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Eric N. Powell

Eleanor A, Bochenek

John M. Klinck
Old Dominion University, jklinck@odu.edu

Eileen E. Hofmann
Old Dominion University, ehofmann@odu.edu

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Influence of short-term variations in food on survival of *Crassostrea gigas* larvae: A modeling study

by Eric N. Powell[1], Eleanor A. Bochenek[1], John M. Klinck[2] and Eileen E. Hofmann[2]

ABSTRACT

A biochemically-based model was developed to simulate the growth, development, and metamorphosis of larvae of the Pacific oyster, *Crassostrea gigas.* The model defines larvae in terms of their protein, lipid, carbohydrate, and ash content and includes variation in growth efficiency and egg quality to better simulate cohort population dynamics. Changes in tissue composition occur as the larva grows and in response to the biochemical composition of the food. The premise behind this modeling study was that certain periods of larval life are more critical than others with respect to the availability of food and that food quality is as important as food quantity. The results of the simulations indicate that critical periods do exist, but that the period of larval life which is critical depends upon the composition of the available food supply and how it varies over time. Overall, the most critical time is late in larval life, near the time of metamorphosis. At this point, some variations in food quality are particularly efficacious, others particularly disastrous. But, under certain circumstances, events early or midway in larval life also dramatically change cohort survival.

Simulations show that cohort survival varies in a relatively predictable way when salinity or food quantity vary. Both control time-integrated food supply to the larva by varying the amount of food ingested. Reduction of time-integrated ingestion reduces survival. Larvae with high growth efficiency are more successful, as are larvae coming from large eggs. The simple effect of time-integrated food presents a stark contrast to the complexity introduced by varying food quality. Simulations indicate that it is late in larval life when larvae are most sensitive to changes in food quality. Increased protein at this time always improves survival. Increased lipid is most efficacious midway in larval life, but also exerts a positive impact late in larval life. Variations in carbohydrate are relatively inconsequential in affecting larval survival. Simulations in which food quantity and food quality vary independently show that cohort survival is sensitive to the exact timing and type of environmental change. Transient changes in food quantity influence survival primarily by varying the length of larval life. Transient changes in food quality, on the other hand, can produce large changes in survivorship by restricting the range of genotypes in the cohort that can survive, as well as by varying larval life span. The simulations support the adaptive advantage of larval cohorts with a relatively wide range of genotypes and suggest the important influence of variations in food quality in maintaining genetic variability.

1. Haskin Shellfish Research Laboratory, Rutgers University, 6959 Miller Avenue, Port Norris, New Jersey, 08349, U.S.A. ***email: eric@hsrl.rutgers.edu***

2. Center for Coastal Physical Oceanography, Crittendon Hall, Old Dominion University, Norfolk, Virginia, 23529, U.S.A.

1. Introduction

Laboratory and hatchery observations (Dupuy *et al.,* 1977; Gallager *et al.,* 1986; Gallager and Mann, 1986; Quayle, 1988; Robinson, 1992), high year-to-year variability in recruitment (Loosanoff, 1966; Hofstetter, 1977; Austin *et al.,* 1996), and the limited evidence for a broodstock-recruitment relationship (Prytherch, 1929; Peterson and Summerson, 1992; Livingston *et al.,* 2000) suggest that the highly-variable survivorship of bivalve larvae in nature is determined by complex interactions of physiology with the physical environment and food supply. Variations in environmental conditions and food supply may affect larval cohort success by reducing survivorship during larval life, by limiting success at metamorphosis, or by slowing growth and thereby increasing larval life span with the consequent increase in exposure of the larvae to predators. Considerable research has been conducted on the general topic of larval survivorship under laboratory and hatchery conditions (Helm *et al.,* 1973; Gallager *et al.,* 1986; His and Seaman, 1992; Haws *et al.,* 1993; Laing, 1995). Issues of food quantity and food quality have received particular attention (Helm *et al.,* 1973; Webb and Chu, 1982; Wikfors *et al.,* 1984; Utting, 1986; Thompson and Harrison, 1992; Thompson *et al.,* 1994, 1996). Application of these results to the study of larvae in the field, however, has been extremely difficult because tracking individual larval cohorts is labor intensive at best and essentially intractable in most environments. Consequently, observations of temporal variation in larval abundance or benthic settlement are often explained by inference (Resnik, 1991). For this reason, larval growth (Rice *et al.,* 1993; Dekshenieks *et al.,* 1993; 1997; 2000) and larval transport models (Jackson, 1986; Hill, 1991; Dekshenieks *et al.,* 1996) have been developed to provide a framework for examining the role of environmental factors in controlling larval survivorship.

A larval growth model developed by Bochenek *et al.* (2001) for *Crassostrea gigas* larvae provides a framework to examine the influence of changes in food quality, as well as food quantity, on larval cohort success. Simulations with the *C. gigas* larval model support the importance of food quantity and food quality in cohort survival (Hofmann *et al.,* 2004). In particular, simulations using different food compositions produce widely varying survivorships at the same food concentration. Some food compositions limit survival because metabolic constraints result in death; others reduce survival by increasing larval life span and consequently loss to predation. Simulations also suggest that, under food-limiting conditions, carbohydrate + lipid-to-protein ratios above 1.2 result in substantially improved survival in comparison to other food compositions (Powell *et al.,* 2002; Hofmann *et al.,* 2004).

Recent work combining field observations and modeling suggests that food content is best assessed using measurements of labile carbohydrate, lipid, and protein (Soniat *et al.,* 1998; Hyun *et al.,* 2001). Field measurements indicate that these measures consistently yield estimates of food concentration considerably above those estimated from chlorophyll content (Heral *et al.,* 1983; Soniat and Ray, 1985; Soniat *et al.,* 1998; Canuel and Zimmerman, 1999; Hyun *et al.,* 2001; Versar, 2001). Crisp *et al.* (1985) demonstrated

experimentally the need of oyster larvae for additional food beyond that provided by phytoplankton. Numerical models similarly suggest that food supply may often limit larval survivorship in estuarine environments (Dekshenieks *et al.,* 2000; Klinck *et al.,* 2002).

Field observations also show that food supply is highly variable over short temporal scales (see also Fegley *et al.,* 1992; Judge *et al.,* 1993; Wilson-Ormond *et al.,* 1997), as is the ratio among the primary constituents, carbohydrate, lipid, and protein, as might be anticipated from studies on temporal variations in phytoplankton abundance and chemical composition (Parsons *et al.,* 1961; Goldman and Stanley, 1974; Marshall and Nesius, 1993; Townsend *et al.,* 1994) and the importance of resuspended benthic algae (Flint and Kalke, 1986; Navarro *et al.,* 1992). Potentially, larvae can be greatly influenced by these short-term variations in the amount and quality of food present during planktonic life. It is essentially this expectation that serves as the basis for the 'critical period' hypothesis proposed for planktotrophic larvae (Lasker, 1975; Cushing and Dickson, 1976; Anger *et al.,* 1981; Cushing, 1990).

In this study, the biochemically-based larval growth model described by Bochenek *et al.* (2001) is used to address issues of larval survivorship in the field, emphasizing the influence of short-term variations in food quantity and food quality. Food rations composed of time-varying carbohydrate, lipid, and protein proportions are input to the model to examine how the interaction of short time-scale variations in environment, food quantity, and food quality might influence the survivorship of bivalve larvae. The following section 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 short-term variations in salinity, food quantity, and food quality on larval survival and success at metamorphosis. The discussion section places these simulations within the context of the current understanding of the influence of the environment on larval survivorship.

2. Model description

a. 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:

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

where L is larval length in μm and α is the rate at which the larva grows in units of d^{-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. 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 components of net production, $\sum_{i=1}^{4} NP$, is positive, when larval condition index is maximal for a given size, and when the restrictions imposed by certain biochemical ratios described subsequently 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 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. Coefficient values for all equations are summarized in Table 1 of Hofmann *et al.* (2004) and their formulation described by Bochenek *et al.* (2001).

b. Parameterization of processes

i. 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 larval 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.

ii. 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 μm, after which filtration rate increases exponentially to its maximum value as the larva reaches 250 μm. At this size, larval behavior changes as the larva nears metamorphosis and filtration rate decreases dramatically. Thus two equations are needed:

$$FR = FR_0 e^{(b_1 + b_2 L + b_3 L^2)}, \qquad \text{for larva} \leq 250\ \mu\text{m} \tag{3}$$

$$FR = FR_0 e^{(c_1 + c_2 L + c_3 L^2 + c_4 L^3)}, \qquad \text{for larva} \geq 250\ \mu\text{m} \tag{4}$$

where FR is filtration rate.

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 and introduced two adjustments to correct growth rate.

The first adjustment was to reduce ingestion efficiency, based on the assumption that not all filtration is associated with feeding, some occurs when larvae swim to maintain position, and on the assumption that the use of saturating food concentrations in experiments designed to measure filtration rate results in more food being filtered from the water column than can be ingested. Adjustment 1 was introduced by the following equation:

$$IE = \frac{\gamma}{Food}\left(\alpha + \beta\left(\frac{L - L_0}{L_f}\right)\right) \tag{5}$$

where ingestion efficiency (IE) is dependent on the ambient food concentration ($Food$) and larval size. The relationship given by Eq. (5) results in reduced feeding efficiency for all larval sizes, but with the maximum reduction associated with smaller larvae.

The second adjustment, necessitated by the fact that larvae use some stored energy for growth early in larval life [Eq. (6), see also Lucas and Rangel (1983)], further reduced filtration rate 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 2 was introduced by the following equation:

$$IE_s = IE\left(\delta + \delta\ min\left(1., \left(\frac{L - L_0}{L_s - L_0}\right)\right)\right) \tag{6}$$

Eq. (6) further reduced ingestion rate for larvae less than 80 μm.

iii. Temperature and salinity effects. His *et al.* (1989) provide measurements of larval growth rate over a range of temperatures and salinities. Bochenek *et al.* (2001) extended the range of conditions to encompass the anticipated environmental range experienced by *C. gigas* larvae, yielding the modification to larval growth rate depicted in their Figure 1.

iv. Food composition and assimilation efficiency. The *C. gigas* larval model allows for differential metabolism of protein, carbohydrate, polar lipid, and neutral lipid. Consequently, ingested food must be defined in terms of the relative contribution of each of these 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 β-linked carbohydrate (e.g., the refractory portion) that cannot be digested by animals and

is therefore not available as food. Multiplication of these assimilation efficiencies by the corresponding food fraction gives an overall assimilation efficiency for *C. gigas* larvae of about 0.7 for optimal food (Bochenek *et al.,* 2001), a value within the range expected for bivalve larvae (estimated from growth efficiency—Jørgensen, 1952; compare also Holland, 1978; Crisp *et al.,* 1985).

Observations show a draw down of neutral lipid reserves during early larval life (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. This process balances the lower ingestion rates for larvae less than 80 μm [e.g., Eqs. (5) and (6)]. In the model, the early life stages of the larva were allowed to use neutral lipid stores to form structural material in the body by calculating a small larva factor (SLF_i) of the form:

$$SLF_i = max\left(0., \lambda \Delta t\left(\frac{SL - L}{SL - L_0}\right)\right) \tag{7}$$

where i indicates protein, carbohydrate, polar lipid, or neutral lipid. This relationship calculates the proportionate length change for larvae 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_0. 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 ($TS\ factor$), food ($Food_i$), the assimilation efficiency (AE_i) and the small larvae factor (SLF_i) as:

$$AI_i = FR \cdot IE_s \cdot TS\ factor \cdot Food_i \cdot AE_i \cdot SLF_i. \tag{8}$$

v. 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. 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. Assimilated carbohydrate is the primary means by which larval respiratory needs are met. 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 is the somatic polar lipid pool in accordance with the protein:polar lipid ratio. Excess assimilated polar lipid goes to the 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. 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 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.

vi. 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:

$$Resp = r_0 W^{\theta} \tag{9}$$

where $Resp$ is given in ml O_2 consumed individual^{-1} hr^{-1} and W is dry tissue weight in mg. The base respiration rate, r_0, is specified using a distribution (described in a following section) that is assumed to reflect genetic variations in metabolic processes that are known to occur for individual *C. gigas* larvae (e.g., Lannan, 1980).

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. 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. 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.

vii. 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 α in Eq. (1). 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.

viii. 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 (Bochenek *et al.,* 2001). 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 one day. This is determined by the inter-relationship 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. Further justification for this modeling approach is given by Powell *et al.* (2002) and Bochenek *et al.* (2001).

The phenomenon of larval searching for appropriate substrate (e.g., Ólafsson *et al.,* 1994; Roegner and Mann, 1990; Hidu and Haskin, 1971) is not included in the model. Successful larvae in the model are assumed to have sufficient reserves to sustain such a search. Varying searching times in the model would simply add a random factor to the success of any larval genotype that would not advance the primary goal of the model, namely to examine the influence of food quantity and food quality during larval life on larval cohort survival. We, therefore, model larvae under the condition that substrate is not a factor limiting survivorship, or, if so, that its influence is not a function of the larval energy budget.

ix. 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 inter-dependencies of the biochemical ratios result 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 the larva is assumed to die (Bochenek *et al.,* 2001). 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 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:lipid or protein:carbohydrate ratios results in death.

c. Model implementation

i. 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 between the volume of a spherical egg and the more ellipsoidal-shaped larva. Therefore, egg diameter was converted to an equivalent larval size using a diameter-to-length conversion factor of 1.096 (Arakawa, 1990). 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 egg dry weight. Neutral lipid content was obtained by difference. A negative value for neutral lipid was assumed to indicate a nonviable egg.

ii. Within-cohort variation in larval growth, mortality, and egg size. A simulated cohort is constructed of a suite of individuals with a size frequency of initial egg sizes and characterized by a range of respiration rates, some of which are less common in the cohort than others and some combinations of which are less viable overall either due to metabolic imbalances, metabolic inefficiencies, or longer larval life spans increasing loss to predation. The distribution of individuals, GE, among the range of possible egg sizes and respiration rates in the cohort is prescribed with a Gaussian function of the form:

$$GE = e^{-\left(\frac{ES-ES_0}{2sd_{egg}}\right)^2} - e^{-\left(\frac{Resp-Resp_0}{2sd_{resp}}\right)^2} \tag{10}$$

where the Gaussian distributions extend for two standard deviations ($2sd_{egg}$, $2sd_{resp}$) about a central egg size and respiration rate given by ES_0 and $Resp_0$, respectively. Thus,

the surviving larval population represents the combined effects of genetics, food composition, and environmental conditions.

iii. Extrinsic 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. Larval survivorship, then, is calculated from the simulated larval results 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. Extrinsic mortality, EM, such as predation, is evaluated at this point with losses increasing in proportion to larval life span:

$$EM(j, k) = e^{-m_0 LD(j,k)} \tag{11}$$

where m_0 is the daily mortality rate 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.

d. Presentation of simulation results

i. Timing of variation. Simulations were designed to evaluate the influence of events that alter food supply at the beginning, middle, and near the end of a typical planktonic life span of 15–20 days (Quayle, 1988; His *et al.,* 1989; Arakawa, 1990; Laing, 1995; Bochenek *et al.,* 2001). Accordingly, transient changes in the environment and food quantity and quality were centered on day 0, 7, and 14, respectively. Hofmann *et al.* (2004) considered events of two durations, 7 and 14 days. The shorter duration event occupies about one-third of larval life; the longer duration event, somewhat more than two-thirds of larval life. Hofmann *et al.* (2004) found that simulations identical except for event duration were qualitatively similar. Consequently, simulations of 7-day duration are the focus of this study.

Generally, only one factor was allowed to vary over the course of a simulation. The non-varying environmental factors used in the simulations were: a temperature of 27.5°C, a salinity of 27.5‰, a food ration of 1.0 mg L^{-1} or 2.0 mg L^{-1}, and a food quality defined by 3 parts protein, 2.5 parts carbohydrate, 0.6 parts polar lipid, and 0.4 parts neutral lipid. This food composition will be referred to as 'standard' food.

ii. Simulation analysis. Larval survival is determined by intrinsic and extrinsic factors. Intrinsic factors issue from the direct influence of environment on larval physiology, growth, and biochemical condition that ultimately increases larval mortality or decreases success at metamorphosis. Extrinsic factors impact survivorship directly by terminating life. The principal source of extrinsic mortality is predation. Losses to predation are determined by the predation rate and by larval life span that controls the exposure time for

the larval cohort. To the extent that intrinsic factors control larval life span, intrinsic and extrinsic factors interact through the predation term.

Analysis of the results of simulations will focus on three metrics. The first of these is total cohort survival through metamorphosis. This metric, integrated across all egg sizes and respiration rates as determined by the Gaussian distribution of genetic traits in the cohort [Eq. (10)], describes the sum of all intrinsic and extrinsic sources of mortality. It is sensitive to physiological constraints as well as cumulative predation pressure controlled by variations in larval life span. Thus, as larval life span increases, total cohort survival decreases because the exposure period to predation increases.

The second metric is the percent of larvae intrinsically capable of survival that do survive. This ratio provides a measure of the importance of predation in total survivorship. As the relative influence of predation declines, the survivorship of larvae intrinsically capable of surviving increases. The ratio is not defined for environmental conditions that do not permit any larvae to survive through metamorphosis (100% intrinsic mortality). These cases are depicted as zeros in the figures that follow.

The third metric focuses on the degree to which different genetic compositions are intrinsically selected for by different environmental conditions. Genetics is implemented in the model by variability in egg size and respiration rate (a surrogate for growth efficiency). The two-dimensional array describing the full range of egg sizes and growth efficiencies used in the model (Fig. 3 in Hofmann *et al.,* 2004) is divided into four quadrants: large (55–73 μm) and small (37–55 μm) eggs each with low (0.628–1.047 KJ d^{-1}) or high (1.047–1.675 KJ d^{-1}) respiration rates and, therefore, high or low growth efficiencies. Hereafter, larvae will be assigned to these four quadrants thusly: quadrant 1, larvae with high respiration rates (low growth efficiencies) from small eggs; quadrant 2, larvae with high respiration rates (low growth efficiencies) from large eggs; quadrant 3, larvae with low respiration rates (high growth efficiencies) from small eggs; and quadrant 4, larvae with low respiration rates (high growth efficiencies) from large eggs. Tallying the number of genetic combinations (54 possible per quadrant, 216 total), in the array defined by the parameterization of Eq. (10), yielding successful larvae in each quadrant provides a measure of the effect of genotype on larval survival.

iii. Food quality and genetics. Changes in food quality, brought about by variations in the composition of phytoplankton, are likely to be common (Tester *et al.,* 1995; Phlips and Badylak, 1996; Canuel and Zimmerman, 1999) and potentially as significant for larval survival as are changes in food concentration. For the simulations used in this study, a range of food qualities was chosen from the carbohydrate, lipid, and protein contents reported for algal species such as *Tetraselmis, Dunaliella, Amphidinium, Monochrysis, Thalassiosira,* and *Isochrysis* (Parsons *et al.,* 1984; Wikfors *et al.,* 1984; Utting, 1986; Roman, 1983; Lee *et al.,* 1971) and from field measurements of carbohydrate, lipid, and protein (Soniat and Ray, 1986; Soniat *et al.,* 1998; Hyun *et al.,* 2001). The measurements of algal biochemical composition and the field assays of food quality were used as food

compositions in the simulations, rather than creating artificial mixtures, so as to reflect realistic changes in the ratio of protein to lipid, protein to carbohydrate, and lipid to carbohydrate.

The reliability of model simulations rests upon the effectiveness of the description of basic larval biochemistry used in creating the model, as discussed in Powell *et al.* (2002) and Bochenek *et al.* (2001). The greatest uncertainty resides in the fate of protein when the food ingested has a high protein-to-lipid ratio. In the model, protein is used only for somatic growth. Larvae fed a high protein diet grow rapidly, but store insufficient lipid to sustain successful metamorphosis. The assumption that excess protein is not metabolized into other biochemical constituents or eliminated in some way leads directly to the low survivorships observed in these simulations. Such simulations underestimate survival if an alternate fate for excess protein is available. Nevertheless, the expectation that larval survival is improved by lower protein ratios as observed in experimental studies (e.g., Helm *et al.,* 1973; Wikfors *et al.,* 1984; Thompson *et al.,* 1994, 1996; see also Garciá-Esquivel *et al.,* 2001) is reproduced by the simulations.

A second uncertainty concerns the availability of essential fatty acids. Substantial research supports the conclusion that oyster larvae have an absolute requirement for certain unsaturated fatty acids (e.g. Webb and Chu, 1982; Thompson *et al.,* 1996; McCausland *et al.,* 1999; Soudant *et al.,* 1999). Modeling larval metabolism at the more detailed level of individual fatty-acid constituents is not presently feasible. In this study, lipids are apportioned into two large pools, the neutrals serving primarily as energy stores, and the polars serving primarily a structural purpose. Simulations are based on the assumption that any of the simulated diets include enough essential fatty acids to minimize the influence of any individual constituent on growth and survival. One can reasonably expect that any fatty acid being limiting in the diet would reduce growth and increase mortality, an uninteresting although potentially important outcome. Consequently simulations were designed to examine cases where individual constituents were not limiting. Transfers between the neutral and polar lipid pools are based similarly on the assumption that any single constituent was not limiting.

A third uncertainty arises from the fate of stored egg reserves in the first few hours of larval life when feeding rate is low (e.g., Lucas and Rangel, 1983). 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. Precisely how the larva mobilizes the reserves to provide necessary structural lipid, carbohydrate, and protein, as well as the requirements of basal metabolism, is unknown. No attempt has been made to formalize this biochemical process beyond the conversion of carbon from one tissue constituent to another. As events in these first few hours critically influence subsequent survival (e.g., Gallager and Mann, 1986), this necessary simplification may be significant.

A fourth uncertainty arises from the role that lipid content is imputed to play in metamorphosis. In the model, variations in neutral and polar lipid dictate competency and

metamorphosis success. Results of simulations agree with literature observations on metamorphosis (Bochenek *et al.,* 2001); however, little is actually known about the biochemical mechanism controlling metamorphosis. Recent data of Garciá-Esquivel *et al.* (2001) indicate that low survival was related to high protein use during metamorphosis, in agreement with model construction that relates higher lipid stores to increased metamorphosis success.

A final uncertainty arises from the meager knowledge about the genetic variation in cohorts of bivalve larvae, at least from the standpoint of the contribution of genetic variation to survival in the field. In the model, genetic variation is introduced through variations in egg size and respiration rate, the latter being a surrogate for 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 any of these would have essentially the same effect. Although the range of genetic compositions implemented in the model is based upon experimental observation (Bochenek *et al.,* 2001), the genetic variation in the simulated larval cohorts cannot be compared with field observations of genetic variation in larval cohorts, of which we know little. Nevertheless, the chosen distribution of genotypes directly affects the outcome of the simulations. The sensitivity of simulated outcomes to this distribution of variation has been explored by Bochenek *et al.* (2001).

3. Results

a. Results of simulations

i. Time-varying salinity. The presumption is that temperature varies relatively little over the course of planktonic life, in comparison to the range necessary to substantially change cohort survival (Hofmann *et al.,* 2004). Salinity, on the other hand, may vary considerably over short periods of time. Transient changes in salinity over a seven day period at optimal temperature and saturating food supply (27.5°C, 2 mg L^{-1}, Fig. 1A) have little effect on cohort success (cases 7–9, Fig. 1B). Reducing food supply to 1 mg L^{-1} (cases 1–3, Fig. 1B) reduces cohort survival by increasing planktonic life span and, thereby, increasing predatory losses. In this figure, and all subsequent figures, cases are listed in order from case 1 on top to case 9, in this example, at the bottom. An increase in salinity minimizes the impact of reduced food supply, especially if the increase occurs near the time of metamorphosis (case 1, Fig. 1B). The increase in filtration rate that accompanies a rise in salinity provides the greatest amount of resources for the larva at this critical time. At lower temperatures, e.g. 22.5°C, the timing is even more critical. Only an increase in salinity near metamorphosis permits any larval survival (case 4 versus cases 5–6, Fig. 1B).

At saturating food conditions (2 mg L^{-1}), larvae with high growth efficiency [quadrants 3 (small eggs) and 4 (large eggs)] are favored (cases 7–9, Fig. 1C). Limiting larval success

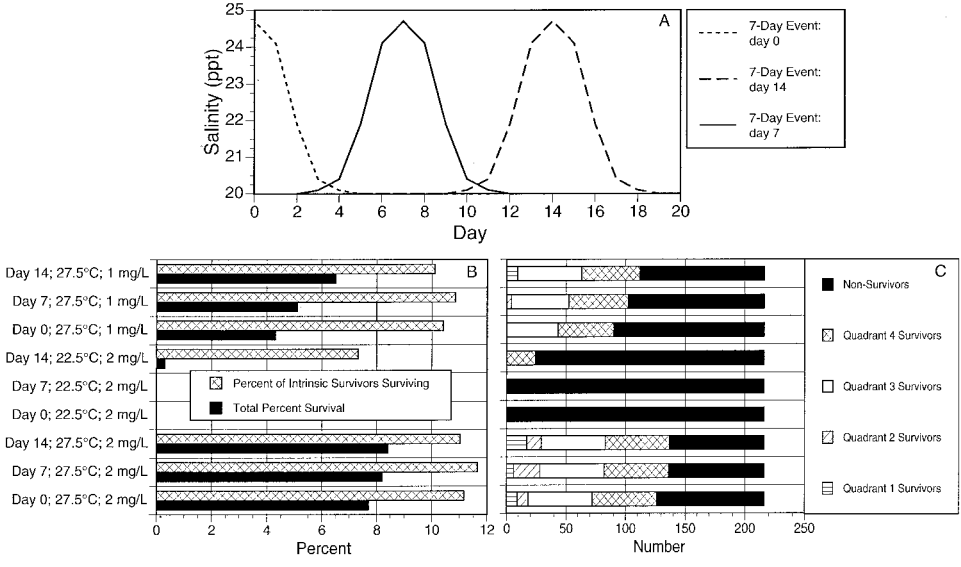

Figure 1. Results of simulations focusing on short-term variations in salinity obtained for 27.5°C and standard food rations of 1 mg L^{-1} or 2 mg L^{-1}. (A) Time variability of salinity was imposed as 7-day events centered on days 0, 7, or 14, during which time salinity rose from 20‰ to 25‰. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrants are defined as in Figure 3 of Hofmann *et al.* (2004): quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the salinity pulse was centered, and the temperature and food concentration used for the simulation. Cases referred to in the text are enumerated with case 1 at the top and case 9 at the bottom.

by reducing food supply or lowering temperature increasingly favors larvae with high growth efficiency (cases 1–6, Fig. 1C). A short-term increase in salinity has little impact on the genetic distribution of successful larvae (Fig. 1C). Overall, the entire range of salinity and temperature conditions tested with the model favor larvae with high growth efficiency (Fig. 1C) under saturating food conditions.

ii. Time-varying food concentration. The timing of a short-term decrease in food supply (Fig. 2A) did not substantially affect larval cohort survival. Survival was limited regardless of the timing (cases 1–3, the top 3 cases, Fig. 2B). At optimal temperatures and salinities as well, the timing of an increase in food concentration (Fig. 2A) produced relatively little effect on larval cohort survival (cases 7–9, the bottom 3 cases, Fig. 2B). Cohort survival was relatively high in every case. In contrast, at suboptimal temperatures (22.5°C), an increase in food supply at metamorphosis was critical for cohort survival (case 4, Fig. 2B).

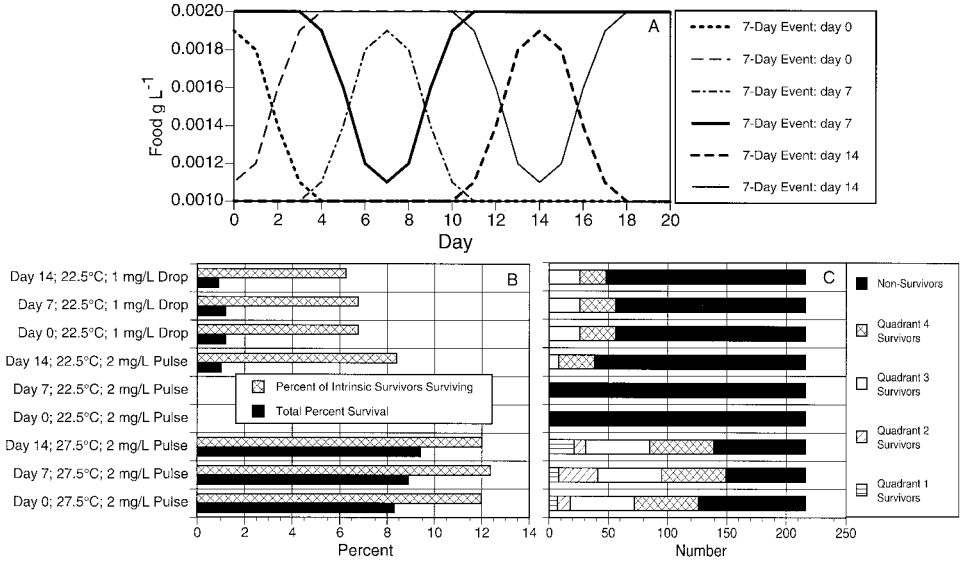

Figure 2. Results of simulations focusing on short-term variations in food concentration obtained for 27.5‰ and a ration of standard food at 27.5°C and 22.5°C. (A) Time variability of food concentration was imposed as 7-day events centered on days 0, 7, or 14, during which time food concentration increased from 1 mg L^{-1} to 2 mg L^{-1} or decreased from 2 mg L^{-1} to 1 mg L^{-1}. Time series are in pairs, with the first in each pair a 7-d increase in food and the second in each pair a 7-d decrease in food. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the food pulse was centered (e.g., Day 14), the temperature used for the simulation (e.g., 22°C) and the magnitude and type of food variation used (e.g., a 1 mg L^{-1} decline). Cases referred to in the text are enumerated with case 1 at the top and case 9 at the bottom.

Short-term restrictions in food supply restrict the success of larvae with low growth efficiency [quadrants 1 (small eggs) and 2 (large eggs), cases 1–3, Fig. 2C]. At optimal environmental conditions, some of these larvae do contribute to cohort survival, whereas none do at lower temperature (cases 4–6, Fig. 2C). Under certain circumstances, such as increasing food toward the end of larval life (cases 4, 7, Fig. 2C), egg size is also important in determining larval survival. Larvae from small eggs [quadrants 1 (low growth efficiency) and 3 (high growth efficiency)] or large eggs [quadrants 2 (low growth efficiency) and 4 (high growth efficiency)] may do better at this time, depending upon temperature. However, the influence of egg size on survival is never equivalent to the effect of growth efficiency.

Increasing the duration of events controlling food availability from 7 days to 14 days (Fig. 3A) does not materially change the pattern of larval survival or the suite of genetic

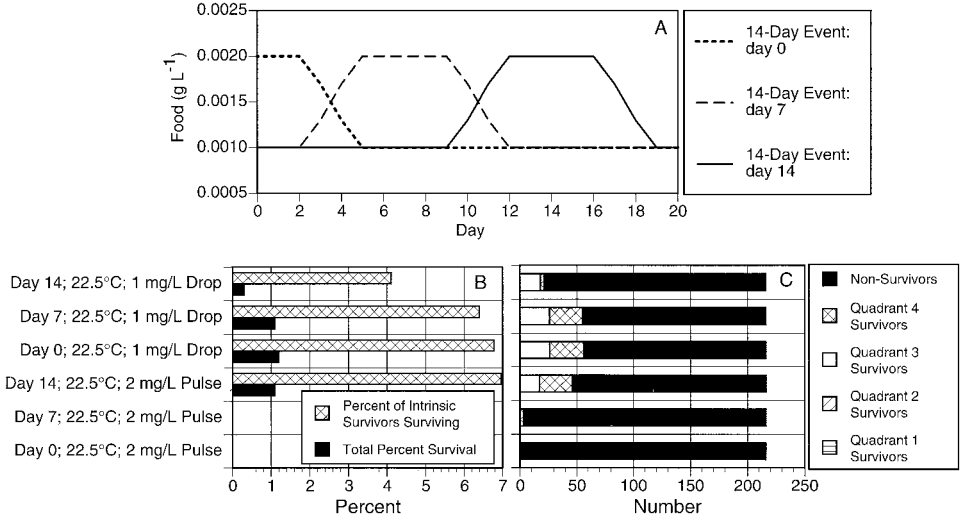

Figure 3. Results of simulations focusing on short-term variations in food concentration obtained for 27.5‰ and a ration of standard food at 27.5°C and 22.5°C. (A) Time variability of food concentration was imposed as 14-day events centered on days 0, 7, or 14, during which time food concentration increased from 1 mg L^{-1} to 2 mg L^{-1} or decreased (not shown) from 2 mg L^{-1} to 1 mg L^{-1}. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the food pulse was centered (e.g., Day 14), the temperature used for the simulation (e.g., 22.5°C), and the magnitude and type of food variation used (e.g., 1 mg L^{-1} decline). Cases referred to in the text are enumerated with case 1 at the top and case 6 at the bottom.

predispositions that facilitate survival (Fig. 3C). Once again, an increase in food near the time of metamorphosis is essential for cohort survival when temperature is low (case 4, Fig. 3B). Genetic variations in growth efficiency [quadrants 1 and 2 (low growth efficiency) vs. 3 and 4 (high growth efficiency), cases 1–4, Fig. 3C] are more important in determining larval survival than variations in egg size [quadrants 1 and 3 (small eggs) vs. 2 and 4 (larger eggs), cases 1–4, Fig. 3C].

iii. Time-varying food quality. Food quality was varied between a very high protein and a high carbohydrate diet (Fig. 4A). A small increase in lipid occurred coincident with the increase in carbohydrate. The very high protein diet did not permit survival, even if a 7-day change to a high carbohydrate diet occurred during larval life (cases 1–4, the top 4 cases, Fig. 4B). In contrast, the high carbohydrate diet resulted in significant cohort survival (case 5, Fig. 4B). A pulse of high protein, low carbohydrate food decreased total survival (cases

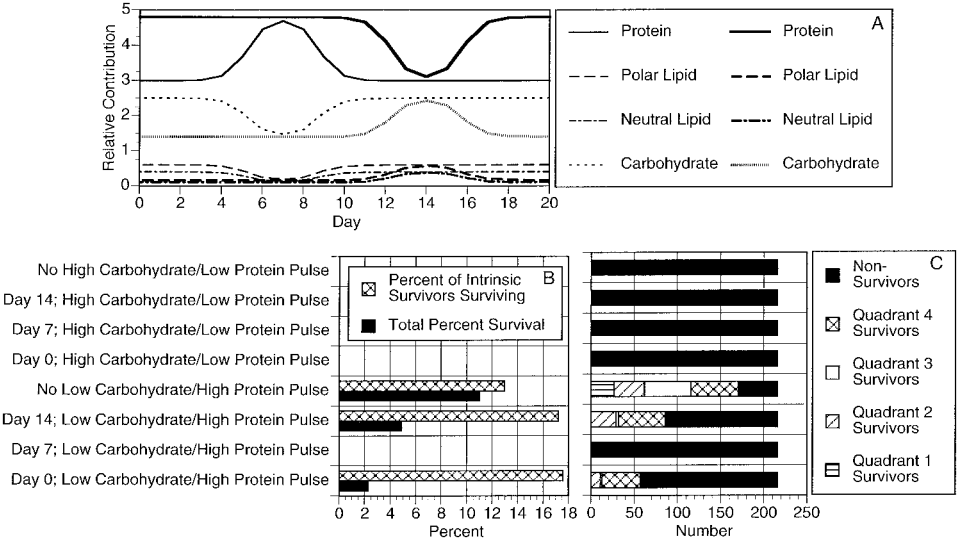

Figure 4. Results of simulations focusing on short-term variations in the proportion of carbohydrate and protein in the diet at 27.5‰, 27.5°C, and a 1.5 mg L^{-1} ration. (A) Time variability of food quality was imposed as 7-day events centered on days 1, 7, or 14. An increase in protein and decrease in carbohydrate centered on Day 7 (left legend, narrow lines) and an increase in carbohydrate and decrease in protein centered on Day 14 (right legend, thick lines) are shown. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percentage of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. Cases referred to in the text are enumerated with case 1 at the top and case 8 at the bottom. Key to the left indicates the day on which the food pulse was centered (e.g., Day 14), and the type of food pulse (e.g., low carbohydrate/high protein). Results for constant food quality over the larva's lifetime designated 'No' pulse, are shown in cases 1 and 5 for comparison.

6–8, the bottom 3 cases, Fig. 4B). Larvae did not survive if the very high protein diet was provided during the middle of larval life (case 7, Fig. 4B). Survival did occur if the very high protein pulse occurred early or late in larval life (cases 6, 8, Fig. 4B), but total cohort survival declined in comparison to the case for a continuous high-carbohydrate diet (case 5, Fig. 4B). In both instances, however, the decrease in total cohort survival was minimized because losses to predation declined. Consequently, more of the intrinsic survivors, those larvae that would have completed metamorphosis successfully in the absence of predation, survived and completed metamorphosis because larval life span decreased (cases 6, 8 vs. 5, Fig. 4B).

The high protein pulse consistently favored larvae coming from large eggs (quadrants 2 and 4, cases 6–8, Fig. 4C). These larvae began life with a higher lipid content that sustained the larvae during the high-protein portion of life. Increased somatic growth from

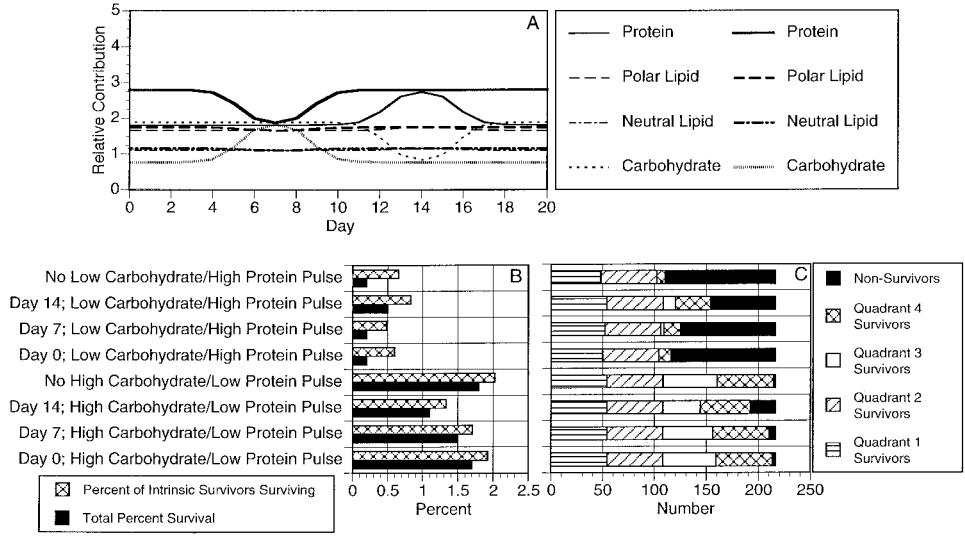

Figure 5. Results of simulations focusing on short-term variations in the proportion of carbohydrate and protein in the diet at 27.5‰, 27.5°C, and a 0.75 mg L^{-1} ration. (A) Variability in food quality was imposed as 7-day events centered on days 1, 7, or 14. An increase in carbohydrate and decrease in protein centered on Day 7 (right legend, thick lines) and an increase in protein and decrease in carbohydrate centered on Day 14 (left legend, thin lines) are shown. Results of simulations show (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quality was centered (e.g., Day 14) and the variation in food quality imposed (e.g., a low carbohydrate/high protein pulse). Results for constant food quality over the larva's lifetime, designated 'No' pulse, are shown in cases 1 and 5 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 8 at the bottom.

the added protein decreased larval life span, so losses to predation were minimized (cases 6–8, Fig. 4B). Total survival did not increase, however, because no survival occurred in larvae coming from small eggs (quadrants 1 and 3, cases 6–8, Fig. 4C).

For comparison, larvae were exposed to a high protein diet, but less extreme than the previously-simulated diet, and a high carbohydrate diet (Fig. 5A). Lipid levels were higher in both diets than either diet used in the previous set of simulations (Fig. 4A). Larval survival is more consistent over this range of diets (Fig. 5B), due to the proportional increase in lipid and decrease in protein. The high, but less extreme, protein diet is inherently better (case 5, Fig. 5B vs. case 1, Fig. 4B). The high protein diet permits all larval genotypes to survive (case 5, Fig. 5C), whereas the high carbohydrate diet only favors larvae with low growth efficiency [quadrants 1 and 2 (both small and large eggs),

case 1, Fig. 5C]. Larvae with low growth efficiency burn excess carbohydrate and, thereby, limit the likelihood of a metabolic imbalance.

A 7-day change in food quality affects larval survival primarily if it occurs late in larval life. An increase in carbohydrate late in larval life decreases survival of larvae heretofore exposed to a diet rich in protein (case 6, Fig. 5B). Higher protein in the food late in larval life increases the survival of larvae heretofore exposed to a diet rich in carbohydrate (case 2, Fig. 5B). The change in total cohort survival is expressed by a varying fraction of survivors with high growth efficiency [compare quadrants 1 and 2 (low growth-efficiency larvae) with 3 and 4 (high growth-efficiency larvae) in cases 2 and 6, Fig. 5C]. Of these, larvae from larger eggs, beginning life with greater lipid reserves, do best [compare quadrants 3 (larvae from small eggs) and 4 (larvae from large eggs), cases 2 and 6, Fig. 5C].

The next set of simulations considered short-term variations in the protein-to-lipid ratio (Fig. 6A). In this diet, an increase in lipid is also accompanied by a moderate decrease in carbohydrate (Fig. 6A). These simulations produced larval survival patterns similar to the previous case in which protein and carbohydrate were varied (Fig. 5), emphasizing the interchangeability of carbohydrate and lipid (Fig. 6). The protein-rich diet produces a higher survivorship than the lipid-rich diet (case 5 vs. case 1, Fig. 6B), principally because the fraction of intrinsic survivors surviving increases. The implication is that this diet reduces larval life span in comparison to the diet richer in lipid and this reduces loss to predation. The diet rich in protein favors larvae with high growth efficiency (quadrants 3 and 4, case 5, Fig. 6C), whereas the lipid-rich diet favors larvae with low growth efficiency (quadrants 1 and 2, case 1, Fig. 6C).

A high protein event during the life span of larvae existing on a lipid-rich diet increases survival, but only if it occurs late in larval life (quadrants 3 and 4, case 2 vs. 3 and 4, Fig. 6B). This event increases the survival of larvae with high growth efficiency (case 2 vs. 3 and 4, Fig. 6C). The influence of a high lipid event for larvae normally exposed to a protein-rich diet is more complex. The total percent survival is low when the event occurs early in larval life (case 8, Fig. 6B). Increased lipid during the last two-thirds of larval life increases total survival (cases 6 and 7, Fig. 6B). Total survival is greatest if the event occurs midway during larval life. Increased survival occurs because intrinsic survival increases; in particular, larvae coming from large eggs (quadrants 2 and 4) and small-egged larvae with high growth efficiency (quadrant 3) are more likely to successfully complete metamorphosis (cases 6 and 7, Fig. 6C). The percent of intrinsic survivors surviving decreases however, as the high-lipid event occurs ever later in larval life (cases 6–8, Fig. 6B). Thus, highest total survival occurs midway in larval life when the increase in intrinsic survival and the decrease in larval life span dovetail to maximize cohort survival.

Simulations that use larger short-term variations in the protein-to-lipid ratio provide further insight into the effect of protein on larval survival (Fig. 7A). The very high protein diet, once again, if present during a substantial portion of larval life, results in no survival (cases 1–4, Fig. 7B). This diet contains an insufficient amount of lipid to cover larval

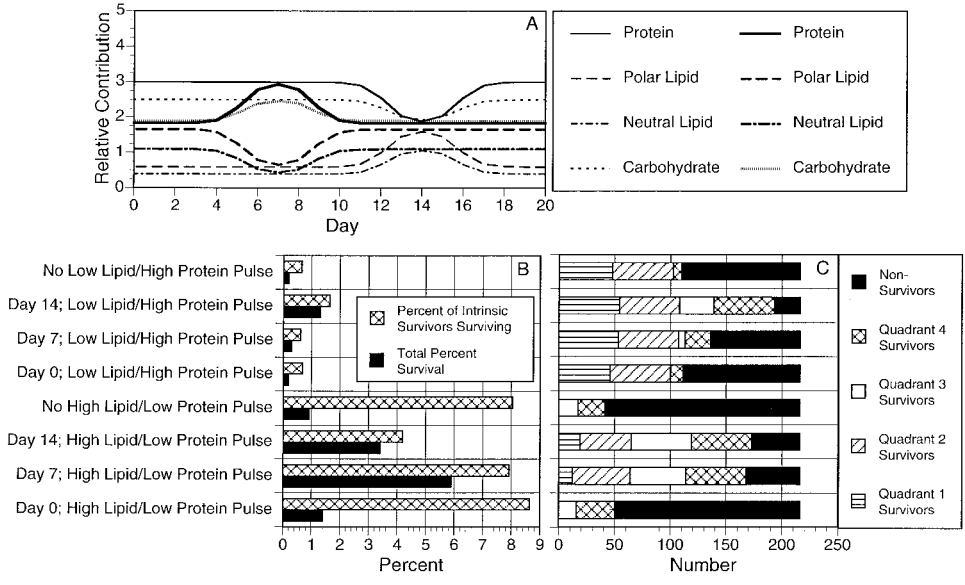

Figure 6. Results of simulations focusing on short-term variations in the proportion of lipid and protein in the diet at 27.5‰, 27.5°C, and a 0.75 mg L^{-1} ration. (A) Time variability in food quality was imposed as 7-day events centered on days 0, 7, or 14. An increase in protein and decrease in lipid centered on Day 7 (right legend, thick lines) and an increase in lipid and decrease in protein centered on Day 14 (left legend, thin lines) are shown. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quality was centered (e.g., Day 14) and the variation in food quality imposed (e.g., a low lipid, high protein pulse). Results for constant food quality over the larva's lifetime, designated 'No' pulse, are shown in cases 1 and 5 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 8 at the bottom.

metabolic needs. A high protein pulse in a diet otherwise high in lipid, however, is particularly advantageous if it occurs late in larval life (case 6, Fig. 7B). This diet dramatically increases larval survivorship. The complement of genotypes yielding successful larvae in this simulation includes all combinations of growth efficiency and egg size (case 6, Fig. 7C) and, as importantly the length of larval life declines. This decline reduces loss to predation.

Interestingly, the occurrence of a high protein event early in larval life selects for larvae from large eggs with low growth efficiency (quadrant 2, case 8, Fig. 7C). Early in larval life, the extra lipid in these eggs sustains the larvae through the period of reduced lipid availability in the food. Later on, the low growth efficiency permits the larva to reduce its lipid-to-protein ratio, thereby permitting successful metamorphosis. The outcome of this

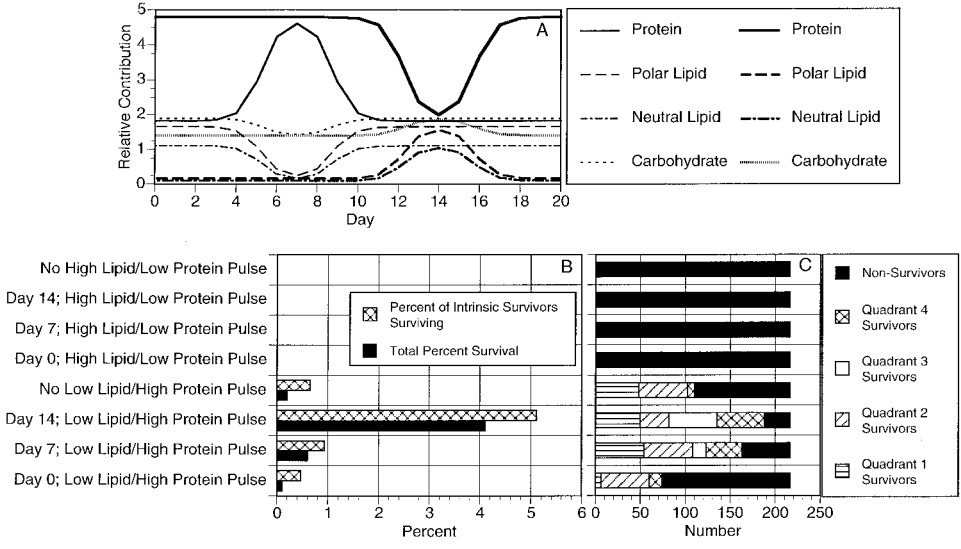

Figure 7. Summary of simulations focusing on short-term variations in the proportion of lipid and protein in the diet at 27.5‰, 27.5°C, and a 0.75 mg L^{-1} ration. (A) Time variability in food quality was imposed as 7-day events centered on days 0, 7, or 14. An increase in protein and decrease in lipid centered on Day 7 (left legend, narrow lines) and an increase in lipid and decrease in protein centered on Day 14 (right legend, thick lines) are shown. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quality was centered and the variation in food quality imposed. Results for constant food quality over the larva's lifetime, designated 'No' pulse, are shown in cases 1 and 5 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 8 at the bottom.

simulation depends, of course, on the mechanism by which metamorphosis is invoked in the model (see Bochenek *et al.,* 2001).

Simulations that use diets with relatively constant protein content, but in which the carbohydrate-to-lipid ratio varies, illustrate the degree of interchangeability of these two biochemical constituents (Fig. 8A). Despite their interchangeability for many metabolic processes, variation in the carbohydrate-to-lipid ratio does affect survivorship (Fig. 8B). Greater total cohort survival occurs with the high lipid diet than the high carbohydrate diet (compare cases 1 and 5, Fig. 8B) because a greater suite of genotypes yields successful larvae (compare cases 1 and 5, Fig. 8C). Larval life span of successful larvae is shorter with the high carbohydrate diet, as is shown by the higher fraction of intrinsic survivors surviving (case 1, Fig. 8B), but only larvae with high growth efficiency survive (quadrants 3 and 4, case 1, Fig. 8C).

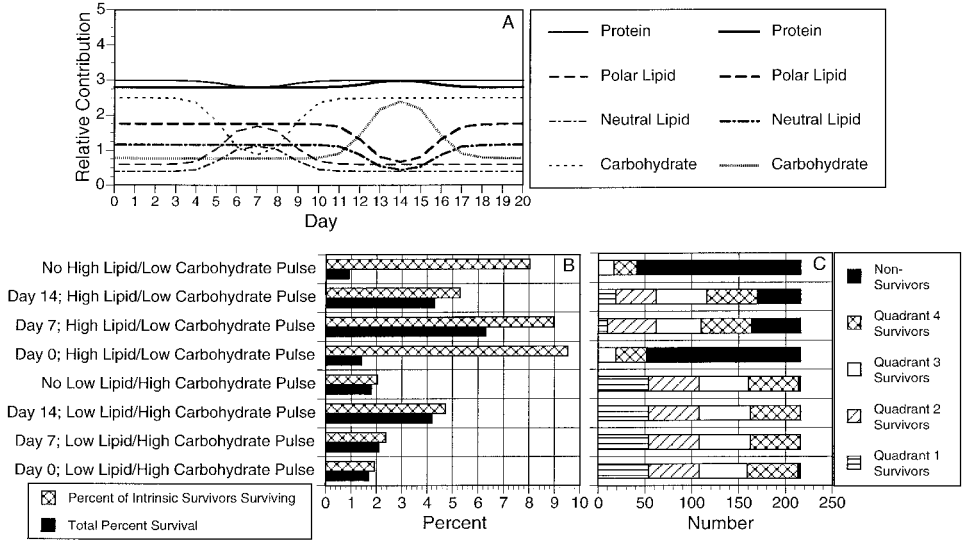

Figure 8. Summary of simulations focusing on short-term variations in the proportion of lipid and carbohydrate in the diet at 27.5‰, 27.5°C, and a 0.75 mg L^{-1} ration. (A) Time variability in food quality was imposed as 7-day events centered on days 0, 7, or 14. An increase in lipid and decrease in carbohydrate centered on Day 7 (left legend, narrow lines) and an increase in carbohydrate and decrease in lipid centered on Day 14 (right legend, thick lines) are shown. Summary of simulation results expressed as (B) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (C) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quality was centered and the variation in food quality imposed. Results for constant food quality over the larva's lifetime, designated 'No' pulse, are shown in cases 1 and 5 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 8 at the bottom.

A short-term increase in carbohydrate content is efficacious only if it occurs late in larval life (case 6, Fig. 8B). Total cohort survival increases because larval life span declines (Fig. 8B) and because more genotypes are successful (case 6, Fig. 8C). High lipid food increases total cohort survival, particularly if the event occurs midway during larval life (case 3, Fig. 8B). Again, the number of genotypes yielding successful larvae increases (case 3, Fig. 8C), as does the fraction surviving predation due to the shortening of larval life span (case 3, Fig. 8B).

iv. Time-varying food quantity and food quality. The simulations described previously (Figs. 4–8) suggest that changes in food quality can substantially influence larval cohort survival. However, food quantity can vary as well as food quality and not necessarily in a

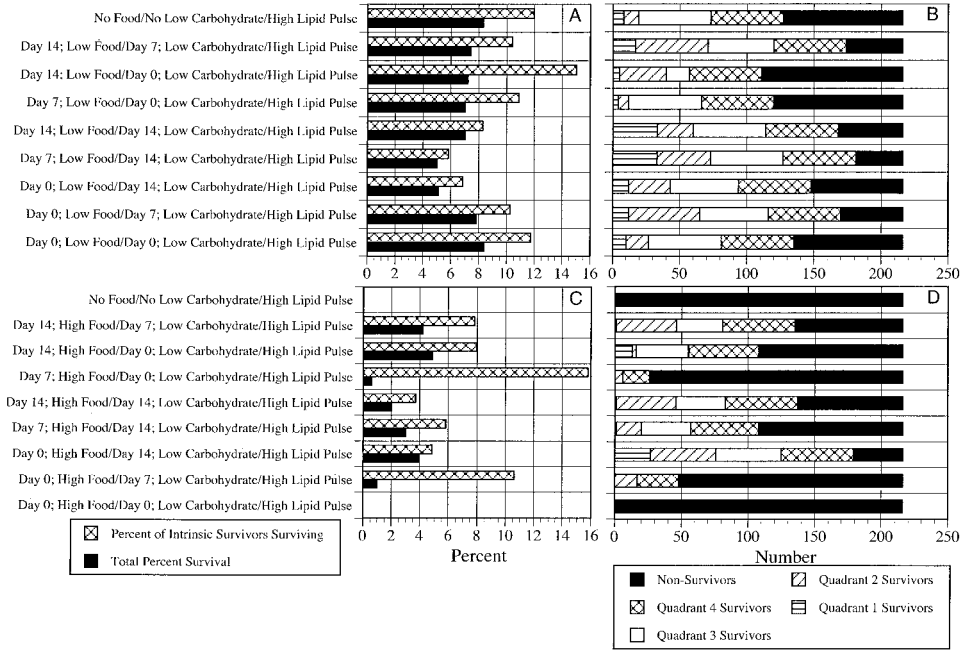

Figure 9. Summary of simulations focusing on short-term variations in the proportion of lipid and carbohydrate in the diet and independent short-term variations in the concentration of food at 27.5‰ and 27.5°C. Time variability in food quality and food concentration was imposed as 7-day events centered on days 0, 7, or 14, as shown in Figure 8. Food concentration varied between 0.5 mg L^{-1} and 1 mg L^{-1}. Summary of simulation results expressed as (A, C) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (B, D) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quantity was centered, the variation in food quantity imposed, the day on which the variation in food quality was centered, and the variation in food quality imposed. As an example, case 2 records the results for a simulation in which food quantity was increased for a 7-day period centered on Day 14 and food quality was varied such that lipid increased and carbohydrate decreased during a 7-day period centered on Day 7. Results for constant food quality and quantity over the larva's lifetime, designated 'No' pulse, are shown in case 1 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 9 at the bottom.

concordant way. Independent variations in food quantity and quality produce a complex array of survival patterns (Figs. 9–11).

A decline in food quantity that occurs out of phase with pulses of low-carbohydrate, high-lipid food produces an array of survival patterns. Overall, the reduction in food quantity increases larval life span and, so, reduces larval survival (cases 2–9, Fig. 9A). As in previous figures, the case numbers referred to in this and subsequent figures are ordered

from top, case 1, to bottom, in this case, case 8. This trend is offset somewhat by an increase in the range of genotypes producing successful larvae (compare case 1 with cases 2–9, Fig. 9B), so that total cohort survival does not vary too much (Fig. 9A). An exception is the case where a food decline earlier in larval life precedes an increase in lipid content late in larval life (case 3, Fig. 9A, B). Survival declines despite a decrease in larval life span because the number of successful genotypes is dramatically reduced (case 3, Fig. 9B).

Low food supply during much of larval life restricts survival (case 1, Fig. 9C). An increase in food supply is efficacious in this circumstance in all but one case (cases 2–8, Fig. 9C). Survival is greatest if increased food occurs late in larval life or if increased food precedes an increase in lipid late in larval life (cases 2, 3, 6, 7, Fig. 9C). Each increases the number of genotypes yielding successful larvae, particularly those larvae from large eggs with high growth efficiency (quadrant 4, same cases, Fig. 9D).

A diet that varies between high protein and high lipid while food quantity varies independently between high and low concentrations substantially affects total cohort survival (Fig. 10). When transient periods of reduced food supply occur and when lipid-rich food is available for most of larval life, survival is increased by a pulse of protein-rich food late in larval life (cases 5–7, Fig. 10A), regardless of the timing of a transient decrease in total food supply. The number of genotypes yielding successful larvae increases (cases 5–7, Fig. 10B), but even more importantly is the decrease in larval life span that increases the fraction of intrinsic survivors surviving predation (cases 5–7, Fig. 10A).

A transient increase in food supply, for larvae experiencing lipid-rich food for much of larval life, also benefits the larvae predominately when the protein component of the food increases late in larval life (cases 5–7, Fig. 10C). Greatest survival occurs when a transient increase in food supply and a transient increase in protein content coincide (case 5, Fig. 10C). All three cases with maximum cohort survival (cases 5–7, Fig. 10D) increase the number of genotypes supporting successful larvae, particularly those coming from small eggs (quadrants 1 and 3). At the same time, larval life span is reduced. The coincidence of a reduction in larval life span and an increase in the survivorship of larvae coming from small eggs produces a large increment in larval cohort survival.

A final set of simulations considers the case of transient shifts in the protein-to-carbohydrate ratio accompanied by independent shifts in total food supply. Transient reductions in food supply reduce total cohort survival to some degree (cases 2–9, Fig. 11A), as they did when the protein-to-lipid content was varied. Nevertheless, total cohort survival remains relatively high, regardless of the timing of a reduction in food or the timing in the switch between carbohydrate and protein content (Fig. 11A). Poorest survivorship occurs when a transient reduction in food occurs early in larval life and the carbohydrate content of the food increases late in larval life (cases 6–7, Fig. 11A). These larvae are the most deprived of protein. Most genotypes yield survivors (Fig. 11B). Variation in total cohort survival is primarily determined by variations in larval life span that influence total loss to predation.

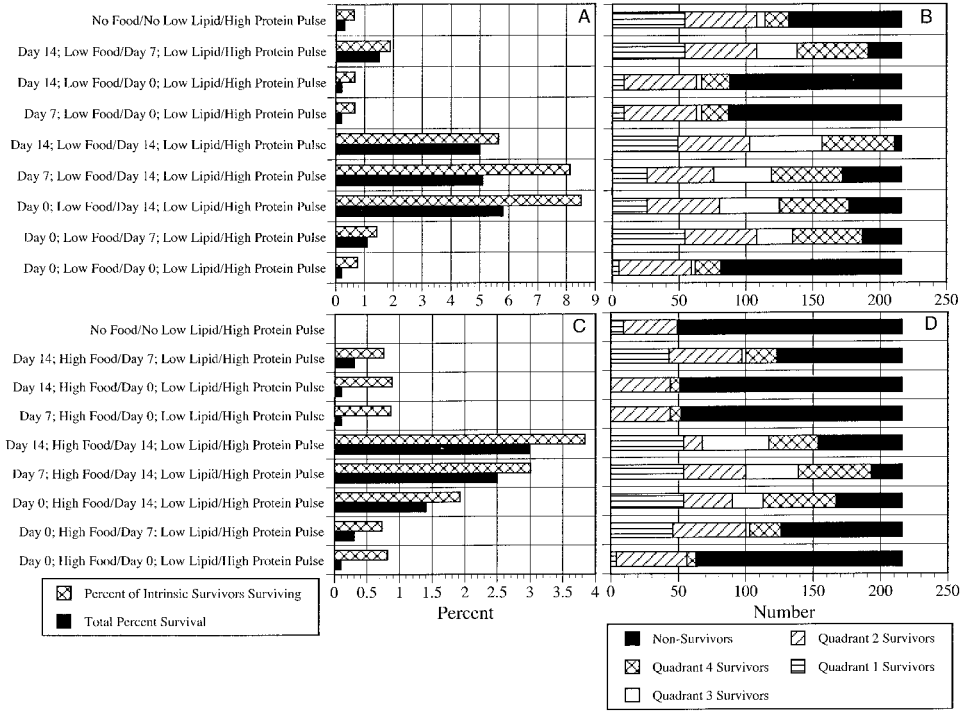

Figure 10. Summary of simulations focusing on short-term variations in the proportion of lipid and protein in the diet and independent short-term variations in the concentration of food at 27.5‰ and 27.5°C. Time variability in food quality and food concentration was imposed as 7-day events centered on days 0, 7, or 14, as shown in Figure 6. Food concentration varied between 0.5 mg L^{-1} and 1 mg L^{-1}. Summary of simulation results expressed as (A, C) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (B, D) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quantity was centered, the variation in food quantity imposed, the day on which the variation in food quality was centered, and the variation in food quality imposed. As an example, case 2 records the results for a simulation in which food quantity was increased for a 7-day period centered on Day 14 and food quality was varied such that protein increased and lipid decreased during a 7-day period centered on Day 7. Results for constant food quality over the larva's lifetime, designated 'No' pulse, are shown in case 1 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 9 at the bottom.

A transient increase in food yields much the same pattern. Survivorship is relatively uniform and most larval genotypes contribute successful larvae (Fig. 11C, D). Variations in larval life span again explain variations in cohort survival (Fig. 11C, D). A transient reduction in protein content of the food late in larval life reduces survivorship by extending larval life span (cases 5–7, Fig. 11C), regardless of food availability.

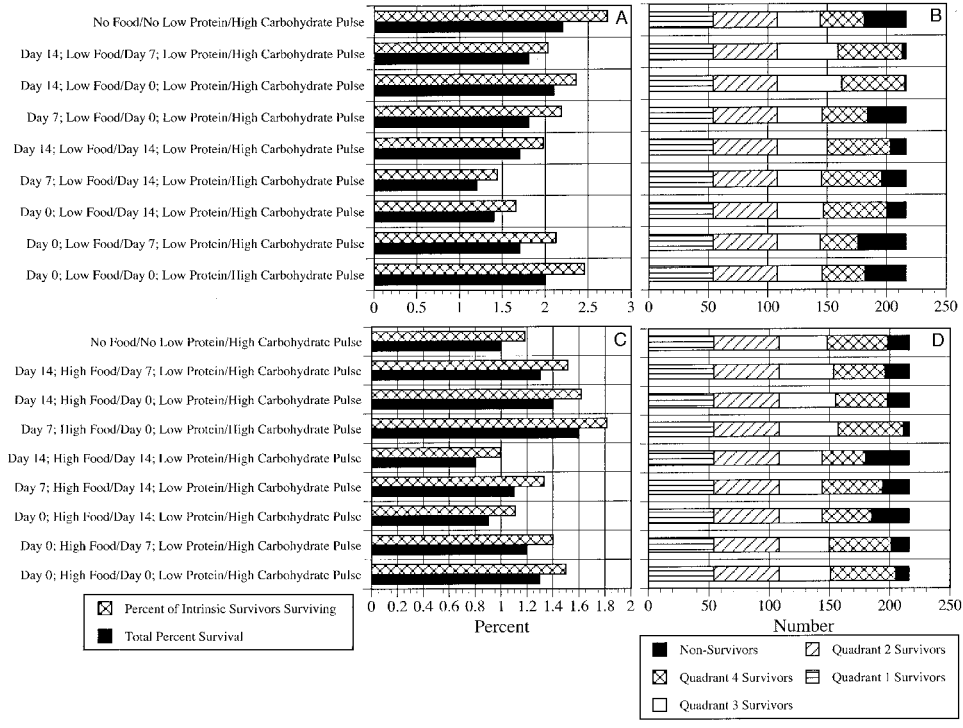

Figure 11. Summary of simulations focusing on short-term variations in the proportion of carbohydrate and protein in the diet and short-term variations in the concentration of food at 27.5‰ and 27.5°C. Time variability in food quality and food concentration was imposed as 7-day events centered on days 0, 7, or 14, as shown in Figure 5. Food concentration varied between 0.5 mg L^{-1} and 1 mg L^{-1}. Summary of simulation results expressed as (A, C) the percent of the larval cohort surviving and the percent of intrinsic survivors surviving and (B, D) the number of genetic combinations of egg size and growth efficiency yielding intrinsic survivors. Quadrant definitions: quadrant 1, low growth-efficiency larvae from small eggs; quadrant 2, low growth-efficiency larvae from large eggs; quadrant 3, high growth-efficiency larvae from small eggs; quadrant 4, high growth-efficiency larvae from large eggs. The key to the left indicates the day on which the variation in food quantity was centered, the variation in the food quantity imposed, the day on which the variation in food quality was centered, and the variation in food quality imposed. As an example, case 2 records the results for a simulation in which food quantity was increased for a 7-day period centered on Day 14 and food quality was varied such that carbohydrate increased and protein decreased during a 7-day period centered on Day 7. Results for constant food quality over the larva's lifetime, designated 'No' pulse, are shown in case 1 for comparison. Cases referred to in the text are enumerated with case 1 at the top and case 9 at the bottom.

4. Discussion

a. Overview

The simulations of cohort survival under varying conditions of food quantity and food quality strongly support the expectation that larval cohort survival varies over a wide range

in what might appears to be an unpredictable manner, if detailed time series of food quantity and quality are unavailable. Short-term variations in food quality, in particular, can dramatically influence cohort survival. Some consistent trends exist in the simulations, such as improved cohort survival at temperatures and salinities that are optimal for feeding. Such simple trends are not so easily discovered when variations in food quantity and food quality are considered. Results depend upon the details of the time history of food supply and food quality. Should this same behavior occur in the field, the likelihood is extremely high that it would manifest itself as the apparently unpredictable variability in settlement success routinely observed from field monitoring of bivalve recruitment. Thus, what may at first appear to be unpredictable temporal variations in recruitment may well be more predictable variations, if the underlying food and environmental conditions are more precisely known.

The simulations indicate that critical feeding periods during larval life do exist. However, the simulations also suggest that a single critical feeding period does not exist. Whether the most critical time is early, midway, or late in larval life depends upon the environment and, in particular, the composition of the food. Some changes in food quality produce much improved or much diminished larval survival if they occur early in larval life; others midway in larval life; and still others late in larval life.

Larval survival represents the sum of intrinsic and extrinsic processes. Environmental conditions that produce low survival generally are those that exacerbate both intrinsic and extrinsic mortality. That is, the range of genotypes yielding successful larvae contracts and larval life span simultaneously increases, permitting increased predatory losses. Conversely, environmental conditions that produce high survival generally minimize both sources of mortality. What is interesting is the frequency at which the two mortality types offset each other. To a degree, intrinsic and extrinsic mortality are independent physiologically. A trend seen in the simulations presented here is that a contraction in the range of genotypes yielding successful larvae may be offset by a shortening of larval life span in those potentially successful larvae with the consequent reduction in predatory losses. Offsetting trends in intrinsic and extrinsic mortality damp out variation in cohort survival over a significant range of potential environmental variability.

The range of genotypes described by variation in egg size and growth efficiency allows some conclusions about the effect of genetic variability on larval survival. Egg size influences survival principally by determining the amount of lipid stores present at the beginning of larval life. Growth rates vary in larval cohorts (e.g., Del Rio-Portilla and Beaumont, 2000) and this difference in growth efficiency influences larval life span, but it also generates a complex interaction between food quality and survivorship. Protein-rich diets tend to favor high growth efficiency to maximize lipid storage. Lipid-rich diets tend to favor low growth efficiency to maximize somatic growth by burning excess carbon. Of course, these inferences necessarily depend on the accurate modeling of the fate of dietary protein and rest on the assumption that certain essential fatty acids will not be limiting, as previously discussed.

The simulations show that most environmental variables, such as salinity and food quantity, influence the survivorship of larvae with low growth efficiency (quadrants 1 and 2, Figs. 1C, 2C). These larvae generally do not survive. Thus, these environmental conditions, by themselves, would tend to reduce the range of genotypes over time. However, food quality provides a counterweighing influence. All combinations of genotypes benefit disproportionately by some sequence of food qualities during larval life. In particular, selected food compositions can selectively benefit larvae with low growth efficiencies, larvae coming from large eggs, or larvae with high growth efficiencies over other genotypes. The single exception is larvae from small eggs. Larvae from small eggs are rarely the most successful larvae. These larvae require a protein-rich diet early in larval life and relatively high food supply to survive until feeding efficiency increases. However, these same conditions also benefit larvae from large eggs, suggesting that unique conditions benefiting larvae from small eggs are uncommon. Presumably, the increase in total fecundity is the driving force maintaining small-egg genotypes (Bochenek *et al.,* 2001). Regardless, the simulations support the adaptive advantage of larval cohorts with a relatively wide range of genotypes.

To a substantial extent, the timing of events is more important than the duration of events, although this aspect of variability was not investigated in depth. Nevertheless, in the simulations that were run, varying event durations from 7 to 14 days produced much less change in cohort survival than varying the timing of events from Day 0 to Day 7 to Day 14 (e.g., Figs. 2, 3). Timing, and the type of event, are critical to larval survival.

b. Salinity and food quantity

The simulation results suggest that complexity in the variation in cohort survival arises primarily from variations in food quality. Simulated cohort survival varies in a relatively predictable way when environmental variations are limited to salinity or changes in food quantity. Both, in effect, control the time-integrated food supply, either by influencing filtration rate or food availability. Reduction of the time-integrated food reduces cohort survival and selects for larvae with high growth efficiency as well as larvae coming from large eggs. Generally, the timing of temporal variability is inconsequential. Increased food, for example, has much the same effect whether it occurs early, midway, or late in larval life (Fig. 2A). An exception occurs under extreme conditions of limiting time-integrated food. In this case, an increase in food supply or filtration rate late in larval life results in survivorship of some larvae, when, without such an increase, no larvae would survive (Figs. 1B, 2B). Surviving larvae almost exclusively are those coming from large eggs and having high growth efficiencies. The increase in food late in larval life provides enough energy stores for these larvae to successfully support metamorphosis, according to model simulations.

c. Food quality

The simplicity of the effect of time-integrated food availability on cohort survival presents a stark contrast to the complexity introduced by variations in food quality. Metabolic requirements vary through larval life, such that some larval genotypes are more sensitive to variations in food composition at certain times. The simulations show that larvae from small eggs, for example, require sufficient protein early in life to rapidly grow to a size where feeding efficiency is increased. This must occur before early lipid stores are exhausted. Similarly, larvae with high growth efficiency require an increase in dietary lipid late in larval life to provide sufficient lipid stores for metamorphosis. As a consequence, the timing of changes in food quality is critical for most genotypes.

Considering the simulations as a whole, the most critical time for larvae is near the end of larval life (Day 14 in the simulations, Table 1). Increased protein at this time always improves survival. As modeled, the feeding efficiency of late stage larvae is declining. Accordingly, increased protein in the diet provides increased resources for somatic growth to counterweigh the loss due to declining feeding efficiency. Also, metamorphosis is assumed to be triggered by a fall in lipid stores. Increased protein in the diet increases the draw down of lipid stores that then triggers metamorphosis. The accuracy of the outcome of these simulations is dependent upon the triggering mechanism for metamorphosis, but the simulations are not at variance with the most recent experimental evidence (Garciá-Esquivel *et al.,* 2001).

When lipid is in sufficient quantity, increased protein early in larval life also increases survival (Table 1). More rapid somatic growth permits increased feeding efficiency earlier in larval life.

A diet enriched in lipid is most efficacious midway in larval life, but such a diet also exerts a positive impact on larval survival late in larval life (Table 1). Larvae require a certain amount of stored lipid to sustain metamorphosis. Most lipid storage occurs midway in larval life when feeding efficiency is highest. On the other hand, increased dietary lipid early in larval life has little influence on cohort survival (Table 1). The early part of larval life is sustained by lipid stores in the egg. In the model, additional lipid does not aid in this process because it does not increase somatic growth.

Variations in carbohydrate are relatively inconsequential. Increased carbohydrate only influences larval survival if the increase occurs late in larval life (Table 1). When accompanied by lower lipid, the event positively affects survivorship because carbohydrate spares the need to draw down lipid stores to cover the needs of tissue maintenance. When accompanied by lower protein, the event negatively affects survivorship because lipid must be mobilized to cover the needs of tissue maintenance.

d. Food quantity and food quality

Simple changes in food quality during larval life produce a moderately complex array of larval survivorships, but some consistent trends do emerge (Table 1). Simple changes in food quantity during larval life produce even simpler and more consistent trends in larval

Table 1. Summary of the influence of variations in food quality on larval cohort survival from Figures 4–8. Row and column categories represent the changes in food quality during a 7-day event. Numbers on the table represent the timing of events. Pluses and minuses represent the relative differences in outcome between the three event timings simulated (on day 0, 7, or 14). ++, survival substantially increased over the two alternatives; +, survival increased; −, survival diminished relative to the two alternatives.

	Low Carbohydrate Pulse	Low Protein Pulse	Low Lipid Pulse
Very-high Protein Pulse	$0^{+}, 7^{-}, 14^{++}$		14^{++}
High Protein Pulse	14^{+}		14^{+}
High Carbohydrate Pulse		14^{-}	14^{+}
High Lipid Pulse	$0^{-}, 7^{++}, 14^{+}$	$0^{-}, 7^{++}, 14^{+}$	

survivorship. Simulations in which the two variables, food quality and food quantity, are allowed to vary independently break down some of these previously identified trends, although, in many cases, the influence of food quality continues to dominate the outcome.

For example, increased protein in the diet late in larval life consistently improved survivorship (Table 1). This signal overwhelms an independent shift in food concentration (Fig. 10). The positive impact of increased protein occurs regardless of the timing or type of event, increase or decrease, introduced by a change in food quantity. Increased dietary carbohydrate with an accompanying decrease in protein diminishes survivorship if the event occurs late in larval life. This trend is unchanged by an earlier increase in food supply (Fig. 11). In contrast, changes in dietary lipid are often offset by changes in food quantity. For example, increased lipid positively affects larval survivorship if it occurs late in larval life (Table 1). A preceding decrease in food, however, negates this effect (Fig. 9). Increased lipid early in larval life diminishes survivorship (Table 1). An increase in food quantity late in life compensated for this effect (Fig. 9). The positive impact of increased dietary lipid midway in larval life essentially is negated by varying food supply, regardless of the timing.

The complexity of outcomes in the simulations independently varying food quantity and food quality originates in the independent effects of food quantity and food quality on extrinsic and intrinsic mortality. A large variation in survival of genotypes is produced depending upon the exact timing and type of events during larval life (cf. Figs. 9B, D, 10B, D, 11B, D). Large increases in survival originate because a large number of genotypes survive and/or because larval life span is shortened, thus minimizing predation (Fig. 10A, B). However, offsetting extrinsic and intrinsic processes offer important exceptions. For example, lipid-rich food early in larval life, with a later increase in food, results in a much shortened larval life span for surviving larvae (Fig. 9C). However, overall survival is low because few genotypes are intrinsically capable of survival (Fig. 9D). In comparison and concordantly, intrinsic survival is much higher for some genotypes under certain conditions (Fig. 11B), yet extended larval life spans reduce survival when a decline in food

precedes a drop in dietary protein (Fig. 11A). Overall, these more complex simulations indicate that changes in food quantity continue to influence survival primarily by varying the length of larval life. Food quality, on the other hand, tends to influence the genetic composition of the cohort, as well as varying larval life, and so produces large changes in survivorship. Some, but not all, of these changes can be compensated for by variations in food concentration.

5. Conclusions

The premise behind this modeling study is that certain periods of larval life are more critical than others with respect to the availability of food and that food quality is as important as food quantity in this respect. The literature on critical periods has focused on the time of first feeding for fish (e.g., Lasker, 1975, 1978; Lasker and Zweifel, 1978). The results of the simulations of *Crassostrea gigas* larvae indicate that critical feeding periods do exist, but that, for these larvae, which period is critical depends upon the time series of food. Overall, the most consistently critical time is late in larval life, near the time of metamorphosis. At this point, some variations in food quality are particularly efficacious, others particularly disastrous. The same is true for variations in food quantity. But, under certain circumstances, timing of events early or midway in larval life also dramatically changes cohort survival.

Clearly, a multitude of possible timing scenarios exist in nature and these will have a wide range of outcomes dictated by the mix of larval genotypes, the composition and quantity of the food, and the range of variation in composition and quantity. Probably, this complexity impedes the search for a clear relationship between broodstock abundance (or fecundity) and recruitment in shellfish. Probably, this complexity promotes the large year-to-year variations in settlement that are observed.

How important are food quantity and food quality in the field? The two variables affect larval survival in rather different ways. Classically, food quantity has been assessed by inference from chlorophyll measurements. Consistently, this measure produces food estimates that are too low to explain the observed success of oyster larvae (Crisp *et al.,* 1985; Dekshenieks *et al.,* 1993; Bochenek *et al.,* 2001), as well as post-settlement animals (Wilson-Ormond *et al.,* 1997; Soniat *et al.,* 1998; Hyun *et al.,* 2001). Alternate measures of food, whether it be total organic nitrogen (Wilson-Ormond *et al.,* 1997) or lipid + protein + labile carbohydrate (e.g., Soniat *et al.,* 1998; Hyun *et al.,* 2001; Versar, 2002) consistently show much higher food values. If these latter measures are appropriate for larvae, then food quantity may rarely be the limiting issue, although the dependency of oyster larvae on food concentrations higher than the adults normally require [compare Dekshenieks *et al.* (1993) and Powell *et al.* (1995)] and higher than required by some other bivalve larvae (Crisp *et al.,* 1985), do not exclude the possibility that food quantity is often a limiting condition.

Field studies measuring the time series of lipid, protein and labile carbohydrate also reveal substantial fluctuations in food composition, however. Typically, the food is lipid

rich with relatively little carbohydrate (Soniat *et al.,* 1998; Hyun *et al.,* 2001; Versar, 2002). High lipid content would tend to favor a wide range of genotypes. Typically, changes in food composition are dictated by changes in protein and lipid. Carbohydrate tends to be a more stable constituent. Accordingly, the simulations of particular noteworthiness, assuming these field measurements do measure the real food supply supporting larval growth, are the simulations in which the protein-to-lipid ratio varies. In these simulations, the most critical period is late in larval life as the larva nears metamorphosis. Changes in the protein-to-lipid ratio at that time can strongly increase or decrease cohort survival. The simulations, as they relate to field observations of food supply, suggest that experimental work should be directed at the influence of protein and lipid compositions on larval biochemistry at field-measured concentrations and in field-measured proportions.

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