

# Effects of short-term food variability on the plasticity of age and size at metamorphosis of porcelain crab larvae

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## Abstract

In a series of three experiments, we tested the effects of short-term food variability on the larval development of *Petrolisthes cabrilloi*. We first reared seven sibling clutches of zoeae at 19°C in 10 constant food rations (ranging 2–40 *Artemia* nauplii d<sup>-1</sup>) to determine maximal and minimal values for age and size at metamorphosis to the megalops stage. Mean age at metamorphosis ranged between 18.3–38.0 d after hatching and correlated negatively with food. Mean dry mass of megalopae ranged between 71.8–296.0 µg and correlated positively with food. The effect of food ration overwhelmed the small variation among clutches. Data from this experiment involving non-varying food rations were applied to a model of metamorphosis in variable environments to make quantitative predictions for more complicated regimes in which food varies during development. We tested the predictions by performing two experiments in which larvae were switched between high-food and low-food rations at various developmental stages and at controlled times. Size at metamorphosis was plastic throughout the entire larval period, but plasticity in the timing of metamorphosis was lost during the final 20–30% of the larval period. More importantly, data from the variable feeding regimes were within 95% confidence intervals for 14 of the 16 model predictions for age and size at metamorphosis. The model allowed the results of relatively simple experiments involving several nonvarying food rations to be extrapolated to more complicated scenarios involving short-term food variability.

Most benthic invertebrates have a complex life history where bottom-living adults produce planktonic larvae (Strathmann 1987). During development, pelagic larvae can encounter varying environmental conditions on a range of spatial and temporal scales (Davis et al. 1991; Seuront et al. 2001) that can lead to variable ages and sizes at metamorphosis. For species that have complex life cycles, duration of the larval period and size at metamorphosis are considered key life-history traits (Werner 1988; Twombly and Tisch 2002). Recent field sampling of benthic settlers underscores the variability in larval size and condition at metamorphosis (Pineda et al. 2002; Jarrett 2003). Marine invertebrate larvae, similar to most other animals with complex life cycles, face a trade-off between larval duration and body size at metamorphosis (Pechenik 1999). Pelagic larvae with a prolonged larval period will experience greater predation risk (Morgan 1995). A larger size at metamorphosis can increase the survival and performance of subsequent stages (Pechenik et al. 2002; Marshall et al. 2003; Phillips 2004).

Variability of food resources is especially relevant to the

life histories of marine invertebrate larvae because pelagic food resources vary in time and space on small scales relative to the development time of larvae (Dekshenieks et al. 2001; Alldredge et al. 2002). The patchiness of plankton has been a cornerstone of oceanography and limnology for decades, and recent technology has revealed spatial patchiness on very small scales. For example, thin layers (on the order of centimeters) of concentrated phytoplankton are common and may persist for several days (Rines et al. 2002).

Swimming behaviors and physical processes likely allow zooplankton to aggregate near dense food patches (Price 1989; Seuront et al. 2001). Metaxas and Young (1998) created algal patches in mesocosms and found that larvae aggregated near patches for several hours. Laboratory experiments with copepods also show that swimming behaviors allow individuals to remain in thin (~3-cm) food patches (Tiselius 1992). Larvae that encounter short-term, high-density food patches probably will experience increased growth and development rates, resulting in phenotypic plasticity in the timing of and size at life-stage transitions.

Most efforts to measure the effects of environmental variability, especially food variability, on the development of invertebrate larvae have cultured larvae in different levels of a resource that are each held constant for the entire larval period (Strathmann 1987; Boidron-Métairon 1995). The majority of studies examining short-term food variability that occurs *during* larval development have focused on periodic starvation in filtered seawater (see McEdward and Qian 2001; Pechenik et al. 2002; Moran and Manahan 2004). Although periodic starvation can provide many insights into larval physiology, larvae in nature will never encounter conditions as extreme as filtered seawater. Drawing ecological conclusions about the effects of short-term food variability on larval development requires experiments involving less

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extreme variability on a range of scales (see Pechenik et al. 1996a; Davis 1998; Hentschel and Emler 2000).

Insights into the effects of short-term food variability on the development of marine invertebrate larvae also can be gained from several ecological models, most of which were initially developed in the context of amphibians (see Wilbur and Collins 1973; Leips and Travis 1994; Day and Rowe 2002). Several of these models and empirical data from experiments designed to test them suggest that plasticity in the timing of metamorphosis can be lost late in the larval period (Smith-Gill and Berven 1979; Leips and Travis 1994; Hentschel 1999). In particular, Hentschel's (1999) model suggests that the timing of this transition from a plastic to a fixed rate of development can be predicted from empirical data on a larva's growth and development in a range of nonvarying, constant conditions. Hentschel's model applies data from the maximal and minimal larval growth trajectories (see fig. 1 in Hentschel 1999) to predict the size and age at metamorphosis if food varies on short time scales during larval development (see fig. 2 in Hentschel 1999). For example, if larvae experience an increase in food concentration, the model assumes they will take full advantage and will metamorphose at the maximal size unless developmental plasticity in the timing of metamorphosis is lost before the food increase is encountered. When the timing of metamorphosis becomes developmentally fixed, future changes in food will continue to affect a larva's size at metamorphosis but will no longer affect the timing of metamorphosis (see fig. 2 in Hentschel 1999). Experiments comparing the effects of different food rations that remain constant during larval development are much easier to design and complete than are experiments that include short-term food variability. Hentschel's (1999) model suggests ecologists can extrapolate from relatively simple experiments to predict the quantitative effects of possible scenarios involving small-scale food variability in nature.

We applied the approach outlined in Hentschel (1999) to study how food variability influences the timing of and size at metamorphosis to the megalops stage of porcelain crab larvae, *Petrolisthes cabrilloi*. In addition to conducting the first empirical test of Hentschel's (1999) model, we addressed several specific questions about the effects of food variability on the development of crab larvae: (1) When zoea larvae are exposed to a wide range of constant food rations, what are the extreme maxima and minima for the timing of and size at metamorphosis to the megalops stage? (2) How does increasing or decreasing food at four different time points during the first and second zoeal stages affect the age and size at metamorphosis? (3) If food is either increased or decreased at exactly the same time point and developmental stage, will larvae consistently alter their rate of development (or show a loss of plasticity) regardless of whether food was increased or decreased? (4) When in the zoeal period is plasticity in the timing of metamorphosis to the megalops stage lost in response to short-term food variability?

## Methods

*Study species and culturing techniques*—The porcelain crab, *P. cabrilloi*, is abundant in intertidal mussel beds from

Morro Bay, California, to Bahia de la Magdalena, Baja California (Haig 1960). Filter-feeding adults produce pelagic larvae that develop as two carnivorous zoeal stages and one filter-feeding megalops stage (Haig 1960). Gravid females can be found ~10 months of the year.

We collected late-stage ovigerous female crabs from mussel beds at Scripps Pier, La Jolla, California. Gravid females were transported to the laboratory and held separately for 1–5 d in 600-ml glass beakers containing 0.22- $\mu$ m-filtered, autoclaved seawater (FASW). All crabs were maintained in an incubator (Percival Scientific) at  $19 \pm 1^\circ\text{C}$  and a 14:10 light:dark (LD) photoperiod. Each gravid female was isolated so we could compare variation among clutches in conjunction with testing for effects due to the feeding regime experienced by larvae. Eggs typically hatch at night, and our larval-feeding experiments began on a night when the eggs of at least two females hatched. Active Zoea I larvae were haphazardly chosen from each clutch and transferred to ~200 individual 50-ml plastic beakers containing 40 ml of FASW.

Food treatments were randomly assigned to individual beakers. *P. cabrilloi* zoeae were fed a controlled number of 2-d-old *Artemia* nauplii (hereafter referred to as *Artemia*; San Francisco Bay Brand). The cultured *Artemia* were fed *Nannochloropsis* sp. (Aquaculture Supply US) and Selco (Florida Aqua Farms). Zoeae were transferred daily to autoclaved beakers containing FASW and the appropriate ration of *Artemia*. Daily monitoring also included counting the number of uneaten *Artemia* in each beaker and checking for molts to Zoea II or to megalops.

Beakers with larvae were held in a plastic storage cabinet containing removable drawers; each drawer contained 20 beakers in a Plexiglas frame. Cabinets were placed in the Percival incubator to maintain temperature at  $19 \pm 1^\circ\text{C}$  and a 14:10 LD period. Beaker position within a drawer and drawer position within the cabinet were randomly changed once each day.

*Age and size measurements*—Age of each larva at metamorphosis to the megalops stage was recorded as the number of days since hatching. We also measured the dry mass of each larva that successfully completed metamorphosis to the megalops stage. Individual megalopae were placed on a 0.22- $\mu$ m filter membrane (13-mm diameter) and rinsed three times with 0.22- $\mu$ m-filtered Nanopure water. Each filter containing a megalopa was placed in an aluminum boat, dried at  $60^\circ\text{C}$  for 24 h, and cooled in a desiccator for 24 h. The mass of each larva was determined by using a Mettler AT21 microbalance (Mettler-Toledo) accurate to 1  $\mu\text{g}$ . The mass of each megalopa was measured three times to compute an average for each individual. During each experiment, we also determined the dry mass of a random subset of Zoea I hatchlings and larvae at the Zoea II molt.

*Experiment 1*—To determine the ranges of size and age at the megalops stage, especially the extreme maximal and minimal growth trajectories (Hentschel 1999), we exposed larvae of seven clutches of *P. cabrilloi* to 10 constant food rations between 2–40 *Artemia* d<sup>-1</sup> (Table 1). This experiment was designed to measure the magnitude of variations in the

Table 1. Design parameters for the three runs of experiment 1. *Petrolisthes cabrilloi* larvae were fed one of 10 daily rations (No. *Artemia* nauplii) that each remained constant from hatching until metamorphosis to the megalops stage. The first two runs included larvae from two different clutches. The third run included larvae from three clutches. Clutch size was determined by counting the number of Zoea I larvae that hatched from each gravid female, and the female's size was determined by measuring carapace width.

Run	Start date	No. <i>Artemia</i> d <sup>-1</sup>	Clutch	Clutch size	Female size (mm)
1	23 Jul 2002	5, 10, 20, 30, or 40	A	>130	8
		5, 10, 20, 30, or 40	B	>130	8
2	05 Sep 2002	5, 10, 20, 30, or 40	C	>130	7
		5, 10, 20, 30, or 40	D	>130	8
3	13 Nov 2002	8, 13, or 20	E	80	7.5
		17 or 20	F	35	5
		2, 3, 17, or 20	G	89	7

timing of and size at metamorphosis of genetically similar larvae (i.e., siblings from the same clutch) and to compare the magnitude of variation within a single clutch to the variation among different clutches.

Because of time and space constraints, the experiment was divided into three runs. The first two runs (July and September 2002) each included larvae from two different clutches (120 larvae per clutch). Larvae in the first two runs were cultured individually at one of five food rations that remained constant throughout the zoeal period (5, 10, 20, 30, or 40 *Artemia* d<sup>-1</sup>). Larvae were randomly assigned to each food-ration treatment. Because preliminary experiments suggested that larvae fed low rations had low survival (~30%), the 5 and 10 *Artemia* d<sup>-1</sup> rations began with 36 and 30 zoeae per clutch, respectively. The 20, 30, and 40 *Artemia* d<sup>-1</sup> rations each began with 18 zoeae per clutch to ensure that 5–10 larvae would survive to the megalops stage in each ration.

We analyzed data from each of the first two runs separately with two-way mixed model ANOVAs to test for differences in the timing of metamorphosis to the megalops stage and differences in dry mass of megalopae due to food ration (fixed factor), clutch (random factor), and an interaction between food and clutch (Zar 1984). Clutch was treated as a random factor because female crabs were haphazardly collected in the field. We also calculated the magnitude of each effect (Graham and Edwards 2001).

In the first two runs, the magnitude of differences among clutches was small relative to the differences among food rations, so we performed a third run (November 2002) that included three clutches and a wider variety of intermediate and low rations (2, 3, 8, 13, 17, or 20 *Artemia* d<sup>-1</sup>; Table 1). There were not enough larvae in any of the three clutches to assign some larvae to every one of the six rations, but at least 14 larvae from each clutch were fed 20 *Artemia* d<sup>-1</sup> to permit controlled comparisons among the three clutches. This also permitted controlled comparisons among all three runs and among all seven clutches of experiment 1 (Table 1).

*Experiment 2*—In nature, larvae will not encounter a single, nonvarying food concentration throughout several weeks of planktotrophic development. Yet, most studies of

marine larval development in “variable environments” are limited to comparing different levels of a resource that are each held constant throughout the larval period (e.g., experiment 1 above) (Boidron-Métairon 1995). In experiment 2, we tested the effects of short-term food variability that occurs during larval development by exposing *P. cabrilloi* larvae to pulses of either increased or decreased food at various times. The design of this experiment is similar to Twombly's (1996) study of copepods and the second experiment of Hentschel and Emlet's (2000) study of barnacle larvae, except we reared larvae individually rather than in batch cultures.

Newly hatched zoeae from a single clutch were randomly divided between a high-food (40 *Artemia* d<sup>-1</sup>) and a low-food ration (10 *Artemia* d<sup>-1</sup>). Larvae in these “control” treatments did not experience a change in food during development (analogous to the 10 and 40 *Artemia* d<sup>-1</sup> treatments in experiment 1). In addition to the two control rations, the experiment included eight other feeding regimes in which larvae that began at either the low-food or high-food ration experienced a shift to the opposite ration on one of four different days. Increases from low to high food occurred on day 6, 11, 17, or 20. Decreases from high to low food occurred on day 5, 9, 13, or 15. We planned the timing of these shifts to be near the end of Zoea I, the start of Zoea II, midway through Zoea II, and late Zoea II (~25%, 45%, 70%, and 80% of the entire zoeal period of the control feeding regime). The increases of food did not occur on the same days as the decreases of food because larvae in the low-food control took longer to complete the zoeal stages than did larvae in the high-food control (25.1 d vs. 18.5 d; Table 2). Once a larva was shifted to a different ration, it continued at that second ration until metamorphosis to the megalops stage.

We performed two runs of this experiment with two clutches per run (Table 3). The first run (October 2002) included a large clutch (>150 larvae) and a small clutch (97 larvae). Larvae from the large clutch were randomly assigned to all 10 feeding regimes, but larvae from the small clutch were not assigned to two of the feeding regimes. In the second run (April 2003) each clutch had >170 larvae, and larvae from both clutches were assigned to all 10 feeding regimes.

Table 2. Mean age and dry mass of *Petrolisthes cabrilloi* larvae that metamorphosed to the megalops stage in experiments 1, 2, and 3 when fed constant rations: 10, 20, or 40 *Artemia* d<sup>-1</sup>. Grand means for age and dry mass at metamorphosis are listed under each set of rations.

Ration	Clutch	Experiment	Start date	<i>n</i>	Age ± 1 SE (d)	Mass ± 1 SE (μg)
10	A	1	23 Jul 2002	2	24.0±0.0	163±2
	B	1	23 Jul 2002	6	23.8±0.2	157±2
	C	1	05 Sep 2002	3	24.7±0.3	137±4
	D	1	05 Sep 2002	4	27.5±0.6	131±6
	H	2	17 Oct 2002	5	24.4±0.4	150±16
	I	2	17 Oct 2002	2	26.0±0.0	160±15
					Mean <sub>10</sub> = 25.1	Mean <sub>10</sub> = 149
20	A	1	23 Jul 2002	11	20.1±0.3	234±6
	B	1	23 Jul 2002	12	19.7±0.5	243±5
	C	1	05 Sep 2002	10	20.0±0.2	229±5
	D	1	05 Sep 2002	10	20.4±0.6	232±6
	E	1	13 Nov 2002	6	21.7±0.4	211±10
	F	1	13 Nov 2002	3	22.0±0.6	192±9
	G	1	13 Nov 2002	10	21.4±0.6	239±6
	L	3	29 Sep 2003	9	21.3±0.3	218±5
					Mean <sub>20</sub> = 20.8	Mean <sub>20</sub> = 225
40	A	1	23 Jul 2002	13	18.5±0.2	292±6
	B	1	23 Jul 2002	11	17.8±0.3	279±4
	C	1	05 Sep 2002	11	18.3±0.2	305±9
	D	1	05 Sep 2002	9	18.4±0.2	308±8
	H	2	17 Oct 2002	6	18.7±0.5	279±7
	I	2	17 Oct 2002	3	17.7±0.3	274±25
	J	2	16 Apr 2003	10	20.1±0.2	278±11
	K	2	16 Apr 2003	8	18.9±0.2	307±10
					Mean <sub>40</sub> = 18.5	Mean <sub>40</sub> = 290

Data from the constant rations of 10 and 40 *Artemia* d<sup>-1</sup> were applied to Hentschel's (1999) model to make predictions about the effects of short-term food variability on the timing of and size at metamorphosis. To make robust predictions about age and size at metamorphosis to the megalops stage in the variable feeding regimes of experiment 2, we pooled data for all of the clutches fed the constant 10 or 40 *Artemia* d<sup>-1</sup> rations in both experiments 1 and 2 (Table 2). By using grand means from experiments 1 and 2 as input for the model, we constructed linear growth trajectories, predicted age and size at metamorphosis to the megalops stage, and predicted the age at which plasticity in the timing of metamorphosis would be lost (Fig. 1). We also used the data

Table 3. Design parameters for the two runs of experiment 2. Each run included larvae from two different clutches. Clutch size is the number of Zoea I larvae that hatched from each gravid female. The number of *Petrolisthes cabrilloi* larvae used from each clutch and the female's carapace width also are reported.

Run	Start date	Clutch	Clutch size	<i>n</i>	Female size (mm)
1	17 Oct 2002	H	202	140	8
		I	97	94	7
2	16 Apr 2003	J	197	160	7
		K	226	160	8

from the six clutches fed the constant 10 *Artemia* d<sup>-1</sup> ration and the eight clutches fed the constant 40 *Artemia* d<sup>-1</sup> ration (Table 2) to determine 95% confidence intervals (CIs) around each prediction for the eight feeding regimes that included shifts between low- and high-food rations.

For larvae that experienced food increases from 10 to 40 *Artemia* d<sup>-1</sup>, we predicted that plasticity in the timing of metamorphosis would be lost on day 12.1 (95% CI = day 10–14; Fig. 1). Therefore, larvae that experienced increases on days 17 or 20 were predicted to metamorphose on the same day as larvae in the 10 *Artemia* d<sup>-1</sup> control (i.e., about day 25; Fig. 1). Larvae that experienced increases on days 6 or 11 were predicted to metamorphose at the maximum size (i.e., ~290 μg; Fig. 1). For larvae that experienced food decreases from 40 to 10 *Artemia* d<sup>-1</sup>, we predicted plasticity in the timing of metamorphosis would be lost on day 8.9 (95% CI = day 8–10; Fig. 1). Therefore, larvae that experienced decreases on days 9, 13, or 15 were predicted to metamorphose on the same day as larvae in the 40 *Artemia* d<sup>-1</sup> control (i.e., about day 18.5; Fig. 1). Larvae experiencing a decrease on day 5 were predicted to metamorphose on the same day as larvae in the 10 *Artemia* d<sup>-1</sup> control (i.e., about day 25).

These predictions were tested by comparing data from the appropriate feeding regimes with *t*-tests. Because the effects of food variability on the age and dry mass of megalopae were much greater than the variation among different clutch-

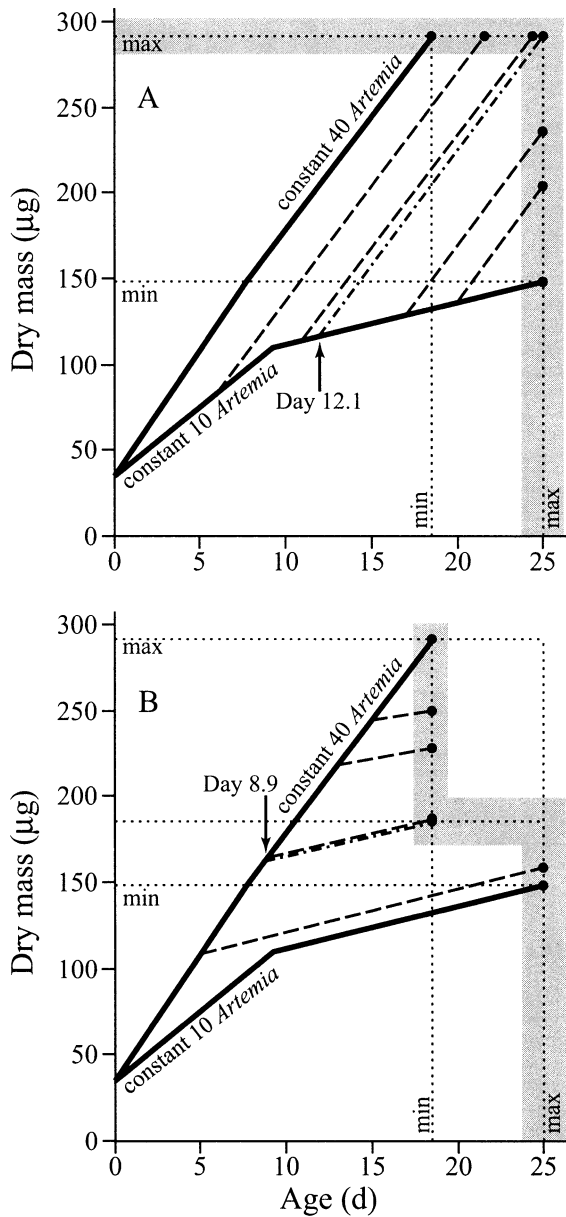


Fig. 1. Predictions for age and size at metamorphosis to the megalops stage for *Petrolisthes cabrilloi* larvae that would experience short-term food variability during experiment 2. Predictions were made by using Hentschel's (1999) model and data from the 10 and 40 *Artemia*  $d^{-1}$  treatments of experiments 1 and 2. Bold lines are the mean growth trajectories for the clutches of larvae fed 10 or 40 *Artemia*  $d^{-1}$  in experiments 1 and 2 (Table 2). (A) Predictions for larvae that experience increased food. Dashed lines are the predicted growth trajectories for larvae that would experience food increases from 10 to 40 *Artemia*  $d^{-1}$  on days 6, 11, 17, or 20. Endpoints of each trajectory indicate the predicted age and size at metamorphosis to the megalops stage. Gray areas are 95% confidence intervals (CI) around the predicted maximum dry mass (290  $\mu g$ ) and the predicted maximum age (day 25.1). Larvae initiated on 10 *Artemia*  $d^{-1}$  were predicted to lose plasticity in the timing of metamorphosis on day 12.1 (95% CI = day 10–14). The growth trajectory depicted by a combination of dashes and dots reveals the transition from a plastic to a fixed rate of development. (B) Predictions for larvae that experience decreased food. Dashed lines are the predicted growth trajectories for larvae that would experience

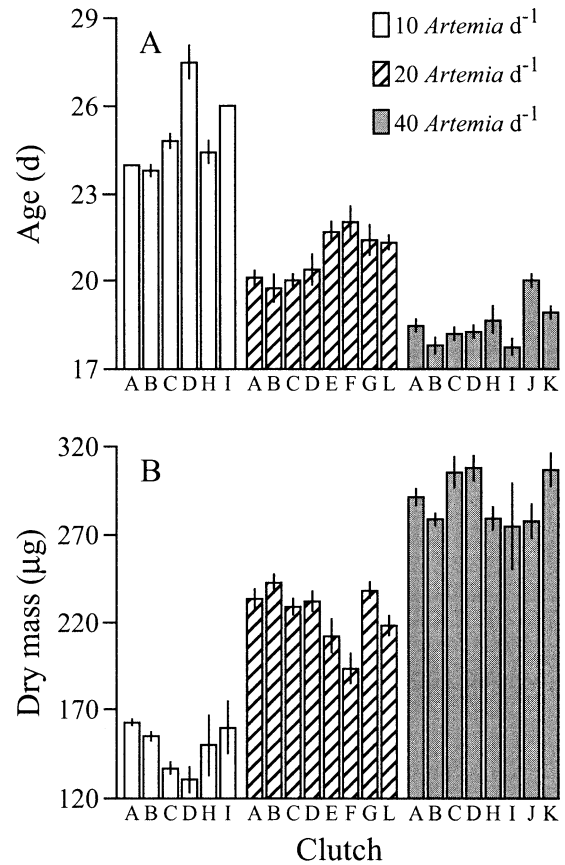


Fig. 2. Clutch to clutch variation in the timing of and size at metamorphosis to the megalops stage of *Petrolisthes cabrilloi* larvae fed constant food rations of either 10, 20, or 40 *Artemia*  $d^{-1}$  in experiments 1, 2, and 3. (A) Mean number of days from hatching to metamorphosis ( $\pm 1$  SE). (B) Mean dry mass of megalopae ( $\pm 1$  SE).

es (Fig. 2; Table 2), we performed the statistical comparisons on data pooled among clutches. Pooling increased statistical power and made it more likely to reject the model's predictions. Howard (2004) also analyzed data from each clutch individually, and the general trends did not differ from the analysis of pooled data.

**Experiment 3**—This experiment tested whether plasticity in age and size at metamorphosis occurs in response to both increases and decreases of food that are experienced at exactly the same time point. The design of this experiment is

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food decreases from 40 to 10 *Artemia*  $d^{-1}$  on days 5, 9, 13, or 15. Gray areas are 95% CIs around the maximum age (day 25.1), the minimum age (day 18.5), and an intermediate size plateau (185  $\mu g$ ) predicted by Hentschel (1999). Larvae initiated on 40 *Artemia*  $d^{-1}$  were predicted to lose plasticity in the timing of metamorphosis on day 8.9 (95% CI = day 8–10). The growth trajectory indicating the transition from a plastic to a fixed rate of development (a combination of dashes and dots) is partially obscured by the dashed trajectory beginning at day 9.

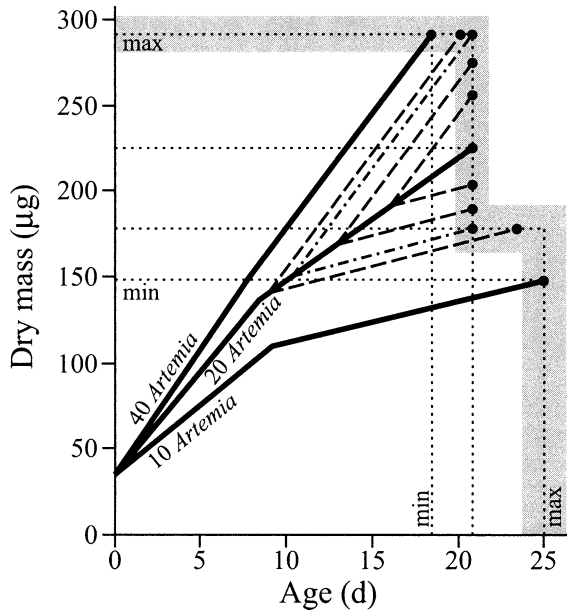


Fig. 3. Predictions for the age and size at the metamorphosis to the megalops stage for *Petrolisthes cabrilloi* larvae in experiment 3. Predictions were made by applying data from the 10, 20, and 40 *Artemia*  $d^{-1}$  treatments of experiments 1, 2, and 3 to Hentschel's (1999) model. The bold line ending at 20.8 d and 225  $\mu g$  is the mean growth trajectory of the eight clutches of larvae fed 20 *Artemia*  $d^{-1}$  (Table 2). Predicted growth trajectories (dashed lines) are shown for larvae that experience food increases from 20 to 40 *Artemia*  $d^{-1}$  or decreases from 20 to 10 *Artemia*  $d^{-1}$  on days 9, 13, or 16. Endpoints of each trajectory indicate the predicted age and size at metamorphosis to the megalops stage. Gray areas are 95% confidence intervals (CIs) around the predicted maximum dry mass (290  $\mu g$ ), the predicted intermediate size plateau (177  $\mu g$ ), the predicted maximum age (day 25.1), and the predicted minimum age (day 18.5). Plasticity in the timing of metamorphosis was predicted to be lost on day 10.4 (95% CI = day 8–13). Trajectories of increases and decreases on day 5 are not shown because they were predicted to be very similar to the mean 40 *Artemia*  $d^{-1}$  and 10 *Artemia*  $d^{-1}$  endpoints, respectively.

similar to the third experiment in Hentschel and Emlet (2000), in which barnacle nauplii were initially raised on an intermediate-food ration followed by symmetrical increases or decreases of food at specific time points.

At the start of experiment 3, all larvae were fed an intermediate ration of 20 *Artemia*  $d^{-1}$ . Larvae randomly assigned to a control regime experienced this ration throughout development. Other subsets of larvae experienced a shift to either 10 or 40 *Artemia*  $d^{-1}$  on one of 4 d after hatching (day 5, 9, 13, or 16), corresponding to ~25%, 45%, 60%, or 75% of the entire zoeal period of the control group. Once a larva was shifted to a different ration, it continued at that second ration until metamorphosis to the megalops stage. This experiment included only one clutch.

We used Hentschel's (1999) model and all available data from the constant rations of 10, 20, and 40 *Artemia*  $d^{-1}$  (Table 2) to make predictions for the age and size of larvae at metamorphosis to the megalops stage in the eight food-switch treatments (Fig. 3). For controls fed 20 *Artemia*  $d^{-1}$ , we predicted that plasticity in the timing of metamorphosis

would be lost on day 10.4 (95% CI = day 8–13; Fig. 3). Therefore, larvae that experienced food shifts on day 13 or 16 were predicted to metamorphose on the same day as larvae in the control (i.e., about day 21; Fig. 3). We tested these predictions by performing *t*-tests on data from the appropriate feeding regimes. Larvae that experienced food increases from 20 to 40 *Artemia*  $d^{-1}$  on day 5 or 9 were predicted to metamorphose at the maximum size (i.e., ~290  $\mu g$ ; Fig. 3). Larvae that experienced food decreases from 20 to 10 *Artemia*  $d^{-1}$  on day 5 were predicted to metamorphose at the maximum age (i.e., about day 25; Fig. 3), but larvae that experienced a food decrease on day 9 were predicted to metamorphose at an age between that of the control larvae and larvae that experienced a food decrease on day 5 (Fig. 3). Because this experiment did not include constant rations of 10 or 40 *Artemia*  $d^{-1}$ , we could only evaluate the predictions of the model for the maximum size and age at metamorphosis by comparing data from the food shifts that began on day 5 or day 9 to the 95% CIs for the predicted maximum size and age at metamorphosis (Fig. 3).

## Results

*Variation among clutches*—To assess the variability among different clutches, we tested for differences in the timing of and size at metamorphosis to the megalops stage among all clutches fed constant rations of either 10, 20, or 40 *Artemia*  $d^{-1}$  in experiments 1, 2, and 3. There were significant differences in the mean age at metamorphosis to the megalops stage among clutches of larvae fed either 10 ( $F_{5,16} = 12.950$ ,  $p < 0.001$ ), 20 ( $F_{7,63} = 3.050$ ,  $p = 0.008$ ), or 40 *Artemia*  $d^{-1}$  ( $F_{7,63} = 9.236$ ,  $p < 0.001$ ). There also were significant differences in the mean dry mass of megalopae among clutches fed 10 ( $F_{5,15} = 5.150$ ,  $p = 0.006$ ), 20 ( $F_{7,63} = 4.4$ ,  $p < 0.001$ ), or 40 *Artemia*  $d^{-1}$  ( $F_{7,63} = 2.475$ ,  $p = 0.026$ ).

The magnitude of the variation among clutches was, however, very small relative to the variation among food rations. Within each ration, the mean age at metamorphosis to the megalops stage varied by only 2–3 d among clutches (Fig. 2A; Table 2). In contrast, age at megalops varied by 6.6 d due to food ration (difference in mean age at megalops between larvae fed either 10 or 40 *Artemia*  $d^{-1}$ ; Table 2). Mean dry mass of megalopae varied by only 30–40  $\mu g$  among clutches within each food ration (Fig. 2B; Table 2). Dry mass of megalopae varied by 141  $\mu g$  due to food ration (mean difference between larvae fed either 10 or 40 *Artemia*  $d^{-1}$ ; Table 2).

*Experiment 1*—The two lowest food levels (two and three *Artemia*  $d^{-1}$ ) did not result in any larvae that successfully metamorphosed to the megalops stage, and only one larva successfully molted to the Zoea II stage. Only two larvae out of 120 fed five *Artemia*  $d^{-1}$  and only one larva of 27 larvae fed eight *Artemia*  $d^{-1}$  successfully metamorphosed to the megalops stage. A ration of 10 *Artemia*  $d^{-1}$  appeared to be the minimal ration that would reliably lead to megalopae.

The mean age at metamorphosis to the megalops stage was negatively correlated with food ration and ranged from 18.3–38.0 d (Fig. 4). Age at metamorphosis to megalops

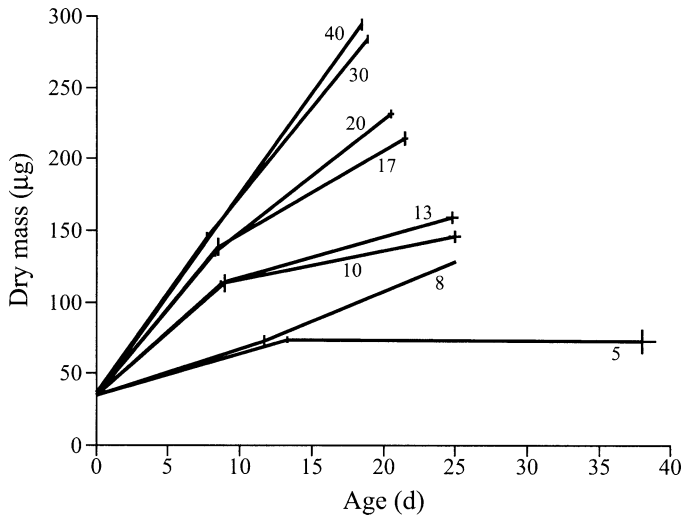


Fig. 4. Plasticity in age and size at metamorphosis to the megalops stage for seven clutches of *Petrolisthes cabrilloi* larvae in experiment 1. Lines are growth trajectories for larvae fed different constant food rations (numbers indicate the daily ration of *Artemia* nauplii). Each trajectory is drawn by using the mean dry mass at hatching, the mean age and dry mass ( $\pm 1$  SE) at the molt to Zoea II (midpoint), and the mean age and dry mass ( $\pm 1$  SE) at metamorphosis to the megalops stage (endpoint).

varied significantly among food rations ( $F_{4,10} = 112.305$ ;  $p < 0.001$ ) and among clutches ( $F_{3,131} = 13.077$ ;  $p < 0.001$ ). There also was a significant interaction between clutch and ration ( $F_{10,131} = 2.935$ ;  $p = 0.002$ ). Although there was a significant clutch effect and a significant interaction, these accounted for only 15% and 2%, respectively, of the total variance. The magnitude of the food effect was much more important, accounting for 83% of the total variance.

The mean dry mass of megalopae correlated positively with food ration, ranging from 71.8–296.0  $\mu\text{g}$  (Fig. 4). Dry mass varied due to food ration ( $F_{4,10} = 74.296$ ;  $p < 0.001$ ) but not due to clutches ( $F_{3,131} = 0.262$ ;  $p = 0.853$ ). The clutch-ratio interaction was significant ( $F_{10,131} = 2.966$ ;  $p = 0.002$ ). The food effect was the most important, accounting for 97% of the total variance.

To apply Hentschel's (1999) model, we identified the maximum growth trajectory for *P. cabrilloi* larvae to be  $\sim 40$  *Artemia*  $\text{d}^{-1}$ . On average, larvae fed 30 *Artemia*  $\text{d}^{-1}$  took 0.6 d longer to metamorphose to megalopae than did larvae fed 40 *Artemia*  $\text{d}^{-1}$  ( $t$ -test,  $p < 0.001$ ). The larvae fed 30 *Artemia*  $\text{d}^{-1}$  also had 13  $\mu\text{g}$  less dry mass at metamorphosis than did larvae fed 40 *Artemia*  $\text{d}^{-1}$  ( $t$ -test,  $p = 0.013$ ). Although these comparisons are statistically significant, the magnitudes of the differences in age and size are very small. Furthermore, daily counts of uneaten *Artemia* indicated that zoeae fed 40 *Artemia*  $\text{d}^{-1}$  often did not consume 10 of the *Artemia* in their beaker, whereas zoeae fed 30 *Artemia*  $\text{d}^{-1}$  rarely had more than three uneaten *Artemia*. We plotted growth trajectories for larvae fed each of the eight nonvarying rations in experiment 1 (Fig. 4). Because the differences among clutches were very small, we pooled the data among clutches. The range of ages and sizes at metamorphosis (i.e., endpoints of

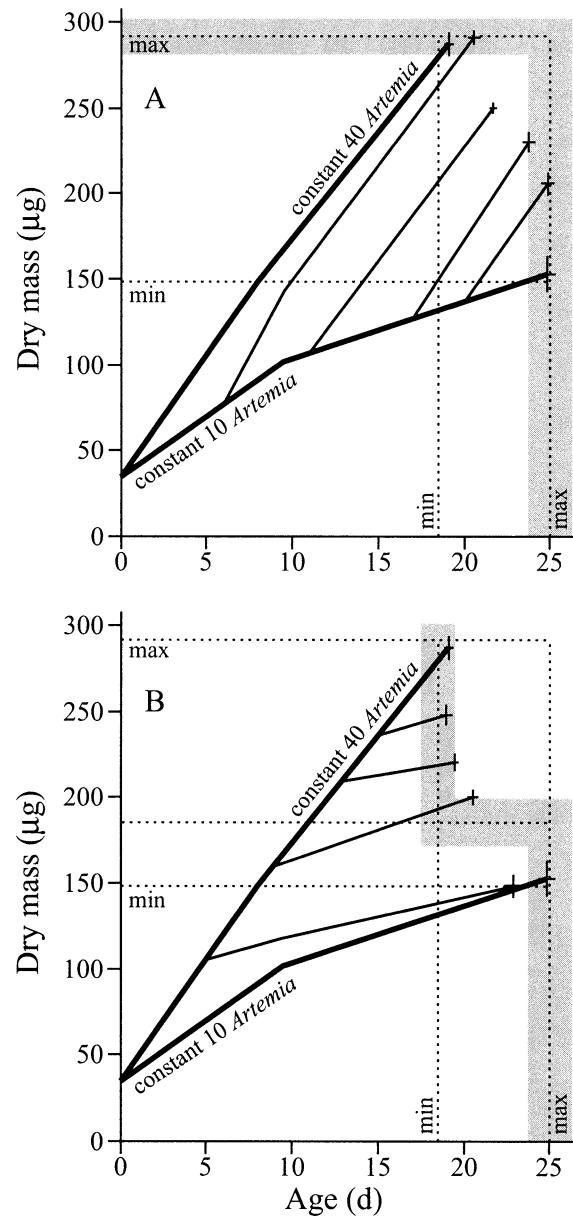


Fig. 5. Age and size of *Petrolisthes cabrilloi* larvae at metamorphosis to the megalops stage in experiment 2. (A) Food increases from 10 to 40 *Artemia*  $\text{d}^{-1}$  on days 6, 11, 17, or 20. (B) Food decreases from 40 to 10 *Artemia*  $\text{d}^{-1}$  on days 5, 9, 13, or 15. Bold growth trajectories represent larvae fed constant food rations (10 or 40 *Artemia*  $\text{d}^{-1}$ ). The endpoint of each trajectory is the mean age and dry mass ( $\pm 1$  SE) at metamorphosis to the megalops stage. Shaded areas are 95% confidence intervals for the age and size predictions (Fig. 1).

the trajectories) form the reaction norm for plasticity in response to the nonvarying food rations (Hentschel 1999).

**Experiment 2**—The mean age at metamorphosis to the megalops stage varied significantly due to feeding regime ( $F_{9,220} = 68.082$ ,  $p < 0.001$ ), ranging from 19.0–24.9 d (Fig. 5). The mean dry mass of megalopae also varied significantly due to feeding regime ( $F_{9,216} = 34.521$ ,  $p < 0.001$ ),

Table 4. Survival through metamorphosis to the megalops stage in experiment 2. The number of *Petrolisthes cabrilloi* Zoea I larvae used at the start of the experiment ( $n_i$ ), number of larvae that successfully metamorphosed to the megalops stage ( $n_f$ ), and number of clutches represented in each feeding regime are reported. Analyses were based on data pooled between the two runs of this experiment (Table 3). Each of the two runs started with two clutches of larvae, but low survival in some feeding regimes resulted in only two or three of the four clutches being represented in  $n_f$ .

Feeding regime	$n_i$	$n_f$	No. of clutches
10 <i>Artemia</i> d <sup>-1</sup> Control	58	7	2
10 <i>Artemia</i> d <sup>-1</sup> increased to 40 <i>Artemia</i> d <sup>-1</sup>			
Day 6	53	32	3
Day 11	42	32	4
Day 17	50	26	4
Day 20	63	15	3
40 <i>Artemia</i> d <sup>-1</sup> Control	38	27	4
40 <i>Artemia</i> d <sup>-1</sup> decreased to 10 <i>Artemia</i> d <sup>-1</sup>			
Day 5	76	6	2
Day 9	74	12	2
Day 13	56	40	4
Day 15	44	33	4

ranging from 149–291  $\mu\text{g}$  (Fig. 5). Survival to the megalops stage was lower when larvae experienced prolonged exposure to low food (Table 4). By applying data from the constant rations of 10 and 40 *Artemia* d<sup>-1</sup> (Table 2) to Hentschel's (1999) model, we made several predictions about metamorphosis in the feeding regimes that included short-term variability.

First, we predicted *P. cabrilloi* larvae experiencing food increases from 10 to 40 *Artemia* d<sup>-1</sup> after day 12.1 would metamorphose at the same age as larvae in the 10 *Artemia* d<sup>-1</sup> control (Fig. 1). Data confirmed this prediction for larvae that experienced a food increase on day 20 (*t*-test,  $p = 0.880$ ; Fig. 5A). Larvae that experienced a food increase on day 17, however, metamorphosed 1.2 d earlier than larvae in the 10 *Artemia* d<sup>-1</sup> control (*t*-test,  $p = 0.029$ ; Fig. 5A).

We also predicted larvae experiencing increases from 10 to 40 *Artemia* d<sup>-1</sup> before day 12.1 would metamorphose at the same size as larvae in the 40 *Artemia* d<sup>-1</sup> control (Fig. 1). Data confirmed this prediction for larvae that experienced a food increase on day 6 (*t*-test,  $p = 0.560$ ; Fig. 5A). Larvae that experienced an increase on day 11, however, metamorphosed with 37  $\mu\text{g}$  less dry mass than larvae in the 40 *Artemia* d<sup>-1</sup> control (*t*-test,  $p < 0.001$ ; Fig. 5A).

Hentschel's (1999) model predicted larvae experiencing food decreases from 40 to 10 *Artemia* d<sup>-1</sup> after day 8.9 would metamorphose at the same age as larvae in the 40 *Artemia* d<sup>-1</sup> control (Fig. 1). Data confirmed this prediction for larvae that experienced a decrease on day 15 (*t*-test,  $p = 0.718$ ; Fig. 5B) and for larvae that experienced a decrease on day 13 (*t*-test,  $p = 0.383$ ; Fig. 5B). Larvae that experienced a decrease on day 9, however, metamorphosed 1.4 d later than larvae in the 40 *Artemia* d<sup>-1</sup> control (*t*-test,  $p = 0.002$ ; Fig. 5B). We also predicted larvae shifted from 40 to 10 *Artemia* d<sup>-1</sup> on day 5 would metamorphose at the same

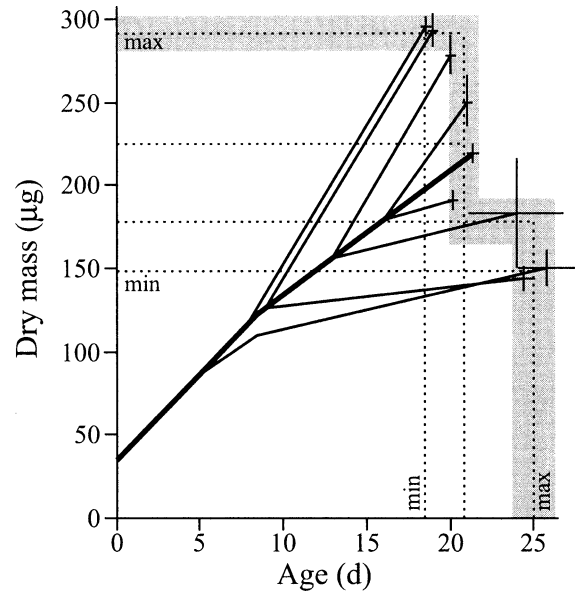


Fig. 6. Age and size of *Petrolisthes cabrilloi* larvae at metamorphosis to the megalops stage in experiment 3. Food was increased from 20 to 40 *Artemia* d<sup>-1</sup> and decreased from 20 to 10 *Artemia* d<sup>-1</sup> on days 5, 9, 13, or 16. The bold growth trajectory represents larvae fed a constant ration (20 *Artemia* d<sup>-1</sup>). Trajectory endpoints are the mean age and dry mass ( $\pm 1$  SE) at metamorphosis to the megalops stage. Shaded areas are 95% confidence intervals for age and size predictions (Fig. 3). The first 3 d of the trajectory for the food increase that began on day 5 are obscured by the bold trajectory representing 20 *Artemia* d<sup>-1</sup>.

age as larvae fed 10 *Artemia* d<sup>-1</sup> (Fig. 1), but larvae that experienced the food decrease on day 5 metamorphosed 1.9 d earlier than larvae in the 10 *Artemia* d<sup>-1</sup> control (*t*-test,  $p = 0.018$ ; Fig. 5B).

**Experiment 3**—The mean age at metamorphosis to the megalops stage varied significantly due to feeding regime ( $F_{8,55} = 23.648$ ,  $p < 0.001$ ), ranging from 18.6–25.6 d (Fig. 6). The mean dry mass of megalopae also varied significantly due to feeding regime ( $F_{8,50} = 22.772$ ,  $p < 0.001$ ), ranging from 144–296  $\mu\text{g}$  (Fig. 6). Survival to the megalops stage was lower when larvae experienced prolonged exposure to low food (Table 5). By applying data from the constant rations of 10, 20, and 40 *Artemia* d<sup>-1</sup> (Table 2) to Hentschel's (1999) model, we made predictions about metamorphosis in the feeding regimes that included short-term variability.

First, we predicted larvae experiencing either food increases from 20 to 40 *Artemia* d<sup>-1</sup> or food decreases from 20 to 10 *Artemia* d<sup>-1</sup> after day 10.4 would metamorphose at the same age as larvae in the 20 *Artemia* d<sup>-1</sup> control (Fig. 3). Larvae that experienced a food increase on day 16 fit the model's prediction (*t*-test,  $p = 0.457$ ), but larvae experiencing a food increase on day 13 did not, metamorphosing 1.3 d earlier than larvae in the control group (*t*-test,  $p = 0.004$ ; Fig. 6). Larvae that experienced a food decrease on day 13 also fit the prediction of the model (*t*-test,  $p = 0.537$ ), but larvae that experienced a food decrease on day 16 did not,

Table 5. Survival through metamorphosis to the megalops stage in experiment 3. Initial sample sizes ( $n_i$ ) and the number of *Petrolisthes cabrilla* larvae that successfully metamorphosed to the megalops stage ( $n_f$ ) in each feeding regime are reported.

Feeding regime	$n_i$	$n_f$
20 <i>Artemia</i> d <sup>-1</sup> Control	19	9
20 <i>Artemia</i> d <sup>-1</sup> increased to 40 <i>Artemia</i> d <sup>-1</sup>		
Day 5	16	12
Day 9	19	12
Day 13	16	8
Day 16	19	8
20 <i>Artemia</i> d <sup>-1</sup> decreased to 10 <i>Artemia</i> d <sup>-1</sup>		
Day 5	24	3
Day 9	27	3
Day 13	16	2
Day 16	19	7

metamorphosing 1.2 d earlier than larvae in the 20 *Artemia* d<sup>-1</sup> control group ( $t$ -test,  $p = 0.008$ ; Fig. 6).

Hentschel's (1999) model predicted that larvae experiencing a food increase before day 10.4 would metamorphose at the maximum size (i.e.,  $\sim 290 \mu\text{g}$ ; Fig. 3), and larvae that experienced food increases on day 5 or day 9 metamorphosed within the 95% CI for the maximum size (Fig. 6). We also predicted that larvae experiencing a food decrease before day 10.4 would metamorphose at the maximum age (i.e.,  $\sim 25$  d; Fig. 3), and larvae that experienced a food decrease on day 5 or day 9 metamorphosed within the 95% CI (Fig. 6).

## Discussion

The minimum food ration that allowed for successful metamorphosis to the megalops stage of *P. cabrilla* larvae was five *Artemia* nauplii d<sup>-1</sup>, and the rate of development and size at metamorphosis reached maxima at  $\sim 40$  *Artemia* d<sup>-1</sup> (Fig. 4). Between these extremes, the timing of metamorphosis ranged from a mean of 18.3–38.0 d, and size at metamorphosis ranged from 71.8–296.0  $\mu\text{g}$  (Fig. 4). The tremendous plasticity in the timing of and size at metamorphosis exhibited by *P. cabrilla* larvae could be an adaptation to the natural environmental variability experienced during development, especially food patchiness in the ocean. A larger size at metamorphosis could lead to greater juvenile performance and fitness (Marshall et al. 2003; Phillips 2004). When food is scarce, larvae would benefit from slowing their development until they might encounter and exploit unpredictable food-rich patches (Dekshenieks et al. 2001; Rines et al. 2002). A slow development rate could, however, increase the risk of planktonic predation (Morgan 1995).

The relationships between the development of marine invertebrate larvae and the performance of subsequent life stages are poorly understood and controversial (Strathmann et al. 2002). Clarifying the consequences of short-term food variability for the timing of and size at metamorphosis will lead to a more quantitative understanding of recruitment variability in marine populations and the dispersal potential of larvae in various oceanographic conditions.

*Responses to short-term food variability*—In general, the responses of *P. cabrilla* larvae to short-term food variability were similar in experiments 2 and 3 (Figs. 5, 6). Both revealed that plasticity in the timing of metamorphosis was lost late in the larval period. This loss of plasticity reflects a larva's developmental commitment to initiate metamorphosis. In experiment 2, plasticity in the timing of metamorphosis was lost by day 20 (i.e.,  $\sim 80\%$  of the 25.2-d zoeal period) for larvae fed 10 *Artemia* d<sup>-1</sup> and by day 13 (i.e.,  $\sim 70\%$  of the 18.8-d period) for larvae fed 40 *Artemia* d<sup>-1</sup> (Figs. 5, 6). In experiment 3, the food increases revealed that plasticity in the timing of metamorphosis was lost by day 16 of the 21.3-d zoeal period (i.e.,  $\sim 75\%$ ) for larvae fed 20 *Artemia* d<sup>-1</sup>. Our estimate that plasticity in the timing of metamorphosis was lost between 70–80% of the larval period is similar to the results of other studies involving frogs (Leips and Travis 1994), mosquitos (Bradshaw and Johnson 1995), copepod nauplii (Twombly 1996), and barnacle nauplii (Hentschel and Emlet 2000). The food decreases on day 16 of experiment 3, however, showed a slight acceleration of development (Fig. 6).

The acceleration of larval development in response to a decrease of food late in the larval period has not been found in previous food-switching experiments. Such acceleration has been predicted by some models of metamorphosis in variable environments (Wilbur and Collins 1973; Day and Rowe 2002). We caution, however, that experiment 2 did not reveal developmental acceleration when food decreased late in the larval period. The last food decrease in experiment 2 occurred on day 15 of the 18.8-d larval period of the 40 *Artemia* d<sup>-1</sup> control; in experiment 3, the last decrease occurred on day 16 of the 21.3-d larval period of the 20 *Artemia* d<sup>-1</sup> control (i.e., 80% and 75% of the respective control periods). Day and Rowe (2002) suggest there is a size threshold for metamorphosis; if food decreases when larvae are larger than the threshold, development should accelerate. The acceleration also should be greater as larvae grow further above the threshold. We did not weigh larvae when food shifts occurred, but the fact that the last food decrease in experiment 2 occurred 5% later than did the last decrease in experiment 3 suggests that larval size relative to a threshold does not explain the lack of acceleration in experiment 2.

Although plasticity in the timing of metamorphosis was lost late in the zoeal period, the size of *P. cabrilla* at metamorphosis to the megalops stage remained plastic throughout development in both experiments 2 and 3 (Figs. 5, 6). In general, earlier food shifts affected size at metamorphosis more than did later food shifts. Other food-switching experiments have consistently shown similar results for diverse larvae (for review, see Hentschel 1999).

*Comparisons to Hentschel's (1999) model*—Experiments 2 and 3 were designed explicitly as quantitative tests of the predictions derived from applying Hentschel's (1999) model to data from larvae fed a range of constant food rations (Fig. 4; Table 2). Overall, data from feeding regimes that included a shift in food concentration during larval development supported most of the model's predictions (i.e., 14 of the 16 endpoints of growth trajectories were within 95% CIs; Figs. 5, 6). The large sample sizes in our  $t$ -test comparisons led

to high statistical power, and data did allow us to reject null hypotheses (i.e., treatment groups that were predicted to have an equivalent age or size at metamorphosis) for some of the feeding regimes. For example, data for feeding regimes that included a food increase near the middle of the larval period consistently showed that larvae metamorphosed at sizes smaller than the predicted maximal size (Figs. 5A, 6). In addition, predictions for when the eventual timing of metamorphosis would shift from developmentally plastic to fixed were consistently a few days earlier than revealed by the data (Figs. 5, 6). In fact, the 95% CIs around the predicted day when plasticity in the timing of metamorphosis would be lost were relatively broad (i.e., 2–5 d or 10–20% of the 19–25-d larval periods for the respective control treatments: Figs. 5, 6). The broad CIs were caused by relatively small variability in the estimates for the maximal size at metamorphosis and the growth rates of larvae in the control trajectories propagating and magnifying variability in the estimate of the day when plasticity in the timing of metamorphosis would be lost. Despite some uncertainty surrounding the predictions for when plasticity in the timing of metamorphosis would be lost, the predicted ages at metamorphosis were always accurate to within 1.5 d of the empirical means (Figs. 5, 6). In general, the results of experiments 2 and 3 demonstrate that data from relatively simple experiments involving a range of nonvarying food rations (i.e., experiment 1) can be applied to Hentschel's (1999) model to generate estimates for complicated feeding regimes that involve short-term food variability.

This is the first *a priori* test of Hentschel's (1999) model, and additional tests are required before the model can be widely applied to analyze the effects of short-term food variability on the ecology of diverse larvae. Applying the model to the larval ecology of most benthic invertebrates requires some care in defining the end of the larval period. Because most planktotrophic larvae of benthic invertebrates metamorphose after encountering a settlement cue, metamorphosis of these larvae depends primarily on the perception of the cue(s) (Hadfield et al. 2001; Hadfield and Koehl 2004). Before being able to metamorphose in response to an external cue, such larvae must first develop a competency to perceive and respond to the cue. For these larvae, the timing of competence represents an end to the portion of the larval period in which the development rate can be influenced by food variability (Pawlik and Mense 1994; Pechenik et al. 1996b; Davis 1998). When studying the larvae of cue-dependent invertebrates, the end of the precompetent period represents an ecologically relevant analogy to the "timing of metamorphosis" discussed in Hentschel (1999).

Diverse taxa also might not meet the simplifying assumptions of Hentschel's (1999) model to the same degree as do *P. cabrilloi* zoea. For example, species that deviate significantly from the assumption of linear growth trajectories will require additional data as input for the model (e.g., measurements of sizes at intermediate points along a growth curve rather than measurements of only the sizes at hatching and metamorphosis). In addition, all of our experiments were conducted at a constant water temperature. If temperature, food concentration, food quality, and other variables that can affect rates of larval growth and development vary indepen-

dently, a simple model based on the variability of one parameter might not yield robust predictions in more complicated scenarios that have yet to be tested in controlled experiments. In particular, sources of larval nutrition such as dissolved organic matter and bacteria (Boiron-Metairon 1995) can be difficult to measure and control in relation to other key variables.

*Potential applications*—The ecology of marine invertebrate larvae has often been considered a "black box" full of uncertainties and hypotheses that are difficult to test experimentally. In recent years, oceanographers and larval biologists have made significant advances toward understanding how larval behaviors and physical processes interact to transport pelagic larvae to benthic recruitment sites (Wing et al. 1998; Shanks et al. 2003a). Although duration of the pelagic larval period is central to any oceanographic model's prediction of dispersal and recruitment (Stockhausen et al. 2000; Gaines et al. 2003; Shanks et al. 2003b; Siegel et al. 2003), few models include any detail beyond an average development time in the plankton. Understanding the supply side of larval recruitment has increased greatly in recent years (Underwood and Keough 2001), but we also are learning that the quality of larvae arriving at settlement sites varies in time and space (Jarrett 2003; Gimenez et al. 2004). The nutrition of planktotrophic larvae before metamorphosis also is known to affect the performance of juveniles after metamorphosis (Pechenik et al. 1996a,b, 2002; Miller and Emlet 1999; Phillips 2004), providing clear evidence that a full understanding of recruitment will require more than knowing the number of larvae arriving at a site.

We have shown how data from relatively simple experiments (e.g., experiment 1) can be applied to Hentschel's (1999) model to generate reliable predictions for more complicated scenarios involving environmental variability during larval development. Whether these predictions can be applied to populations in nature depends primarily on an ability to measure the environmental parameters likely to affect larval development significantly. Recent advances in ocean observing systems (Schofield et al. 2003; Isern and Clark 2003) promise to provide the environmental data needed to set boundary conditions for the temporal and spatial variability of key parameters. With adequate data on how larvae will respond to environmental variability, we can explore larval development in a variety of scenarios based on real-world data. Such an approach should be able to improve predictions of recruitment variability in marine populations and the management of fisheries and marine protected areas.

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