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Parameterisation of bivalve functional traits for mechanistic eco-physiological dynamic energy budget (DEB) models

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ABSTRACT: Mechanistic models such as those based on dynamic energy budget (DEB) theory are emergent ecomechanics tools to investigate the extent of fitness in organisms through changes in life history traits as explained by bioenergetic principles. The rapid growth in interest around this approach originates from the mechanistic characteristics of DEB, which are based on a number of rules dictating the use of mass and energy flow through organisms. One apparent bottleneck in DEB applications comes from the estimations of DEB parameters which are based on mathematical and statistical methods (covariation method). The parameterisation process begins with the knowledge of some functional traits of a target organism (e.g. embryo, sexual maturity and ultimate body size, feeding and assimilation rates, maintenance costs), identified from the literature or laboratory experiments. However, considering the prominent role of the mechanistic approach in ecology, the reduction of possible uncertainties is an important objective. We propose a revaluation of the laboratory procedures commonly used in ecological studies to estimate DEB parameters in marine bivalves. Our experimental organism was *Brachidontes pharaonis*. We supported our proposal with a validation exercise which compared life history traits as obtained by DEBs (implemented with parameters obtained using classical laboratory methods) with the actual set of species traits obtained in the field. Correspondence between the 2 approaches was very high (>95%) with respect to estimating both size and fitness. Our results demonstrate a good agreement between field data and model output for the effect of temperature and food density on age–size curve, maximum body size and total gamete production per life span. The mechanistic approach is a promising method of providing accurate predictions in a world that is under increasing anthropogenic pressure.

KEY WORDS: Mechanistic models · Dynamic energy budget · Bivalve · Parameterisation methods · *Brachidontes pharaonis* · Mediterranean Sea

INTRODUCTION

Organismal fitness (i.e. the total amount of gametes produced per life span; the so-called Darwinian fitness in Bozinovic et al. 2011) is a prominent concept in biology and evolutionary ecology (Roff 1992). It is considered to be one of the important key measures of ecosystem functioning, and of predicting response and resilience to human pressure (Hughes et al. 2005, Loreau 2010). A novel line of research proposes mechanistic models such as those based on the dynamic energy budget theory (DEB; Kooijman 2010), as reliable tools to investigate the extent of fitness (Bozinovic et al. 2011) in organisms through changes in life history traits (LH; Stearns 1992) as explained by bioenergetics principles (Nisbet et al. 2010, Kear-

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ney 2012). The biological coherence of the DEB approach (Kooijman 2010) in describing how organisms use energy from food, and which rules they follow when allocating that energy to growth and reproduction (Sousa et al. 2010) has already been demonstrated in ecology (Nisbet et al. 2000), biogeography (sensu Buckley et al. 2010) and global change sciences (Kearney et al. 2010, Kearney 2012). Recent research based on DEB models have had implications in many applied fields, such as aquaculture (e.g. Sarà et al. 2012a, Saraiva et al. 2012), fishery research (e.g. Jusup et al. 2011), terrestrial conservation ecology (e.g. Kearney 2012), eco-toxicology (e.g. Jager et al. 2010) and in studies of climate change (e.g. Sarà et al. 2011a, 2012b, V. Montalto et al. unpubl.).

The rapidly growing interest in this approach centers around the mechanistic characteristics of DEB, which are based on certain rules (first principles: the conservation laws) that dictate the use of mass and energy through organisms. Even in its simplest form, the standard DEB model is able to capture the functional response of all organisms (Nisbet et al. 2010), thereby providing mechanistic predictions of their growth and fitness. Therefore, if we are able to provide a common functioning scheme based on these rules for each organism, we could use such principles to predict their responses under environmentally varying conditions (sensu Benedetti-Cecchi 2000).

The DEB model contextualises organismal functioning under local environmental conditions. To date, this approach can be used to predict what might happen in terms of fitness if local conditions (such as temperature and food) changed. These important and positive aspects of DEB applications in a wide range of contexts are likely to make it a popular approach in the future, as the scientific community is faced with the challenge of accurately forecasting the effects of global climate change (Hoegh-Guldberg & Bruno 2010).

A potential limitation of the DEB approach is the methods used to obtain parameters for relevant or key species. This is an important step in mechanistic modelling and consists of the incorporation of real-world knowledge (i.e. an observation of the organism in the wild or in the laboratory) into model parameters. To run DEB models effectively, we need to estimate the parameters of target species which are species-specific (e.g. Arrhenius relationships, the way to describe the link between temperature and metabolic functioning in each species, etc.). The intrinsic mechanistic nature of DEB models means that if we start with a few known eco-physiological variables (e.g. those previously measured in the lab or wild) of target species, we can then mechanistically derive the remaining parameters needed to run DEBs. In simple terms, this is the core of the recent covariation method (Lika et al. 2011, Kearney 2012) which, starting from a few experimental data primarily extrapolated from literature and more rarely from direct experiments in the laboratory (e.g. van der Meer 2006, Saraiva et al. 2011a, Freitas et al. 2011), integrates mathematical and statistical tools to obtain DEB parameters. This means, however, that mathematical estimations of model parameters must rely on real knowledge of organismal traits, which in turn are derived from experimental procedures. At the current stage of mechanistic DEB research and modelling, it is not possible to model functional traits of organisms in total absence of information about the relevant organism. Fortunately, sets of DEB parameters for most living species can be approximately derived from current experimental knowledge, as most are based on classical eco-physiological variables commonly measured in laboratories worldwide. For instance, the area-specific maximum ingestion rate (J_{Xm}; J h^{-1} cm^{-2}), is the combination of (1) ingestion rate as commonly measured in most organisms, i.e. the feeding rate multiplied by the amount of organic matter in the food, such as the clearance rate in bivalves (Widdows & Staff 2006) or the feeding rate in fish (Du et al. 2006), (2) the scaled functional response f (Holling 1959) including an expression of food density (e.g. µg chl *a* l^{-1} in the case of bivalves, or amount of pelleted food in cultivated fish) and the parameter X_K, (i.e. the half saturation coefficient which is derivable from measurements of ingestion rates estimated at different food concentrations) and lastly, (3) the structural body volume based on the organismal shape, easily derivable from common biometric and gravimetric measurements.

As a result of the increasing prominence of mechanistic models in current scientific research, this study aims to adapt a set of classically established laboratory procedures, widely used in investigations of the ecological responses of organisms at individual level, to bridge the apparent gap in the standardisation of DEB parameters. To validate this approach, we have provided a dataset to test the confidence of our procedures, with the intent of making the use of mechanistic models more generalizable in future ecological studies. The long term objective is to standardise the experimental approach, based on mechanistic rules and an eco-physiological set of procedures to provide accurate estimates of energy allocation by organisms (Kearney et al. 2010, Sarà et al. 2011a, Kearney 2012, Sarà et al. 2012a).

This study involved the following steps: (1) a brief description of the standard DEB model (Kooijman 2010), (2) an examination of the agreement between the DEB parameters and the classical eco-physiological parameters usually adopted in physiological and behavioural studies of animals, (3) the identification of life history traits and the extent of fitness in a DEB context, (4) the specific description of experimental laboratory procedures used to estimate eco-physiological rates that are in turn used to derive the equivalent DEB parameters, (5) the execution of DEB models using DEB parameters (estimated using Step 4) with the inclusion of local temperature and food density into the model of *Brachidontes pharaonis* living in western Sicily, and lastly, (6) validation of the model output, using the age–size curve calculated from *B. pharaonis* collected in the field and the results of a gonadal output experiment carried out in the laboratory.

MATERIALS AND METHODS

Model species and study area

The pharaonic mussel *Brachidontes pharaonis* was used as a model species for this study. *B. pharaonis* is an invasive Lessepsian species that entered the Mediterranean Sea from the Red Sea through the Suez Canal, and which is able to out-compete native organisms for space and resources (Sarà et al. 2000). The large body of companion research data available on *B. pharaonis* (Sarà et al. 2000, 2003, 2008a,b, in press, Sarà 2007) was of great use to our validation exercise. *B. pharaonis* is sufficiently small (3–4 cm) to be easily maintained in aquaria, and robust enough to be handled for experiments. This makes it an ideal experimental species for use in ecological studies. Between 2009 and 2010, we collected more than 3000 *B. pharaonis* from the Ettore Pond of Stagnone di Marsala (Trapani, Western Sicily; 37° 52′ N, 12° 28′ E), our main study area. *B. pharaonis* is so abundant in the Ettore Pond (>8000 ind. m^{-2}; Sarà et al. 2000) that our collection did not disrupt local population equilibria or the ecological community. Once collected, organisms were brought back to the laboratory where they were cleaned of epibionts. All animals selected for experimental treatment were left to acclimatize for at least 1 mo in 300 l tanks with running seawater, and fed ad libitum with fresh algal cells (*Isochrysis galbana*) cultivated in the Experimental Ecology & Behaviour Laboratory at the University of Palermo.

Step 1: Standard DEB model

This section comprises a brief description in order to avoid repetition of the considerable amount of literature already published on this topic, as listed at the DEB website (www.bio.vu.nl/thb/deb/index.html; Kooijman 2010).

DEB incorporates whole-organism bioenergetics, connecting individual behaviours to population growth via estimates of reproductive output, i.e. Darwinian fitness (Fig. 1). The mechanistic nature of the DEB theory provides an exceptionally powerful tool for predicting the physiological performance of organisms according to first principles. Apart from food, the most important factor driving the metabolic machinery of an organism is its body temperature. In ectotherms such as aquatic invertebrates, this is a direct function of the external environment (Lima et al. 2011).

The following concepts, well summarized by Jager (2012), are very general and represent the main pillars of the standard DEB model (Kooijman 2010). (1) The standard animal grows isomorphically (i.e. it does not change shape over its life cycle) and the body is composed of (i) reserves fuelling metabolism, and (ii) structures requiring maintenance. (2) The feeding process is composed of food searching, handling and digestion. Food digested and energy assimilated are assumed to be proportional to the organism's surface area following the Type II functional response (Holling 1959). (3) Once assimilated, energy and matter are stored in the reserve compartment (e.g. carbohydrate, lipid, protein). (4) In the standard DEB model, the animal has one reserve and one structural compartment. (5) With constant amounts of food, the reserves are a constant proportion of the structure from embryo to death. (6) A fixed fraction (κ) of the mobilised flux of energy from the reserve is allocated to growth and somatic maintenance, while the remaining $1-\kappa$ is allocated to reproduction (gamete production and maturity maintenance). (7) Somatic maintenance has priority over growth, and growth ceases when all reserves are required for somatic maintenance. Maintenance costs are proportional to structural body volume. (8) Maturity maintenance has priority over maturation and reproduction, and maturity maintenance costs (albeit small) are proportional to the reserve invested into maturation. (9) Stage transitions such as fertilization, settlement and adult maturation are triggered by fixed maturity thresholds. Maturity has no mass or energy, but has the status of 'information', and is quantified by the amount of reserve needed to build

it up. After sexual maturation, maturity does not increase further. (10) At the onset of sexual maturation, the energy flux to maturity maintenance is redirected to a reproduction buffer, which is then converted into a discrete number of eggs or sperm prior to spawning events.

It is important to highlight that all members of a species tend to have common life history traits, and this supports the idea that the value of DEB parameters is genetic. If none of the above-mentioned assumptions are violated, it thereby stands to reason that each species differs from others mainly in parameter values, rather than in the structure of the DEB model. However, it is well known that stressful conditions caused by anthropogenic sources are able to restrict organismal functioning; so much so that organisms depart from the main assumptions and optimal performance (Jager 2012).

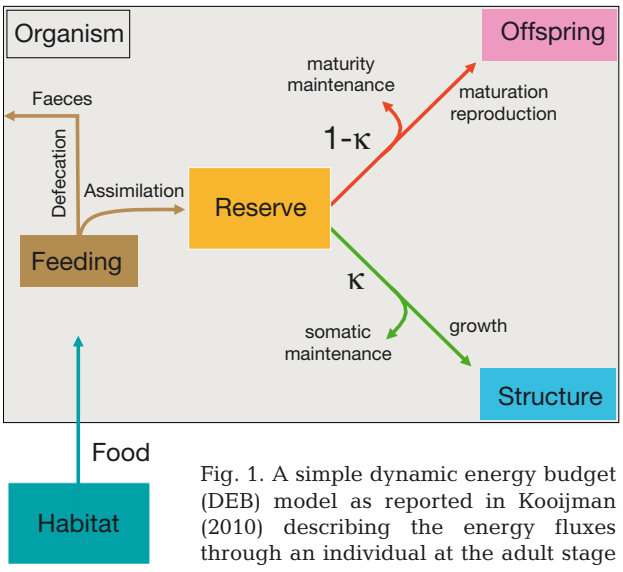

Fig. 1. A simple dynamic energy budget (DEB) model as reported in Kooijman (2010) describing the energy fluxes through an individual at the adult stage

Step 2: DEB and eco-physiological parameters

Bivalves meet most of the main assumptions of the standard DEB model — they are isomorphic, and they can be considered as having one reserve and one structure. Accordingly, we can use the standard DEB model (Fig. 1), which is a rather simple version of DEB theory (and of reality) but does adequately describe the life processes of most aquatic ectotherms (i.e. polychaetes, crustaceans, molluscs and fish; sensu Kooijman 2010, Nisbet et al. 2010). Several DEB parameters are not directly measurable. In such cases, we suggest that the most appropriate approach would be an integration of mathematical tools and experimental procedures, whereby energy acquisition and metabolic rates are preferably estimated under varying temperature, feeding conditions and food intake (Widdows et al. 1979, 1985, Widdows & Staff 2006, Filgueira et al. 2011). In Table 1, we report the agreement between the most important eco-physiological variables commonly representing the base of a bivalve's bioenergetics and DEB parameters.

Table 1. DEB parameters and physiological variables; physiological rates and relative mean values that were measured under standard laboratory conditions according to the equations given in the last column are shown in **bold**. V: structural volume; C_{t_0}: initial concentration; C_{t_1}: final concentration; Vol: chamber volume; t: time; k: physiological rate; T: temperature; J_X: ingestion rate; f: functional response; POM: particulate organic matter; µx: reserve density; pA: assimilation rate; AE: assimilation efficiency; F: food; E: faeces

Parameter	Unit	DEB formula	Formula with experimental rate	Physiological rate	Experimental mean value	Equation
$[\dot{p}M]$, Volume-specific maintenance costs	J h^{-1} cm^{-3}	$[\dot{p}M] = \boldsymbol{\dot{p}M}/V$	$[\dot{p}M] = \mathbf{RR} \times 0.456/V$	Respiration rate, RR	**15.4** µmol O_2 h^{-1}	$RR = (C_{t_0} - C_{t_1}) \times Vol \times 60(t_1 - t_0)^{-1}$
T_A, Arrhenius temperature	K	$T_A = \ln\lvert\mathbf{k}(_T)/\mathbf{k}(_1)\rvert \times (T_1 \times T)/(T - T_1)$	$T_A = \ln\lvert\mathbf{RR}(_T)/\mathbf{RR}(_1)\rvert \times (T_1 \times T)/(T - T_1)$	Respiration rate, RR	**12.7** µmol O_2 h^{-1}	$RR = (C_{t_0} - C_{t_1}) \times Vol \times 60(t_1 - t_0)^{-1}$
$\{\dot{J}_{Xm}\}$, Maximum surface area-specific ingestion rate	J h^{-1} cm^{-2}	$\{\dot{J}_{Xm}\} = \boldsymbol{\dot{J}_X}/f\,V^{2/3}$	$\{\dot{J}_{Xm}\} = \mathbf{CR} \times$ mg POM $\times\ 18.5 / f\,V^{2/3}$	Clearance rate, CR	**0.39** l h^{-1}	$CR = Vol \times (\ln C_1 - \ln C_2)/\Delta t$
ae, Assimilation efficiency	–	$\boldsymbol{ae} = (\mu x\,\dot{J}_X)/\dot{p}A$	$\mathbf{AE} = (\mu x\,\dot{J}_X)/\dot{p}A$	Conover ratio, ae	**0.75**	$AE = (F - E)/[(1 - E)F]$

In the following paragraphs, we describe the various DEB parameters required to estimate the energetics of bivalves (and other animals in general). We use the typical notation and symbols commonly adopted in a standard DEB model (van der Veer et al. 2006, Kooijman 2010).

Shape coefficient δ and structural volume

In a DEB context, body volume and surface area play crucial roles in energetic exchanges and fluxes. For instance, food acquisition rates are considered to be proportional to surface area (and are usually displayed in curly brackets), while maintenance rates are usually related to volume of biomass (and displayed in square brackets). In the standard DEB model, we assume that the volumes and surface areas of most organisms (including bivalves) increase during growth according to the rules of isomorphism, with the implication that an organism does not change its shape during the growth (Kooijman 2010).

Shape coefficients and structural volumes are abstract quantities. Some errors were previously made attempting to equate measured volume to structure volume; the main difficulty is distinguishing between structure and reserves. Following DEB theory, the volume of an animal can be measured under laboratory conditions using the most common procedures used to measure length (e.g. length of umbonal axis and width in bivalves, of columella in gastropods, of carapax in crustaceans, or total body length in fishes). Consequently, we need to transform the common measurement of length (L; cm) into structural volume (V; cm^3) as done, for example, with length data at birth and sexual maturity that allow us to estimate the corresponding volume (i.e. V_b and V_p; see Table 2). For this, a correction coefficient is required, known in the DEB context as a shape coefficient, δ (delta). The shape coefficient depends on the type of length measurements that have been taken; and in bivalves, we usually derive it from length or height and width of shells, and wet weight from flesh with no shells (van der Meer 2006). Thus, the shape coefficient is estimated using the following formula: $\delta = (WW\, d_{Vw}^{-1})^{1/3} \times L^{-1}$, where WW is wet weight of bivalves (i.e. flesh weight, g), d_{Vw} is the specific density for structure that we can assume, in most benthic invertebrates, to be equal to 1 g cm^{-3} and L is the total shell length (cm). Shape coefficients are dimensionless, and in mussels usually assume a value ranging from 0.2 to 0.5. To transform the measurement of length to a structural volume, the following formula is applied: $V = (\delta \times L)^3$.

Feeding process

Feeding is a species-specific behavioural trait and, in general, can include 2 mutually exclusive activities: food acquisition and food processing (handling + digestion + assimilation). In some species, food processing may prevent or limit subsequent food acquisition; however in bivalves, food acquisition and processing occur simultaneously. All food acquisition processes are proportional to the surface area of the animal. In bivalves, the complete feeding process involves 3 steps and has been well described in a mechanistic context by Saraiva et al. (2011) as (1) filtration, (2) ingestion and (3) digestion and absorption (assimilation). To avoid repetition, here we briefly describe (2) and (3).

Ingestion rate J_X

The ingestion rate J_X is the process of moving the food to the gut. It is dependent on food availability, body size and temperature, and typically includes the functional response f (Type II; Holling 1959) as follows:

$$J^{\cdot}_X = \{J^{\cdot}_{Xm}\} \times f \times V^{2/3} \quad (1)$$

where $\{J^{\cdot}_{Xm}\}$ is the area-specific maximum ingestion rate, expressed in J h^{-1} cm^{-2}; $f = X\,(X_K + X)^{-1}$ being the scaled functional response (ranging from 0–1) with X = food density (µg food l^{-1}) and X_K = the half-saturation coefficient (Fig. 2); and V is the structural body volume expressed as the cube of the shape coefficient and the organism's shell length, i.e. $V = \delta \times L^3$.

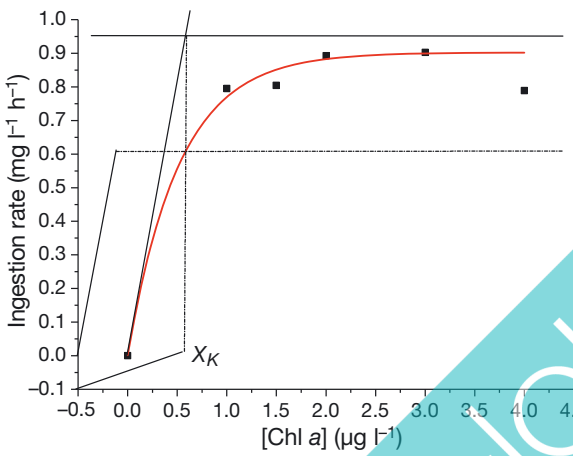

Fig. 2. Relationship between ingestion rate (mg l^{-1} h^{-1}) and food density ([chl *a*], µg l^{-1}). For most animals, this relationship can be described by Holling's Type II functional response. X_K: the saturation coefficient, the food density at which the ingestion rate is half the maximum

The function f is the Type II Holling functional response (Holling 1959). The saturation coefficient X_K depends on food quality, and in this paper we express it as a concentration of chlorophyll *a* (µg chl *a* l^{-1}), where the ingestion rate is half of the maximum (X_K; Fig. 2). Although it is not generalizable (e.g. estuarine bivalves), we have chosen to use chl *a*, as it has been commonly used in the literature to calculate food-linked DEB parameters (Pouvreau et al. 2006), especially in open coastal waters (Sarà et al. in press). However, the choice of food depends on the organism type (e.g. artificial pellet or fish scraps in cultivated fish, Mazzola et al. 2000; or copepods and/or *Artemia* spp. in zooplanktivores, Edmunds 2011). In suspension feeders such as bivalves, the maximum ingestion rate $\{\dot{J}_{Xm}\}$ can be derived from ingestion rate measurements (IR; µg l^{-1} h^{-1}) based on typical clearance rate experiments. Hence, $\{\dot{J}_{Xm}\}$ = IR $(f \times V^{2/3})^{-1}$, where IR is the maximum ingestion rate below a threshold of pseudofaeces production (e.g. Widdows et al. 1979, Widdows & Staff 2006, Sarà et al. 2008a,b).

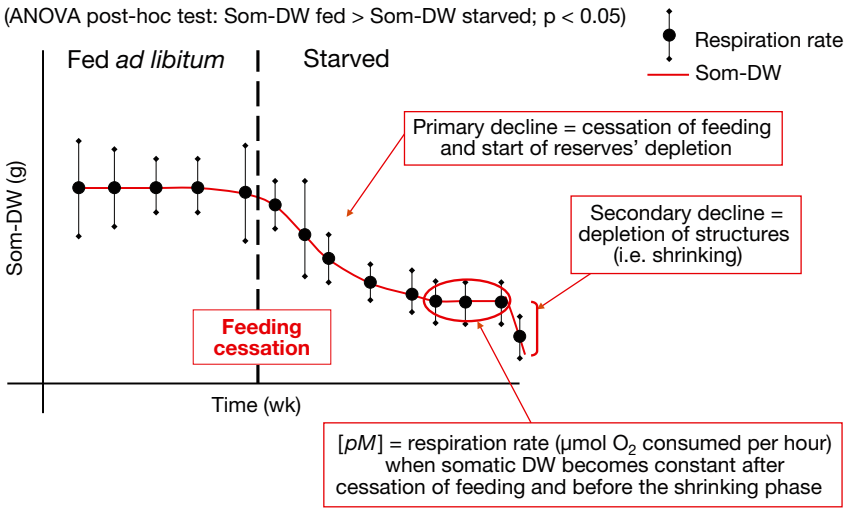

Fig. 3. Temporal changes in somatic dry weight (Som-DW) pattern of *Brachidontes pharaonis* following the cessation of feeding: constant Som-DW curve during ad libitum feeding showing typical respiration rates (i.e. not significantly different from controls), and depletion of reserves following feeding cessation. Som-DW decline can be quick (i.e. days) and respiration rates become significantly lower than controls; Som-DW curve levels off after a few weeks and corresponding respiration rates become constant (always significantly lower than controls); usually immediately preceding the beginning of structural depletion (shrinking). The mean oxygen consumption rate measured during the third phase (circled) should correspond to *pM*

Assimilation rate J_{EA}

Assimilation is the final step of food processing and is defined as the process whereby food is absorbed and converted into the organism's reserves (Kooijman 2010). The assimilation of food is assumed to be independent of the feeding rate per se, but proportional to the ingestion rate. The rate of assimilated energy J_{EA} is explicitly related to the food density through a functional response curve: $J_{EA} = f \times \{J_{Am}\} \times V^{2/3}$, where f is the scaled functional response (see above), $\{J_{Am}\}$ (J d^{-1} cm^{-2}) is the maximum assimilation rate per unit surface area, and V is the structural body volume (see above).

Operationally, we obtain $\{J_{Am}\}$ as follows: $\{J_{Am}\}$ = AE × µx × $\{J_{Xm}\}$ where AE (ranging from 0–1) is the assimilation efficiency calculated via the Conover ratio (Conover 1966), as is traditionally done across the current literature (Sarà et al. 2008a); µx is a conversion factor of food into energy (J mg^{-1}) and $\{\dot{J}_{Xm}\}$ is the area-specific maximum ingestion rate, expressed in J h^{-1} cm^{-2} (see above).

Volume-specific maintenance costs [pM]

Maintenance costs depend on the processes needed by the organism to survive, including all the biochemical processes necessary for basal metabolism — particularly costly protein synthesis / turnover (Widdows & Hawkins 1989). Maintenance costs are assumed by the standard DEB model to always scale with the volume of the individual ($\dot{p}M = [\dot{p}M] \times V$); whether the animal is fed or unfed, and other activities such as feeding and digestion are assumed not to be included in this average value of maintenance. In a DEB context, volume-specific maintenance costs might be estimated in an indirect way from experiments in which animals are starved and individual oxygen consumption and dry weight profiles are compared with those of well-fed animals (i.e. controls) where oxygen consumption includes the cost of feeding, digestion and growth / synthesis (Fig. 3; see also Fig. 5). While the parameter V is the structural volume (see above), $[\dot{p}M]$ is indirectly obtained and corresponds to the value of oxygen consumption before the dry weight of starved organisms declines significantly. Starvation experiments are useful to estimate pM because it is possible to link the use of oxygen with maintenance alone. Indeed, although several processes contribute to the value of

oxygen consumption (an indirect measure of *pM*; Ren & Schiel 2008), in comparison to direct calorimetry (Widdows & Hawkins 1989), if growth and feeding processes are switched off, oxygen consumption can only be due to changes in the maintenance rate. However, additional research will be needed to clarify the extent of each metabolic contribution to *pM*.

Maximum storage density [E_m] and volume-specific costs for growth [E_G]

The maximum storage density [E_m] and volume-specific costs of growth [E_G] correspond to the amount of energy that is stored in reserves in readiness to be used for reproduction and growth. They cannot be estimated directly, but it is possible to derive them from the *pM* experimental setup (see Step 4). If seasonal patterns of the species are known, these parameters can be calculated from the balance of the energy content before and after the growing season (van der Veer et al. 2006, Cardoso 2007).

Kappa (κ)

The κ-rule described in the DEB theory asserts that a fixed fraction of assimilated energy (κ) is allocated to maintenance and somatic growth, and that the remaining fraction (1 – κ) is available for gamete production (Fig. 1). The value of κ and the reproduction efficiency k_R; see Table 2) can only be estimated through specific mathematical routines (i.e. DEB tools, available online at www.bio.vu.nl/thb/deb/index.html) and following the Add-my-pet document (Kooijman 2010), having determined all other DEB parameters (e.g. J_X, *pM*, Arrhenius temperatures).

Arrhenius temperature (T_A)

Physiological rates all depend on body temperature, and in the same species all rates should change as a function of temperature in the same way (Fig. 4). For a species-specific range of temperatures, the description proposed by S. Arrhenius (1889) usually fits well (Kooijman 2010):

$$k_{(T)} = k_{(T1)} \times \exp\left(T_A\,[T_{ref}]^{-1} - T_A\,[T]^{-1}\right) \quad (2)$$

where $k_{(T)}$ is a physiological rate at ambient temperature T; T is the absolute temperature (in Kelvin); $k_{(T_{ref})}$ is the physiological rate at the reference temperature T_1; and T_A is the Arrhenius temperature.

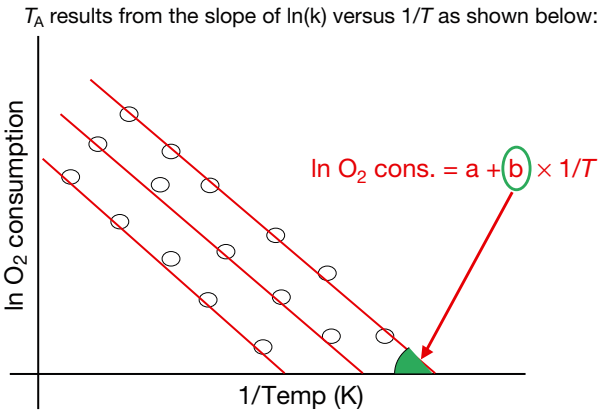

Fig. 4. Representation of Arrhenius temperature (T_A): the slope of the straight line that results from plotting the ln of rate k (preferably O_2 consumption) against the inverse of temperature (in Kelvin). The Arrhenius relationship usually provides a good explanation for the variation in temperature dependence of metabolic rates across species, and implies that each species can only obtain positive rates of growth within a specific range of tolerable temperatures (i.e. the optimum temperature for enzymatic reactions; see Kooijman 2010 for further details)

Estimates of T_A and of the lower and upper boundaries of the tolerance range are typically based on experiments of oxygen consumption carried out at different acclimation temperatures. While most authors extrapolate the thermal tolerance range from published data (Cardoso et al. 2006, Pouvreau et al. 2006), the most reliable approach is a direct calculation of physiological rates (e.g. feeding, excretion, heartbeat, respiration) obtained from representative samples of the population through laboratory experiments. Since the thermal optimum is species-specific and often linked to the geographical area in which the species lives (e.g. ~17–20°C for Mediterranean or 13–15°C or less for North Atlantic species), we suggest the use of the calculated, species-specific value. A typical approach depicts the entire thermal window of organismal functioning (Angilletta et al. 2010, Kearney et al. 2010). Once the optimal thermal window (e.g. 0–40°C) of the species has been established and physiological rates obtained at different steps (e.g. every 5°C step), it is possible to calculate the Arrhenius temperatures by applying the formula above (but see later for details).

Step 3: Obtaining life history traits from DEB

Through the mechanistic DEB approach, we are able to quantify the major life history traits of organisms. Such an operation is relatively simple with ec-

totherms, as they have a direct link to the environment through the habitat temperature (see 'Step 5' for how to incorporate temperature into the model). Such a quantitative mechanistic approach in contrast to a correlative approach (Buckley et al. 2010) represents an important advance deserving of future attention (sensu Kearney 2012, G. Sarà et al. unpubl. data).

The current, standard DEB model allows us to quantify (1) maximum individual habitat size, (2) time required to reach sexual maturity, (3) number of reproductive events per life span, (4) total number of eggs per life span, and (5) number of eggs per spawning event.

Maximal individual habitat size (MIHS)

To explain the link between energy budgets and body size, Kooijman (2010) suggested the use of the following formula: MIHS = $\kappa \times \{pAm\}\,[pM]^{-1}$, where κ is the fraction allocated to somatic maintenance and growth (i.e. the κ-rule; Koojiman 2010); $\{pAm\}$ is the surface area-specific assimilation rate, which depends on the Holling (1959) scaled functional response f; and $[pM]$ is the volume-specific maintenance rate (Kooijman 2010). For details of all parameters see Step 2. MIHS is dependent on energy allocated to growth (as the numerator) and maintenance requirements (as the denominator). Locally, individuals will establish their asymptotic size if all assimilated energy is constantly used for maintenance as no more energy is available for growth (i.e. growth ceases when these 2 terms are equal). Thus, MIHS will be a direct function of the amount of food available for consumption, through its relationship with the Holling scaled functional response f (Kearney 2012). Implicitly, this means that the energy available from food (inside the fundamental thermal niche of a species, as expressed by the tolerance of thermal limits; Saraiva et al. 2012) is the main determinant of fitness in ectotherms.

Maturation time (MT)

Although there may be several ways to explain the point at which an organism changes from juvenile (feeds but does not reproduce) to adult (feeds and reproduces), a species-specific threshold in structural size seems to be the best approach (Jager 2012). Indeed, one of the most important principles of the standard DEB model is that sexual maturity is an event that generally happens once a fixed amount of structure is obtained (Kooijman 2010). This implies that organisms tend to allocate energy to somatic growth and body reserves according to the κ-rule from fertilisation/settlement onwards, and they develop, maintain and replenish their energy reserves until a certain size threshold is reached. Once an organism reaches this threshold (which is typically genetically fixed; Jager 2012), it starts to invest in gamete development and reproduction. When the amount of energy reaches a sufficient level in maturity, gametes start to be packaged and spawned. Thus, sexual maturity occurs when adults stop investing in their maturation, and instead allocate this flow of energy to the production of offspring (Jager 2012). A primary requirement for modelling MT is thus the species-specific information of the smallest size at sexual maturity. This means that we need to allocate experimental effort either in field studies, laboratory experiments, or by searching the current literature, to establish the smallest individual with mature gonads (i.e. size at sexual maturity). Such information can usually be extracted from the literature, as it is an essential part of many classical biological and ecological studies. Therefore, if we know the smallest size possible for sexual maturity, the energy flux $1 - \kappa$ coming from existing reserves and/or the amount of food energy assimilated (minus costs of maintenance, digestion and growth/protein synthesis), and assuming that energy is used for reproductive purposes (i.e. gamete development and maturation), we can estimate the time needed to reach maturity. MT is therefore the time (in days) required to reach the minimal size that allows for gamete development and maturation. MT is strictly habitat-specific (i.e. thermal conditions and available food abundance) as it depends on the specific time required to reach the minimal size threshold for sexual maturity and first spawning.

Number of reproductive events per life span (RE)

RE is another basal life history trait of animals. Most evolutionary strategies seem to be dependent on this parameter (Stearns 1992). Reproduction is considered to be dependent on the amount of reserve energy which is ultimately shared between growth and reproduction, according to the κ-rule. DEB rules for predicting RE assume that each time the amount of energy has reached a certain density in the reproduction buffer, it will overflow as gametes.

Thus, the rules essentially concern the content of the reproduction buffer which will be filled during the adult life of the organism, depending on the food

conditions (the 1 – κ fraction from the mobilization flux minus the maturity maintenance; S. Saraiva pers. comm.). In the case of bivalves, when the reproduction buffer content reaches a certain threshold (as described by the gonado-somatic ratio, reproduction buffer/total mass of the organism) and the temperature is above a certain point, then the organism spawns (Gabbott & Bayne 1973). Larger amounts of food will, in principle, lead to more spawning events, since the reproduction buffer can then be filled more easily (as long as temperature is above the threshold). Conversely, less food will lead to fewer spawning events; not only because the filling of the reproduction buffer is slower but because bivalves can use their gonads for maintenance if there is not enough food (S. Saraiva pers. comm.). In general, the trigger for conversion of the buffer into eggs or sperm, and the age at which the organism spawns is species-specific.

Total reproductive output (TRO)

When the energy of a reproductive buffer reaches a threshold, it is packaged into gametes which are produced in a discrete number of spawning events. This implies that the continuous flow of energy allocated to reproduction needs to be collected in a buffer (see above). Since DEB assumes that the amount of energy required to build one gamete is usually constant (e.g. 0.0019 J for one egg in mussels; van der Veer et al. 2006) and that amount is species-specific, TRO will depend on the amount of energy available for reproduction coming from reserves and stored into a reproduction buffer (whose extent is species-specific). TRO is an important factor in studying Darwinian fitness (Bozinovic et al. 2011, Sarà et al. in press) of species, and plays an important role in population connectivity and ecosystem resilience (Hughes et al. 2005).

By dividing TRO and RE, we obtain the number of eggs for reproductive event (ERE). This is another important factor, as ERE may help us infer many other aspects of population dynamics, such as the pace of colonisation of an area rather than the temporal individual (population) contribution to a metapopulation.

Step 4: Laboratory experiments to estimate DEB parameters in bivalves

In this section we report all the experimental procedures that can be used to obtain DEB parameters, which are in turn required to run DEB models in bivalves. Whilst we stress the correspondence between every experiment to obtain ecophysiological and DEB parameters, we wish to highlight that most of these procedures are applicable to nearly all benthic aquatic ectotherms, with modifications as necessary for species type (e.g. feeding rate: in fish it is according to Petit et al. 2003; while in crabs it is according to Stevens 2012).

Length–weight coefficient (L–W) to obtain the shape coefficient, δ

The relationship between total shell length (L) and somatic wet weight (WW) of *Brachidontes pharaonis* was derived from the biometrics of either well-fed animals or animals that had been collected in the field. The total length (TL, cm) of mussels collected from pristine sites (i.e. in good health and not subjected to stressful conditions) was measured by means of a digital calliper (DIGI-Kanon; ± 0.001 cm), while the somatic wet weight (WW, g) was measured using an analytical balance (Mettler Toledo PL 602-5). For these measurements, animals should be post-spawning (spawning usually occurs between late spring and early summer; Sarà et al. 2000) and in the growth phase. The L–W relationship is species-specific, but there is individual variability. To reduce this potential interference, we measured more than 1000 individuals collected from the same location (Ettore pond) in late summer 2009, when we were sure that most animals lacked gonadal tissue (Sarà et al. 2008b). Generally speaking, however, animals should be collected from different sites following the common procedures of experimental design (Underwood 1997). This should make the L–W relationship as species-representative as possible.

Ingestion rate (J_X)

The DEB parameter J_X includes $\{\dot{J}_{Xm}\}$ and the functional response involving the half saturation coefficient, X_K. $\{\dot{J}_{Xm}\}$ and X_K might normally be derived from current measurements of ingestion rates. In bivalves, the common way to measure the ingestion rate is through measurements of clearance rates (CR; l h^{-1}; defined as the volume of water cleared of suspended particles h^{-1}; Table 1). This can be carried out by means of either an open flow-

through or closed system, where the removal of suspended algal cells added to filtered seawater is measured (Widdows & Staff 2006). Previous studies have shown that there are no significant differences between the methods (Widdows 1985). Although a flow-through system has been used routinely in field monitoring programmes (Widdows et al. 1995, 2002), most laboratory studies (e.g. Ezgeta-Balić et al. 2011, Romano et al. 2011) use a simple closed system to avoid the need for filtered sea water and having to dispose of large quantities of water containing toxic chemicals. For reasons of simplicity, it is recommended that CR be calculated from the exponential decline in cell concentration in a closed system (beaker or tank of water) over a period of 1.5 to 2 h (Widdows & Staff 2006). For subtidal bivalves where there may be greater temporal variability in CR, the number of replicates should be increased (e.g. from 16 to 24 individuals) and measurements should be taken twice in order to overcome the variability of individual clearance response (sensu Underwood 1997, Sarà et al. 2008b). To obtain the J_{Xm}, we ran experiments of clearance rates with an initial food concentration of 25000 cells ml^{-1} of *Isochrysis galbana* (numerous other algae species are also appropriate for this purpose, as long as the cell size is within the normal feeding range of target bivalves; Dame 1996). The ingestion rate is derived by multiplying CR by the amount of suspended particulate organic matter in the water of the mussel's habitat (e.g. ~4.9 µg l^{-1} POM) (Widdows & Staff 2006).

Obtaining ingestion rates to derive the half saturation coefficient makes for longer experiments. Indeed, the X_K is a function of food concentration and to draw the experimental functional response hyperbolic curve (Fig. 2), we need to obtain ingestion rates through clearance experiments at different fixed concentrations of food density and at the constant temperature representing the theoretical optimum for the target species (e.g. 20°C). In the present paper, to study the functional response of *Brachidontes pharaonis*, we used 5 chl *a* concentrations (µg l^{-1}). The number of different concentrations and the extent of the concentration range depend on the trophic status of the marine habitats where the target species lives. In the Mediterranean, which is characterised by strong oligotrophy (Sarà et al. 2011b), an appropriate range is from 0.5 to 4.0 µg l^{-1}; this also applies to more eutrophic sites in the Adriatic Sea, whose chl *a* concentrations often spike at over 3–4 µg l^{-1}. With other seas, like some North American Pacific sites, the range should be enlarged to include higher values and spikes (e.g. Strawberry Hill, Oregon, 50–100 µg l^{-1}; Petes et al. 2008).

Our experiments, both J_{Xm} and X_K estimations, were carried out in 2 sessions, with 16 mussels per session (n = 16 × 2) at each food concentration according to Widdows & Staff (2006) and Sarà et al. (2008a). Sixteen acclimated animals (3 wk) were individually placed in beakers containing 1 l of filtered thermo-regulated seawater (20°C), positioned on a stirrer base plate to keep the water thoroughly mixed and oxygenated (Romano et al. 2011). After 20 min, when bivalves started to gape, algal cells (*Isochrysis galbana*) were added to each beaker using a syringe. For J_{Xm}, we started with an initial concentration of 25000 cells ml^{-1}; for X_K, we used increasing cell concentrations resembling different food concentrations, from 0.5 to 4.0 µg chl *a* l^{-1}. At 30 min intervals over a period of 2 h, 20 ml aliquots were sampled from each beaker, in order to assess the exponential decrease of cell numbers as a result of filtering by mussels. The decline in cell concentration was monitored using a Coulter Counter (Beckman Coulter© Model Z2). Two beakers without bivalves were used as controls and they showed no significant decline throughout the experimental period. The clearance rate (l h^{-1}) was then calculated using the following equation (Coughlan 1969): $CR = Vol(\ln C_1 - \ln C_2)/\Delta t$, where Vol = volume of water (e.g. 1 l) and C_1 and C_2 are the cell concentrations at the beginning and end of each time increment (i.e. every 0.5 h) (Ezgeta-Balić et al. 2011).

Assimilation rate J_{EA}

To estimate the assimilation rate J_{EA} ($= f \times \{J_{Am}\} \times V^{2/3}$), we need to calculate the individual assimilation efficiency, AE. This is a qualitative measure and is commonly obtained by comparing the proportion of organic matter in the algal cells and mussel faeces according to the Conover (1966) equation: $AE = (F - E)\,[(1 - E) \times F]^{-1}$, where F is the relationship between dry weight and ash free dry weight of algal food, and E is a relationship between dry weight and ash-free dry weight (AFDW) of faecal pellets. In our experiments, algal food and faecal pellets were collected and filtered through GF/C filters (washed, combusted and pre-weighed). The filters were dried at 90°C and weighed, then combusted in a furnace at 450°C for 4 h, and then re-weighed.

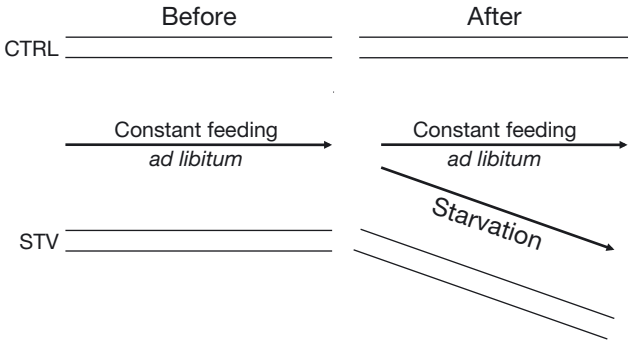

Fig. 5. A possible experimental design to measure *pM*. CTRL: control (animals fed ad libitum for the whole experimental period); STV: starvation treatment (animals are initially fed ad libitum and later starved)

Volume-specific maintenance costs [*pM*]

The estimation of volume-specific maintenance costs is based on the measurements of the oxygen consumption and dry weights of well-fed and starved individuals (Ren & Schiel 2008). For this experiment, we used 624 animals; however, the number of animals required to run *pM* experiments would depend on species characteristics (i.e. local abundance, size, handling resistance, level of protection). Our experimental design is shown in Fig. 5; animals were divided into tanks and fed ad libitum for at least 6–8 wk (hereafter called 'feeding period'). Ad libitum feeding was constantly assured with the use of a simple drip system, like those adopted in human healthcare. The algae we used was *Isochrysis galbana*; the culture was kept at an intense green (density of over 1 billion cells ml^{-1}) and the density was measured twice a week using a Coulter Counter (Beckman Coulter © Model Z2).

Every week, a sample of 16 animals was randomly collected from the tanks and oxygen consumption was measured. The rate of decrease in oxygen concentration was measured in a respirometric chamber (0.7 l; Pierron) completely filled with filtered water and stirred by a magnetic bar. The decline was recorded every 10 min for 1 h by an oxygen probe (Hanna Instruments, HI 76407/4) connected to an oxygen meter (Hanna Instruments, model HI 9145). Respiration rate (RR, µmol O_2 h^{-1}) was calculated according to Widdows & Staff (2006): $RR = (C_{t_0} - C_{t_1}) \times Vol_r \times 60(t_1 - t_0)^{-1}$, where C_{t_0} is oxygen concentration at the beginning of the measurement, C_{t_1} is the oxygen concentration at the end of the measurement, and Vol_r is the volume of water in the respirometric chamber. Following the respirometry, each animal was dissected and shell valves separated from somatic tissue. Shell valves and flesh were dried for 24 h at 90°C in pre-weighed aluminium trays to obtain the dry weight (DW, mg) and then combusted at 450°C to estimate, AFDW (mg). After the feeding period, animals were divided into 2 groups: 312 were continually well-fed, while the remaining 312 were starved in 0.45 µm filtered water. Oxygen consumption and dry weight were measured weekly in animals from both treatments (well-fed vs. starved) and the experiment finished when starved animals started to utilize their structural tissues (i.e. entered the shrinking phase; Fig. 5 and Fig. 1 in Ren & Schiel 2008).

Maximum storage density [E_m] and volume-specific costs for growth [E_G]

A common procedure is to estimate the difference in energy content of the structural mass of an organism in well-fed condition and after starvation, but before death. The formula is $[E_m] = (SMI_{fed} - SMI_{starved}) \times 23\ (\delta^3)^{-1}$ where SMI is the somatic mass index of both starved and well-fed animals, expressed as somatic ash free dry mass (AFDM, mg) divided by the cube of total length (Cardoso 2007). The volume-specific costs for growth [E_G] are linked to the complexity of the cell (Kooijman 2010), which implies that it might be similar for phylogenically related organisms (e.g. mussels and oysters). To calculate [E_G], it is necessary to measure the SMI of starved animals and apply the following equation: $[E_G] = SMI_{starved} \times 23\ (\delta^3)^{-1}$.

Arrhenius temperature (T_A)

Any physiological rate can be a good proxy to estimate the Arrhenius temperature. This is based on the theory asserting that in every species, the link between metabolic rate and body temperature follows a specific trend within the thermal window of a species' metabolic functioning (Fig. 4). The idea is that a certain rate is controlled by an enzyme(s) that has an inactive configuration at low and high temperatures, below and above the optimum temperature, respectively (Kooijman 2010).

On this basis, we measured the oxygen consumption rates in *Brachidontes pharaonis* at different temperatures. The method was the same as reported for the *pM* estimation (see above). Operationally, we ran respirometry experiments with at least 8 animals which were acclimated for at least 2–3 wk at each temperature, from 5°C to 40°C, at each 5°C increment. The appropriate 'thermal window' depends on the species. Once the metabolic rate (i.e. respiration, ex-

cretion) has been obtained, it can be log-transformed and plotted against the inverse of temperature (in Kelvin); the slope of the resulting straight line is the Arrhenius temperature (Fig. 4; Freitas et al. 2010). To run the DEB model, we needed a further 4 parameters: T_L and T_H which were, respectively, the lower and upper boundaries of the tolerance range where 69% of the enzymes are active; and T_{AL} and T_{AH}, which are the Arrhenius temperatures for the rate of decrease at both boundaries (Kooijman 2010).

In the current literature on benthic invertebrate ecology, the heart beat rate (HBR, beats s^{-1}) in invertebrates is becoming a valid and complementary (and sometimes alternative) method to oxygen consumption. The simplicity and economic convenience of this method is due to the introduction of the non-disruptive cardio-plethysmographic technique (Depledge & Andersen 1990) for measuring HBR. It provides the opportunity to assess the metabolic response of organisms under varying physical and chemical conditions. It has been successfully employed to investigate the ecological aspects of many intertidal benthic organisms, such as gastropods (De Santini et al. 1999, Dong & Williams 2011), crabs (De Fur & Mangum 1979) and particularly mussels in different intertidal habitats worldwide, and under different levels of exposure to pollutants (Curtis et al. 2000, Halldórsson et al. 2008) and varying salinities (Sarà & De Pirro 2011). It consists of gluing inexpensive infra-red sensors externally onto the valves (e.g. Williams et al. 2011), very close to the heart position of the target organism. Heart signals are usually amplified and filtered using a special amplifier card (Newshift Lda; Sarà & De Pirro 2011) and then detected by means of a portable oscilloscope such as Fluke™ 125 or USB PicoScope devices (series 3000 and over; PicoScope Inc.) connected to a laptop computer equipped with heartbeat reading software. HBR is recorded at intervals from 1 to 5 min as a function of target species throughout a 1 h experimental session (Sarà & De Pirro 2011). HBR changes as a function of temperature with high confidence. In addition, HBR allows us to consistently increase the sample size, in order to reduce possible interference due to individual variability, which could enhance the likelihood of generalisation at the species level of these important DEB parameters.

Step 5: Running models in an environmentally explicit context

In DEB models, the main driver of bivalve life history is represented by body temperature (here expressed as mean seawater temperature; Lima et al. 2011) and food density (Pouvreau et al. 2006, Kearney et al. 2010, Kooijman 2010, Sarà et al. 2011a). To obtain local temperature, we put 4 temperature loggers (iBCod Type 22L; Alpha Mach) into the Ettore pond. We obtained the hourly sea water subtidal temperatures over 4 yr (2006–2009), which were then introduced into the model (Kearney et al. 2010) through the Arrhenius relationships. This is one of the most important mechanistic steps of DEB modelling. Temperature enters the model through its contribution to ingestion $\{J_{Xm}\}$ (mg h^{-1} cm^{-2}) and maintenance $[pM]$ rates. For example, Arrhenius temperatures will be entered into the model as shown in the following equation (made with the surface-area specific ingestion rate):

$$\{J_{Xm}\} = \{J_{Xm}\} \times \exp\{T_A \times [1/(273 + 20) - 1/(273 + BT)]\} / \left(1 + \exp\{T_{AL} \times [1/(273 + BT) - 1/T_L]\} + \exp\{T_{AH} \times [1/T_H - 1/(273 + BT)]\}\right)$$

where T_A = Arrhenius temperature (determined from the slope of plots of ln(k) against $1/T$; T_{AL} = lower boundary of the Arrhenius temperature; T_L = lower tolerance temperature; T_{AH} = upper boundary of the Arrhenius temperature; T_H = upper tolerance temperature; BT = body temperature. To provide accurate predictions, the temporal resolution of body temperature should be as tight as possible (sensu Kearney et al. 2012, Sarà et al. in press): preferably hourly, but at least at 6 h intervals. For submerged organisms living in subtidal habitats, BT closely approximates the temperature of the surrounding water, but for intertidal animals exposed to air at low tide, BT is driven by multiple interacting factors including air temperature, solar radiation, and wind speed (Helmuth 1998, 1999, Sarà et al. 2011a, in press). To obtain information on the amount of food available for bivalves, water samples were taken bi-monthly (6 samples per year; in 2009 only) to estimate the amount of phyto-pigments (chl *a*) according to Sarà (2009). Although food was estimated on a bi-monthly basis, the data was incorporated into the model on an hourly basis through the functional response: f = food (food + X_K)$^{-1}$, where food was chl *a* (µg l^{-1}) and X_K was the half-saturation coefficient as estimated according to methods given in Step 4. With these local data, we obtained all life history traits and the extent of fitness as reported above (i.e. MIHS, TRO, ERE).

Step 6: Validation exercise

We validated the DEB output using a comparison of the animal's ultimate body size (estimated with the

age analysis obtained from animals collected in the field) and the number of eggs / sperm experimentally produced in the laboratory by acclimated animals. For this step, animals were collected in both March and April of 2009 and 2010 from the Ettore pond, where this species has established highly dense populations (Sarà et al. 2000). Collection was carried out in 3 plots of 20 × 20 cm quadrats. All animals were brought back to the laboratory and analysed to determine age. Age was estimated using the analysis of shell rings proposed in Peharda et al. (2012) by cutting shells with a Dremel rotary (Series 4000; Robert Bosch Tool Corporation) and counting the number of rings through use of a stereomicroscope (Leica Z4). An example of the image obtained is shown in Fig. 6.

In addition, we induced spawning in *Brachidontes pharaonis* individuals collected from the pond. Before starting with gonadal induction, every animal was measured and weighed. By selecting a thermal shock of 10°C (departing from the rearing condition of 20°C) and hydrogen peroxide concentrations (Helm et al. 2004), we obtained the mass spawned by 10 specimens in a known volume of seawater (Fig. 7). Once the sex of each animal had been visually identified with the use of a stereomicroscope (Leica Z4), gametes were counted and their volume measured with a Beckman Coulter Counter Z2.

RESULTS

The DEB model was run using environmental data (food and temperature; 2006–2009) from the Ettore pond, together with parameters of *Brachidontes pharaonis* estimated using the classical experimental approach (Table 2) integrated and optimised by a Matlab routine. The model predicted a maximum size of ~3.64 cm (Fig. 8), attainable 4 yr after recruitment with 2006–2009 temperature data.

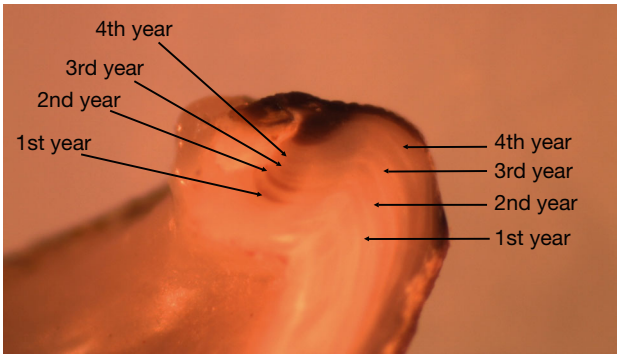

Fig. 6. Section of a *Brachidontes pharaonis* shell showing banding

Fig. 7. Induced spawning of a female *Brachidontes pharaonis* in the lab

This size corresponded to a wet weight of 2.21 g, a total amount of 5.9 million eggs in 4 yr, and 16 separate reproductive events. The first reproductive event was about 2 mo (66 d) after recruitment. The growth curve simulated with the DEB model is reported in Fig. 8. Our field validation exercise with the age-size curves based on the analysis of valve rings returned a maximal size of 3.73 cm at 4 yr in 2009, and 3.69 cm in 2010 (Fig. 9). DEB model estimates deviated from real life measurements by ~2.4% in 2009 (a yearly difference of less than ~0.60%) and by ~1.4% in 2010 (a yearly difference of less than ~0.34%). The induction of spawning in 10 animals (Table 3) showed that females produced on average 441 069 ± 243 009 eggs per bout, ranging between 20 and 30 µm in diameter, while males produced 437 378 ± 173 456 sperm per bout with a size ranging between 7.8 and 20 µm (probably reflecting clumping of sperm). Although the DEB model's predicted number of eggs per single reproductive event was 371 368, this value fell well within the range of the measured average for females, even though the prediction was about 15% less (~3.8% year^{-1}).

DISCUSSION

The outcome of our study is highly encouraging for the further development of a standard set of techniques that can be used to estimate mechanistic parameters required for modelling life processes in animals. Life history traits and fitness of a standard *Brachidontes pharaonis* individual that were pre-

Table 2. *Brachidontes pharaonis.* DEB parameters estimated from experiments

Parameter	Unit	Definition	Value
$\{\dot{J}_{Xm}\}$	J h^{-1} cm^{-2}	Maximum surface area-specific ingestion rate	17.88 ± 14.30
$[\dot{p}M]$	J h^{-1} cm^{-3}	Volume-specific maintenance costs	9.29 ± 4.16
$[E_m]$	J cm^{-3}	Maximum storage density	1967 ± 190
$[E_G]$	J cm^{-3}	Volume-specific costs of growth	1118 ± 73
κ		Fraction of utilised energy spent on maintenance plus growth	0.80
δm		Shape coefficient (length–weight relationship)	0.288 ± 0.039
V_b	cm^3	Volume at birth	0.00000049
V_p	cm^3	Volume at sexual maturity	0.01008
AE		Assimilation efficiency (Conover ratio)	0.75 ± 0.12
X_K	µg chl *a* l^{-1}	Saturation coefficient	0.62
k_R		Reproduction efficiency	0.95
T_A	K	Arrhenius temperature	8232 ± 2923
T_{ref}	K	Reference temperature	293
T_L	K	Lower tolerance temperature	284
T_H	K	Upper tolerance temperature	305
T_{AH}	K	Upper boundary of the Arrhenius temperature	6005 ± 1049
T_{AL}	K	Lower boundary of the Arrhenius temperature	17957 ± 1795

dicted by the DEB model and improved with classical experimental eco-physiological techniques were very close to reality, as shown by our validation. We detected only a small annual departure from reality: less than 1% for size, and less than 4% for the amount of eggs produced per life span (i.e. fitness). We regard the extent of such a departure negligible at the present stage of DEB research, however, it deserves further attention in order to clarify possible causes. Although there is ample scope for improvement of techniques to reduce error margins, identifying the potential sources of bias contributing to the differences between field observations and the model output is an important objective. There are 2 levels of potential imprecision leading to the slight departure of the model from reality: (1) the resolution of habitat information used in our modelling exercise, and (2) the experimental approach used to estimate the animals' major physiological traits.

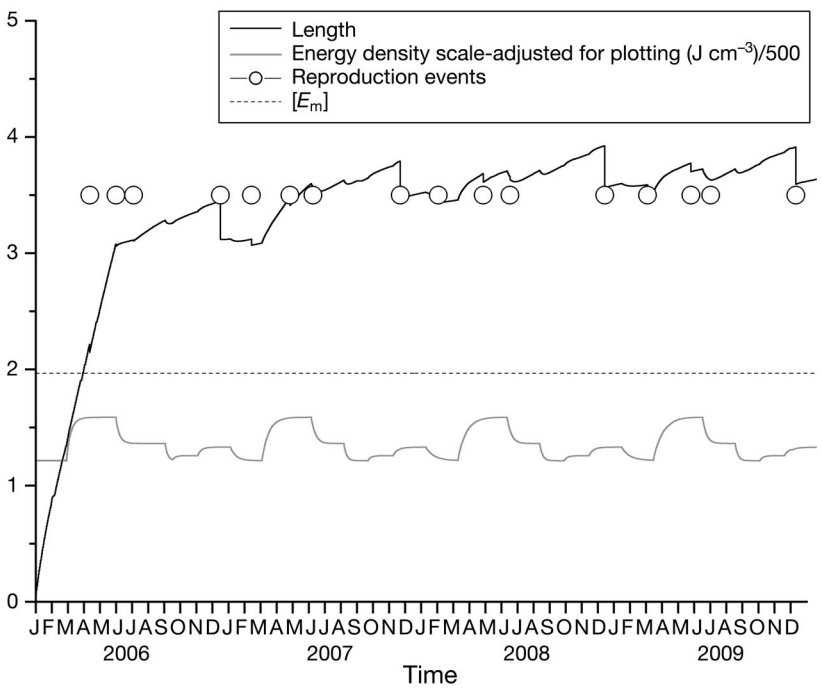

Fig. 8. *Brachidontes pharaonis*. Predicted growth in length (cm) derived from dynamic energy budget model. Predictions are based on hourly sea water temperatures and bi-monthly concentrations of food (chl *a*). $[E_m]$ = maximum storage density; energy density scale (J cm^{-3}) = body condition-reserve density; circles = spawning events

Potential source of bias at the habitat level

The habitat level was described in our model by temperature and food, as in other similar studies (Pouvreau et al. 2006, Cardoso 2007, Kearney et al. 2010, Sarà et al. 2011a, 2012a, in press). The resolution of temperature was hourly while resolution of food was bi-monthly. It is unlikely that the temperature resolution could have been responsible for the model's departure — the hourly data seems sufficient to ensure a very good response (Kearney et al. 2012). However, the temporal resolution of food

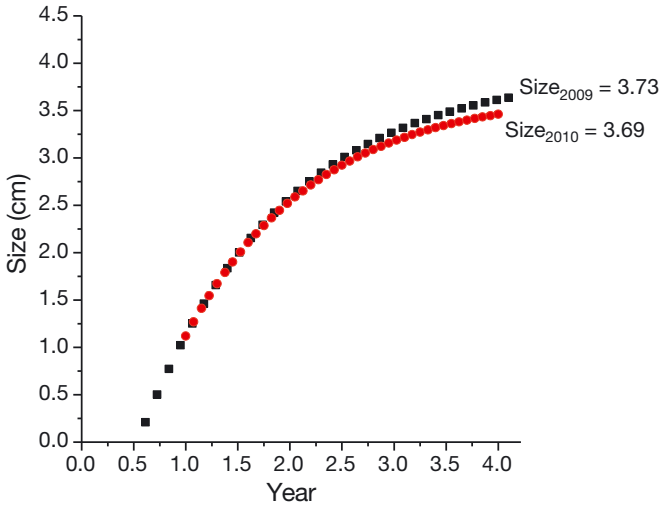

Fig. 9. Estimated *Brachidontes pharaonis* growth curves from field data (2009 and 2010) through the relationship between age (estimated by the shell ring analysis of animals collected in the field) and measured shell length. The 6-mo age interval is due to limitations of shell ring analysis (i.e. not more accurate than 6 mo)

density could be a major factor in any departure, especially in less oligotrophic and more variable temperate coastal environments. In particular, better estimates of food density could have important implications for the predicted number of eggs produced, which was slightly underestimated by the model. It is likely that bi-monthly data were not sufficiently tight to capture the daily and weekly fluctuations of food available to our suspension feeders, and this may have generated the slight underestimation. On the other hand, as already stated, the extent of LH traits (e.g. size, and consequently fitness; Stearns 1992) is dependent on the ratio between energy allocated to growth and maintenance requirements: the greater the assimilated energy, the more the growth. As a consequence, infrequent sampling of temporal data on available food energy (e.g. at annual, seasonal or monthly timescales) may lead to a reduction of variance around means, thereby reducing the likelihood of appreciating the whole repertoire of variability of real-world conditions.

Table 3. *Brachidontes pharaonis.* Gonadal output as measured in 10 animals by the Beckman Coulter Counter. ID: identification number; TL: total length; No. gametes: number of gametes ml^{-1} as counted by the Coulter Counter; SE: standard error; Fitness: total number of gametes released per mass spawning event

ID	TL (mm)	No. gametes Mean	SE	Cell volume Mean	SE	Fitness
Female						
22	19.87	370	13.34	10184	198	185056
10	23.42	124	2.67	8939	216	61778
5	23.95	573	7.56	10980	108	286278
23	24.07	1212	35.58	11102	127	606000
1	24.21	1374	36.28	12160	95	686944
Male						
14	18.16	541	18.29	906	27	270500
12	23.06	711	72.41	1239	97	355722
3	24.58	1192	107.96	942	57	596056
2	25.73	1301	80.05	919	35	650556
6	25.80	628	52.94	1139	47	314056

This aspect should be considered in future research; and larger amounts of data (at least monthly, if not weekly) to determine the amount of food available to animals would be desirable. However, another aspect may have contributed to the departure from reality. Here, we expressed the amount of food for bivalves only with suspended chl *a*. These organisms usually rely on a mixture (Sarà 2006) comprised of both fresh organic matter (such as that derived from primary production, represented by chl *a* from both phytoplankton (Sarà et al. 2003) and resuspended microphytobenthic alga (Sarà 2006, 2007), and detrital matter of both autotrophic and heterotrophic origins (Sarà 2006, 2007). Thus, although we used chl *a* to estimate *Brachidontes pharaonis* feeding parameters (e.g. ingestion, assimilation, X_K), using only this type of food could have contributed to the slight underestimation of available food and life history traits provided by the model. This underestimation would be far greater in more eutrophic and hydrodynamic estuarine environments compared to the oligotrophic waters of the Mediterranean.

This hypothesis still needs to be tested (with *Brachidontes pharaonis* as well as other species), and further research is required to clarify the effect of food quality on DEB outcomes. However, if the choice of chl *a* as food quantifier leads to a departure of DEB model outcome, large scale predictions such as those needed in the climate change context could take advantage of this fact. Thus, the reduced quality of predictions due to the choice of chl *a* is counterbalanced by chl *a* being the only trophic variable measured on large scales (e.g. Sarà et al. 2011b,c, 2012 in press) by satellite imagery and by buoy networks worldwide (e.g. the Italian ISPRA or the Californian Ocean Observing Node [BOON] maintained at UC Davis). It has also been the most common trophic variable measured in pelagic habitats for many decades, involving hundreds of oceanographic cruises worldwide. This has led to the presence of a very large chl *a* database from many sites worldwide, which is useful if we want to investigate spatial-

temporal variability of ecological responses mechanistically simulated by DEB (Kearney et al. 2010, Sarà et al. 2011a).

High quality experimental data and estimates of DEB parameters

The most important part of compiling a good dataset for DEB modelling and quantifying LH traits and fitness is to obtain precise estimates of the characteristics of experimental animals (e.g. feeding, assimilation, costs). In the last decade, when mechanistic models began to be consolidated across the literature, most organismal characteristics were compiled from the available literature. There are many examples of this in bivalves (Kearney et al. 2010, Sarà et al. 2012a), fish (van der Meer et al. 2011, Jusup et al. 2011) and terrestrial reptiles (Kearney 2012). In this context, it is clear that a lack of precision in estimating functional traits can create significant errors, which in turn can reduce the accuracy of DEB predictions of LH traits and fitness. Using the most accurate experimental procedures possible should ensure a good estimate of the organismal functional trait in order to derive most DEB parameters. Moreover, the most important issue in obtaining accurate estimates of key functional traits is likely to be obtaining an adequate measure of individual variability through the use of appropriate sample sizes in experimental studies (sensu Underwood 1997). Each procedure requires a specific protocol (which is adaptable to any species), that depends on e.g. the ease of collecting animals from the wild, their ability to be maintained and to grow under mesocosm conditions, their degree of protection and their rarity. With invertebrates such as bivalves, which are readily maintained in laboratories, it is easy to adopt moderately large sample sizes to control the individual variability of physiological responses from which DEB parameters are derived. For example, an important component of the energy budget is doubtless the surface-specific assimilation rate {*pAm*} that in a DEB context includes the assimilation efficiency (AE) as measured here by the Conover ratio (Conover 1966). Even very small departures of AE estimates, due to negligible imprecisions of experimental procedures, can have important repercussions on fitness estimates. Fig. 10 illustrates the outcome of a sensitivity analysis simulating the effect of small changes of AE (from 0.70 to 0.80, in 0.1 increments) on *Brachidontes pharaonis* size (and then fitness) in our study area. Prediction of size was subjected to a variable error (from −7% [L_{max} = 3.40 cm] to +7% [L_{max} = 3.90 cm]) departing consistently from the mean size value (L_{max} = 3.64 cm) obtained using an AE mean (0.75 ± 0.12, n = 84 animals). This is a simplistic example to demonstrate that if we incorporate small changes in estimates of one functional trait (viz. AE), there is a risk of introducing an important systematic error in our modelling, which may have significant consequences on the extent of all LH traits. Such a risk becomes even more likely if poor experimental estimates of more than one trait are incorporated into the model. While such considerations may sound discouraging in terms of the use of mechanistic models, we do strongly support such an approach and its reliability in predicting distributions of key species and testing ecological hypotheses. In order to increase the accuracy of data to be incorporated into models (both functional traits and spatial-temporal resolution of environmental data), it is necessary to ensure that strict sampling protocols are adopted, and that these protocols meet all the assumptions of experimental designs (e.g. correct sample size, experimental power, independence; Underwood 1997).

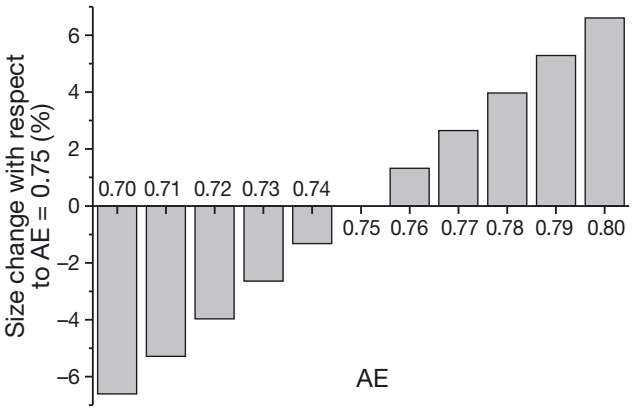

Fig. 10. Sensitivity analysis carried out to test the effect of small changes of assimilation efficiency (AE) on *Brachidontes pharaonis* size. DEB parameters were those reported in Table 2. The simulations were carried out with AE from 0.70 to 0.80, in 0.1 increments, to show the percent change in shell length compared to that obtained with the standard AE (0.75)

This is a challenge for the future and, if met, the mechanistic approach will probably be the only pursuable path towards providing accurate forecasts in a changing world under increasing anthropogenic pressure.

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