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A BIOCHEMICALLY BASED MODEL OF THE GROWTH AND DEVELOPMENT OF *CRASSOSTREA GIGAS* LARVAE

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ABSTRACT A biochemically based model was developed to simulate the growth, development and metamorphosis of larvae of the Pacific oyster, *Crassostrea gigas*. The model is unique in that (1) it defines larvae in terms of their protein, neutral lipid, polar lipid, carbohydrate, and ash content; (2) it tracks weight separately from length to follow larval condition index; and (3) it includes genetic variation in growth efficiency and egg quality to better simulate cohort population dynamics. The model includes parameterizations for larval filtration, ingestion, and respiration, which determine growth rate, and processes controlling larval mortality and metamorphosis. The initial biochemical content of the larva is determined by the composition of the egg. Changes in the initial ratios of protein, carbohydrate, neutral lipid, and polar lipid occur in response to the biochemical composition of available food as the larva grows. Modeling the process of metamorphosis requires a series of size-based and biochemically based triggers: (1) larvae become potentially competent to metamorphose at 275 μm, following a decrease in filtration rate at 250 μm; (2) larvae become competent to metamorphose when a daily decline in neutral lipid of 25% or more occurs; and (3) larvae metamorphose successfully if neutral lipid stores exceed polar lipid stores. Although based on simple biochemistry, the model succeeds in simulating such basic characteristics of *C. gigas* larval development and metamorphosis as larval life span and size structure at metamorphosis and the influence of egg and food quality and food quantity on survival. These results suggest that simple biochemical constructs may encompass the biochemical transitions most prominent in determining cohort success. Simulations of larval development show that for the smallest larvae, assimilation does not provide adequate resources to explain observed growth, although measured filtration rates would indicate otherwise. Egg lipid stores are needed to sustain the larva. The simulations also identify egg sizes in the range 37–73 μm to be viable, very similar to observations. Egg sizes outside this range are predicted to be nonviable due to lipid deficiencies in early larval life. Similarly, simulations identify upper and lower genetic limits on growth efficiency beyond which larvae cannot acquire sufficient neutral lipid stores to successfully metamorphose. As food supply declines, animals with high growth efficiencies are selected in the simulation. Low-protein food diets are predicted to increase larval survival. High-protein diets result in insufficient carbohydrate and neutral lipid to cover metabolic and storage needs. Thus, the influence of growth efficiency is nonrandomly distributed across egg size and respiration rate and the influence seems to be mediated in part by food quantity and to a larger measure by food quality.

KEY WORDS: *Crassostrea gigas*, larvae, Pacific oyster, growth, development model, biochemical, food

INTRODUCTION

For benthic species that have a planktonic larval phase of their life history, survivability of the larvae is the key determinant of recruitment to the adult population. Thus, much research has gone into identifying factors affecting the growth, development, metamorphosis, and settlement of larvae, especially for species with commercial value, such as the Pacific oyster, *Crassostrea gigas*.

Larvae of *C. gigas* undergo growth and development that is typical of bivalve larvae, with progression through D-shaped, umbo, and pediveliger stages (Fig. 1). The fraction of developmental time spent in each stage is variable and the rate at which the larvae progress through each is affected by local temperature, salinity, and food conditions (Helm & Millican 1977, Malouf & Breese 1977, Nascimento 1980, Gerdes 1983a, His & Maurer 1988, Nell & Holliday 1988; His et al. 1989, Pauley et al. 1988, Arakawa 1990, His & Seaman 1992, Robinson 1992, Thompson & Harrison 1992, Laing 1995, Thompson et al. 1996). The range of fractional development times reported for the small umbo to pediveliger stages (Fig. 1) is a reflection of these effects on larval growth.

Once spawned, the ultimate fate of *C. gigas* larvae is determined by the interaction of a number of factors. The first is the initial biochemical composition of the egg released by the adult, in particular the initial egg lipid content. Studies have shown correlations between egg size, lipid content, and bivalve larval development (Helm et al. 1973, Gallager et al. 1986, Gallager & Mann 1986a, Lee & Heffernan 1991). An implication of these studies is that initial egg lipid content is a determinant of larval survivorship and success at metamorphosis.

A second factor is the ability of the larvae to grow and develop so that total time spent in the plankton is minimized, thereby reducing exposure to mortality from predation. Because spawning pulses by adult populations, in nature, occur at widely variable times throughout the spawning season, larvae experience a widely varying set of environmental conditions. The timing of environmental conditions relative to a particular phase of larval life can greatly affect the total time needed for larvae to complete development (Dekshenieks et al. 1993). The concept of a critical period immediately post-hatch during which many planktonic larvae are particularly susceptible to low food supply is now well established (Cushing & Dickson 1976, Anger et al. 1981, Taggart & Leggett 1987), and the available data for bivalve larvae support the sensitivity of larvae to periods of low food supply (Gallager et al. 1986, His and Seaman 1992, Laing 1995). Studies also suggest that food quality, not just food quantity, is a critical issue (Thompson et al. 1994, 1996). Insufficient dietary lipid, for example, significantly limits larval growth and survival (Helm et al. 1973, Gallager et al. 1986).

A third factor affecting larval survival is the ability to acquire sufficient internal energy stores to successfully complete metamorphosis and set. Studies have shown that larvae can reach the size

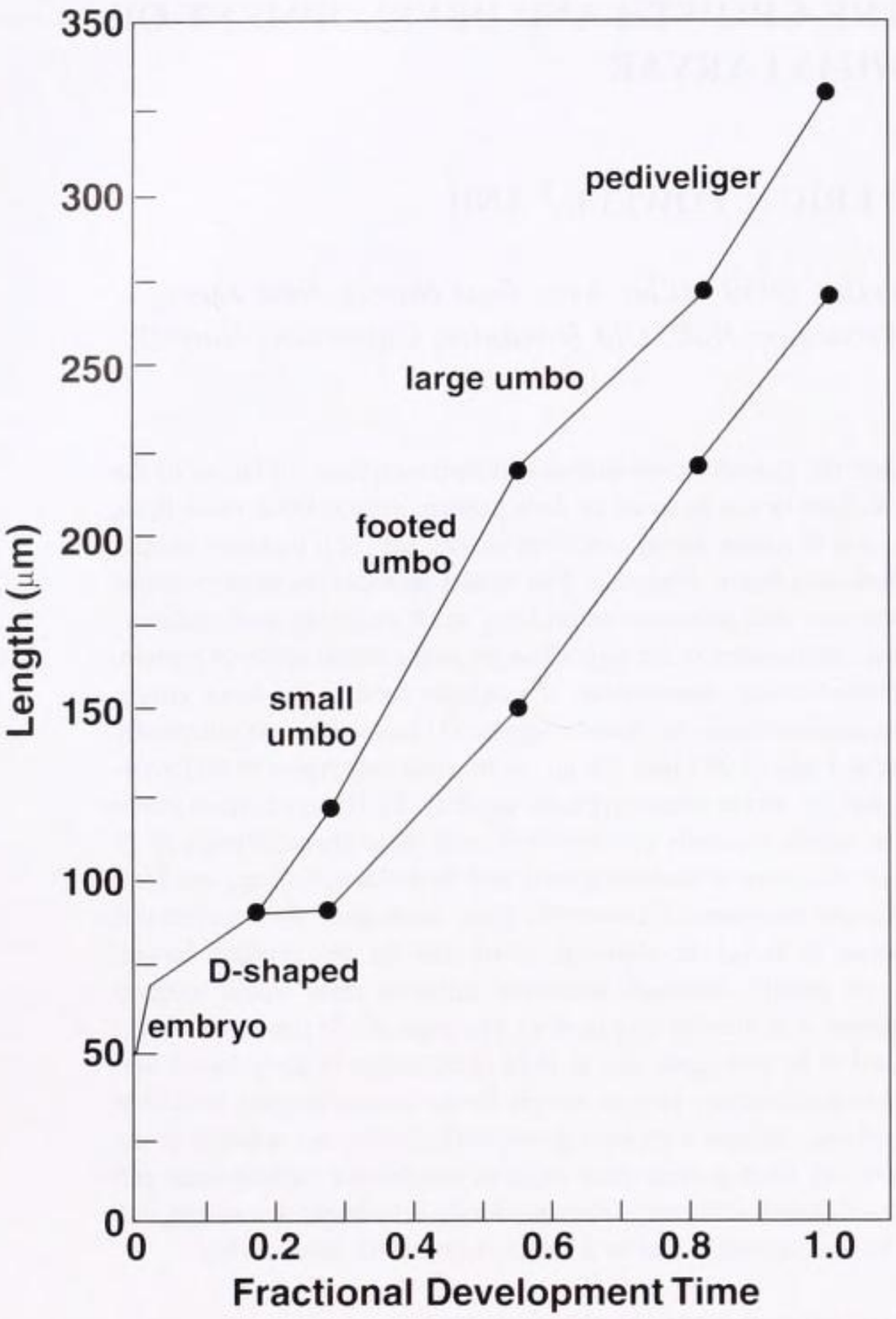


Figure 1. Schematic of the developmental stages of *Crassostrea gigas* larvae as a function of fraction of development time. The two curves bracket the range of sizes for the different stages, as reported in Arakawa (1990).

needed for metamorphosis but be unable to complete this step (Robinson 1992, Haws et al. 1993, Laing 1995). The possibility that a short-term deprivation of food early in larval life may reduce metamorphosis success can be inferred from a variety of studies that emphasize the critical importance of adequate food throughout larval life.

The increasing emphasis on the importance of an adequate diet, including quantity and quality, in determining a larval cohort's success and the recognition that adequate energy resources are needed for successful metamorphosis suggest the need to incorporate biochemical transfers into models of larval growth and development. The existing larval models determine growth from ingestion rate, decremented by the energy losses due to respiration and incomplete digestion (e.g., Carlotti & Sciandra 1989, Dekshenieks et al. 1993, 1996). These types of models, however, cannot be used to examine issues of food quality, nor can they be used to simulate the energy-reserve hypothesis underlying metamorphosis success. To investigate the influences of food quality and quantity on growth, development, and successful metamorphosis of *C. gigas* larvae, a biochemically based model was developed that includes explicit parameterizations for the metabolism of protein, carbohydrate, polar lipid, and neutral lipid within the standard parameterizations of energy flow via ingestion, assimilation, and respiration.

The following section provides a description of the *C. gigas* larval model and the parameterizations used in this model. This is followed by simulations that illustrate the effect of initial egg size, food quality, food quantity, and environmental conditions on larval growth, survival, and success at metamorphosis. The discussion section places these simulations within the context of the current understanding of the effect of environmental conditions and food quality on larval growth, survival, and metamorphosis.

MODEL DESCRIPTION

Model Structure and Governing Equation

The change in length for an individual larva over time is given by:

$$\frac{dL}{dt} = \alpha L \tag{1}$$

where L is larval length in μm and α is the rate at which the larva grows and has units of day^{-1}. Larval growth rate (α) is based on formulations that allow differential metabolism of the protein, carbohydrate, and lipid content of the food ingested by the larva. Thus, net production is expressed as the difference between assimilated ingestion (AI) and respiration (R):

$$NP_i = AI_i - R_i \tag{2}$$

where i represents the four basic biochemical components included in the model: protein, polar lipid, neutral lipid, and carbohydrate. An increase in larval length occurs when the sum of the four, $\sum_{n=1}^{4} NP_i$ is positive; when larval condition index is maximal for a given size; and when the restrictions imposed by certain biochemical ratios are simultaneously met. Thus, excess net production, *ENP*, is the basic quantity responsible for larval growth.

The specification of *ENP*, which determines α, is based on filtration, the metabolism of carbohydrates, polar lipids, neutral lipids, and proteins within the larva, and the conversion of the metabolized food into structural components of the larva versus the conversion into storage material. A basic assumption in this model is that the formation of structural components determines the increase in larval length. Material converted into storage components (i.e., neutral lipids) does not result in increased larval length. The conversions and parameterizations used to model these processes are described in the following sections.

The *C. gigas* larval model given by Eq. (1) was solved numerically using a third-order Adams-Bashforth scheme (Canuto et al. 1988) with a time step of 0.1 day.

Model Parameterizations

Observations from field and laboratory studies were used to derive the relationships that describe the processes affecting net production of *C. gigas* larvae. The basic units used in the model are grams, joules, and μm, and these are not necessarily always consistent with the units used for measurements. Thus, the first step in developing the larval model was to obtain conversions that allow the model calculations and output to be consistent with measurements and to be compared with observations. Also, many of the larval processes vary in amplitude or even form with larval size, requiring that relationships used to describe these be size dependent.

Length-to-Dry Tissue Weight Conversion

Numerous field and laboratory data sets exist that can be used to derive a length-to-dry tissue weight relationship for *C. gigas* larvae. However, the reporting of these data sets is quite variable, with some reported in terms of dry tissue weight (Gerdes 1983a) and others reported in terms of whole animal weight (His & Maurer 1988, Nascimento 1980, Waldock & Nascimento 1979). Dry tissue weight is the desired unit for deriving this relationship, so the data sets reported as whole animal weight were multiplied by a factor of 0.25, which corrects for the shell being 75% of the animal weight (His & Maurer 1988). The resultant data set (Fig. 2) was used to obtain the length-to-dry tissue weight relationship:

$$W = aL^b \quad (3)$$

where W is larval weight in ng and L is larval length in μm. The coefficients a and b are given in Table 1. The correspondence between Eq. (1) and the data sets is shown in Figure 2.

Length-to-Ash Weight Conversion

His and Maurer (1988) provide measurements of the percent of total dry weight as a function of larval size. These values were obtained by summing the percent organic matter represented by larval protein, carbohydrate, and lipids that were determined independently. As a comparison, His and Maurer (1988) also determine the percent total dry weight of organic matter by combustion of the whole animal. These data form the basis for developing a relationship to relate larval length to ash weight.

Comparison of the organic matter values determined by the sum of the components and those obtained from the heating method differ by about 5%. The difference was assumed to be due to protein, which is more completely measured by the heating method, so this difference was added to the protein value for a given size larva. The percent total dry weight for the largest larval size measured by His and Maurer (1988), 274 μm, was reduced from 29.1% to 26.2% because attempts at using the higher value to produce a relationship between larval length and ash weight gave unrealistic results. The reduction in percent total dry weight was obtained by maintaining the same proportional difference in the heating and sum of component determinations of percent dry weight as reported for other larval sizes. The rationale for the reduction in value is that the measured value included effects of metamorphosis that are not explicitly included in the larval model. The percent total dry weight for the 274 μm larva was also applied to the measured dry weight (7000 ng) for 320 μm larvae to provide an additional data point at the larger size.

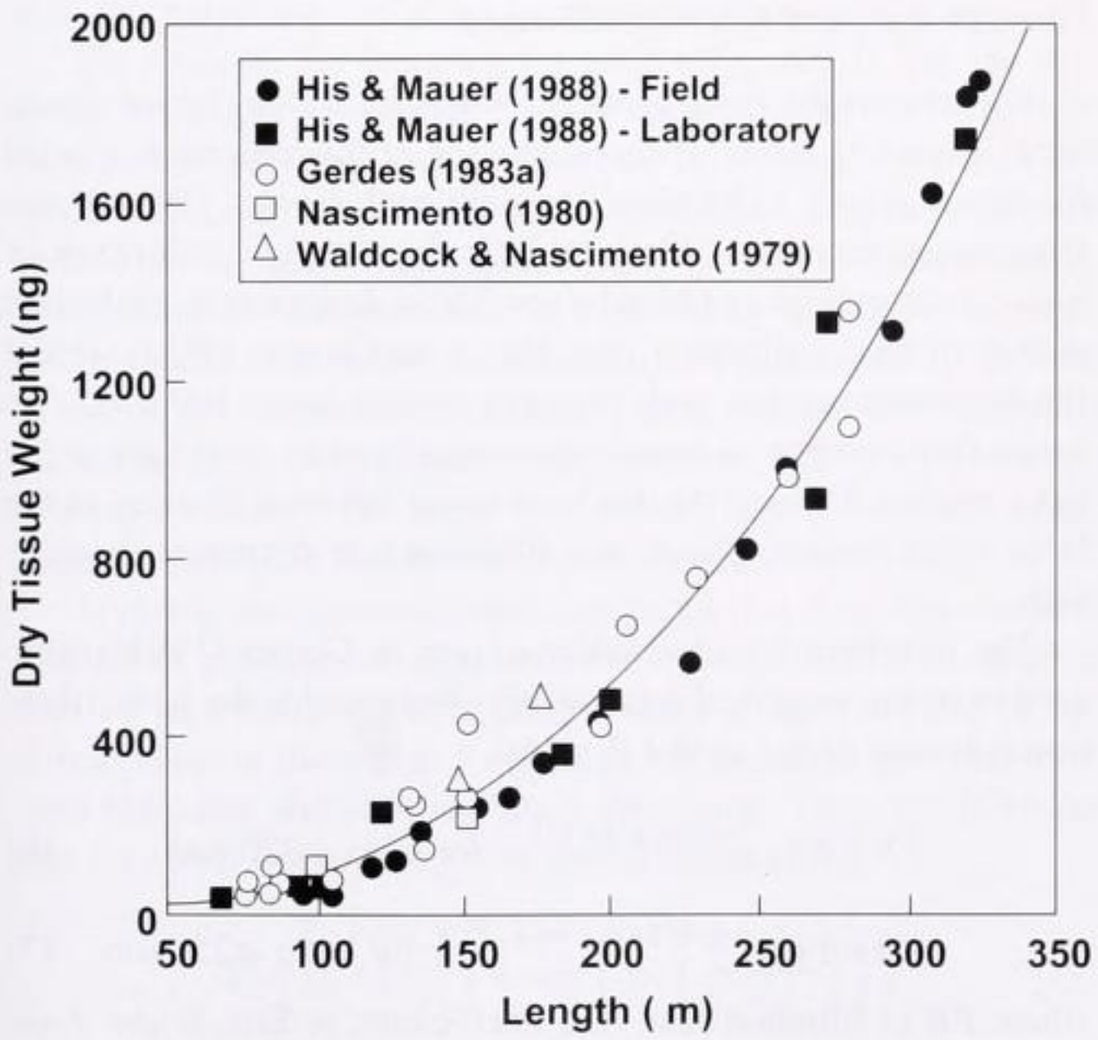


Figure 2. Dry tissue weight as a function of *Crassostrea gigas* larval length.

TABLE 1.

Definition, units, and values for the variables used in the *Crassostrea gigas* larva model equations.

Variable	Definition	Units	Value
a	constant	ng	6.745×10^{-4}
b	constant	none	2.557
c	constant	ng larva^{-1}	3.931×10^{-3}
d	constant	none	2.440
a_1	constant	none	0.3436
a_2	constant	μm^{-1}	–0.00347
a_3	constant	μm^{-2}	2.0323×10^{-5}
a_4	constant	μm^{-3}	-3.1107×10^{-8}
FR_o	constant	1 day^{-1}	24×10^{-6}
b_1	constant	none	–2.0909
b_2	constant	μm^{-1}	0.0335
b_3	constant	μm^{-2}	-3.2798×10^{-5}
c_1	constant	none	96.9948
c_2	constant	μm^{-1}	–1.1011
c_3	constant	μm^{-2}	4.3×10^{-3}
c_4	constant	μm^{-3}	-5.5985×10^{-6}
γ	constant	none	0.0025
α	constant	none	0.35
β	constant	none	0.08
L_o	initial larva length	μm	calculated
L_f	larva length	μm	100
δ	constant	none	0.5
L_s	larva length	μm	0.5
λ	maximum neutral lipid usage	% day^{-1}	75.
SL	upper larval size for neutral lipid use	μm	80.
r_o	base respiration rate	KJ day^{-1}	calculated
θ	constant	none	0.96
m_o	daily mortality rate	day^{-1}	
ES_o	central egg size	μm	50
$Resp_o$	central respiration rate	KJ day^{-1}	1.046
$2sd_{egg}$	standard deviation, egg size	μm	0.2
$2sd_{resp}$	standard deviation, respiration rate	KJ day^{-1}	0.2

The length-to-ash weight data set was fit with a regression of the form

$$AW = cL^d \quad (4)$$

where AW is larval ash weight in ng larva^{-1} and the coefficients c and d are given in Table 1. The correspondence between the data and fitted curve is shown in Figure 3.

Length-to-Protein-to-Ash Ratio Conversion

The protein values for the different sized larva reported in His and Maurer (1988) were divided by the corresponding larval ash

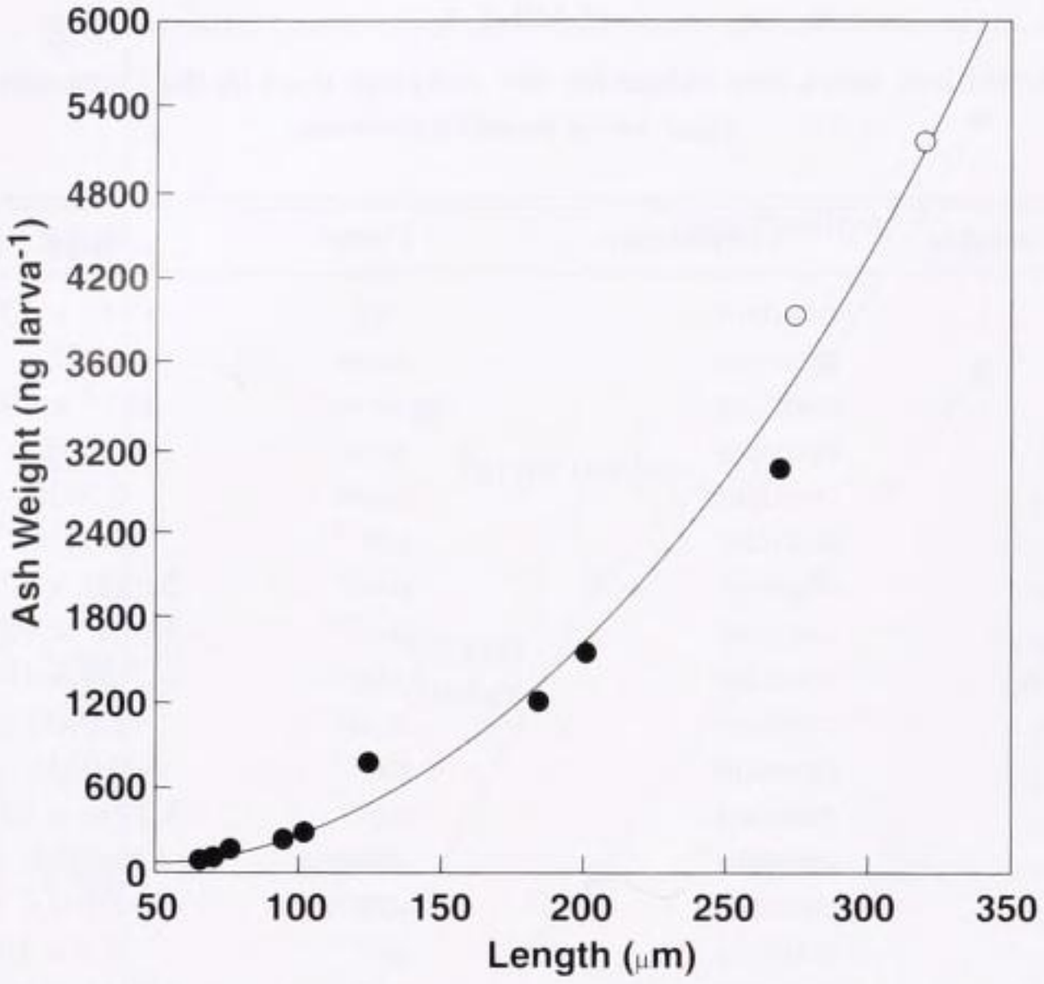


Figure 3. Ash weight as a function of *Crassostrea gigas* larval length. The solid circles are measurements given in His and Maurer (1988). The open circles represent measurements given in His and Maurer (1988) that were modified as described in the text.

weight values, determined as described in the previous section, to obtain protein-to-ash ratios as a function of larval size (Fig. 4). The curve fit to these data is of the form:

$$PAR = a_1 + a_2L + a_3L^2 + a_4L^3 \quad (5)$$

where *PAR* is the protein-to-ash ratio and the coefficient values are given in Table 1. This relationship describes a ratio that decreases from a value of 0.27 to a minimum of 0.18 at a larval size of about 100 μm, after which it again increases. The initial decrease is associated with the development of shell. The increase in the ratio as the larva gets larger reflects the decrease in the surface-to-volume ratio of the larva as it grows.

Carbohydrate, Protein, Polar Lipid, and Neutral Lipid Conversions

In the model, conversions between carbohydrate, lipid, and protein were needed to account for the debiting of the biochemical pools to cover the metabolic cost of respiration and the movement of carbon between different tissue types. The needed conversions are of two types. Those for respiratory demands are expressed in terms of energy (e.g., joules); those for tissue conversions are expressed in terms of moles carbon.

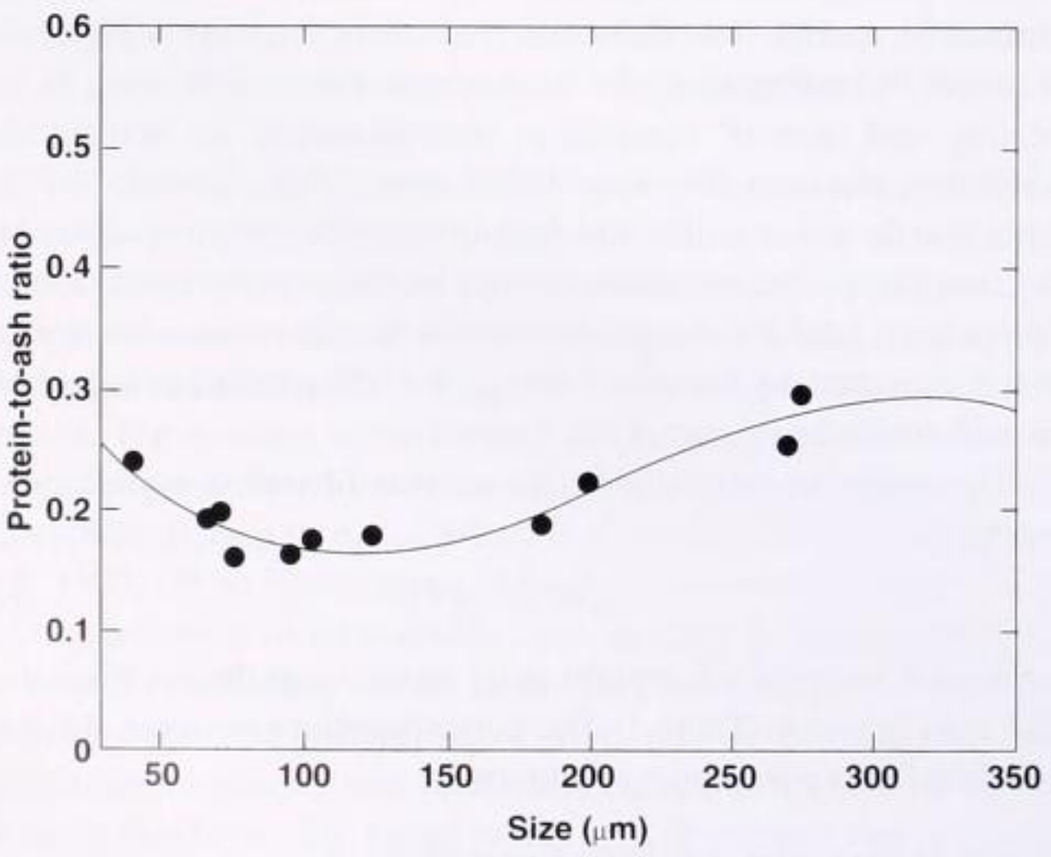


Figure 4. Protein-to-ash ratio as a function of *Crassostrea gigas* larval length. The solid circles are the ratios calculated from the protein and ash values given in His and Maurer (1988).

Respiratory demands were converted to equivalent energy values using 39.5 kJ g^{-1} for lipids, 23.6 kJ g^{-1} for protein, and 17.2 kJ g^{-1} for carbohydrates. These conversions are based on data given in Mann and Gallager (1985), Trytek and Allen (1980), and Crisp (1971).

Interconversion of biochemical constituents was based on carbon equivalent weights using palmitic acid, serine, and glucose residues, respectively, to define the ratio between moles carbon and molecular weight. In this way, carbon atoms were conserved, but the total constituent weight changed depending upon the contribution of carbon to the molar weight. So, for example, the conversion of tissue carbohydrate to tissue lipid is based on a ratio of 1.929, which expresses the relative weight of lipid and carbohydrate based on the equivalency of carbon atoms.

In addition, certain weight ratios between structural constituents were associated with healthy larvae. When in sufficient quantity, assimilated food constituents were allocated to tissue pools using these ratios. Deviations in the resulting tissue composition from these ratios resulted in mortality. The relationship between tissue lipid and protein was obtained from His and Maurer (1988), under the assumption that the total lipid content of *C. gigas* larvae, like *C. virginica* larvae, is about evenly split between neutral and polar lipids (Gallager et al. 1986, Whyte et al. 1987). This yielded a preferred tissue polar lipid-to-tissue protein ratio of 0.11 for healthy larvae. Information from the same sources was used to establish the equivalent ratio between tissue carbohydrate, most of which was assumed to be structural since neutral lipid is the primary storage constituent, and tissue protein. The value of the tissue carbohydrate-to-tissue protein ratio was set at 0.01. Both ratios were independent of larval size.

Parameterization of Processes

Filtration Rate and Filtration Efficiency

Filtration is the basic process by which *C. gigas* larvae obtain food. Therefore, accurate representation of this process is needed for larval growth to be correctly simulated. Gerdes (1983a) presents measurements of filtration rates for *C. gigas* at a range of food concentrations and larval sizes. These data show three distinct phases of larval filtration (see Fig. 8 in Gerdes 1983a). Initial filtration rates are low until the larva reaches about 100 μm, after which filtration rate increases exponentially to its maximum as the larva reaches 250 μm. At this size, larval behavior changes as the larva nears metamorphosis and filtration rate decreases dramatically.

The filtration rate observations given in Gerdes (1983a) were used to derive empirical relationships that provide the basic filtration structure of the model (Fig. 5):

$$FR = FR_o\, e^{(b_1+b_2 L+b_3 L^2)}, \quad \text{for larva} \leq 250\ \mu\text{m} \quad (6)$$

$$FR = FR_o e^{(c_1+c_2 L+c_3 L^2+c_4 L^3)}, \quad \text{for larva} \geq 250\ \mu\text{m} \quad (7)$$

where *FR* is filtration rate. The coefficients in Eqs. 6 and 7 are given in Table 1.

Initial simulations that used the above relationships for larval

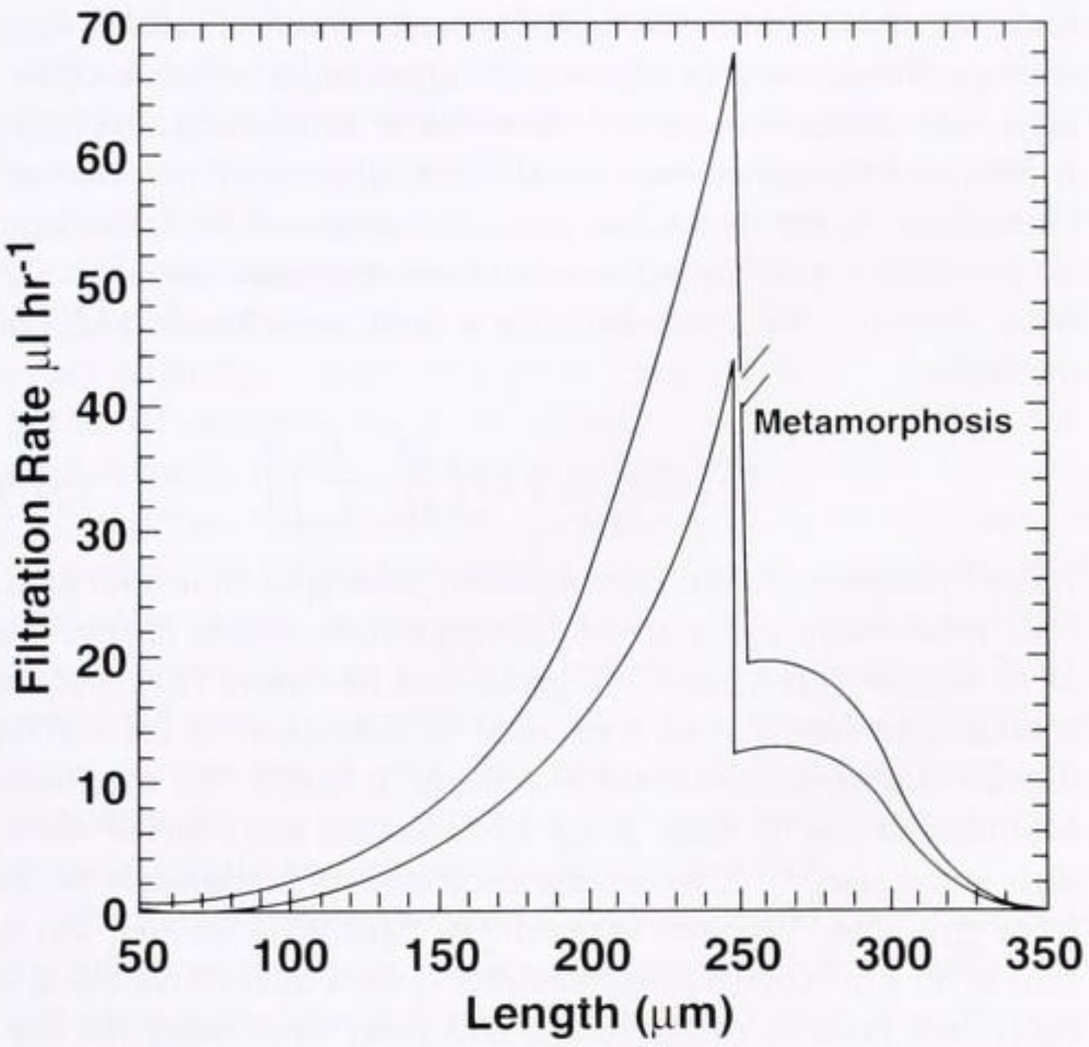


Figure 5. Upper curve, filtration rate as a function of *Crassostrea gigas* larval length calculated from Eqs. 6 and 7 as described in the text. Lower curve: filtration rate after corrections described by Eqs. 8 and 9 (for food concentration of 2 mg L^{-1}).

filtration rate resulted in growth rates that were too high. Reduction of the growth rate was accomplished by adding a factor to the filtration rate that reduces ingestion efficiency. This factor is of the form:

$$IE = \frac{\gamma}{Food}\left[\alpha + \beta\left(\frac{L - L_o}{L_f}\right)\right] \tag{8}$$

where ingestion efficiency (IE) is a nondimensional quantity that depends on the ambient food concentration ($Food$) and larval size. Coefficient values are given in Table 1. The relationship given by Eq. 8 results in reduced feeding efficiency for all larval sizes, but with the maximum reduction associated with smaller larvae.

The rationale for reducing filtration efficiency is that the vellum is a multipurpose organ, so there must be some inefficiency in each of the activities and functions of this organ; otherwise the larvae could only swim at the rate that allows maximum ingestion and dispersal, and escape capabilities would be compromised. Thus, filtration rates measured in a laboratory setting for *C. gigas* larvae should be regarded as measures of vellum activity and not as measures of ingestion. In addition, most research has been conducted using saturating food concentrations. This would exacerbate any tendency for more food to be filtered from the water column than could be ingested by the larva.

Applying the ingestion efficiency factor (Eq. 8) to Eqs. 6 and 7 resulted in realistic larval growth rates, except for larvae smaller than 80 µm. Early in larval life the rapid changes leading to the development of the organs for feeding and digestion should further limit ingestion and/or assimilation efficiency. Thus, the filtration rate for small larvae (FR_s) was further reduced by:

$$IE_s = IE \cdot \delta \cdot \left(1 + min\left(1., \left(\frac{L - L_o}{L_s - L_o}\right)\right)\right) \tag{9}$$

The above approach is based on the assumption that the vellum in small larvae is very inefficient at capturing food particles and/or that digestion is less efficient in small larvae. Coefficient values and definitions are given in Table 1.

A relationship between low food supply and increased feeding efficiency due to enlargement of the vellum (Strathmann et al. 1993) was not included in the model because the influence of vellum enlargement on filtration rate is unknown. The model may underestimate growth rate at low food supply.

Temperature and Salinity Effects

As with most bivalve larvae, the metabolic processes controlling growth in *C. gigas* larvae are affected by temperature and salinity (Lee & Lee 1968, Helm & Millican 1977, Ventilla 1984). His et al. (1989) provide measurements of larval growth rate for salinities between 20‰ and 35‰ and temperatures between 15°C and 30°C. These measurements start with larvae with a mean shell length of 57 µm, which is prior to the development of the D-shaped stage (Fig. 1), and extend through the first seven days of larval growth. The differences in the measured larval growth, for the fed larvae, between day 0 and day 7 were calculated for each temperature and salinity. The resultant values were linearly interpolated to obtain growth rates at intermediate temperature and salinity values. Normalizing this matrix of growth rates to the growth rate at 25°C and 30‰ provides the fractional change in larval growth rate at a given temperature and salinity (Fig. 6).

The measured growth rates given in His et al. (1989) were extended to the entire range of temperature encountered by *C. gigas* larvae by assuming that larval growth rate decreases in a linear fashion to zero between 15°C and 0°C and to zero between 30°C and 35°C. This pattern in growth rate is based on observations of increased larval abnormalities in these temperature ranges (Arakawa 1990). Similarly, for salinity, the growth rate at 20‰ was linearly reduced to zero at 10‰, after which growth rate remained zero. This was based on observations of increased larval abnormalities at low salinities (Arakawa 1990). For salinities above 35‰, larval growth rate at 40‰ was assumed to be one-half

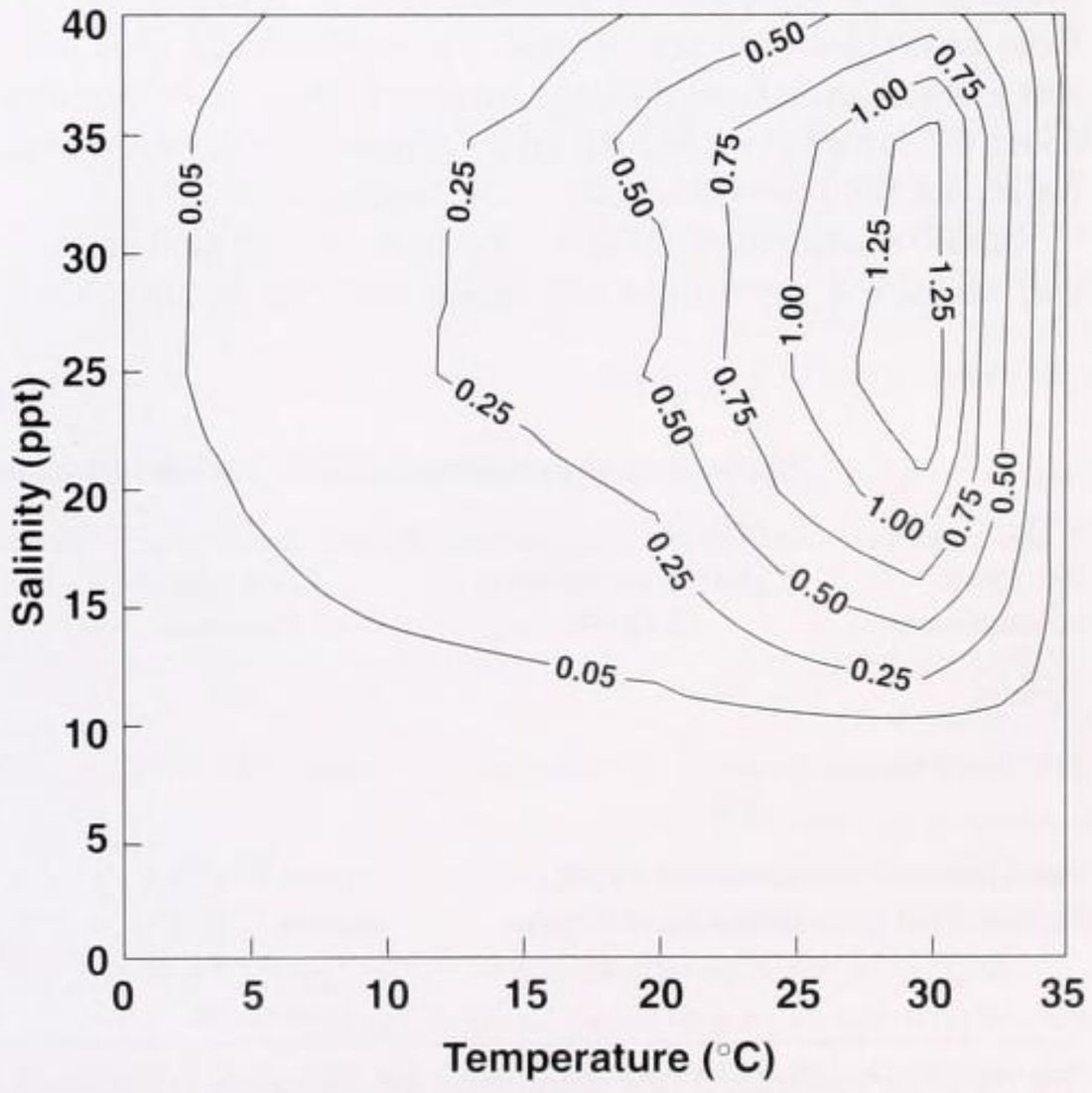


Figure 6. Fractional change in *Crassostrea gigas* larval growth rate as a function of temperature and salinity.

the larval growth rate at 35‰, and growth rates at intermediate salinities were obtained by linear interpolation. The assumption of reduced growth rate at 40‰ is based on measurements (Nell & Holiday 1988) that show *C. gigas* growth rate at 39‰ being one-half the value at 35‰.

The fractional change in larval growth over the entire range of temperature and salinity was verified by comparing simulated larval length with reported larval lengths measured at known temperatures and salinities (Helm & Millican 1977, Nell & Holiday 1988, His et al. 1989, Robinson 1992). These comparisons showed excellent agreement between simulated and observed larval length, except for lengths between 150 and 200 μm. At these sizes, mismatches of 10 to 45 μm occurred for the higher temperature and salinity values. Larvae of this size are growing rapidly at salinities above 24‰ and temperatures above 25°C, and hence the growth curves are steep. Therefore, slight mismatches in the reporting of measured length for a given larval age can greatly affect comparisons with simulated lengths.

Food Composition and Assimilation Efficiency

The *C. gigas* larval model allows for differential metabolism of protein, carbohydrate, polar lipid, and neutral lipid. For this to occur, the food ingested by the larva must be expressed in terms of the relative contribution of each of these biochemical constituents. Based upon measurements reported in Utting (1986), Roman (1983), and Lee et al. (1971), the average biochemical composition of marine algae, in terms of ash-free dry weight, was taken to be 3 parts protein, 2.5 parts carbohydrate, 0.6 parts polar lipid, and 0.4 parts neutral lipid. This basic structure defines the food reservoir for the larvae, for most simulations.

Handa (1969) provides assimilation efficiencies for plant material of 1.0 for protein, 1.0 for polar and neutral lipids, and 0.2 for carbohydrates. The reduced assimilation efficiency for carbohydrates arises because 80% of plant carbohydrate is structural or β-linked carbohydrate (e.g., the refractory portion) that cannot be digested by animals and is therefore not available as food. The available 20% represents labile carbohydrate. Multiplication of these assimilation efficiencies with the corresponding food fraction gives an overall assimilation efficiency for *C. gigas* larvae of about 0.7, which is within the range expected for bivalve larvae (estimated from growth efficiency by Jørgensen 1952).

Initial simulations showed that the above assimilation efficiencies resulted in growth rates for larvae less than 80 μm, which were too small. Observations show a drawdown of neutral lipid reserves during early larval life in *C. gigas* and *C. virginica* (Gallager et al. 1986, Gallager and Mann 1986a, Whyte et al. 1987, His & Maurer 1988), presumably to fill the carbon needs not covered by feeding. In the model, the early life stages of the larva were allowed to use neutral lipid stores to form structural material in the body. This was done by calculating a small larva factor (SLF_i) of the form:

$$SLF_i = max\left[0., \lambda\, \Delta t \left(\frac{SL - L}{SL - L_o}\right)\right] \qquad (10)$$

where i indicates protein, carbohydrate, polar lipid, or neutral lipid. This relationship calculates the proportionate length change for larva smaller than 80 μm in a given time increment (Δt), and the neutral lipid reserves are then used in proportion to the carbon requirement needed to sustain the change in length. The maximum neutral lipid that is used, given by λ, occurs when larvae are at their initial size, L_o. This amount decreases proportionately as the larva grows and becomes increasingly capable of feeding, and is zero at 80 μm. The mobilized neutral lipid is then converted into equivalent protein, carbohydrate, and polar lipid using the biochemical conversions given previously. This is the only instance in the model where protein is created *de novo*, rather than being obtained from food.

Thus, the assimilated ingestion (AE_i) can be expressed as the product of the filtration rate (FR), the ingestion efficiency (IE_s), temperature and salinity effects ($TSfactor$), food ($Food_i$), the assimilation efficiency (AE_i), and the small larvae factor (SLF_i) as:

$$AI_i = FR \cdot IE_s \cdot TSfactor \cdot Food_i \cdot AE_i \cdot SLF_i \qquad (11)$$

Fate of Assimilated Ingestion

The assimilated ingestion obtained from Eq. (11) is parameterized in terms of protein, neutral lipid, polar lipid, and carbohydrate, and the fate of each of these biochemical constituents differs within the larva (Table 2). Protein assimilated in a given time interval has, as its primary destination, the somatic protein pool. Protein may also be used to cover a respiratory deficit (discussed below) in accordance with the appropriate protein-to-carbohydrate-to-polar lipid ratio.

The carbohydrate needs of the larva are determined by the amount needed to maintain tissue carbohydrate in its proper proportion and that needed to cover the metabolic process of respira-

TABLE 2.

Destination of assimilated protein, carbohydrate, polar lipid, and neutral lipid in *Crassostrea gigas* larvae.

Food constituent	Primary destination in larva	Food deficit response	Food surplus response	Tissue maintenance deficit	Early life (<80 μm)
Protein	somatic P	NA	NA	respiration (P:C:PL)	NA
Carbohydrate	somatic C & respiration (P:C)	somatic PL (P:PL)	neutral lipid reserve	respiration (P:C:PL)	NA
Polar lipid	somatic PL (P:PL)	somatic C (P:C)	neutral lipid reserve	respiration (P:C:PL)	NA
Neutral lipid	neutral lipid reserves	somatic C (P:C); somatic PL (P:PL) respiration	NA	somatic C (P:C); somatic PL (P:PL); respiration	somatic C; somatic PL; somatic P; respiration

The particular biochemical ratio determining the conversion to individual reservoirs is indicated. Table columns two, three, and four indicate the fate of the food; column five indicates the fate of the tissue. Transfers of food that do not occur in response to deficit or surplus conditions are indicated by NA. Protein, carbohydrate, and polar lipid are indicated by P, C, PL, respectively.

tion (Table 2). Assimilated carbohydrate is the primary means by which larval respiratory needs are met (Table 2). The required somatic carbohydrate is determined so as to maintain the carbohydrate-to-protein ratio (0.01), and this amount is debited from the available assimilated carbohydrate and added to the carbohydrate pool. Excess carbohydrate (food surplus response in Table 2) becomes part of the larval neutral lipid reserve. When tissue imbalances occur (e.g., insufficient polar lipid to meet the tissue compositional requirements of the larvae), somatic carbohydrate is used to maintain larval polar lipid in its proper proportion.

The primary destination of assimilated polar lipid in the larva is the somatic polar lipid pool in accordance with the protein-to-polar lipid ratio (Table 2). Excess assimilated polar lipid goes to the larval neutral lipid pool. When carbohydrate imbalances occur, polar lipid reserves are mobilized to produce somatic carbohydrate in an amount that is consistent with maintaining the protein-to-carbohydrate ratio. Polar lipids are also used to cover tissue maintenance deficits arising from respiratory demands.

The primary destination of assimilated neutral lipid is the neutral lipid pool (Table 2). This internal pool is mobilized to maintain somatic carbohydrate and somatic polar lipid pools in accordance with the appropriate ratios when assimilated protein, carbohydrate, and polar lipid are not present in the proper proportions in the food. The neutral lipid pool can also be used to cover respiratory needs during periods of carbohydrate deficit. This pool also provides a means for small larvae, less than 80 μm, to produce somatic carbohydrate, polar lipid, and protein as well as cover respiratory costs early in larval life.

At any point in the development of the larva, the inability to maintain one of the biochemical constituent ratios or the inability to remove a deficit in a biochemical pool results in death of the larva.

Respiration

Respiration provides the only metabolic loss of assimilated energy in the *C. gigas* model. Measurements show that respiration rate for *C. gigas* larvae increases with larval size and with temperature (Fig. 7). Laboratory measurements of respiration rate for *C. gigas* larvae cover a range of larval sizes measured at 25°C (Gerdes 1983b) and 20°C (Hoegh-Guldberg & Manahan 1995) can be described by the relationship:

$$Resp = r_o W^{\theta} \tag{12}$$

where *Resp* is given in mL O_2 consumed ind^{-1} h^{-1} and W is animal dry tissue weight in mg. The base respiration rate, r_o, is assumed to reflect genetic variations in metabolic processes that are known to occur for individual *C. gigas* larvae (e.g., Lannan 1980). Hence, this parameter is specified using a distribution (described in a following section) that is assumed to represent metabolic variability within the larval population. Other coefficients are defined in Table 1. The respiration rates measured at 25°C (Fig. 7) were used along with the fractional changes in growth rate (Fig. 6) to obtain the full range of temperature and salinity effects on larval respiration. Respiration rate was converted to an energy demand using 20.21 J (mL O_2 consumed)$^{-1}$ to determine the metabolic cost of respiration.

Equation (12) provides the metabolic cost of respiration that must be met by the larva. As discussed in the previous section, the assimilated carbohydrate pool provides the first biochemical reservoir that is used to meet this demand (Table 2). This pool is converted to equivalent energy units using the conversions given previously, and the needed carbohydrate is removed from the pool. Any excess is added to the neutral lipid pool in an amount that is consistent with the carbohydrate-to-lipid ratio (Table 2).

If the assimilated carbon pool is insufficient to meet the cost of respiration, then the remaining deficit is taken from the neutral lipid pool and any remaining deficit is then taken proportionately from the structural components of the larva (Table 2). Periods during which the larva resorts to using structural material to cover metabolic costs result in reduction of larval condition index defined in the model as a reduction in the protein-to-ash ratio.

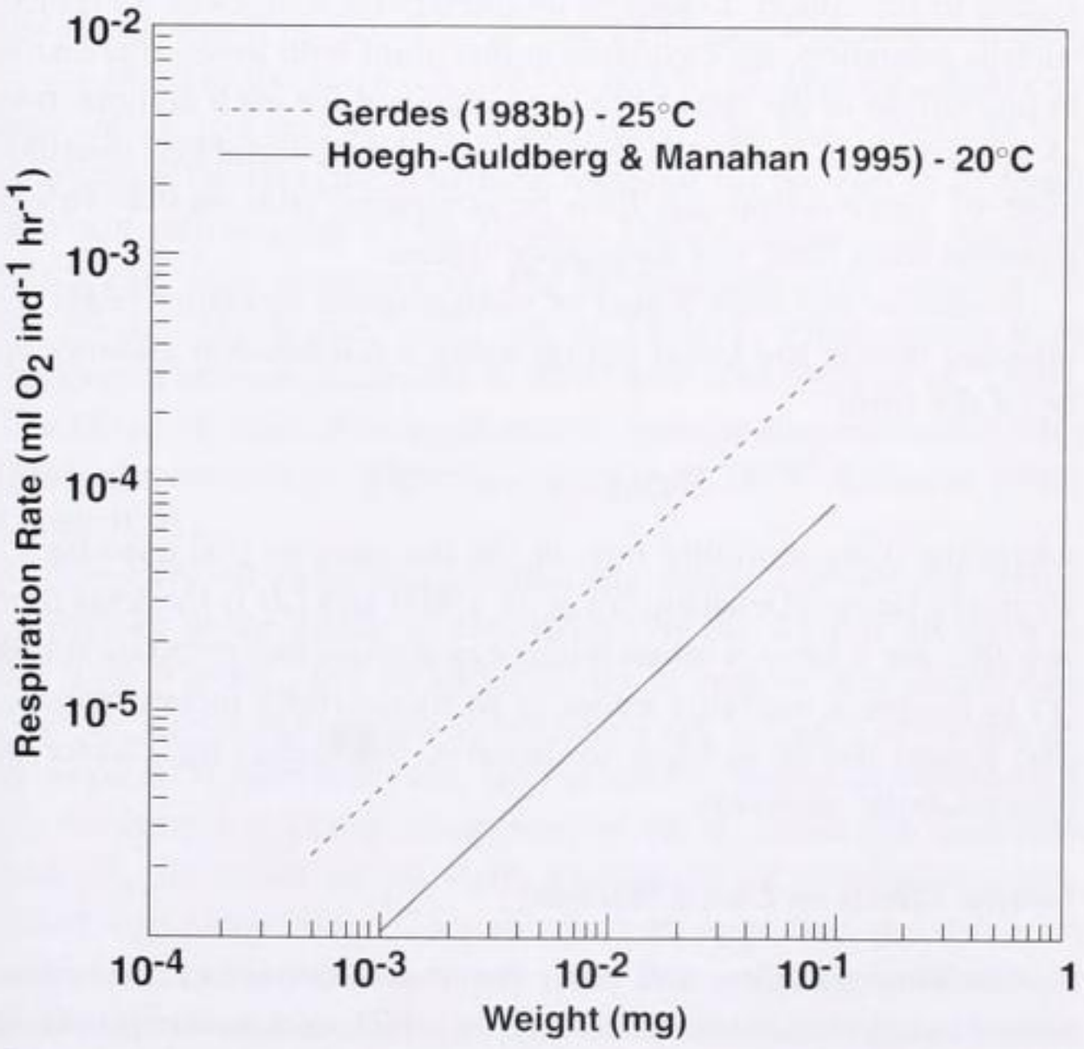


Figure 7. ***Crassostrea gigas*** **larval respiration rate measured as a function of larval weight at two temperatures.**

Larval Growth

Larval growth in a given time interval is based on maintaining the protein-to-ash ratio (Fig. 4, Eq. 5) for a given larval length. Larval growth resulting in an increase in length is assumed to occur when the protein, carbohydrate, and polar lipid pools are in excess of what is needed to maintain the protein-to-ash ratio at a given size. This is the excess net production (*ENP*) that determines α in Eq. 1.

Excess protein is obtained by subtracting from the protein pool the amount that is needed to maintain the ash weight at a given larval length, as is determined by the protein-to-ash ratio. The excess polar lipid and carbohydrate pools are computed from the excess protein pool based on the required structural ratios of these constituents. The excess net production for a given time interval is the sum of the excess protein, polar lipid, and carbohydrate. This gives the excess net production in a given time interval in terms of an increment in larval weight. The weight increment is then used with the length-to-dry tissue weight relationship (Fig. 2) to obtain an incremental increase in length for the increase in weight.

During times of protein deficit with respect to ash weight (low condition index), the larva can have a positive net production that increases organic mass and condition index, but produces no excess net production and, hence, no increase in length.

Larval Metamorphosis

Observations suggest that once *C. gigas* larvae reach 275 μm they may initiate metamorphosis and this process may or may not be successful (Ventilla 1984, Kusaki 1991, Laing 1995). Thus, in the model, the larva is assumed to have the potential of becoming competent for metamorphosis at 275 μm. Just prior to this point, at a length of 250 μm, filtration rate declines. Observations show that *C. gigas* larvae can become competent for metamorphosis over a range of sizes. This implies that the switch between Eqs. 6 and 7 controlling the point where filtration rate changes might contain a size dependency, determined by some metabolic process that is, as yet, unknown. Not having this information, the point at which the larva can become competent for metamorphosis was fixed at a size a little larger than the size observed by Gerdes (1983a) for the change in filtration rate. Simulations discussed subsequently support this decision.

Once the larva reaches 275 μm, it becomes competent to metamorphose if it experiences a 25% drop in neutral lipid stores in one day. This is determined by the interrelationship of food supply, filtration rate, and respiration rate. Competency is triggered by a decrease in neutral lipid that, if continued, would impair successful metamorphosis. Once competent, the larva immediately attempts metamorphosis. Successful completion of metamorphosis occurs if the larval neutral lipid pool is greater than the polar lipid pool. This establishes a minimum storage requirement needed to sustain metamorphosis. If this condition is not satisfied, then metamorphosis is unsuccessful and the larva dies.

Biochemically Determined Metabolic Mortality

The simulated larval growth prior to metamorphosis is based on maintaining specific ratios between protein, polar lipid, carbohydrate, and ash weight. Small variations in these ratios are allowed, consistent with changes that occur in the larva as it grows (cf. Fig. 4). However, large changes are not permissible. The interdependencies of the biochemical ratios results in the protein-to-ash ratio being a good indicator of the biochemical state of the larva. If this ratio is reduced at any time to 70% or less of its needed value, then larval condition index is too low and the larva is assumed to die. This condition is termed starvation in the model.

During the initial stages of larval growth, about the first two days, the larva does not filter efficiently (Fig. 5), and hence food ingestion is not usually sufficient to cover metabolic costs. During this period, it is assumed that the larva survives by using its stored neutral lipid supply. However, if during this period the neutral lipid supply approaches zero, the larva is assumed to have reached its metabolic point of no return and dies. Also, inability of the larva to maintain its required protein-to-lipid and protein-to-carbohydrate ratios results in death.

Model Implementation

Initial *C. gigas* Egg Size, Including Genetic Variability

The eggs spawned by *C. gigas* adults have an average size of 50 μm (Quayle 1988, Arakawa 1990). However, using this as the initial condition for the model resulted in mismatches in the initial simulated and observed length-to-weight relationships, which are based on larval size. Thus, simple egg diameter is not the appropriate metric for use with the length-to-weight and other conversions. The discrepancy arises because of the mismatch in volume of a spherical egg and the more ellipsoidal-shaped larva. Therefore, it was necessary to convert initial egg diameter to an equivalent larval size. This was done using a diameter-to-length conversion factor of 1.096 (Arakawa 1990) that conserves volume in going from a spherical egg to an ellipsoidal-shaped larva. Thus, a 50 μm egg is equivalent to a 54.8 μm larva.

C. virginica egg size is observed to range between 30 and 80 μm (Gallager et al. 1986). More limited information is available for *C. gigas*, but a similar range of egg sizes can be inferred (Breese & Malouf 1975). This variation was assumed to represent genetically or environmentally determined variability in the spawning population. Therefore, for each simulation, the initial conditions included a range of egg sizes.

To establish the initial biochemical composition of the egg, the larval size immediately post-hatch was used with the length-to-dry tissue weight relationship (Fig. 2) to calculate an initial dry weight, which in turn was used to obtain an initial ash weight value (Fig. 3). The protein component of the egg was then determined by multiplying the ash weight by the protein-to-ash ratio. The egg polar lipid content was determined by multiplying the protein content by the polar lipid-to-protein ratio. The carbohydrate content was taken to be 1% of the dry weight of the modified egg. Egg neutral lipid content was obtained by difference through subtracting the protein, polar lipid, and carbohydrate weight from the initial dry weight value. If this calculation resulted in a negative value of neutral lipid, which can occur for very small eggs, the egg was assumed to be nonviable.

Predation and Other Nonmetabolic Sources of Larval Mortality

The larval model provides, as output variables, the total time for larval development, larval size at the end of the simulation, and a description of why the simulation ended. Termination of a simulation occurs because of successful metamorphosis, unsuccessful metamorphosis, inappropriate metabolic ratios, and starvation. The simulated larval results are then examined with a submodel that calculates larval survivorship based on the timing of mortality and the larval life span of the survivors for each combination of egg size and respiration rate represented by the genetic variability assigned to the cohort. Losses to nonmetabolic sources of mortality, such as predation, are evaluated at this point with losses increasing in proportion to the larval life span obtained for each combination of egg size and respiration rate. The resultant simulated distributions of survivorship can then be compared with similar values reported from field and laboratory studies.

Predation and other forms of nonmetabolic mortality (EM) are imposed during the larval period using a relationship assumed to be of the form

$$EM(j,k) = e^{-m_o LD(j,k)} \tag{13}$$

where the daily mortality rate, m_o, is the same as that used for *C. virginica* larvae (Dekshenieks et al. 1997) and LD is the total time required for a larva with an initial egg size (j) and respiration rate (k) to trigger a mortality event or to successfully metamorphose. The longer the larva takes to develop, the higher the chance of nonmetabolic mortality.

Genetic Effects on Larval Mortality

Growth, mortality, and other population processes are apportioned based on genetic variability, in which certain combinations of initial egg size and respiration rate are less common in the cohort and in which certain combinations are less viable overall,

either due to metabolic imbalances, metabolic inefficiencies, or longer larval life spans increasing nonmetabolic mortality. This type of genetically determined outcome, *GE,* is prescribed with a Gaussian function of the form:

$$GE = e^{-\left(\frac{ES - ES_o}{2sd_{egg}}\right)^2} - e^{-\left(\frac{Resp - Resp_o}{2sd_{resp}}\right)^2} \quad (14)$$

where the Gaussian distributions extend for two standard deviations ($2sd_{egg}$, $2sd_{resp}$) about a central egg size and respiration rate that are given by ES_o and $Resp_o$, respectively. Coefficient definitions and values are given in Table 1. Equation 14 weights mortality or any other population process by a population distribution that is characterized by a certain range of egg sizes and respiration rates. Thus, the surviving larval population represents the combined effects of genetics, food composition, and environmental conditions.

RESULTS

Reference Simulation

The reference simulation was run with near-optimal environmental conditions of 25°C, 30‰, a food concentration of 2 mg L^{-1}, and food with a protein, polar lipid, neutral lipid, carbohydrate ratio of 3:0.6:0.4:2.5. Development of *C. gigas* larvae over the first few days of larval life is primarily sustained by egg neutral lipid stores. The drawdown of neural lipid stores results in a decrease in the neutral lipid-to-protein ratio (Fig. 8A). The decrease in this ratio is most pronounced for eggs with small initial sizes. For initial egg sizes of 40 to 50 μm, the neutral lipid-to-protein ratio approaches zero. All larvae, independent of initial egg size, reach a maximum ratio value of between 0.15 and 0.165 and a size of ≥275 μm (Fig. 8B). Larvae arising from larger eggs reach these values earlier, and hence can metamorphose earlier. Once a size of 250 μm is reached, filtration rate declines and so, too, does the neutral lipid to protein ratio. However, growth continues and most larvae metamorphose at about 300 μm, regardless of initial egg size (Fig. 8B).

The simulated larval growth is exponential and independent of initial egg size (Fig. 8B). Larvae reach 100 μm, which corresponds to the small umbo stage (cf. Fig. 1), in 4 to 12 days for initial egg sizes of 70 μm and 40 μm, respectively. The rate of growth accelerates after 100 μm. The time required for the larvae to reach 300 μm ranges from 13 to 19 days for the largest and smallest initial egg sizes, respectively. The development time required for the 50 μm egg to reach 300 μm is 17 days, which agrees with development rates measured at 25°C and 30‰ (His et al. 1989). The range of simulated development times is also consistent with those reported for *C. gigas* larvae (Quayle 1988, Arakawa 1990, Laing 1995).

The effect of variations in initial egg size and growth efficiency on the fate of the larvae is summarized by the state of the larva at the time it either dies or successfully completes metamorphosis (Fig. 9). Variations in growth efficiency are modeled as variations in respiration rate; however, similar results would be obtained if the variation was in any component of Eq. 2. Initial egg sizes less than 37 μm result in nonviable larvae for all respiration rates. Initial egg sizes above 73 μm result in eggs that do not have sufficient neutral lipid stores after day 2 to continue development at all respiration rates. The large initial size of these eggs results in an imbalance in neutral lipids that cannot be corrected subsequently (cf. Fig. 8A). A similar fate occurs for initial egg sizes

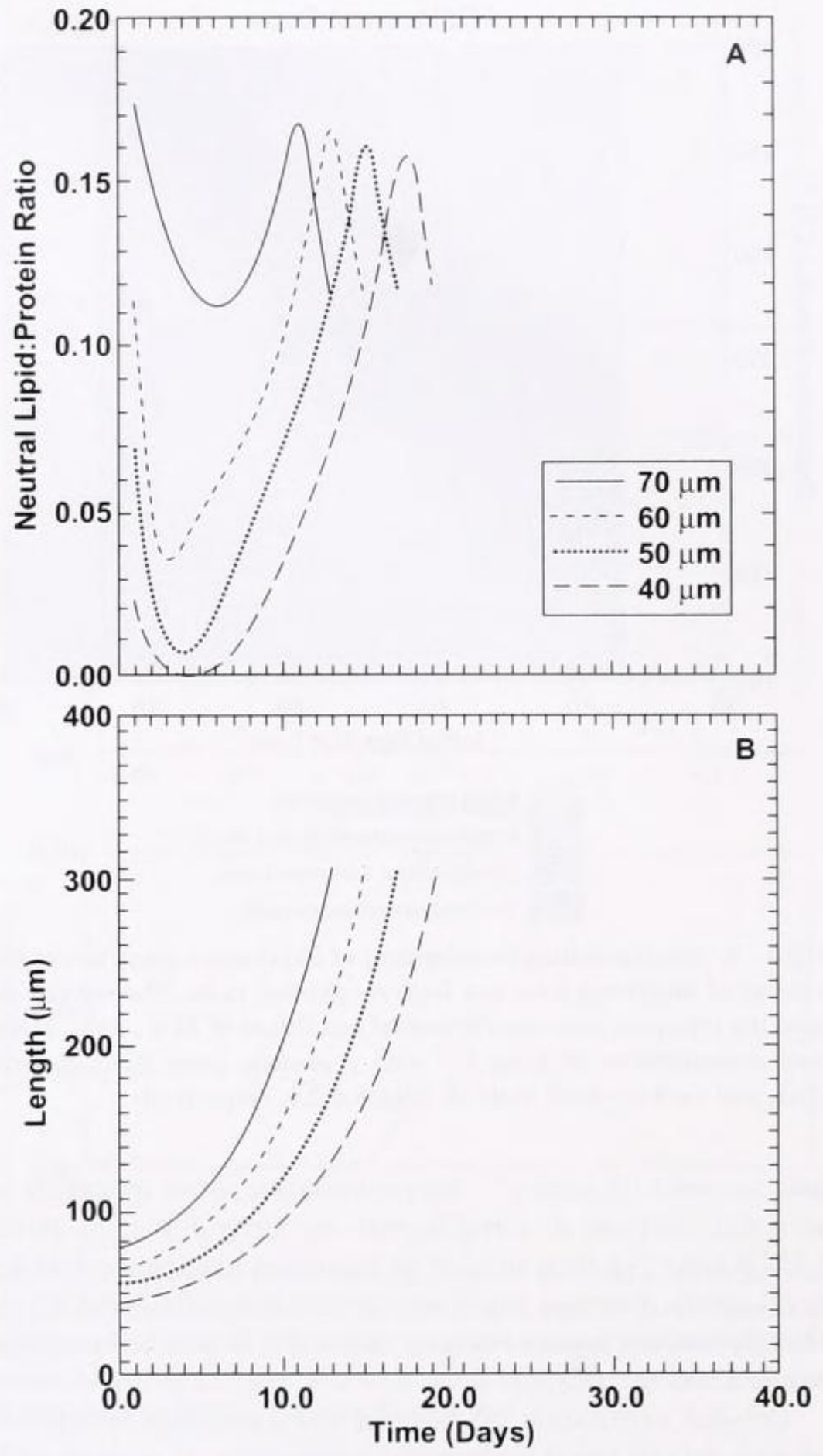


Figure 8. Simulated time development of the (A) neutral lipid-to-protein ratio and (B) *Crassostrea gigas* length obtained for a selection of initial egg sizes from 40 μm to 70 μm.

between 40 and 50 μm that yield larvae with high base respiration rates. For these larvae, too much of the neutral lipid pool is required to cover respiratory demand, and this produces a metabolic imbalance from which the larva cannot recover. Above and below the region of initial egg sizes and base respiration rates that produce successful metamorphosis are regions where the larva develops to the point of attempting metamorphosis, but is unable to do so successfully. A drop in neutral lipid triggers metamorphosis in these larvae, but they have insufficient lipid stores to cover the metabolic costs of metamorphosis.

At the population level, the fraction of the larvae that survive to complete metamorphosis is dependent on the initial egg size (Fig. 10A), with the distribution of survivorship centered around an initial egg weight of 50 μm, which is the center of the egg size distribution. Survivorship tapers off toward larger egg sizes, with essentially no survival to metamorphosis at egg sizes greater than 68 μm. Survivorship at smaller egg sizes decreases abruptly with essentially no survival at sizes less than 40 μm. Larval survivorship as a function of base respiration rate (Fig. 10B) is maximal at

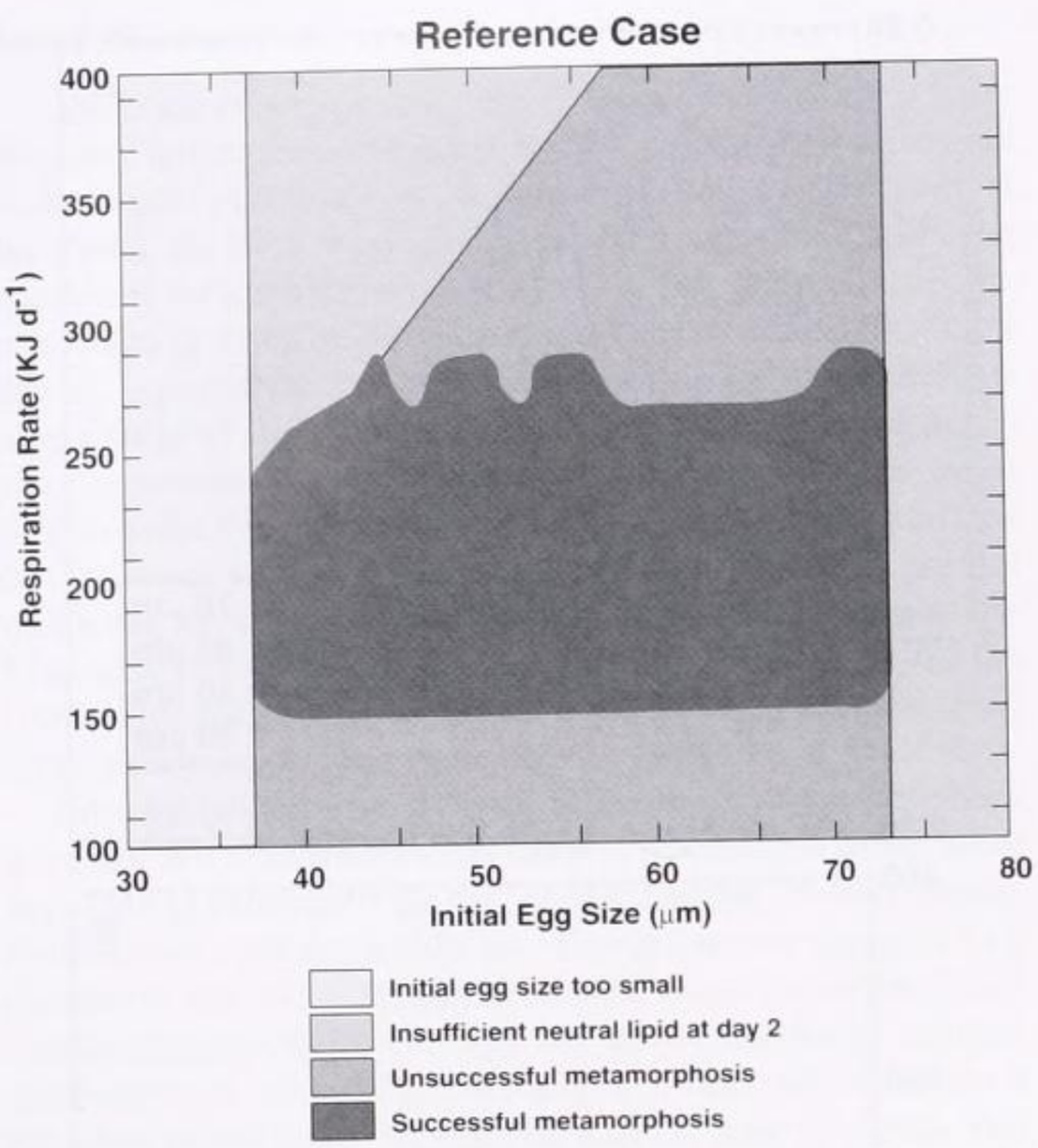


Figure 9. Simulated time development of *Crassostrea gigas* larvae for a range of initial egg sizes and base respiration rates. The simulation used the reference case environmental conditions of 25°C, 30‰, and a food concentration of 2 mg L^{-1} with a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0.4:2.5, respectively.

rates around 1.05 KJ day^{-1}. Respiration rates above this result in an abrupt decrease in survival, with no survival at rates above 1.254 KJ day^{-1}. Larval survival at respiration rates below 1.05 KJ day^{-1} slowly decreases and is zero at rates below 0.628 KJ day^{-1}. Most larvae reach sizes between 300 and 325 μm before metamorphosis (Fig. 10C).

Detailed verification of the reference simulation is difficult because data on larval biochemical composition, as it varies with egg size, development time, and environmental conditions, are meager. General trends in larval success as measured by survivorship, larval size, and larval life span are much better known. Four such trends are observed in the simulation. (1) Adequate neutral lipid stores are a prerequisite of high survival during the critical period a few days post-hatch and at metamorphosis. The reference simulation demonstrates both effects (Figs. 8A, 10B). (2) Typically, egg size ranges from 40–60 μm. The reference simulation identifies this range as optimal for *C. gigas*, based on changes in biochemical composition dictated by larval energetics (Fig. 10A). (3) Successful metamorphosis occurs for larvae of 295–340 μm in the reference simulation. This is a frequently observed size range (Ventilla 1984, Kusaki 1991, Laing 1995). (4) Larval life span for the most successful egg sizes varies from about 14 to 17 days, a range that approximates the norm in observation (Ventilla 1984, Arakawa 1990, Laing 1995).

Effect of Larval Filtration

The parameterization for larval filtration (Eqs. 6 and 7, Fig. 5) is based on the assumption that the filtration response changes abruptly at 250 μm. *C. gigas* larvae are observed to metamorphose at a range of lengths. The change in filtration rate is an important

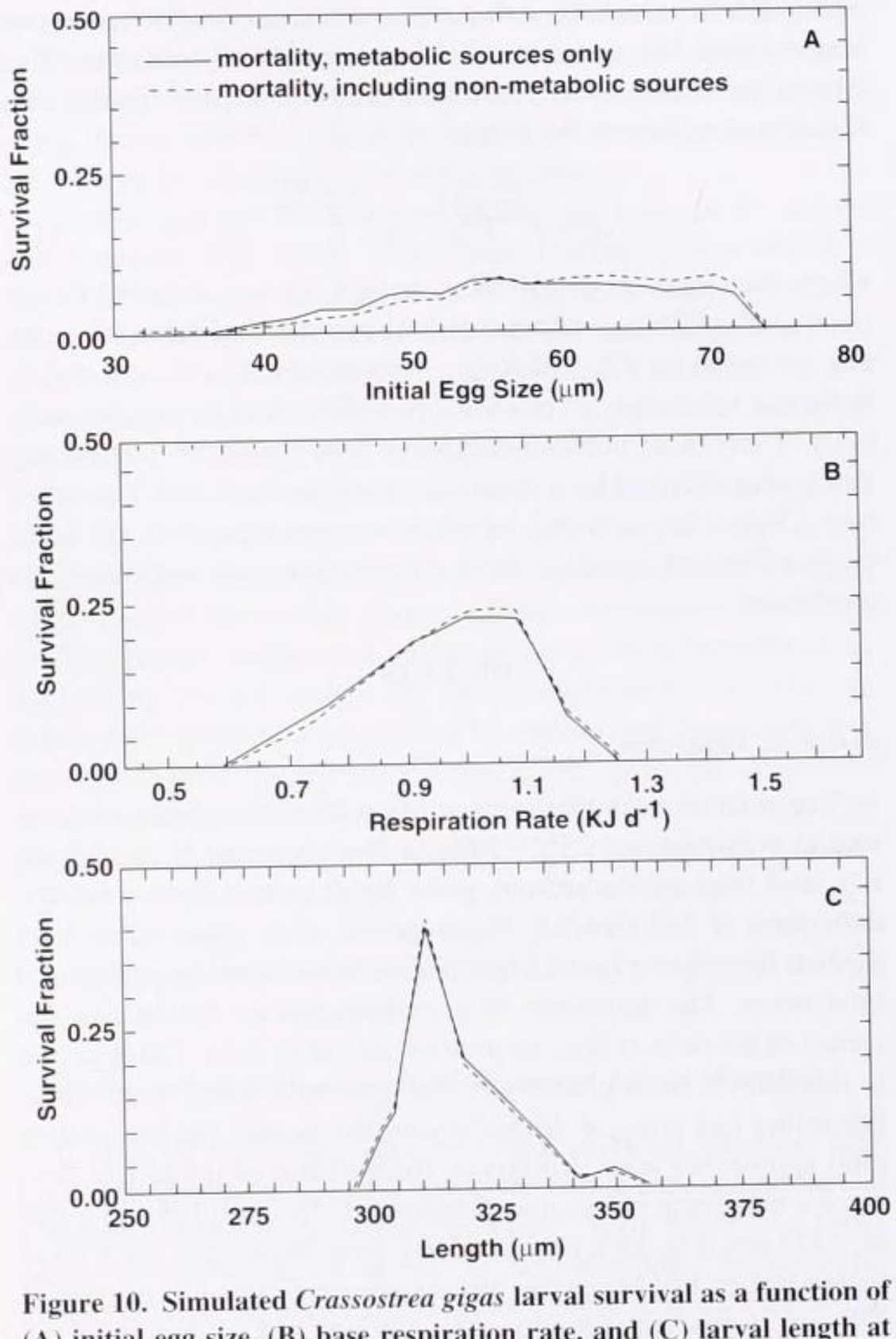


Figure 10. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis, using the reference case environmental conditions given in Figure 9.

contributor to the mechanisms by which the model determines the onset and success of metamorphosis. Therefore, the larval length at which the change in filtration response occurs was varied.

Varying the onset of a decline in filtration rate does not impact the range of viable egg sizes because this range is determined by events that predate this point in the larva's life span (Figs. 11A, 11B). However, if filtration rate changes at 270 μm, larvae spend a longer time at a high filtration rate. These animals metamorphose successfully over a wider range of base respiration rates than those that support successful metamorphosis when filtration rate begins to decline at 250 μm (Fig. 12B). Also, the overall population survivorship is higher; 78.8% of the cohort versus 62.4% in the reference simulation (Figs. 9, 10), and the range of lengths at which larvae metamorphose is wider (Fig. 12C).

Moving the larval length at which the filtration rate response changes to a smaller size, 230 μm, results in a limited range of base respiration rates at which successful metamorphosis can occur (Figs. 11B, 13B). Many of the animals survive to attempt metamorphosis, but are unsuccessful at completing the process. The smaller size at which the filtration rate is reduced results in the larvae not storing enough neutral lipid to cover the metabolic needs associated with metamorphosis. Larval length at metamorphosis (Fig. 13C) is now limited to a small range of sizes.

Total population survivorship drops from 62.4% in the refer-

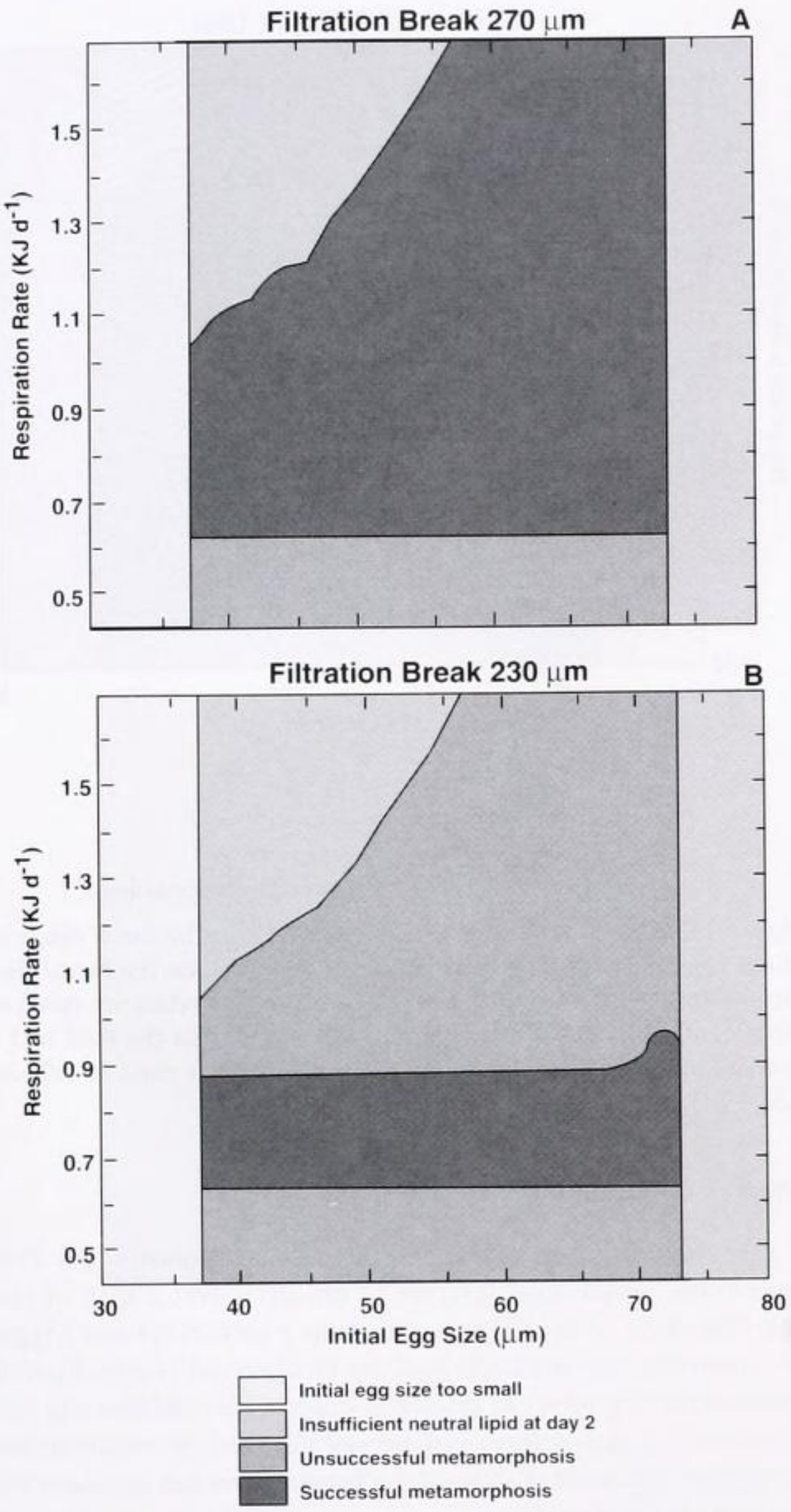


Figure 11. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and base respiration rates in which the change in larval filtration rate response is at (A) 270 μm and (B) 230 μm. The simulation used the reference case environmental conditions given in Figure 9.

ence case to 8.1% with a size trigger of 210 μm rather than 250 μm. Cohort survivorship declines dramatically at trigger sizes below 250 μm (Table 3) and asymptotes rapidly above 250 μm. At trigger sizes above 250 μm, some larvae do not metamorphose until reaching sizes much larger than normally observed (e.g., 375–400 μm, Fig. 12C), however. Accordingly, larval growth, as modeled, requires a filtration rate trigger near 250 μm to obtain observed levels of cohort survivorship and size at metamorphosis. This approximates the size where filtration rate declines are observed to take place (Gerdes 1983a).

Effect of Diet

Information on the effects of diet on growth and survival of *C. gigas* larvae indicate that high-lipid and low-protein diets are usu-

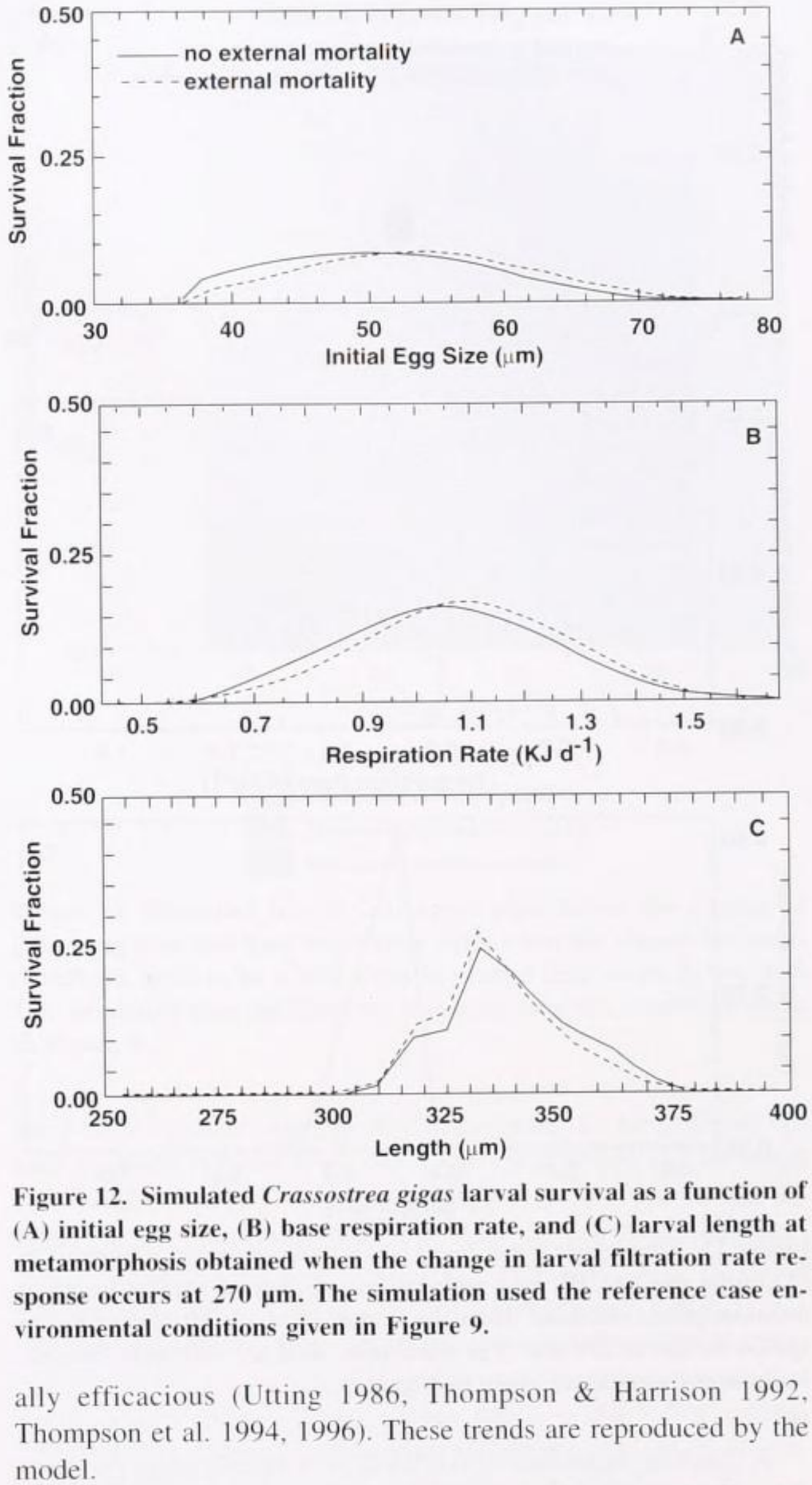


Figure 12. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis obtained when the change in larval filtration rate response occurs at 270 μm. The simulation used the reference case environmental conditions given in Figure 9.

ally efficacious (Utting 1986, Thompson & Harrison 1992, Thompson et al. 1994, 1996). These trends are reproduced by the model.

A larval diet that is lacking in neutral lipid but contains the correct ratios of protein, polar lipid, and carbohydrate (3:0.6:0:2.5) results in animals that are unable to successfully complete metamorphosis at all ranges of initial egg size and base respiration rate (Fig. 14). Lack of neutral lipids results in more of the protein and carbohydrate pools being used to cover the demands of respiration and growth and, so, lipid stores are insufficient to sustain metamorphosis. In addition, the range of egg sizes and base respiration rates in which the neutral lipid store at day 2 is insufficient for further development is greatly expanded relative to what is obtained for a diet containing neutral lipid (Fig. 14 versus Fig. 9).

A diet in which the protein content is 50% higher relative to the standard diet (4:0.6:0.4:2.5) produces a similar result in that no combination of initial egg size or respiration rate results in successful metamorphosis (Fig. 15A). Larvae either have insufficient neutral lipid reserves at day 2 to continue development or attempt metamorphosis but fail to complete the process. A high-protein diet requires that more of the neutral lipid stores be used to cover tissue structural needs, and hence less lipid is stored for later use in metamorphosis.

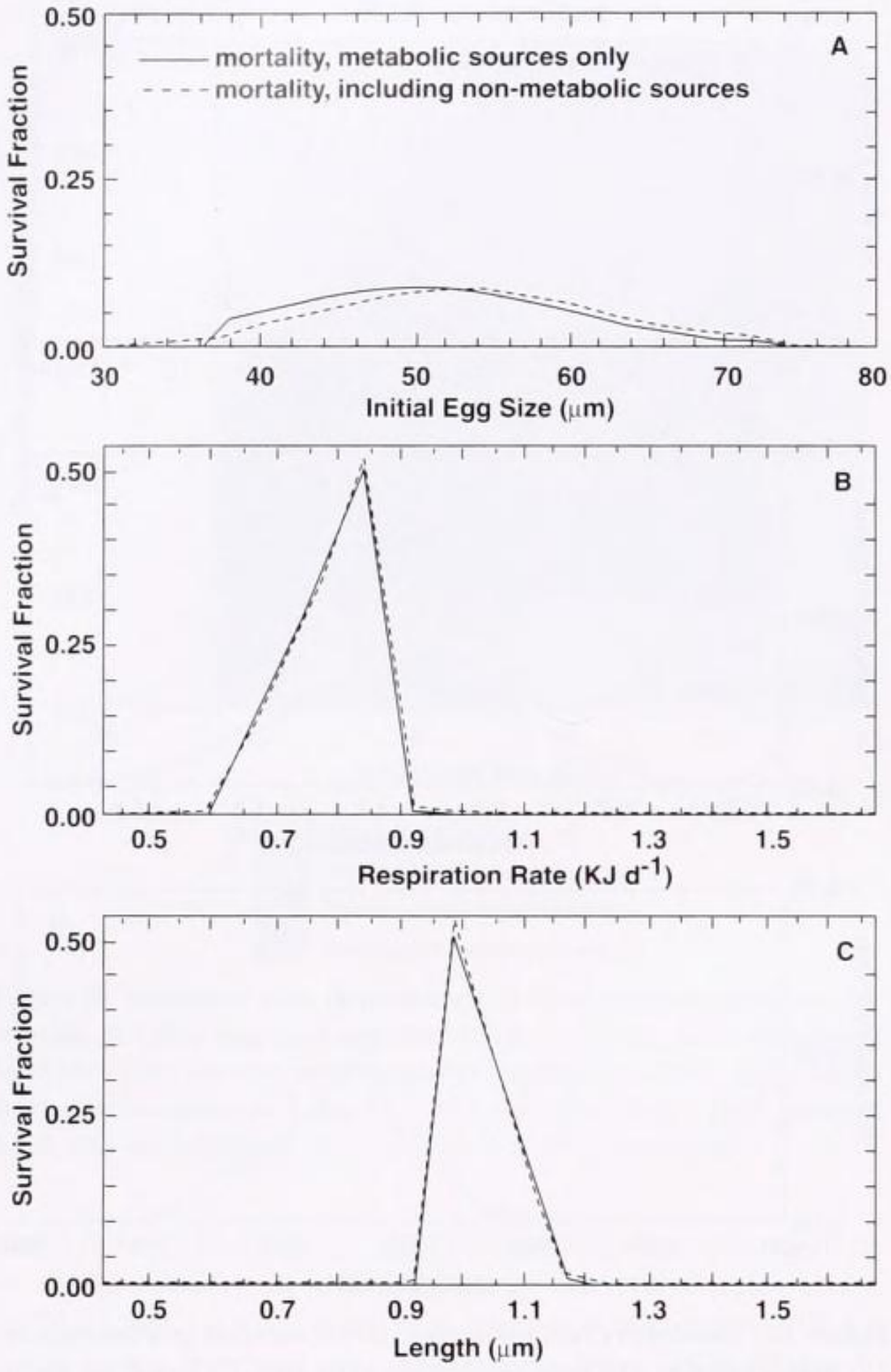


Figure 13. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis obtained when the change in larval filtration rate response occurs at 230 μm. The simulation used the reference case environmental conditions given in Figure 9.

A diet low in protein (2:0.6:0.4:2.5) is beneficial to the larva (Fig. 15B). Ingestion of low-protein food extends the range of respiration rates that result in successful metamorphosis (Fig. 15B versus Fig. 9). More polar lipid and carbohydrate is available to cover metabolic costs and to increase neutral lipid stores. This increases metamorphosis success. Simulations indicate that a low-protein diet increases overall population survivorship under hatchery conditions where nonmetabolic causes of mortality are minimized (88.8% from 62.4% in the reference simulation), but decreases survivorship under field conditions, from 6.1% in the reference simulation to 4.7%, due to slower growth and longer planktonic times increasing losses to predation.

TABLE 3.

Total population survivorship for simulated cohorts of *Crassostrea gigas* larvae in which the larval size triggering a change in filtration rate was varied from 210 μm to 290 μm.

Trigger size	210	230	250	270	290
Cohort survivorship	8.1%	17.2%	62.4%	78.8%	78.8%

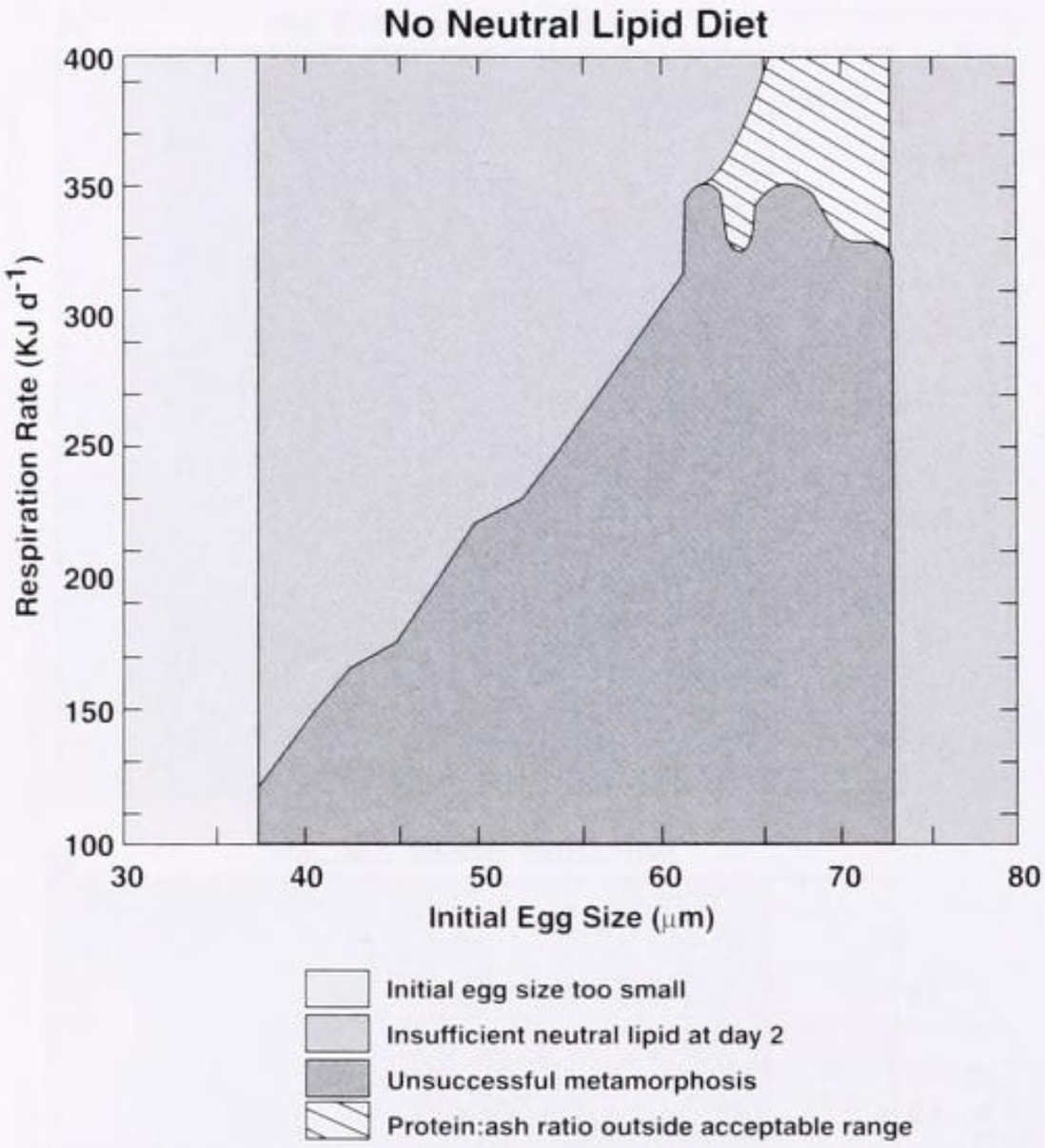


Figure 14. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and respiration rates resulting when the larval diet includes no neutral lipid. The simulation used the reference case environmental conditions given in Figure 9, except that the food had a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0:2.5.

Effect on Metamorphosis

The cue that is assumed to initiate metamorphosis is a 25% drop in the neutral lipid reserves of the larva over a span of one day. This level of decline was chosen as a metamorphosis trigger by comparing the simulated size and biochemical composition of metamorphosing larvae to measured values. The condition and size of larvae at metamorphosis is quite variable, and the metamorphosis trigger value used in the model represents one that simulates the average of these conditions.

Requiring only a 10% drop in neutral lipids in one day to trigger metamorphosis results in essentially all combinations of initial egg size and base respiration rate producing larvae that successfully undergo metamorphosis (Fig. 16 versus Fig. 9). The lower trigger value allows larvae to have a greater energy store at the time metamorphosis is attempted, and hence the probability of success is increased. At the population level, a wide range of egg sizes and base respiration rates results in survival (Figs. 17A & 17B), but larval length at metamorphosis is shifted to smaller larvae (Fig. 17C). The population mode in this case extends from 285 μm to about 305 μm, a size range predominately comprising sizes smaller than typically observed and smaller than the 295 μm to about 335 μm size range observed in the reference simulation (Fig. 9C). Increasing the trigger to a 40% reduction in neutral lipids in one day (not shown) results in attempted, but failed, metamorphosis at all initial egg sizes and base respiration rates. Thus, the value of 25% produces observed success rates for metamorphosis at a range of observed egg sizes not achieved by higher or lower trigger values.

Metamorphosis success is determined by the ratio of neutral

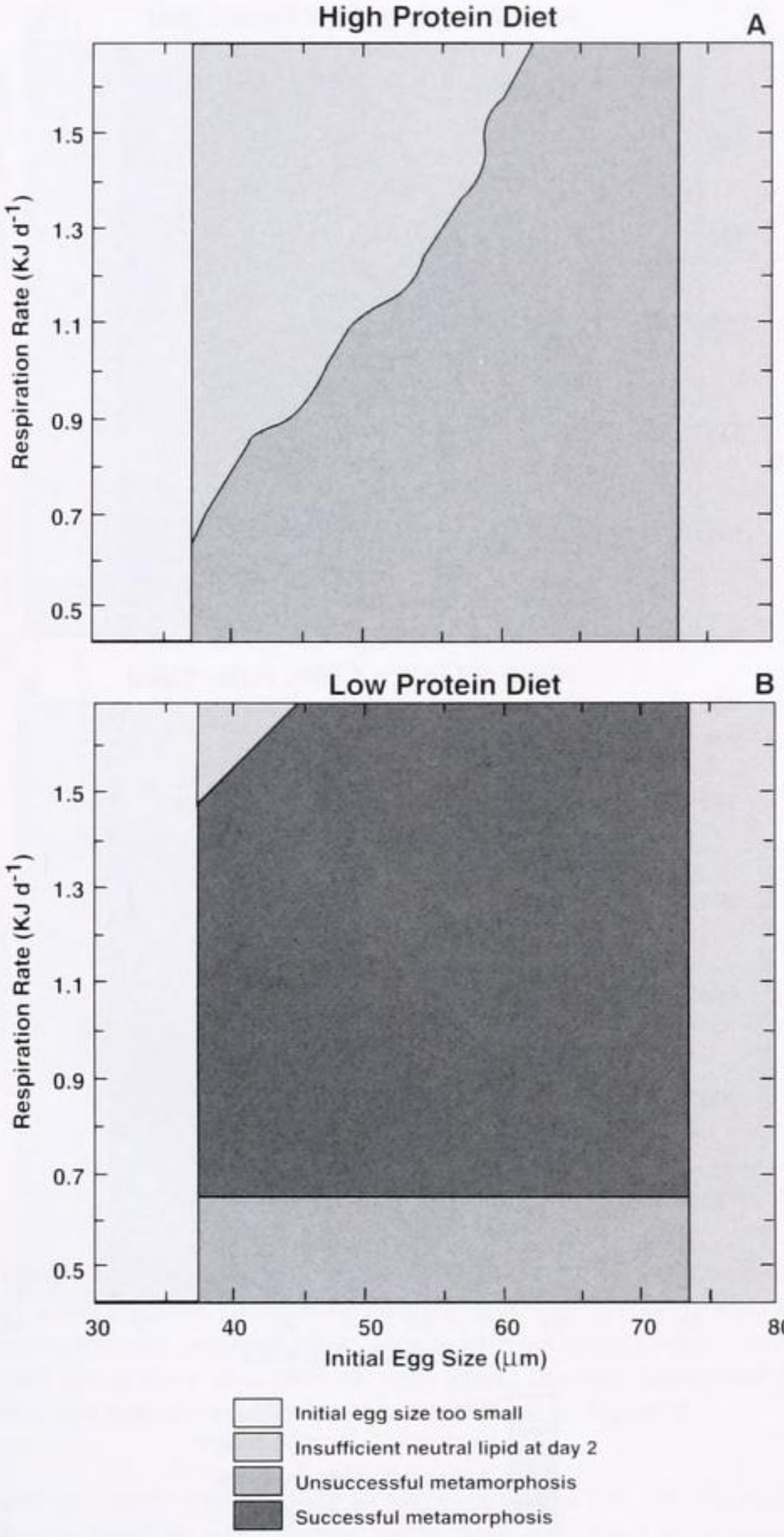


Figure 15. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and respiration rates resulting when the larval diet is (A) high in protein and (B) low in protein. The simulation used the reference case environmental conditions given in Figure 9, except that the food had a protein, polar lipid, neutral lipid, and carbohydrate ratios of 4:0.6:0.4:2.5 and 2.5:0.6:0.4:2.5.

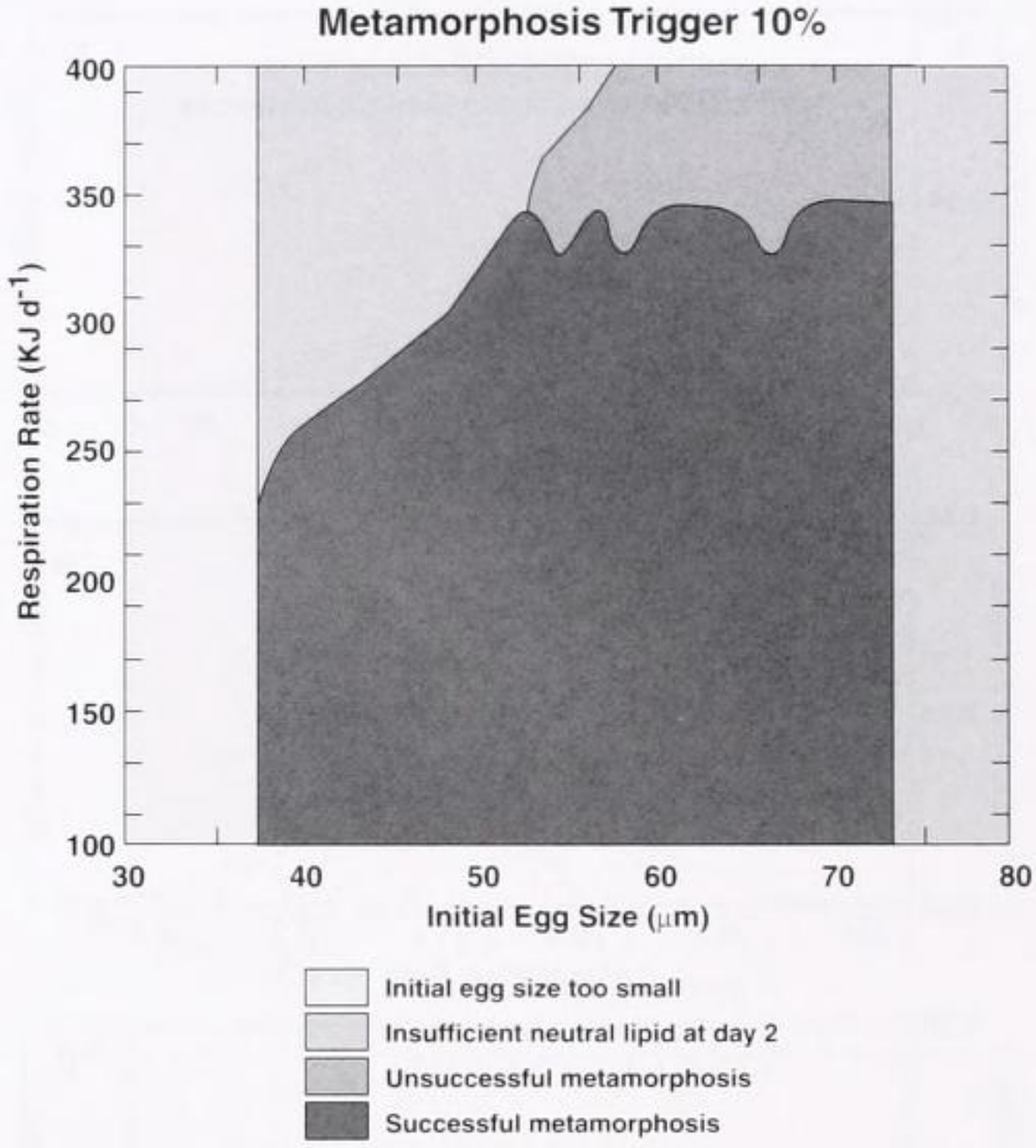


Figure 16. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and base respiration rates when the trigger for metamorphosis is set to be a 10% drop in neutral lipid stores in one day. The simulation used the reference case environmental conditions given in Figure 9.

lipid to polar lipid in the animal at the time that metamorphosis is triggered. In the reference case (Fig. 9), successful metamorphosis occurs if the content of neutral lipid exceeds the content of polar lipid. Allowing metamorphosis to be successful when the larval neutral lipid reserves are greater than 80% of the polar lipid content produces successful metamorphosis over a large range of initial egg sizes and respiration rates (Fig. 18A). At the population level, larval survival is enhanced over a wide range of initial egg sizes and base respiration rates (Fig. 19A, 19B). The length at metamorphosis also extends over a wide range (Fig. 19C), wider than normally observed, but the mode of the population is still around 308 μm, as seen in the reference simulation (cf. Fig. 10C).

If metamorphosis is successful only when the neutral lipid pool is greater than 110% of the polar lipid pool, the range of initial egg sizes and respiration rates at which successful metamorphosis occurs is greatly reduced (Fig. 18B). The effect of egg size on larval survivorship is only somewhat modified (Fig. 20A); however, the range of base respiration rates that result in successful metamorphosis is greatly reduced (Fig. 20B), as is the range of larval lengths (Fig. 20C). Overall, trigger values above 100%, a 1:1 neutral lipid-to-polar lipid ratio, generate rates of successful metamorphosis that are too low and larval sizes at metamorphosis that are too large (Table 4). Trigger values below 100% at first seem to be defensible; however, survivorships are unusually high. Hence, model conditions chosen for the reference simulation include, as the minimally required neutral lipid stores for success at metamorphosis, a quantity greater than the polar lipid content of the larva (>1:1). Recall that the polar lipid content is constrained to a predetermined ratio with protein and structural carbohydrate, and so the same results would have occurred had neutral lipid been compared to any structural constituent.

Temperature and Salinity Effects

The temperature and salinity used for the previous simulations, 25°C and 30‰, is near optimal for the growth and development of *C. gigas* larvae (cf. Fig. 6). The optimal range of these environmental variables is narrow, and therefore relatively small changes in these conditions have the potential of causing large changes in larval growth and development. Population survivorship is nonzero over a relatively narrow temperature range and a somewhat wider salinity range (Table 5). A 5°C reduction in temperature, from 25°C to 20°C for example, results in no combination of initial egg size or base respiration rate that produces successful metamorphosis (Fig. 21A). A reduction in salinity to 20‰ extends the

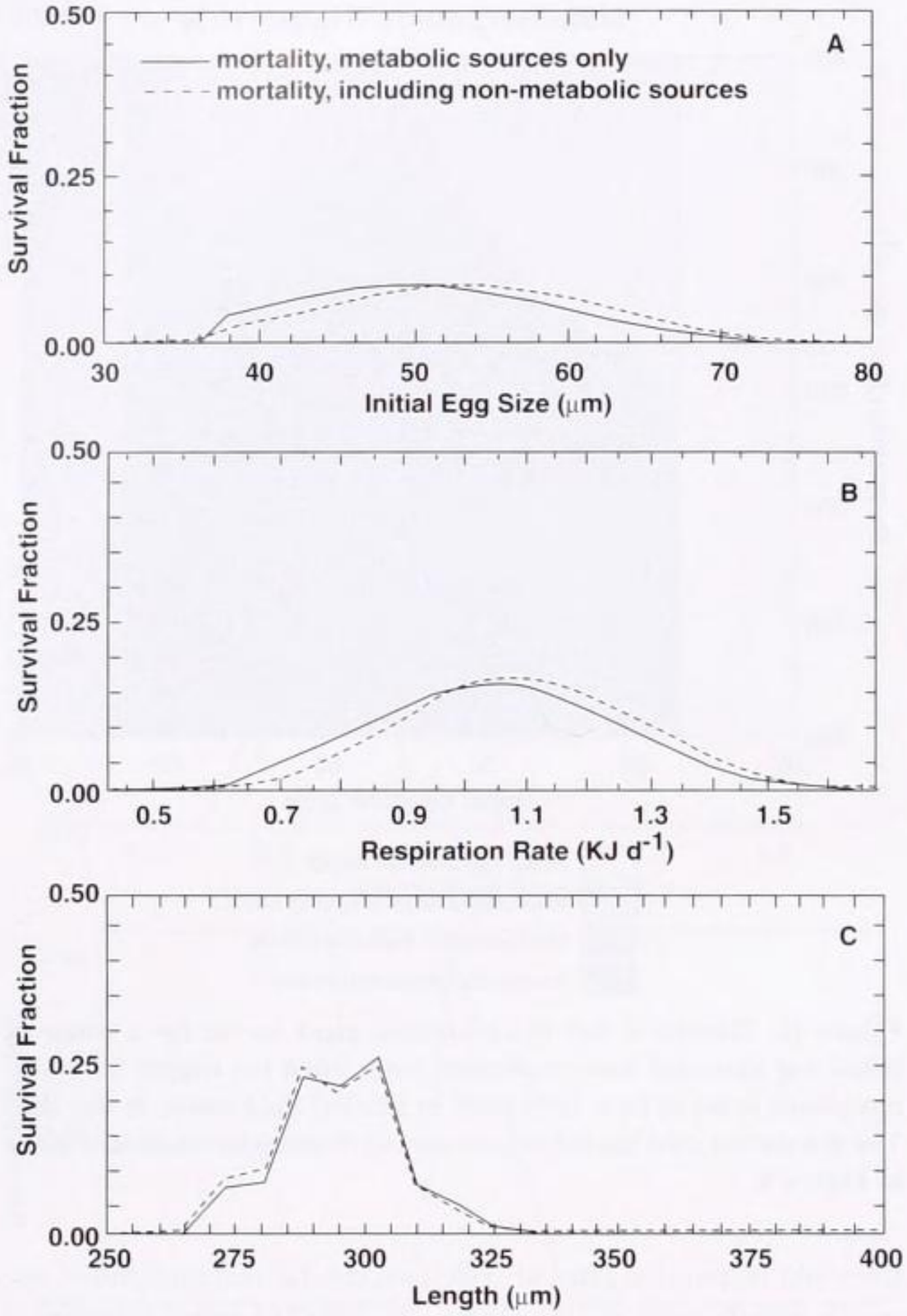


Figure 17. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) respiration rate, and (C) larval length at metamorphosis obtained when metamorphosis occurs in response to a 10% drop in neutral lipid stores in one day. The simulation used the reference case environmental conditions given in Figure 9.

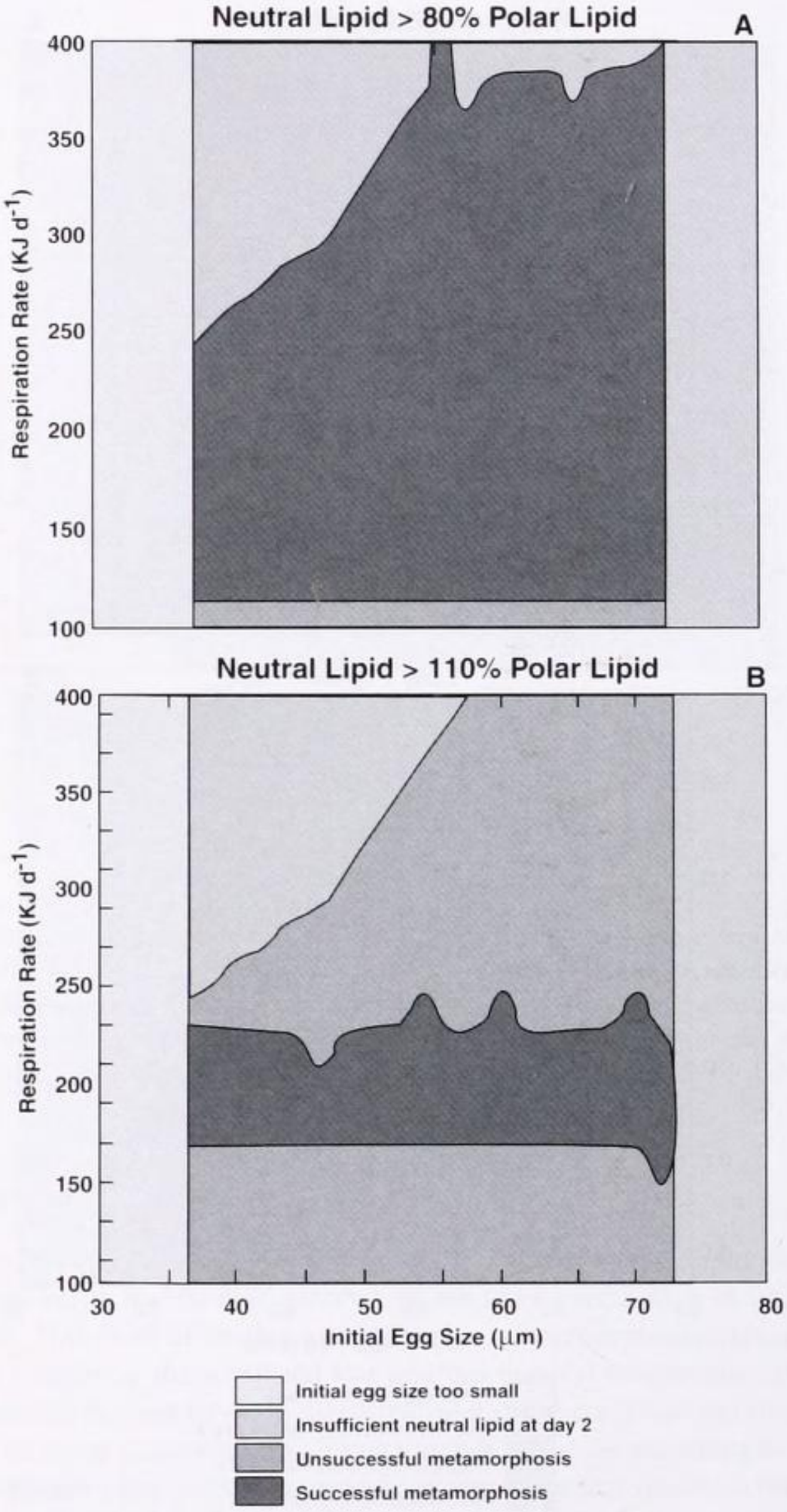


Figure 18. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and respiration rates when the metamorphosis trigger occurs when (A) neutral lipids are greater than 80% of the polar lipid stores and (B) neutral lipids are greater than 110% of the polar lipid stores. The simulation used the reference case environmental conditions given in Figure 9.

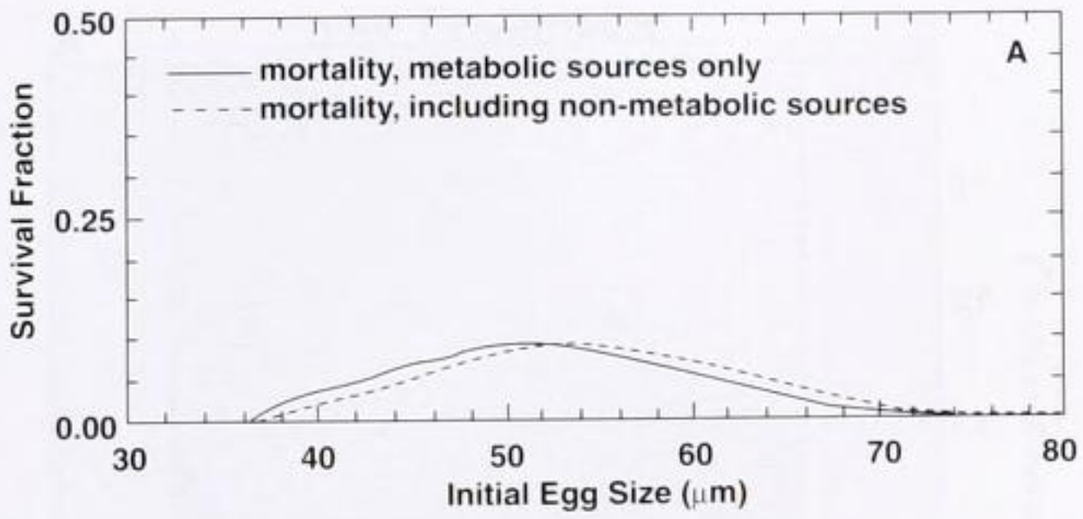
range of initial egg weights and base respiration rates at which the larva exceeds its neutral lipid constraint at day 2 of development (Fig. 21B). Half as many animals exposed to this salinity successfully complete metamorphosis as in the reference case.

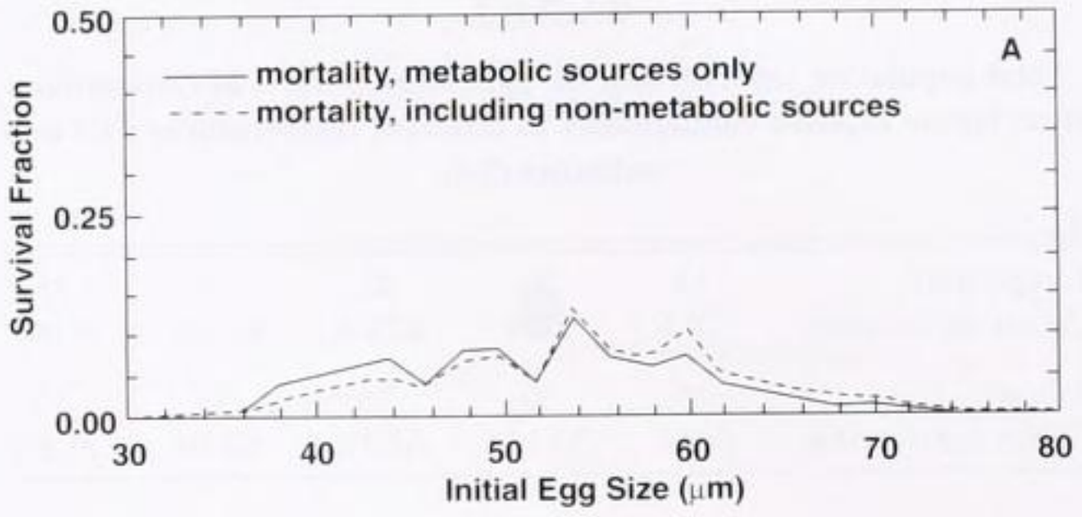
Effect of Variations in Food Resources

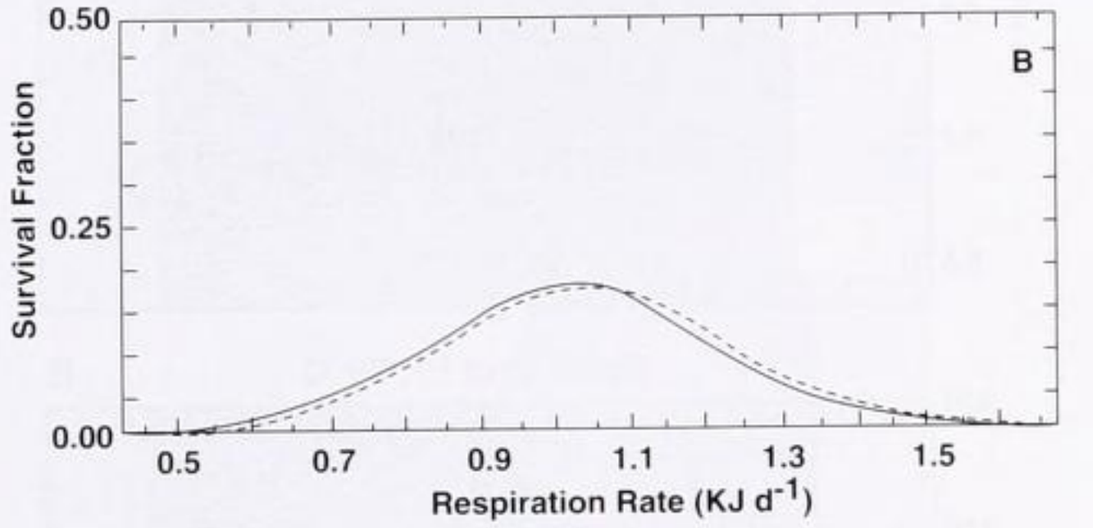
Environmental food concentration can vary over a wide range. Food concentration in excess of 2 mg L^{-1} used for the reference simulation (cf. Fig. 9) only extends somewhat the range of initial egg weights and base respiration rates that result in successful metamorphosis because 2 mg L^{-1} food is a saturating food concentration. However, a 50% reduction in food concentration, from 2 mg L^{-1} to 1 mg L^{-1}, significantly narrows the range of base respiration rates that result in successful metamorphosis (Fig. 22A). The strong coupling between respiration rate and food availability is not surprising since respiration is the primary metabolic loss that the larva must cover through ingestion. This result is further substantiated when looking at population level survival trends (Table 6). Survivorship declines rapidly at food concentrations below 2 mg L^{-1} and reaches zero at food concentrations of about 0.5 mg L^{-1}.

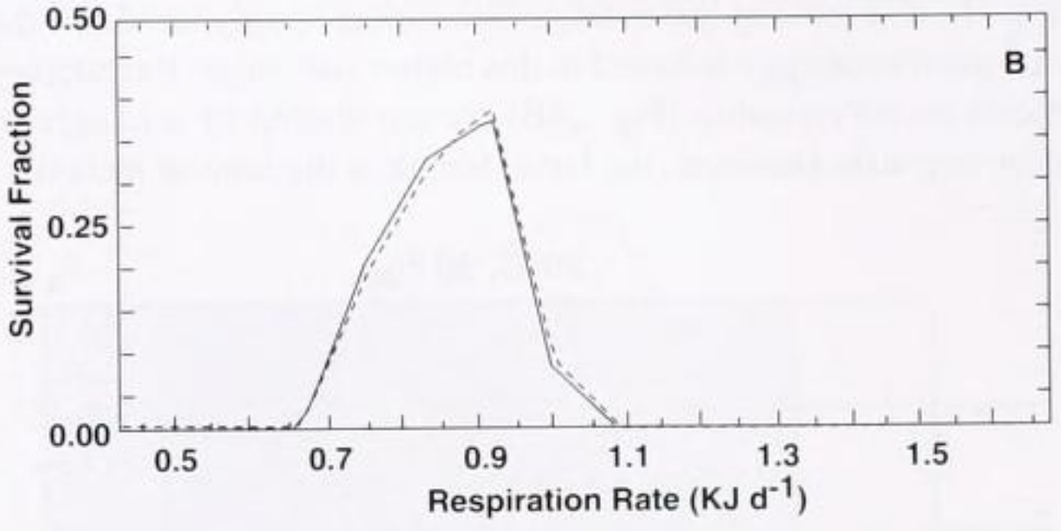
Certain environmental conditions may spare a decrease in food supply by permitting an increase in filtration rate. Increasing temperature in the previous simulation to 30°C, for example, increases survival by permitting some larvae with high respiratory demands to survive (Fig. 22B). Total population survival increases from 27.2% to 37.6% at the higher temperature.

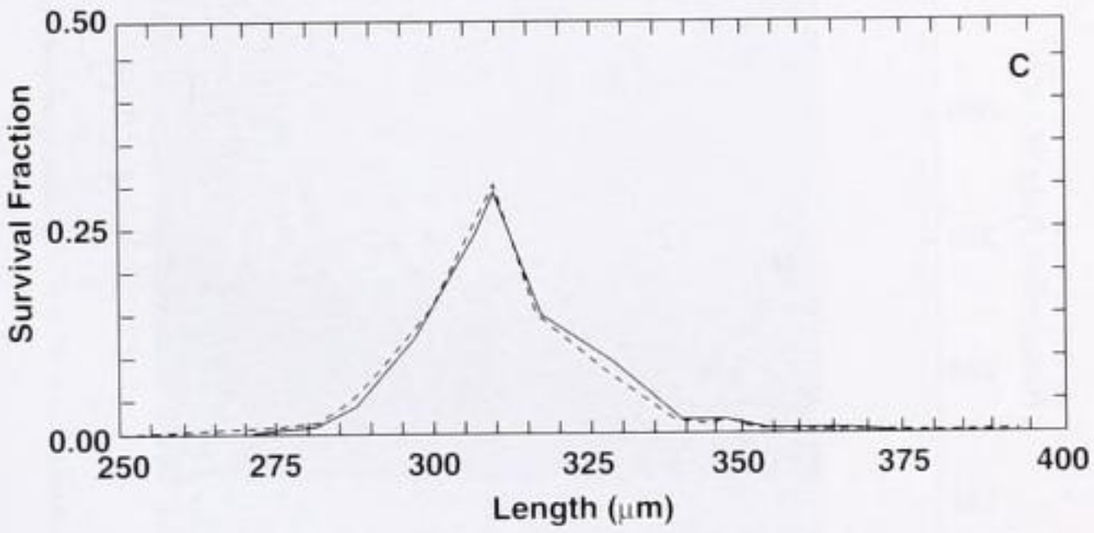
Planktonic values of food of 0.5 mg L^{-1} or less are not unusual. Consequently, larvae may experience times of starvation due to significantly reduced food concentrations. A simulation in which food is available at a concentration of 2 mg L^{-1} for days 1 to 4 of larval development and unavailable after day 5 shows that no combination of initial egg size and base respiration rate results in successful metamorphosis (Fig. 23A). Death results from poor condition in some cases, but more frequently from metabolic imbalances between principal biochemical constituents. Simulated











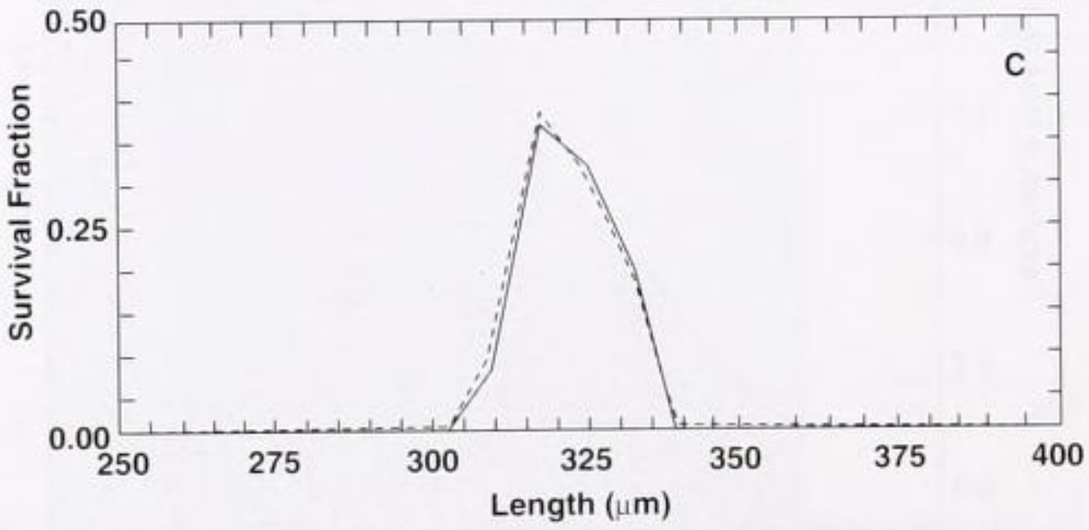


Figure 19. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis obtained when metamorphosis occurs when neutral lipids are greater than 80% of polar lipid. The simulation used the reference case environmental conditions given in Figure 9.

Figure 20. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg-size, (B) base respiration rate, and (C) larval length at metamorphosis obtained when metamorphosis occurs when neutral lipids are greater than 110% of polar lipid. The simulation used the reference case environmental conditions given in Figure 9.

survival times were similar to those observed for starved larvae (His & Seaman 1992, Laing 1995). Survival times of simulated larvae starved after day 4 were two to eight days, with most dying within two days. Larvae starved after day 9 survived two to ten days, with most dying in the first four days. Thirty-three percent of larvae starved after day 14 completed metamorphosis. These were larvae that came from relatively large eggs with low base respiration rates (Fig. 23B). Such larvae were much closer to metamorphosis on day 14 when starvation began than other larvae and were able to complete metamorphosis using their energy stores.

This last simulation is particularly interesting because a narrower range in egg size produced successful larvae. In other simulations, varying base respiration rate was much more significant in determining survival than varying egg size. The simulation supports the conclusion of Gallager et al. (1986) and Gallager and Mann (1986b) that egg quality is important in minimizing losses due to low food supply during larval life.

Genetics of Egg Size and Respiration Rate

As discussed previously, it was necessary to model larval cohorts characterized by a range of egg sizes and growth efficiencies to obtain the observed ranges in larval life span, size at metamorphosis, and survivorship seen in experimental studies of *C. gigas* larvae. One important choice, then, was the mean of the frequency distribution chosen for these two variable characters.

The average egg size was taken to be 50 μm in the reference simulation. With a few exceptions, varying the conditions of the simulation did not vary the range of viable egg sizes to a great degree (Figs. 9–22). The range of viable egg sizes is dictated by the most basic constraints imposed by biochemical composition at birth and by environment (e.g., food supply) rather than by genetics. Not surprisingly, changing the initial egg distribution so that it is centered on a 60 μm egg, rather than a 50 μm egg, results in survivorship that is skewed toward larger egg sizes (Fig. 24A versus 9A) because most eggs of 40 to 70 μm in size are viable

TABLE 4.

Total population survivorship for simulated cohorts of *Crassostrea gigas* larvae in which successful metamorphosis occurs when neutral lipid reserves exceed by a given fraction the polar lipid content.

Neutral lipid-to-polar lipid ratio	0.8:1	1.0:1	1.1:1	1.2:1
Cohort survivorship	80.5%	62.4%	26.5%	0.0%

TABLE 5.

Total population survivorship for simulated cohorts of *Crassostrea gigas* larvae exposed continuously to different temperatures (°C) and salinities (‰).

Temperature	15	20	25	30	35
Cohort survivorship	0.0%	0.0%	62.4%	63.3%	0.0%
Salinity	15	20	25	30	35
Cohort survivorship	0.0%	33.1%	63.3%	62.4%	31.8%

(Fig. 9), and moving the average size higher simply increases the total number of eggs spawned in this higher size range. Respiratory effects on survivorship (Fig. 24B) are not altered by a change to mean egg size. However, the larval length at the time of metamorphosis is somewhat larger (Fig. 24C). Total survivorship declines somewhat from 64% to 60% because more of the cohort that is spawned falls into egg sizes above 70 μm. Overall, however, the simulation shows that a moderate change in mean egg size does not materially change the outcome of the simulation. As the simu-

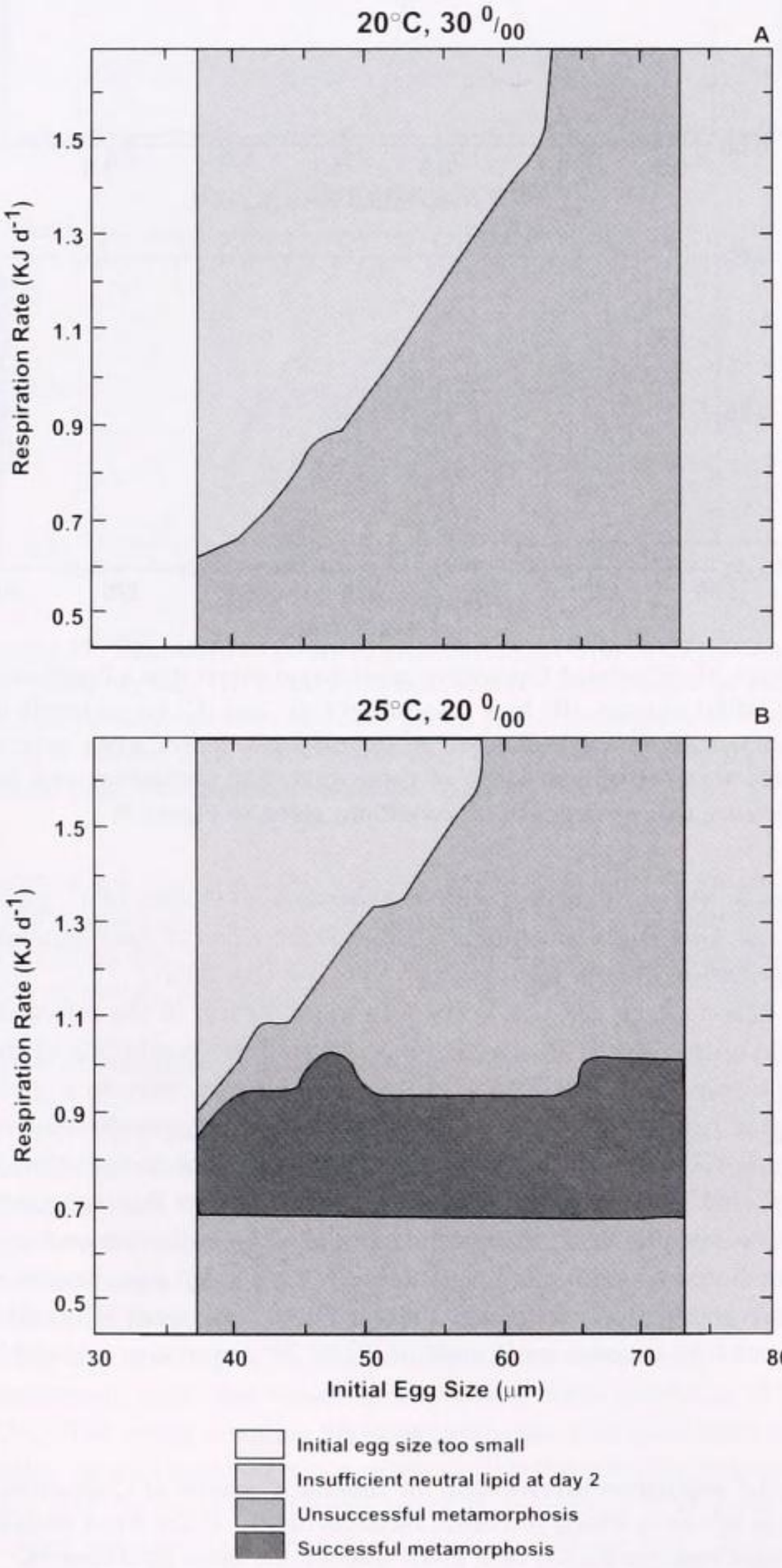


Figure 21. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and base respiration rates at (A) 20°C, 30‰ and (B) 25°C, 20‰. Food concentration was 2 mg L^{-1} with a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0.4:2.5.

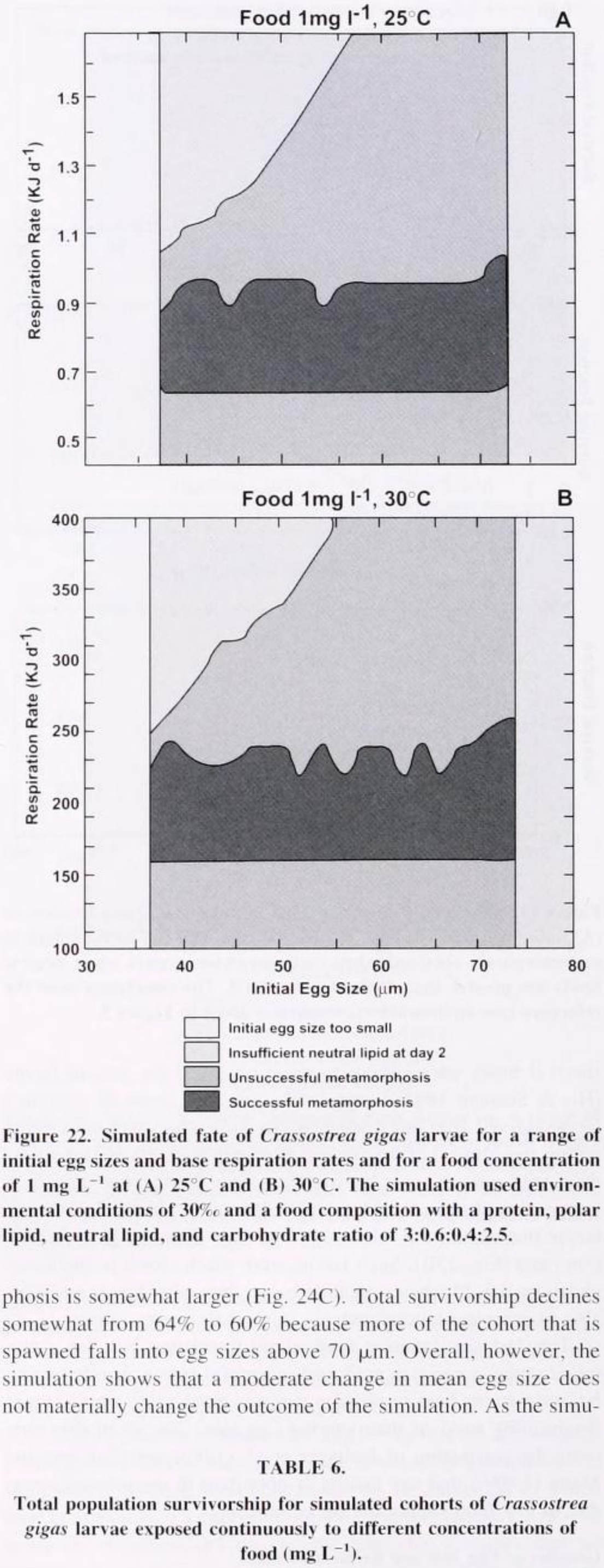


Figure 22. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and base respiration rates and for a food concentration of 1 mg L^{-1} at (A) 25°C and (B) 30°C. The simulation used environmental conditions of 30‰ and a food composition with a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0.4:2.5.

TABLE 6.

Total population survivorship for simulated cohorts of *Crassostrea gigas* larvae exposed continuously to different concentrations of food (mg L^{-1}).

Food	0.5	1.0	2.0	4.0
Cohort survivorship	0.0%	27.2%	62.4%	64.4%

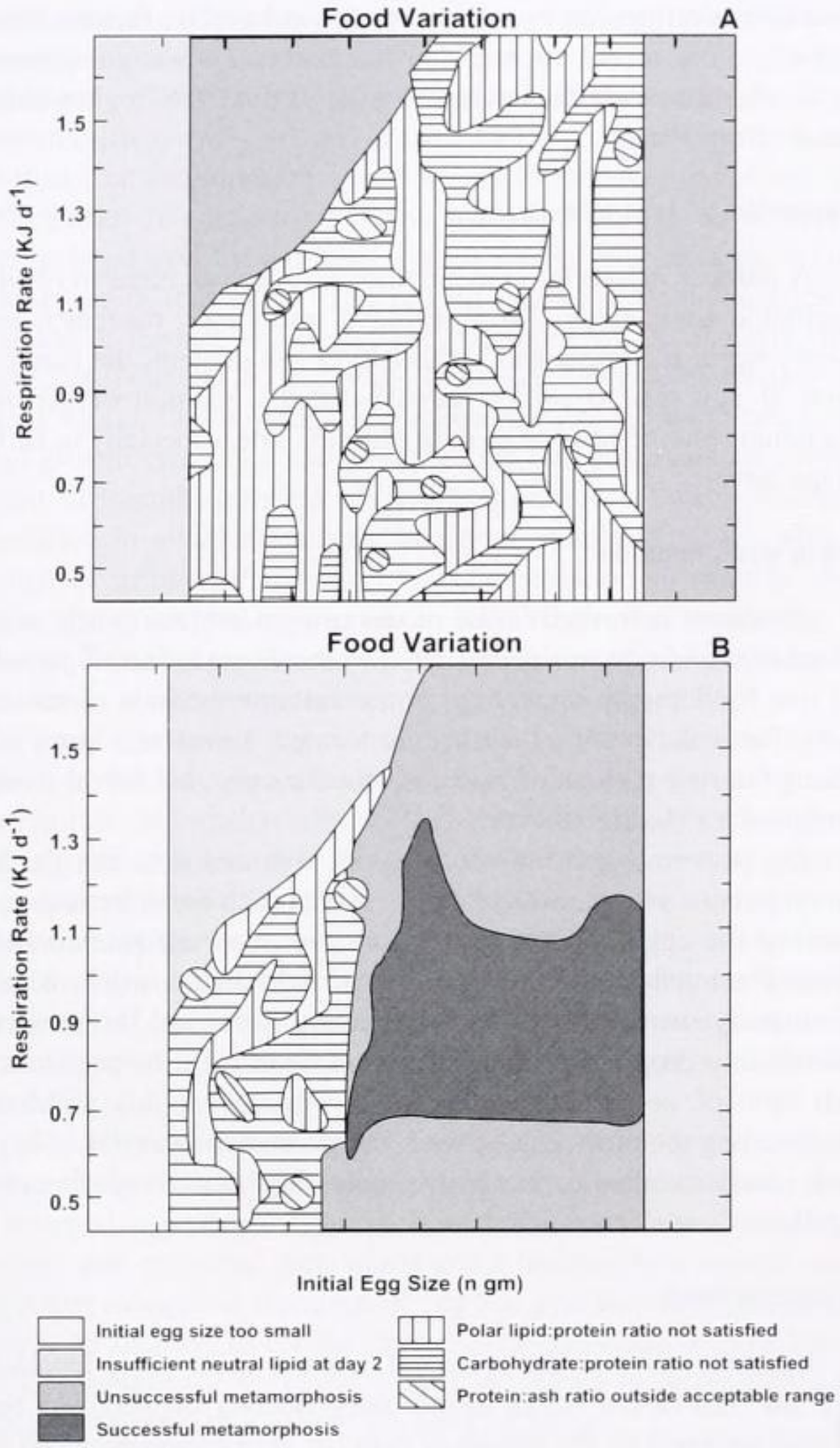


Figure 23. Simulated fate of *Crassostrea gigas* larvae for a range of initial egg sizes and base respiration rates and (A) a food concentration of 2 mg^{-1} available for days 1 to 4 and zero afterward and (B) a food concentration of 2 mg^{-1} available for days 1 to 14 and zero afterward. The simulation used environmental conditions of 25°C, 30‰ and a food composition with a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0.4:2.5.

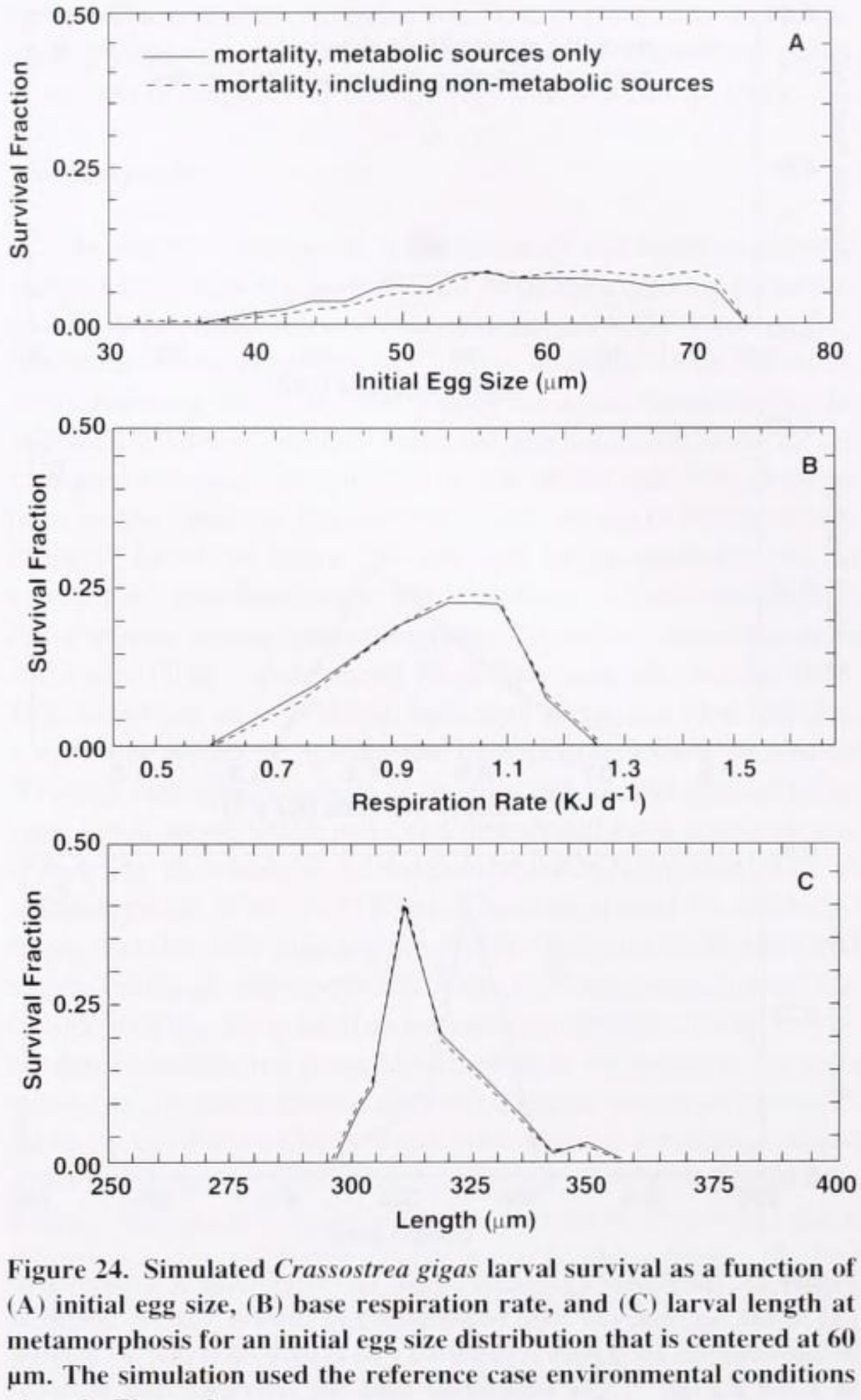


Figure 24. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis for an initial egg size distribution that is centered at 60 μm. The simulation used the reference case environmental conditions given in Figure 9.

lation depicted in Figure 23B shows, this outcome may not be repeated in cases of nonsaturating food supply.

Average base respiration rate for larvae was set at 1.05 KJ day^{-1}. Increasing the mean base respiration rate by 20% to 1.25 KJ day^{-1} results in larval survival skewed toward lower respiration rates (Fig. 25B) and smaller egg sizes (Fig. 25A), with a rapid decrease in survival above respiration rates of 1.05 KJ day^{-1} and egg sizes above 60 μm. Larval survival peaks at a metamorphosis length of 308 μm, which is similar to that obtained in the reference simulation (Fig. 9C). However, larval survivorship decreases rapidly for larger larvae (Fig. 25C). Total survivorship declines dramatically from 64% to 33%. Higher base respiration rates penalize larger larvae because insufficient excess neutral lipid can be obtained under the food supply provided to cover tissue maintenance and provide the requisite energy stores for metamorphosis. Thus, the model is considerably more sensitive to moderate changes in base respiration rate than in mean egg size. Conversely, the influence of environment on the range of viable base respiration rates is much greater than on the range of viable egg sizes and, so, a change in mean respiration rate might easily produce an alternate result under different environmental conditions.

DISCUSSION

Perspective

The model described here is unique in that it seeks to recreate many of the growth and mortality phenomena observed in *C. gigas* larvae from basic biochemical principals. The approach was dictated by a desire to model the influence of food quality and short-term changes in food supply on larval growth and survival and the influence of egg size and composition on ultimate success at metamorphosis.

Although biochemically based, the model contains only the crudest biochemical constructions. The larva is modeled as a four-constituent organism composed of protein, carbohydrate, neutral lipid, and polar lipid. Each constituent and the transitions between constituents are modeled using the simplest of flow schemes. So, for example, lipid and carbohydrate are interchangeable, carbohydrate covers respiratory demand when in sufficient supply, and

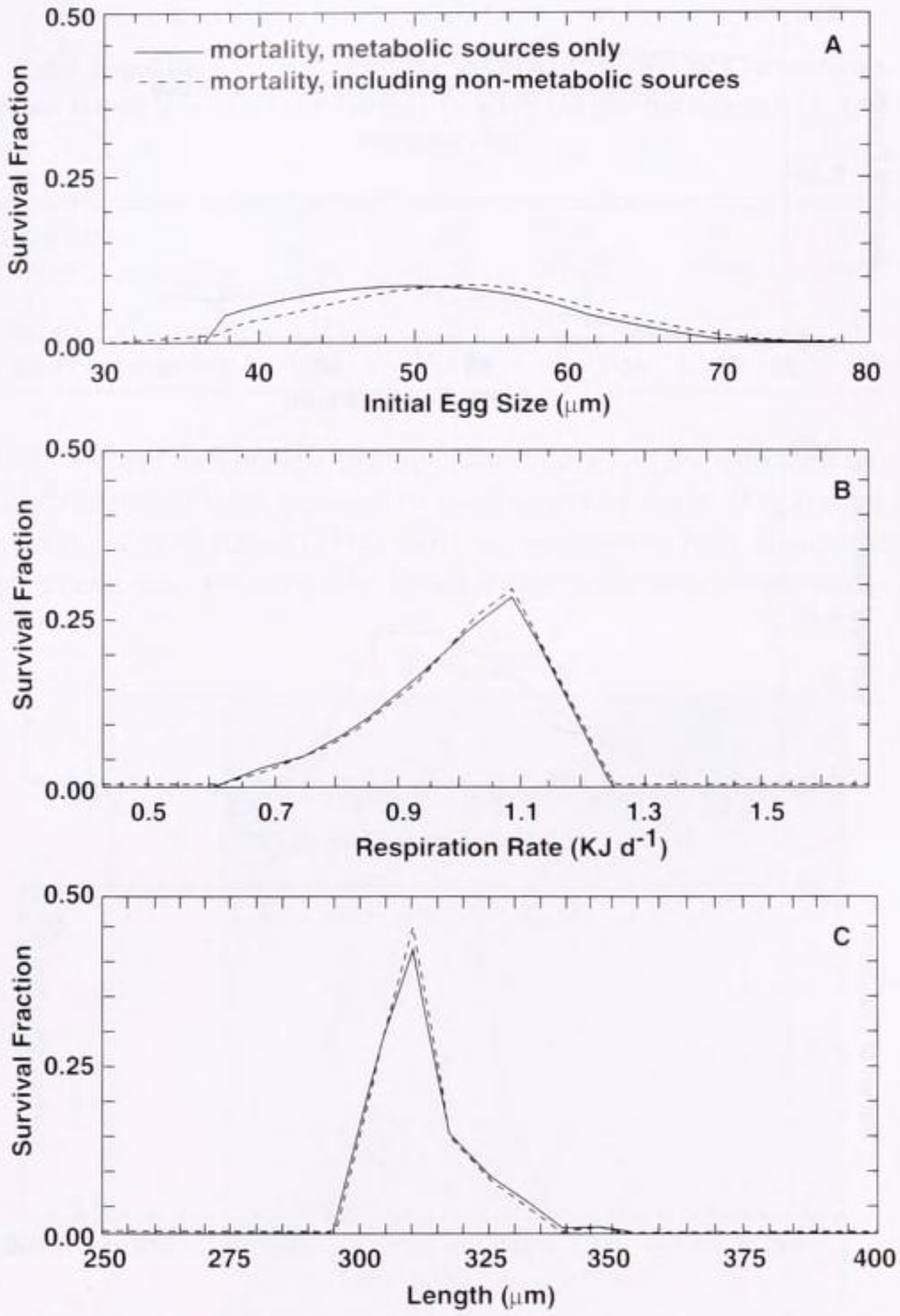


Figure 25. Simulated *Crassostrea gigas* larval survival as a function of (A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis for a base respiration rate distribution that is centered at 1.254 KJ day^{-1}. The simulation used the reference case environmental conditions given in Figure 9.

neutral lipid is the primary storage component. Assimilated protein is used only to create tissue protein, and this necessitates the formation of a certain amount of structural carbohydrate and polar lipid. Failure to supply these other components in the amounts required by protein assimilation results in structural imbalances and eventually death. More complex biochemical transformations are excluded. So, for example, although many amino acids can be synthesized, protein, in the model, comes only from protein ingested as food, with the one exception early in larval life when neutral lipid is used to sustain growth in the first few days after birth. Carbohydrate and protein, though potentially used as energy reserves for metamorphosis or to sustain the larva during periods of negative scope for growth, are only used as the last resort and, then, only in proportion to their fractional contribution to structural tissues.

Although based on a simplistic biochemistry, the model succeeds in simulating some of the basic observations of *C. gigas* eggs and larvae, suggesting that simple biochemical constructs can be successful and may encompass the biochemical transitions most prominent in determining cohort success. Verifications of many of the details in the simulations cannot be accomplished because of limited information on the details of larval biochemical composition as it is influenced by environmental and genetic factors. Nevertheless, the model succeeds in simulating known population-level characteristics that permit verification at this higher-order level of integration.

Necessities of Model Construction

A number of special model constructs were required to obtain verifiable simulations. These included resolving a mismatch between egg ash content and earliest larva ash content, the conversion of egg size to earliest larval size, the addition of genetic variation, and the need to modify filtration rate, especially in early stage larvae.

Birth and Condition

Condition is tracked in the model using a protein-to-ash ratio. Tracking condition was necessitated by the desire to model periods of low food supply, including, in the extreme, periods of starvation. Particularly once the shell is formed, larval size does not change during periods of restricted food supply, but larval tissue weight does (Laing 1995).

The protein-to-ash ratio for eggs is high and does not fit the larval pattern. Hence, initializing the model with the protein-to-ash ratio of the egg consistently failed to produce verifiable simulations. Presumably, as the egg develops and hatches, ash is added from inorganic solutes in the surrounding water and the protein-to-ash ratio drops. The model was initialized with the protein-to-ash ratio of newly hatched larvae to circumvent this problem. Reconciling the mismatch between the protein-to-ash ratio of eggs and newly hatched larvae will require additional experimental studies.

Length at Birth

Eggs are more or less spherical. Larvae, even newly hatched, are not. The model tracks length independently of weight, a necessity imposed by the wealth of data for verification provided in terms of length and the need to follow condition. Initializing the model with egg "length" (diameter) fails because the increase in length during egg development is not representative of growth, but simply a result of tissue reorganization. Consequently, a growth model cannot account for this process. We used a conversion from egg diameter to earliest larval length to circumvent this problem.

Genetic Variation

One of the key observations recorded in the literature is the success rate at metamorphosis and the size of metamorphosing larvae. Considerable variability exists depending upon the conditions of larval culture, and egg quality provides a sufficiently strong signal that variations in egg quality should influence success at metamorphosis in simulated spawns. Considerable variability also exists within cohorts. Such variability cannot exist if all larvae in the cohort are equivalently affected by environmental conditions. Consequently, it was necessary to add some variation between larvae to the model.

The observations of Gallager and Mann (1986a) and Gallager et al. (1986) provide a basis for describing a range of egg sizes with a simple Gaussian function to define the frequency of a given egg size in a cohort. This range in egg sizes also produced a range in egg qualities in that larger eggs were relatively more lipid rich. The resulting simulations showed an improved fit to observation in

that a range of larval sizes and success rates at metamorphosis were obtained.

However, the range in predicted larval size and success at metamorphosis was still too constrained in comparison to observation. The obvious next option was to include a range of growth efficiencies. Genetic variation in growth efficiency is well described and may accrue from any number of processes including variations in respiration rate, protein turnover, assimilation efficiency, or feeding efficiency (e.g., Garton 1984, Koehn and Hilbish 1987, Garton & Berg 1989, Koehn & Bayne 1989, Garton & Haag 1991). In the model, respiration rate and filtration rate control growth efficiency and, although the two processes are somewhat differently affected by temperature and salinity, inserting variation in either effectively generates simulated larvae with a range of growth efficiencies. Genetic variation in growth efficiency was inserted as a range in base respiration rates using a simple Gaussian construction. This addition produced the range in outcomes at metamorphosis expected from observation.

Simulations were run to examine the influence of varying the mean of the Gaussian distribution describing egg size and respiration rate. Simulations did not change markedly with a variation in egg size because the range of viable egg sizes was tightly constrained, as discussed later. The model was more sensitive to variations in the mean base respiration rate. Here, however, simulations showed that little leeway existed for varying the central tendency of base respiration rate because substantial changes in cohort survival occurred with relatively small changes in central tendency.

These results are compared, for the most part, to observations taken under saturating conditions of food and near-optimal environmental conditions. Optimal base respiration rates, to a large extent, and egg sizes, particularly under limiting food supply, suggest that changes in the range of egg size and base respiration rate might be adaptive in certain cases that might routinely exist under field conditions. One might expect variations in respiration rate (=growth efficiency) to be the most adaptive.

Filtration Rate

Measured filtration rates always provided growth rates higher than observed. The mismatch was largest for smallest larvae. In these animals, observed reductions in neutral lipid clearly indicated that assimilation does not provide adequate resources to explain observed growth, although measured filtration rates would indicate otherwise. It seems likely that filtration rate and ingestion rate are not equivalent in larvae or that assimilation efficiency is size-dependent.

In the model, a size dependency on ingestion rate or assimilation efficiency is effectively equivalent, so no attempt was made to distinguish between the two. One might reasonably conclude that both feeding and digestion should be affected by larval development processes, particularly during early larval life, and that this might lower the amount of energy realized at a given filtration rate. One might also conclude that filtration efficiency in part is a function of the larva's use of the vellum to maintain its position in the water column as well as to feed and, so, particularly under conditions of saturating food supply where most filtration rates are measured, a tendency to filter more material than can be ingested should exist. Regardless of the cause, to lower growth rates from levels predicted from observed filtration rates, we imposed a size-dependent penalty on ingestion that was largest for the smallest larvae. The mismatch between observed growth rates and simulated growth rates from measured filtration rates, however, points to an area of early larval biology that warrants further study.

Metamorphosis

The simple biochemical construction of the model required a simple explanation for the metabolic basis for triggering metamorphosis. A full explanation of how endogenous and exogenous factors control metamorphosis (e.g., Coon & Bonar 1986, Fitt et al. 1990, Berias & Widdows 1995) does not exist. Accordingly, the approach used was derived within the limitations imposed by the four-pool biochemical construct of the model and five observations in the literature that directly related to it. (1) Filtration rate drops in larvae of about 250 μm and larger, probably due to changes in either behavior or the beginnings of tissue reorganization that must presage metamorphosis. The former option would be sufficient. Older larvae spend more time near the bottom (e.g., Dekshenieks et al., 1997) and, thus, may spend less time filtering, a fact that would be interpreted in experiment as a decline in filtration rate. The reduction in feeding rate should ultimately reduce larval scope for growth, and this should have consequences concerning the decision to metamorphose. (2) Smallest size at metamorphosis is about 275 μm. This size should be somewhat larger than the size triggering the decline in filtration rate. (3) Lipid stores decline at metamorphosis. This could be a consequence of a decline in scope for growth as well as a consequence of the energy needed to reorganize tissue. (4) Literature information suggests that larvae require a certain amount of stored energy to metamorphose successfully. Although any kind of tissue constituent might provide this energy, the reliance of larvae on neutral lipid as the primary energy store suggests that the proportion of neutral lipid is a good measure of energy available for metamorphosis. (5) The quantity of lipid present in the egg influences larval survival. Thus some information on the status of neutral lipid reserves should pertain to the decision to undergo metamorphosis.

The process of metamorphosis was modeled using these five observations to generate explicit triggers for certain steps in the process, as follows. (1) Larvae were assumed to become potentially competent to metamorphose at 275 μm, following a decrease in filtration rate at 250 μm. Simulations showed that the filtration rate decline could not be set at 230 μm or 275 μm. The minimum size for metamorphosis, in most simulations, did not fall below 285 μm, so that the 275 μm size limit was rarely invoked. That is, invoking a change in filtration rate at 250 μm normally resulted in larvae metamorphosing at sizes above 275 μm. (2) Larvae were assumed to become competent to metamorphose when a daily decline in neutral lipid of a certain level occurred. Our assumption was that larvae might be expected to continue to grow and store lipid as long as a sufficiently positive scope for growth was present and this would enhance success, but that a decline in neutral lipid would reduce success. Accordingly, metamorphosis should occur when scope for growth dropped significantly below zero. The range of observed sizes at metamorphosis suggests that some process of this sort does occur. Although the decline in filtration rate at 250 μm predestined larvae to eventually reach the trigger point defined by a significant neutral lipid decline, food quantity and quality and biochemical composition can permit growth much in excess of 250 μm before scope for growth drops to substantially negative values. Typically, in the model, metamorphosis occurred at sizes of 300–330 μm, as observed in culture. (3) Larvae were

assumed to metamorphose successfully if neutral lipid supplies were adequate. Adequacy was judged as a ratio between energy stores and structural components.

We cannot evaluate how accurately the modeled mechanism for metamorphosis approaches reality, not having available an adequate understanding of the biochemistry of the process. However, the simulations reveal some interesting trends. The choice of 250 μm as the point where filtration rate declines is based on observation, but the model also indicates that this trigger is tightly constrained to this size. Neither 230 μm nor 270 μm sizes offered verifiable results. The choice of a 25% daily decline in neutral lipid triggering competency is also tightly constrained. Values of 10% and 40% did not provide results equivalent to observations. Both larval size distributions and success rates at metamorphosis varied from observations. The choice of a ≥1:1 ratio of neutral lipid to polar lipid is also tightly constrained. Values of 0.8:1 and 1.1:1 produce unrealistic size distributions and success rates at metamorphosis.

Verification of this construction for modeling metamorphosis was directed at evaluating performance in simulating four important phenomenon: (1) variations in egg quality significantly influenced success at metamorphosis, (2) variations in food quality and quantity significantly influenced success at metamorphosis, (3) larval life span as predicted was well within the range of observations, and (4) larval size structure at metamorphosis was well within the range of observation. Obtaining these four results requires a reasonably accurate rendition of growth and survival at the biochemical resolution of the model. This suggests that the approach to modeling metamorphosis must reflect, in some significant way, the process as it actually proceeds in the larva.

Consequences of Model Construction

Larval success is determined by intrinsic and extrinsic factors. Intrinsic factors include egg size and quality and genetic makeup. Extrinsic factors include temperature, salinity, food quality, and food quantity.

Implications of Egg Size

Oyster eggs are about 50 μm in diameter, with a size range typically of 40–60 μm. The model identifies viable egg sizes in the range 37–73 μm, very similar to observations. Egg sizes outside this range are predicted to be nonviable due to lipid imbalances in early larval life. Very likely, the lower limit of 37 μm represents a packaging problem. Egg size is simply too small to provide adequate resources for the structural changes required in forming the first larval stage. In the model, the required structural tissue ratios cannot be achieved and still provide any neutral lipid reserves. In effect, the larva is never born. The upper limit of about 73 μm yields a larva that has insufficient neutral lipid reserves to cover metabolic needs immediately post-hatch. During this time, feeding is inefficient, and some of the larva's carbon needs for growth and tissue maintenance must be met by using neutral lipid reserves. The larger larvae, coming from eggs >73 μm in diameter, essentially starve to death before they can become competent filter feeders. This may provide one explanation for the small size of most planktotrophic eggs.

Presumably, the upper limit on egg size could be extended by increasing neutral lipid reserves; however, the bet hedging mode of life (e.g., Stearns 1976) would limit the amount of energy invested in any one embryo. The trade-off between additional energy expenditure and increased success at metamorphosis is clearly indicated in Figures 11, 15, and 23. Larger eggs yield successful larvae over a much larger range of respiration rates and environmental conditions than do smaller eggs. Larger eggs yield larvae that reach metamorphosis faster (shorter planktonic time), thus minimizing loss to predation and the chance of reduced survival from transient reductions in food supply. Thus, the simulations suggest that the average egg size of 50 μm minimizes the chance of reproductive failure, which increases rapidly at smaller egg sizes, while still permitting the spawning of a large number of eggs. As an example, increasing average egg size to 60 μm reduces total egg output by 31% at a given total energy expenditure. An equivalent increase in larval success is not achieved in our simulations.

The model also indicates, however, that transient reductions in food supply during larval life may increase the success rate for large eggs relative to small eggs. In this circumstance, the extra energy required to produce large eggs may be better repaid. Whether an increase in fitness is adaptively advantageous requires a better understanding of food supply under field conditions and how this influences larval survival.

Respiration (=Growth Efficiency) Effects

In the model, varying respiratory rate is equivalent to varying growth efficiency. Larvae with high growth efficiency have low respiration rates. The model identified an upper and lower limit to growth efficiency under defined environmental conditions. The upper limit varies widely depending upon environmental conditions, whereas the lower limit is relatively fixed. Simulations show that the upper limit on base respiration rate (e.g., ~1 kJ day^{-1} in Fig. 9) is determined by the point at which larvae cannot acquire sufficient neutral lipid stores to successfully metamorphose. Smaller eggs are also less viable because insufficient neutral lipid can be stored to cover larval needs over a few days post-hatch. Interestingly, very low respiration rates also normally result in unsuccessful larvae. These animals put too much assimilated carbon into somatic structural tissue and so have insufficient neutral lipid reserves. We are unaware of experimental data upon which to verify this last result.

Condition and Mortality

Many models do not explicitly follow length and weight independently (e.g., Powell et al. 1992, Dekshenieks et al. 1993). In bivalves, tracking condition permits observation of larval performance during periods of low food supply. This requires tracking length and weight independently such that not all increases in weight result in changes in length and such that no decreases in weight result in decreases in length. The performance of the model was evaluated under conditions of food deprivation by simulating the process of starvation. Although the mechanisms of death under these conditions are described in the model, death occurs due to a variety of biochemical imbalances, depending upon the initial status of the larva. Whether such a degree of complexity actually exists requires more information on the changes in larval biochemical composition under conditions of low food supply. However, the higher-level effects that integrate biochemical processes

were simulated by the model, including a decrease in weight (condition), a drop in neutral lipid content, a nonlinear time-dependent increase in mortality, and the still-successful metamorphosis of older larvae.

In addition to inadequate food supply, larvae can die if food of inadequate composition is ingested. Thus, rigorous criteria were set for biochemical compositions not allowed in viable larvae. Food having inadequate lipid or being too protein-rich resulted in mortality, even if the quantity of food remained high. These parameterizations describing mortality under such conditions are essentially *ad hoc* constructs (literature observations not being available), but they did produce cohort mortality rates that appeared to be realistic.

Effect of Diet

Most experimental studies on *C. gigas* larvae have used food supplies of ≥ 2 mg L^{-1}. This level of food saturates feeding and, in fact, raising food quantity from 2 mg L^{-1} to 4 mg L^{-1} in the model has little influence on simulated larval success. However, as in *Crassostrea virginica* (Dekshenieks et al. 1993), food quantities below 1 mg L^{-1} dramatically restrict larval growth and survival. As food supply declines, animals with high growth efficiencies are selected for in the model. At high food content, larger eggs with lower growth efficiencies also survive to metamorphosis. With rare exceptions, small eggs with low growth efficiencies never do. Thus, the influence of growth efficiency is nonrandomly distributed across egg size, and the influence seems to be mediated in part by food quantity and to a larger measure by food quality.

The influence of food content on *C. gigas* larval growth and survivorship has received considerable attention (e.g., Wilson 1978, Waldock & Nascimento 1979, Helm & Laing 1987). Although not all studies agree, low-protein diets and high-lipid diets often show improved growth and survivorship. The simulations show the positive effect of a low-protein diet on larval growth and survivorship. With this diet, a relatively larger portion of ingested energy is allocated to energy stores that in turn sustain the larva through metamorphosis. With a high-protein diet, larvae grow too fast and fail to store enough energy to sustain them through metamorphosis. The destination of protein within the larva is limited in terms of building tissue and covering metabolic needs (Table 2) if insufficient carbohydrate is ingested. Any transfer of excess amino acid into other tissue components is not permitted. Potentially, this allocation of ingested protein is too simplistic, although the simulations do provide some insight into the value of a low-protein diet.

Simulations with no neutral lipid gave similar results in terms of larval survivorship and growth. Thus, the relative amounts of protein and neutral lipid in larval food are important determinants of growth and survival.

A number of studies have identified specific components of the lipid pool as important dietary constituents (e.g., Thompson et al. 1994, 1996). The model could be expanded to track more complex biochemical pools such as polyunsaturated fatty acids (PUFAs) or sterols. The fact that the model achieves realistic simulations over a relatively wide range of environmental and dietary conditions indicates that the approach used to model larval biochemistry, including the subsuming of a diversity of lipid compounds into two pools, polar and neutral, is sufficient to provide realistic simulations of larval growth, metamorphosis and survival.

Temperature and Salinity

C. gigas is known to be relatively stenotopic for the genus. Temperature and salinity conditions describing optimal growth circumscribe a narrow range. The model reproduces this behavior. In this contribution, most simulations were run under optimal conditions of 25°C and 30‰. Lower temperatures result in insufficient neutral lipid storage and metamorphosis because feeding rate is low. Temperatures above 30°C result in biochemical imbalances due to high respiratory demand. Low salinity also results in insufficient food ingestion to meet the demands of metamorphosis. Again, data to verify the accuracy of predicted cause and effect on biochemical composition are not available.

Growth rate is a complex function dependent upon the balances of ingestion and respiration. The ability of positive environmental conditions to offset a reduction in food supply and vice versa depends upon the relative scaling of their affects on respiration and ingestion. Thus, increased temperature can "spare" a reduction in food, permitting the same growth rate, if the influence of temperature on ingestive processes scales with a larger exponent than the influence of temperature on respiration. The importance of differential scaling in the energy balance of bivalve molluscs and other animals is well known (e.g., Newell et al. 1977, Powell et al. 1992, Brown et al. 1993). Given the sensitivity of growth and survival to decreases in food supply, the fact that a decrease in food supply often occurs during summer months in *C. gigas* habitat (Kobayashi et al. 1997, Hyun et al., in press), when an increase in temperature is likely to be of significance in increasing ingestion rate, suggests that the differential scaling of ingestive processes and respiration is likely of significance for the reproductive success of the species.

CONCLUSIONS

A model that simulates the growth, development, and metamorphosis of *Crassostrea gigas* larvae has been developed. The model is the first of its kind in that it (1) tracks length separately from weight so that changes in condition can be followed and (2) predicts growth from the ingestion and transformation of biochemical constituents, thus permitting the simulation of the effects of changes in food quality. Food quality and feeding rate are important constraints in larval culture, so the model might be used to optimize culture conditions for *C. gigas* larvae as well as to investigate the influence of critical periods of food supply in larval development in the field. Of particular importance is the investigation of "teleconnections" during larval life in which events occurring at one point in larval life have consequences at another, temporally distant, point. The model has a crude depiction of the biochemistry of *C. gigas* larvae. However, the model works well even with this limited biochemistry and indicates that the formulation of sophisticated biochemically based models offers the promise of substantially improving the population modeling of marine larvae.

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A BIOCHEMICALLY BASED MODEL OF THE GROWTH AND DEVELOPMENT OF CRASSOSTREA GIGAS LARVAE

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ABSTRACT A biochemically based model was developed to simulale the grow th. development and metamorphosis of larvae of the

Pacific oyster, Crassosrrea gigas. The model is unique in that (1) it defines larvae in terms of their protein, neutral lipid, polar lipid,

carbohydrate, and ash content; (2) it tracks weight separately from length to follow larval condition index; and (3) it includes genetic

variation in growth efficiency and egg quality to better simulate cohort population dynamics. The model includes parameterizations

for larval filtration, ingestion, and respiration, which determine growth rate, and processes controlling larval mortality and metamor-

phosis. The initial biochemical content of the larva is determined by the composition of the egg. Changes in the initial ratios of protein,

carbohvdrate. neutral lipid, and polar lipid occur in response to the biochemical composition of available food as the larva grows.

Modeling the process of metamorphosis requires a series of size-based and biochemically based triggers: (I) larvae become potentially

competent to metamorphose at 275 |a.m. following a decrease in filtration rate at 2.50 (im; (2) larvae become competent to metamor-

phose when a daily decline in neutral lipid of 25'7f or more occurs; and (3) larvae metamorphose successfully if neutral lipid stores

exceed polar lipid stores. Although based on simple biochemistry, the model succeeds in simulating such basic characteristics of C.

gigas larval development and metamorphosis as larval life span and size structure at metamorphosis and the influence of egg and food

quality and food quantity on survival. These results suggest that simple biochemical constructs may encompass the biochemical

transitions most prominent in determining cohort success. Simulations of larval development show that for the smallest larvae,

assimilation does not provide adequate resources to explain observed growth, although measured filtration rates would indicate

otherwise. Egg lipid stores are needed to sustain the larva. The simulations also identify egg sizes in the range 37-73 [xm to be viable,

very similar to observations. Egg sizes outside this range are predicted to be nonviable due to lipid deficiencies in early larval life.

Similarly, simulations identify upper and lower genetic limits on growth efficiency beyond which larvae cannot acquire sufficient

neutral lipid stores to successfully metamorphose. As food supply declines, animals with high growth efficiencies are selected in the

simulation. Low-protein food diets are predicted to increase larval survival. High-protein diets result in insufficient carbohydrate and

neutral lipid to cover metabolic and storage needs. Thus, the influence of growth efficiency is nonrandomly distributed across egg size

and respiration rate and the influence seems to be mediated in part by food quantity and to a larger measure by food quality.

KEY WORDS: Cnusoslrea gigas. larvae. Pacific oyster, growth, development model, biochemical, food

INTRODUCTION

For benthic species that have a planktonic larval phase (if their

life history, survivability of the larvae is the key deterniinunt of

recruitment to the adult population. Thus, much research has gone into identifying factors affecting the growth, development, metamorphosis, and settlement of larvae, especially for species with commercial value, such as the Pacific oyster, Crassostreu gigas.

Larvae of C. gigas undergo growth and development that is typical of bivalve larvae, with progression through D-shaped. umbo, and pediveliger stages (Fig. 1 1. The fraction of developmental time spent in each stage is variable and the rate at which the larvae pi-ogress through each is affected by local temperature, salinity, and food conditions (Helm & Millican 1977, Malouf & Breese 1977, Nascimento 1980, Gerdes 1983a, His & Maurer 1988. Nell & Holliday 1988; His et al. 1989, Pauley et al. 1988, Arakawa 1990. His & .Seaman 1992. Robinson 1992. Thompson & Harrison 1992, Laing 1993, Thompson et al. 1996). The range of fractional development times reported for the small umbo to pediveliger stages (Fig. 1) is a reflection of these effects on larval growth.

Once spawned, the ultimate fate of C. gigas larvae is determined by the interaction of a number of factors. The first is the initial biochemical composition of the egg released by the adult, in particular the initial egg lipid content. Studies have shown correlations between egg size, lipid content, and bivalve larval development (Helm et al. 1973, Gallager et al, 1986, Gallager & Mann 1986a, Lee & Heffeman 1991). An implication of these studies is that initial egg lipid content is a determinant of larval survivorship and success at metainorphosis.

A second factor is the ability of the larvae to grow and develop so that total time spent in the plankton is minimized, thereby reducing exposure to mortality from predation. Because spawning pulses by adult populations, in nature, occur at widely variable times throughout the spawning season, larvae experience a widely varying set of environmental conditions. The timing of environiTiental conditions relative to a particular phase of larval life can greatly affect the total time needed for larvae to complete development (Dekshenieks et al. 1993). The concept of a critical period immediately post-hatch during which many planktonic larvae are particularly susceptible to low food supply is now well established (Gushing & Dickson 1976, Anger et al. 1981, Taggart & Leggett 1987), and the available data for bivalve larvae support the sensitivity of larvae to periods of low food supply (Gallager et al. 1986, His and Seaman 1992, Laing 1995). Studies also suggest that food quality, not just food quantity, is a critical issue (Thompson et al. 1994, 1996), Insufficient dietary lipid, for example, significantly limits larval growth and survival (Helm et al. 1973. Gallager et al. 1986).

A third factor affecting larval survival is the ability to acquire sufficient internal energy stores to successfully complete metamorphosis and set. Studies have shown that larvae can reach the size

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350

300

250-

-§ 200

S 150

100

50

0.2 0.4 0.6 0.8

Fractional Development Time

1.0

Figure 1. Schematic of the de\el()pniental stages of Crassiislrea gigas lar>ae as a function of fraction of dexelopnient time. The two curves hraclvet the range of sizes for the different stages, as reported in Ar-

akawa (1990).

needed for metamorphosis but be unable to complete this step (Robinson 19')2. Haws et al. 1993. Laing 1993). The possibility that a shoit-terni deprivation of food early in larval life may reduce metamorphosis success can be inferred from a variety of studies that emphasize the critical importance of adequate food throughout larval life.

The inci'easing emphasis on the importance of an adequate diet, mcluding quantity and quality, in determining a larval cohort's success and the recognition that adequate energy resources are needed for successful metamorphosis suggest the need to incorporate biocheinical transfers into models of larval growth and development. The existing lar\al models determine growth tYom ingestion rate, decremented by the energy losses due to respiration and incomplete digestion (e.g., Carlotti & Sciandra 1989, Dekshenieks etal. 1993, 1996). These types of models, however, cannot be used to examine issues of food quality, nor can they be used to simulate the energy-reserve hypothesis underlying metamorphosis success. To investigate the influences of food quality and quantity on growth, development, and successful metamorphosis of C. gigas larvae, a biochemically based model was developed that includes explicit parameteri/ations for the metabolism of protein, carbohydrate, polar lipid, and neutral lipid within the standard parameteiizations of energy flow via ingestion, assimilation, and respiration.

The following section provides a description of the C. gigcis

larval model and the pai'ameterizations used in this model. This is followed by simulations that illustrate the effect of initial egg size, food quality, food quantity, and environmental conditions on larval growth, survival, and success at metamorphosis. The discussion section places these simulations within the context of the current understanding of the effect of environmental conditions and food quality on larval growth, survival, and metamorphosis.

MODEL DESCRIPTION

Model Structure and Governing Equation

The change in length for an individual larva over time is given by:

It'

aL

(1)

whei'e L is larval length in jxm and a is the rate at which the larva grows and has units of day'". Larval growth rate (a) is based on formulations that allow differential metabolism of the protein, carbohydrate, and lipid content of the food ingested by the larva. Thus, net production is expressed as the difference between assimilated ingestion (AI) and respii^alion [R):

NP,

Al, - R,

(2)

where / represents the four basic biochemical components included in the model: protein, polar lipid, neutral lipid, and carbohydrate. An increase in larval length occurs when the sum of the four, Y.t= i NP. is positive: when larval condition index is maximal for a given size: and when the restrictions imposed by certain biochemical ratios are simultaneously met. Thus, excess net pioduction, ENP. is the basic quantity responsible for larval growth.

The specification of ENP. which determines a, is based on filtration, the metabolism of carbohydrates, polar lipids, neutral lipids, and proteins within the larva, and the conversion of the metabolized food into structural components of the larva versus the conversion into storage material. A basic assumption in this model is that the formation of structural components determines the increase in larval length. Material converted into storage components (i.e., neutral lipids) does not result in increased larval length. The conversions and parameterizations used to model these processes are described in the following sections.

The C. gigas laival model given by Eq. (1) was solved numerically using a third-order Adains-Bashforth scheme (Canuto et al. 1988) with a time step of 0.1 day.

Model Parameterizations

Observations from field and laboratory studies were used to derive the relationships that describe the processes affecting net production of C. gigas larvae. The basic units used in the model are grams, joules, and (jtm. and these are not necessarily always consistent with the units used for measurements. Thus, the first step in developing the lar\ al model was to obtain conversions that allow the model calculations and output to be consistent with measurements and to be compared with observations. Also, many of the larval processes vary in amplitude or even form with larval size, requiring that lelationships used to describe these be size dependent.

[Begin Page: Page 245]

Lcngth-I()-I)r\ Tissue Weight Conversion

Numerous field and laboratory data sets exist that can be used to derive a length-to-dry tissue weight relationship for C. gigas larvae. However, the reporting of these data sets is quite variable, with some reported in terms of dry tissue weight (Gerdes 1983a) and others reported in terms of whole animal weight (His & Mau-

rer 1988. Nascimento 1980. Waldock & Nascimento 1979). Dry tissue weight is the desired unit for deriving this relationship, so the data sets reported as whole animal weight were multiplied by a factor of 0.25. which corrects for the shell being 757c of the animal weight (His & Maurer 1988). The resultant data set (Fig. 2) was used to obtain the length-to-dry tissue weight relationship:

at"

W

(3)

where IV is larval weight in ng and L is larval length in (a.m. The coefficients a and h are given in Table I . The correspondence between Eq. (I) and the data sets is shown in Figure 2.

Length-to-.\sh Weight Conversion

His and Maurer (1988) provide measurements of the percent of total dry weight as a function of larval size. These values were obtained by summing the percent organic matter represented by larval protein, carbohydrate, and lipids that were determined independently. As a comparison. His and Maurer (1988) also determine the percent total dry weight of organic matter by combustion of the whole animal. These data form the basis for developing a relationship to relate larval length to ash weight.

Comparison of the organic matter values determined by the

sum of the components and those obtained from the heating method differ by about 5%. The difference was assumed to be due to protein, which is more completely measured by the heating method, so this difference was added to the protein value for a given size larva. The percent total dry weight for the largest larval size measured by His and Maurer (1988). 274 p.m, was reduced from 29. 1 % to 26.2% because attempts at using the higher value to produce a relationship between larval length and ash weight gave

2000

1600

G)

C

5, 1200

'5

5

0)

3

(A

</)

800

Q

400

50

100

300

350

150 200 250

Length (m)

Figure 2. Dry tissue weight as a function of Crassostrea gigas larval length.

TABLE 1.

Dennition, units, and values for the variables used in the Crassostrea gigas larva UKxIel equations.

unrealistic results. The reduction in percent total dry weight was obtained by maintaining the same proportional difference in the heating and sum of component determinations of percent dry

weight as reported for other larval sizes. The rationale for the reduction in value is that the measured value included effects of metamorphosis that are not explicitly included in the larval model. The percent total dry weight for the 274 p.m larva was also applied to the measured dry weight (7000 ng) for 320 ixm larvae to provide an additional data point at the larger size.

The length-to-ash weight data set was fit with a regression of the form

AW = cL''

(4)

where A IV is larval ash weight in ng larva'' and the coefficients c and (/ are given in Table I . The coirespondence between the data and fitted curve is shown in Figure 3.

Length-to-Protein-to-Ash Ratio Conversion

The protein values for the different sized larva reported in His and Maurer (1988) were divided by the corresponding larval ash

[Begin Page: Page 246]

300

350

150 200 250

Length (um)

Figure 3. Ash weight as a function of Crassostrea gigas larval length. The solid circles are measurements given in His and Maurer (19881. The open circles represent measurements given in His and Maurer (1988) that were modified as described in the text.

weight values, delermined as described in tlie previous section, to obtain protein-to-ash ratios as a function of larval size (Fig. 4). The curve fit to these data is of the form:

PAR

+ ii-.L + a,L- + a_iL^

(^)

where PAR is the protein-lo-ash ratio and the coefficient \alues are given in Table I. This relationship describes a ratio that decreases from a value of 0.27 to a minimum of 0. 1 8 at a larval size of about 100 |xm, after which it again increases. The initial decrease is

associated with the development of shell. The increase in the ratio as the larva gets larger reflects the decrease in the surface-to-volume ratio of the larva as it grows.

Carbohydrate, Protein, Polar Lipid, and Neutral Lipid Conversions

In the model, conversions between carbohydrate, lipid, and protein were needed to account for the debiting of the biochemical

0.6

0.5

o

SO.4

o 0.3

0.2

0.1

50

100

250

Figure 4. Protein-to-ash ratio as a function of Crassostrea gigas larval length. The solid circles are the ratios calculated from the protein and ash values given in His and Maurer (1988).

pools to cover the metabolic cost of respiration and the movement of carbon between different tissue types. The needed conversions are of two types. Those for respiratory demands are expressed in terms of energy (e.g.. joules); those for tissue conversions are expressed in terms of moles carbon.

Respiratory demands were converted to equivalent energy values using 39.5 kJ g"' for lipids. 23.6 kJ g~' for protein, and 17.2 kJ g~' for carbohydrates. These conversions are based on data given in Mann and Gallager (1985). Trytek and Allen (1980). and Crisp (1971).

Interconversion of biochemical constituents was based on carbon equivalent weights using palmitic acid, serine, and glucose residues, respectively, to define the ratio between moles carbon and molecular weight. In this way. carbon atoms were conserved,

but the total constituent weight changed depending upon the contribution of carbon to the molar weight. So, for example, the conversion of tissue carbohydrate to tissue lipid is based on a ratio of 1.929, which expresses the relative weight of lipid and carbohydrate based on the equivalency of carbon atoms.

In addition, certain weight ratios between structural constituents were associated with healthy larvae. When in sufficient quantity, assimilated food constituents were allocated to tissue pools using these ratios. Deviations in the resulting tissue composition from these ratios resulted in mortality. The relationship between tissue lipid and protein was obtained from His and Maurer (1988), under the assumption that the total lipid content of C. gigas larvae, like C. rirginica larvae, is about evenly split between neutral and polar lipids (Gallager el al. 1986. Whyte et al. 1987). This yielded a preferred tissue polar lipid-to-tissue protein ratio of 0.11 for healthy larvae. Information from the same sources was used to establish the equivalent ratio between tissue carbohydrate, most of which was assumed to be structural since neutral lipid is the primary stoiage constituent, and tissue piotein. The value of the tissue carbohydrate-to-tissue protein ratio was set at 0.01. Both ratios were independent of larval size.

Parameterization of Processes

Filtration Rate and Filtration Efficiency

Filtration is the basic process by which C. gigas lai^ae obtain food. Therefore, accurate representation of this process is needed

for larval growth to be correctly simulated. Gerdes (1983a) presents measurements of filtration rates for C. gigas at a range of food concentrations and larval sizes. These data show three distinct phases of larval filtration (see Fig. 8 in Gerdes 1983a). Initial filtration rates are low until the larva reaches about 100 ixm. after which filtration rate increases exponentially to its maximum as the larva reaches 250 p.m. At this size, larval behavior changes as the larva nears metamorphosis and filtration rate decreases dramatically.

The filtration rate observations given in Gerdes (1983a) were used to derive empirical relationships that provide the basic filtration structure of the model (Pic. 5):

FR = FR„ e''''*''' '-*''' '''. for larva <250

|j.m

(6)

FR = FR,/''*- '-^'^ '•'''' ' ', for larva >250 (xm (7)

wheie FR is filtration rate. The coefficients in Eqs. 6 and 7 are given in Table 1 .

Initial simulations that used the above relationships for larval

[Begin Page: Page 247]

Biochemical Model of C. g/gas Larvae

247

Metamorphosis -

50 100

150 200 250

Length (i^im)

300 350

Figure 5. L'pper ciir\ e. nitration rate as a t'unction of Crassostrea gigas larval length calculated from Eqs. 6 and 7 as described in the text. Lower curve: nitration rate after corrections described by Eqs. 8 and 9 (for food concentration of 2 nig 1^ ').

tlllratioii rate resulted in growth rates that were too high. Reduction of the growth rate was accomphshed by adding a factor to the fikratioii rate that reduces ingestion efficiency. This factor is of the form:

IE--

7

Food

a + p

L-L

(8)

where ingestion efficiency (/£) is a nondimensional quantity that depends on the ambient food concentration (Food) and larval size. Coefficient values are given in Table 1 . The relationship given h\ Eq. 8 results in reduced feeding efficiency for all larval sizes, but with the maximum reduction associated with smaller larvae.

The rationale for reducing filtration efficiency is that the vellum is a multipurpose organ, so there must be some inefficiency in each of the activities and functions of this organ; otherwise the larvae could only swim at the rate that allows maximum ingestion and dispersal, and escape capabilities would be compromised. Thus, filtration rates measured in a laboratory setting for C. gij^as larvae should be regarded as measures of vellum activity and not as measures of ingestion. In addition, most research has been conducted using saturating food concentrations. This would exacerbate any tendency for more food to be filtered from the water column than could be ingested by the larva.

Applying the ingestion efficiency factor (Eq. 8) to Eqs. 6 and 7

resulted in realistic larval growth rates, except for larvae smaller than 80 (Jini. Early in larval life the rapid changes leading to the development of the organs for feeding and digestion should further limit ingestion and/or assimilation efficiency. Thus, the filtration rate for small larvae (FR^) was further reduced by:

IE=IE-?>{ 1 + mill 1

L-L,

L - L,

(9)

The above approach is based on the assumption that the vellum in small larvae is very inefficient at capturing food particles and/or

that digestion is less efficient in small larvae. Coefficient values and definitions are given in Table I .

A relationship between low food supply and increased feeding efficiency due to enlargement of the vellum (.Strathmann et al. 1993) was not included in the model because the influence of vellum enlargement on filtration rate is unknown. The model may underestimate growth rate at low food supply.

Temperature and Salinity Effects

As with most bivalve larvae, the metabolic processes controlling growth in C. gigas larvae are affected by temperature and

salinity (Lee & Lee 1968, Helm & Millican 1977, Ventilla 1984). His et al. (1989) provide measurements of larval growth rate for salinities between 20%o and 35%c and temperatures between 15°C and 30"C. These measurements start with larvae with a mean shell length of .'^7 ixm, which is prior to the development of the D-shaped stage (Fig. 1). and extend through the first seven days of larval growth. The differences in the measured larval growth, for the fed larvae, between day and day 7 were calculated for each temperature and salinity. The resultant values were linearly interpolated to obtain growth rates at intermediate temperature and salinity values. Normalizing this matrix of growth rates to the growth rate at 25°C and 30%c provides the fractional change in larval growth rate at a given temperature and salinity (Fig. 6).

The measured growth rates given in His et al. (1989) were extended to the entire range of temperature encountered by C. gigas larvae by assuming that larval growth rate decreases in a linear fashion to zero between 15°C and 0°C and to zero between 30°C and 35°C. This pattern in growth rate is based on observations of increased larval abnormalities in these temperature ranges (Arakawa 1990). Similarly, for salinity, the growth rate at 207ic was linearly reduced to zero at lO^fr, after which growth rate remained zero. This was based on observations of increased larval abnormalities at low salinities (Arakawa 1990). For salinities above 35%?. larval sirowlh rate at 40%? was assumed to be one-half

Temperature (°C)

Figure 6. Fractional change in Crassostrea gigas larval growth rate as a function of temperature and salinity.

[Begin Page: Page 248]

the larval growth rate at 35%c. and growth rates at intermediate salinities were obtained by linear interpolation. The assumption of reduced growth rate at 40%c is based on measurements (Nell & Holiday 1988) that show C. gigas growth rate at 3%', being one-half the value at 35Vcc.

The fractional change in larval growth over the entire range of temperature and salinity was verified by comparing simulated larval length with reported larval lengths measured at known temperatures and salinities (Helm & Millican 1977. Nell & Holiday 1988. His et al. 1989. Robinson 1992). These comparisons showed excellent agreement between simulated and observed larval length, except for lengths between 150 and 200 ^x.m. At these sizes, mismatches of 10 to 45 |jim occurred for the higher teinperature and salinity values. Larvae of this size are growing rapidly at salinities above 24'^t and temperatures above 25"C, and hence the growth

curves are steep. Therefore, slight mismatches in the reporting of measured length for a given larval age can greatly affect comparisons with simulated lengths.

Food Composition and Assimilation Efficiency

The C. gigas larval model allows for differential metabolism of protein, carbohydrate, polar lipid, and neutral lipid. For this to occur, the food ingested by the larva must be expressed in terms of the relative contribution of each of these biochemical constituents. Based upon measurements reported in Utting (1986). Roman (1983). and Lee et al. (1971). the average biochemical composition of marine algae, in terms of ash-free dry weight, was taken to be 3 parts protein, 2.5 parts carbohydrate, 0.6 parts polar lipid, and 0.4 parts neutral lipid. This basic structure defines the food reservoir for the larvae, for most simulations.

Handa (1969) provides assimilation efficiencies for plant material of 1 .0 for protein, 1 .0 for polar and neutral lipids, and 0.2 for carbohydrates. The reduced assimilation efficiency for carbohydrates arises because 80% of plant carbohydrate is structural or P-linked carbohydrate (e.g.. the refractory portion) that cannot be digested by animals and is therefore not available as food. The available 20% represents labile carbohydrate. Multiplication of these assimilation efficiencies with the corresponding food fraction gives an overall assimilation efficiency for C. gigas larvae of about 0.7. which is within the range expected for bixahe lar\ae (estimated from growth efficiency by Jorgensen 1952).

Initial simulations showed that the above assimilation efficiencies resulted in growth rates for lar\ae less than 80 p.m. which

were too small. Observations show a drawdown of neutral lipid reserves during early larval life in C. gigas and C. virgiiiica (Gallager etal. 1986, Gallager and Mann 1986a, Whyte et al. 1987. His & Maurer 1988). presumably to fill the carbon needs not covered by feeding. In the model, the early life stages of the larva were allowed to use neutral lipid stores to form structural material in the body. This was done by calculating a small larva factor iSLF,) of the form:

SLF, = iiuu

0.. \ Ar

SL-L

SL - L,

(10)

where / indicates protein, carbohydrate, polar lipid, or neutral lipid. This relationship calculates the proportionate length change for larva smaller than 80 p-m in a given time increment (At), and the neutral lipid reserves are then used in proportion to the carbon requirement needed to sustain the change in length. The maximum neutral lipid that is used, given by X. occurs when larvae are at their initial size. L,,. This amount decreases proportionately as the

larva grows and becomes increasingly capable of feeding, and is zero at 80 p.m. The mobilized neutral lipid is then converted into equivalent protein, carbohydrate, and polar lipid using the biochemical conversions given previously. This is the only instance in the model where protein is created de novo, rather than being obtained from food.

Thus, the assimilated ingestion (AE,) can be expressed as the product of the filtration rate iFR). the ingestion efficiency (/£,). temperature and salinity effects (TSfaclor). food {Food,), the assimilation efficiency iAE,). and the small larvae factor (SLF,) as:

At, = FR lE^ TSfactor Food, AE, SLF,

(11)

Fate of Assimilated Ingestion

The assimilated ingestion obtained from Eq. (1 1) is parameterized in terms of protein, neutral lipid, polar lipid, and carbohydrate, and the fate of each of these biochemical constituents differs within the lar\a (Table 2). Protein assimilated in a given time interval has, as its primary destination, the somatic protein pool. Protein may also be used to cover a respiratory deficit (discussed below) in accordance with the appropriate protein-to-carbohydrate-to-polar lipid ratio.

The carbohydrate needs of the larva are determined by the amount needed to maintain tissue carbohydrate in its proper pro-

portion and that needed to co\er the metabolic process of respira-

TABLE 2.

Destination of assimilated protein, carbohydrate, polar lipid, and neutral lipid in Crassostrea gigas larvae.

The particular biochemical ratio determining the conversion to individual reservoirs is indicated. Table columns two. three, and four indicate the fate of

the food; column five indicates the fate of the tissue. Transfers of food that do not occur in response to deficit or surplus conditions are indicated by NA.

Protein, carbohydrate, and polar lipid are indicated by P. C. PL, respectively.

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tion (Table 2). Assimilated carbohydrate is the primary means by which larval respiratory needs are met (Table 2). The required somatic carbohydrate is determined so as to maintain the carbo-hydrate-to-protein ratio (0.01). and this amount is debited from the available assimilated carbohydrate and added to the carbohydrate pool. Excess carbohydrate (food surplus response in Table 2) be-comes part of the larval neutral lipid reserve. When tissue imbal-ances occur (e.j;.. insufficient polar lipid to meet the tissue com-positional requirements of the larvae), somatic carbohydrate is used to maintain larval polar lipid in its proper proportion.

The primary destination of assimilated polar lipid in the larva is the somatic polar lipid pool in accordance with the protein-to-polar lipid ratio (Table 2). Excess assimilated polar lipid goes to the lar\al neutral lipid pool. When carbohydrate imbalances occur, polar lipid reserves are mobilized to produce somatic carbohydrate in an amount that is consistent with maintaining the protein-to-carbohydrate ratio. Polar lipids are also used to cover tissue maintenance deficits arising from respiratory demands.

The primarv destination of assimilated neutral lipid is the neutral lipid pool (Table 2). This internal pool is mobilized to maintain somatic carbohydrate and somatic polar lipid pools in accordance with the appropriate ratios when assimilated protein, carbohydrate, and polar lipid are not present in the proper proportions in the food. The neutral lipid pool can also be used to cover respiratory needs during periods of carbohydrate deficit. This pool also provides a means for small larvae, less than 80 (j,m, to produce somatic carbohydrate, polar lipid, and protein as well as cover respiratory costs early in larval life.

At any point in the development of the larva, the inability to maintain one of the biochemical constituent ratios or the inability to remove a deficit in a biochemical pool results in death of the larva.

Respiration

Respiration provides the only metabolic loss of assimilated

energy in the C. gigas model. Measurements show that respiration

10-4

Figure 7. Cra

tion of larval

10-3

10-1

10-2

Weight (mg)

ssostrea gigas lar>al respiration rate measured as a func-

weight at two temperatures.

rate for C. gigas larvae increases with larval size and with tem-
perature (Fig. 7). Laboratory measurements of respiration rate for
C. gigas larvae cover a range of larval sizes measured at 25°C
(Gerdes 1983b) and 20°C (Hoegh-Guldberg & Manahan 1995) can
be described by the relationship:

Resp

.W"

(12)

where Resp is given in mL O, consumed ind~' h"' and W is animal dry tissue weight in mg. The base respiration rate, /„, is assumed to reflect genetic variations in metabolic processes that are known to occur for individual C. ^i,'/,i;((i larvae (e.g.. Lannan 1980). Hence, this parameter is specified using a distribution (described in a following section) that is assumed to represent metabolic variability within the larval population. Other coefficients are defined in Table 1. The respiration rates measured at 25°C (Fig. 7) were used along with the fractional changes in growth rate (Fig. 6) to obtain the full range of temperature and salinity effects on larval respiration. Respiration rate was converted to an energy demand using 20.21 J (niL O, consumed)^' to determine the metabolic cost of respiration.

Equation (12) provides the metabolic cost of respiration that must be met by the larva. As discussed in the previous section, the assimilated carbohydrate pool provides the first biochemical reservoir that is used to meet this demand (Table 2). This pool is converted to equivalent energy units using the conversions given previously, and the needed carbohydrate is removed from the pool. Any excess is added to the neutral lipid pool in an amount that is consistent with the carbohydrate-to-lipid ratio (Table 2).

If the assimilated carbon pool is insufficient to meet the cost of respiration, then the remaining deficit is taken from the neutral lipid pool and any remaining deficit is then taken proportionately from the structural components of the larva (Table 2). Periods

during which the larva resorts to using structural material to cover metabolic costs result in reduction of larval condition index defined in the model as a reduction in the protein-to-ash ratio.

Larval Growth

Larval growth in a given time interval is based on maintaining the protein-to-ash ratio (Fig. 4. Eq. 5) for a given larval length. Larval growth resulting in an increase in length is assumed to occur when the protein, carbohydrate, and polar lipid pools are in excess of what is needed to maintain the protein-to-ash ratio at a given size. This is the excess net production (ENP) that determines a in Eq. 1.

Excess protein is obtained by subtracting from the protein pool the amount that is needed to maintain the ash weight at a given larval length, as is determined by the protein-to-ash ratio. The excess polar lipid and carbohydrate pools are computed from the excess protein pool based on the required structural ratios of these constituents. The excess net production for a given time interval is the sum of the excess protein, polar lipid, and carbohydrate. This gives the excess net production in a given time interval in terms of an increment in larval weight. The weight increment is then used with the length-to-dry tissue weight relationship (Fig. 2) to obtain an incremental increase in length for the increase in weight.

During times of protein deficit with respect to ash weight (low condition index), the larva can have a positive net production that increases organic mass and condition index, but produces no ex-

cess net production and. hence, no increase in length.

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Larval Metamorphosis

Observations suggest that once C. i>ii;(is larvae reach 275 ^l.m they may initiate metamorphosis and this process may or may not be successful (Ventilla 1984. Kusaki 1991. Laing 1995). Thus, in the model, the larva is assumed to have the potential of becoming competent for metamorphosis at 275 |JLm. Just prior to this point, at a length of 250 |xm. filtration rate declines. Observations show that C. gigas larvae can become competent for metamorphosis over a range of sizes. This implies that the switch between Eqs, 6 and 7 controlling the point where filtration rate changes might contain a size dependency, determined by some metabolic process that is. as yet. unknown. Not having this information, the point at which the larva can become competent for metamoiphosis was fixed at a size a little larger than the size observed by Gerdes (1983a) for the change in filtration rate. Simulations discussed subsequently support this decision.

Once the larva reaches 275 \xm, it becomes competent to meta-

morphose if it experiences a 25% drop in neutral lipid stores in one day. This is determined by the interrelationship of food supply, filtration rate, and respiration rate. Competency is triggered by a decrease in neutral lipid that, if continued, would impair successful metamorphosis. Once competent, the larva immediately attempts metamorphosis. Successful completion of metamorphosis occurs if the larval neutral lipid pool is greater than the polar lipid pool. This establishes a minimum storage requirement needed to sustain metamorphosis. If this condition is not satisfied, then metamorphosis is unsuccessful and the larva dies.

Biochemically Determined MetaboUc Mortality

The simulated larval growth prior to metamorphosis is based on maintaining specific ratios between protein, polar lipid, carbohydrate, and ash weight. Small variations in these ratios are allowed, consistent with changes that occur in the larva as it grows (cf. Fig. 4). However, large changes are not permissible. The interdependencies of the biochemical ratios results in the protein-to-ash ratio being a good indicator of the biochemical state of the larva. If this ratio is reduced at any time to 70% or less of its needed value, then larval condition index is too low and the larva is assumed to die. This condition is termed starvation in the model.

During the initial stages of larval growth, about the first two days, the larva does not filter efficiently (Fig. 5). and hence food ingestion is not usually sufficient to cover metabolic costs. During this period, it is assumed that the larva survives by using its stored

neutral lipid supply. However, if during this period the neutral lipid supply approaches zero, the larva is assumed to have reached its metabolic point of no return and dies. Also, inability of the larva to maintain its required protein-to-lipid and protein-to-carbohydrate ratios results in death.

Model Implementation

Initial C. gigas Egg Size, Including Genetic Variability

The eggs spawned by C. gigas adults have an average size ot 50 jjLm (Quayle 1988. Arakawa 19<-)()). However, using this as the initial condition for the model resulted in mismatches in the initial simulated and observed length-to-weight relationships, which are based on larval size. Thus, simple egg diameter is not the appropriate metric for use with the length-to-weight and other conversions. The discrepancy arises because of the mismatch in volume of a spherical egg and the more ellipsoidal-shaped larva. Therefore, it was necessary to convert initial egg diameter to an equivalent larval size. This was done using a diameter-to-length conversion factor of 1.096 (Arakawa 1990) that conserves volume in going from a spherical egg to an ellipsoidal-shaped larva. Thus, a 50 p-m egg is equivalent to a 54.8 p.m larva.

C. viri;inica egg size is observed to range between 30 and 80 Ijim (Gallager et al. 1986). More limited information is available For C. gigas. but a similar range of egg sizes can be interred (Breese & Malouf 1975). This variation was assumed to represent

genetically or environmentally determined variability in the spawning population. Therefore, for each simulation, the initial conditions included a range of egg sizes.

To establish the initial biochemical composition of the egg. the larval size immediately post-hatch was used with the length-to-dry tissue weight relationship (Fig. 2) to calculate an initial dry weight, which in turn was used to obtain an initial ash weight value (Fig. 3). The protein component of the egg was then determined by multiplying the ash weight by the protein-to-ash ratio. The egg polar lipid content was determined by multiplying the protein content by the polar lipid-to-protein ratio. The carbohydrate content was taken to be 1% of the dry weight of the modified egg. Egg neutral lipid content was obtained by difference through subtracting the protein, polar lipid, and carbohydrate weight from the initial dry weight value. If this calculation resulted in a negative value of neutral lipid, which can occur for very small eggs, the egg was assumed to he non\ iable.

Predation and Other Nonmetabolic .Sources of Larval Mortality

The larval model provides, as output variables, the total time for larval development, larval size at the end of the simulation, and a description of why the simulation ended. Termination of a simulation occurs because of successful metamorphosis, unsuccessful metamorphosis, inappropriate metabolic ratios, and starvation. The simulated larval results are then examined with a submodel that calculates larval survivorship based on the timing of mortality and

the larval life span of the survivors for each combination of egg size and respiration rate represented by the genetic variability assianed to the cohort. Los.ses to nonmetabolic sources of mortality, such as predation. are evaluated at this point with losses increasing in proportion to the larval life span obtained for each combination of egg size and respiration rate. The resultant simulated distributions of survivorship can then be compared with similar values reported from field and laboratory studies.

Predation and other forms of nonmetabolic mortality (EM) are imposed during the larval period using a relationship assumed to be of the form

EM(j.k)

i.MDU.k)

(13)

where the daily mortality rate, iii,, is the same as that used for C. virginica larvae (Dekshenieks et al. 1997) and LD is the total time required for a larva with an initial egg size (7) and respiration rate (k) to trigger a mortality event or to successfully metamorphose. The longer the larva takes to develop, the higher the chance of nonmetabolic mortality.

(;enetic P'.ffects on Larval Mortality

Growth, mortality, and other population processes are appor-

tioned based on genetic variability, in which certain combinations of initial egg size and respiration rate are less common in the cohort and in which certain combinations are less viable overall.

[Begin Page: Page 251]

either due to uietahiilic imbalances, metabolic inetYicieneies, or longer lar\;il life spans increasing nonmetabolic mortality. This type of genetically determined outcome. GE. is prescribed with a Gaussian function of the form:

GE-.

(14)

where the Gaussian distributions extend for two standard deviatiiins (2.sy/,.^,^,. 2,v(y,_,,,,) about a central egg size and respiration rate that are given by £5„ and Resp,,. respectively. Coefficient definitions and values are given in Table 1. Equation 14 weights mortality or any other population process by a population distribution that is characterized by a certain range of egg sizes and respiration rates. Thus, the surviving larval population represents the combined effects of genetics, food composition, and environmental

conditions.

RESULTS

Reference Simiilution

The reference simulation was run with near-optimal en\ ironmental conditions of 25"C. 30'^ff, a food concentration of 2 mg L'". and food with a protein, polar lipid, neutral lipid, carbohydrate ratio of 3:0.6:0.4:2.5. Development of C. gigas larvae over the first few days of larval life is primarily sustained by egg neutral lipid stores. The drawdown of neural lipid stores results in a decrease in the neutral lipid-to-protein ratio (Fig. 8A). The decrease in this ratio is most pronounced for eggs with small initial sizes. For initial egg sizes of 40 to 50 |ji.m. the neutral lipid-to-protein ratio approaches zero. All larvae, independent of initial egg size, reach a maximum ratio value of between 0.15 and 0.165 and a size of >275 |a.ni (Fig. 8B). Larvae arising from larger eggs reach these values earlier, and hence can metamorphose earlier. Once a size of 250 (xni is reached, filtration rate declines and so. too. does the neutral lipid to protein ratio. However, growth continues and most larvae metamorphose at about 300 fjLin. regardless of initial egg size (Fig. 8B).

The simulated larval growth is exponential and independent of initial egg size (Fig. 8B). Larvae reach 100 p,m. which corresponds to the small umbo stage (cf. Fig. 1). in 4 to 12 days for Initial egg sizes of 70 (j,m and 40 p.m. respectively. The rate of growth ac-

celerates after 100 p,ni. The time required for the larvae to reach 300 |j.m ranges from 13 to 19 days for the largest and smallest initial egg sizes, respectively. The development time required for the 50 (j.m egg to reach 300 jxm is 17 days, which agrees with development rates measured at 25°C and iVAt (His et al. 1989). The range of simulated development times is al.so consistent with those reported for C. gigas larvae (Quayle 1988. Arakawa 1990. Laing 1995).

The effect of variations in initial egg size and growth efficiency on the fate of the larvae is summarized by the state of the larva at the time it either dies or successfully completes metamorphosis (Fig. 9). Variations in growth efficiency are modeled as variations in respiration rate; however, similar results would be obtained if the variation was in any component of Eq. 2. Initial egg sizes less than 37 p-ni result in nonviable larvae for all respiration rates. Initial egg sizes above 73 (jim result in eggs that do not have sufficient neutral lipid stores after day 2 to continue development at all respiration rates. The large initial size of these eggs results in an iinbalance in neutral lipids that cannot be corrected subsequently (cf. Fig. 8A). A similar fate occurs for initial egg sizes

0.20

= 0.15

ra

q:

c

a)

^*

o

a.

o

'5.

0.10-

0.05

0.00

I I I I I 1 I I I I I I I 1 I I I

I I I I I I I I I I I I I I I

A

- 70 pm

- 60 pm

• 50 pm

40 pm

0.0

10.0

30.0

40.0

20.0

Time (Days)

Figure 8. Simulated time development of the (A) neutral lipid-to-protein ratio and (B) Crassoslrea gigas lenjith obtained for a selection of initial egg sizes from 40 ytm to 70 pni.

between 40 and 50 p.ni that yield larvae with high base respiration rates. For these larvae, too much of the neutral lipid pool is required to cover respiratory demand, and this produces a metabolic imbalance from which the larva cannot recover. Above and below the region of initial egg sizes and base respiration rates that produce successful metamorphosis are regions where the larva develops to the point of attempting metamorphosis, but is unable to do so successfully. A drop in neutral lipid triggers metamorphosis in these larvae, but they have insufficient lipid stores to cover the

metabolic costs of metamorphosis.

At the population level, the fraction of the larvae that survive to complete metamorphosis is dependent on the initial egg size (Fig. lOA). with the distribution of survivorship centered around an initial egg weight of 50 p-m. which is the center of the egg size distribution. Survivorship tapers off toward larger egg sizes, with essentially no survival to metamorphosis at egg sizes greater than 68 (xm. Survivorship at smaller egg sizes decreases abruptly with essentially no survival at sizes less than 40 p.m. Larval survivorship as a function of base respiration rate (Fig. lOBj is maximal at

[Begin Page: Page 252]

Reference Case

400

350 -

300

250 -

200

150

100

50 60

Initial Egg Size(iim

I I Initial egg size too small

I I Insufficient neutral lipid at day 2

[^^. I Unsuccessful metamorphosis

^^1 Successful metamorphosis

Figure 9. Sinuilated time development of Ciassostrea gigas larvae for a range of initial egg sizes and base respiration rates. The simulation used the reference case environmental conditions of 25 C, Wr<. and a food concentration of 2 mg L' with a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0.4:2.5, respectively.

rates around 1.05 KJ day"'. Respiration rates above this result in an abrupt decrease in survival, with no survival at rates above 1.254 KJ day"'. Larval survival at respiration rates below 1.05 KJ day"' slowly decreases and is zero at rates below 0.628 KJ day" . Most larvae reach sizes between .^00 and 325 ixin before meta-morphosis (Fig. IOC).

Detailed verification of the reference simulation is difficult because data on larval biochemical composition, as it varies with eoa size, development time, and environmental conditions, are meager. General trends in larval success as measured by survivorship. lar%'ai size, and larval life span are much better known. Four such trends are observed in the simulation. (1) Adequate neutral lipid stores are a prerequisite of high survival during the critical period a few days post-hatch and at metamorphosis. The reference simulation demonstrates both effects (Figs. 8A. lOB). (2) Typically, egg size ranges from 40-60 p.m. The reference simulation identifies this range as optimal for C. gigas. based on changes in biochemical composition dictated by larval energetics (Fig. lOA). (3) Successful metamorphosis occurs for larvae of 295-340 (iiii in the reference simulation. This is a frequently observed size range (Ventilla 1984. Kusaki 1991. Laing 1995). (4) Larval life span for the most successful egg sizes varies from about 14 to 17 days, a range that approximates the norm in observation (Ventilla l'-)S4. Arakawa 1990. Laing 1995).

Effect of Uirval Filtration

The parameterization for larval filtration (Eqs. 6 and 7. Fig. 51 is based on the assumption that the filtration response changes abruptly at 250 p.m. C. gigas larvae are observed to metamorphose at a range of lengths. The change in filtration rate is an important

0.9 1.1 1.3

Respiration Rate (KJ d"")

0.50

re 0.25

0.00

250

275

300

350

375

400

325

Length (um)

Figure 10. Simulated Crassostrea gigas larval survival as a function of (Al initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis, using the reference case environmental conditions given in Figure 9.

contributor to the mechanisms by which the model determines the onset and success of metamorphosis. Therefore, the larval length at which the change in filtration response occurs was varied.

Varying the onset of a decline in filtration rate does not impact the range of viable egg sizes because this range is determined by events that predate this point in the larva's life span (Figs. I lA. 1 IB). However, if filtration rate changes at 270 |xm. larvae spend a longer time at a high filtration rate. These animals metainorphose successfully over a wider range of base respiration rates than those that support successful metamorphosis when filtration rate begins to decline at 250 |JLm (Fig. 128). Also, the overall population survivorship is higher; 78.8% of the cohort versus 62.4% in the reference simulation (Figs. 9. 10). and the range of lengths at which larvae metamorphose is wider (Fig. 12C).

Moving the larval length at which the filtration rate response changes to a smaller size. 230 |xm. results in a limited range of base respiration rates at which successful metamorphosis can occur (Figs. IIB. 13B). Many of the animals survive to attempt metamorphosis, but are unsuccessful at completing the process. The smaller size at which the filtration rate is reduced results in the larvae not storing enough neutral lipid to cover the metabolic needs associated with metamorphosis. Larval length at metamorphosis (Fig. 13C) is now limited to a small range of sizes.

Total population survivorship drops from 62.4% in the reter-

[Begin Page: Page 253]

Biochemical Model of C. gigas Larvae

253

Filtration Break 230 urn

1.5

1.3

a. 0.9

0.7

0.5

30

40

50 60

Initial Egg Size (Llm)

70

80

I I Initial egg size too small

I I Insufficient neutral lipid at day 2

I I Unsuccessful metamorptiosis

^^1 Successful metamorpfiosis

Figure 11. Simulated fate of Crassostiea gigas larvae for a range of Initial egg sizes and base respiration rates in which the change in larval nitration rate response is at (A) 27(1 pui and (Bl 230 pm. The simulation used the reference case environmental conditions given in Figure 9.

ence case to 8.1% with a size trigger of 21(1 \xm rather than 2.'il) (xm. Cohort sur\ ivorship declines dramatically at trigger sizes below 250 fxm (Table 3) and asymptotes rapidly above 250 p.m. At trigger sizes above 250 fj.m. some larvae do not metamorphose until reaching sizes much larger than normally observed (e.g.. 375^00 (Xin. Fig. I2C). however. Accordingly, larval growth, as modeled, requires a filtration rate trigger near 250 (xm to obtain observed levels of cohort survivorship and size at metamorphosis. This approximates the size where filtration rate declines are observed to take place (Gerdes 1983a).

Effect of Diet

Information on the effects of diet on growth and survival of C.

gigas larvae indicate that high-lipid and low-protein diets are usu-

30

40

50 60

Initial Egg Size (^m)

70

80

0.50

15 0.25

0.00

0.50

re 0.25

Respiration Rate (KJ d' ')

0.00

Figure 12. .Simulated Crassoslrea gigii\ lar>al survival as a function of (.\) initial egg size, (B) base respiration rate, and (Cl larval length at metamorphosis obtained when the change in larval Filtration rate response occurs at 270 pm. The simulation used the reference case environmental conditions given in Figure 9.

ally efficacious (Utfing 1986, Thompson & Harrison 1992. Thompson et al. 1994. 1996). These trends are reproduced by the model.

A larval diet that is lacking in neutral lipid but contains the conect ratios of protein, polar lipid, and carbohydiate (3;().6:0:2.5) results in animals that are unable to successfully complete metamorphosis at all ranges of initial egg size and base respiration rate (Fig. 14). Lack of neutral lipids results in more of the protein and carbohydrate pools being used to cover the demands of respiration and growth and, so, lipid stores are insufficient to sustain metamorphosis. In addition, the range of egg sizes and base respiration

rates in which the neutral lipid store at day 2 is insufficient for further development is greatly expanded relative to what is obtained for a diet containing neutral lipid (Fig. 14 versus Fig. 9).

A diet in which the protein content is SO'/r higher relative to the standard diet (4:0.6:0.4:2.5) produces a similar result in that no combination of initial egg size or respiration rate results in successful metamorphosis (Fig. 15A). Larvae either have insufficient neutral lipid reserves at day 2 to continue development or attempt metamorphosis but fail to complete the process. A high-protein diet requires that more of the neutral lipid stores be used to cover tissue structural needs, and hence less lipid is stored for later use in metamorphosis.

[Begin Page: Page 254]

0.50

™ 0.25

0.00

- mortality, metabolic sources only

- mortality, including non-metabolic sources

400

No Neutral Lipid Diet

0.50-

75 0.25

0.00

0.50

a 0.25

0.00

0.9 1.1

Length (|im)

Figure 13. Simulated Crassoslrea gigas larval survival as a functitin of (Al initial egg size, (Bl base respiration rate, and (C'l larval length at metamorphosis obtained when the change in larval nitration rate response occurs at 230 urn. The simulation used the reference case environmental conditions given in Figure 9.

A diet low in protein (2:0.6:0.4:2.5) is beneficial to the larva (Fig. I5B). Ingestion of low-protein food extends the range of respiration rates that resuU in successful metamorphosis (Fig. 15B versus Fig. 9). More polar lipid and carbohydrate is available to cover metabolic costs and to increase neutral lipid stores. This increases metamorphosis success. Simulations indicate that a low-protein diet increases overall population survivorship under hatchery conditions where nonmetabolic causes of mortality are minimized (88.8% from 62.4% in the reference simulation), but decreases survivorship under field conditions, from 6.19^^ in the reference simulation to 4.7%. due to slower growth and longer planktonic times increasing losses to predation.

TABLE 3.

Total population survivorship for simulated cohorts of Crassoslrea gigas larvae in which the larval size triggering a change in filtration rate was varied from 210 pm to 290 (ini.

Trigger size	Cohort survivorship
210	8.1%
230	

17.2%

250

62.4%

.270

78.8%

290

78.8%

50 60

Initial Egg Size (um)

[I Initial egg size loo small

I I Insufficient neutral lipid al day 2

I I Unsuccessful melamorphosis

P^^^ Protein:asfi ratio outside acceplable range

Figure 14. Simulated fate of Crassoslrea gigas larvae for a range of initial egg sizes and respiration rates resulting when the larval diet includes no neutral lipid. The simulation used the reference case en-

vironmental conditions given in Figure 9, except that the food had a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:0.6:0:

Effect on Metamorphosis

The cue that is assumed to initiate metamorphosis is a 25% drop in the neutral lipid reserves of the larva over a span of one day. This level of decline was chosen as a metamorphosis trigger by comparing the simulated size and biochemical composition of metamorphosing larvae to measured values. The condition and size of larvae at metamorphosis is quite variable, and the metamorphosis trigger value used in the model represents one that simulates the average of these conditions.

Requiring only a 10% drop in neutral lipids in one day to trigger metamorphosis results in essentially all combinations of initial egg size and base respiration rate producing larvae that successfully undergo metamorphosis (Fig. 16 versus Fig. 9). The lower trigger value allows larvae to have a greater energy store at the time metamorphosis is attempted, and hence the probability of success is increased. At the population level, a wide range of egg sizes and base respiration rates results in survival (Figs. 17A & I7B1. but larval length at metamorphosis is shifted to smaller larvae (Fig. 17C). The population mode in this case extends from 285 10.111 to about 305 p.m. a size range predominately comprising sizes smaller than typically observed and smaller than the 295 p.m to about 335 |xni size range observed in the reference simulation (Fig. 9C). Increasing the trigger to a 40% reduction in neutral lipids in one day (not shown) results in attempted, but failed,

metamorphosis at all initial egg sizes and base respiration rates. Thus, the value of 25% produces observed success rates for metamorphosis at a range of observed egg sizes not achieved by higher or lower trigger values.

MetaiTiorphosis success is determined by the ratio of neutral

[Begin Page: Page 255]

BiocHtMicAL Model of C. gigas Larvae

255

Metamorphosis Trigger 10%

30

40

50 60

Initial Egg Size (urn)

80

I I Initial egg size too small

I I Insufticjent neutral lipid at day 2

I j Unsuccessful metamorphosis

^^1 Successful metamorphosis

Fiiiurt' 15. Simulated fate of Crassoslrca gigas lar\ae for a range of initial v^^ .sizes and respiration rates resulting when the larval diet is (.\) high in protein and (B) lo« in protein. The simulation used the reference case environmental conditions given in Kigure t, except that the food had a protein, polar lipid, neutral lipid, and carbohydrate ratios of 4:0.6:(l.4:2.5 and 2.5:0.6:(l.4:2.5.

lipid to polar lipid in the animal at the time that metanwrphosis is triggered. In the reference case (Fig. 9). successful metamorphosis occurs if the content of neutral lipid exceeds the content of polar lipid. Allowing metamorphosis to be successful when the larval neutral lipid reserves are greater than 80'vf of the polar lipid content produces successful metamorphosis over a large range of initial egg sizes and respiration rates (Fig. 18A|. At the population level, larval survival is enhanced over a wide range of initial egg sizes and base respiration rates (Fig. 19A. 19B). The length at metamorphosis also extends over a wide range (Fig. 19C). wider than normally observed, but the mode of the population is still around 308 jxm. as seen in the reference simulation (cf. Fig. IOC). If metamorphosis is successful only when the neutral lipid pool is greater than 1 lO^c of the polar lipid pool, the range of initial egg

400

350

300

250

200

150

100

50 60

Initial Egg Size (urn)

I I Initial egg size too small

I I Insufficient neutral lipid at day 2

[I Unsuccessful metamorphosis

^^1 Successful metamorphosis

Figure 16. Simulated fate of Crassostrea gigas larvae for a range of initial egg sizes and base respiration rates when the trigger for metamorphosis is set to be a 10% drop in neutral lipid stores in one day.

The simulation used the reference case environmental conditions given in Figure 9.

sizes and respiration rates at which successful metamorphosis occurs is greatly reduced (Fig. 18B). The effect of egg size on larval survivorship is only somewhat modified (Fig. 20A); however, the range of base respiration rates that result in successful metamorphosis is greatly reduced (Fig. 20B). as is the range of larval lengths (Fig. 20C). Overall, trigger values above 100%. a 1:1 neutral lipid-to-polar lipid ratio, generate rates of successful metamorphosis that are too low and larval sizes at metamorphosis that are too large (Table 4). Trigger values below 1009f at first seem to be defensible; however, survivorships are unusually high. Hence, model conditions chosen for the reference simulation include, as the minimally required neutral lipid stores for success at metamorphosis, a quantity greater than the polar lipid content of the larva (>1:1). Recall that the polar lipid content is constrained to a predetermined ratio with protein and structural carbohydrate, and so the same results would have occurred had neutral lipid been compared to any structural constituent.

Temperature and Salinity Effects

The temperature and salinity used for the pre\ ious simulations. 25"C and SO'Xt. is near optimal (or the growth and development of C. gigas larvae (cf. Fig. 6). The optimal range of these environmental variables is narrow, and therefore relatively small changes in these conditions have the potential of causing large changes in

larval growth and development. Population survivorship is non-zero over a relatively narrow temperature range and a somewhat wider salinity range (Table 5). A 5^C reduction in temperature, from 25°C to 20°C for example, results in no combination of initial egg size or base respiration rate that produces successful meta-morphosis (Fig. 21A). A reduction in salinity to 207cc extends the

[Begin Page: Page 256]

0.50

^ 0.25

3

cn

0.00

— 1 — I — I — I—

-T 1 1 1 p

- mortality, metabolic sources only

-mortality, including non-metabolic sources

30

40

50 60

Initial Egg Size (^m)

70

80

0.00

250

275

300

325

Length (pm)

350

375

Figure 17. Simulated Crassostrea gigas larval survival as a function of (A) initial egg size, (Bl respiration rate, and (C) larval length at metamorphosis obtained when metamorphosis occurs in response to a 10% drop in neutral lipid stores in one dav. The simulation used the reference case environmental conditions given in Figure 9.

range of initial egg weights and base respiration rates at which the larva exceeds its neutral lipid constraint at day 2 of development (Fig. 21B). Half as many animals exposed to this salinity successfully complete metamorphosis as in the reference case.

Effect of I ariations in Food Resources

Environmental food concentration can vary over a wide range. Food concentration in excess of 2 mg L"' used for the reference simulation (cf. Fig. 9) only extends somewhat the range of initial egg weights and base respiration rates that result in successful metamorphosis because 2 mg L"' food is a saturating food concentration. How ever, a 50% reduction in food concentration, from 2 mg L~' to 1 mg L"'. significantly narrows the range of base respiration rates that result in successful metamorphosis (Fig. 22A|. The strong coupling between respiration rate and food availability is not surprising since respiration is the primary metabolic loss that the larva must cover through ingestion. This result is further substantiated when looking at population level survival

trends (Table 6). Survivorship declines rapidly at food concentrations below 2 ing L'" and reaches zero at food concentrations of about 0.5 mg L'".

Certain environmental conditions inay spare a decrease in food

400

350

300

Neutral Lipid > 80% Polar Lipid

200

150

30

40

50 60

Initial Egg Size (urn)

70

I Inrtial egg size too small

I] Insufficient neutral lipid at day 2

j I Unsuccessful metamorphosis

m Successful metamorphosis

Figure 18. Simulated fate of Crassostrea gigas larvae for a range of initial egg sizes and respiration rates v\hen (he metamorphosis trigger occurs when {A) neutral lipids are greater than 80% of the polar lipid stores and IB) neutral lipids are greater than 1 10% of the polar lipid stores. The simulation used the reference case environmental conditions given in Figure 9.

supply by permitting an increase in filtration rate. Increasing temperature in the previous simulation to 30°C. for example, increases survival by permitting some larvae with high respiratory demands to survive (Fig. 22B). Total population survival increases from 27.2% to 37.6% at the higher temperature.

Planktonic values of food of 0.5 mg L"' or less are not unusual. Consequently, larvae may experience times of starvation due to significantly reduced food concentrations. A simulation in which food is available at a concentration of 2 mg L~' for days 1 to 4 of larval development and unavailable after day 5 shows that no combination of initial egg size and base respiration rate results in successful metamorphosis (Fig. 23A). Death results from poor condition in some cases, but more frequently from metabolic im-

balances between principal biochemical constituents. Simulated

[Begin Page: Page 257]

Biochemical Model of C. gigas Larvae

257

0.50

S 0.25

— I — [— I 1 1 — I — i — r 1 1 1 i ^ I I 1 i r-

- mortality, metabolic sources only

-mortality, including non-metabolic sources

0.5

0.7

0.9 1.1

Respiration Rate (KJ d

1.3

1i

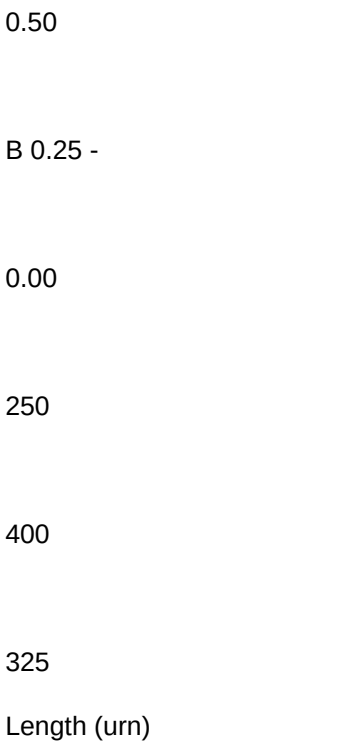
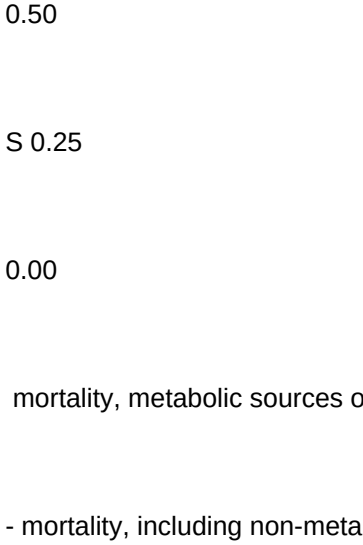


Figure 19. Simulated Crassoslrea gigas larval survival as a function of (.A) initial egg size, (B) base respiration rate, and (C) larval length at metamorphosis obtained when metamorphosis occurs when neutral lipids are greater than 80 9f of polar lipid. The simulation used the reference case environmental conditions given in Figure 9.



30

50 60

Initial Egg Size (Mm)

80

0.50

75 0.25

0.00

0.50

ra 0.25

Respiration Rate (KJ d")

-1 — I — I — I — I — I — i — I — I I I I I I r

0.00 '-^

250

275

300

375

400

325

Length (um)

Figure 20. Simulated Crassostrea gigas larval survival as a function of (Al initial egg-size. (B) base respiration rale, and (Cl larval length at metamorphosis obtained when metamorphosis occurs when neutral lipids are greater than UO'^r of polar lipid. The simulation used the reference case environmental conditions given in Figure 9.

survival times were similar to Uiose observed tor starved larvae (His & Seaman 1992. Lainy 1993). Survival times of simulated larvae starved after day 4 were two to eight days, with most dying within two days. Larvae starved after day 9 survived two to ten days, with most dying in the first four days. Thirty-three percent of larvae starved after day 14 completed metamorphosis. These were larvae that came from relatively large eggs with low base respiration rates (Fig. 2.^B). Such larvae were much closer to metamorphosis on day 14 when starvation began than other larvae and were able to complete metamorphosis using their energy stores.

This last simulation is particularly interesting because a narrower range in egg size produced successful larvae. In other simulations, varying base respiration rate was much more significant in

determining survival than varying egg size. The simulation supports the conclusion of Gallager et al. (1986) and Gallager and Mann (1986b) that egg quality is important in minimizing losses due to low food supply during larval life.

Genetics of Egg Size and Respiration Kate

As discussed previously, it was necessary to model larval cohorts characterized by a range of egg sizes and growth efficiencies to obtain the observed ranges in larval life span, size al metamorphosis, and survivorship seen in experimental studies of C. gigas larvae. One important choice, then, was the mean of the frequency distribution chosen for these two variable characters.

The average egg size was taken to be 50 p-ni in the reference simulation. With a few exceptions, varying the conditions of the simulation did not vary the range of viable egg sizes to a great degree (Figs. 9-22). The range of viable egg sizes is dictated by the most basic constraints imposed by biochemical composition at birth and by environment (e.g.. food supply) rather than by genetics. Not surprisingly, changing the initial egg distribution so that it is centered on a 60 p.m egg, rather than a 50 p-m egg, results in survivorship that is skewed toward larger egg sizes (Fig. 24A versus 9A) because most eggs of 40 to 70 p,m in size are viable

TABLE 4.

Total population survivorship for simulated cohorts of Crassostrea

gigas larvae in which successful metamorphosis occurs when neutral

lipid reserves exceed by a given fraction the polar lipid content.

Neutral lipid-to-polar lipid ratio

Cohort survivorship

0.8:1

80.5'7r

1.0:1

62.4%

1.1:1

26.5'7r

1.2:1

0.0%

[Begin Page: Page 258]

TABLE 5.

Total population sur\ ivorship for simulated cohort.s of Crassostrea

gigas Ian at' exposed continuously to different temperatures (C) and

salinities (%c).

Temperature 15 20 25 30 35

Cohort survivorshi|i O.O'r 0.0"f 62.4% 63.3% 0.0%

Salinity 15 20 25 30 35

Cohort survivorship 0.0% 33.1% 63.3% 62.4% 31.8%

(Fig. 9), and moving the average size higher simply increases the total number of eggs spawned in this higher size range. Respiratory effects on survivorship (Fig. 24B) are not aUered by a change to mean egg size. However, the larval length at the time of metamor-

30

40

50 60

Initial Egg Size (^m)

I Initial egg size too small

I I Insufficient neutral lipid at day 2

I . . I Unsuccessful metamorphosis

1^1 Successful metamorphosis

Figure 21. Simulated fate of Crassostrea gigas larvae for a range of initial egg sizes and base respiration rates at (.\1 2(1 C, MVit and (Bl 25C 2(1';.. Food concentration was 2 mg [" with a protein, polar lipid, neutral lipid, and carbohydrate ratio of 3:U.6:().4:2.5.

30 40 50 60 70 80

Initial Egg Size (um)

Initial egg size too small

I I Insufficient neutral lipid at day 2

I I Unsuccessful metamorphosis

^^1 Successful metamorphosis

Figure 22. Sinudated fate of Crassostrea gigas lar>ae for a range of initial egg sizes and base respiration rates and for a food concentration of 1 mg L'" at (A) 25°C and (Bl 30C. The simulation used environmental conditions of iO"i(and a food composition with a protein, polar

lipid, neutral lipid, and carbohydrate ratio of 3:11.6:11.4:2.5.

phosis is somewhat larger (Fig. 24C). Total survivorship declines somewhat from 64% to 60% because more of the cohort that is

spawned falls into egg sizes above 70 |xm. Overall, however, the simulation shows that a moderate change in mean egg size does not malerially change the outcome of the simulation. As the simu-

TABLE 6.

Total population survivorship for simulated cohorts of Crassostrea

gigas larvae exposed ccmtinuously to different concentrations of

food img F ').

Food	Cohort survivorship
0.5	0.0%
1.0	27.2%
2.0	

62.4%.

4.0

64.4%.

[Begin Page: Page 259]

Biochemical Model of C. gigas Larvae

Food Variation A 0.50

259

Initial Egg Size (n gm)

I I Initial egg size too small | | | | Polar lipidprotein ratio not satisfied

I I Insufficient neutral lipid at day 2

j I Unsuccessful metamorphosis

^^H Successful metamorphosis

Carbohydrate protein ratio not satisfied

I Protein ash ratio outside acceptable range

Figure 23. Siniululed fate of Crassostrea gigas larMie fur a range of

initial egg sizes and base respiration rates and I A) a food concentration of 2 nig~' available for days 1 to 4 and zero afterward and (B) a food concentration of 2 mg~' available for days 1 to 14 and zero afterward. The simulation used environmental conditions of 25C, MVir and a food composition with a protein, polar lipid, neutral lipid, and carbohydrate ratio of .1:().6:(l.4:2.5.

lation depicted in Figure 23B sliows. this outcome may not be repeated in cases of nonsaturating food supply.

Average base respiration rate for larvae was set at 1 .03 KJ day"'. Increasing the mean base respiration rate by 20% to 1.25 KJ day"' results in larval survival skewed toward lower respiration rates (Fig. 25B) and smaller egg sizes (Fig. 25A), with a rapid decrease in survival above respiration rates of 1.05 KJ day"' and egg sizes above 60 |jLm. Larval survival peaks at a metamorphosis length of 30ii iJim. which is similar to that obtained in the reference simulation (Fig. 9C). However, larval survivorship decreases rapidly for larger larvae (Fig. 25C). Total survivorship declines dramatically from 64% to 339f . Higher base respiratiim rates penalize larger larvae because insufficient excess neutral lipid can be obtained under the food supply provided to cover tissue maintenance and provide the requisite energy stores for metamorphosis. Thus, the model is considerably more sensitive to moderate changes in base respiration rate than in mean egg size. Conversely, the influ-

>

'>

CO

0.00

30

0.50

- mortality, fitetabolic sources only

-mortality, including non-metabolic sources

40

50 60

Initial Egg Size (i^im)

70

80

n 0.25

0.00

"T — I — I — I — r

0.5 0.7 0.9 1.1 1.3 1.5

Respiration Rate (KJ d'^)

0.00

250

275

300

325

Length (^m)

350

375

400

Figure 24. Simulated Crassostrea gigas larval survival as a function of (.\1 initial egg size, (B) base respiration rate, and (C'l larval length at metamorphosis for an initial egg size distribution that is centered at 60 pm. The simulation used the reference case environmental conditions given in Figure 9.

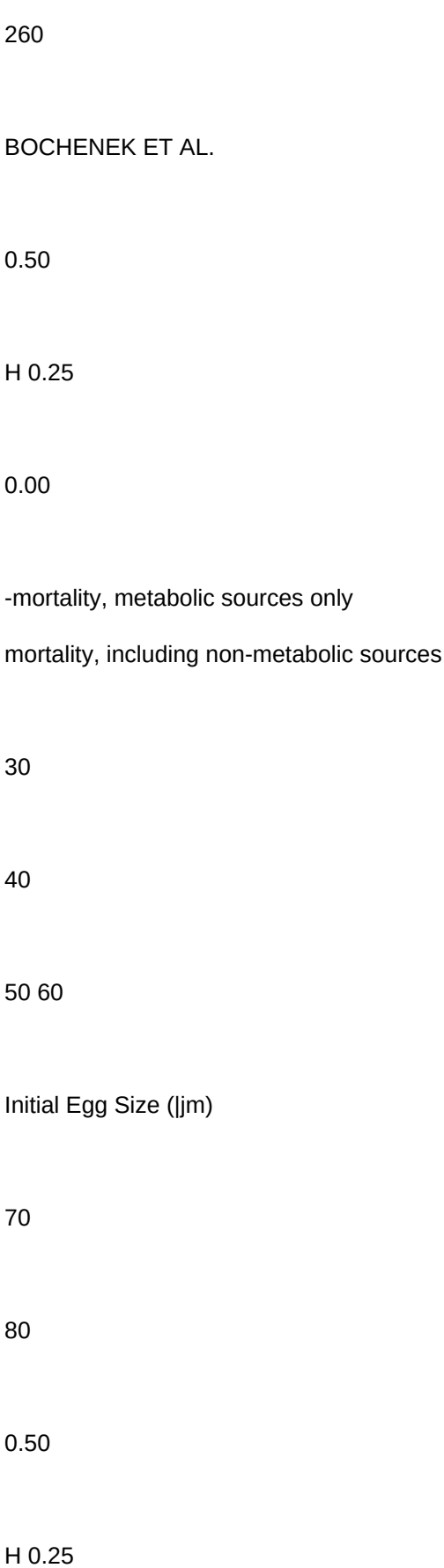
ence of environment on the range of viable base respiration rates is much greater than on the range of viable egg sizes and. so. a change in mean respiration race might easily produce an alternate result under different environmental conditions.

DISCUSSION

Perspective

The model described here is unique in that it seeks to recreate many of the growth and mortality phenomena observed in C. gigas larvae from basic biochemical principals. The approach was dictated by a desire to model the influence of food quality and short-term changes in food supply on larval growth and survival and the influence of egg size and composition on ultmiate success at metamorphosis.

Although biochemically based, the model contains only the crudest biochemical constructions. The larva is modeled as a four-constituent organism composed of protein, carbohydrate, neutral lipid, and polar lipid. Each constituent and the transitions between constituents are modeled using the simplest of flow schemes. So, for example, lipid and carbohydrate are interchangeable, carbohydrate covers respiratory demand when in sufficient supply, and


0.50
H 0.25
0.00
-mortality, metabolic sources only
mortality, including non-metabolic sources
30
40
50 60
Initial Egg Size (|jm)
70
80
0.50
H 0.25

0.00""

0.50

0.7 0.9 1.1 1.3 1.5

Respiration Rate (KJ d"^)

TO 0.25

0.00

250

275

350

375

400

300 325

Length (^im)

Figure 25. Simulated Crassoslrea gigas larval survival as a function of

(A) initial egg size, (Bl base respiration rate, and IC) larval length at

metamorphosis for a base respiration rate distribution that is centered at 1.254 KJ day"'. The simulation used the reference case environmental conditions given in Figure 9.

neutral lipid is the primary storage component. Assimilated protein is used only to create tissue protein, and this necessitates the formation of a certain amount of structural carbohydrate and polar lipid. Failure to supply these other components in the amounts required by protein assimilation results in structural imbalances and eventually death. More complex biochemical transformations are excluded. So. for example, although many amino acids can be synthesized, protein, in the model, comes only from protein ingested as food, with the one exception early in larval life when neutral lipid is used to sustain growth in the first few days after birth. Carbohydrate and protein, though potentially used as energy reserves for metamorphosis or to sustain the larva during periods of negative scope for growth, are only used as the last resort and, then, only in proportion to their fractional contribution to structural tissues.

Although based on a simplistic biochemistry, the model succeeds in simulating some of the basic observations of C. gigas eggs and larvae, suggesting that simple biochemical constructs can be successful and may encompass the biochemical transitions most prominent in determining cohort success. Verifications of many of the details in the simulations cannot be accomplished because of limited information on the details of larval biochemical composi-

tion as it is influenced by environmental and genetic factors. Nevertheless, the model succeeds in simulating known population-level characteristics that permit verification at this higher-order level of integration.

Necessities of Model Constnietion

A number of special model constructs were required to obtain verifiable simulations. These included resolving a mismatch between egg ash content and earliest larva ash content, the conversion of egg size to earliest larval size, the addition of genetic variation, and the need to modify filtration rate, especially in early stage larvae.

Birth and Condition

Condition is tracked in the model using a protein-to-ash ratio. Tracking condition was necessitated by the desire to model periods of low food supply, including, in the extreme, periods of starvation. Particularly once the shell is formed, larval size does not change during periods of restricted food supply, but larval tissue weight does (Laing 1993).

The protein-to-ash ratio for eggs is high and does not fit the larval pattern. Hence, initializing the model with the protein-to-ash ratio of the egg consistently failed to produce verifiable simulations. Presumably, as the egg develops and hatches, ash is added from inorganic solutes in the surrounding water and the protein-to-ash ratio drops. The model was initialized with the protein-to-

ash ratio of newly hatched larvae to circumvent this problem. Reconciling the mismatch between the protein-to-ash ratio of eggs and newly hatched larvae will require additional experimental studies.

Length at Birth

Eggs are more or less spherical. Larvae, even newly hatched, are not. The model tracks length independently of weight, a necessity imposed by the wealth of data for verification provided in terms of length and the need to follow condition. Initializing the model with egg ""length"" (diameter) fails because the increase in length during egg development is not representative of growth, but simply a result of tissue reorganization. Consequently, a growth model cannot account for this process. We used a conversion from egg diameter to earliest larval length to circumvent this problem.

Genetic Variation

One of the key observations recorded in the literature is the success rate at metamorphosis and the size of metamorphosing larvae. Considerable variability exists depending upon the conditions of larval culture, and egg quality provides a sufficiently strong signal that variations in egg quality should influence success at metamorphosis in simulated spawns. Considerable variability also exists within cohorts. Such variability cannot exist if all larvae in the cohort are equivalently affected by environmental conditions. Consequently, it was necessary to add some variation

between larvae to the model.

The observations of Gallager and Mann (1986a) and Gallager et al. (1986) provide a basis for describing a range of egg sizes with a simple Gaussian function to define the frequency of a given egg size in a cohort. This range in egg sizes also produced a range in egg qualities in that larger eggs were relatively more lipid rich. The resulting simulations showed an improved fit to observation in

[Begin Page: Page 261]

that a range of larval sizes and success rales at iiictaniorpliosis were obtained.

However, the range in predicted lar\al si/e and success at metamorphosis was still too constrained in comparison to observation. The obvious next option was to include a range of growth efficiencies. Genetic variation in growth efficiency is well described and may accrue from any number of processes including variations in respiration rate, protein turnover, assimilation efficiency, or feeding efficiency (e.g.. Garton 1984. Koehn and Hilbish 1987. Garton & Berg 1989. Koehn & Bayne 1989. Garton & Haag 1991). In the model, respiration rate and filtration rate con-

trol growth efficiency and. although the two processes are somewhat differently affected by temperature and salinity, inserting variation in either effectively generates simulated larvae with a range of growth efficiencies. Genetic variation in growth efficiency was inserted as a range in base respiration rates using a simple Gaussian construction. This addition produced the range in outcomes at metamorphosis expected from observation.

Simulations were run to examine the influence of varying the mean of the Gaussian distribution describing egg size and respiration rate. Simulations did not change markedly with a variation in egg size because the range of viable egg sizes was tightly constrained, as discussed later. The model was more sensitive to variations in the mean base respiration rate. Here, however, simulations showed that little leeway existed for varying the central tendency of base respiration rate because substantial changes in cohort survival occuiTed with relatively small changes in central tendency.

These results are compared, for the most part, to observations taken under saturating conditions of food and near-optimal environmental conditions. Optimal base respiration rates, to a large extent, and egg sizes, particularly under limiting food supply, suggest that changes in the range of egg size and base respiration rate might be adaptive in certain cases that might routinely exist under tleld conditions. One might expect variations in respiration rate (= growth efficiency) to be the most adaptive.

Filtration Rate

Measured filtration rates always provided growth rates higher than observed. The mismatch was largest for smallest larvae. In these animals, observed reductions in neutral lipid clearly indicated that assimilation does not provide adequate resources to explain observed growth, although measured filtration rates would indicate otherwise. It seems likely that filtration rate and ingestion rate are not equivalent in larvae or that assimilation efficiency is size-dependent.

In the model, a size dependency on ingestion rate or assimilation efficiency is effectively equivalent, so no attempt was made to distinguish between the two. One might reasonably conclude that both feeding and digestion should be affected by larval development processes, particularly during early larval life, and that this might lower the amount of energy realized at a given filtration rate. One might also conclude that filtration efficiency in part is a function of the larva's use of the vellum to maintain its position in the water column as well as to feed and. so. particularly under conditions of saturating food supply where most filtration rates are measured, a tendency to filter more material than can be ingested should exist. Regardless of the cause, to lower growth rales from levels predicted from observed filtration rates, we imposed a size-dependent penalty on ingestion that was largest for the smallest

larvae. The mismatch between observed growth rates and simulated growth rates from measured filtration rates, however, points to an area of early lar\ al biology that warrants further study.

Metamorphosis

The simple biochemical construction of the model required a simple explanation for the metabolic basis for triggering metamorphosis. A full explanation of how endogenous and exogenous factors control metamorphosis (e.g.. Coon & Bonar 1986. Fitt et al. 1990, Berias & Widdows 1995) does not exist. Accordingly, the approach used was derived within the limitations imposed by the four-pool biochemical construct of the model and five observations in the literature that directly related to it. (1) Filtration rate drops in larvae of about 230 [xni and larger, probably due to changes in either behavior or the beginnings of tissue reorganization that must presage metamorphosis. The former option would be sufficient. Older larvae spend more time near the bottom (e.g.. Dekshenieks et al.. 1997) and. thus, may spend less time filtering, a fact that would be interpreted in experiment as a decline in filtration rate. The reduction in feeding rate should ultimately reduce larval scope for growth, and this should have consequences concerning the decision to metamorphose. (2) Smallest size at metamorphosis is about 275 (j,m. This size should be somewhat larger than the size triggering the decline in filtration rate. (3) Lipid stores decline at metamorphosis. This could be a consequence of a decline in scope for growth as well as a consequence of the energy needed to reorganize tissue. (4) Literature information suggests that larvae require a certain amount of stored energy to metamorphose successfully. Although any kind of tissue constituent might provide this energy, the reliance of larvae on neutral lipid as the primary energy store suggests that the proportion of neutral lipid is

a good measure of energy available for metamorphosis. (5) The quantity of lipid present in the egg influences larval survival. Thus some information on the status of neutral lipid reserves should pertain to the decision to undergo metamorphosis.

The process of metamorphosis was modeled using these five observations to generate explicit triggers for certain steps in the process, as follows. (I) Larvae were assumed to become potentially competent to metamorphose at 275 ptm, following a decrease in filtration rate at 250 jjim. Simulations showed that the filtration rate decline could not be set at 2M) |xm or 275 ixm. The minimum size for metamorphosis, in most simulations, did not fall below 285 (Jim. so that the 275 iJ-m size limit was rarely invoked. That is. invoking a change in filtration rate at 250 p.m normally resulted in larvae metamorphosing at sizes above 275 |xm. (2) Larvae were assumed to become competent to metamorphose when a daily decline in neutral lipid of a certain level occurred. Our assumption was that larvae might be expected to continue to grow and store lipid as long as a sufficiently positive scope for growth was present and this would enhance success, but that a decline in neutral lipid would reduce success. Accordingly, metamorphosis should occur when scope for growth dropped significantly below zero. The range of observed sizes at metamorphosis suggests that some process of this sort does occur. Although the decline in filtration rate at 250 jjim predestined larvae to eventually reach the trigger point defined by a significant neutral lipid decline, food quantity and quality and biochemical composition can permit growth much in excess of 250 p.m before scope for growth drops to substantially

negative values. Typically, in the model, metamorphosis occurred at sizes of 300-330 |j,m, as observed in culture. (3) Larvae were

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assumed to metamoqjhose successfully if neutral lipid supplies were adequate. Adequacy was judged as a ratio between energy stores and structural components.

We cannot evaluate how accurately the modeled mechanism for metamoiphosis approaches reality, not having available an adequate understanding of the biochemistry of the process. However, the simulations reveal some interesting trends. The choice of 250 (Jim as the point where filtration rate declines is based on observation, but the model also indicates that this trigger is tightly constrained to this size. Neither 230 [x.m nor 270 |xm sizes offered verifiable results. The choice of a 25% daily decline in neutral lipid triggering competency is also tightly constrained. Values of 10% and 40% did not provide results equivalent to observations. Both larval size distributions and success rates at metamorphosis varied from observations. The choice of a >1:1 ratio of neutral lipid to polar lipid is also tightly constrained. Values of 0.8: 1 and 1.1:1 produce unrealistic size distributions and success rates at meta-

morphosis.

Verification of this construction for modeling metamorphosis was directed at evaluating performance in simulating four important phenomenon: (1) variations in egg quality significantly influenced success at metamorphosis. (2) variations in food quality and quantity significantly influenced success at metamoiphosis. (3 1 larval life span as predicted was well within the range of observations, and (4) larval size structure at metamorphosis was well within the range of observation. Obtaining these four results requires a reasonably accurate rendition of growth and survi\al at the biochemical resolution of the model. This suggests that the approach to modeling metamorphosis must reflect, in some significant way. the process as it actually proceeds in the larva.

Conseqiienccs of Model Coiistnictioii

Larval success is determined by intrinsic and extrinsic factors. Intrinsic factors include egg size and quality and genetic makeup. Extrinsic factors include temperature, salinity, food quality, and food quantity.

Implications of Egg Size

Oyster eggs are about 50 |jLm in diameter, with a size range typically of 40-60 |j.m. The model identifies viable egg sizes in the range 37-73 |j.m, very similar to observations. Egg sizes outside this range are predicted to be nonviable due to lipid imbalances in early larval life. Very likely, the lower limit of 37 p,m represents

a packaging problem. Egg size is simply too small to provide adequate resources for the structural changes required in forming the first larval stage. In the model, the required structural tissue ratios cannot be achieved and still provide any neutral lipid reserves. In effect, the larva is never born. The upper limit of about 73 |j,m yields a larva that has insufficient neutral lipid reserves to cover metabolic needs immediately post-hatch. During this time, feeding is inefficient, and some of the larva's carbon needs for growth and tissue maintenance must be met by using neutral lipid reserves. The larger larvae, coming from eggs >73 |jim in diameter. essentially starve to death before they can become competent filter feeders. This may provide one explanation for the small size of most planktotrophic eggs.

Presumably, the upper limit on egg size could be extended by increasing neutral lipid reserves; however, the bet hedging mode of

life (e.g.. Steams 1976) would limit the amount of energy invested in any one embryo. The trade-off between additional energy expenditure and increased success at metamorphosis is clearly indicated in Figures 11. 15, and 23. Larger eggs yield successful larvae over a much larger range of respiration rates and environmental conditions than do smaller eggs. Larger eggs yield larvae that reach metamorphosis faster (shorter planktonic time), thus minimizing loss to predation and the chance of reduced survival from transient reductions in food supply. Thus, the simulations suggest that the a\erage egg size of 50 [j,ni minimizes the chance of reproductive failure, wliich increases rapidly at smaller egg sizes,

while still permitting the spawning of a large number of eggs. As an example, increasing average egg size to 60 |xm reduces total egg output by 31% at a given total energy expenditure. An equivalent increase in larval success is not achieved in our simulations. The model also indicates, however, that transient reductions in food supply during larval life may increase the success rate for large eggs relative to small eggs. In this circumstance, the extra energy required to produce large eggs may be better repaid. Whether an increase in fitness is adaptively advantageous requires a better understanding of food supply under field conditions and how this influences larval survival.

Respiration (=Gro\vth Efficiencyl Effects

In the model, varying respiratory rate is equivalent to varying growth efficiency. Larvae with high growth efficiency have low respiration rates. The model identified an upper and lower limit to growth efficiency under defined environmental conditions. The upper limit varies widely depending upon environmental conditions, whereas the lower limit is relali\ely fixed. Simulations show that the upper limit on base respiration rate (e.g., -1 kJ day"' in Fig. 9) is determined by the point at which larvae cannot acquire sufficient neutral lipid stores to successfully metamorphose. Smaller eggs are also less viable because insufficient neutral lipid can be stored to cover larval needs over a few days post-hatch. Interestingly, very tow respiration rates also normally result in unsuccessful larvae. These animals put too much assimilated carbon into somatic structural tissue and so have insufficient neutral lipid reserves. We are unaware of experimental data upon which to

verify this last result.

Condition and Mortality

Many models do not explicitly follow length and weight independently (e.g., Powell et al. 1992, Dekshenieks et al. 1993). In bivalves, tracking condition permits observation of larval performance during periods of low food supply. This requires tracking length and weight independently such that not all increases in weight result in changes in length and such that no decreases in weight result in decreases in length. The performance of the model was evaluated under conditions of food deprivation by simulating the process of starvation. Although the mechanisms of death under these conditions are described in the model, death occurs due to a variety of biochemical imbalances, depending upon the initial status of the larva. Whether such a degree of complexity actually exists requires more information on the changes in larval biochemical composition under conditions of low food supply. However, the higher-level effects that integrate biochemical processes

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were simulated by the model, including a decrease in weight (con-

dition), a drop in neutral lipid content, a nonlinear time-dependent increase in mortality, and the still-sLicccssful metamorphosis of older hir\ae.

In addition to inadequate food suppl\ . larvae can die if food of inadequate composition is ingested. Thus, rigorous criteria were set for biochemical compositions not allowed in viable larvae. Food having inadequate lipid or being too protein-rich resulted in mortality, even if the quantity of food remained high. These pa-raiiieterizations describing mortality under such conditions are es-sentially aJ hoc constructs (literature observations not being avail-able), but they did produce cohort mortality rates that appeared to be realistic.

Effect of Diet

Most experimental studies on C. gigcis larvae have used food supplies of >2 mg L '. This level of food saturates feeding and. in fact, raising food quantity from 2 mg L'" to 4 mg L~' in the model has little influence on simulated larval success. However, as in Crassosirea virginica (Dekshenieks et al. 199.^). food quantities below 1 ing L'" dramatically restrict larval growth and survival. As food supply declines, animals with high growth efficiencies are selected for in the iriodel. At high food content, larger eggs with lower growth efficiencies also survive to metamorphosis. With rare exceptions, small eggs with low growth efficiencies never do. Thus, the influence of growth efficiency is nonrandomly distrib-uted across egg size, and the influence seems to be mediated in part

by food quantity and to a larger measure by food quality.

The influence of food content on C. gigas larval growth and survivorship has received considerable attention (e.g.. Wilson 1978. Waldock & Nascimento 1979. Helm & Laing 1987). Although not all studies agree, low-protein diets and high-lipid diets often show improved growth and survivorship. The simulations show the positive effect of a low-protein diet on larval growth and survivorship. With this diet, a relatively larger portion of ingested energy is allocated to energy stores that in turn sustain the larva through metamorphosis. With a high-protein diet, larvae grow too fast and fail to store enough energy to sustain them through metamorphosis. The destination of protein within the larva is limited in terms of building tissue and covering metabolic needs (Table 2) if insufficient carbohydrate is ingested. Any transfer of excess amino acid into other tissue components is not permitted. Potentially, this allocation of ingested protein is too simplistic, although the simulations do provide some insight into the value of a low-protein diet.

Simulations with no neutral lipid gave similar results in terms of larval survivorship and growth. Thus, the relative amounts of protein and neutral lipid in larval food are important determinants of growth and sur\ i\al.

A number of studies have identified specific components of the lipid pool as important dietary constituents (e.g.. Thompson et al. 1994, 1996). The model could be expanded to track inore complex biochemical pools such as polyunsaturated fatty acids (PUFAs) or sterols. The fact that the model achieves realistic simulations over

a relatively wide range of environmental and dietary conditions indicates that the approach used to model larval biochemistry, including the subsuming of a diversity of lipid compounds into two pools, polar and neutral, is sufficient to provide realistic simulations of larval growth, metamorphosis and survival.

Temperature and Salinity

C gigas is known to be relatively stenotopic for the genus. Temperature and salinity conditions describing optimal growth circumscribe a narrow range. The model reproduces this behavior. In this contribution, most simulations were run under optimal conditions of 25°C and 30%c. Lower temperatures result in insufficient neutral lipid storage and metamorphosis because feeding rate is low. Temperatures above .30'C result in biochemical imbalances due to high respiratory demand. Low salinity also results in insufficient food ingestion to meet the demands of metamorphosis. Again, data to verify the accuracy of predicted cause and effect on biochemical composition are not available.

Growth rate is a complex function dependent upon the balances of ingestion and respiration. The ability of positive environmental conditions to offset a reduction in food supply and vice versa depends upon the relative scaling of their affects on respiration and ingestion. Thus, increased temperature can "spare" a reduction in food, permitting the same growth rate, if the influence of temperature on ingestive processes scales with a larger exponent than the influence of temperature on respiration. The importance of differ-

ential scaling in the energy balance of bivalve molluscs and other animals is well known (e.g.. Newell et al. 1977, Powell et al. 1992. Brown et al. 1993). Given the sensitivity of growth and survival to decreases in food supply, the fact that a decrease in food supply often occurs during summer months in C. gigas habitat (Kobayashi et al. 1997. Hyun et al.. in press), when an increase in temperature is likely to be of significance in increasing ingestion rate, suggests that the differential scaling of ingestive processes and respiration is likely of significance for the reproductive success of the species.

CONCLUSIONS

A model that simulates the growth, development, and metamorphosis of Crassosirea gigas larvae has been developed. The model is the first of its kind in that it (1) tracks length separately from weight so that changes in condition can be followed and (2) predicts growth from the ingestion and transformation of biochemical constituents, thus permitting the simulation of the effects of changes in food quality. Food quality and feeding rate are important constraints in larval culture, so the model might be used to optimize culture conditions for C. gigas larvae as well as to investigate the influence of critical periods of food supply in larval development in the field. Of particular importance is the investigation of "teleconnections" during larval life in which events occurring at one point in larval life have consequences at another, temporally distant, point. The model has a crude depiction of the biochemistry of C. gigas larvae. However, the model works well even with this limited biochemistry and indicates that the formulation of sophisticated biochemicallv based models offers the

promise of substantially improving the population modeling of marine larvae.

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