Sublethal toxicant effects with dynamic energy budget theory: application to mussel outplants

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Abstract We investigate the effectiveness of a sublethal toxic effect model embedded in Dynamic Energy Budget (DEB) theory for the analysis of field data. We analyze the performance of two species of mussels, *Mytilus galloprovincialis* and *M. californianus*, near a diffuser discharging produced water in the Southern California Bight, California. Produced water is a byproduct of oil production consisting of fossil water together with compounds added during the extraction process, and generally contains highly elevated levels of pollutants relative to sea water. Produced water negatively affects the production of somatic and reproductive biomass in both mussel species; we show that these negative effects can be quantified with our DEB-based modeling framework through the estimation of toxic effect scaling parameters. Our analyses reveal that the toxic impact of produced water on growth and reproduction of *M. californianus* is substantially higher than for *M. galloprovincialis*. Projections of the expected lifetime production of gonad biomass indicate that the environmental impact of produced water can be as large as 100%, whereas short-term assessment without the use of DEB theory projects a maximum effect of only 30%.

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Introduction

Sublethal toxic effects are difficult to assess in field systems. Classic measures from standard toxicity tests, such as the No Observed Effect Concentration (NOEC) and EC_{50}, are of limited value, since test species or strains may not be representative of the species in the system under study, environmental conditions influence the bioavailability of toxic compounds, and organisms in real systems are typically concomitantly exposed to a variety of stressors, including a mixture of toxicants rather than a single one. Indeed, it is well known that relating classic toxicity measures to ecological impacts is challenging. For example, EC_{50} values indicate disastrously high contamination levels, whereas more conservative measures, such as the EC_1 or EC_5 and NOEC are fraught with statistical problems (Alvarez et al. 2006; Crane and Newman 2000; Kooijman et al. 2008b). On a more fundamental note, even if a classic measure could be accurately and precisely estimated from field data, its usefulness is still of limited value as it cannot be used to predict the effect of a pollutant on the performance of organisms and populations, unless this measure has a meaningful interpretation in a process-based modeling framework.

Dynamic energy budget (DEB) theory provides a process-based modeling framework that has been successfully applied to ecotoxicological questions (Billoir et al. 2007; Ducrot et al. 2007; Jager and Kooijman 2005; Kooijman and Bedaux 1996). DEB models describe the ingestion and assimilation of food by individual organisms and its utilization for maintenance, growth and reproduction (Kooijman

2000; Nisbet et al. 2000). Toxic compounds alter the performance of organisms by changing parameters of the DEB model. DEB theory offers at least four important advantages over the classic methods of analysis in ecotoxicology. First, toxicity assessments in DEB theory are independent of experimental protocol, i.e., toxicity parameters estimated within the DEB framework are independent of exposure time and choice of toxicant test concentration (Alvarez et al. 2006; Billoir et al. 2008; Jager et al. 2004). Second, with information about several organisms and toxicant combinations, it is possible to make inferences about other combinations via quantitative structure-activity (QSAR) and body-size scaling relationships (Kooijman et al. 2007). Third, environmental conditions are explicit drivers in DEB theory, implying that, in principle, the impact of variable resources and multiple stressors can be investigated in a single, coherent framework. Fourth, different endpoints (e.g., respiration, reproduction and growth) are the cumulative result of shared processes in DEB theory. This not only implies that different endpoints can be meaningfully compared (Alvarez et al. 2006), but also that the theory can be used to make predictions of the impact of toxicants on populations and potentially on ecosystem processes (Billoir et al. 2007; Kooijman 2000).

Using previously developed methodology (Muller et al. 2009), we analyze the performance of two species of mussels, *Mytilus galloprovincialis* and *M. californianus*, near a diffuser discharging produced water in the Southern California Bight near Santa Barbara, California. Produced water is a byproduct of oil production consisting of fossil water from subsurface geological formations and waste water generated during oil production; the volume of produced water often is an order of magnitude higher than that of the gas and oil obtained (Benko and Drewes 2008). The composition of produced water is highly variable, as it depends on the source geological formation, but compared to seawater it generally contains highly elevated levels of pollutants, including heavy metals, volatile aromatic compounds, naphthalenes and aliphatic hydrocarbons (Benko and Drewes 2008; Stephenson 1992). Produced water extracted in the Santa Barbara region reduces gametogenesis and gamete performance in sea urchins (Krause 1994, 1995), settlement success of red abalone larvae (Raimondi and Schmitt 1992) and production in blue and California mussels (Osenberg et al. 1992); it also affects infaunal community structure (Osenberg et al. 1992).

Our main goal is to investigate the effectiveness of our toxic effect model (Muller et al. 2009) for the analysis of field data. The data analyzed in this study originate from an outplant study in the Southern California Bight (Osenberg et al. 1992) in which the production of somatic and gonad tissue in mussels declined with decreasing distance from the discharge. Sites near the diffuser had production rates

that were 30% lower than the most distant sites. Although this decline is statistically significant, the biological relevance of this short-term decline is unknown. Here we show that by estimating toxicity parameters we can quantify the sublethal impacts of produced water on the production of both mussel species, compare the sensitivity of both species, and estimate the long-term effects of produced water exposure on mussel reproductive output.

Model

Dynamic Energy Budget (DEB) theory uses a modular approach to describe production, toxicant exchange and toxicant effects. Because a detailed discussion of model assumptions and derivation can be found elsewhere (Kooijman 2000, 2001; Kooijman et al. 2008a, b; for an introduction to DEB theory, see Nisbet et al. 2000), we confine this presentation to the essentials. Figure 1 outlines the energy and material flows. Table 1 lists the equations used in this paper, and Table 2 explains symbols and conventions.

The DEB model distinguishes three types of biomass in a heterotrophic organism, i.e., structure, reserves and biomass set aside for reproduction (including sperm and eggs), and has, in its original formulation, two state variables, i.e., the amount of structural mass and the density of reserves (i.e., the amount of reserves as a fraction of the amount of structure). We assume that structural biomass is proportional to the shell length cubed (i.e., mussel shape remains constant through ontogeny), so that we can use shell length (see Eq. (1) in Table 1), which is readily measured, to describe structural mass, which is not available from the field data. In DEB theory, the reserve density approaches a steady state in constant food environments. Because the model assumes that each type of biomass has a constant chemical composition, in steady state the absolute amount of reserves is proportional to the amount of structure. Consequently, the amount of somatic biomass (i.e., the amount of structural and reserve biomass combined) in constant food environments is proportional to the amount of structural mass (i.e., to shell length cubed; see Eq. (2) in Table 1). The dynamics of the third type of biomass, i.e., reproductive material, are given by Eq. (3). The lengthy solution to Eq. (4), which can be found analytically, represents the amount of reproductive material formed as a function of shell length.

In addition to data on shell length, somatic mass and gonadal mass (available from Osenberg et al. 1992), we have data on the barium content in the shell matrix. Barium is one of many contaminants in produced water that may be responsible for impaired production of mussels. We do not have data on these other pollutants, but DEB theory predicts
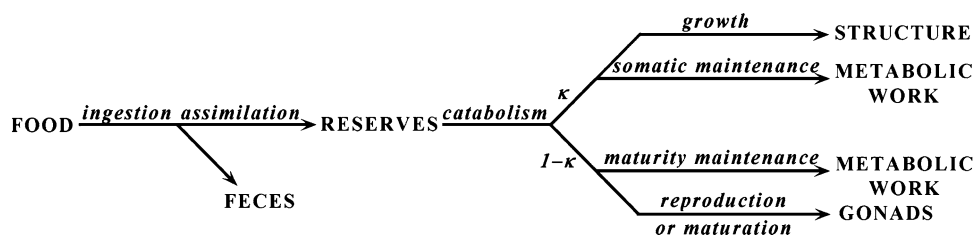
Fig. 1 Material and energy flows in a heterotrophic metazoan according to DEB theory (Kooijman 2000). The compositions of reserves and structure are constant, implying that conversion efficiencies for assimilation and growth, and the maintenance rate for a unit of structure are constant. Food is ingested at a rate proportional to the surface area of the organism and the scaled food density (type II functional response). Under non-starvation conditions, a constant fraction of mobilized reserves is used for somatic maintenance and growth, with maintenance having priority over growth; the remaining fraction is used for maturity maintenance and maturation (juveniles) or reproduction (adults), with maturity maintenance having priority over maturation and reproduction. The sublethal toxic effect mechanisms considered in this paper affect the rates of feeding and maintenance

Table 1 Equations (see Table 2 for an explanation of symbols)

Shell length (constant environment only)

$$L = L_\infty - (L_\infty - L_0)e^{-r_B t} \text{ with } r_B = [p_M]/3(\kappa f[E_m] + [E_G]) \text{ and } L_\infty = \kappa f \kappa_X \mu_{XA}\{J_{XAm}\}/[p_M] \tag{1}$$

Somatic mass

$$W_{d,S} = \alpha L^3 \text{ with } \alpha = \left(\delta_M^3 d_{Vd} + \frac{\delta_M^3 w_{Ed}[E]}{\mu_E}\right) \tag{2}$$

Reproduction

$$\frac{dW_{d,R}}{dt} = p_{d,R} r_B\left(gL_\infty L^2 + fL^3 - (f+g)L_p^3\right) \text{ with } p_{d,R} = 3w_R[E_m](1-\kappa)/\mu_{CR} \tag{3}$$

$$\frac{dW_{d,R}}{dL} = \frac{p_{d,R}\left(gL_\infty L^2 + fL^3 - (f+g)L_p^3\right)}{L_\infty - L} \tag{4}$$

Toxicant effect on asymptotic size

$$L_{\infty,Q} = L_\infty\left(1 + \frac{Q_{Sh}}{K_{Sh}}\right)^{-2} \tag{5}$$

Toxic effect on von Bertalanffy growth rate

$$r_{B,Q} = r_B\left(1 + \frac{Q_{Sh}}{K_{Sh}}\right) \tag{6}$$

Expected lifetime gonad production

$$\overline{W}_{d,R} = \int_0^\infty \frac{dW_{d,R}}{dt}e^{-ht}dt \tag{7}$$

that in a constant environment, the ratio of pollutants in various body compartments is constant, and that the body burden of toxicants that are depurated rapidly equilibrate relatively rapidly with their respective ambient concentrations (Kooijman 2000; Kooijman et al. 2008a). Many of the compounds in produced water are volatile aromatic and aliphatic hydrocarbons with a relatively low molecular mass (Benko and Drewes 2008; Stephenson 1992), implying that these compounds are exchanged relatively rapidly between mussels and the environment. Therefore, we assume, rather boldly, that the body burdens of the various pollutants in produced water are proportional to the barium content of the shell, and that these body burdens equilibrate rapidly relative to the duration of the exposure to produced water.

Because we do not know which components in produced water cause the decline in biomass production in the mussels, although barium is implicated (Higashi et al. 1992), we assume that the effects of different toxicants work additively and that the barium content of the shell is an indicator of the total burden of toxicants in the mussels. Furthermore, we use the most general sublethal toxic effect

model developed by (Muller et al. 2009). This model assumes that toxicants affect feeding rates and maintenance costs, which leads to changes in two compound parameters: the von Bertalanffy growth rate and the ultimate length. Eqs. (5) and (6) describe the change in the values of those parameters as a function of the barium content of the shell.

To investigate the longer-term ecological implications of sub-lethal effects of produced water discharge, we calculate the expected lifetime production of gonad mass [Eq. (7)]. This extrapolation to longer time-scales requires assumptions about mortality rates. We assume there were no lethal toxic effects, because mortality rates in the out-plant study neither depended on the distance to the point of discharge nor the barium content of the shell (Osenberg et al. 1992). We further assume a constant mortality rate, leading to an exponential distribution of survival times. Although a constant mortality rate, independent of age and/or size, is unrealistic, we justify the use of this mathematically convenient distribution with the argument that we merely wish to illustrate the impact of sublethal effects on the expected lifetime reproductive effort of mussels.

Table 2 Symbols (see Table 1 for equations)

Symbol	Dimension*	Interpretation
C	$\#/V$	Ambient toxicant concentration
d_{vd}	m/L^3	Density of structure (dry mass)
$[E]$	e/L^3	Density of energy reserves
$[E_g]$	e/L^3	Volume-specific cost of growth
$[E_m]$	e/L^3	Maximum energy reserve density
f	–	Scaled functional response
g	–	Energy investment ratio
h	$1/t$	Specific mortality rate
$\{J_{XAm}\}$	$\#/L^2 t$	Surface-specific maximum ingestion rate
k_d	$V/L^2 t$	Surface-specific uptake rate of ambient toxicants
k_e	L/t	Toxicant elimination rate
k_{Sh}	$\#/m$	Toxicant scaling body burden for effects on maintenance
L	L	Body length
L_0	$L**$	Initial length
L_∞	$L**$	Asymptotic or ultimate length
$L_{\infty,Q}$	L	Asymptotic or ultimate length with barium
L_p	L	Length at puberty
$[M_Q]$	$\#/L^3$	Body burden
$\{p_{Am}\}$	$e/L^2 t$	Surface-specific maximum assimilation rate
$p_{d,R}$	m/L^3	Compound parameter for reproduction
$[p_M]$	$e/L^2 t$	Volume-specific maintenance rate
P_{ShV}	L^3/m	Partitioning coefficient of barium between shell and structure
Q_{Sh}	$\#/m$	Barium density in shell
r	$1/t$	Specific population growth rate
r_B	$1/t$	Von Bertalanffy growth rate
$r_{B,Q}$	$1/t$	Von Bertalanffy growth rate with barium
R	$\#/t$	Reproduction rate
t	t	Time
w_{Ed}	$m/\#$	Molar mass of reserves (on C-1 basis)
w_R	$m/\#$	Molar mass of gonads (on C-1 basis)
$W_{d,S}$	m	Mass of somatic biomass
$W_{d,R}$	m	Mass of gonad biomass
$\overline{W}_{d,R}$	m	Expected lifetime production of gonad biomass
α	m/L^3	Compound parameter for somatic biomass
δ_M	–	Shape coefficient
κ	–	Fraction of catabolic power energy spent on maintenance and growth
κ_X	–	Assimilation efficiency
μ_E	$e/\#$	Chemical potential of reserves
μ_{XA}	$e/\#$	Chemical potential of food

*, # is number; e energy; L body length; m mass; V ambient volume

Materials and methods

The data analyzed in this study originate from an outplant study in the Southern California Bight off the California coast (Osenberg et al. 1992). On 7 June 1990 marked mussels (range in size: 20–62 mm shell length) of two species, *M. galloprovincialis* and *M. californianus*, were placed at distances ranging from 1 to 1,000 m from an outfall discharging produced water from oil production.

Shell length of each mussel was measured at the start of the outplant period, as was the somatic and gonad biomass of a subset of mussels from the same population. After 119 days of exposure, each surviving mussel was remeasured to obtain its final length, as well the somatic and gonadal dry mass.

In addition, the shells of these mussels were archived for later analysis of barium, which can substitute for calcium during shell formation. Because barium is at high

concentration in the produced water, barium in the shell matrix constitutes a measure of integrated exposure to produced water. Mussel shell fragments from the growing edge of ~ 10 shells per distance were chipped off, dried, and pulverized into a fine powder. Approximately 0.5 g of powder per mussel was digested in concentrated nitric acid, which was later evaporated. The residue was redissolved in 8 ml of 2% nitric acid and centrifuged to remove particulates, and the digest was then subjected to inductively coupled plasma-atomic emission spectrometry to measure Ba and Ca content (using wavelengths of 455.4 and 315 nm, respectively). Standards were run every 15–30 samples; all standard curves had a linear correlation coefficient of >0.999.

Analysis and results

To quantify the biological significance of the decline in mussel performance with decreasing distance to the produced water diffuser, we analyze the data with the sublethal toxic effect module of the Dynamic Energy Budget (DEB) model outlined in Table 1. To apply the model given the available data, we assume that all mussels experienced the same constant food environment, that pollutant levels at each location remained unchanged for the duration of the study, and that the exchange of toxicants between mussels and the environment was sufficiently fast that body burdens quickly equilibrated. This last assumption implies that production during the transient period of toxicant accumulation was minor relative to the total production accrued over the outplant period. We will return to this assumption in the "Discussion".

Our analysis requires known initial amounts of somatic and gonad biomass. Because those amounts could not be determined experimentally, we use the data on shell length, gonad mass and somatic mass from the non-outplanted mussels to estimate the initial amounts of biomass in each of the outplanted mussels. Eq. (2) provides a good description of the length—somatic mass relationships for both species (see Fig. 2). To relate the amount of gonad mass to shell length with the solution to Eq. (4), we assume that the source mussels never spawned, or if they did, that they spawned only once prior to the outplant study at a fixed length. The curve fits are quite satisfactory for our purpose (see Fig. 3).

We take two approaches to analyze the sublethal effects of produced water on both mussel species. In the first, we consider effects on growth of shells. Shell lengths after 119 days of exposure are fitted as a function of initial length and shell barium content by Eq. (1). In the second approach, we analyze the effects of produced water on the production of somatic and gonad biomass. The amount of somatic biomass after 119 days of exposure and the
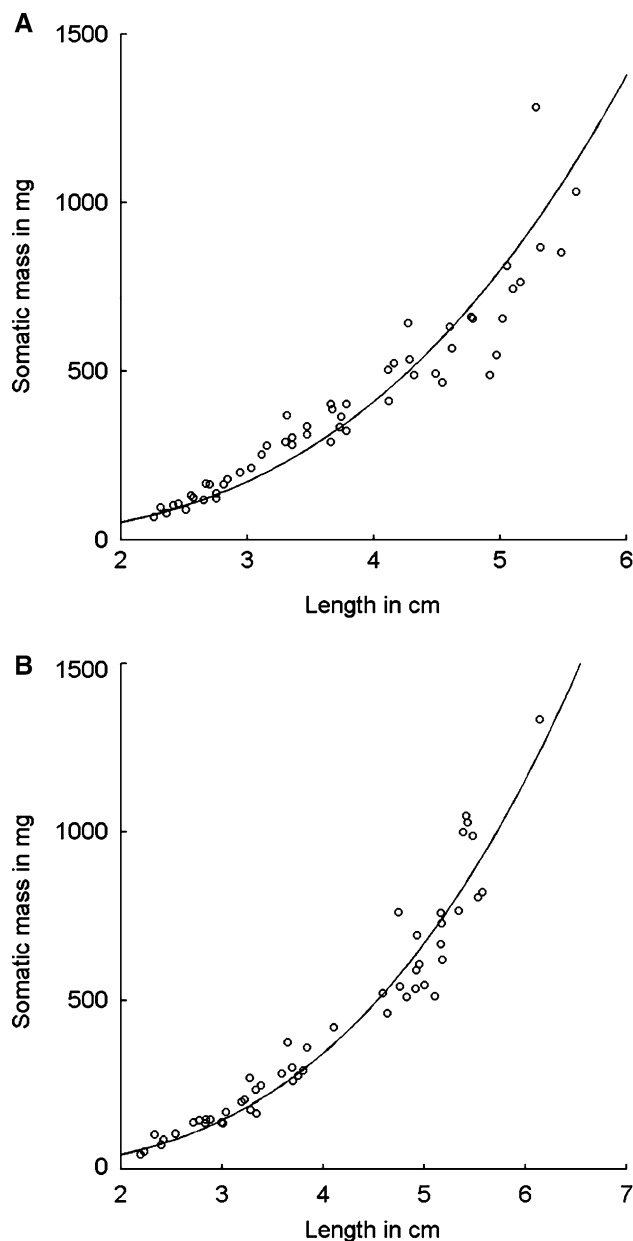

Fig. 2 Initial amount of somatic biomass in *Mytilus galloprovincialis* (**a**) and *M. californianus* (**b**) as a function of shell length. Data are fitted with Eq. (7) in Table 1 ($\alpha_{edu} = 6.39 \pm 0.17$ mg cm^{-3} and $\alpha_{cal} = 5.36 \pm 0.13$ mg cm^{-3})

amount of gonad mass produced during this period are concomitantly fitted as a function of initial length and estimates of the initial somatic and gonad masses (see previous paragraph). We weight each squared residual with the inverse of the dependent variable squared. With this crude way of weighting, we avoid that the minimization process is dominated by errors in the measurements of the somatic mass of larger individuals, as absolute errors in somatic mass tend to be higher than those in gonad biomass and absolute errors in data from larger individuals are larger than those of smaller ones.
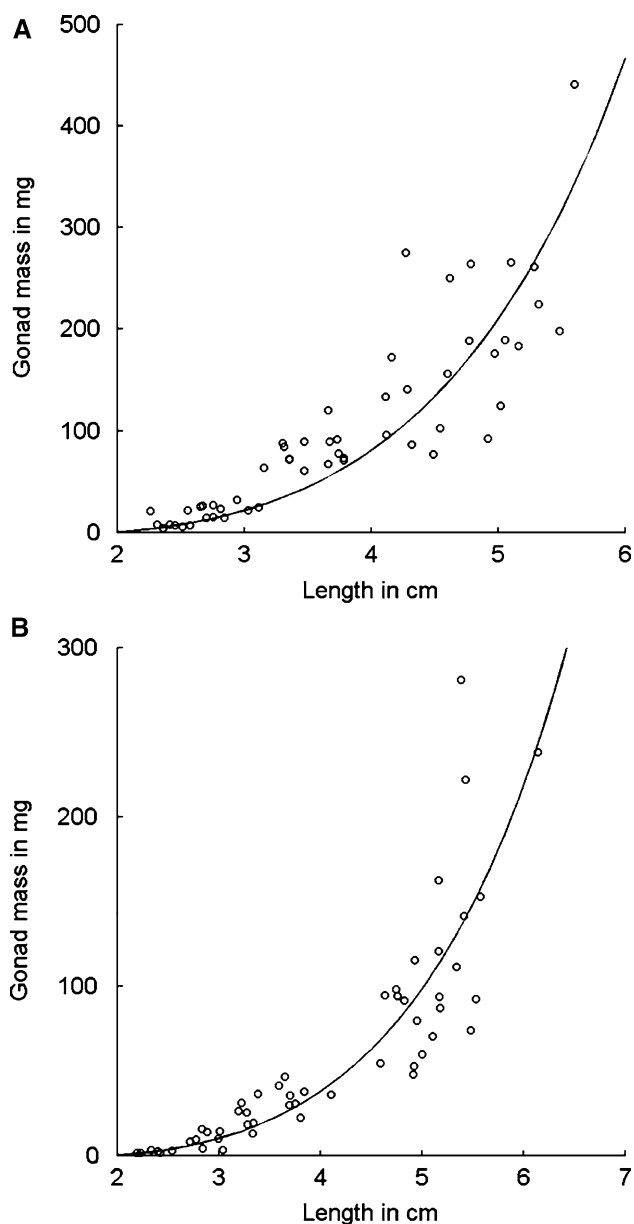
Fig. 3 Initial amount of gonad biomass in *Mytilus galloprovincialis* (**a**) and *M. californianus* (**b**) as a function of shell length. Data are fitted with the solution of Eq. (6) in Table 1; $3[E_m] (1 - \kappa) \eta_{RC} = 2.52 \pm 0.13$ (**a**) and 1.18 ± 0.06 mg cm^{-3} (**b**); $L_\infty = 10$ cm; $L_0 = 2$ cm; $L_p = 2$ cm; $f = 1$; $g = 1$. The last three parameter values hardly affect curve fits. L_0 can be estimated by eye with reasonable accuracy. L_∞ does impact curve fits to some extent, but cannot be estimated reliably from these data (note that L_∞ depends of food density, i.e., the values in Table 3 cannot be used)

Fig. 4 Production in *M. galloprovincialis* exposed for 119 days to produced water in the Southern California Bight; a higher shell barium content means a closer proximity to the discharge. Panel **a** shows the somatic biomass content of individual mussels after 119 days of exposure as a function of shell length prior to exposure and shell barium content; panel **b** shows the amount of gonad biomass produced during the 119 days of exposure as a function of the amount of somatic biomass after 119 days of exposure and shell barium content. The surfaces represent model fits to both data sets simultaneously; parameter values are listed in Table 3

Given the scatter in these field data, the model fits to the biomass data are quite satisfactory (Figs. 4 and 5); the model fits to the final lengths are equally satisfactory (results not shown). However, there is a marked discrepancy between the parameters estimated from the length and the biomass data (see Table 3). For both mussel species,
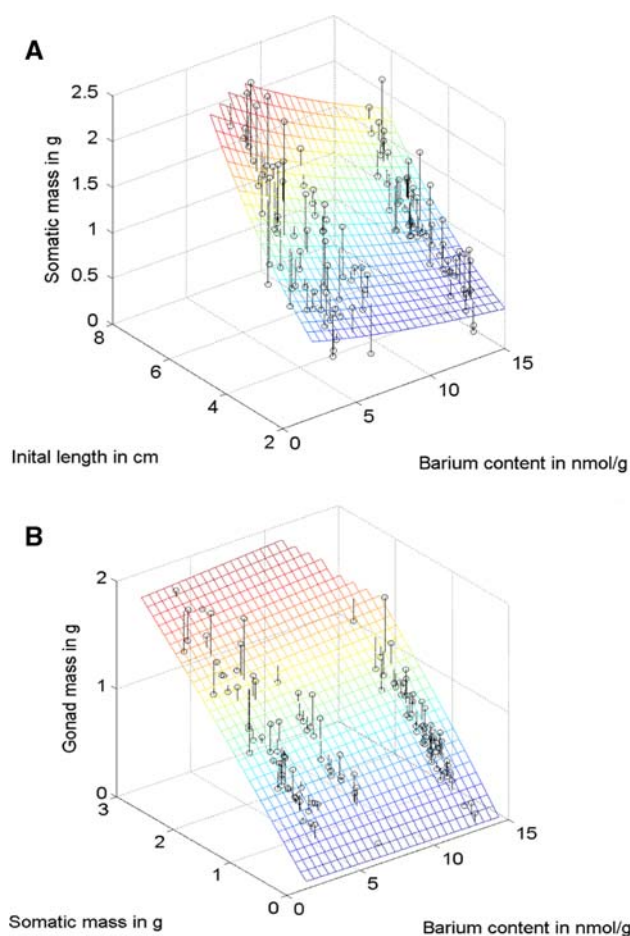
the toxicant scaling parameters estimated from shell length data alone are higher than those estimated from the data on somatic and gonad biomass, implying that the growth of shells is less affected by produced water than the production of somatic and gonad biomass. A related finding is that the ultimate lengths estimated from the two approaches differ substantially. For both species, the ultimate lengths estimated from biomass data alone are high, but the standard errors are large, implying that the tissue data contain little information about the ultimate lengths. This indicates that mussels away from the discharge had relatively more reserves or, more likely, that the amount of structural biomass is not simply proportional to the shell length cubed, implying that the calcification rate is not only a
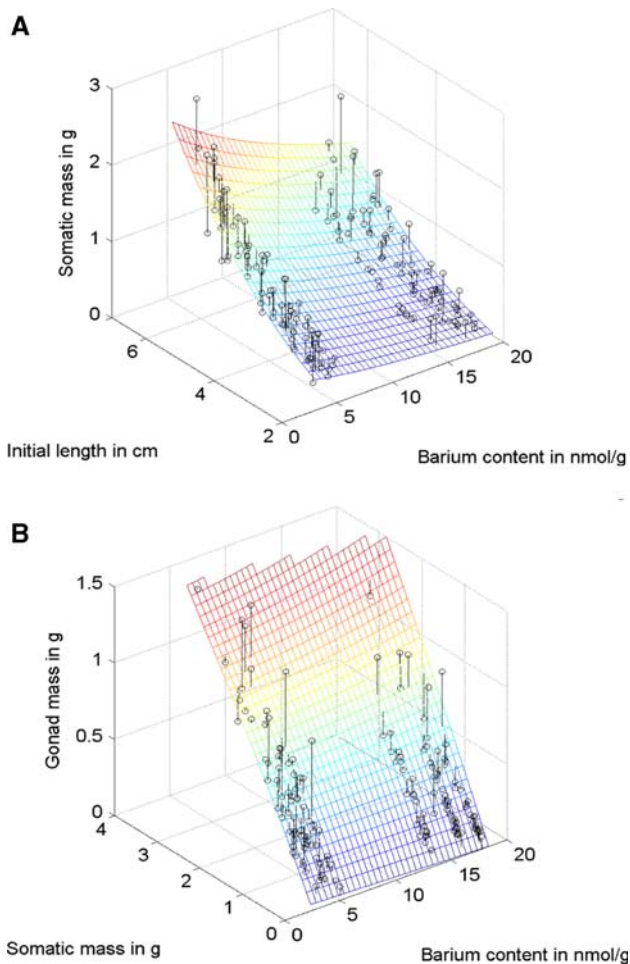
Fig. 5 Production in *M. californianus* exposed for 119 days to produced water in the Southern California Bight; a higher shell barium content means a closer proximity to the discharge. Panel **a** shows the somatic biomass content of individual mussels after 119 days of exposure as a function of shell length prior to exposure and shell barium content; panel **b** shows the amount of gonad biomass produced during the 119 days of exposure as a function of the amount of somatic biomass after 119 days of exposure and shell barium content. The surfaces represent model fits to both data sets simultaneously; parameter values are listed in Table 3

function of the growth rate, but may also depend on the assimilation rate, as is the case in scleractinian corals (Gattuso et al. 1999).

The toxicant scaling parameters estimated for *M. californianus* are lower than those estimated for *M. galloprovincialis* (see Table 3), which indicates that the former species is more sensitive to the toxicants in produced water than the latter. This difference in sensitivity becomes more pronounced when the accumulation of barium in the mussel shells is taken into account. At the same distance from the discharge, *M. californianus* tends to accumulate more barium in its shell than *M. galloprovincialis* in this outplant. The mussel species also differ slightly in their response of gonad versus somatic biomass production to produced water exposure. In *M. galloprovencialis*, individuals of a given final somatic mass have a relatively constant final gonad mass as shell barium content increases (Fig. 4b). This implies that the production of gonad and somatic biomass in this species are affected approximately equally by toxicants in produced water. They both decline with exposure to produced water, and at approximately equal rates. In contrast, in *M. californianus,* individuals of large somatic mass but with higher shell barium contents contain larger amounts of gonads (Fig. 5b). This implies that the production of somatic biomass in larger individuals is more affected than the production of gonads by toxicants in produced water. The increase in gonad mass (with barium content: Fig. 5b) arises because gonad mass declines less slowly with produced water exposure than does somatic mass.

We calculate the long-term effect of produced water on the expected lifetime production of gonad biomass with the fitted parameters (Table 3). The one additional parameter that is needed is the per capita death rate. The expected lifetime reproduction increased nearly linearly with the mean lifetime (i.e., the inverse of the death rate) for mean lifetimes greater than 1 year (results not shown). Consequently, the mean lifetime has little effect on the percent decline in expected lifetime gonad biomass production due to produced water. For the purposes of presentation, we therefore assume a per capita death rate of 0.25 a^{-1}, corresponding to a mean lifetime of 4 years. The percent decline in expected lifetime gonad production steeply increases as a function of the barium content of the shell (see Fig. 6), implying that even at the lowest barium

Table 3 Parameter estimated from shell length and biomass with standard errors (assuming $f = 1$)

Parameter	*M. galloprovincialis* Shell length	*M. galloprovincialis* Somatic and gonad biomass	*M. californianus* Shell length	*M. californianus* Somatic and gonad biomass
L_∞ (cm)	8.23 (0.58)	11.83 (8.21)	8.07 (0.52)	24.05 (24.14)
L_p (cm)	–	3.20 (2.60)	–	3.69 (2.08)
r_B (a^{-1})	1.50 (0.22)	1.24 (0.26)	0.91 (0.11)	0.37 (0.15)
K_{Sh} (nmol g^{-1})	252.13 (139.51)	52.30 (10.45)	97.02 (19.91)	26.03 (7.59)
$p_{d,R}$ (g cm^{-3})	–	0.11 (0.34)	–	1.77 (0.73)
g (–)	–	10.59 (34.63)	–	0.80 (0.34)

contents measured at 1 km from the point of discharge, the reduction in expected lifetime gonad production is quite substantial: about 35 and 50% for *M. galloprovincialis* and *M. californianus*, respectively. Another interesting observation is that as long as food availability supports growth to a size well beyond the size at maturation, the patterns in percent decline are relatively insensitive to food density (although the absolute amount of expected lifetime reproduction obviously depends strongly on food density). Finally, produced water compromises the reproductive effort of *M. californianus* more than that of *M. galloprovincialis*.

Discussion

Patterns in ecological and ecotoxicological data from the field are often difficult to characterize quantitatively. Temporal and spatial variation in environmental factors

Fig. 6 Percent reduction in expected lifetime gonad production in *M. galloprovincialis* (**a**) and *M. californianus* (**b**) as a function of shell barium content and scaled food density with the parameter values listed in Table 3 and a life expectancy of 4 years. The shell barium contents on the *y*-axis reflect the approximate range of contents measured in shells in the outplant studies

combine with ecological interactions to obscure connections between endpoints and stressors of interest. A clearly defined mechanistic framework relating endpoint to environmental variables is often lacking. As a result, the analysis of data is usually confined to finding data sets that have statistically significant differences from some reference, and, if possible, to summarize the relationship between endpoint and toxicant level with some metric, such as the NOEC or EC_{50}.

This approach has two major drawbacks. First, the power of a test is constrained by the variability of the data, the magnitude of the underlying cause of a pattern, and the number of independent sampling events (Osenberg et al. 1994). In our example this means that the estimate of NOEC depends on the choice of test concentrations, and that, in principle, the estimate of NOEC could be higher than the EC_{50}. Second, these metrics do not take full advantage of the information contained in the data. For example, Osenberg et al. (1992) established that mussels near the discharge had *statistically* significantly lower production than mussels further away from the discharge. This alone says little about the *biological* significance of the effects. Even though they emphasized the observed magnitude of reduction on tissue production to be large (50–75%), this was based only on short-term results and lacked any method to extrapolate those short-term measurements into longer-term consequences.

Those drawbacks can be circumvented by following the mechanistic approach used in this paper. We have interpreted different types of data (somatic growth and gonad production) in a single framework and summarized the impact of produced water on mussel performance through a single metric with physiological relevance, i.e., the toxicant scaling parameter. This metric also predicts the impact of produced water on other quantities not analyzed in this paper (or measured by Osenberg et al. 1992), such as the rates of respiration and feeding. The credibility of the predictions of course rests on the credibility of the underlying DEB model, but this can be evaluated in the context of a very broad collection of studies involving an impressive, rapidly growing number of studies of different organisms and environments. By treating different species within a common framework, our approach permits interspecific comparisons. Our metric suggests that *M. californianus* is affected more strongly by produced water than *M. galloprovincialis*. We speculate that the latter species has a higher capacity to transform organic toxicants in produced water than the former species, as smaller species typically demonstrate higher relative monooxygenase activities than larger species (Walker et al. 2006, p 68). Also, *M. galloprovincialis* may be naturally more exposed to toxins, and therefore have a higher biotransformation potential, than *M. californianus*, as *M. galloprovincialis*
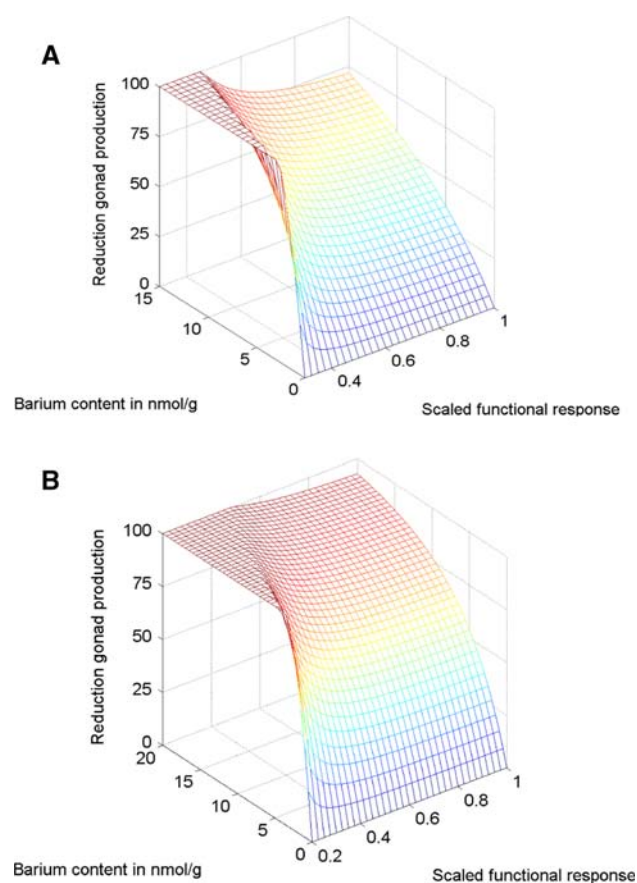
tends to dwell in or near estuaries, whereas the latter is more prevalent in open waters. It is likely that the *M. galloprovincialis* used by Osenberg et al. had been exposed to higher levels of organic pollutants before the outplant study than *M. californianus*, as the former were harvested at a location closer to natural oil seep areas than the latter.

Another advantage of our mechanistic approach is that it allows us to make projections of the impact of stress on lifetime reproductive output, as in the example worked out in this paper. Our aim has been to demonstrate the power of a DEB-based approach to interpreting field data rather than to characterize the effects of produced water on *natural* mussel populations, which would obviously require further ecological and environmental information. Indeed, the potential to link individual physiology to population growth rates motivated the earliest, pioneering application of DEB-like theory to ecotoxicology (Kooijman and Metz 1984). It is impossible to infer population level effects from projected values of lifetime reproductive output, without knowledge of other factors, such as fertilization success, larval dispersal and survival. Moreover, produced water affects larval development in mussels (Spangenberg and Cherr 1996) and possibly fertilization success and larval survival, as it does in sea urchins and abalones (Krause 1994; Raimondi and Schmitt 1992). However, the initially strong decline in expected lifetime gonad production with increasing shell barium content (Fig. 6) suggests that the potential environmental impacts of produced water are even stronger than commonly believed (e.g. Osenberg et al. 1992; Stephenson 1992; Stromgren et al. 1995).

With the DEB modeling framework, more information can be extracted from data than with traditional methods. Because toxic effect parameters in DEB theory refer to impacts on physiological processes, effects on other quantities than those measured can be calculated. Furthermore effects on physiological processes can be used to estimate population level effects (Billoir et al. 2007; Jager et al. 2004; Lopes et al. 2005; Muller et al. 2009). We expect that analyses of data about the impact of other stressors would similarly benefit from the approach followed in this study.

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