorphosis in Experiment 2 (Table 4); the reasons for this response are unknown.

Covariation of age and size at metamorphosis

The relationship between age and size at metamorphosis varied with environmental conditions (Table 7). Individuals raised under constant high food (presumed high growth rates) or switched from high to low food (presumed decreased growth rates) showed an inverse relationship between age and size that was always highly significant. High food concentrations early in larval development appeared to stimulate growth, so that the youngest metamorphs were the largest. In contrast, individuals raised under constant low food (presumed reduced growth rates) or low food switched to high food (presumed increased growth rates) showed no significant correlation between age and size at metamorphosis, although the relationship between these traits was usually (with one exception) positive. Low-food conditions early in larval development appeared to prolong growth, so that the youngest metamorphs were the smallest and the oldest were the largest. These qualitative patterns suggest that the relationship between age and size at metamorphosis in *M. edax* is determined by food concentrations experienced early in larval life. Because age and size covaried significantly only when food concentrations were high early in development, the effects of food on growth may be asymmetric.

DISCUSSION

The aim of this research was to gain a better understanding of the larval-to-juvenile transition (metamorphosis) in a freshwater copepod by examining plasticity of larval growth and development. Both growth

TABLE 6. Partitioning of treatment sums of squares (SS) for size at metamorphosis into orthogonal comparisons between control and treatment groups and among treatments, followed by Dunnett's test to compare each treatment with its appropriate control and Tukey's HSD test to discriminate among treatment groups.

Source	df	SS	MS	*F*	*P*
Experiment 1: HL					
Control vs. Treatment	1	0.079	0.079	28.03	<0.001
Among treatments	2	0.03	0.015	5.32	<0.02
Dunnett's test:	HC HLN5 HLN3 HLN4				
Tukey's HSD test:	HLN5 HLN3 HLN4				
Experiment 2: LH					
Control vs. Treatment	1	0.0297	0.0297	23.46	<0.001
Among treatments	2	0.0159	0.00795	6.28	<0.01
Dunnett's test:	LHN4 LHN3 LHN5 LC				
Tukey's HSD test:	LHN4 LHN3 LHN5				

Notes: For Dunnett's and Tukey's HSD tests, treatments connected by the same underscore are not significantly different at $P = 0.05$. Treatment codes are arranged in descending order.

TABLE 7. Pearson product-moment correlations (r) between age and size at metamorphosis for each experimental treatment, and level of statistical significance (P).

Treatment	r	P
HC	−0.34	0.0008
HLN5	−0.54	0.0001
HLN4	−0.41	0.0001
HLN3	−0.56	0.0001
LC	0.15	0.21
LHN5	0.21	0.05
LHN4	−0.07	0.56
LHN3	0.14	0.22

and development remained plastic over the first half of the larval period in *Mescyclops edax* but age and size at metamorphosis became fixed during the latter third of the larval period. This study extends a previous one which showed that variation in age and size at metamorphosis in *M. edax* could not be explained solely by variation in newborn size (indicative of differential maternal provisioning), and which suggested that larval growth and development were at least partly responsible for the variation observed (Twombly 1995). More generally, the present study is one of a few to test metamorphic models (especially fixed-rate models) for invertebrates and may be the first to examine these models experimentally with an organism whose rigid exoskeleton constrains growth. My results show that metamorphosis in crustaceans differs in some important ways from patterns predicted for and found in other organisms. Comparing metamorphic patterns among different organisms, including crustaceans, may help to clarify the factors affecting this phenomenon and the role it plays in an individual's life history. The relevance of particular aspects of the present study to a more general understanding of metamorphosis is examined in more detail in separate sections of the following discussion.

Predictions of anuran models

Both the Wilbur–Collins and Leips–Travis Dynamic Allocation metamorphic models are based on a minimum size threshold that must be attained for metamorphosis to occur. This threshold has not yet been quantified for copepods or other crustaceans. In my experiments, food concentration was changed at three different stages prior to the point when individuals were developmentally competent to metamorphose. Because size increases with developmental stage, these individuals were also likely to be smaller than the minimum required size for metamorphosis. Under these conditions (food changed before metamorphic competency), model predictions for both age and size at metamorphosis are straightforward. The Wilbur–Collins model predicts significant differences in age at metamorphosis among all treatments when food is either decreased or increased, while size at metamorphosis should be similar among treatments in each case (Fig. 1A, B). In contrast, the Leips–Travis model predicts that ages at metamorphosis will not differ between treatments and their relevant control when food concentrations are changed after development has become fixed, although size at metamorphosis will respond to food conditions throughout the larval period (Fig. 1C, D).

Individual *M. edax* experiencing increased food concentrations at three different larval stages all differed from control (LC) individuals in age at metamorphosis, but larval development appeared to be fixed by the time that 60% of the larval period had passed. Individuals switched to low food at the fifth naupliar stage metamorphosed at the same ages as HC control individuals. These results generally support the Leips–Travis Dynamic Allocation model. Individuals switched to low food concentrations appeared to delay metamorphosis and nauplii in the highest food concentrations accelerated metamorphosis, in contrast to predictions of the Wilbur–Collins model. Size at metamorphosis in *M. edax* differed among treatments representing early (N3 and N4 stage) vs. later (N5 stage) switches. This result more closely matches predictions of the fixed-rate model. However, late changes (increases or decreases) in food had no effect on size at metamorphosis, indicating that size (along with age) is fixed after 60% of the larval period has passed. This result is not predicted by either of the models and represents one of the most interesting ways in which crustaceans differ from other organisms.

While results for both age and size at metamorphosis more closely match predictions of fixed-rate models, some of the predictions of the Wilbur–Collins model are difficult to test without detailed information on the minimum critical size for metamorphosis in copepods or growth patterns once this size is achieved. For example, although nauplii in high food concentrations did not appear to delay metamorphosis, they may have reached the size threshold relatively earlier than nauplii reared in low food concentrations, and delayed metamorphosis relatively longer than individuals in other treatments (supporting Wilbur–Collins). Growth and development are difficult to quantify non-destructively for copepods because the larval stages are both small and fragile. Use of exuviae or molt skins (Twombly and Burns 1996) promises a way to collect the data needed to test amphibian models more precisely.

Both models predict that variation in size at metamorphosis should be low in control treatments (HC and LC) and highest among individuals switched from low to high food concentrations (Travis 1984). In *M. edax,* variation in size at metamorphosis (coefficients of variation) was generally low among all treatments, and actually was lower for LH treatments than it was for either control or HL treatments. Copepod size at metamorphosis responded differently to environmental conditions than does amphibian body size.

Flexible and fixed rate models often predict similar

correlations between size and age at metamorphosis. For example, both the Wilbur–Collins and the Leips–Travis model predict a positive correlation between age and size at metamorphosis when individuals are reared in low food concentrations or are switched from high to low food. Comparing correlations obtained for *M. edax* with specific model predictions is thus not very explanatory. This comparison is interesting, however, because *M. edax* differs from amphibians in the patterns exhibited. Age and size at metamorphosis were negatively correlated for individuals reared in high constant food concentrations as well as for all individuals switched from high to low food. These patterns of covariation can be explained by high food concentrations experienced by all individuals early in larval development. A similar dependence on early larval conditions was observed for individuals reared initially in low food concentrations and then either maintained at low food or switched to high food concentrations. With one exception, these treatments all showed a positive correlation between age and size at metamorphosis (opposing the predictions of both models) although none of the correlations was significant. In amphibians, food conditions (and growth history) early in the larval period often have little or no influence on metamorphic parameters (Alford and Harris 1988, Leips and Travis 1994). The correlations measured for *M. edax* are more similar to the developmental inertia found in the pitcher-plant mosquito *Wyeomyia smithii.* In this insect, the effects of food conditions experienced by the first larval instar extend into subsequent instars, although not always to metamorphosis (Bradshaw and Johnson 1995). Patterns of covariation in *M. edax* may reflect the fact that both age and size at metamorphosis are fixed during development and are thus insensitive to environmental conditions later in the larval period.

Late fixation of larval duration

Age at metamorphosis appeared to have become fixed late in the larval period in *M. edax,* but before the ultimate larval stage was reached. Empirical data from diverse species show a similar pattern. Fifth instar larvae of the milkweed bugs *Oncopeltus fasciatus* and *O. cingulifer cingulifer* that have attained a minimum critical size initiate the requisite endocrinological processes and metamorphose within a fixed time irrespective of food supplies or recent growth history (Blakley and Goodner 1978). Larvae that encounter good food supplies after the commitment to metamorphosis is made are large when they metamorphose, while those fed on poor food are small. A similar response occurs in the tobacco hornworm, *Manduca sexta,* (Nijhout and Williams 1974*a,* Truman and Riddiford 1974) but is not true for all insects. Allegret (1964) showed that the program for the onset of metamorphosis in the moth *Galleria* was flexible in that the larva is committed to metamorphosis during the presumptive penultimate instar, but the decision to metamorphose can be modified by the amount of growth that occurs early in this instar. In the pitcher-plant mosquito, *Wyeomyia smithii,* age at metamorphosis does not become fixed until the last larval instar, and metamorphosis is contingent upon some growth during this instar (Bradshaw and Johnson 1995). Late fixation of larval development also occurs in amphibians (Hensley 1993, Leips and Travis 1994). The point at which development becomes fixed most likely coincides with an endocrine commitment to metamorphosis (Nijhout and Williams 1974*b,* Hensley 1993, Bradshaw and Johnson 1995).

Size at metamorphosis

The timing of metamorphosis is size dependent in some insects (e.g., Blakley and Goodner 1978, Nijhout 1975, Allegret 1964), but not all (e.g., Clarke and Langley 1962, Beck 1971). Blakley (1981) suggested three ways in which body size might affect the timing of metamorphosis: (1) size determines the molt at which metamorphosis occurs, but does not initiate the molting cycle (e.g., *Galleria mellonella,* Allegret 1964); (2) both the molt at which metamorphosis occurs and initiation of the molt cycle depend on larval size (e.g., *Manduca sexta,* Nijhout and Williams 1974*a, b*); (3) the metamorphic molt is developmentally predetermined, and size provides the stimulus for activating this molt (e.g., *Oncopeltus,* Riddiford 1970; Blakley and Goodner 1978; *Wyeomyia smithii,* Bradshaw and Johnson 1995). In each case, a critical *minimum* size is the key stimulus for molting or metamorphosis and appears to have been the target of natural selection. Blakley's third model may be the best description of metamorphosis in permanent-pond copepods, as there are no records of supernumery instars when food is limiting in either field or laboratory situations (Elgmork and Langeland 1970 and Czaika 1982 report fixed instar numbers for copepods). Rather, instar duration and larval development are prolonged under these conditions, possibly until the size requisite to trigger molting has been reached.

Size at metamorphosis may be less important to adult survival and reproduction in *M. edax* than it is in insects like *Oncopeltus* or *Wyeomyia* because juvenile copepods continue to grow for several stages before reproductive maturity, whereas the insects cease growth and reproduce immediately after metamorphosis. Nevertheless, as Blakley (1981) discusses, size-determined molt cycles in organisms with exoskeletons ensure that exoskeleton dimensions during subsequent development do not impose limitations on growth, which would ultimately result in small adult sizes. Size at metamorphosis appears to be tightly constrained in freshwater copepods (Twombly 1995) and may not respond to changes in food conditions in ways analogous to amphibians or other invertebrates.

Potential selection pressures

Crustacean larvae face fundamentally different constraints on body size than those faced by amphibians

or by invertebrates without rigid exoskeletons. Delay of metamorphosis by prolongation of the ultimate larval stage in order to achieve larger (maximum) body size is an unlikely if not impossible strategy. In *M. edax,* metamorphosis was delayed and the sixth larval stage prolonged in low food concentrations, and this delay was associated with smaller sizes at metamorphosis. Size at metamorphosis is constrained in copepods, and late changes in food concentrations had no effect on size at metamorphosis in *M. edax.* These results indicate that models placing a fitness premium on delay of metamorphosis to achieve maximum possible body sizes may not apply to crustaceans because the potential for growth in any stage is limited by a rigid exoskeleton.

Plasticity in age and size at metamorphosis is often interpreted as an adaptive response to temporary environments (e.g., Werner and Gilliam 1984, Newman 1992, Perrin 1992), which are potentially more variable than permanent environments for factors affecting larval growth and development. This interpretation has recently been challenged by Leips and Travis (1994), who showed comparable levels of plasticity in species from permanent and temporary ponds and suggested that plasticity in the timing or size of metamorphosis could be an adaptation to life in permanent environments. The limited data available for metamorphosis in copepods show more plasticity in permanent pond populations than in temporary ones (Twombly 1995 and *unpublished data*). Size-determined metamorphosis is often advanced as a response to unpredictable environments (e.g., Blakley 1981, Policansky 1983), and the metamorphic patterns exhibited by *M. edax* suggest that the conditions experienced by larval copepods may be variable and unpredictable even though the environment is permanent. Environmental uncertainty could be due to temperature fluctuations, as Durbin and Durbin (1992) have shown that even small temperature fluctuations have a large effect on growth and subsequent population dynamics of *Acartia* spp. Naupliar food supplies are also likely to be variable and unpredictable, as are predation pressures.

In general, the timing of metamorphosis in amphibians is understood as a compromise between the conflicting pressures of achieving maximum body size and minimizing risks of dessication or predation. The appropriate pressures for freshwater copepods, which do not undergo such a dramatic shift in habitats, are still unknown, but they are likely to include both food supplies (affecting larval vs. juvenile growth rates) and size-dependent predation that influences mortality rates in the two developmental phases. The profit (increased body size) gained in amphibians by delaying metamorphosis is reduced in copepods, and selection pressures on copepod larval growth and development, which determine timing of metamorphosis, are likely to differ from selection pressures identified in other organisms.

Conclusions

Metamorphosis is a critical life cycle transition that has been well studied in some organisms but only sporadically examined in others. It is a common life cycle phenomenon in crustaceans. Ecological studies of crustacean metamorphosis have focused on environmental cues or stimuli for settlement (and thus metamorphosis), primarily in marine decapods (e.g., Herrnkind and Butler 1986, Cobb et al. 1989, Harms 1992). Variation in age and size at metamorphosis has not often been studied directly, and there have been few attempts to examine the ecological consequences of this variation in crustaceans or to apply existing models to these organisms. The results reported here show that age and size at metamorphosis were variable and suggest that metamorphosis is determined by size in a common freshwater crustacean. Both growth and development remained plastic during the first half of the larval period in *M. edax,* but age and size at metamorphosis were fixed sometime during the latter 40% of larval life. Age and size at metamorphosis in *M. edax* generally supported predictions of the Dynamic Allocation model, although fixation of size at metamorphosis is a novel result. The Wilbur–Collins flexible development model may not apply to *M. edax* due to size constraints imposed by a rigid exoskeleton, although this model is appropriate for other organisms. The generality of my results among copepods and other crustaceans remains to be determined; the degree of developmental plasticity may vary interspecifically and among populations of one species, as well as within a single population over time. Investigating the factors affecting metamorphosis in crustaceans will contribute directly to a more general understanding of ontogenetic changes in the relationship between larval growth and development for individuals with complex life cycles.

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