Ecological Modelling
November 2017, Volume 363 Pages 221-233
http://dx.doi.org/10.1016/j.ecolmodel.2017.09.003
http://archimer.ifremer.fr/doc/00400/51137/

Integrating the effects of salinity on the physiology of the eastern oyster, *Crassostrea virginica*, in the northern Gulf of Mexico through a Dynamic Energy Budget model

Lavaud Romain [1, 2], La Peyre Megan K. [3, *], Casas Sandra M. [1], Bacher Cédric [4], La Peyre Jérôme F. [1]

[1] Louisiana State Univ, Ctr Agr, Sch Renewable Nat Resources, Baton Rouge, LA 70803 USA.
[2] Gulf Fisheries Ctr, Dept Fisheries & Oceans Canada, Moncton, NB, Canada.
[3] Louisiana State Univ, Ctr Agr, US Geol Survey, Louisiana Cooperat Fish & Wildlife Res Unit,Sch R, Baton Rouge, LA 70803 USA.
[4] IFREMER, Ctr Bretagne, Dynam Coastal Ecosyst Res Unit, Plouzane, France.

* Corresponding author : Megan K. La Peyre, email address : MLapeyre@agcenter.lsu.edu

Abstract :

We present a Dynamic Energy Budget (DEB) model for the eastern oyster, *Crassostrea virginica*, which enables the inclusion of salinity as a third environmental variable, on top of the standard foodr and temperature variables. Salinity changes have various effects on the physiology of oysters, potentially altering filtration and respiration rates, and ultimately impacting growth, reproduction and mortality. We tested different hypotheses as to how to include these effects in a DEB model for *C. virginica*. Specifically, we tested two potential mechanisms to explain changes in oyster shell growth (cm), tissue dry weight (g) and gonad dry weight (g) when salinity moves away from the ideal range: 1) a negative effect on filtration rate and 2) an additional somatic maintenance cost. Comparative simulations of shell growth, dry tissue biomass and dry gonad weight in two monitored sites in coastal Louisiana experiencing salinity from 0 to 28 were statistically analyzed to determine the best hypothesis. Model parameters were estimated through the covariation method, using literature data and a set of specifically designed ecophysiological experiments. The model was validated through independent field studies in estuaries along the northern Gulf of Mexico. Our results suggest that salinity impacts C. virginica's energy budget predominantly through effects on filtration rate. With an overwhelming number of environmental factors impacting organisms, and increasing exposure to novel and extreme conditions, the mechanistic nature of the DEB model with its ability to incorporate more than the standard food and temperature variables provides a powerful tool to verify hypotheses and predict individual organism performance across a range of conditions.

Highlights

► We developed a Dynamic Energy Budget model for the oyster Crassostrea virginica. ► The DEB model was extended to include salinity as a third environmental variable. ► We tested 2 hypotheses to account for the effect of salinity on oysters physiology. ► Salinity affects C. virginica physiology through filtration rather than maintenance. ► The model was validated against independent data from the northern Gulf of Mexico.

Keywords : DEB theory, Bioenergetics, Filtration, Maintenance, Growth, Estuary, Louisiana

1. Introduction

Variable environmental conditions define estuaries, controlling the distribution of many species, their individual performance (growth, reproduction, etc.) and their population dynamics. Understanding how individuals respond and handle these variable and changing environmental conditions through bioenergetics approaches provides a means to predict the performance of an organism. A foundation species found in estuaries along the northern Gulf of Mexico (nGoM), the eastern oyster, *Crassostrea virginica*, is controlled by multiple environmental variables, with salinity and temperature often cited as the dominant factors controlling individual performance (Galtsoff, 1964; Shumway and Koehn, 1982; La Peyre et al., 2009; Rybovich et al., 2016). With significant interest in managing this species to maximize production and restoration efforts, the need for general and robust prediction tools is critical. Numerical modelling, which accounts for important environmental controls, enables predictions of organism performance under more extreme or novel conditions (Nisbet et al., 2012; Fonseca and Gallucci, 2016). This paper integrates bioenergetics information from field and laboratory studies and uses the Dynamic Energy Budget (DEB) framework to develop a mechanistic model that includes salinity as a forcing variable, and enables predictions of *C. virginica* performance to changing and predicted conditions in the nGoM.

A number of modeling tools have been developed in recent decades to predict growth of shellfish in response to environmental variables. For *C. virginica* in particular, several regionally-based regression models have been developed to estimate oyster harvest (Ulanowicz et al., 1980), habitat suitability indices (Cake, 1983; Soniat et al., 2014), effects of temperature and salinity on larval development (Dekshenieks et al., 1993) and population dynamics (Livingston et al., 2000). More mechanistic approaches have also been used (Powell et al., 1992; Cerco and Noel, 2005; Wang et al., 2008). However, few of these models are transferable outside the region and conditions for which they were created as they use a detailed sequence of steps to model physiological processes, based on specific allometric relationships (Pouvreau et al., 2006). These approaches produce locally accurate results, yet are only descriptive and very hard to generalize, even towards animals of the same species but different in size (van der Meer, 2006). In contrast, Dynamic Energy Budget (DEB) models (Kooijman, 2010) provide an explicit understanding of factors controlling physiology and life history traits of organisms, especially in changing environments (Kooijman, 2010). DEB theory aims for a generic and mechanistic approach applicable to all species, but parameter calibration requires precisely quantifying

rates of key physiological processes under different environmental conditions. DEB models have been applied to various economically important bivalve species over the last decade (Pouvreau et al., 2006; Flye-Sainte-Marie et al., 2009; Saraiva et al., 2011; Lavaud et al., 2014). Filgueira et al. (2014) proposed a set of DEB parameters for *C. virginica*, derived from other studies mainly dealing with another oyster species, *Crassostrea gigas*. This approach however potentially induces physiological inconsistency in the set of parameters (van der Meer, 2006; Lika et al., 2011). The use of empirical datasets in a standardized single step procedure is thus necessary to estimate a consistent set of parameters.

Standard DEB models operate with only two forcing variables: temperature and food availability. However, in the case of *C. virginica* living in estuaries along the nGoM, large salinity variations critically control *C. virginica* physiology; low salinity is well known to be associated with reduced growth rates and delayed gonad development (Loosanoff, 1953; La Peyre et al., 2013; Leonhardt et al., 2017) and to change population dynamics by affecting mortality (Casas et al., 2015; Rybovich et al., 2016; Leonhardt et al., 2017) larval survival, development and settlement (Calabrese and Davis, 1970). The incorporation of salinity as a third variable in a DEB model therefore seems necessary. However, the mechanisms behind these effects are still not fully understood.

While some studies showed that lower salinities stimulate oyster respiration (Percy et al., 1971; Shumway and Koehn, 1982), others reported no or a limited effect of salinity on this physiological rate (van Winkle, 1972; Galtsoff, 1964; Casas et al., in review). As osmoconformers, oysters do not maintain a constant body fluid and cell osmolarity and as a result, when seawater salinity changes, water moves across plasma membranes in the direction that tends to equalize osmolarity outside and inside cells. To contend with the change in cell volume due to water movements and to conform to the changing osmolarity, oysters rely on a few mechanisms: 1) the intracellular regulation of osmolytes comprised of organic molecules and inorganic ions (Pierce and Greenberg, 1973; Baginski and Pierce, 1975) which likely involves a metabolic cost (Liu et al., 1990), and/or 2) the closure of the shell valves in order to seclude their soft body from the surrounding environment, directly leading to the shutdown of energy input through food (Galtsoff, 1964; Shumway et al., 1977).

A few current models incorporate the effects of salinity on physiological processes of *C. virginica*, generally focusing on food intake and respiration. Cerco and Noel (2005), in their report on the Chesapeake Bay oyster population, implemented the effect of salinity on filtration rate exclusively through a sigmoidal function. Other models applied a correction factor to both filtration and respiration rates to account for low salinity effects (Powell et al., 1992). Most recently, Maar et al. (2015) proposed an equation which invokes changes in metabolic cost, filtration rate and morphology to model the effect of low salinity on the energy budget of *Mytilus edulis* in the Baltic Sea. This last study is the first one to attempt to integrate salinity in a DEB model.

In this paper we developed a DEB model for *C. virginica* accounting for low salinity effects on their physiology. We present a set of DEB parameters estimated through the covariation method (Lika et al., 2011) using data observed from across the distribution range of *C. virginica*. We tested two mechanisms to include the low salinity effect in the bioenergetic model: 1) through an effect on filtration and 2) through an effect on somatic maintenance (measured by respiration). Different

modeling scenarios (no effect, effect on filtration only, effect on somatic maintenance only, and effect on both of these physiological processes) were examined to simulate observed growth and reproduction at two study sites in coastal Louisiana and statistically determine the best way to implement salinity effect. The model was then validated using eight independent field monitoring data sets from the nGoM.

2. Material and methods

The individual bioenergetic model developed in this study is based on Dynamic Energy Budget (DEB) theory (Kooijman, 2010). This work builds on the model for *Crassostrea gigas*, originally developed by Pouvreau et al. (2006) and improved by Bernard et al. (2011). For a detailed description of DEB models the reader is referred to the above mentioned publications. The present study describes the development and adaptation of this model for *C. virginica*, including the addition of salinity effects on physiology.

Briefly, the model describes the dynamics of four state variables: the energy in reserve E, the energy allocated to structure V (including somatic growth), the energy allocated to development and reproduction in the reproduction buffer E_R and the energy used to create gametes E_{Go} (Figure 1). A major difference from previous DEB models is the use of three forcing variables: temperature, food availability and salinity, as opposed to just two (temperature, food availability). Furthermore, as for all bivalves, the model includes metabolic acceleration between birth and metamorphosis (Kooijman et al., 2011), resulting in a proportional increase of assimilation and mobilization parameters with length during larval development.

DEB models require detailed information about the physiology of the organism. While numerous studies have been done on *C. virginica* physiology, and growth and mortality recorded in various conditions, having explicit data using the same oyster population within the range of conditions encountered by the organism enables more accurate model parameterization and validation. We collected data on *C. virginica* for the purpose of this project through experimental field and laboratory studies in order to parameterize the model accurately, and validate the model.

2.1. DEB model parameter estimation

Most DEB parameters cannot be determined directly through experiments, requiring the use of compound parameters (basically amalgams of the core DEB parameters) which are more easily estimated from typical empirical data sets (van der Meer, 2006; Lika et al., 2011). Estimates of many of the DEB parameters for *C. virginica* were thus obtained through the covariation method, performed in GNU Octave software (see Supporting Information for a full description of the process). Prior to applying the covariation method, we generated several primary DEB parameters, listed below, using a combination of published and unpublished datasets, and laboratory experiments.

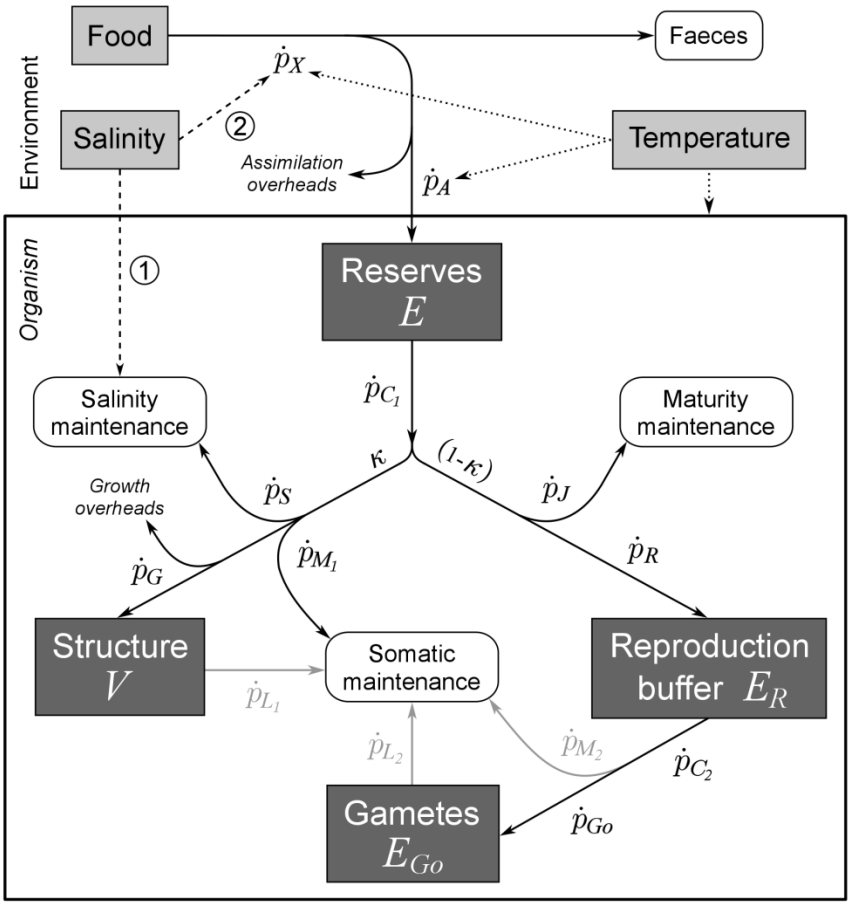

Figure 1. Conceptual scheme of the DEB model applied to *C. virginica*. Plain arrows illustrate energy fluxes, and broken arrows symbolize the effect of environmental variables on energy fluxes. State variables are presented in square boxes. The effect of salinity on physiological rates is tested through two hypotheses: (1) an additional surface related maintenance cost and (2) a direct inhibitory effect on filtration (see text for detail). Gray arrows represent the energy mobilized to pay somatic maintenance when $\dot{p}_{M1}$ is not sufficient.

2.1.1. Arrhenius temperature T_A and shape coefficient δ_M

Two primary parameters were estimated using information from the published literature according to van der Veer et al. (2006): the Arrhenius temperature T_A (Menzel, 1955; Loosanoff, 1958; Galtsoff, 1964; Feng, 1965; Dame, 1972; Shumway and Koehn, 1982) and the shape coefficient δ_M (Powell et al., 1995; La Peyre et al., 2013; Casas et al., 2015; Rybovich et al., 2016). Following the same method, we determined the Arrhenius temperature at the upper and lower boundaries outside the tolerance range of *C. virginica*.

2.1.2. Maximum surface-specific ingestion rate $\{\dot{p}_{Xm}\}$

Two physiological rates (the maximum clearance rate and the pseudofeces production threshold) were determined through concurrent laboratory work, described in Casas et al. (in review), which led to the estimation of another primary parameter, the maximum surface-specific ingestion rate $\{\dot{p}_{Xm}\}$. Ingestion

rate is considered to be at a maximum when the production of pseudofeces begins (Winter, 1978). Therefore, the estimation of $\{\dot{p}_{Xm}\}$ (in J d^{-1} cm^{-2}) was calculated as:

$$\{\dot{p}_{Xm}\} = \{\dot{C}_R\}\, X_P\, E_X$$

with $\{\dot{C}_R\}$ being the clearance rate (L d^{-1} cm^{-2}) at food density X_P (cell L^{-1}), i.e. immediately below the pseudofeces production threshold (Casas et al., in review), and E_X the average energetic content of a microalgae (J $cell^{-1}$), derived from Newell (1982) and Enright et al. (1986).

2.1.3. Estimation of volume-specific somatic maintenance costs $[\dot{p}_M]$ *and maximum reserve density* $[E_m]$

Volume-specific somatic maintenance costs $[\dot{p}_M]$ are defined as the energy requirement of an individual to stay alive, which excludes investments in the production processes of growth, reproduction and development (Kooijman, 2010). Only indirect approaches can be used to estimate this parameter, through changes in energy content by starvation over time (van der Meer, 2006) or measurements of respiration rate of starved organisms (van der Veer et al., 2006). Energy content data can also be used to estimate the maximum reserve density $[E_m]$, which is described as the difference between the energy content of a well-fed individual and the one after starvation (i.e., depletion of all reserve). We conducted a laboratory starvation experiment to estimate somatic maintenance and reserve density (Ren and Schiel 2008).

All oysters used to estimate $[E_m]$ were the progeny of broodstock collected in November 2013 in Breton Sound, one of Louisiana public oyster grounds, and spawned at the Louisiana Sea Grant oyster hatchery, in Grand Isle, Louisiana, in May 2014. The progeny were grown in aquaculture bags held in an adjustable long line system (ALS, BST oyster Supplies Co., Cowell, SA, Australia). We collected 2050 oysters in January 2015 with mean shell height and tissue dry weight (± standard deviation) of 5.70 ± 1.10 cm and 0.88 ± 0.48 g respectively (n = 50). Oysters were scrubbed, divided equally into 15 groups, and placed in 400 L tanks located at Louisiana State University filled with 0.5 µm filtered water at a salinity of 15 and temperature of 20 °C. Temperature and salinity in each of the 15 tanks were then adjusted at a rate of 2 °C and 3 ppt per day until reaching a desired combination of fifteen conditions with five different salinities: 3, 6, 9, 15 and 25 ppt and three different temperatures: 10, 20 and 30 °C. Oysters were maintained unfed and water quality was monitored throughout the experiment. After three weeks of acclimation, fifteen individuals from each tank were randomly sampled every month for the first three months and then every two weeks thereafter. Shell heights (cm) were measured, as well as tissue dry weight (g), obtained by drying tissue at 60 °C until constant weights were reached after about 48 h. Weights were standardized to an animal of standard shell height $H_{std} = 6$ cm using the relationship $W_{std} = (H_{std}/H)^3 * W$, with W and H the measured weight and height respectively. The effect of the two forcing variable (temperature and salinity) on weight variation was assessed by conducting a two-factor ANOVA using R 3.0.1 software (R Development Core Team, 2012). Energy content (J cm^{-3}) was obtained through bomb calorimetry analysis of dry tissue (Parr Isoperibol oxygen bomb calorimeter, Model 6200). For this analysis, individuals were pooled whenever necessary (up to 5 individuals) in order to reach a sample mass of 1 g dry weight. Twenty oysters were sacrificed at the

beginning of the experiment in order to get the initial energy content. Monitoring of weight loss was continued until Day 195 but all reserves were considered depleted at Day 125 when dry weight loss stabilized over time (Ren and Schiel, 2008) and data collected beyond this point were not included in the study as structural mass was potentially being broken down as a source of energy. The difference in energy content between starved animals and the initial sampling gives the maximum reserve density $[E_m]$.

2.2. Implementation of salinity as a forcing variable: hypotheses and simulation plan

2.2.1. Salinity DEB hypotheses

The standard DEB model with temperature and food availability as forcing variables had to be modified to incorporate the effects of salinity on *C. virginica* physiology. Low salinity has been hypothesized to affect oyster growth through 1) filtration rates, and 2) somatic maintenance.

In the first hypothesis, low salinity affects *C. virginica* by limiting the energy input through reduced filtration. In fact, laboratory and field research have suggested *C. virginica* reduce or stop feeding at low salinity (Powell et al., 1992; McFarland et al., 2013; Casas et al., in review). In order to address this mechanism, we tested the approach employed by Powell et al. (1992), applying a salinity correction factor on filtration (c_S; Eq. 2, Table 1):

$$c_s = \begin{cases} 1, & at\ S \geq S_H \\ \dfrac{S - S_L}{S_H - S_L}, & at\ S_L < S < S_H \\ 0, & at\ S \leq S_L \end{cases}$$

where S_H stands for the higher salinity threshold over which salinity has no observed effect, S_L is the lower salinity threshold at which filtration is stopped and S is the current salinity (values of all parameters are given in Table 2). The value of these thresholds was taken from a review of the literature (Galtsoff, 1964; Soniat et al., 2013; Casas et al., in review) and set at 10 ppt for the S_H and 3 ppt for S_L.

The second hypothesis is based on the potentially significant cost of equilibrating the internal osmolarity to match that of the surrounding seawater, which is achieved through the excretion of metabolites (Pierce and Greenberg, 1973; Baginski and Pierce, 1975; Liu et al., 1990). However, oyster cells must maintain certain levels of metabolites to survive the stress of low-salinity environment. These levels of solutes attract water molecules because of simple diffusion, leading cells to continuously spend energy moving water out to control their volume. In DEB theory, metabolic work such as maintenance of concentration gradients across membranes or the turnover of proteins is accounted for through maintenance fluxes (Kooijman, 2010). On that account, as salinity changes, more energy would be required to adjust cell osmolarity and one would expect the costs of somatic maintenance to be greater. While DEB theory states that osmosis related energy fluxes can be accounted for through surface-area specific maintenance costs (Kooijman, 2010), no equation is provided to compute these costs (and salinity is not considered as a forcing variable in standard DEB models).

Table 1. Equations of the fluxes of energy implemented in *C. virginica* DEB model.

N°	Description	Equation
(1)	Temperature correction factor	$c_T = exp\left\{\frac{T_A}{T_1} - \frac{T_A}{T}\right\} \left(1 + exp\left\{\frac{T_{AL}}{T_1} - \frac{T_{AL}}{T_L}\right\} + exp\left\{\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_1}\right\}\right) \left(1 + exp\left\{\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right\} + exp\left\{\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right\}\right)^{-1}$
(2)	Salinity correction factor on filtration	$c_s = \begin{cases} 1, & at\ S \geq S_H \\ \frac{S - S_L}{S_H - S_L}, & at\ S_L < S < S_H \\ 0, & at\ S \leq S_L \end{cases}$
(3)	Ingestion rate	$\dot{p}_X = \{\dot{p}_{Xm}\}\ f\ V^{2/3}\ c_T\ c_S$
(4)	Assimilation rate	$\dot{p}_A = \kappa_X\ \dot{p}_X$
(5)	Reserve mobilization rate	$\dot{p}_{C1} = [E]\frac{c_T\ [E_G]\ \dot{v}\ V^{2/3} + c_T\ [\dot{p}_M]}{\kappa\ [E] + [E_G]}$, with $[E] = \frac{E}{V}$
(6)	Maintenance rate related to salinity	$\dot{p}_S = \sigma\ max(S_H - S, 0)\ V^{2/3}$
(7)	Structural maintenance rate	$\dot{p}_{M1} = ([\dot{p}_M]\ V + \dot{p}_S)\ c_T$
(8)	Structural growth rate	$\dot{p}_G = \kappa\ \dot{p}_{C1} - \dot{p}_{M1}$
(9)	Maturity maintenance rate	$\dot{p}_J = min(V, V_P)\ [\dot{p}_M]\ \left(\frac{1-\kappa}{\kappa}\right)\ c_T$
(10)	Maturation and reproduction rate	$\dot{p}_R = (1-\kappa)\ \dot{p}_{C1} - \dot{p}_J$
(11)	Lysis of structure rate	$\dot{p}_{L1} = max(\dot{p}_{M1} - (\kappa\ \dot{p}_{C1} + \dot{p}_{M2} + \dot{p}_{L2}), 0)$
(12)	Gamete mobilization rate	$\dot{p}_{C2} = E_R\left(\frac{\{\dot{p}_{Xm}\}\ \kappa_X\ c_T}{[E_m]\ V^{1/3}} + \frac{[\dot{p}_M]\ c_T}{[E_G]}\right)\left(\{1-\kappa\}\ \frac{E}{[E_G]\ V + \kappa\ E}\right)$
(13)	Emergency maintenance rate	$\dot{p}_{M2} = min(\dot{p}_{M1} - \kappa\ \dot{p}_{C1}, \dot{p}_{C2})$
(14)	Gonad allocation rate	$\dot{p}_{Go} = \dot{p}_{C2} - \dot{p}_{M2}$
(15)	Gamete resorption rate	$\dot{p}_{L2} = max\left(\frac{\dot{p}_{M1} - \kappa\ \dot{p}_{C1} + \dot{p}_{M2}}{Y_{Go}}, 0\right)$
(16)	Total dry flesh mass calculation	$DFM = \frac{E + E_R}{\mu_E} + V\ d_V + \frac{E_{Go}\ d_{Go}}{[E_{Ggo}]}$
State variable differential equations		
(17)	Reserves	$\frac{dE}{dt} = \dot{p}_A - \dot{p}_{c1}$
(18)	Structural volume	$\frac{dV}{dt} = \frac{\dot{p}_G - \dot{p}_{L1}}{[E_G]}$
(19)	Development/reproduction	$\frac{dE_R}{dt} = \dot{p}_R - \dot{p}_{c2}$
(20)	Gametes	$\frac{dE_{GO}}{dt} = \dot{p}_{GO} - \dot{p}_{L2} - spawning$

Table 2. List of the DEB parameters estimated for *C. virginica*. All values are given for post-metamorphosis organisms ($\{\dot{p}_{Xm}\}$ must be divided by an acceleration factor of $s_M = 3.28$ to obtain the value before metabolic acceleration).

Parameter description	Symbol	Value	Unit	Source
Main parameters				
Shape coefficient	δ_M	0.2	-	This study
Maximum surface-specific ingestion rate	$\{\dot{p}_{Xm}\}$	249.5	J d^{-1} cm^{-2}	$= \{\dot{C}_R\} X_P E_X$
Volume-specific somatic maintenance costs	$[\dot{p}_M]$	38	J d^{-1} cm^{-3}	This study
Volume-specific cost for structural growth	$[E_G]$	5230	J cm^{-3}	This study
Maximum reserve density	$[E_m]$	5420	J cm^{-3}	This study
Allocation fraction to somatic growth and maintenance	κ	0.82	-	This study
Assimilation efficiency	κ_X	0.75	-	This study
Reproduction efficiency	κ_R	0.95	-	Kooijman, 2010
Maturity threshold at puberty	$E_H{}^p$	369.9	J	This study
Additional and compound parameters				
Clearance rate at X_P	$\{\dot{C}_R\}$	56	L d^{-1} cm^{-2}	Casas et al., in review
Pseudofeces production threshold	X_P	3 x 10^7	cell L^{-1}	Casas et al., in review
Calorific content of a microalgae	E_X	1.49 x 10^{-7}	J $cell^{-1}$	Average value from Newell, 1982 and Enright et al., 1986
Energy content of 1 g of reserve	μ_E	23000	J g^{-1}	Kooijman, 2010
Dry mass ratio of structure	d_V	0.2	g_{dw} $g_{ww}{}^{-1}$	This study
Dry mass ratio of gonad	d_{Go}	0.31	g_{dw} $g_{ww}{}^{-1}$	Bernard et al., 2011
Volume specific cost for gonad	$[E_{Ggo}]$	7500	J cm^{-3}	Bernard et al., 2011
Yield of gonad tissue used for maintenance	Y_{Go}	0.25	-	Bernard et al., 2011
Temperature effect				
Arrhenius temperature	T_A	6700	K	This study
Reference temperature	T_1	293	K	This study
Lower boundary tolerance range	T_L	283	K	Galtsoff, 1964
Upper boundary tolerance range	T_H	303	K	Galtsoff, 1964
Arrhenius temperature for lower boundary	T_{AL}	21820	K	This study
Arrhenius temperature for upper boundary	T_{AH}	45380	K	This study
Salinity effect				
Upper salinity threshold	S_H	10	ppt	Casas et al., in review
Lower salinity threshold	S_L	3	ppt	Casas et al., in review
Maintenance coefficient related to salinity	σ	5-40	J d^{-1} ppt^{-1} cm^{-2}	This study

Nevertheless, Maar et al. (2015), formulated an equation based on this DEB concept to formalize energy costs related to salinity changes in blue mussels, *Mytilus edulis*. We used this approach

to test the hypothesis of an additional energy cost on maintenance, and computed a maintenance flux ($\dot{p}_S$, in J d^{-1}) in addition to the somatic maintenance costs $[\dot{p}_M]$; this maintenance always has priority over growth (Eq. 6, Table 1):

$$\dot{p}_S = \sigma\, max(S_H - S, 0)\, V^{2/3}$$

with σ the maintenance coefficient related to salinity (J d^{-1} ppt^{-1} cm^{-2}) and V the structural volume (cm^3). Maar et al. (2015) estimated a value of σ = 12.55 J d^{-1} ppt^{-1} cm^{-2} for *M. edulis*. In our simulations we decided to test a range of values for this parameter, in order to understand and illustrate the effect of this assumption on the energy budget of *C. virginica*.

In order to test the two hypotheses we evaluated the performance of four different scenarios: 1) no salinity effect, 2) an effect on filtration only, 3) an effect on somatic maintenance only, at values of σ ranging from 5 to 40 J d^{-1} ppt^{-1} cm^{-2} and 4) a combined effect of these two mechanisms, using data sets we collected at a low and a mid-salinity site in coastal Louisiana, and described below. The degree of correspondence between observations and predictions, using the four different scenarios, was evaluated on the basis of the three following statistics plotted in a Taylor diagram (Taylor, 2001): the correlation coefficient and the centered root-mean-squared differences (RMSD) between observed and predicted variables, along with the ratio of the standard deviations of the two variables. The hypothesis yielding the best fit was then selected and used in the validation process of the model.

2.2.2. Field data and hypothesis testing

From November 2014 through November 2015, we monitored *C. virginica* growth, mortality, tissue and gonadal condition and water quality at two locations in coastal Louisiana. These data were generated to evaluate the inclusion of salinity as a third variable in the model and test our four model hypotheses described previously.

This study was conducted at two locations in coastal Louisiana which were selected to represent different salinity regimes. Grand Isle, a barrier island in Barataria Bay, was our moderate salinity site (yearly average over the last 7 years: 19.6 ± 4.3 ppt; USGS station number 073802516, http://waterdata.usgs.gov/la/nwis/nwis). Salinity conditions in Grand Isle are generally considered optimal for *C. virginica* growth in Louisiana. The low salinity site was located adjacent to the Louisiana Universities Marine Consortium (LUMCON) in Cocodrie (yearly average over the last 15 years: 10.2 ± 5.3 ppt; https:// http://weather.lumcon.edu). Both sites have continuous data recorders measuring salinity and temperature.

Oysters used in this study were from the same cohort as the ones used in the starvation experiment (see section 2.2.1.). Briefly, oysters were the progeny of Breton Sound broodstock spawned in May 2014, and maintained in the Adjustable Line System (ALS) at the Louisiana Sea Grant oyster research hatchery in Grand Isle until deployment in November 2014. For deployment, at each site, 740 oysters (mean size: 4.64 ± 1.12 cm) were distributed in ten aquaculture bags in an ALS. To follow individual growth, 100 oysters were tagged and their shell height was measured monthly from November 2014 through November 2015. In addition, 20 individuals were sampled for shell height (cm),

tissue dry weights (g) and 10 individuals for histological analysis every month at each site; mortality was also recorded by counting dead oysters in the ten bags. Shell height was determined by measuring from shell umbo to distal edge using digital calipers. Gonad dry weight was determined according to the following procedure: using histological slides of the entire body, the proportion G of active gonad area relative to total tissue area (excluding gills) was quantified as described by Quintana et al. (2011). Gonad area was first converted to gonad volume (V_{Go}, cm^3) using the measured shell height (H, cm) for each individual:

$$V_{Go} = V\ G = (H\ \delta_M)^3\ G$$

and then converted to gonad dry weight (W_{Go}, g) using the measurement for total tissue wet weight (W_w, g) measured for each individual, assuming that the ratio of gonad wet weight to total tissue wet weight equals the ratio of gonad volume to total tissue volume:

$$W_{Go} = \frac{W_w\ V_{Go}}{V}\ d_{Go}$$

with d_{Go} the dry weight to wet weight ratio of gonad tissues.

Water quality (temperature and salinity) at each site was retrieved from the continuous data recorders in Grand Isle (USGS station number 073802516, http://waterdata.usgs.gov/la/nwis/nwis) and Cocodrie (https://stormcentral.waterlog.com). Chlorophyll-*a* (chl-*a*) concentration was also continuously measured in Cocodrie remained very high throughout the year (yearly average: 14.8 ± 10.5 µg L^{-1}). High concentration of chl-*a* in the vicinity of the Mississippi River is a well-known characteristic of the nGoM (Lane et al., 2007). Therefore, we considered that food was not a limiting factor in this study, which simplifies the computations in the model by assuming that the functional response $f = 1$.

2.3. Model validation

Simulations were performed using GNU Octave software. Growth data sets from studies in the literature conducted along the nGoM were used to validate the model performance after selecting the hypothesis. We evaluated the model according to the guidelines provided by Piñeiro et al. (2008) on model performance testing. We performed linear regressions between measured and predicted shell height (cm), tissue dry weight (g) and gonad dry weight (g) of *C. virginica* growing in eight different locations along the coast of nGoM (Figure 2). The coefficient of determination (r^2) was calculated to measure the proportion of the variance in observed values that was explained by the predicted values and the slope and intercepts were used to describe the consistency and the model bias respectively (Piñeiro et al., 2008). RMSD were calculated to estimate the mean deviation of predicted values with respect to the observed ones, in the same units as the model variable under evaluation. All statistics were produced using R 3.0.1 software (R Development Core Team, 2012).

Figure 2. Location of the study sites used to calibrate (*in italic*) and validate the DEB model for *C. virginica*. Calibration sites were used to test the implementation of salinity in the model. Data source for the validation sites are: 1. Pollack et al. (2011); 2–4. Casas et al. (2015); 7. La Peyre et al. (2013); 8–9. Casas et al. (2017); 10. Ingle and Dawson (1952). Data from sites 5 and 6 were used to calibrate the effect of salinity in the DEB model.

3. Results

3.1. DEB model parameters estimation

The DEB parameters for *C. virginica* were estimated from ecophysiological studies on individuals from the northern Gulf of Mexico and the U.S. Atlantic coast (Table 2). A few parameters were estimated through controlled experiments; other primary parameters of the DEB model were estimated through the covariation method (see Supporting Information). The statistical evaluation of this set of parameters in its capacity to simulate the data provided to their estimation resulted in a goodness of fit of 7.68 on a scale from 0 (poor) to 10 (good).

Six independent studies on the effect of temperature on different physiological rates were used to estimate the Arrhenius temperature (Figure 3), resulting in a value of $T_A = 6\,700$ K. Arrhenius temperatures at both lower (T_L, 283 K, i.e. 10 °C) and upper (T_H, 303 K, i.e. 30 °C) boundaries were estimated at $T_{AL} = 21\,820$ K and $T_{AH} = 45\,380$ K respectively.

Casas et al. (in review) measured a maximum clearance rate of 56 L d^{-1} cm^{-2} for Louisianan oysters which, combined with a pseudofeces production threshold of 3 x 10^7 cells L^{-1} and a conversion factor of 1.49 x 10^{-7} J $cell^{-1}$ for common marine microalgae (Newell, 1982; Enright et al., 1986) resulted in an estimated maximum surface-specific ingestion rate $\{\dot{p}_{Xm}\}$ of 249.5 J d^{-1} cm^{-2}.

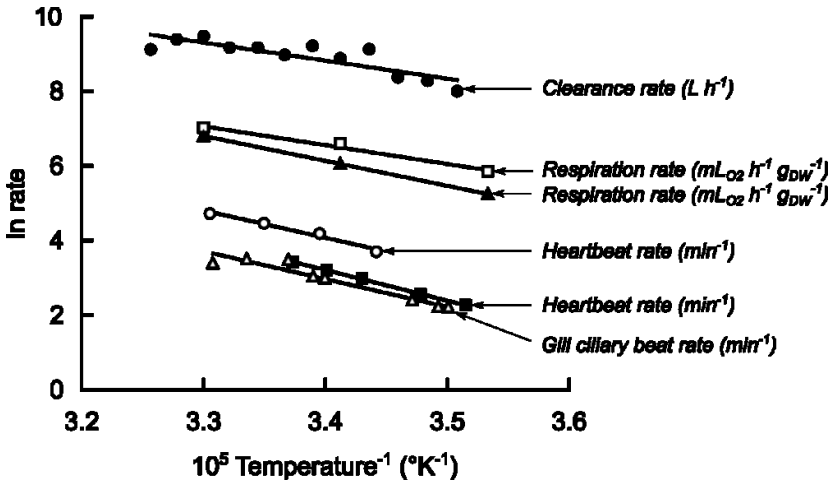

Figure 3. Arrhenius plot for various standardized physiological rates (natural logarithm) of *C. virginica* resulting in an Arrhenius temperature of $T_A = 6\,700$ °K. The pumping rate data (black dots) are from Loosanoff (1958), the first set of respiration rate data (white squares) is from Dame (1972), the second respiration rate data (black triangles) set is from Shumway and Koehn (1982), the first set of heartbeat rate data (white dots) is from Feng (1965), the second one (black squares) is from Menzel (1955) and gill ciliary beat rate data (white triangles) are from Galtsoff (1964).

The results from the starvation experiment conducted for this study are presented in Figure 4. Figure 4a shows that oysters starved at different temperature and salinity conditions experienced a loss of biomass correlated to temperature (two-way ANOVA *p-value* < 0.001) whereas salinity did not significantly affect the rate at which weight decreased (two-way ANOVA *p-value* = 0.31). Moreover no interaction was observed between these two factors (two-way ANOVA *p-value* = 0.46). Respiration rates measured at the beginning and at the end of the experiment showed similar results (Figure 4b) with temperature significantly affecting the oxygen consumption (two-way ANOVA *p-value* < 0.001) whereas salinity had no significant effect (two-way ANOVA *p-value* = 0.23). No clear difference in respiration was observed between day 15 and day 125 (pairwise t test p-value = 0.51).

Bomb calorimetry was used to measure the energy content of dry tissue before and after starvation (in control conditions, i.e. temperature of 20 °C and salinity of 15). The calorific content difference between the beginning and the end of the experiment resulted in an estimated maximum reserve density $[E_m]$ of 5 420 J cm^{-3}. Following the method described by van der Veer et al. (2006) we also estimated a value of 38 J d^{-1} cm^{-3} for the volume-specific maintenance costs $[\dot{p}_M]$.

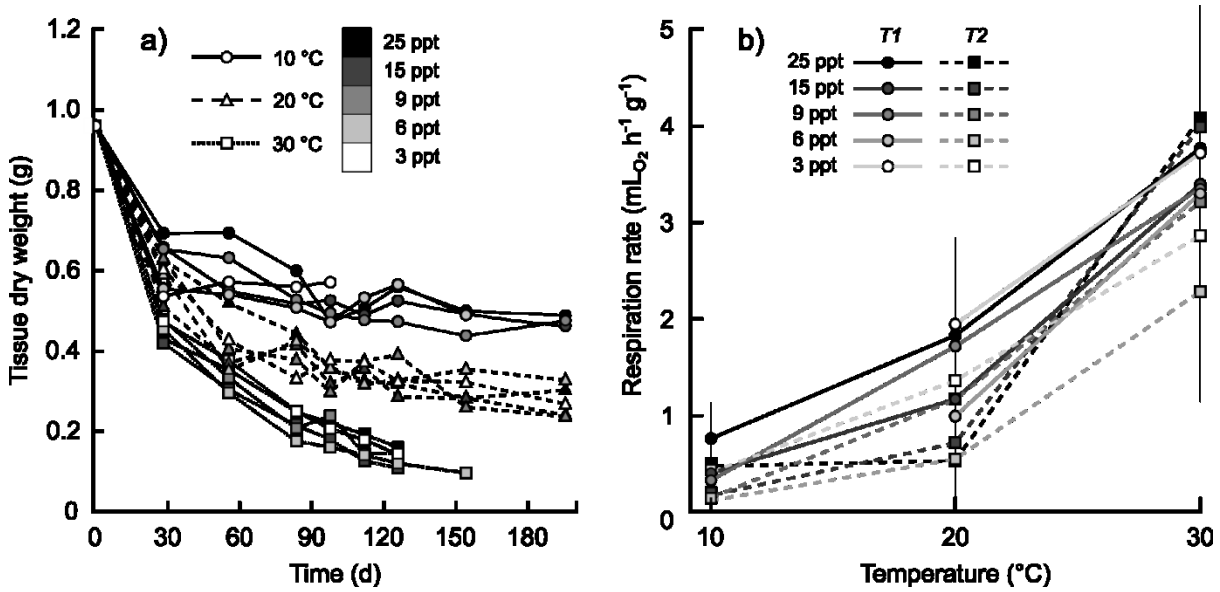

Figure 4. Results from the starvation experiment on *C. virginica*. (a) Changes in tissue dry weights during the starvation experiment, under 15 combinations of temperature and salinity conditions. (b) Respiration rate (in mL_{O2} h^{-1} $g^{-1}_{dry\ weight}$) at the beginning (T1; individuals already starved for an acclimation period of 15 days) and at the end of the starvation experiment (T2; day 125) under 15 combinations of temperature and salinity conditions. Data at T1 10°C 3 and 6 ppt were not included as oxygen concentrations did not vary during the measurements (oysters in these treatments remained closed for several days).

3.2. Implementation of salinity as a forcing variable

Environmental conditions from the two locations used to test the inclusion of salinity in the model showed contrasting salinity, with an average of 20.4 ppt (± 7.9 sd) in Grand Isle and 9.1 ppt (± 7.1 sd) in Cocodrie (Figure 5). Temperature regimes, however, were very similar. *C. virginica* mortality in Grand Isle remained very low with a monthly average of 1.2 % (maximum of 4.1 % in October) while in Cocodrie monthly mortality averaged 11 %, and peaked at 47 % in August. Overall, oysters placed at Cocodrie showed a lower increase in shell height (reaching 6.8 ± 0.8 cm after one year; Figure 6a,d) compared to the individuals located in Grand Isle (9.4 ± 1.0 cm), corresponding to a growth of 70 and 135 µm d^{-1} respectively. Similar patterns were observed in the tissue dry weight variation (Figure 6b,e): initial biomass of 0.26 ± 0.20 g increased to 0.87 ± 0.41 g after one year for oysters placed in Cocodrie and 3.14 ± 1.1 g for oysters placed in Grand Isle. Finally, differences in the reproductive biomass dynamics were also observed between the two sites: one spawning event can be identified in Cocodrie while two sharp decreases in the average gonad dry weight of oysters from Grand Isle indicate two major spawning events (Figure 6c,f). The range of the gonad mass variations was also greater in the latter, reaching 0.69 ± 0.53 g on Day 181, while the maximum value observed in Cocodrie did not exceed 0.20 ± 0.16 g on Day 146.

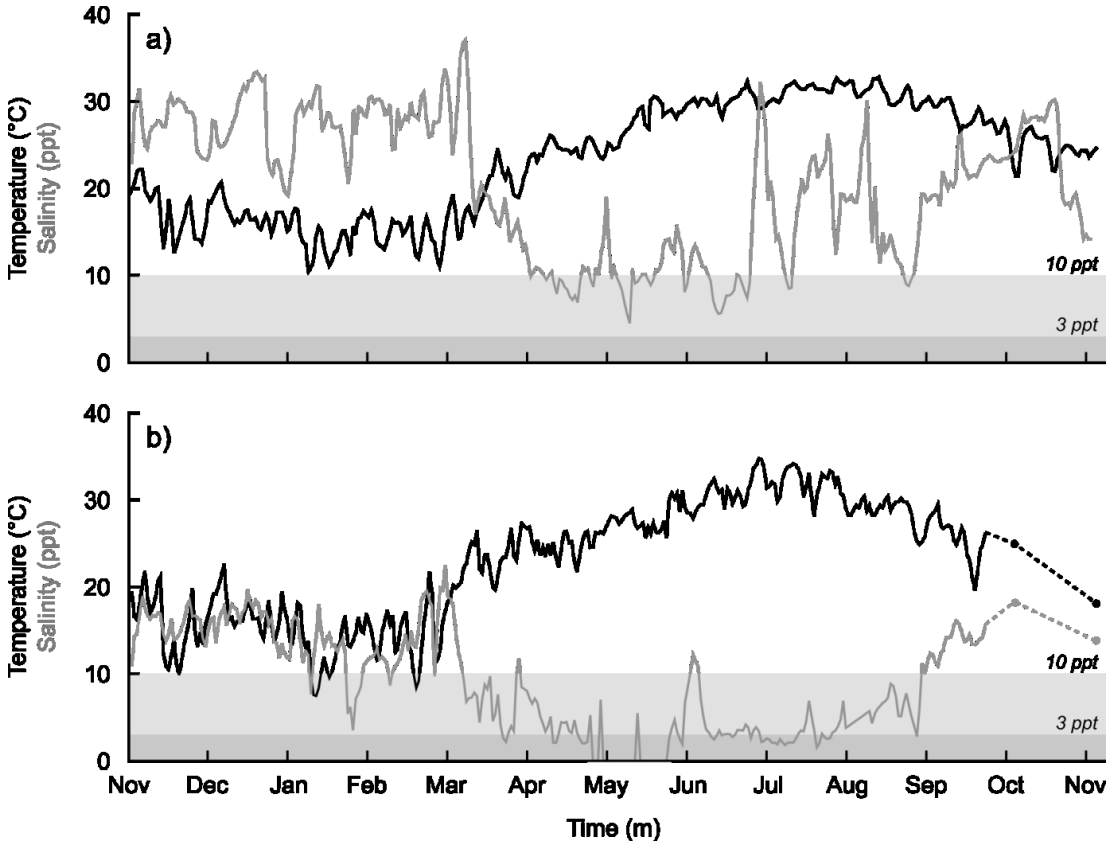

Figure 5. Daily water temperature (°C) and salinity (ppt) at two Louisiana sites starting on November 7th 2014. Grand Isle data (a) are from a USGS continuous water quality recorder: USGS station 073802516. Cocodrie data (b) are from continuous monitoring by the Louisiana Universities Marine Consortium (LUMCON). Dotted lines represent missing data completed by single point measurements (dots) obtained in parallel to the continuous monitoring every month.

Predicted growth (in height and weight, Figure 6a,b,d,e) and reproductive activity (Figure 6c,f) were examined using four alternate DEB modeling scenarios at the two sites. The first modeling scenario tested was that salinity had no effect at all on the individual bioenergetics. The results show a clear overestimation of all physiological rates (black line), especially in Cocodrie. The second scenario presenting the effect of salinity on filtration rate produced the best fit for all three physiological variables (red line). In the third scenario (5 different shades of blue lines), consisting of an increased somatic maintenance costs with decreasing salinity, no acceptable value of the salinity coefficient parameter was identified that fit the data. A flux of 5 J d^{-1} cm^{-3} produced a good fit for growth rates but led to predictions far off the observed gonad dry weight data. Finally, the combination of the effects on filtration and somatic maintenance did not permit to fit the data satisfactorily (green line), especially in Cocodrie. Overall, the model using a salinity effect on filtration only provided the best fit (Figure 7).

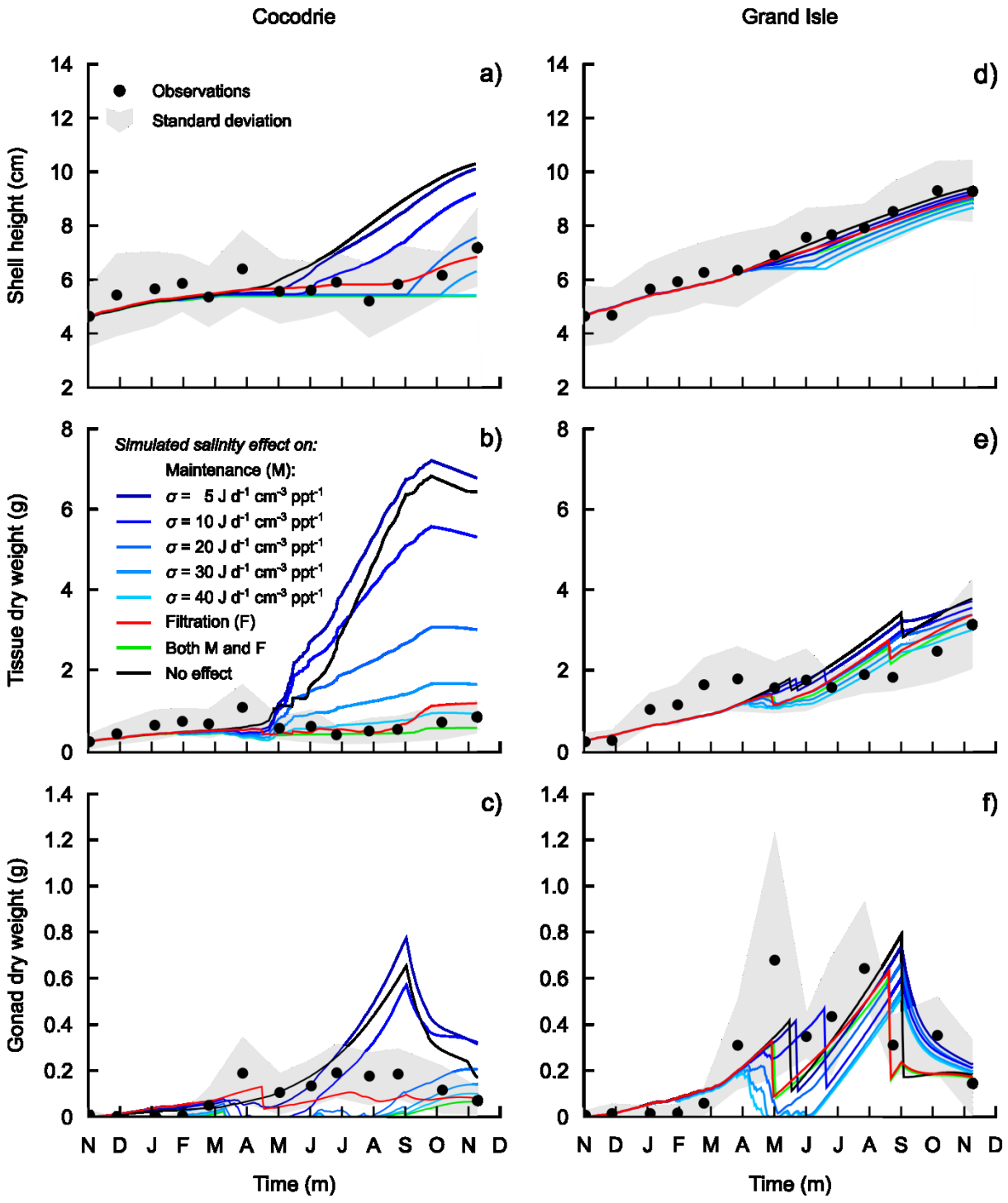

Figure 6. Observations (black dots) ± standard deviation (gray area) and simulations of *C. virginica* shell height (a, d), tissue dry weight (b, e) and gonad dry weight (c, f) in Cocodrie (left) and Grand Isle (right) under different implementations of the effect of salinity on *C. virginica*'s energy budget: no effect (dark line), effect on ingestion (red line, Eq. 2 Table 1), effect on somatic maintenance for which 5 intensities of the σ value were tested (blue lines, Eq. 6 Table 1) or both effects combined (green line).

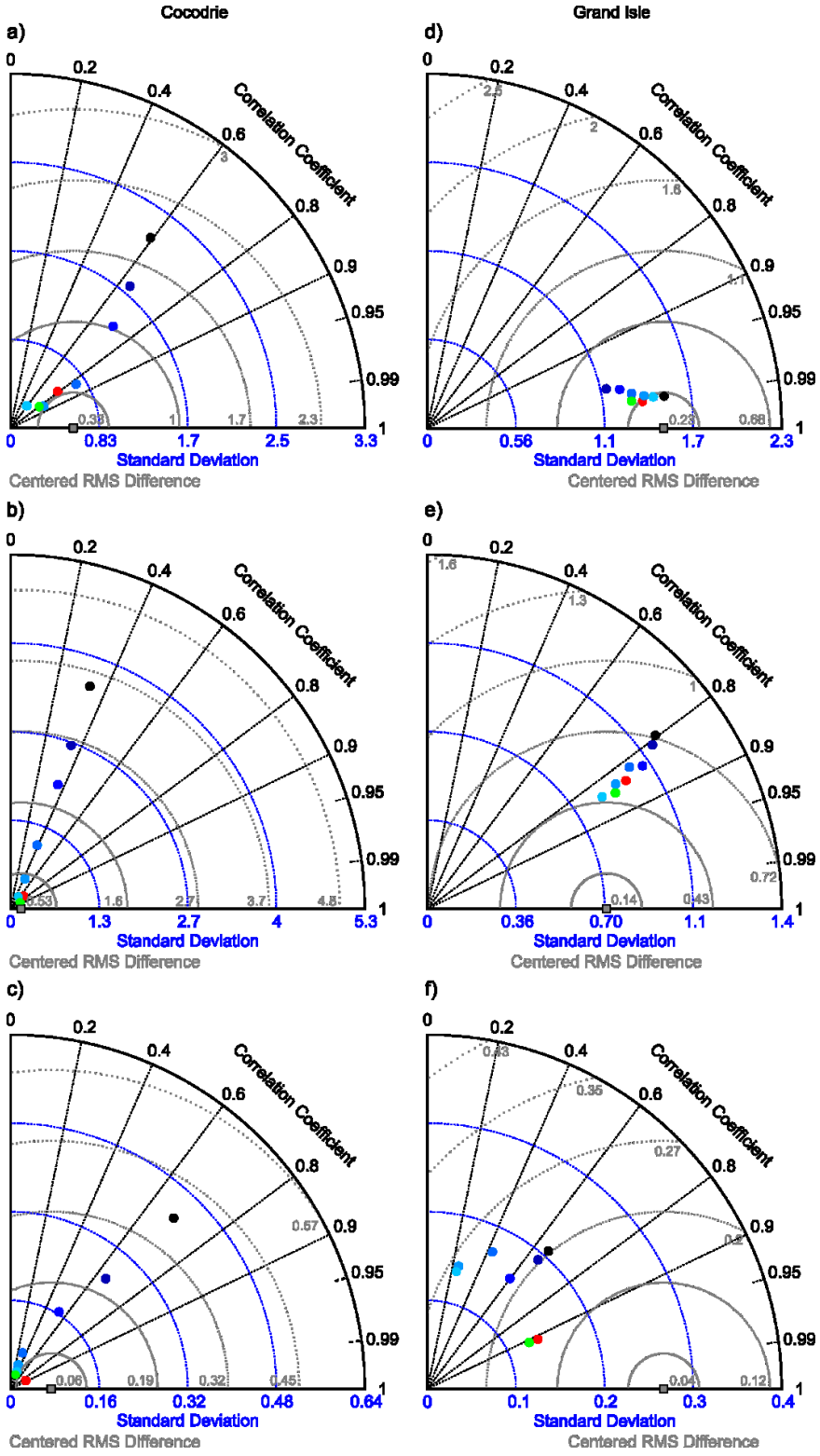

Figure 7. Taylor diagrams displaying the correlation coefficients, standard deviations and centered root mean squared (RMS) differences between observed and simulated shell height (a, d), tissue dry weight (b, e) and gonad dry weight (c, f) of *C. virginica* in Cocodrie (left) and Grand Isle (right). Each dot corresponds to one of the implementations of the effect of salinity in the model (as in Figure 6).

3.3. Model validation

Using the best fit model (including an effect of salinity on filtration rate), the modified DEB model was validated using field data from eight other sites along the nGoM (Figure 2 and 8). The best predictions were obtained for the simulation of shell height (Figure 8a) as the slope of the regression between observed and predicted values was not significantly different from 1 (*p-value* = 0.304). Model predictions of tissue dry weight, however, lack consistency with observed values (*p-value* = 10^{-11}; Figure 8b). Nonetheless, the high r^2 of these regressions (0.8977 and 0.7551 respectively) indicates a strong linear covariance of observed and predicted values (i.e. much of the linear variation of observed values is explained by the variation of predicted values).

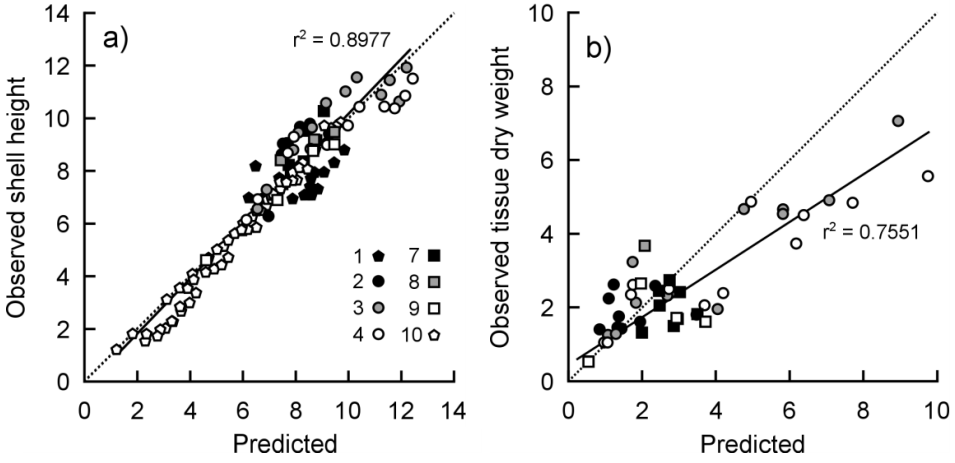

Figure 8. Observed vs. predicted regression scatter plot of *C. virginica* a) shell height and b) tissue dry weight in the different locations used to validate the DEB model. The dotted line represents the 1:1 regression line for perfect correspondence between observed and simulated data. Sites number: 1. Copano Bay, TX; 2,3,4. Sister Lake North, South and West, LA; 7. Bay Gardene, LA; 8. Sandy Bay, AL; 9. Dauphin Island, AL; 10. Apalachicola Bay, FL (Figure 2).

4. Discussion

Temperature and salinity control virtually every aspect of the performance of *C. virginica* across its range (Galtsoff, 1964), but models incorporating salinity often invoke different physiological mechanisms (i.e., Cerco and Noel, 2005; Powell et al., 1992; Maar et al., 2015). In this study we integrate laboratory and field data to develop a mechanistic model to predict the consequences of low and varying salinity on the energy budget of *C. virginica*. We used the conceptual framework presented through DEB theory, and estimated, for the first time, a set of DEB parameters through the covariation method for *C. virginica*, using data from across this species range. Moreover, the developed model includes the implementation of an additional environmental factor, salinity, on the oyster's energy budget. The integration of field and laboratory studies enabled testing and validation of the model, examining several hypothesized pathways by which low salinity may impact *C. virginica* performance (growth, reproduction). The addition of a third forcing variable (salinity) based on energy flow,

demonstrates the robustness and flexibility of such a mechanistic modeling approach, and enables better prediction of *C. virginica* bioenergetics.

4.1. Salinity effect on filtration validated in the DEB model

Salinity changes put an energetic burden on aquatic organisms whether they can actively regulate their internal osmolarity or not (Galtsoff, 1964; Pierce and Greenberg, 1973; Shumway et al., 1977; Rybovich et al., 2016). The various studies used in the calibration of the DEB model all emphasize the fact that low salinity conditions decrease growth rates and increase mortalities, especially at higher temperature (Ingle and Dawson, 1952; Pollack et al., 2011; La Peyre et al., 2013; Rybovich et al., 2016). Our simulations show that salinity mostly affects the bioenergetics of *C. virginica* through the filtration process rather than maintenance costs, which are the two mechanisms that have been postulated to cause energy losses. By comparing the physiological response of *C. virginica* to changing salinity in mechanistically different scenarios (Figure 6), we demonstrated that a decline in energy input through decreased filtration rates was the best way to account for the negative effect of lower salinity on the bioenergetics of *C. virginica*. The implementation of such an effect of salinity on filtration originated from the works of Powell et al. (1992). In their model they implemented an effect of salinity on both filtration and respiration rate, and reached similar conclusion by claiming that negative net production of *C. virginica* in low salinity regimes is derived mainly from an effect on filtration rate via a decrease in food supply.

In the present model, the filtration rate is affected by temperature, just like other energy fluxes in DEB theory. The salinity correction factor for filtration c_S, however, does not depend on temperature and the salinity thresholds S_H and S_L are set constant. It is still not clear whether the effects of temperature and salinity on the physiological response of *C. virginica* are cumulative, synergistic or antagonistic (Shumway and Koehn, 1982) and how S_H and S_L could be affected by temperature. In future work, one might also consider organism age in setting thresholds as it has been shown that salinity optimum (for growth mainly) varies through ontogeny (Galtsoff, 1964; Calabrese and Davis, 1970).

Behavioral and physiological studies have shown that an oyster's first response to a drop in salinity is the closure of the valves (Galtsoff, 1964; Shumway et al., 1977; McFarland et al., 2013), as it can be observed for numerous bivalves to avoid osmotic stress. This behavioral response aids in preventing osmotic stress by secluding the animal from the external environment but obviously prevent individuals to feed. However, in an interesting study on Dungeness crabs, Curtis and McGaw (2010) showed that the likelihood that animals would feed in low salinity increased with starvation. Feeding therefore represents a trade-off between acquiring more energy to maintain biological functions and facing osmotic stress consequences (cell damage, death).

Finally, even if organisms feed during these periods of low salinity, it is very likely that the composition of the available food would be of less value for *C. virginica* (Fulford et al., 2007). In fact, microalgae usually have low tolerance to salinity changes (Brand, 1984) and the reduction in salinity exposes estuaries to more frequent and longer freshwater phytoplankton blooms, including noxious and

toxic forms of fresh water species (Ren et al., 2009). Recent observations of decreases and changes of phytoplankton communities less desirable for *C. virginica* (e.g. cyanobacteria) in Louisiana estuaries with decreasing salinity support this claim (Schaeffer et al., 2012; Riekenberg et al., 2015). In addition to the indirect effects of food quality, laboratory studies have shown a clear and direct negative effect of decreasing salinity on clearance rates of acclimated oysters (Casas et al., in review) while Galtsoff (1964) stated an optimal salinity for clearance rate between 15 and 25. Decreases in the amount of time oyster valves are opened and in the amplitude of the valve openings at salinity of 6 and below were also observed (Casas et al., in review).

4.2. No significant increase in maintenance costs due to low salinity

The simulations using the hypothesis of an increased maintenance cost at lower salinities was not supported by the data, regardless of scaling used (Figure 6). Interestingly, this approach was successfully applied to the case of blue mussels from the Baltic Sea (Maar et al., 2015). However these authors focused on somatic growth and did not look at the effect of salinity on the reproductive activity of mussels. Our field data show lower gonad tissue biomass at the lower salinity site (Cocodrie). While our model allows for the re-allocation of energy from gametes to fuel potentially increased maintenance costs, histological analysis did not display any proof of gamete atresia before the end of the spawning season. Reproductive activity simply was less important for *C. virginica* exposed to low salinity as compared to those at the high salinity site (Grand Isle). This means that lower salinities not only impact the somatic branch (structure) of the metabolic organization, but also reproduction (Figure 1). We must therefore consider that salinity affects the energy fluxes within the organism before the partition of energy from the reserve compartment.

It is widely thought that exposure to changing salinity must have an energy cost in term of solute transport through cell membranes. Some studies have shown that low salinity increases oxygen consumption which would support the idea of an increased metabolic cost (Percy et al., 1971; Shumway and Koehn, 1982). Others, however, reported no effect on *C. virginica* (Galtsoff, 1964; Casas et al., in review, in winter) or decreasing oxygen consumption at low salinity (van Winkle, 1972; Casas et al., in review, in summer). If an acute change of salinity requires the cell to either synthesize or excrete solutes, once the adjustment to surrounding conditions is complete, no more energy should be spent when salinity stabilizes. A recent study using juvenile oysters has also demonstrated that levels of ATP, the major energy metabolite of the cell, were not affected by exposure to changing salinity, suggesting that the metabolic adjustments (fluxes of various solutes) to low salinity are sufficient to prevent ATP depletion and severe cellular energy deficiency (Dickinson et al., 2012). It is possible that in a continuously changing salinity environment where oysters constantly have to adjust and equilibrate their osmolarity to that of the surrounding water, the costs in terms of loss of solutes might have a larger effect on the organism's fitness.

The starvation experiment also failed to provide evidence indicating increased energy costs related to somatic maintenance at different salinities. In fact, temperature was found to be most related to somatic maintenance (as measured by variations in biomass and respiration rates). While our laboratory and modeling results fail to support the hypothesis that lowered salinity increases somatic

maintenance costs, the oysters used in the starvation experiment were acclimated for at least three weeks and maintained at constant salinities which would not have required any changes in intracellular osmolyte concentrations. In contrast, many previous physiological studies showing increased metabolic work in *C. virginica* used data from oysters exposed to acute changes of salinity and rarely account for long term trends. Osmoconformers use a series of biochemical and behavioral responses to cope with a change in the environmental osmolarity and reach a new osmotic equilibrium (Shumway et al., 1977; Pierce and Greenberg, 1973; McFarland et al., 2013). However, the duration of such reactions is usually no longer than a couple hours (Percy et al., 1971). Bayne (1973) showed that mussels exhibit a short term response to a change in salinity which could have non-significant costs of energy due to the rapidity of the response. Furthermore, he emphasized that after a short period of acclimation there are no more or few costs related to this change. This would explain the stable respiration rates observed in the starvation experiment (Figure 4b). Moreover, Pierce and Greenberg (1973) demonstrated that the more euryhaline an animal, the more rapid the recovery of cell volume. *C. virginica*, especially in the nGoM, experience tremendous ranges of salinity, so these populations have likely adapted with response mechanisms enabling them to handle changing environments. If salinity changes become more rapid, or extreme, it is possible there may be some energetic cost.

4.3. Conclusions

We demonstrated that low salinity did not increase the maintenance cost on the energy budget of *C. virginica*, but that reduced energy input through feeding as the bivalves close their shells to seclude themselves from the adverse conditions (i.e., lower salinity) explained variations in growth and reproduction. Specifically, the starvation laboratory study confirmed that salinity alone failed to explain the decrease of biomass through time while combined lab and field studies provided support that decreased energy intake through reduced feeding provided better explanations of observed changes in growth and reproduction.

While this work focused on selecting mechanisms for understanding effects of lower salinity on *C. virginica* performance, the same approach could be tested in the opposite case in which high salinity might affect *C. virginica* metabolism. Few estuaries on the coast of nGoM are identified as inverse estuaries, where evaporation greatly exceeds the inflow of fresh water. These conditions can be encountered for instance in Laguna Madre, TX. However, it is likely that other factors linked to higher salinity alter oyster's fitness before this physical component had any impact on their physiology – namely, predation (Wilber, 1992) and diseases development (Chu et al., 1993; La Peyre et al., 2003; La Peyre et al., 2009). Incorporating these impacts requires developing population based models and additional modules accounting for interactions between pathogens and their host (Flye-Sainte-Marie et al., 2009).

It is well recognized that the state of a biological organism depends on physical, chemical and biological conditions of its environment. However when we describe these complex interactions, we often try to simplify and generalize what happens in the real world. This is especially true in biological modeling where scientists face the trade-off between simplicity, reproducibility and accounting for individual variability or the various environmental variables that affect organisms. In this context,

mechanistic models can help us determine which factors to include or which way to implement their action on biological functions. The ability to implement a salinity effect within a DEB model demonstrates the utility of this approach in the face of multiple stressors, and provides insight in understanding the physiological response of organisms, in this case *C. virginica*, to these stressors. Ultimately, a DEB model approach provides a potentially powerful tool enabling predictions of organism performance under both commonly experienced as well as more extreme or novel conditions. As management of riverine flows, along with changes in freshwater inflow and precipitation are expected to increase, impacting estuarine salinity regimes, the model developed provides a tool to better predict impacts on *C. virginica* performance.

Acknowledgments

The authors want to thank Stephanie Bernasconi for the help in field sampling and laboratory experiments. We thank Dr. John Supan for spawning Breton Sound broodstock, and Dr. Jack Losso for access to the bomb calorimeter. This research was funded by Louisiana Sea Grant and LabexMer French project (ANR-10-LABX-19-01). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Baginski, R.M. and Pierce, S.K. (1975). Anaerobiosis: a possible source of osmotic solute for high salinity acclimation in marine molluscs. *J. Exp. Biol.* 62, 589–598.

Bayne, B.L. (1973). The response of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comp. Biochem. Physiol. Part A Physiol.* 45(3), 793–806.

Bernard, I., De Kermoysan, G. and Pouvreau, S. (2011). Effect of phytoplankton and temperature on the reproduction of the Pacific oyster *Crassostrea gigas*: Investigation through DEB theory. *J. Sea Res.* 66(4), 349–360.

Brand, L.E. (1984). The salinity tolerance of forty-six marine phytoplankton isolates. *Estuarine, Coastal Shelf Sci.* 18(5), 543–556.

Cake Jr, E.W. (1983). Habitat suitability index models: Gulf of Mexico American oyster. US Department of Interior Fish and Wildlife Service. FWS/0BS-82/10.57. 37 pp.

Calabrese, A. and Davis, H.C. (1970). Tolerances and requirements of bivalve molluscs. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 20(1), 553.

Casas, S.M., La Peyre, J.F. and La Peyre, M.K. (2015). Restoration of oyster reefs in an estuarine lake: population dynamics and shell accretion. *Mar. Ecol. Prog. Ser.* 524, 171–184.

Casas S.M., Walton W., Chaplin G., Rikard S., Supan J. and La Peyre J.F. (2017). Performance of oysters selected for dermo resistance compared to wild oysters in northern Gulf of Mexico estuaries. *Aquacult. Environ. Interact.* 9, 169–180.

Casas, S.M., La Peyre, M.K., Lavaud, R., Comeau, L.A., Filgueira, R. and La Peyre, J.F. (in review). Physiological effects of salinity on Eastern oyster form the North Gulf of Mexico during winter and summer. Submitted for publication in *Mar. Biol*.

Cerco, C.F. and Noel, M.R. (2005). Assessing a ten-fold increase in the Chesapeake Bay native oyster population. A Report to the EPA Chesapeake Bay Program. Annapolis, Maryland, USA. 82 pp.

Chu, F.L.E., La Peyre, J.F. and Burreson, C.S. (1993). *Perkinsus marinus* infection and potential defense-related activities in eastern oysters, *Crassostrea virginica* : salinity effects. *J. Invertebr. Pathol.* 62(3), 226–232.

Curtis, D.L. and McGaw, I.J. (2010). Respiratory and digestive responses of postprandial Dungeness crabs, *Cancer magister*, and blue crabs, *Callinectes sapidus*, during hyposaline exposure. *J. Comp. Physiol. B.* 180(2), 189–198.

Dame, R.F. (1972). The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. *Mar. Biol.* 17(3), 243–250.

Dekshenieks, M.M., Hofmann, E.E. and Powell, E.N. (1993). Environmental effects on the growth and development of eastern oyster, *Crassostrea virginica* (Gmelin, 1791), larvae: a modeling study. *J. Shellfish Res.* 12(2), 241–254.

Dickinson, G.H., Ivanina, A.V., Matoo, O.B., Pörtner, H.O., Lannig, G., Bock, C., Beniash, E. and Sokolova, I.M. (2012). Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters, *Crassostrea virginica*. *J. Exp. Biol.* 215(1), 29–43.

Enright, C.T., Newkirk, G.F., Craigie, J.S. and Castell, J.D. (1986). Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. *J. Exp. Mar. Biol. Ecol.* 96(1), 1–13.

Feng, S.Y. (1965). Heart rate and leucocyte circulation in *Crassostrea virginica* (Gmelin). *The Biological Bulletin*, 128(2), 198–210.

Filgueira, R., Guyondet, T., Comeau, L.A. and Grant, J. (2014). A fully-spatial ecosystem-DEB model of oyster (*Crassostrea virginica*) carrying capacity in the Richibucto Estuary, eastern Canada. *Journal of Marine Syst.* 136, 42–54.

Flye-Sainte-Marie, J., Jean, F., Paillard, C. and Kooijman, S.A.L.M. (2009). A quantitative estimation of the energetic cost of brown ring disease in the Manila clam using Dynamic Energy Budget theory. *J. Sea Res.* 62, 114–123.

Fonseca, G. and Gallucci, F. (2016). The need of hypothesis-driven designs and conceptual models in impact assessment studies: An example from the free-living marine nematodes. *Ecol. Indic.* 71, 79–86.

Fulford, R.S., Breitburg, D.L., Newell, R.I.E., Kemp, W.M. and Luckenbach, M. (2007). Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. *Mar. Ecol. Prog. Ser.* 336, 43–61.

Galtsoff, P.S. (1964). The American oyster *Crassostrea virginica* Gmelin. US Fish and Wildlife Services. *Fishery bulletin*, 64, 480 pp.

Ingle, R.M. and Dawson Jr, C.E. (1952). Growth of the American oyster, *Crassostrea virginica* (Gmelin) in Florida waters. *Bull. Mar. Sci.* 2(2), 393–404.

Kooijman, S.A.L.M. (2010). Dynamic Energy Budget theory for metabolic organization. Cambridge, UK, third edition. University Press, 532 pp.

Lane, R.R., Day, J.W., Marx, B.D., Reyes, E., Hyfield, E. and Day, J.N. (2007). The effects of riverine discharge on temperature, salinity, suspended sediment and chlorophyll *a* in a Mississippi delta estuary measured using a flow-through system. *Estuarine, Coastal Shelf Sci.* 74(1), 145–154.

La Peyre, M.K., Nickens, A.D., Volety, A.K., Tolley, G.S. and La Peyre, J.F. (2003). Environmental significance of freshets in reducing *Perkinsus marinus* infection in eastern oysters *Crassostrea virginica*: potential management applications. *Mar. Ecol. Prog. Ser.* 248, 165–176.

La Peyre, M.K., Gossman, B. and La Peyre, J.F. (2009). Defining optimal freshwater flow for oyster production: Effects of freshet rate and magnitude of change and duration on eastern oysters and *Perkinsus marinus* infection. *Estuaries Coasts* 32(3), 522–534.

La Peyre, M.K., Eberline, B.S., Soniat, T.M. and La Peyre, J.F. (2013). Differences in extreme low salinity timing and duration differentially affect eastern oyster (*Crassostrea virginica*) size class growth and mortality in Breton Sound, LA. *Estuarine, Coastal Shelf Sci.* 135, 146–157.

Lavaud, R., Flye-Sainte-Marie, J., Jean, F., Emmery, A., Strand, Ø. and Kooijman, S.A.L.M. (2014). Feeding and energetics of the great scallop, *Pecten maximus*, through a DEB model. *J. Sea Res.* 94, 5–18.

Leonhardt, J.M., Casas, S., Supan, J.E. and La Peyre J.F. (2017). Stock assessment for eastern oyster seed production and field grow-out in Louisiana. *Aquaculture* 466, 9–19.

Lika, K., Kearney, M.R., Freitas, V., van der Veer, H.W., van der Meer, J., Wijsman, J.W.M., Pecquerie, L. and Kooijman, S.A.L.M. (2011). The “covariation method” for estimating the parameters of the standard Dynamic Energy Budget model I: Philosophy and approach. *J. Sea Res.* 66(4), 270–277.

Liu, L.L., Stickle, W.B. and Gnaiger, E. (1990). Normoxic and anoxic energy metabolism of the southern oyster drill *Thais haemastoma* during salinity acclimation. *Mar. Biol.* 104(2), 239–245.

Livingston, R.J., Lewis, F.G., Woodsum, G.C., Niu, X.F., Galperin, B., Huang, W., Christensen, J.D., Monaco, M.E., Battista, T.A., Klein, C.J., Howell IV, R.L. and Ray, G.L. (2000). Modelling oyster population response to variation in freshwater input. *Estuarine, Coastal Shelf Sci.* 50(5), 655–672.

Loosanoff, V.L. (1953). Behavior of oysters in water of low salinities. *Proc. Natil. Shellfisheries Assoc.* 43, 135–151.

Loosanoff, V.L. (1958). Some aspects of behavior of oysters at different temperatures. *Biol. Bull.* 114(1), 57–70.

Maar, M., Saurel, C., Landes, A., Dolmer, P. and Petersen, J.K. (2015). Growth potential of blue mussels (*M. edulis*) exposed to different salinities evaluated by a Dynamic Energy Budget model. *J. Marine Syst.* 148, 48–55.

McFarland, K., Donaghy, L. and Volety, A.K. (2013). Effect of acute salinity changes on hemolymph osmolality and clearance rate of the non-native mussel, *Perna viridis*, and the native oyster, *Crassostrea virginica*. *Southwest Florida Aquat. Invasions* 8(3), 299–310.

Menzel, R.W. (1955). The effect of temperature on the ciliary action and other activities of oysters. *Florida State Univ. Stud.* 22, 25–36.

Newell, R.I.E. (1982). An evaluation of the wet oxidation technique for use in determining the energy content of seston samples. *Can. J. Fish. Aquat. Sci.* 39(10), 1383–1388.

Nisbet, R.M., Jusup, M., Klanjscek, T. and Pecquerie, L. (2012). Integrating dynamic energy budget (DEB) theory with traditional bioenergetic models. *J. Exp. Biol.* 215(6), 892–902.

Percy, J.A., Aldrich, F.A. and Marcus, T.R. (1971). Influence of environmental factors on respiration of excised tissues of American oysters, *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 49(3), 353–360.

Pierce, S.K. and Greenberg, M.J. (1973). The initiation and control of free amino acid regulation of cell volume in salinity stressed marine bivalves. *J. Exp. Biol.* 59, 435–440.

Piñeiro, G., Perelman, S., Guerschman, J.P. and Paruelo, J.M. (2008). How to evaluate models: observed vs. predicted or predicted vs. observed? *Ecol. Modell.* 216(3), 316–322.

Pollack, J.B., Kim, H.C., Morgan, E.K. and Montagna, P.A. (2011). Role of flood disturbance in natural oyster (*Crassostrea virginica*) population maintenance in an estuary in South Texas, USA. *Estuaries Coasts* 34(1), 187–197.

Pouvreau, S., Bourles, Y., Lefebvre, S., Gangnery, A. and Alunno-Bruscia, M. (2006). Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. *J. Sea Res.* 56(2), 156–167.

Powell, E.N., Hofmann, E.E., Klinck, J.M. and Ray, S.M. (1992). Modeling oyster populations I. A commentary on filtration rate. Is faster always better? *J. Shellfish Res.* 11, 387–398.

Powell, E.N., Klinck, J.M., Hofmann, E.E., Wilson-Ormond, E.A. and Ellis, M.S. (1995). Modeling oyster populations V. Declining phytoplankton stocks and the population dynamics of American oyster (*Crassostrea virginica*) populations. *Fish. Res.* 24(3), 199–222.

Quintana, R., Burnside, W.M., Supan, J.E., Lynn, J.W. and Tiersch, T.R. (2011). Rapid estimation of gonad-to-body ratio in eastern oysters by image analysis. *North Am. J. Aquacult.* 73(4), 451–455.

R Development Core Team (2012). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Ren, J.S. and Schiel, D.R. (2008). A dynamic energy budget model: parameterisation and application to the Pacific oyster *Crassostrea gigas* in New Zealand waters. *J. Exp. Mar. Biol. Ecol.* 361(1), 42–48.

Ren, L., Rabalais, N.N., Turner, R.E., Morrison, W. and Mendenhall, W. (2009). Nutrient limitation on phytoplankton growth in the upper Barataria Basin, Louisiana: microcosm bioassays. *Estuaries Coasts* 32(5), 958–974.

Riekenberg, J., Bargu, S. and Twilley, R. (2015). Phytoplankton community shifts and harmful algae presence in a diversion influenced estuary. *Estuaries Coasts* 38(6), 2213–2226.

Rybovich, M., La Peyre, M.K., Hall, S.G. and La Peyre, J.F. (2016). Increased temperatures combined with lowered salinities differentially impact oyster size class growth and mortality. *J. Shellfish Res.* 35(1), 101–113.

Saraiva, S., van der Meer, J., Kooijman, S.A.L.M. and Sousa, T. (2011). Modelling feeding processes in bivalves: A mechanistic approach. *Ecol. Modell.* 222(3), 514–523.

Schaeffer, B.A., Kurtz J.C. and Hein, M.K. (2012). Phytoplankton community composition in nearshore coastal waters of Louisiana. *Marine Pollut. Bull.* 64(8), 1705–1712.

Shumway, S.E. and Koehn, R.K. (1982). Oxygen consumption in the American oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 9(1), 59–68.

Shumway, S.E., Gabbott, P.A. and Youngson, A. (1977). The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin-positive substances in the adductor muscles of eight species of bivalve molluscs. *J. Exp. Mar. Biol. Ecol.* 29(2), 131–150.

Soniat, T.M., Cozelmann, C.P., Byrd, J.D., Roszell, D.P., Bridevaux, J. L., Suir, K.J. and Colley, S.B. (2013). Predicting the effects of proposed Mississippi River diversions on oyster habitat quality: Application of an oyster habitat suitability index model. *J. Shellfish Res.* 32 (3), 629–638.

Soniat, T.M., Cooper, N., Powell, E.N., Klinck, J.M., Abdelguerfi, M., Tu, S., Mann, R. and Banks, P.D. (2014). Estimating sustainable harvests of eastern oysters, *Crassostrea virginica*. *J. Shellfish Res.* 33(2), 381–394.

Taylor, K.E. (2001). Summarizing multiple aspects of model performance in a single diagram. *J. Geophys. Res. Atmos.* 106(D7), 7183–7192.

Ulanowicz, R.E., Caplins, W.C. and Dunnington, E.A. (1980). The forecasting of oyster harvest in central Chesapeake Bay. *Estuarine Coastal Marine Sci.* 11(1), 101–106.

van der Meer, J. (2006). An introduction to Dynamic Energy Budget (DEB) models with special emphasis on parameter estimation. *J. Sea Res.* 56(2), 85–102.

van der Veer, H.W., Cardoso, J.F. and van der Meer, J. (2006). The estimation of DEB parameters for various Northeast Atlantic bivalve species. *J. Sea Res.* 56(2), 107–124.

van Winkle, W. (1972). Ciliary activity and oxygen consumption of excised bivalve gill tissue. *Comp. Biochem. Physiol. Part A Physiol.* 42(2), 473–485.

Wang, H., Huang, W., Harwell, M.A., Edmiston, L., Johnson, E., Hsieh, P., Milla, K., Christensen, J., Stewart, J. and Liu, X. (2008). Modeling oyster growth rate by coupling oyster population and hydrodynamic models for Apalachicola Bay, Florida, USA. *Ecological Modelling*, 211(1), 77–89.

Wilber, D.H. (1992). Associations between freshwater inflows and oyster productivity in Apalachicola Bay, Florida. *Estuarine, Coastal Shelf Sci.* 35(2), 179–190.

Winter, J.E. (1978). A review on the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture* 13(1), 1–33.

SUPPLEMENTARY DATA

Integrating the effects of salinity on the physiology of the eastern oyster, *Crassostrea virginica,* in the northern Gulf of Mexico through a Dynamic Energy Budget model

Romain Lavaud, Megan K. La Peyre, Sandra M. Casas, Jérôme F. La Peyre and Cédric Bacher

Appendix – Covariation method for DEB parameter estimation

Five parameters of the DEB model for *Crassostrea virginica* were calculated from experiments and data available in the literature. The rest of the DEB parameters had to be estimated, and as advised by van der Meer (2006), all available physiological data sets should be combined in a standard procedure to estimate parameters through simultaneous regression. We used the robust and widely accepted "covariation method" described by Lika et al. (2011). This approach identifies the best set of parameters to simulate various types of observable data in one single step: "zero-variate" data (single numbers such as the age at metamorphosis, length at puberty or ultimate body dry weight), and "uni-variate" data (referring to the evolution of a dependent variable such as weight or respiration rate over an independent variable, e.g. time). The main goal of this approach is to avoid physiologically inconsistent parameter values by setting physiological constraints and rules for parameter covariation, as implied by the physical laws on which DEB theory has been built. Regression routines provided in the package DEBtool (http://www.bio.vu.nl/thb/deb/) were used in software GNU Octave 4.2.0. (Eaton et al., 2016) to estimate the remaining parameters values by minimizing the weighted sum of squared deviations between observations and model predictions. The use of parameter's value already calculated through experiments is an integral part of the estimation process, therefore exploiting the covariation of parameters and adding constraint to the possible value of the parameters to be estimated.

The collection of data gathered from the literature used in the estimation of the DEB parameters through the covariation method is presented in Table S1. Predicted values are in close agreement with observations despite an underestimated age at metamorphosis a_j (7 days compared to 20 days observed in average). Predicting larval stage duration of marine bivalves can be troublesome given the influence of other environmental variable such as salinity, oxygen concentration or hydrodynamics (Dekshenieks et al., 1993). Similar underestimation has is observed in other bivalve species such as *Crassostrea gigas*, *Perna viridis*, *Pecten maximus*, *Argopecten purpuratus* and *Arctica islandica* (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/species_list.html). Predicted reproduction rate was also slightly underestimated but still falls in the same order of magnitude as the observation. Prediction for uni-variate data are presented in Figure S1. Good agreement was found between observations and predictions. The set of parameters estimated through the covariation method thus allows a very satisfactory prediction of a wide range of observations.

Table S1. Source of zero-variate data used to estimate the DEB parameters for *C. virginica* through the covariation method (Lika et al., 2011). Length data correspond to larvae diameter at birth and metamorphosis stages and to the distance from shell umbo to distal edge for later stages.

Description	Symbol	Value	Predicted	Unit	Reference
Age at birth	a_b	1.50	1.76	d	Gallager et al. (1986)
Age at metamorphosis	a_j	20	9	d	Carriker (2009)
Age at puberty	a_p	84	74	d	La Peyre (unpublished data)
Life span	a_m	7300	7299	d	Galtsoff (1964)
Length at birth	L_b	0.0075	0.0057	cm	Gallager et al. (1986)
Length at metamorphosis	L_j	0.035	0.035	cm	Carriker (2009)
Length at puberty	L_p	3.00	3.25	cm	La Peyre (unpublished data)
Ultimate length	L_i	20.6	20.9	cm	Galtsoff (1964)
Dry weight at birth	W_b	1.5×10^{-7}	3.0×10^{-8}	g_{DW}	Gallager et al. (1986)
Dry weight at metamorphosis	W_j	7.5×10^{-6}	7.2×10^{-6}	g_{DW}	Carriker (2009)
Dry weight at puberty	W_p	0.12	0.12	g_{DW}	La Peyre (unpublished data)
Ultimate dry weight	W_i	35.8	31.7	g_{DW}	Galtsoff (1964)
Reproduction rate	R_i	8×10^{-5}	7×10^{-5}	# d^{-1}	Loosanoff and Davies (1963)

Most of the estimated parameter values are comparable to previous DEB parameters estimated for other marine bivalve species (van der Veer et al., 2006; Flye-Sainte-Marie et al., 2009; Saraiva et al., 2011; Lavaud et al., 2014). Moreover, they are very similar to those proposed by Filgueira et al. (2014) although resulting from different estimation methods. Two main differences occur in the values of the volume-specific costs for growth $[E_G]$ and the maximum reserve density $[E_m]$, estimated by Filgueira et al. (2014) at 1521 and 2586 J cm^{-3} respectively whereas we found much higher values of 5230 and 5420 J cm^{-3} respectively. The calculation of $[E_G]$ is probably the cause of the observed discrepancy between the two values as they used the indirect method described in van der Veer et al. (2006). These authors admit the weakness of their approach as being only based on information on the energy efficiency in endotherms and the conversion used being only a reflection of the expected order of magnitude. The covariation method, on the other hand, relies on the assumption that the growth efficiency κ_G should be around 0.8 (meaning that 20 % of energy is lost through overheads during the growth process). The relationship between $[E_G]$ and κ_G through the DEB formula: $\kappa_G = M_V \, \mu_V \, / \, [E_G]$ (Kooijman, 2010), with M_V the volume-specific mass of structure (8.3862 mmol cm^{-3}) and μ_V the chemical potential for structure (500 kJ mol^{-1}) allows the estimation of $[E_G]$. The estimated value of the maximum reserve density $[E_m]$ is twice as high as the one found by Filgueira et al. (2014). This is unlikely to come from the difference of methods used although we directly measured the energy content of oyster tissues while they estimated it through the calculation proposed by van der Veer et al. (2006). The most likely explanation to this discrepancy comes from the origin of oysters used in the two studies: we worked on Louisianan animals while Filgueira et al. (2014) used a data set from Eastern Canada.

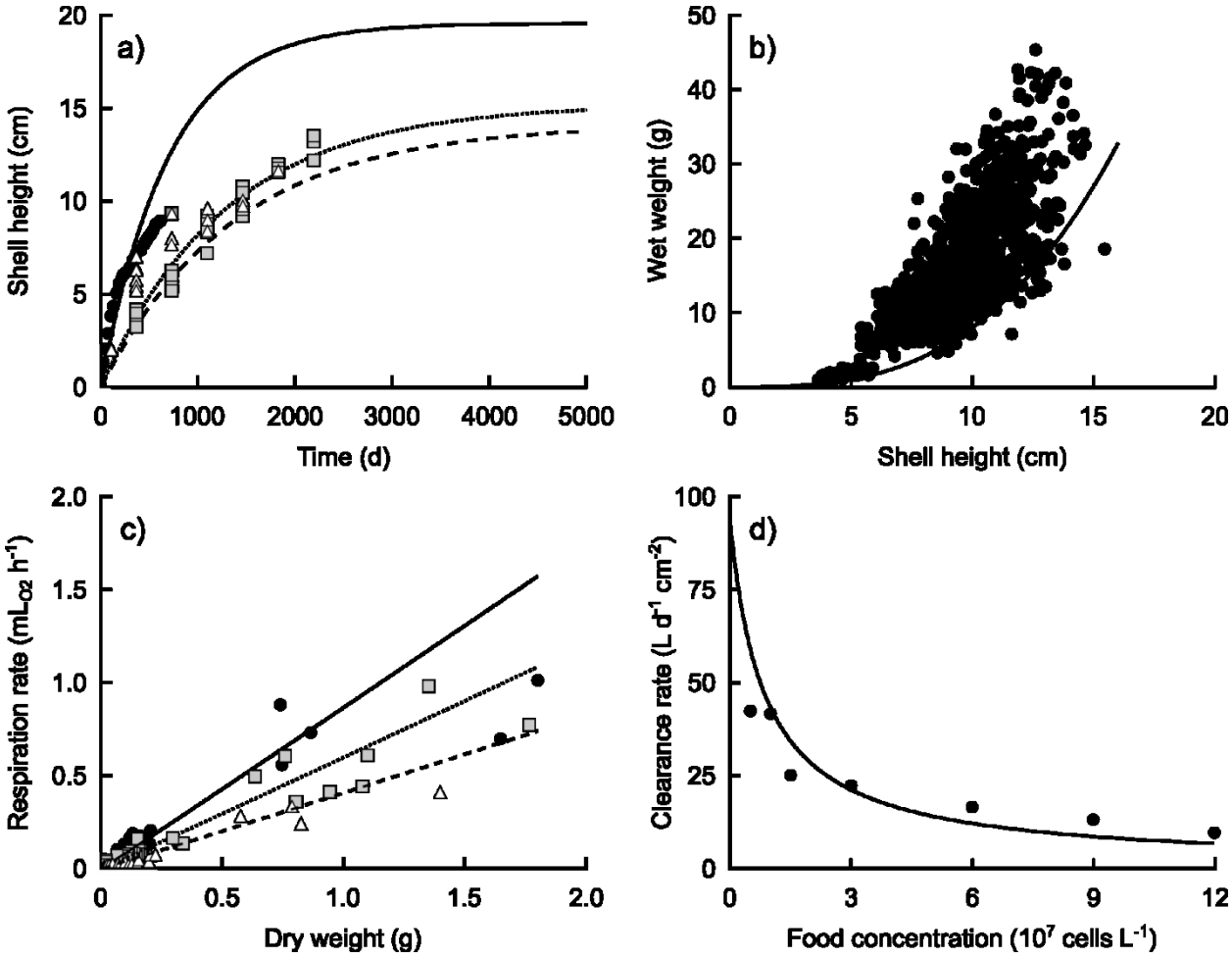

Figure S1. Model predictions vs. observation of the uni-variate data used to estimate the DEB parameters for *C. virginica* through the covariation method (Lika et al., 2011): a) growth data from Hopkins et al. (1953; black dots), Mann et al. (2009; gray squares), Paynter et al. (2010; white triangles) and model predictions (solid, dashed and dotted line respectively), b) wet weight of tissue vs. shell height from Powell et al. (1995), LaPeyre et al. (2013), Casas et al. (2015) and Rybovich et al. (2016) (all black dots) and model predictions (solid line), c) respiration rate data from Dame (1972) at 30 °C (black dots), 20 °C (gray squares), 10 °C (white triangles) and model predictions (solid, dashed and dotted line respectively) and d) clearance rate data vs. food concentration from Casas et al. (in review; black dots) and model predictions (solid line).

References

Carriker, M.R. (2009). The Shell and Ligament. In The Eastern Oyster *Crassostrea virginica* (Chapter 3). Maryland Sea Grant College, University of Maryland, College Park, Maryland, 75-168.

Eaton, J.W., Bateman, D., Hauberg, S. and Wehbring, R. (2016). GNU Octave version 4.2.0 manual: a high-level interactive language for numerical computations.

Gallager, S.M., Mann, R. and Sasaki, G.C. (1986). Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture* 56(2), 81-103.

Hopkins, S.H., Mackin, J.G. and Menzel, R.W. (1953). The annual cycle of reproduction, growth and fattening in Louisiana oysters. *Proc. Natl. Shellfish Assoc.* 44, 39-50.

Kooijman S.A.L.M. (2014). Metabolic acceleration in animal ontogeny: An evolutionary perspective. *J. Sea Res.* 94, 128-137.

Loosanoff, V.L. and Davis, H.C. (1963). Rearing of bivalve mollusks. *Adv. Mar. Biol.* 1, 1-136.

Mann, R., Southworth, M., Harding, J.M. and Wesson, J.A. (2009). Population studies of the native eastern oyster, *Crassostrea virginica* (Gmelin, 1791) in the James River, Virginia, USA. *J. Shellfish Res.* 28(2), 193-220.

Paynter, K.T., Politano, V., Lane, H.A., Allen, S.M. and Meritt, D. (2010). Growth rates and prevalence of *Perkinsus marinus* in restored oyster populations in Maryland. *J. Shellfish Res.* 29(2), 309-317.