Influence of food quality and quantity on the growth and development of *Crassostrea gigas* larvae: a modeling approach

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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 it (1) defines larvae in terms of their protein, neutral lipid, polar lipid, carbohydrate and ash content; (2) tracks weight separately from length to follow larval condition index and (3) 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 that determine growth rate and processes controlling larval mortality and metamorphosis. Changes in tissue composition occur as the larva grows and in response to the biochemical composition of the food. The simulations show that genetically determined variations in growth efficiency produce significant changes in larval survival and success at metamorphosis. Larvae with low growth efficiency are successful under a much narrower range of culture conditions than larvae with high growth efficiency. The impact of low growth efficiency is primarily controlled by the ability of larvae to store lipid for metamorphosis. Culture conditions that provide increased dietary lipid counterweigh low growth efficiency. Changes in food quantity and quality had little effect on size at metamorphosis. On the other hand, larval life span and success rate at metamorphosis varied over a wide range depending upon the conditions of the simulation. Food quality and food availability both influence larval life span and, hence, larval survival. As ingestion rate decreases, larval life span increases and cohort survival declines. Increased lipid or decreased protein in the diet improves cohort survival. Changes in carbohydrate content are less influential. If cohort success is significantly affected by mortality during larval life rather than success at metamorphosis, the influence of food quality becomes more

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complex. The range of food compositions yielding high survival is restricted by a balance between improved success at metamorphosis obtained by increased lipid storage and the shortening of larval life span as a result of more rapid growth, a function of protein availability. These simulations illustrate the strength and utility of numerical models for evaluating and designing hatchery protocols for optimizing yield of *C. gigas* larvae. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** *Crassostrea gigas*; Hatchery; Larvae; Oyster; Numerical model; Biochemical composition; Metamorphosis; Genetics

### 1. Introduction

*Crassostrea gigas* is cultured world-wide (Ventilla, 1984; Kusaki, 1991; Kobayashi et al., 1997; Hyun et al., 2001). Although many locales depend on natural set, hatchery-based culture is well established (Helm and Millican, 1977; Quayle, 1988; Collet et al., 1999). Optimizing conditions in the hatchery is, therefore, an important task because the economics of hatchery production necessitate limiting mortality in the larvae and in the newly settled seed. Generally, hatcheries minimize total cohort mortality by attempting to maximize larval growth and success at metamorphosis. One of the most important aspects of hatchery operations is feeding. Food quantity and food quality are both important in larval cohort success (e.g., Wilson, 1978; Waldock and Nascimento, 1979; Nascimento, 1980; Helm and Laing, 1987; Laing, 1995), as are feeding frequency and larval density.

Optimizing hatchery production, particularly at commercial scales, is difficult because the process does not lend itself easily to experimental design protocols, particularly in that the range over which an experimental variable can vary is often large, necessitating a complex experimental design. Accordingly, a numerical model of larval development provides a useful and inexpensive framework for evaluating a wide range of possible hatchery protocols prior to or without resorting to experimental manipulation. Such a model, however, must be biochemically based, because food quality is a significant issue in hatchery optimization and food quality is, essentially, a biochemical parameter.

The existing larval growth models determine growth from ingestion rate, decremented by the energy losses due to respiration and incomplete digestion (e.g., Carlotti and Sciandra, 1989; Dekshenieks et al., 1993, 1997; Hofmann and Lascara, 2000). These types of models, however, cannot be used to examine issues of food quality. Accordingly, we developed a biochemically based model 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. This model can be used to examine issues of food quality.

Section 2 provides a brief description of the *C. gigas* larval growth model and the parameterizations used. This is followed by simulations that illustrate the effect of food quantity, food quality, larval density and feeding frequency on larval growth, survival and success at metamorphosis (Section 3). Section 4 places these simulations within the context of the current understanding of the effects of food and feeding on larval growth, survival and metamorphosis.
2. Model description

2.1. Model structure and governing equation

A detailed description of the C. gigas larval model is presented in Bochenek et al. (2001). The change in length for an individual larva over time is given by:

\[
\frac{dL}{dt} = \alpha L
\]

where \(L\) is larval length in μm and \(\alpha\) is the rate at which the larva grows in units of day\(^{-1}\). Larval growth rate \((\alpha)\) 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):

\[
NP_i = \frac{AI_i}{C_0} - R_i
\]

where \(i\) represents the four basic biochemical components included in the model: protein, polar lipid, neutral lipid and carbohydrate. Excretion is assumed to be a minor carbon loss in comparison to respiration. An increase in larval length occurs when the sum of the four, \(\Sigma_{n=1}^4 NP\), 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 production, ENP, is the basic quantity responsible for larval growth.

The specification of ENP, which determines \(\alpha\), is based on filtration rate, the metabolism of carbohydrate, polar lipid, neutral lipid and protein within the larva and the conversion of the metabolized food into structural components and 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 growth. 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. This time resolution is sufficient to avoid numerical diffusion as the larva grows.

2.2. Parameterization of processes

2.2.1. Preferred biochemical composition

Certain weight ratios between structural constituents are assumed to be 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 and tissue protein. Most tissue
carbohydrate was assumed to be structural because neutral lipid is the primary storage constituent. The value of the tissue carbohydrate-to-tissue protein ratio was set at 0.01. Both ratios were independent of larval size.

2.2.2. Filtration rate and filtration efficiency

The filtration rate observations given in Gerdes (1983a) were used to derive empirical relationships that provide the basic filtration structure of the model. Initial filtration rates are low until the larva reaches about 100 \( \mu m \), after which filtration rate increases exponentially to its maximum value as the larva reaches 250 \( \mu m \). At this size, larval behavior changes as the larva nears metamorphosis and filtration rate decreases dramatically. Thus, two equations are needed:

\[
FR = FR_0 e^{(b_1 + b_2 L + b_3 L^2)}, \quad \text{for larvae} \leq 250 \, \mu m \tag{3}
\]

\[
FR = FR_0 e^{(c_1 + c_2 L + c_3 L^2 + c_4 L^3)}, \quad \text{for larvae} \geq 250 \, \mu m \tag{4}
\]

where \( FR \) is filtration rate. The coefficients in Eqs. (3) and (4) are given in Table 1.

Bochenek et al. (2001) found that larval filtration rate calculated from Eqs. (3) and (4) gave growth rates that were too high, particularly for small larvae. Bochenek et al. (2001) introduced two adjustments to filtration rate to correct growth rate. (1) Ingestion efficiency

<p>| Table 1 |
| Definition, units and values for the variables used in the <em>Crassostrea gigas</em> larva model equations |</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( FR_0 )</td>
<td>constant</td>
<td>litres day(^{-1})</td>
<td>( 24 \times 10^{-6} )</td>
</tr>
<tr>
<td>( b_1 )</td>
<td>constant</td>
<td>none</td>
<td>( -2.0909 )</td>
</tr>
<tr>
<td>( b_2 )</td>
<td>constant</td>
<td>( \mu m )</td>
<td>( 0.0335 )</td>
</tr>
<tr>
<td>( b_3 )</td>
<td>constant</td>
<td>( \mu m^2 )</td>
<td>( -3.2798 \times 10^{-5} )</td>
</tr>
<tr>
<td>( c_1 )</td>
<td>constant</td>
<td>none</td>
<td>( 96.9948 )</td>
</tr>
<tr>
<td>( c_2 )</td>
<td>constant</td>
<td>( \mu m^{-1} )</td>
<td>( -1.1011 )</td>
</tr>
<tr>
<td>( c_3 )</td>
<td>constant</td>
<td>( \mu m^{-2} )</td>
<td>( 4.3 \times 10^{-3} )</td>
</tr>
<tr>
<td>( c_4 )</td>
<td>constant</td>
<td>( \mu m^{-3} )</td>
<td>( -5.5985 \times 10^{-6} )</td>
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<tr>
<td>( \gamma )</td>
<td>constant</td>
<td>none</td>
<td>( 0.0025 )</td>
</tr>
<tr>
<td>( z )</td>
<td>constant</td>
<td>none</td>
<td>( 0.35 )</td>
</tr>
<tr>
<td>( \beta )</td>
<td>constant</td>
<td>none</td>
<td>( 0.08 )</td>
</tr>
<tr>
<td>( L_0 )</td>
<td>initial larva length</td>
<td>( \mu m )</td>
<td>calculated</td>
</tr>
<tr>
<td>( L_f )</td>
<td>larva length</td>
<td>( \mu m )</td>
<td>100</td>
</tr>
<tr>
<td>( \delta )</td>
<td>constant</td>
<td>none</td>
<td>0.5</td>
</tr>
<tr>
<td>( L_s )</td>
<td>larva length</td>
<td>( \mu m )</td>
<td>0.5</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>maximum neutral lipid usage</td>
<td>% day(^{-1})</td>
<td>75</td>
</tr>
<tr>
<td>( SL )</td>
<td>upper larval size for neutral lipid use</td>
<td>( \mu m )</td>
<td>80</td>
</tr>
<tr>
<td>( r_0 )</td>
<td>base respiration rate</td>
<td>kJ day(^{-1})</td>
<td>calculated</td>
</tr>
<tr>
<td>( \theta )</td>
<td>constant</td>
<td>none</td>
<td>0.96</td>
</tr>
<tr>
<td>( m_0 )</td>
<td>daily mortality rate</td>
<td>day(^{-1})</td>
<td></td>
</tr>
<tr>
<td>( ES_0 )</td>
<td>central egg size</td>
<td>( \mu m )</td>
<td>50</td>
</tr>
<tr>
<td>( Resp_0 )</td>
<td>central respiration rate</td>
<td>kJ day(^{-1})</td>
<td>1.046</td>
</tr>
<tr>
<td>( 2sd_{egg} )</td>
<td>standard deviation, egg size</td>
<td>( \mu m )</td>
<td>0.2</td>
</tr>
<tr>
<td>( 2sd_{resp} )</td>
<td>standard deviation, respiration rate</td>
<td>kJ day(^{-1})</td>
<td>0.2</td>
</tr>
</tbody>
</table>
was reduced assuming that not all filtration is associated with feeding, some occurs when larvae swim to maintain position, and assuming that the use of saturating food concentrations to measure filtration rate results in more food being filtered from the water column than can be ingested. (2) Ingestion was further reduced early in larval life because the rapid changes leading to the development of the organs for feeding and digestion should further limit ingestion and/or assimilation efficiency during that time.

Adjustment 1 was introduced by the following equation:

\[ \text{IE} = \frac{\gamma}{\text{Food}} \left( \alpha + \beta \left( \frac{L - L_0}{L_f} \right) \right) \]  

where ingestion efficiency (IE), which is a nondimensional quantity, is dependent on the ambient food concentration (Food) and larval size. Coefficient values are given in Table 1. The relationship given by Eq. (5) results in reduced feeding efficiency for all larval sizes, but with the maximum reduction associated with smaller larvae.

Adjustment 2 was introduced by the following equation:

\[ \text{IE}_s = \text{IE} \left( \delta + \delta \min \left( 1, \left( \frac{L - L_0}{L_s - L_0} \right) \right) \right) \]  

Eq. (6) further reduced ingestion rate for larvae less than 80 \( \mu \)m. Coefficient values and definitions are given in Table 1.

2.2.3. Temperature and salinity effects

His et al. (1989) provide measurements of larval growth rate over a range of temperatures and salinities, 20% and 35% and 15 and 30 °C. The range of conditions was extended to encompass the anticipated environmental range experienced by \( C. \ gigas \) larvae as described in Bochenek et al. (2001), yielding the modification to larval growth rate depicted in Fig. 1.

2.2.4. 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. 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 \( \beta \)-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) (Jørgensen, 1952).

Initial simulations showed that the above assimilation efficiencies resulted in growth rates for larvae less than 80 \( \mu \)m that were too small. Observations show a draw down of neutral lipid reserves during early larval life in \( C. \ gigas \) and \( C. \ virginica \) (Gallager et al., 1986; Gallager and Mann, 1986; Whyte et al., 1987; His and 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 (SLFᵢ) of the form:

\[
SLFᵢ = \max \left( 0, \lambda \Delta t \left( \frac{SL - L}{SL - L₀} \right) \right)
\]

where \( i \) indicates protein, carbohydrate, polar lipid or neutral lipid. This relationship calculates the proportionate length change for larva smaller than 80 \( \mu \)m in a given time increment (\( \Delta t \)) 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 \( \lambda \), occurs when larvae are at their initial size, \( L₀ \). This amount decreases proportionally as the larva grows and becomes increasingly capable of feeding, and is zero at 80 \( \mu \)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 (\( AEᵢ \)) can be expressed as the product of the filtration rate (\( FR \)), the ingestion efficiency (\( IEᵢ \)), temperature and salinity effects (\( TS \) factor), food (\( Foodᵢ \)), the assimilation efficiency (\( AEᵢ \)) and the small larva factor (\( SLFᵢ \)) as:

\[
AIᵢ = FR \cdot IEᵢ \cdot TS \text{ factor} \cdot Foodᵢ \cdot AEᵢ \cdot SLFᵢ
\]
2.2.5. Fate of assimilated ingestion

The assimilated ingestion obtained from Eq. (8) 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/carbohydrate/polar lipid ratio.

Next, the carbohydrate needs of the larva are determined in terms of the amount required to maintain tissue carbohydrate in its proper proportion and to cover the metabolic process of respiration (Table 2). Assimilated carbohydrate is the primary means by which larval respiratory needs are met (Table 2). The required somatic carbohydrate is determined based on maintaining the carbohydrate/protein ratio (0.01) and this amount is debited from the available assimilated carbohydrate and added to the carbohydrate pool. Excess carbohydrate 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/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/carbohydrate ratio. Polar lipids are also used to cover 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 carbohydrate and polar lipid are not present in the proper proportions in the food. The neutral lipid pool can also

<table>
<thead>
<tr>
<th>Food constituent</th>
<th>Primary destination in larva</th>
<th>Food deficit response</th>
<th>Food surplus response</th>
<th>Tissue maintenance deficit</th>
<th>Early life (&lt; 80 μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>somatic P</td>
<td>NA</td>
<td>NA</td>
<td>respiration [P/C/PL]</td>
<td>NA</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>somatic C and respiration [P/C]</td>
<td>NA</td>
<td>NA</td>
<td>respiration [P/C/PL]</td>
<td>NA</td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>neutral lipid reserves</td>
<td>somatic C [P/C],</td>
<td>somatic C [P/C],</td>
<td>somatic C,</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>somatic PL [P/PL],</td>
<td>somatic PL [P/PL],</td>
<td>somatic PL,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>respiration</td>
<td>respiration</td>
<td>respiration</td>
<td></td>
</tr>
</tbody>
</table>

The particular biochemical ratio determining the conversion to individual reservoirs is indicated. 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.
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.

2.2.6. Respiration

Laboratory measurements of respiration rate for *C. gigas* larvae covering a range of larval sizes measured at 25 °C (Gerdes, 1983b) and 20 °C (Hoegh-Guldberg and Manahan, 1995) can be described by the relationship:

\[
\text{Resp} = r_0 W^\theta
\]

where \( \text{Resp} \) is given in ml O\(_2\) consumed individual \(^{-1}\) h \(^{-1}\) and \( W \) is dry tissue weight in mg. The base respiration rate, \( r_0 \), 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.

Eq. (9) 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). If the assimilated carbohydrate 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, because the somatic tissue pool shrinks with respect to shell weight.

2.2.7. Larval growth

Larval growth in a given time interval is based on maintaining the protein/ash ratio 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/ash ratio at a given length. This is the excess net production (ENP) that determines \( \alpha \) 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/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 the larval weight. The weight increment is then used with the length-to-dry tissue weight relationship 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.

2.2.8. Larval metamorphosis

Observations suggest that *C. gigas* larvae may initiate metamorphosis once they reach 275 μm 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. Once the larva reaches 275 μm, it becomes competent to metamorphose if it experiences a 25% drop in neutral lipid stores in 1 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.

2.2.9. 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. However, large changes are not permissible. The interdependencies of the biochemical ratios results in the protein/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 2 days, the larva does not filter efficiently 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. In addition, inability of the larva to maintain its required protein/lipid or protein/carbohydrate ratios results in death.

2.3. Model implementation

2.3.1. 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 measure to use with the length-to-weight relationship 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) to
maintain conservation of 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. 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 to calculate an initial dry weight, which in turn was used to obtain an initial ash weight value. The protein component of the egg was then determined by multiplying the ash weight by the protein/ash ratio. The egg polar lipid content was determined by multiplying the protein content by the polar lipid/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.

2.3.2. 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:

$$GE = e^{-\left(\frac{ES - ES_0}{2s_{egg}}\right)^2} - e^{-\left(\frac{Resp - Resp_0}{2s_{resp}}\right)^2}$$

where the Gaussian distributions extend for two standard deviations (2s_{egg}, 2s_{resp}) about a central egg size and respiration rate that are given by ES_0 and Resp_0, respectively. Coefficient definitions and values are given in Table 1. Eq. (10) 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.

2.3.3. 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 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.

Nonmetabolic mortality, EM, was imposed during the larval period using a relationship assumed to be of the form:

\[ EM(j, k) = e^{-m_0LD(j, k)} \]

where the daily mortality rate, \( m_0 \), 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.

3. Results

All simulations were run at near-optimal temperatures and salinities (25 °C, 30‰).

3.1. Reference simulation

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 food composition was used in the reference simulation at a ration of 2 mg l\(^{-1}\). Early development of \( C. \) gigas larvae is primarily sustained by egg neutral lipid stores over the first few days. The draw-down of neutral lipid stores results in a decrease in the neutral lipid/protein ratio (Fig. 2A). The decrease in this ratio is most pronounced for eggs with small initial sizes. For initial egg sizes of 40–50 μm, the neutral lipid/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. 2A). 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 lengths ≥300 μm, regardless of initial egg size (Fig. 2B).

The simulated larval growth is exponential and independent of initial egg size (Fig. 2B). The rate of growth accelerates after 100 μm. Larvae reach 100 μm in 4–12 days for initial egg sizes of 70 and 40 μm, respectively. 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 developmental rates measured at 25 °C and 30‰ (His et al., 1989; 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. 3). 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 the energy budget. 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. A similar fate occurs for initial egg sizes 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 successfully do so. 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. 4A), with the distribution of survivorship centered around an initial egg size of 50 μm. Survivorship tapers off towards 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. 4B) is maximal at rates around 1.05 kJ day^{-1}. Respiration rates above 1.05 kJ day^{-1} result in an abrupt decrease in survival. 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. 4C).
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. These trends are reproduced by 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. 2A and 4B). (2) Typically, egg size ranges from 40 to 60

Fig. 4. Simulated *C. 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 Fig. 2.
μm. The reference simulation identifies this range as optimal for *C. gigas*, based on changes in biochemical composition dictated by larval energetics (Fig. 4A). (3) Most larvae successfully metamorphose at lengths of 300–325 μm in the reference simulation. This is a frequently observed size range (Ventilla, 1984; Kusaki, 1991; Laing, 1995). (4) Larval life spans for the most successful egg sizes vary from about 14–17 days, a range that approximates the norm in observation (Ventilla, 1984; Arakawa, 1990; Laing, 1995). Further aspects of model verification are discussed by Bochenek et al. (2001).

### 3.2. Food quality

Simulations were run varying food quality so as to provide food enriched and depleted in protein, carbohydrate and neutral lipid. These simulations were compared to the reference simulation in which larvae were fed food with a protein, polar lipid, neutral lipid, carbohydrate ratio of 3:0.6:0.4:2.5. For these simulations, larvae were assumed to feed ad libitum, so that food was maintained continuously at a concentration of 2 mg l$^{-1}$. Variations in neutral lipid and protein were more significant in determining the success of larvae at metamorphosis than variations in carbohydrate (Figs. 5–7). A low-protein diet increased the frequency of successful metamorphosis, as did a high-lipid diet (Table 3). The converse resulted in very low larval survivorship. High-protein and low-lipid diets yielded larvae that failed to metamorphose successfully because insufficient lipid was stored during larval life (Figs. 6 and 7). Varying carbohydrate in the diet had a much lesser effect, although high-carbohydrate diets increased the frequency of successful metamorphosis in comparison to low-carbohydrate diets. High-carbohydrate diets expanded the range of base respiration rates that produced surviving larvae (Fig. 5).

Size at metamorphosis was affected significantly only at the lowest protein and highest neutral lipid compositions. However, the influence of size at metamorphosis was significant if a nonmetabolic source of mortality was present. In this case, total cohort survival declined because the larvae had longer life spans and metamorphosed at a larger size (Table 3). Thus, an important difference in survival occurred depending upon whether cohort mortality occurred primarily from metabolic (food-dependent) or nonmetabolic sources. Higher survival occurred, for example, with low-protein diets if metabolic sources of mortality were most important, but lower survival occurred with the same diet if nonmetabolic sources of mortality were most important.

### 3.3. Larval density

Simulations were run varying larval density from 100 to 3000 larvae l$^{-1}$. For these simulations, larvae were fed twice daily with the initial food concentration set at 2 mg l$^{-1}$ and a standard food composition of 3 parts protein, 2.5 parts carbohydrate, 0.6 parts polar lipid and 0.4 parts neutral lipid. Between feedings, food concentration declined in proportion to its use by the larvae.

Larval survival through metamorphosis declined precipitously when larval densities were raised above 1000 larvae l$^{-1}$ (Table 4). High density reduced the survival of larvae with low growth efficiency (the high respirers) (Fig. 8). Initial egg size exerted little influence.
Fig. 5. Simulated fate of *C. gigas* larvae for a range of initial egg sizes and base respiration rates in which the amount of carbohydrate in the diet was varied between (A) 47% and (B) 27%. Additional details are provided in Table 3.
Fig. 6. Simulated fate of *C. gigas* larvae for a range of initial egg sizes and base respiration rates in which the amount of neutral lipid in the diet was varied between (A) 3.2% and (B) 10.3%. Additional details are provided in Table 3.
Fig. 7. Simulated fate of *C. gigas* larvae for a range of initial egg sizes and base respiration rates in which the amount of protein in the diet was varied between (A) 42% and (B) 50%. Additional details are provided in Table 3.
3.4. Food quantity and feeding frequency

Simulations were run varying food quantity from 1.0 to 2.0 mg l$^{-1}$. For these simulations, larvae at a density of 100 l$^{-1}$ were fed continuously, twice daily, or once daily using a standard food composition of 3 parts protein, 2.5 parts carbohydrate, 0.6 parts polar lipid, and 0.4 parts neutral lipid. Between feedings, food concentration declined in proportion to its use by the larvae. Nonmetabolic mortality was computed based on larval life span using Eq. (11).

Table 4
Comparisons of cohort success determined from simulations in which larval density was varied from 100 to 3000 larvae l$^{-1}$

<table>
<thead>
<tr>
<th>Density (larvae l$^{-1}$)</th>
<th>Incidence of successful metamorphosis (%)</th>
<th>Modal size at metamorphosis (µm)</th>
<th>Relative survival with added mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>62.4</td>
<td>310</td>
<td>1.53</td>
</tr>
<tr>
<td>500</td>
<td>60.3</td>
<td>310</td>
<td>1.45</td>
</tr>
<tr>
<td>1000</td>
<td>43.1</td>
<td>310</td>
<td>1.00</td>
</tr>
<tr>
<td>2000</td>
<td>2.1</td>
<td>270/330</td>
<td>0.05</td>
</tr>
<tr>
<td>3000</td>
<td>0.0</td>
<td>–</td>
<td>0.00</td>
</tr>
</tbody>
</table>

All simulations were run at near-optimal temperatures and salinities (25 °C, 30%). Larvae were fed ad libitum. Food was maintained continuously at a concentration of 2 mg l$^{-1}$. Food quality was varied from the base case food quality that had a protein, polar lipid, neutral lipid, carbohydrate ratio of 3:0.6:0.4:2.5. These ratios were: low protein, 2.5:0.6:0.4:2.5; medium low protein, 2.5:0.6:0.4:2.5; medium high protein, 3.5:0.6:0.4:2.5; high protein, 4.0:0.6:0.4:2.5; low lipid, 3.0:6.0:2.5:0.4; medium low lipid, 3.0:6.0:2.5:0.4; medium high lipid, 3.0:6.0:2.5:0.4; high lipid, 3.0:6.0:2.5:0.4; low carbohydrate, 3.0:6.0:4:1.5; medium low carbohydrate, 3.0:6.0:4:1.5; medium high carbohydrate, 3.0:6.0:4:3.5; high carbohydrate, 3.0:6.0:4:3.5. Nonmetabolic mortality was computed based on larval life span using Eq. (11). Relative survival is referenced to the 1000 larvae l$^{-1}$ simulation.
Fig. 8. Simulated fate of *C. gigas* larvae for a range of initial egg sizes and base respiration rates in which culture density was varied between (A) 1000 l\(^{-1}\) and (B) 100 larvae l\(^{-1}\). Additional details are provided in Table 4.
parts polar lipid and 0.4 parts neutral lipid (Table 5). Between feedings, food concentration declined in proportion to its use by the larvae.

Decreasing feeding frequency reduces survival and incidence of successful metamorphosis even if a relatively low larval density of 100 l⁻¹ is used (Table 5). Again, growth efficiency influenced the chance of survival through metamorphosis. Larvae with low growth efficiency only survived well under conditions of high food concentration fed frequently to avoid transient decreases in food availability. Initial egg size exerted little influence.

3.5. Food quality and culture density

Simulations were run varying larval density between 1000 and 2000 larvae l⁻¹ using a series of diets varying in protein, carbohydrate and lipid content. For these simulations, larvae were fed twice daily at a beginning concentration of 2 mg l⁻¹. Between feedings, food concentration declined in proportion to its use by the larvae.

Incidence of successful metamorphosis and larval survival varied according to the relationship between larval density and food quality in these simulations (Table 6). For example, a low-protein diet permitted high larval success rates at higher densities. Incidence of successful metamorphosis, at a larval density of 2000 larvae l⁻¹, was twice as high with a low-protein diet or a high-lipid diet than observed using the standard (medium protein/medium lipid) food source with a lower larval density of 1000 l⁻¹.

The influence of changes in dietary carbohydrate content was minor in comparison to the influence of changes in dietary protein or neutral lipid content. Increased neutral lipid also shortened larval life span over a range of larval densities and, so, yielded increased larval survival when nonmetabolic sources of mortality were also important. Low protein, that in some respects is the same dietary type as high neutral lipid because decreasing protein in the diet results in a proportional increase in neutral and polar lipid, achieved the same incidence of successful metamorphosis. However, a low-protein diet was distinctly

<table>
<thead>
<tr>
<th>Feeding regimen</th>
<th>Food supply (mg l⁻¹)</th>
<th>Incidence of successful metamorphosis (%)</th>
<th>Modal size at metamorphosis (µm)</th>
<th>Relative survival with added mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>2.0</td>
<td>62.4</td>
<td>310</td>
<td>1.00</td>
</tr>
<tr>
<td>Twice daily</td>
<td>2.0</td>
<td>59.3</td>
<td>310</td>
<td>0.95</td>
</tr>
<tr>
<td>Twice daily</td>
<td>1.5</td>
<td>46.1</td>
<td>310</td>
<td>0.70</td>
</tr>
<tr>
<td>Twice daily</td>
<td>1.0</td>
<td>0.1</td>
<td>315</td>
<td>0.00</td>
</tr>
<tr>
<td>Once daily</td>
<td>1.5</td>
<td>18.0</td>
<td>315</td>
<td>0.26</td>
</tr>
<tr>
<td>Once daily</td>
<td>1.0</td>
<td>0.0</td>
<td>–</td>
<td>0.00</td>
</tr>
</tbody>
</table>

All simulations were run at near-optimal temperatures and salinities (25 °C, 30%) and used a larval density of 100 larvae l⁻¹. Larvae were fed continuously, twice daily, or once daily using a standard food composition with a protein, polar lipid, neutral lipid, carbohydrate ratio of 3:0.6:0.4:2.5. Between feedings, food concentration declined in proportion to its use by the larvae. Nonmetabolic mortality was computed based on larval life span using Eq. (11). Relative survival is referenced to the continuous feeding simulation.
inferior to the high neutral lipid diet when nonmetabolic sources of mortality were important.

Considering the 10 simulations reported in Table 6 in which larval density and food quality were varied, the range of larval survival when nonmetabolic sources of mortality are included was much wider at a larval density of 2000 larvae $l^{-1}$ than at a larval density of 1000 $l^{-1}$. At a density of 2000 larvae $l^{-1}$, larval survival varied from 0.8 to 1.9 times the reference case (top simulation in Table 6 in this case). At 2000 larvae $l^{-1}$, larval survival varied from 0.03 to 1.63. The extended range at the higher larval density occurred because some diets resulted in much poorer larval survival at the higher larval density. Thus, food quality increases in importance when culture density is high.

4. Discussion

4.1. Conditions for cohort success

The influence of changes in larval density, food quantity and quality and feeding frequency were investigated using a biochemically based larval model for *C. gigas*. Besides survival, the model provided estimates of larval life span, size at metamorphosis and success rate at metamorphosis for those larvae surviving larval life. Over the suite of simulations presented here, variation in food availability and food quality resulted in little variation in size at metamorphosis, although the model permitted larvae to metamorphose at all sizes above 270 $\mu m$. On the other hand, larval life span and success rate at metamorphosis varied over a wide range depending upon the conditions of the simulation.
The model includes genetic variation at birth, incorporated into the model by variations in egg size and lipid content, and genetic variation in larval physiology as it determines growth efficiency. Genetic variation in growth efficiency is well described and it 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 and Berg, 1989; Koehn and Bayne, 1989; Garton and Haag, 1991). In the model, variation in growth efficiency was introduced by using a range of respiration rates within the cohort. Overall, the simulations showed that variations in egg size were relatively unimportant in controlling cohort success, whereas variations in growth efficiency produced significant changes in larval survival and success at metamorphosis. Larvae with low growth efficiency (higher respiration rates as a function of body mass) were successful under a much narrower range of culture conditions than larvae with high growth efficiencies (lower respiration rates). The simulations show, however, that the impact of low growth efficiency is primarily controlled by the ability of larvae to store lipid for metamorphosis. Culture conditions that provide increased dietary lipid counterweigh the influence of low growth efficiency.

Survival to metamorphosis and the success rate at metamorphosis is increased in these simulations by decreased larval density, increased food supply, increased feeding frequency and variations in food quality that increase the proportion of lipid in the diet. The influences of each of these culture conditions is nonlinear. That is, thresholds occur at which relatively small changes in culture conditions result in large changes in larval survival and success at metamorphosis. As an example, increasing larval density from 100 to 1000 larvae l\(^{-1}\) in the simulations described in Table 4 produces relatively little change in survival. A further increase to 2000 larvae l\(^{-1}\) dramatically reduces cohort success. Thus, culture conditions are not uniformly critical over the entire range of choices of food quality, food quantity, feeding frequency and larval density.

4.2. Interaction of larval density and diet

The complex interplay of the four variables evaluated in the simulations presented in Tables 3–6, larval density, food concentration, feeding frequency and food quality, show that the interaction of these nonlinear processes generates a number of different combinations of culture conditions that yield relatively equivalent levels of cohort success. Increasing feeding frequency permits a higher culture density without reducing survival and success at metamorphosis, for example (see also Malouf and Breese, 1977). Increased lipid or decreased protein content in the diet also permits a higher culture density without a decrease in survival and success at metamorphosis. Changes in dietary carbohydrate content are less influential. Although a range of conditions exist that permit successful culture at relatively high larval density, higher density reduces the total range of successful culture conditions. Thus, culture conditions must be more critically constrained as culture density increases to achieve a desired level of cohort success.

Larval density and food quantity are, in a sense, equivalent variables. However, the simulations show the importance of monitoring food concentration. Feeding frequency, that, with density, controls the time-averaged food concentration available to the larvae, significantly affects cohort survival and success at metamorphosis. The importance of
feeding frequency increases with increased larval density and with a decreased initial concentration of food. Significant differences exist in the simulations between once-daily, twice-daily and continuous feeding regimens.

4.3. Food quality

Simulations were conducted using diets that varied in protein, neutral lipid and carbohydrate content. Changes in carbohydrate content affected larval survival and success at metamorphosis, but the degree of variation was small over a relatively wide range of dietary carbohydrate. Larval survival and success at metamorphosis was much more sensitive to changes in dietary protein and lipid. High-lipid diets and low-protein diets were particularly efficacious. Larval survival was greatly reduced if the diet was low in lipid or high in protein.

In the simulated larvae, high-lipid diets increase cohort success because more lipid is stored for metamorphosis. Low-lipid diets restrict lipid stores and most larvae attempt metamorphosis, but fail. Low-protein diets limit growth rate, thereby permitting the storage of a higher quantity of lipid and, as a consequence, the frequency of successful metamorphosis increases. High-protein diets result in more rapid larval growth because, in the model, all ingested protein is used to build somatic tissue. Increased growth rate results in larvae reaching a size conducive to metamorphosis without the necessary lipid stores and, as a consequence, successful metamorphosis rarely occurs. High-carbohydrate diets minimize the use of other structural and storage components for respiration and, as a result, survival at metamorphosis increases. The converse is true for low-carbohydrate diets. However, carbohydrate exerts a relatively limited influence because all other tissue components can spare carbohydrate to meet respiratory demand and carbohydrate accounts for only a small fraction of structural tissue and is not used for energy storage.

The relationship of growth and survival to diet is demonstrated in a number of studies referenced throughout this text and the simulations presented here conform with published experimental results. However, the biochemistry underlying the experimental results is poorly known. Whether the modeled biochemistry correctly portrays the biochemistry of the larvae that yields the experimental results cannot be confirmed without additional experimental data.

4.4. Mitigation of nonmetabolic mortality

Small variations in larval density, feeding regimen and food quality can result in large variations in larval survival. Larval survival can be decreased further if nonmetabolic sources of mortality are present. By nonmetabolic mortality, we refer to any mortality not ascribed directly to the adequacy of available food. In the model, this type of mortality exerts a steady daily toll that decreases survival as larval life span increases.

If nonmetabolic sources of mortality are limited and if food does not drop to critically low levels resulting in a negative scope for growth that produces metabolic imbalances leading to death (His and Seaman, 1992; Laing, 1995; Bochenek et al., 2001), then cohort success is controlled primarily by the success rate at metamorphosis and this success rate is controlled primarily by the ability of the larva to store sufficient lipid reserves during its
larval lifetime to support successful metamorphosis (Robinson, 1992; Haws et al., 1993; Bochenek et al., 2001; see also Holland and Spencer, 1973; Robert et al., 1999). However, if nonmetabolic sources of mortality are important, then larval life span can become the more critical determinant of cohort success and larval life span is influenced by factors that are, in some ways, very different from the factors influencing success at metamorphosis. Accordingly, if nonmetabolic sources of mortality are important, culture conditions may have to be varied from those producing optimal success rates at metamorphosis.

Food quality and food availability both influence larval life span and, hence, larval survival when nonmetabolic sources of mortality are important. Larval density, food concentration and feeding frequency influence larval life span more or less in direct proportion to realized ingestion rate. As ingestion rate decreases, larval life span increases and cohort survival declines.

Food quality also affects the influence of nonmetabolic sources of mortality. In this case, however, cohort survival is increased over a range of food compositions that include moderately increased neutral lipid and moderately decreased protein. Extremes in protein and neutral lipid content of the diet, whether high or low, reduced cohort survival. This was not the case if success at metamorphosis was the primary determinant of cohort success. Increased survival with intermediate diets comes from a balance between improved success at metamorphosis obtained with higher lipid stores and the shortening of larval life span, attained by more rapid growth that, in the model, is a function of protein availability. Thus, food quality exerts a complex influence on cohort success.

4.5. Evaluation of the biochemical approach

The biochemical basis for this model is unique among larval models. The model also is unusual in its inclusion of genetic variability. The development of a biochemically based model was necessary to evaluate issues relating to food quality. Food quality has been a focus of study in larval shellfish biology (e.g., Utting, 1986; Baldwin and Newell, 1991; Min, 1994). Such studies have revealed that low-protein and high-lipid diets normally improve cohort survival and success at metamorphosis (e.g., Helm et al., 1973; Wikfors et al., 1984; Thompson et al., 1994, 1996). These trends are also observed in model simulations, suggesting that food quality issues can be addressed with a relatively simple biochemistry. The model defines four tissue components: protein, neutral lipid, polar lipid and structural carbohydrate. Carbon moves from food into these components, between these components to maintain tissue structural integrity and foster lipid storage and from these components to respired carbon by relatively simple rules. Successful modeling of key observations of the influence of food quality on larval survival and success at metamorphosis suggests that the simple biochemical basis used in the model is adequate for evaluating how food quality influences larval growth and metamorphosis.

Although high-protein diets reduce cohort success (Utting, 1986; Thompson and Harrison, 1992; Thompson et al., 1994, 1996), the model may provide unrealistically low survival rates. In the model, larvae store too little lipid to successfully carry out metamorphosis principally because their growth rate is too rapid. Probably, larvae can limit tissue growth rate when an excess of protein is present by either dumping amino acid or converting protein carbon into other structural components and excreting ammonia.
That amino acids can be released into the medium by oysters is well described (Hammen, 1969; Pierce and Greenberg, 1976; Powell et al., 1982). The movement of carbon from protein into carbohydrate and lipid is also a standard biochemical capability. Under normal circumstances, larval growth rates are high enough that most ingested protein becomes structural protein, as provided for in the model (see also Kreeger et al., 1996). At what point an excess of protein is reached that would necessitate alternative fates for some of the ingested amino acids is not yet well enough understood to be included in the model. However, the low survival obtained with the highest protein diets in these simulations suggests that strategies for limiting growth rate under high-protein diets will be found to be important, once experimental studies are conducted.

5. Conclusions

Over the last several decades, larval growth models have become increasingly complex. These models, however, have utilized the standard processes governing energetics, including ingestion, assimilation and respiration, to simulate growth and survival. Such models have proven to be effective in addressing a wide variety of issues. However, the issue of food quality is not directly amenable to models of this sort. The influence of food quality is manifested through the biochemistry as the animal processes ingested carbon, whereas other factors, such as food quantity, simply influence ingestion rate. The results of simulations using a biochemically based model to evaluate issues of food quality provide insights into how food quality affects cohort survival. Of particular importance is the fact that some diets counterweigh the negative effects of restrictions in food supply, in that lower rates of ingestion do not always result in decreased cohort success. Some diets perform better in maintaining relatively high growth rates when ingestion rate is limited. Thus, the simulation of animals, like molluscan larvae, that are sensitive to changes in food content is improved by inclusion in the model of an underlying biochemical structure supporting the basic processes of ingestion, assimilation, growth and respiration.

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