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Physiological recovery from prolonged 'starvation' in larvae of the Pacific oyster *Crassostrea gigas*

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Abstract

Previous studies of energy metabolism in larvae have described a developmental "point of no return" (PNR), a time by which larvae of planktotrophic marine species must feed in order to survive and grow. This study investigated the effects of long-term food deprivation on developing larvae of the oyster *Crassostrea gigas* with the goal of providing a biochemical and metabolic description of larvae at the PNR in this species. Mortality of unfed larvae was low for the first 14 days without the addition of phytoplankton foods. Even after 33 days without food, larvae were still swimming. Unfed larvae did not lose their ability to capture and digest algal cells when provided with food after 33 days. Growth, metabolic rate and biochemical constituents all increased at the same or greater rates in larvae whose feeding was delayed for 5, 8, 11, 14 or 17 days compared to larvae fed at 2 days old, when feeding was possible. These results show that larvae of *C. gigas* can survive long feeding delays while maintaining a constant rate of metabolism. These results suggest that oyster larvae have the capacity to survive 'starvation' using alternative sources of energy. If there is a "point of no return" beyond which larvae of *C. gigas* must feed on microalgae to survive, our findings suggest this point may be set by the availability of detrital material or dissolved organic carbon that can fuel maintenance metabolism for extended periods equivalent to over four times the predicted lifespan.

Keywords: Larva; Veliger; Physiology; Metabolism; Energy budget; *Crassostrea gigas*; Pacific oyster

1. Introduction

Survival of larvae in the plankton is widely regarded as one of the most important factors shaping both the evolution of life histories and, through larval availability and

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recruitment, the size and distribution of populations in the sea (Thorson, 1950; May, 1973; Strathmann, 1985; Roughgarden et al., 1987; Underwood and Keough, 2000). Yet, the identity and relative importance of the multiple environmental factors that might affect larval survival have remained elusive. One factor often implicated in larval mortality among species with feeding larval stages ('planktotrophic') is the patchy and unpredictable nature of phytoplankton abundance in the ocean; phytoplankton is traditionally thought to be the major source of food for nonpredatory larvae (Thorson, 1950; Strathmann, 1985). As evidence for the importance of phytoplankton, low concentrations of phytoplankton at certain key times of the year can lead to mass starvation of larvae (Erlich et al., 1976; Lucas, 1982; Pechenik, 1987; Starr et al., 1990; Platt et al., 2003). Likewise, phytoplankton concentrations needed to yield maximum growth rates of larvae in the laboratory are often orders of magnitude higher than 'normal' food concentrations in the ocean (Loosanoff and Davis, 1963; Anger and Dawirs, 1981; Lucas, 1982; Crisp et al., 1985; Paulay et al., 1985; Olson and Olson, 1989). Finally, some larvae captured in the field show the morphological characteristics typical of larvae reared on low food rations in the laboratory (Fenaux et al., 1994), suggesting that larvae in the ocean may indeed be food-limited.

The temporal and spatial patchiness of phytoplankton is thought to impose strong selection on the timing of reproductive events in marine species with feeding larval stages. There is strong evidence that many organisms time their spawning to coincide with phytoplankton blooms (Cushing, 1990; Starr et al., 1990). This synchronicity, when it occurs, is thought to strongly benefit larvae because the availability of food is particularly important in early development. Numerous studies of larvae of fish (Blaxter and Hempel, 1963; May, 1973), crustaceans (Anger and Dawirs, 1981; Anger, 1995) and other taxa (e.g. oysters, His and Seaman, 1992; Laing, 1995) have described a 'critical period' in early larval development that is determined by depletion of the energy reserves in the egg. Larvae that do not feed before this ''point of no return'', or PNR (Hjort, 1914; Blaxter and Hempel, 1963), cannot subsequently recover even when provided with food. The existence and length of a PNR in larval life cycles is particularly important in aquatic systems, because organisms that release larvae that need to feed during times of low planktonic productivity are likely to experience poor recruitment if their larvae cannot survive until feeding conditions improve. Reports on the length of time between fertilization and the PNR vary among different taxa and studies. In fish and in crustaceans, the two groups in which the PNR has been most extensively studied, the interval between fertilization and the PNR ranges from days to weeks and is largely dependent on the initial quantity of energy reserves in the egg and the metabolic rates of larvae (May, 1973; Anger et al., 1981; Anger, 1995).

Far less is known about the PNR in other taxa of marine larvae. The larval biology of *Crassostrea gigas*, the Pacific oyster, has received considerable attention because of this species' tremendous economic importance as one of the most widely harvested aquaculture species (Tacon, 2003). His and Seaman (1992) reported a short PNR interval for *C. gigas*, finding that very few larvae recovered from a greater than 4-day feeding delay after fertilization. This critical dependence on the availability of food early in larval development has been built into models of the dynamics of larval transport and survival to metamorphosis in this species (e.g. Bochenek et al., 2001; Powell et al., 2002). Eggs of *C.*

gigas are relatively small (50–60 μm, Strathmann, 1987), from which it is reasonable to infer that larvae could not survive long solely on endogenous reserves. There is, however, some evidence that soft-bodied marine larvae can survive for very long periods after fertilization even when not provided with particulate food. Manahan (1989) reported that unfed larvae of *C. gigas* in bacteria-free culture were, surprisingly, still actively swimming even 6 weeks after fertilization. The mechanisms by which larvae of planktotrophic species can survive long periods without particulate foods are unknown. Also the physiological 'quality' of recovery after feeding is poorly understood for larvae that undergo periods of prolonged starvation. In this study, we investigated the length of the feeding delay that newly developed larvae of *C. gigas* could experience and yet still survive and grow when subsequently fed. We also explored the physiological and biochemical changes that occurred in unfed larvae to determine whether there was a physiological index for larvae that had reached the PNR for this species. Our results indicate a surprising capacity in larvae of *C. gigas* for growth after feeding delays of weeks. Larvae can also survive in the absence of phytoplankton food sources for periods over four times longer than the predicted lifespan based on utilization of endogenous energy reserves.

2. Methods

2.1. General larval culturing conditions

Adult *C. gigas* were obtained from a commercial hatchery (Taylor Shellfish Farms, WA, USA). Larval cultures were started with gametes stripped from the gonads of females and males. All culturing was performed in pristine, off-shore seawater obtained from the Pacific Ocean off Santa Catalina Island, CA. Seawater was filtered to 0.2-μm pore size. Larvae were cultured at 23 ± 2 °C in large-volume containers (200 l) at 10 larvae ml^{-1}. Cultures were gently aerated to aid mixing.

2.2. Delayed feeding experiments

To test the effects of long-term food deprivation on larvae of *C. gigas* during early development, larvae were maintained in filtered seawater without the addition of phytoplankton foods (herein defined as the "unfed" treatment). Seawater was changed every third day starting at day 2, when the first veligers (D-stage larvae) developed from the fertilized egg. To determine whether larvae could subsequently feed and grow after increasing periods without particulate food, a series of "fed" treatments was started at intervals of 3 days from the onset of feeding ability at day 2 through to day 17. These fed treatments were designated as Fed@2, Fed@5, Fed@8, Fed@11, Fed@14 and Fed@17 (days). The numeral indicates the number of days of delay before food was added to cultures. For each fed treatment, three replicates of 200,000 larvae were removed from the unfed treatment and placed into three 20-l culture vessels (i.e., still maintained at 10 larvae ml^{-1}) and fed with *Isochrysis galbana* at 30,000 cells ml^{-1}. Larvae from each different feeding-delay treatment were sampled periodically for

survivorship, growth, metabolic rate and biochemical constituents (carbohydrate, lipid and protein content).

2.3. Survivorship

At specified sampling intervals, all larvae in a given culture were siphoned onto a 50-μm mesh screen at each water change, resuspended in a known volume and enumerated by taking replicate aliquots of a known volume. Survivorship of larvae in the unfed treatment was estimated in two different ways. First, larvae were counted at each sampling interval and the numbers of surviving larvae corrected for the experimental removal of larvae for other measurements (e.g., setting up the feeding treatments). Second, the numbers of live and dead larvae were enumerated. In this second method, living larvae could be readily distinguished from the empty shells of dead larvae. This ratio of live to dead larvae provided an independent method of determining survivorship that did not rely on calculated adjustments for the large numbers of larvae removed over the course of experiments. These two methods gave similar estimates of survivorship (see Section 3). For the fed treatments, survivorship was measured every 2 days. Survivorship in all fed treatments (Fed@2 to Fed@17) after 8 days of continuous feeding was compared using a one-way analysis of variance (8 days of continuous feeding was the longest feeding interval tested across all treatments).

2.4. Feeding ability

To assess whether larvae retained the ability to capture and digest algal cells after increasing periods of food deprivation, unfed larvae were periodically removed from the 200-l cultures and fed with algae at a concentration of 30,000 cells ml^{-1}. After 24 h, larvae were examined with a compound fluorescence microscope under UV excitation to determine uptake and digestion by visualizing algal cells and pigments in the digestive system. Individual larvae ($n = 20–25$) were categorized based on the extent of algal ingestion and digestion. Individuals were classified as (1) not feeding, with empty guts; (2) ingesting, but not digesting, algal cells, with guts containing intact algal cells that emitted red fluorescence under UV excitation; and (3) ingestion and digestion, where larvae had partially digested cells in their stomach and digestive glands that emitted chlorophyll fluorescence. Feeding ability of larvae from unfed cultures was assessed daily by these methods from day 2 through 23, then on days 26, 28, 29 and 33 (see Fig. 4).

2.5. Growth, metabolism and biochemical analysis

Shell length was measured as the greatest distance across the shell on 25 randomly selected animals from each sampling interval and feeding treatment. Larvae were also measured for protein, lipid and carbohydrate content.

Protein—Known numbers of larvae (1500–5000, depending on treatment and developmental stage) were aliquoted into 1.7-ml microcentrifuge tubes, centrifuged, and the seawater was removed. Samples were frozen at −80 °C and later analyzed with the methods of Holland and Gabbott (1971), using the Bradford protein assay as modified by

Jaeckle and Manahan (1989). Protein content was calculated by dividing the total protein in a sample by the total number of larvae in that sample.

Lipid—Known numbers of larvae were processed as above for protein samples and frozen at −80 °C. For analysis, lipids were extracted using the methanol, chloroform and water extraction method of Bligh and Dyer (1959) as modified by Holland and Gabbott (1971), Holland and Spencer (1973) and further refined by Moran and Manahan (2003). In short, lipids were extracted in 1:1:0.5 (v/v/v) water/methanol/chloroform to which stearyl alcohol had been added as an internal standard. Stearyl alcohol had been previously determined not to interfere with native lipid peaks in oyster larvae (Fig. 1A). Lipid content of larvae of *C. gigas* consisted mainly of phospholipid and triacylglycerol. Cholesterol and hydrocarbon were present in trace amounts and were not included in the biochemical analyses of energy content presented

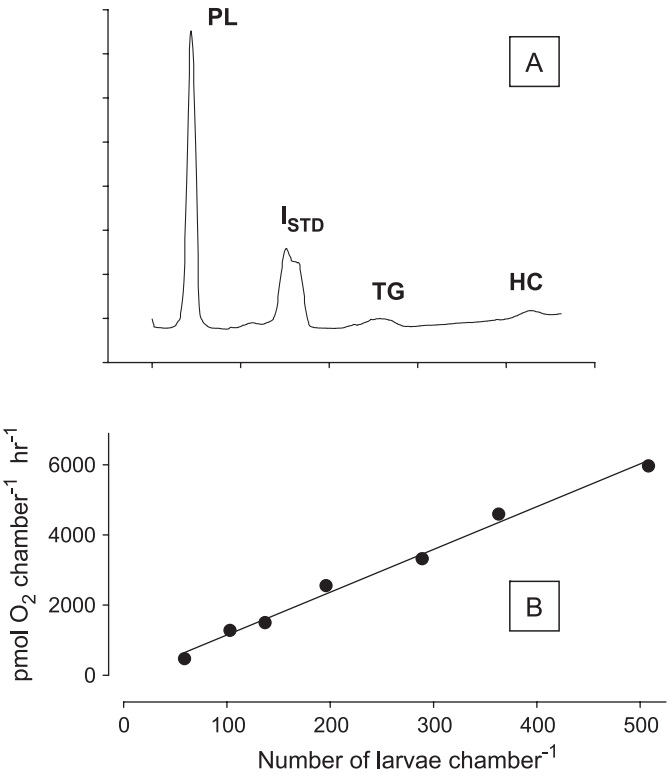

Fig. 1. (A) Chromatograph showing lipid classes in 21-day-old larvae of *C. gigas* fed for 4 days following a 17-day delay of feeding after fertilization. Peak heights are in volts (arbitrary units) on the ordinate axis; units on the abscissa are elution times. PL, phospholipid; I_{STD}, stearyl alcohol internal standard; TG, triacylglycerol; HC, hydrocarbon. (B) Respiration measurement of 12-day-old larvae of *C. gigas* fed for 4 days following an 8-day feeding delay. Each data point represents the oxygen consumed in a single respiration chamber plotted against the number of larvae in that chamber. Respiration of an individual larva is calculated as the slope of the least-squares regression line ($b = 12.2$, $r^2 = 0.99$). Error is calculated as the standard error of the slope (S.E. = 0.51).

here. Phases were separated by the addition of water and chloroform to a final ratio of 1:0.5:0.75 water/methanol/chloroform. To quantify the amounts of each lipid class, three 1-μl replicates of each sample were applied to thin-layer chromatography Chromarods of 1-mm diameter (Iatron Laboratories) with a 1.5-μl gastight syringe (Precision Sampling). Chromarods were developed in a solvent system of 60:6:0.1 hexane/diethyl ether/formic acid to separate major lipid classes, dried and immediately analyzed with an Iatroscan MK-5 flame ionization detector. The Iatroscan was calibrated for each compound class with L-α-phosphatidylcholine (phospholipid), cholesterol, tripalmitin (triacylglycerol), nonadecane (aliphatic hydrocarbon) and stearyl alcohol (fatty alcohol internal standard). Lipid content per sample for each class was calculated as the mean of each of three replicates. Total lipid content was calculated as the sum of major lipid classes per individual.

Carbohydrate—Total carbohydrate content was quantified using the methods of Holland and Gabbott (1971). In brief, eggs and larvae were extracted with cold, 5% trichloroacetic acid and the supernatant was hydrolyzed for two hours at 95 °C in 1 M HCl. Carbohydrates were then spectrophotometrically quantified with a ferricyanate reduction reaction using glucose as a standard. Carbohydrate analyses were performed on 1-day-old trochophores and 11-day-old unfed larvae. Subsequently, 11-day-old unfed larvae were provided with food for 8 and 10 days and were again analyzed for carbohydrate content.

Respiration—Larval respiration was measured at 23 °C using the end-point determination methods of Marsh and Manahan (1999). In brief, larvae were removed from cultures and suspended in 0.2-μm filtered seawater in small (~ 500 μl) respiration chambers. A range of concentrations from 10 to 1000 larvae per respiration chamber was tested (exact number depended on respiratory rates of larvae in a particular treatment). Replicates of seven to eight respiration chambers containing larvae were used for each set of stage-specific respiration measurements. Larvae were incubated for 2–5 h, after which 300-μl subsamples were taken from each chamber with a temperature-equilibrated glass-tight syringe. Oxygen tension was measured in each sample with a polarographic oxygen sensor (Model 1302, Strathkelvin). Larvae were then counted and oxygen consumption per animal was calculated as the slope of the regression line of oxygen consumed per hour against number of larvae in each respiration chamber (Fig. 1B). The error of each estimate was calculated as the standard error around the slope of the regression line.

Citrate synthase activity—Activity of citrate synthase (CS), an enzyme involved in mitochondrial activity, is an indicator of aerobic metabolism (Hochachka and Somero, 1984). The amount of CS was measured in homogenates of larvae of *C. gigas* (Srere, 1969, as modified for measurements of larvae by Marsh et al., 1999). The Q_{10} of the enzyme was 1.7 over the range of temperatures tested (10–30 °C, $n = 10$). Since a histidine buffer (50 mM at pH 8.0) was used for this assay, and that buffer's pH is temperature-dependent, we tested the activity of CS over a pH range (7.8–8.2 at 25 °C) and found no pH effect on enzyme activity over the range tested. Results presented here for CS activity in larvae were from measurements made at 23 ± 1 °C (i.e., the culturing temperature and the temperature at which respiration measurements were made).

3. Results

3.1. Survivorship

Unfed treatment—After an initial decrease between days 2 and 5, survivorship of unfed larvae remained constant to day 14, after which mortality increased (Fig. 2). On day 23, the last day of mortality counts, 15% of larvae were still actively swimming (equivalent to 274,000 D-stage veliger larvae that had survived over 3 weeks of 'starvation'). A similar estimate of 19% survival was obtained by enumeration of empty shells.

Fed cultures—After a range of feeding delays from 2 to 17 days, larvae were fed and their survival determined after 8 days of continuous feeding. There were no significant differences in subsequent survivorship among the different treatments for up to 2 weeks of feeding delay (Fig. 3, days 2–14), although the difference between Fed@2 and Fed@14 was marginally nonsignificant ($p = 0.056$). The only statistically significant decrease in survivorship occurred in larvae 'starved' for 17 days prior to feeding (one-way ANOVA of all six feeding-delay treatment: $df = 5, 12$, $f = 7.91$, $p > 0.002$; followed by a post-hoc Tukey HSD test, $p < 0.05$).

3.2. Feeding capacity

When unfed larvae of 2 to 33 days in age were given algal cells for the first time, close to 100% of the larvae had algal cells in their guts when observed 24 h after first exposure to food (Fig. 4). Unfed larvae showed no trend towards a loss of the ability to capture or ingest algae over the 33-day period of 'starvation'. Likewise, there was no apparent trend towards a loss of the ability to digest algal cells (Fig. 4).

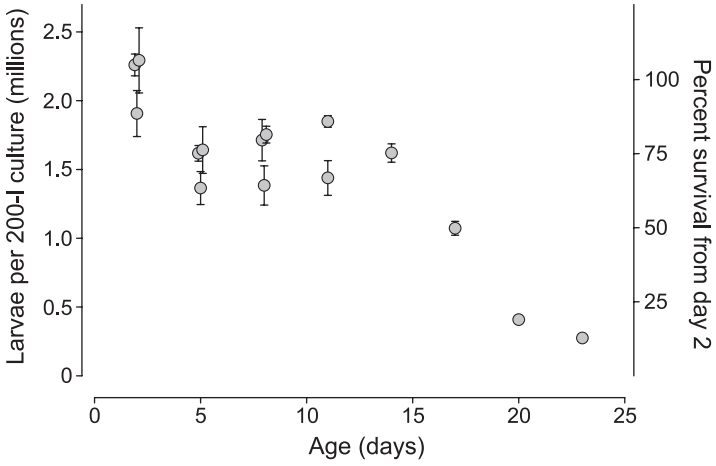

Fig. 2. Survivorship of larvae in the unfed treatments. Data points represent the mean and standard errors of survivorship counts, each from a different 200-l culture. For ease of visual presentation data are also presented as percent survival, where the 100% starting value was calculated from the mean number of larvae initially present in each of the three different 200-l culture vessels at the start of the experiment.

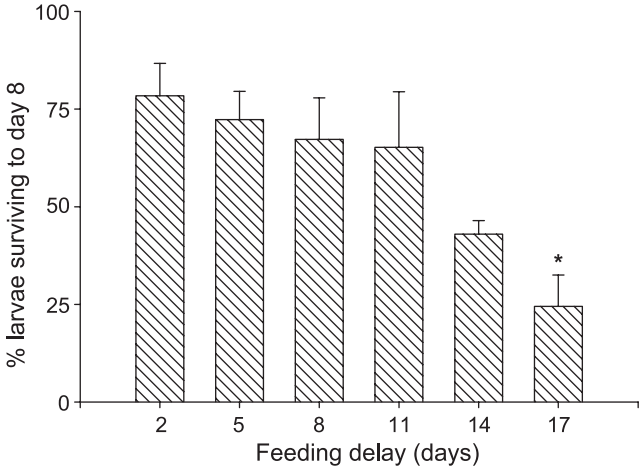

Fig. 3. Percent survivorship of larvae from each feeding-delay treatment (2–17 days) after 8 days of subsequent feeding. Error bars are standard errors. Data are presented as percent survival for ease of graphical presentation; however, ANOVA was performed on the raw count data. Asterisk = day 17 was significantly different from all other treatments at $p < 0.05$.

3.3. Growth, respiration and biochemical analysis

Shell length—Fig. 5 shows the ability of larvae to grow when provided with algal food following increasing periods of feeding delay. In all fed treatments, shell length (growth) increased with time and there was no evident decrease in shell growth rate with increased feeding delay. For instance, the growth of larvae fed after a 2-day feeding delay was

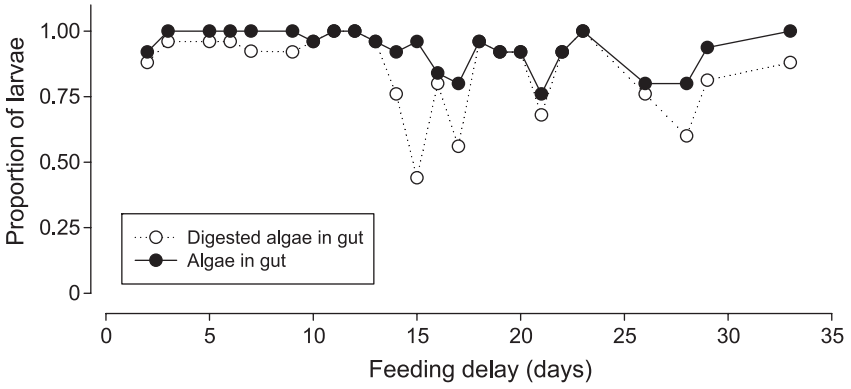

Fig. 4. Ability of unfed larvae to capture and digest algal cells over increasing numbers of days of 'starvation'. Black circles represent proportion of larvae with algal cells in their guts 24 h after exposure to *Isochrysis galbani* at 30,000 cells ml^{-1}. White circles represent proportion of larvae that were digesting algal cells after the same interval measured by chlorophyll fluorescence (see text for details).

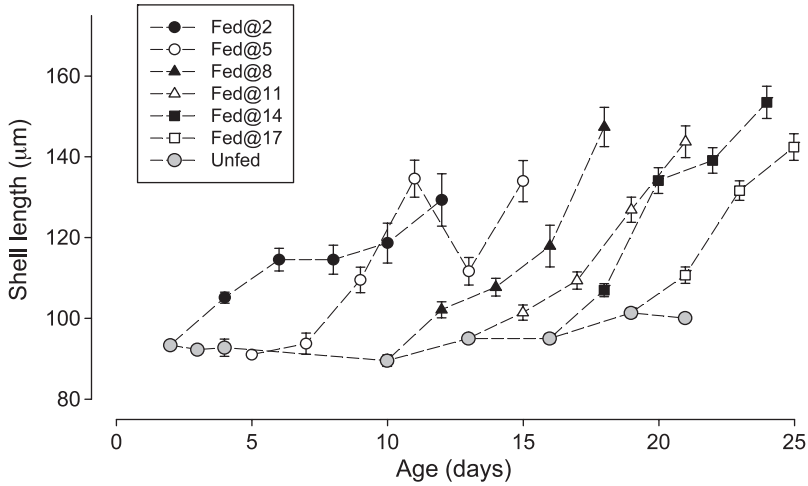

Fig. 5. Shell lengths of unfed larvae and larvae that were fed algae, following a range of feeding delays from 2 to 17 days. Each point represents the mean larval shell length ($n = 25$). Error bars are standard errors. The lower value on the ordinate axis is set at 80 μm, near the size of a newly formed D-veliger larva.

similar to that following a 17-day feeding delay (Fig. 5). The unfed larvae, acting as a control, showed no substantial increase in size over the 21 days tested.

Oxygen consumption—The respiration rates of unfed larvae remained relatively low and constant for the 25 days of measurements (Fig. 6). In all cases where larvae were fed following various feeding delays, respiration rates substantially increased as larvae grew

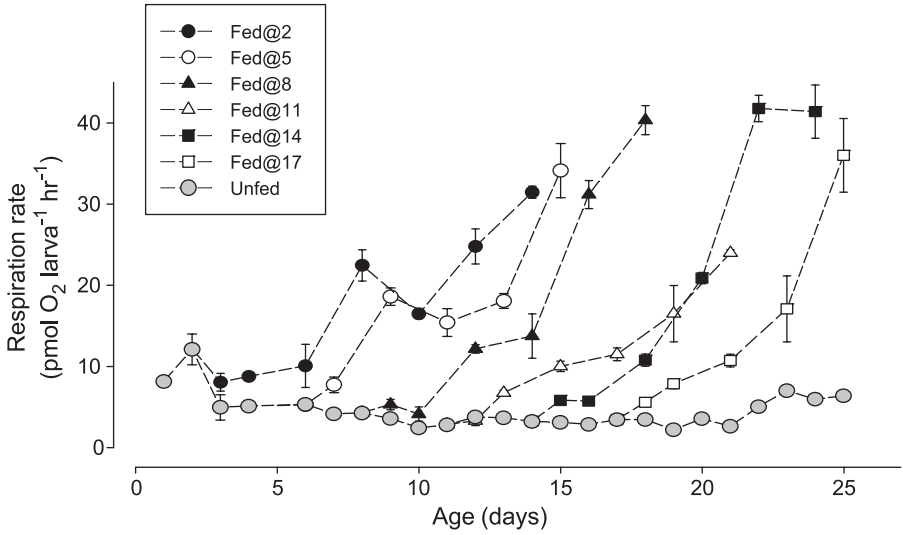

Fig. 6. Respiration rates of unfed larvae and larvae that were fed algae after a range of feeding delays from 2 to 17 days. Error bars are standard errors of the estimate of respiration rate per individual larva (see Fig. 1B).

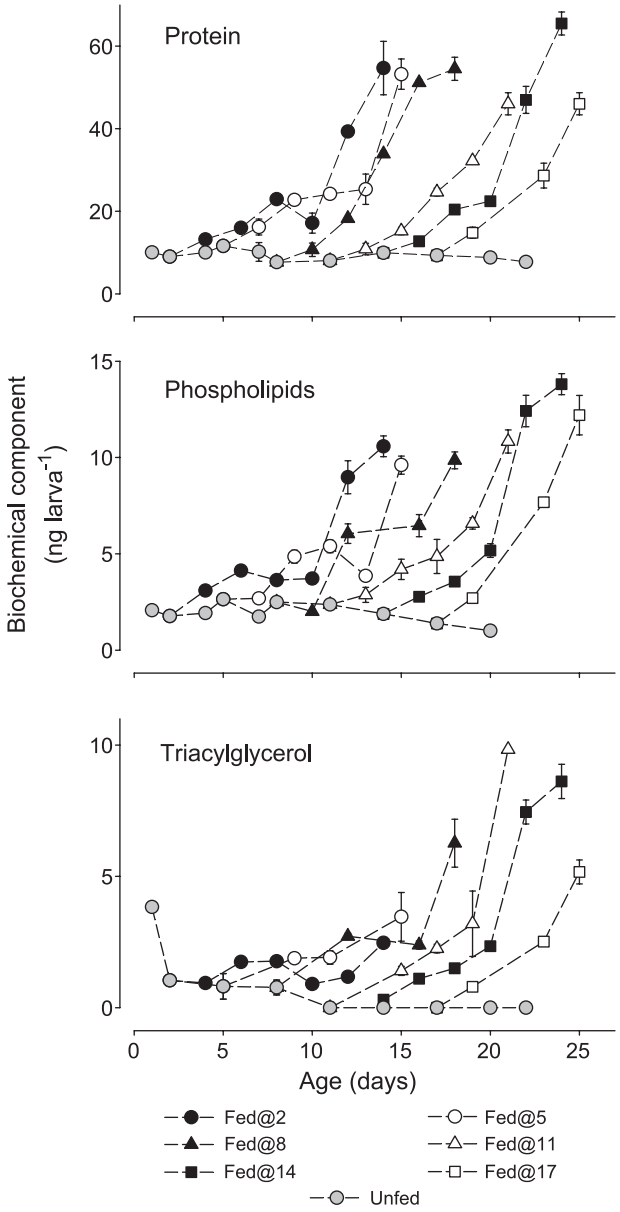

Fig. 7. Protein, phospholipid and triacylglycerol content of unfed larvae and larvae that were fed algae following a range of feeding delays from 2 to 17 days. Each point represents the mean and standard error ($n = 3$ and 4 independent samples for lipid and protein, respectively).

(cf. growth data in Fig. 5). Again, there was no obvious effect of increasing feeding delay on the subsequent physiological performance of fed larvae.

Biochemical analysis—The protein, phospholipid and triacylglycerol contents of unfed larvae and of larvae from all the different feeding-delay treatments are given in Fig. 7. In

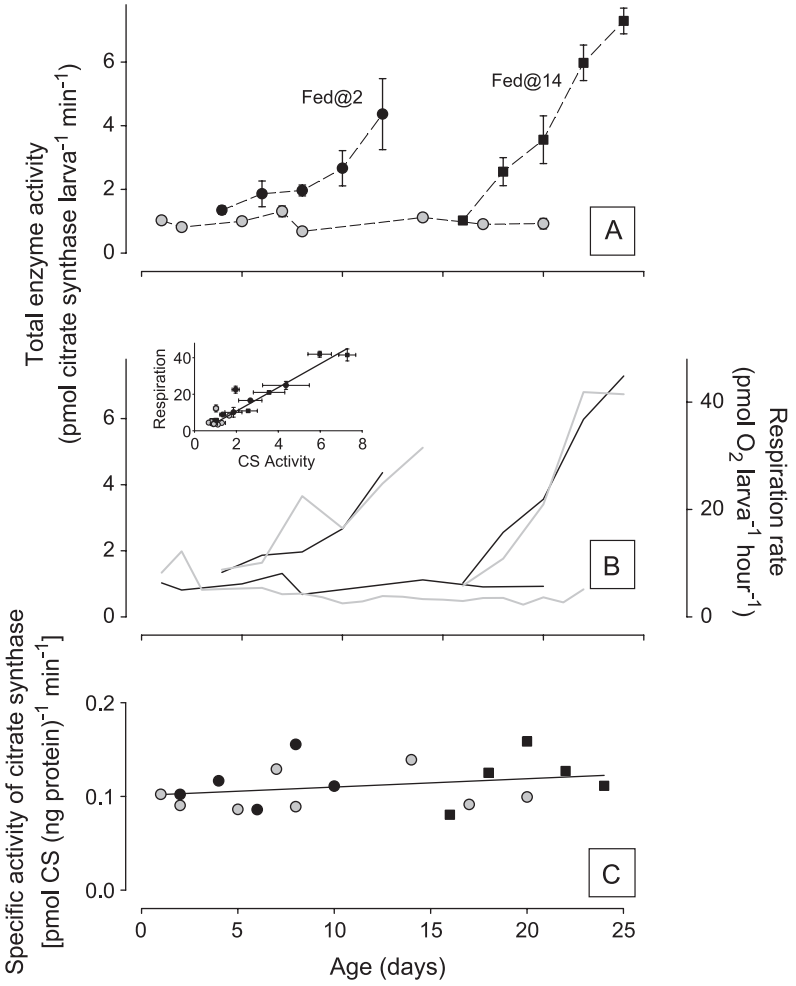

Fig. 8. (A) Total enzyme activity of CS in larvae of *C. gigas* from three treatments: unfed (grey circles); delay of 2 days prior to being fed algae (Fed@2, black circles) and delay of 14 days prior to feeding (Fed@14, squares). Each point represents the mean and standard errors for $n = 3$ independent samples. Measurements of CS for larvae in the fed treatments began 48 h after the onset of feeding (i.e., day 4 for Fed@2 and day 16 for Fed@14). (B) Comparison between total CS activity and respiration rate as a function of feeding treatment (defined in A). Black line shows the same data as A; grey line represents per-larva O_2 consumption for the same larval cohorts (data from Fig. 6). The inset shows the close relationship between total CS activity and per-larva O_2 consumption for treatments in Figure B (linear regression: $y = 6.5x - 2.2$, $r^2 = 0.95$). (C) Protein-specific CS activity for the same feeding treatments (symbols as in A). There was no significant relationship between protein-specific CS activity and age when fed and unfed larvae were pooled ($df = 1, 17$, $F = 2.07$, $p = 0.17$).

early-stage larvae of *C. gigas* (trochophore, 1 day old), protein is the dominant biochemical component, being present in higher amounts than phospholipid or triacylglycerol. Carbohydrate was present in low amounts in all samples tested. On a per-larva basis, 1-day-old larvae had 0.7 ± 0.1 ng carbohydrate and 11-day-old unfed larvae had 0.3 ± 0.1 ng carbohydrate. Larvae fed for 8 days contained 3.4 ± 1.2 ng carbohydrate and larvae fed for 10 days had 4.6 ± 0.3 ng carbohydrate. These amounts or carbohydrate were small, equivalent to 4.2%, 2.8%, 7.5% and 6.5% of the sum of measured constituents (lipids, proteins and carbohydrates) of each group, respectively.

The protein content of unfed larvae did not change over the 3 weeks tested (Fig. 7, top graph). By analysis of variance, the slope of the change in protein content with age was not significantly different from zero (variance ratio = 2.68, $F_{0.05\ (1,\ 39)} = 5.42$). Phospholipid content did not change significantly in unfed larvae over the 20-day period of measurement (variance ratio = 4.16, $F_{0.05\ (1,8)} = 7.57$). Triacylglycerol content decreased rapidly from the trochophore stage (day 1) to the veliger stage (day 2), and then had a steady decline to below analytical detection limits by day 11 (shown as zero points on the graph). In all fed cultures, protein, phospholipid and triacylglycerol content each increased steadily over time after initiation of feeding (Fig. 7). There was no detrimental effect of delayed feeding on increases in biochemical constituents, even after 17 days of 'starvation'.

Citrate synthase activity—The total amount of citrate synthase remained low in unfed larvae (Fig. 8A). Once larvae were fed after a 2- and 14-day feeding delay, the amount of enzyme increased, e.g., for the treatment with a 14-day delay in feeding, citrate synthase activity increased eight-fold after feeding, indicating an increase in mitochondria as growth proceeded. The change in oxygen consumption with and without food mirrored the corresponding changes in citrate synthase activities (Fig. 8B and B inset). This increase in the amount of enzyme corresponded to an increase in total protein content, as the protein-specific activity of citrate synthase remained constant, independent of age or feeding history (Fig. 8C).

4. Discussion

Survival of larvae is one of the most important factors determining recruitment into adult populations of marine organisms, and the availability of food to planktotrophic larvae is critical for survival. The concept of a 'critical period' during early development, in which food must be available soon after larvae become capable of feeding, has received considerable attention (Hjort, 1914; Blaxter and Hempel, 1963; Starr et al., 1990; His and Seaman, 1992; Bochenek et al., 2001; Platt et al., 2003). The relationships among egg size, energy content and larval life history strategies have also been studied extensively (Vance, 1973; Caswell, 1981; Strathmann, 1985; Hart, 1995; McEdward, 1997; Levitan, 2000). Less attention has been focused, however, on the physiological capacity of planktotrophic larval forms of marine invertebrates to resist 'starvation'. Such larval forms often develop from small eggs with low energy content (Vance, 1973; Jaeckle, 1995) and are presumed to be highly dependent on exogenous foods soon after the first feeding stage is reached (Anger, 1987; Starr et al., 1990; His and Seaman, 1992; Bochenek et al., 2001). These

models use the assumption that prior to the onset of feeding, or in the absence of particulate food sources such as algae or prey items, larvae behave as 'closed' metabolic systems that are predominantly fuelled by endogenous energy reserves rather than energetic inputs from the environment.

In our study, larvae of *C. gigas* survived for extended periods after fertilization without having access to algal foods. Larval survivorship decreased between days 2 and 5, but then remained constant until day 14 with 75% of larvae still alive (Fig. 2). Even after 33 days of 'starvation', many larvae were still swimming actively and could both capture and digest algal cells (Fig. 4). At this point, cultures were discontinued, but there was no visible indication that 'starved' 33-day-old larvae were close to death. Not only did unfed larvae survive and retain feeding abilities (Fig. 4), but, even after a 17-day feeding delay, larvae also retained full physiological capacities to increase in size (Fig. 5) and biochemical components (Fig. 7). Likewise, after a similarly long feeding delay, larvae showed increases in metabolic rate when fed (Fig. 6) and corresponding increases in citrate synthase activity that were equal to or greater than increases seen in larvae fed early in development, at day 2 (Fig. 8A). The strong relationship between oxygen consumption and citrate synthase activity across treatments (Fig. 8B) suggests that larval metabolic rate is directly related to mitochondrial density. The protein-specific activity of citrate synthase is also independent of age and feeding history (Fig. 8C), which in turn suggests that mitochondrial division rates were not adversely affected by prolonged feeding delays. Clearly, these larvae are not fully dependent upon their endogenous reserves to sustain maintenance metabolism in the absence of algal foods. Herein, we refer to larvae under these conditions as being 'unfed' rather than truly 'starving'.

To determine how long endogenous reserves could theoretically sustain larvae of *C. gigas*, we calculated an energy budget for unfed larvae using the energy equivalents of protein, lipid and carbohydrate content of 1-day-old trochophore larvae as an estimate of initial larval energy reserves. We used the biochemical composition of 1-day-old larvae rather than egg values because the larvae used in these experiments were started from eggs that were 'stripped' from ripe gonads of adult oysters. Such stripped egg samples are likely to be contaminated with immature oocytes and adult tissue; thus, it is difficult to obtain an accurate estimate of the actual endogenous materials available in eggs obtained in this manner. One-day-old larvae of *C. gigas* contained 10.0 ± 0.7 ng protein, 3.8 ± 0.1 ng triacylglycerol, 2.1 ± 0.1 ng phospholipid (Fig. 7) and 0.7 ± 0.1 ng carbohydrate (data for carbohydrate not shown on figure). Converting these amounts to energy equivalents with values of 24.0, 39.5 and 17.5 kJ g^{-1} for protein lipid, and carbohydrate respectively (values obtained from Gnaiger, 1983) yields a total energy content of 485 μJ $larva^{-1}$ that could potentially be used to fuel metabolic requirements.

We calculated the cumulative cost of metabolism for a larva of a particular age from the summed daily oxygen consumption from days 1 to 25 (day 25 was the last day on which respiration rates were measured in these experiments). By summing the daily energetic costs of metabolism, we estimate that larvae exceeded their entire maternal energetic endowment of 485 μJ $larva^{-1}$ between days 7 and 8, a small fraction of their observed lifespan (Fig. 9: calculation based on protein and lipid oxidation at 484 kJ mol^{-1} O_2). Our measurements of biochemical constituents showed that unfed larvae were not in fact using their entire initial endogenous energy reserves of 485 μJ. For example by day 14, protein

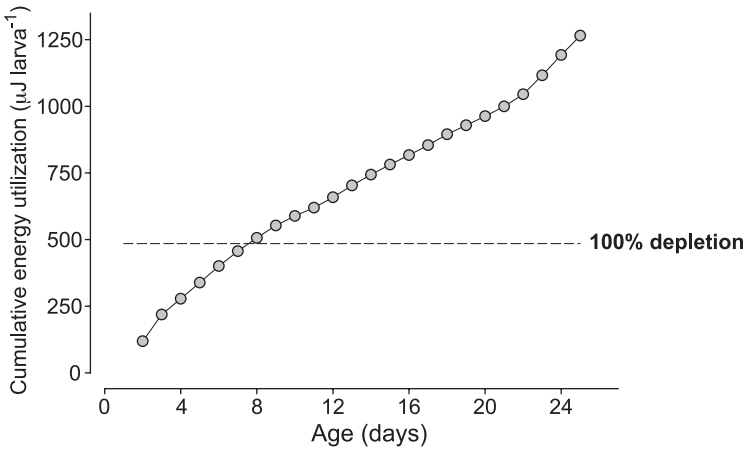

Fig. 9. Cumulative energy use plotted against age for unfed larvae. Cumulative energy was calculated using average respiration rates over 24-h periods and an oxyenthalpic conversion factor of 484 kJ (mol O_2)$^{-1}$. Dashed line represents the 100% depletion of endogenous reserves of protein, lipid and carbohydrate present in 1-day-old larvae if all of larval metabolism was fuelled by endogenous reserves (i.e., larvae were 'starving' and had no input of any exogenous energy).

and phospholipid (Fig. 7; 9.9 ± 0.3 and 1.9 ± 0.2 ng larva^{-1}, respectively) had not decreased from the initial content of 1-day-old larvae, while by day 11 triacylglycerol decreased to undetectable levels (< 0.5 ng larva^{-1}). Thus, the energy potentially available to larvae from the depletion of endogenous reserves came only from the neutral lipid fraction and was equivalent to only 150 μJ larva^{-1} over 11 days assuming complete depletion of triacylglycerol (i.e., 3.8 ng lipid at 39.5 kJ g^{-1} = 150 μJ).

At the average metabolic rate of 1- and 2-day-old larvae (Fig. 6, mean of 10.2 pmol O_2 larva^{-1} h^{-1}), a total of 108 μJ were expended to reach the D-veliger stage at day 2 (calculation based on lipid oxidation at 441 kJ mol^{-1} O_2, the only reserve depleted). These 108 μJ would theoretically represent 72% of the total energy (150 μJ) available from depletion of neutral lipid reserves initially present in 1-day-old larvae. From the actual measurement of triacylglycerol utilization 3.8 ng was present in 1-day-old larvae and 1.0 ng in 2-day-old larvae (Fig. 7), which represents a 74% depletion. Within experimental error, the theoretical and measured values for depletion of lipid reserves are indistinguishable in early larval development. For later larval stages, however, the maintenance of a constant metabolic rate and the survival of larvae for several weeks after reaching the D-veliger stage cannot be explained from utilization of endogenous reserves.

Some of our energy calculations are in agreement with the general conclusions for larvae of *C. gigas* that endogenous reserves can only last a few days (His and Seaman, 1992; Laing, 1995; Bochenek et al., 2001). Our empirical measurements, however, of subsequent larval survival in the absence of algal foods suggest that these larvae can withstand long periods of 'starvation'. The greater than four-fold difference between the theoretical total utilization of energy reserves by day 8 and the actual survival time observed for larvae (at least 33 days) cannot be explained by considering reasonable propagation of the accumulated errors with the suite of physiological and biochemical

measurements used. Clearly, larvae of *C. gigas* are capable of surviving for several weeks past the theoretical 'point of no return', if this point were calculated only from available energy reserves and respiratory costs in larvae. Utilization of endogenous energy reserves is insufficient to explain larval metabolic costs. The largest energy reserve by mass, protein, was not used by larvae during food deprivation (Fig. 7) (cf. other studies with later, metamorphic stages of *C. gigas*—Haws et al., 1993; Garcia-Esquivel et al., 2002). In our study, the amount of energy available from neutral lipid could only supply a small fraction (~ 12%) of the metabolic costs required for 25 days (1265 μJ larva^{-1}: summing O_2 consumption of unfed larvae from Fig. 6, calculation based on protein and lipid oxidation at 484 kJ (mol O_2)$^{-1}$). In the absence of complete metabolic 'shut-down' (which was not observed—see respiration data, Fig. 6), we conclude that larvae must have exploited other exogenous sources of energy to fuel maintenance metabolism after the first few days of development.

What other sources of energy in seawater might be available to larvae? This has been a matter of much debate over many decades (Loosanoff and Davis, 1963; Olson and Olson, 1989). In our experiments, larvae were maintained in 0.2 μm (pore size) filtered seawater and could not have supplemented their energy needs with phytoplankton or other large detrital material. Larvae might augment their nutrition with alternative sources of energy such as dissolved organic material in seawater (Stephens, 1988; Manahan, 1990; Gomme, 2001) or bacteria (Douillet, 1993a,b; Gallager et al., 1994). In nature, the relative importance of different sources of potential nutrition for larvae is not clear. In part, this is because of the lack of detailed information on the fine-scale patchiness of dissolved and particulate sources of food on the microenvironmental scales that are relevant to larvae in the ocean. In our laboratory experiments, the guts of unfed larvae always appeared empty under light and fluorescence microscopy. With the larval mortality that occurred in the culture vessels, populations of bacteria or heterotrophic protists may have grown in the larval cultures between water changes and might have provided a possible food source. Larvae of *C. gigas* are capable of ingesting and assimilating carbon from some strains of bacteria (Baldwin and Newell, 1991; Douillet, 1993a,b; Douillet and Langdon, 1993), and at high bacterial concentrations the carbon input from bacterivory is theoretically sufficient to meet the metabolic needs of small larvae (Douillet, 1993b). Likewise, larvae of the closely related species *C. virginica* readily consumed heterotrophic ciliates and flagellates in the laboratory (Baldwin and Newell, 1991).

We did not measure the concentrations of bacteria or heterotrophic protists in our cultures. However, microscopic observations of all unfed cultures on all days revealed that protists occurred on only one sampling date (day 14) and were at low abundance (<0.5 ml^{-1}). The frequent water changes, the clarity and filtration of the offshore seawater used in the unfed cultures and the empty-appearing guts of unfed larvae, all suggest that concentrations of bacteria and heterotrophic protists were not likely to be present in sufficient amounts to entirely support larval metabolism. The observations of Manahan (1989) that unfed larvae of *C. gigas* reared in bacteria-free culture were actively swimming 6 weeks after fertilization further support the suggestion that larvae can survive in the absence of bacterial or heterotrophic protistan food sources.

Another possible source of energy for larvae in unfed cultures is dissolved organic material (DOM) in seawater. Uptake of DOM from seawater has been documented for

many species of marine invertebrates (Stephens, 1988; Gomme, 2001) and DOM has been suggested as a potentially important source of energy for larval forms (Manahan, 1990), including larvae of *C. gigas* (Manahan and Crisp, 1982; Manahan, 1983, 1989). The ability of larvae of *C. gigas* in our study to maintain maintenance metabolism for long periods in the absence of phytoplankton foods (Fig. 6), while not fully using their endogenous energy reserves (Fig. 7), suggests that DOM might supply an important supplementary source of energy. Under conditions of below-sustenance concentrations of particulate foods in nature, DOM could assist in maintaining the viability of marine invertebrate larvae past theoretical points of no return, pending an improvement in environmental food availability.

In contrast to growth and metabolism, survivorship of larvae of *C. gigas* was negatively affected by feeding delay. Larval mortality was significantly higher in fed cultures that had experienced the longest feeding delay (Fig. 3; see treatment Fed@17) than in cultures that received food earlier (Fed@2 to Fed@14). The highest mortality in larvae that were fed after 17 days occurred in the first few days post-feeding, suggesting that while many larvae were fully capable of recovering, others could not take advantage of food after this prolonged feeding delay. This suggests the presence of variation in the ability of larvae to survive feeding delays, which may be related to genotype. Genotype-dependent variation in larval performance (measured as growth rate and protein deposition) has been documented in *C. gigas* (Hedgecock et al., 1996). It is possible that all newly developed veliger larvae could feed and grow if immediately provided with food, whereas after the physiological stress of a prolonged feeding delay, some larvae with 'inferior' genotypes would be unable to recover. The genetic underpinnings of performance are receiving attention in adults and larvae of *C. gigas* (see Hedgecock et al., 1995, 1996), making this an excellent species for future studies on the interrelationships of genetics, environment and physiological performance of larvae.

Thorson (1950) commented that given the high metabolic needs of larvae and the perception of limited food availability in the oceans, ''it might be expected that most pelagic larvae living under natural conditions would starve and perish''. Later studies, based on better methods of measurement decades later, still supported that view. Crisp et al. (1985, p. 773) stated that their results from studies on bivalve larvae ''reinforce the paradox that food levels in the sea seem insufficient to support optimal growth, or indeed any growth at all''. In support of these arguments, small animals such as larvae generally have greater metabolic demands than large animals due to high mass-specific metabolic rates (e.g., Zeuthen, 1953; Widdows, 1991); likewise, there is considerable support for the long-standing view that the amount of phytoplankton in the ocean is notoriously patchy (Mackas et al., 1985; Villafane et al., 1995) and, based on laboratory experiments (e.g., Paulay et al., 1985; Olson and Olson, 1989), is rarely present at concentrations necessary to sustain maximum larval growth. Low phytoplankton availability is a particularly important issue for planktotrophic species in low-productivity environments such as the tropics (Lucas, 1982) and the deep sea, and in strongly seasonal environments such as the polar oceans (Clarke, 1983, 1992; Pearse et al., 1991). Yet, larvae of soft-bodied marine invertebrates raised in natural (unfiltered and frequently changed) seawater do not starve (Fenaux et al., 1994). Numerous studies have failed to find evidence for poor nutritional condition in field-captured larvae (reviewed by Olson and Olson, 1989), although field-

captured larvae may exhibit morphological indicators of food-limited growth (Fenaux et al., 1994). The resolution to this apparent paradox may lie in the ability of these organisms to utilize other, alternative sources of nutrition to fuel maintenance metabolism in the absence of phytoplankton foods.

Our study demonstrates that larvae of *C. gigas* have the capacity to survive without particulate phytoplankton food for much longer periods than previously supposed (see His and Seaman, 1992) or than energetic modeling would predict (Fig. 9). When larvae that had experienced long (up to 17 day) feeding delays were provided with algal foods, they were able to immediately up-regulate growth and metabolism at rates equivalent to larvae that experienced no feeding delay (Figs. 5–8). This ability is not limited to feeding larval stages of *C. gigas*. Shilling and Manahan (1990) estimated that, based on biochemical constituents in the egg and measured larval metabolic rates, larvae of the planktotrophic echinoid *Strongylocentrotus purpuratus* would utilize their entire egg reserves in under 14 days if all larval metabolic needs were met endogenously. Preliminary experiments we conducted on larvae of *S. purpuratus* showed that when larvae were first fed (the alga *Rhodomonas lens*) after a feeding delay of 22 days after fertilization, they were still able to grow and reach metamorphosis (data not shown).

This ability of larvae of *C. gigas* and other species to tolerate complete deprivation of phytoplankton for long periods suggests that variability in phytoplankton production may not pose as difficult an ecological quandary for some planktotrophic marine species as has been supposed previously. During periods of low phytoplankton availability, larvae of *C. gigas*, and likely other species as well, have the ability to sustain maintenance metabolism on other external sources of energy and to commence feeding and growth when food becomes available. Hence, the presence of a "point of no return" in larval development is likely to be strongly influenced by (1) the extent to which larvae can exploit alternative nutritional pathways such as bacterivory, omnivory and DOM uptake; and (2) the quantity and character of these materials in the larval environment.

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References

Anger, K., 1987. The D_O threshold: a critical point in the larval development of decapod crustaceans. J. Exp. Mar. Biol. Ecol. 108, 15–30.

Anger, K., 1995. Starvation resistance in larvae of a semiterrestrial crab, *Sesarma curacaoense* (Decapoda: Grapsidae). J. Exp. Mar. Biol. Ecol. 187, 161–174.

Anger, K., Dawirs, R.R., 1981. Influence of starvation on the larval development of *Hyas areneus* (Decapoda: Majidae). Helgol. Meeresunters. 34, 287–311.

Anger, K., Dawirs, R.R., Anger, V., Costlow, J.D., 1981. Effects of early starvation periods on zoeal development of brachyuran crabs. Biol. Bull. 161, 199–212.

Baldwin, B.S., Newell, R.I.E., 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 78, 285–301.

Blaxter, J.H.S., Hempel, G., 1963. The influence of egg size on herring larvae (*Clupea harengus* L.). J. Cons.-Cons. Int. Explor. Mer 28, 211–240.

Bligh, E.G., Dyer, W.F., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bochenek, E.A., Klinck, J.M., Powell, E.N., Hofmann, E.E., 2001. A biochemically based model of the growth and development of *Crassostrea gigas* larvae. J. Shellfish Res. 20, 243–265.

Caswell, H., 1981. The evolution of "mixed" life histories in marine invertebrates and elsewhere. Am. Nat. 117, 529–536.

Clarke, A., 1983. Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr. Mar. Biol. Annu. Rev. 21, 341–453.

Clarke, A., 1992. Reproduction in the cold: Thorson revisited. Invertebr. Reprod. Dev. 22, 175–184.

Crisp, D.J., Yule, A.B., White, K.N., 1985. Feeding by oyster larvae—the functional-response, energy budget and a comparison with mussel larvae. J. Mar. Biol. Assoc. U.K. 65, 759–783.

Cushing, D.H., 1990. Plankton production and year-class strength in fish populations—an update of the match–mismatch hypothesis. Adv. Mar. Biol. 26, 249–293.

Douillet, P.A., 1993a. Bacterivory in Pacific oyster *Crassostrea gigas* larvae. Mar. Ecol. Prog. Ser. 98, 123–134.

Douillet, P.A., 1993b. Carbon contribution through bacterivory in larvae of the Pacific oyster *Crassostrea gigas*. Mar. Ecol. Prog. Ser. 102, 303–314.

Douillet, P.A., Langdon, C.J., 1993. Effects of marine bacteria on the culture of axenic oyster *Crassostrea gigas* (Thunberg) larvae. Biol. Bull. 184, 36–51.

Erlich, K.F., Blaxter, J.H.S., Pemberton, R., 1976. Morphological and histological changes during the growth and starvation of herring and plaice larvae. Mar. Biol. 35, 105–118.

Fenaux, L., Strathmann, M.F., Strathmann, R.R., 1994. Five tests of food-limited growth of larvae in coastal waters by comparison of rates of development and form of echinoplutei. Limnol. Oceanogr. 39, 84–98.

Gallager, S.M., Waterbury, J.B., Stoecker, D.K., 1994. Utilization of the marine cyanobacterium *Synechococcus* sp. by larvae of the bivalve *Mercenaria mercenaria*. Mar. Biol. 119, 251–259.

Garcia-Esquivel, Z., Bricelj, V., Felbeck, H., 2002. Metabolic depression and whole-body response to enforced starvation by *Crassostrea gigas* postlarvae. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 133, 63–77.

Gnaiger, E., 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger, E., Forstner, H. (Eds.), Polarographic Oxygen Sensors: Aquatic and Physiological Applications. Springer-Verlag, New York, NY, pp. 337–345.

Gomme, J., 2001. Transport of exogenous organic substances by invertebrate integuments: the field revisited. J. Exp. Zool. 289, 254–265.

Hart, M.W., 1995. What are the costs of small egg size for a marine invertebrate with feeding planktonic larvae? Am. Nat. 146, 415–426.

Haws, M.C., DiMichele, L., Hand, S.C., 1993. Biochemical changes and mortality during metamorphosis of the Eastern oyster, *Crassostrea virginica*, and the Pacific oyster, *Crassostrea gigas*. Mol. Mar. Biol. Biotechnol. 2, 207–217.

Hedgecock, D., McGoldrick, D.J., Bayne, B.L., 1995. Hybrid vigor in Pacific oysters: an experimental approach using crosses among inbred lines. Aquaculture 137, 285–298.

Hedgecock, D., McGoldrick, D.J., Manahan, D.T., Vavra, J., Appelmans, N., Bayne, B.L., 1996. Quantitative and molecular genetic analyses of heterosis in marine bivalve molluscs. J. Exp. Mar. Biol. Ecol. 203, 49–59.

His, E., Seaman, M.N.L., 1992. Effects of temporary starvation on the survival, and on subsequent feeding and growth, of oyster (*Crassostrea gigas*) larvae. Mar. Biol. 114, 277–279.

Hjort, J., 1914. Fluctuations in the great fisheries of northern Europe. J. Rapp. Conserv. Explor. Mer 20, 1–228.
Hochachka, P.W., Somero, G.N., 1984. Biochemical Adaptation. Princeton Univ. Press, Princeton, NJ.
Holland, D.L., Gabbott, P.A., 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. J. Mar. Biol. Assoc. U.K. 51, 659–668.
Holland, D.L., Spencer, B.E., 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L., during larval development, metamorphosis and early spat growth. J. Mar. Biol. Assoc. U.K. 53, 287–298.
Jaeckle, W.B., 1995. Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward, L.R. (Ed.), Ecology of Marine Invertebrate Larvae. CRC Press, Boca Raton, FL, pp. 49–78.
Jaeckle, W.B., Manahan, D.T., 1989. Growth and energy imbalance during the development of a lecithotrophic molluscan larva (*Haliotis rufescens*). Biol. Bull. 177, 237–246.
Laing, E., 1995. The lipid content, spatfall and subsequent growth of early and late settling hatchery-reared Pacific oyster, *Crassostrea gigas* Thunberg, larvae. Aquac. Res. 29, 19–25.
Levitan, D.R., 2000. Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. Am. Nat. 156, 175–192.
Loosanoff, V.L., Davis, H.C., 1963. Rearing of bivalve mollusks. In: Russell, F.S. (Ed.), Advances in Marine Biology, vol. 1. Academic Press, London, pp. 1–136.
Lucas, J.S., 1982. Quantitative studies of feeding and nutrition during larval development of the coral reef asteroid *Acanthaster planci* (L.). J. Exp. Mar. Biol. Ecol. 65, 173–193.
Mackas, D.L., Denman, K., Abbott, M.R., 1985. Plankton patchiness: biology in the physical vernacular. Bull. Mar. Sci. 37, 652–674.
Manahan, D.T., 1983. The uptake and metabolism of dissolved amino acids by bivalve larvae. Biol. Bull. 164, 236–250.
Manahan, D.T., 1989. Amino acid fluxes to and from seawater in axenic veliger larvae of a bivalve (*Crassostrea gigas*). Mar. Ecol. Prog. Ser. 53, 247–255.
Manahan, D.T., 1990. Adaptations by invertebrate larvae for nutrient acquisition from seawater. Am. Zool. 30, 147–160.
Manahan, D.T., Crisp, D.J., 1982. The role of dissolved organic material in the nutrition of pelagic larvae: amino-acid uptake by bivalve veligers. Am. Zool. 22, 635–646.
Marsh, A.G., Manahan, D.T., 1999. A method for accurate measurements of the respiration rates of marine invertebrate embryos and larvae. Mar. Ecol. Prog. Ser. 184, 1–10.
Marsh, A.G., Leong, P., Manahan, D.T., 1999. Energy metabolism during embryonic development and larval growth of an Antarctic sea urchin. J. Exp. Biol. 202, 2041–2050.
May, R.C., 1973. Larval mortality in marine fishes and the critical period concept. Proc. Int. Symp. Scot. Mar. Biol., 3–19.
McEdward, L.R., 1997. Reproductive strategies of marine benthic invertebrates revisited: facultative feeding by planktotrophic larvae. Am. Nat. 150, 48–72.
Moran, A.L., Manahan, D.T., 2003. Energy metabolism during larval development of two abalone species, *Haliotis fulgens* and *H. sorenseni*. Biol. Bull. 204, 270–277.
Olson, R.R., Olson, M.H., 1989. Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? Ann. Rev. Ecolog. Syst. 20, 225–248.
Paulay, G., Boring, L., Strathmann, R.R., 1985. Food limited growth and development of larvae: experiments with natural seawater. J. Exp. Mar. Biol. Ecol. 93, 1–10.
Pearse, J.S., McClintock, J.B., Bosch, I., 1991. Reproduction of Antarctic marine invertebrates: tempos, modes and timing. Am. Zool. 31, 65–80.
Pechenik, J.A., 1987. Environmental influences on larval survival and development. In: Pechenik, J.A., Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), General Aspects: Seeking Unity in Diversity: Reproduction of Marine Invertebrates, vol. IX. Blackwell, Palo Alto, CA, pp. 551–608.
Platt, T., Fuentes-Yaco, C., Frank, K.T., 2003. Marine ecology: spring algal bloom and larval fish survival. Nature 423, 398–400.
Powell, E.N., Bochenek, E.A., Klinck, J.M., Hofmann, E.E., 2002. Influence of food quality and quantity on the growth and development of *Crassostrea gigas* larvae: a modeling approach. Aquaculture 210, 89–117.
Roughgarden, J., Gaines, S.D., Pacala, S., 1987. Supply side ecology: the role of physical transport processes.

In: Giller, P., Gee, J. (Eds.), Organization of Communities: Past and Present. Proceedings of the British Ecological Society Symposium, Aberystwyth, Wales. Blackwell, London, pp. 491–518.

Shilling, F.M., Manahan, D.T., 1990. Energetics of early development for the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* and the crustacean *Artemia* sp. Mar. Biol. 106, 119–128.

Srere, P.A., 1969. Citrate synthase. In: Lowenstein, J.M. (Ed.), Methods in Enzymology, Citric Acid Cycle. Academic Press, New York, NY, pp. 3–11.

Starr, M., Himmelman, J.H., Therriault, J.C., 1990. Direct coupling of marine invertebrate spawning with phytoplankton blooms. Science 247, 1070–1074.

Stephens, G.C., 1988. Epidermal amino acid transport in marine invertebrates. Biochim. Biophys. Acta 947, 113–138.

Strathmann, R.R., 1985. Feeding and non-feeding larval development and life-history evolution in marine invertebrates. Ann. Rev. Ecolog. Syst. 16, 339–361.

Strathmann, M.F., 1987. Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast. University of Washington Press, Seattle, 670 pp.

Tacon, A.J., 2003. Aquaculture production trends analysis. Review of the State of World Aquaculture. Food and Agriculture Organization of the United Nations Fisheries Circular, vol. 886 Rev. 2, pp. 5–29. ftp://ftp.fao.org/docrep/fao/oos/y4490E/y4490E01.pdf.

Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25, 1–45.

Underwood, A.J., Keough, M.J., 2000. Supply side ecology: the nature and consequences of variations in recruitment of intertidal organisms. In: Bertness, M.D., Gaines, S.D., Hay, M. (Eds.), Marine Community Ecology. Sinauer, Sunderland, MA, pp. 183–200.

Vance, R.R., 1973. On reproductive strategies in marine benthic invertebrates. Am. Nat. 107, 339–352.

Villafane, V.E., Helbling, E.W., Hom-Hansen, O., 1995. Spatial and temporal variability of phytoplankton biomass and taxonomic composition around Elephant Island, Antarctica, during the summers of 1990–1993. Mar. Biol. 123, 677–686.

Widdows, J., 1991. Physiological ecology of mussel larvae. Aquaculture 94, 147–163.

Zeuthen, E., 1953. Oxygen uptake as related to body size in organisms. Q. Rev. Biol. 28, 1–12.