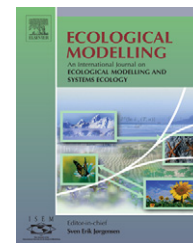




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Size-dependent variations on the nutritional pathway of *Bathymodiolus azoricus* demonstrated by a C-flux model

Irene Martins^{a,*}, Ana Colaço^b, Paul R. Dando^c, Inês Martins^b, Daniel Desbruyères^d, Pierre-Marie Sarradin^d, João Carlos Marques^a, Ricardo Serrão-Santos^b

^a IMAR—Institute of Marine Research, Coimbra Interdisciplinary Centre, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal

^b IMAR—Institute of Marine Research, Department of Oceanography and Fisheries, University of the Azores, Cais de Santa Cruz, 9901-862 Horta, Portugal

^c Marine Biological Association of the UK, Citadel Hill, Plymouth PL12PB, UK

^d IFREMER, Centre de Brest, Dep. DEEP/LEP, BP70 29280 Plouzané, France

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ABSTRACT

Bathymodiolus azoricus is a mussel from vent fields in the south-west of the Azores Triple Junction (Mid-Atlantic Ridge-MAR). Experimental evidence indicates that *B. azoricus* is a mixotrophic organism, which obtains energy from a dual endosymbiosis and filter-feeding. Yet the relative contribution of symbiosis and filter-feeding to *B. azoricus* nutrition is still unclear. To address this question, we developed an individual-based model which describes sulphide and methane uptake by endosymbionts, the energy gained through microbial oxidations, the transfer of energy from endosymbionts to *B. azoricus*, filter-feeding of particulate organic matter (POC) by *B. azoricus* and the energetic wastes of the mytilid with respiration. The model accounts for size-dependent relationships obtained from empirical data. External concentrations of H₂S and CH₄ correspond to estimated values for the Menez Gwen vent field, maximal and minimal values measured at MAR. From *in situ* observed densities of *B. azoricus*, productivity predictions at the individual level were upscale to the mytilid population at Menez Gwen and compared to estimated values. Predicted biomass of *B. azoricus* and its endosymbionts show a very high fitting level with estimated values. Results suggest that the relative contribution of filter-feeding and endosymbiosis varies with *B. azoricus* size, with small mytilids being strongly dependent on filter-feeding, whilst larger mussels obtain a significant portion of its energy from endosymbiosis. This is related with the variation of gill weight with total weight. Results also suggest that, an individual of a certain size can potentially regulate the relative contribution of filter-feeding and endosymbiosis according to external conditions. However, large *B. azoricus* exhibit a higher level of nutritional flexibility than small mytilids. The relative contribution of endosymbiosis and filter-feeding to the total energy budget of *B. azoricus*, as well as the mytilid particulate organic matter requirements, are assessed and discussed under several scenarios.

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* Corresponding author. Tel.: +351 239 836386; fax: +351 239 823603.

E-mail address: imartins@ci.uc.pt (I. Martins).

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1. Introduction

Bathymodiolus azoricus is a bivalve that dominates the communities at the shallower Atlantic vent fields, south-west of the Azores Triple Junction (Mid-Atlantic Ridge): Menez Gwen (850 m) and Lucky Strike (1700 m) (Colaço et al., 1998; Desbruyères et al., 2001) (Fig. 1). Several studies revealed that *B. azoricus* host both thio- and methanotrophic symbionts in their gills (e.g. Distel et al., 1995; Fiala-Médioni et al., 1986) indicating that the energy obtained through microbial oxidations of the reduced compounds (sulphide and methane) released by the vents plays a significant role in the nutrition of *B. azoricus* (Cavanaugh et al., 1992; Pond et al., 1998; Fiala-Médioni et al., 2002). Additionally, *B. azoricus* like other *Bathymodiolus* species also shows characteristics of a functional digestive system, such as the ciliation of the filaments which does not differ from that of littoral species, the presence of a functional feeding groove and well developed labial palps, which indicates that *B. azoricus* also filters and digests organic matter particles (Le Pennec et al., 1990; Fiala-Médioni et al., 1986). Uptake of dissolved organic matter (DOM) by *B. azoricus* may also occur as it has been proven in many marine invertebrates, including bivalves (Siebers and Winkler, 1984; Manahan, 1983; Wendt and Johnson, 2006). However, the factors that determine and control endosymbiosis and filter-feeding processes in *B. azoricus*, as well as the relative contribution of endosymbiosis and filter-feeding to the total energy budget of the Atlantic vent mussel are still unclear. Food web characterization is required as an initial step in understanding an ecosystem (Link, 2002), but in vent mussel communities the complex balance of heterotrophy and autotrophy still remains to be explored (Pile and Young, 1999; Goffredi and Barry, 2002). One possible way to address this question is through ecological models that describe the

uptake of energy from different sources and discriminate for the contribution of each source in the final energetic balance of the organism in question. Moreover, models dealing with species- or population-bioenergetics are useful in clarifying the dynamics of species or populations in relation to environmental variables (Ren and Ross, 2005; Megrey et al., 2007).

2. Objectives

The aim of this study was to understand the energetic balance of *B. azoricus* and its endosymbionts, with the general goal of bringing more insight into food web functioning at hydrothermal vents. Specifically, we wanted to assess the relative contribution of endosymbiosis and filter-feeding to the total energetic budget of *B. azoricus* under different external conditions of sulphide, methane and particulate organic matter (POC). These questions were addressed through a carbon (C)-flux model, which was upscale to the population level by incorporating quantified densities of *B. azoricus* at the Menez Gwen vent field.

3. Material and methods

3.1. Model conceptualisation

A carbon-flux model was developed to describe the energy flow through *B. azoricus* and its endosymbionts. Taking into consideration *in situ* observed densities (Colaço et al., 1998), the model was subsequently used to assess the productivity of *B. azoricus* at the Menez Gwen vent field. Simultaneously, assuming that endosymbionts correspond to 4% of the gill wet weight of *Bathymodiolus* (Powell and Somero, 1986), the

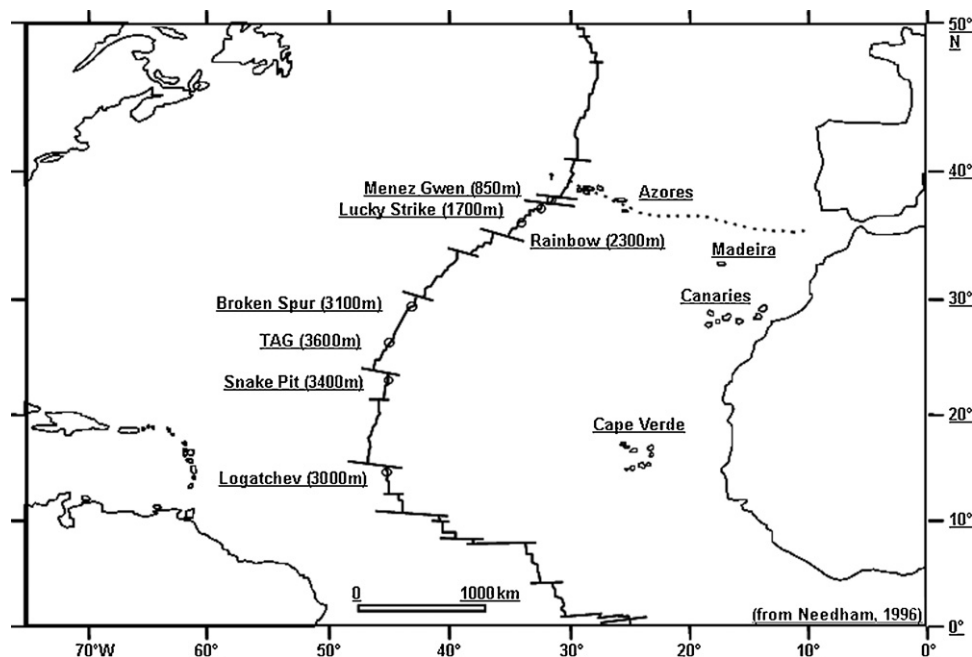


Fig. 1 – Location and depth of hydrothermal vent fields south-west the Azores Triple Junction at the Mid-Atlantic Ridge (MAR).

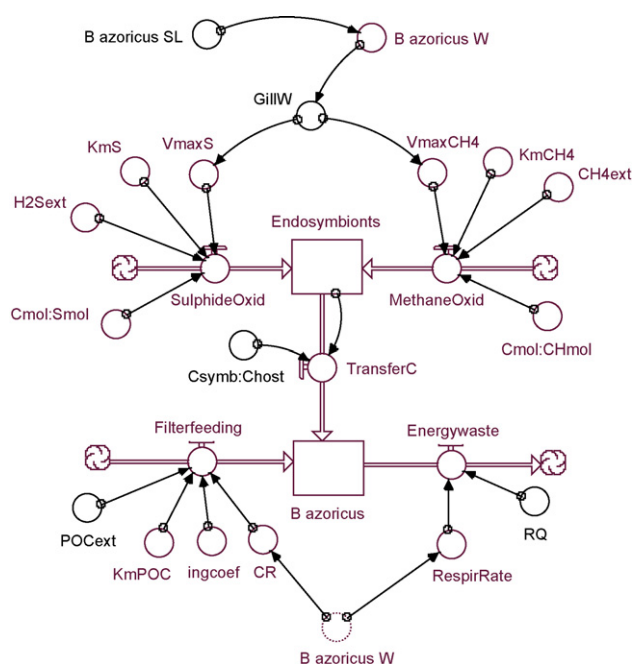


Fig. 2 – Simplified conceptual diagram of the *Bathymodiolus azoricus*-endosymbiont C-flux model. SL—shell length, W—weight, H_2S_{ext} , CH_4 and POC_{ext} —environmental concentrations of sulphide, methane and particulate organic matter, respectively. SulphideOxid and MethaneOxid—sulphide and methane oxidation by endosymbionts, respectively. TransferC—transfer of carbon from endosymbionts to *B. azoricus*. See Table 2 and text for parameter definition.

biomass of endosymbionts was also estimated. The model accounts for the uptake of sulphide (H_2S) and methane (CH_4), the oxidation of H_2S and CH_4 by thio- and methanotrophs, respectively, the filtering of particulate organic matter by *B. azoricus*, the transfer of energy from the symbionts to the host and the energetic wastes of *B. azoricus* (Fig. 2). The flow units of the model are $mgC(carbon)d^{-1}$. The considered average biomass of *B. azoricus* was $550\text{ ind }m^{-2}$ according to local observations by Colaço et al. (1998). The model assumes no limiting conditions of O_2 or DIC.

3.2. Mathematical equations and parameters

The biomass variation of endosymbionts (E) and *B. azoricus* (B) is expressed by Eqs. (1) and (2), respectively:

$$\frac{dE}{dt} = S + M - T \quad (1)$$

$$\frac{dB}{dt} = T + F - R \quad (2)$$

S is the energy gained from sulphide oxidation, M is the energy gained from methane oxidation, T is the transfer of energy from endosymbionts to *B. azoricus*, F is the energy obtained by filter-feeding, R is the energy wastes.

3.2.1. Sulphide- and methane-uptake by endosymbionts

The uptake of substrates by living organisms has physiological constraints often caused by saturation when maximum thresholds are reached. In the case of bacteria, experimental evidence suggests that the uptake of substrates frequently follows a Michaelis–Menten equation, with either single, double or biphasic kinetics (e.g. Ingvorsen et al., 1984; Lovley, 1985; Unanue et al., 1999). In accordance with this, the uptake of sulphide (S) and methane (M) by endosymbionts ($V_{S,M}$) was described by a single Michaelis–Menten kinetics:

$$V_{S,M} = V_{max_{S,M}} \cdot \frac{[S, M]}{K_{m_{S,M}} + [S, M]} \quad (3)$$

$V_{max_{S,M}}$ is the maximum uptake rate of sulphide (S) or methane (M) ($\mu\text{mol }g^{-1} \text{ gill dry wt }d^{-1}$ using a gill dry wt: gill wet wt = 0.162 based on *B. azoricus* from the Menez Gwen ($N = 39$)), $K_{m_{S,M}}$ is the half-saturation constant for the uptake of sulphide or methane ($\mu\text{mol }l^{-1}$), $[S, M]$ is the external concentration of sulphide or methane ($\mu\text{mol }l^{-1}$).

In the available literature, we found no values for maximum uptake rates of sulphide or methane by *B. azoricus*, therefore, we had to use values reported for similar species. Like vent mussels, methane mussels also uptake reduced substrates, mostly through their gills (e.g. Le Pennec et al., 1990) at rates ranging from 96 to $240 \mu\text{mol }g^{-1} \text{ wet wt }d^{-1}$ (Kochevar et al., 1992). Methane mussels only have methanotrophs in their gills, while *B. azoricus* has both thiotrophs and methanotrophs. Possibly due to this, the uptake of methane by methane mussels is higher than the uptake of methane by *B. azoricus*, as the last can also uptake sulphide. For this reason, we assumed that the average rate of methane uptake by methane mussels corresponds to the maximum methane uptake rate by *B. azoricus* (i.e., $120 \mu\text{mol }g^{-1} \text{ wet wt gill }d^{-1}$).

The maximum uptake rate of sulphide was estimated through model routine while assuming that:

- $V_{max_M} = 120 \mu\text{mol }CH_4 \text{ }g^{-1} \text{ wet wt gill }d^{-1}$.
- A maximum productivity value for *B. azoricus* of a certain size based according to estimated values based on empirical data.
- Endosymbiont biomass correspond to 4% of the gill weight; because endosymbiont biomass depends on V_{max_S} , the 4% value acts as a constraint that limits the variation of V_{max_S} .

The value that fulfilled the above pre-requisites was $743 \mu\text{mol }H_2S \text{ }g^{-1} \text{ wet gill }d^{-1}$.

3.2.2. Carbon gain from microbial oxidations

The carbon gained from chemoautotrophic microbial oxidations is referred as the biomass yield for the chemotrophic growth of microorganisms (expressed in C-mol: mol). According to Heijnen and Van Dijken (1992), the maximum biomass yield for sulphide and methane oxidation is 0.3 and 0.55, respectively. Due to constraints of several orders, it is expected that biomass yield in nature is significantly lower than the former values. In accordance with this, empirical evidence indicates that per mole of CH_4 consumed, mussels with functional symbionts produce about 0.3 mol CO_2 (Kochevar et al., 1992) and, according to the proportion 0.3:0.55 for $S: CH_4$, the

Table 1 – Parameter definition, values and mathematical expressions used in the model and information about literature range and methods used to obtain the final values

Parameters	Definition	Used value/expression	Lit. range	Obs.	References
CR ($l\ h^{-1}\ g^{-1}$)	Clearance rate	$CR = 7.45\ W^{0.66}$	–	Obt. for <i>M. edulis</i>	Järnegren and Altin (2006)
V_{max_S} ($\mu\text{mol}\ g^{-1}\ \text{wet wt gill d}^{-1}$)	Sulphide maximum uptake rate	743	14–96	Empirical + calibration	Dando et al. unpublished
K_{m_S} ($\mu\text{mol l}^{-1}$)	Sulphide half-saturation constant	20	–	Calibration	–
γ_S (C-mol: S-mol)	Carbon gained from sulphide oxidation	0.16	0.013–0.3	Empirical + calibration	Tuttle (1985), Heijnen and Van Dijken (1992)
$V_{max_{CH_4}}$ ($\mu\text{mol}\ g^{-1}\ \text{wet wt gill d}^{-1}$)	Methane maximum uptake rate	120	96–240	Empirical; obt. for a cold seep mussel	Kochevar et al. (1992)
$K_{m_{CH_4}}$ ($\mu\text{mol l}^{-1}$)	Methane half-saturation constant	1	–	Empirical; obt. for a cold seep mussel	Kochevar et al. (1992)
γ_M (C-mol:CH ₄ -mol)	Carbon gained from methane oxidation	0.3	0.3–0.55	Empirical; obt. for a cold seep mussel	Heijnen and Van Dijken (1992), Kochevar et al. (1992)
δ (C _{symb} :C _{host})	Carbon transferred from symbionts to host	0.425	0.25–0.65	Empirical + calibration	Fiala-Médioni and Felbeck (1990)
R ($\mu\text{mol C g}^{-1}\ \text{dry wt h}^{-1}$)	Energetic losses due to respiration	$e^{2.69}\ W^{0.76}$	–	Experimental	Dando et al. unpublished
RQ	Respiration coefficient	0.9	0.85–1	Experimental + calibration	Smith (1985), Conway et al. (1992)
μ (mol cm^{-3})	Organic matter ingestion coefficient	6.69×10^{-5}	–	Obtained for <i>P. canaliculus</i>	Ren and Ross (2005) and references therein
DE (%)	Organic matter digestion efficiency	0.753	0.26–0.9	Obtained for <i>M. edulis</i>	Bayne et al. (1989)
POC_{sat} (mol l^{-1})	Half-saturation constant for organic matter	1.63×10^{-5}	–	Obtained for <i>P. canaliculus</i>	Ren and Ross (2005) and references therein

Used conversion factors: wet wt = 0.1745 dry wt (based on *B. azoricus* (N=35) from Menez Gwen). Gill dry wt: gill wet wt = 0.162 (based on *B. azoricus* (N=35) from Menez Gwen), C: dry wt for *B. azoricus* = 0.39 (experimentally obtained by Colaço, unpublished), C: dry wt for endosymbionts = 0.5 (Bratbak, 1985), W = *B. azoricus* dry weight (g), μ and POC_{sat} were subsequently converted to mgC.

Table 2 – Estimated biomass *B. azoricus* and corresponding endosymbionts at the Menez Gwen, assuming an average density of 550 ind m⁻² (Colaço et al., 1998) and based on a significant shell length–dry weight regression for *B. azoricus* (N = 47)

Shell length (mm)	10	30	50	70	90	110
Endosymbionts (mg C m ⁻²)	13	284	1184	3031	6118	10719
<i>B. azoricus</i> (kg wet wt m ⁻²)	0.01	0.23	1.01	2.67	5.51	9.84

biomass yield of sulphide was set at 0.16 mol CO₂. These processes were described by:

$$S = V_S \gamma_S \quad (4)$$

S is the carbon gain from sulphide oxidation, V_S is the sulphide uptake, γ_S is the biomass yield of sulphide and

$$M = V_M \gamma_M \quad (5)$$

M is the carbon gain from methane oxidation, V_M is the methane uptake, γ_M is the biomass yield of methane.

3.2.3. Carbon transfer from symbionts to *B. azoricus*

Only part of the energy obtained from microbial oxidations is transferred to the host mussel as the symbionts require some energy for their own metabolism. According to Fiala-Médioni and Felbeck (1990), between 25 and 65% of the carbon fixed by the symbionts (δ) is for the host nutrition. Thus, T in Eqs. (1) and (2) is defined as:

$$T = (S + M) \cdot \delta \quad (6)$$

The value 43% of carbon transferred from symbionts to *B. azoricus* was obtained through model calibration (see Section 3.4). For the scenario (see Section 3.6), which accounts for the digestion of symbionts by *B. azoricus* according to some experimental evidence (Fiala-Médioni et al., 1986; Fisher and Childress, 1992; Raulfs et al., 2004), δ was set to 90%.

3.2.4. *B. azoricus* filter-feeding

Filter-feeding by mussels was described in accordance to Ren and Ross (2005):

$$F = \mu \cdot CR \cdot p \cdot DE \quad (7)$$

μ is the ingestion coefficient (mol cm⁻³ converted to mg l⁻¹), CR is the clearance rate (l d⁻¹), p is the functional response of particulate organic matter, DE is the digestion efficiency (%).

$$p = \frac{POC}{POC + POC_{sat}} \quad (8)$$

POC is the organic matter concentration (mg l⁻¹), POC_{sat} is the half-saturation constant for POC (mg l⁻¹).

3.2.5. *B. azoricus* energy wastes

In the present model, the energy wastes of *B. azoricus* were described by an allometric relationship, which accounts for respiration assuming a molar ratio of CO₂ produced to oxygen

consumed (RQ) of 0.9:

$$R = aW^b RQ \quad (9)$$

W is the weight of *B. azoricus* (g dry wt), a and b is the empirical coefficients (Table 1). Coefficients a and b are in accordance with experimental values obtained for *B. azoricus* (Dando et al. unpublished) (Table 2). The respiration coefficient (RQ) was set at 0.9 based on the average value of the reported range of 0.85–1 (Smith, 1985; Conway et al., 1992).

3.3. Biomass of *B. azoricus* and its endosymbionts at Menez Gwen

Estimations of *B. azoricus* biomass were based on a shell length (mm)–dry weight (g) regression obtained from individuals of different sizes collected from Menez Gwen vent field (N = 47)

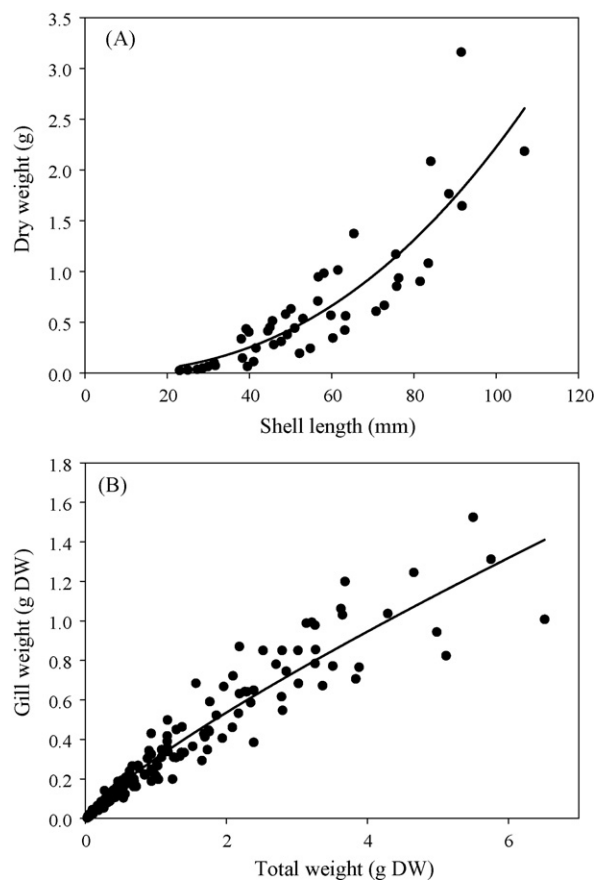


Fig. 3 – Shell length (mm) versus weigh (g dry wt of soft tissue) (N = 47) (A) and gill weight (g dry wt) versus total weight (g dry wt) (N = 153) (B) of *B. azoricus* collected at Menez Gwen.

(Fig. 3A) and on an average value of 550 ind m^{-2} of *B. azoricus* (Colaço et al., 1998). To compare with published data, *B. azoricus* biomass was converted to kg wet wt m^{-2} . The relationship between gill weight (GillW – g dry wt) and total weight (TotalW – g dry wt) of *B. azoricus* from the Menez Gwen ($N = 153$) is described by the following expression (Fig. 3B):

$$\text{GillW} = 0.2754 \cdot \text{TotalW}^{0.9681} \quad (10)$$

Endosymbiont biomass was estimated assuming that endosymbionts correspond to 4% of the gill wet weight of *B. azoricus* according to the value estimated for *B. thermophilus* (Powell and Somero, 1986), using a gill wet wt:gill dry wt = 6.2 based on *B. azoricus* from the Menez Gwen ($N = 39$) and a C: dry wt for endosymbionts of 0.5 (Bratbak, 1985).

3.4. Calibration

The model was calibrated through the trial-error method, against the estimated biomass of endosymbionts and *B. azoricus* at the Menez Gwen. As previously mentioned, throughout the calibration process, the constraint that endosymbionts correspond to 4% of *B. azoricus* gill wet weight was respected.

3.5. Sensitivity analysis

Sensitivity analysis was performed after imposing variations within the range $\pm 10\%$ to each parameter, while all the others were kept unchanged. Sensitivity to external conditions was also tested by the series of performed simulation scenarios (see Section 3.6).

To estimate the sensitivity of parameters, the following expression (Jørgensen, 1994) was used:

$$S = \frac{[\partial X/X]}{[\partial P/P]} \quad (11)$$

X is the state variable (endosymbiont and *B. azoricus* biomass, in the case of the present model), P is the parameter, ∂ is the variation between the final and the initial values.

3.6. Simulations

In every simulation, a certain initial weight of *B. azoricus* (in mg C) and the corresponding weight of endosymbionts (in mg C) were considered to initialize the model. At each run, the model assesses if an individual mytilid, with a certain weight and harbouring the corresponding biomass of endosymbionts, can survive relying on imposed external concentrations. External concentrations of H_2S and CH_4 used in the model are based on values estimated for the Menez Gwen or measured at MAR, while POC concentrations were estimated by model resolution, i.e., every time a certain individual *B. azoricus* could not sustain productivity relying on imposed H_2S and CH_4 concentrations, the exact amount of POC needed to compensate for energetic wastes was estimated by trial-error method. For these purposes, a simulation length of 300 days was considered adequate.

In the initial simulations (scenarios 1, 2 and 3), the model was used to assess if *B. azoricus* and its endosymbionts

would be sustainable when relying solely on endosymbiosis or endosymbiosis coupled to ingestion of symbionts. The following simulations (scenarios 4–7) were used to estimate the POC requirements of *B. azoricus* and the relative contribution of endosymbiosis and filter-feeding to *B. azoricus* productivity.

The tested scenarios were:

- (1) Only endosymbiosis with H_2S and CH_4 concentrations estimated for Menez Gwen: $60 \mu\text{M}$ of H_2S and $100 \mu\text{M}$ of CH_4 (Sarradin, unpublished) – ENDO-MG.
- (2) Only endosymbiosis with H_2S and CH_4 concentrations corresponding to maximal values measured at Menez Gwen, Lucky Strike and Rainbow vent fields: $303 \mu\text{M}$ of H_2S and $177 \mu\text{M}$ of CH_4 (Desbruyères et al., 2001) – ENDO-MAX.
- (3) Endosymbiosis and symbiont digestion with H_2S and CH_4 concentrations corresponding to maximal values measured at Menez Gwen, Lucky Strike and Rainbow vent fields: ENDODIGEST-MAX.
- (4) Endosymbiosis and filter-feeding with external concentrations of H_2S and CH_4 estimated for the Menez Gwen: ENDOFILTER-MG.
- (5) Endosymbiosis and filter-feeding with maximal measured concentrations of H_2S and CH_4 : ENDOFILTER-MAX.
- (6) Endosymbiosis and filter-feeding with H_2S and CH_4 concentrations corresponding to minimal values measured at Menez Gwen, Lucky Strike and Rainbow vent fields: $0.3 \mu\text{M}$ of H_2S and $0.3 \mu\text{M}$ of CH_4 (Desbruyères et al., 2001): ENDOFILTER-MIN.
- (7) Only filter-feeding: FILTER.

A carbon to dry weight ratio of *B. azoricus* (C: dry wt) of 0.39 was assumed (Colaço, unpublished).

4. Results

4.1. Predicted versus estimated biomass values of *B. azoricus* and endosymbionts

According to estimations, at the Menez Gwen vent site, *B. azoricus* biomass varies between 0.01 and $9.84 \text{ kg wet wt m}^{-2}$ for mussels with sizes between 10- and 110-mm SL, respectively, and the corresponding endosymbiont biomass variation is 13–10719 mg C m^{-2} (Table 2). The fitting level between estimated and predicted values is very high for both *B. azoricus* and endosymbionts (ANOVA, $F_{1,4} = 2 \times 10^8$, $P < 0.001$, $r^2 = 1$ and ANOVA, $F_{1,4} = 1 \times 10^{11}$, $P < 0.001$, $r^2 = 1$, respectively) (Fig. 4A and B).

4.2. Contribution of endosymbiosis and filter-feeding to the nutrition of *B. azoricus*

Results indicate that, if exclusively depending on endosymbionts for nutrition and at external concentrations estimated for the Menez Gwen (ENDO-MG), *B. azoricus* cannot keep the estimated productivity levels and show a decreasing tendency over time. This pattern is verified for mussels of all sizes but the % of decreasing productivity over time varies inversely with the size of the mussels. At maximal concentrations measured at MAR and, either for exclusive dependency

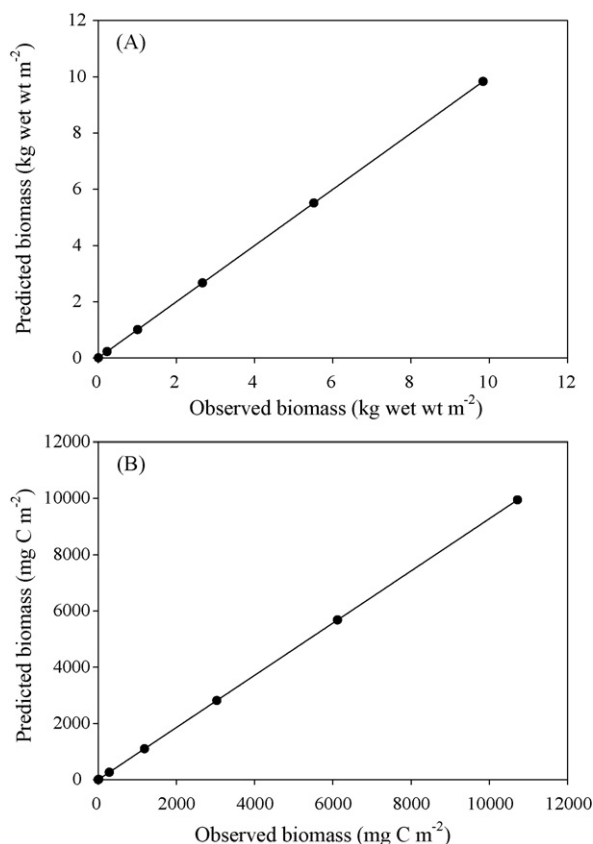


Fig. 4 – Predicted versus observed biomass of *B. azoricus* (kg wet wt m⁻²) (A) and endosymbionts (mg C m⁻²) (B). The two regressions are highly significant: ANOVA, $F_{1,4} = 2 \times 10^8$, $P < 0.001$, $r^2 = 1$ and ANOVA, $F_{1,4} = 1 \times 10^{11}$, $P < 0.001$, $r^2 = 1$, respectively.

on symbiosis (ENDO-MAX) or endosymbiosis coupled to symbiont digestion (ENDODIGEST-MAX), only the largest mussels (SL = 110 mm) can sustain (Fig. 5).

According to results, at H₂S and CH₄ concentrations estimated for Menez Gwen, *B. azoricus* must couple endosymbiosis with filter-feeding to reach the estimated productivity values (Fig. 6A). However, the relative contribution of endosymbiosis and filter-feeding to the total nutrition of *B. azoricus* varies with the size of mytilids, with the contribution of filter-feeding decreasing from 81 to 16% in relation to endosymbiosis, from the smallest to the largest *B. azoricus*, respectively (Fig. 6B).

The ratio filter-feeding: endosymbiosis also varies with external conditions. For maximal concentrations of sulphide and methane measured at MAR (ENDOFILTER-MAX), the previous pattern of nutritional strategy variation with mussel size is kept but the contribution of filter-feeding to the mytilid nutrition decreases, with the largest mussels being able to meet all their nutritional requirements via symbiosis (Fig. 7A). If external concentrations of H₂S and CH₄ decrease to minimal values (ENDOFILTER-MIN), *B. azoricus* must increase filter-feeding rates to compensate for their energetic needs. Compared to the previous scenario, the increase of filter-feeding is much more significant in larger animals, which previously could rely more on endosymbiosis (Fig. 7B).

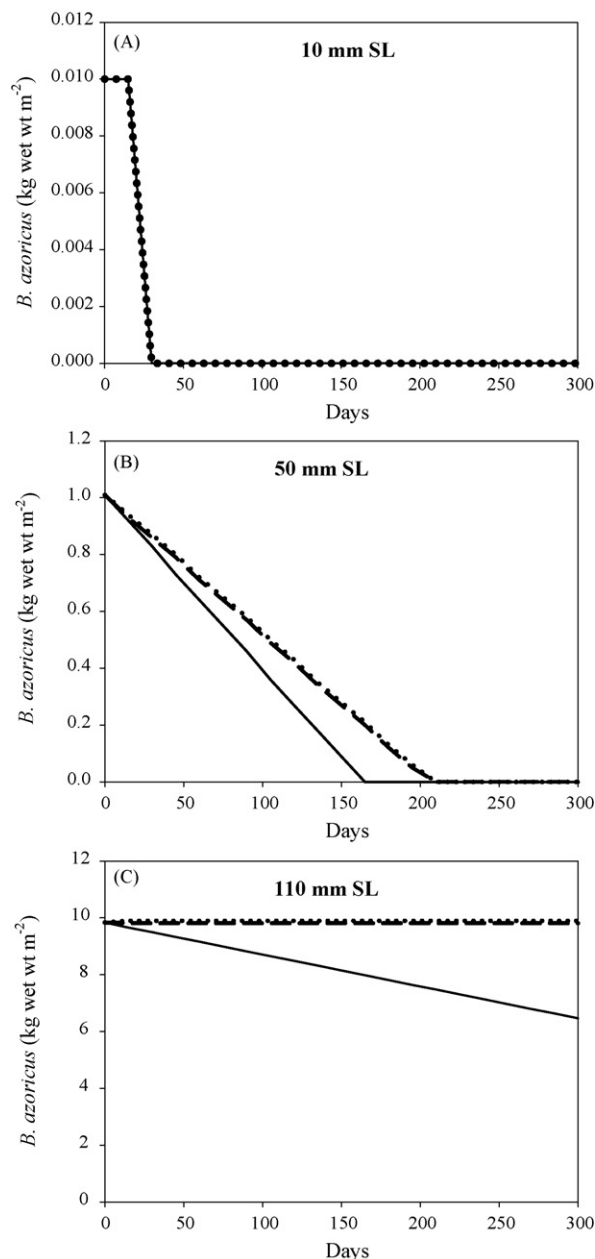


Fig. 5 – Model predictions for biomass variation of *B. azoricus* of different sizes (shell length—SL): 10, 50 and 110 mm (A, B and C, respectively), with endosymbiosis as the only carbon source and under different conditions: H₂S and CH₄ concentrations estimated for Menez Gwen—ENDO-MG (—), maximal H₂S and CH₄ concentrations measured at MAR—ENDO-MAX (---) and maximal concentrations with digestion of symbionts – ENDODIGEST-MAX (· · ·).

In the scenario testing filter-feeding as the only nutritional pathway available for *B. azoricus* (FILTER), results suggest that mytilids must filter between 0.05 and 9 mg of POC l⁻¹ d⁻¹, depending on body size (Fig. 8). According to the present results, the POC requirements of *B. azoricus* also vary with body size and external conditions. For concentrations of sulphide and methane estimated for the Menez Gwen, *B.*

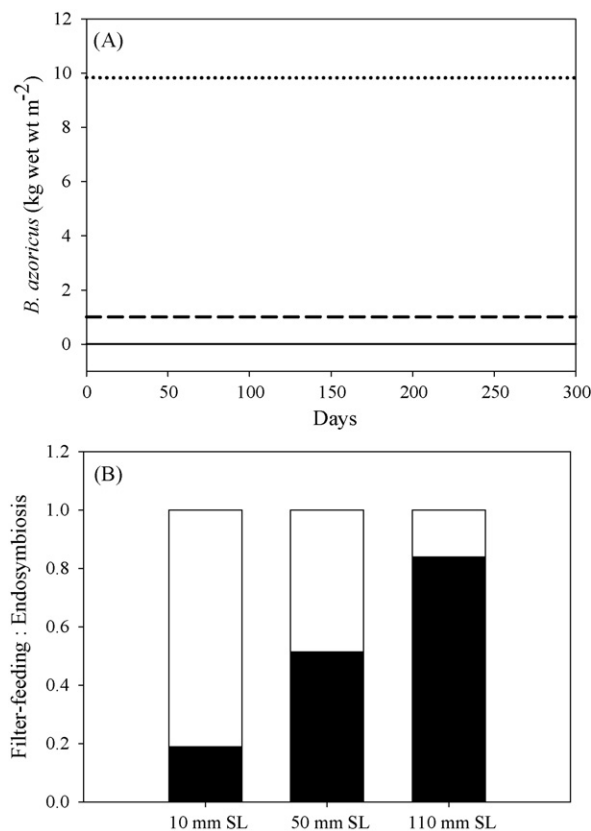


Fig. 6 – Model predictions for biomass variation of *B. azoricus* of different sizes: 0.01 kg wet wt m⁻² corresponding to 10 mm SL (—), 1.01 kg wet wt m⁻² corresponding to 50 mm SL (---) and 9.84 kg wet wt m⁻² corresponding to 110 mm SL (···) with endosymbiosis and filter-feeding as carbon sources and under external concentrations of H₂S and CH₄ estimated for Menez Gwen (ENDOFILTER-MG) (A). The relative contribution of filter-feeding (□) and endosymbiosis (■) varies with the size of *B. azoricus*.

azoricus must filter between 0.04 and 1.4 mg POC l⁻¹ d⁻¹ for the smallest and the biggest considered mussels, respectively. However, for minimal concentrations or absence of reduced substrates available for microbial oxidations, the POC requirements of *B. azoricus* can be as high as 9 mg POC l⁻¹ d⁻¹ for the largest animals, corresponding to a concentration of 0.008 mg l⁻¹ POC (Fig. 9).

4.3. Sensitivity analysis

B. azoricus exhibited a higher sensitivity to parameter variations than endosymbionts (Table 3). In fact, endosymbionts did not show significant sensitivity to imposed variations ($\pm 10\%$ to the initial values of parameters). The parameter that caused the highest impact on the variation of symbionts biomass was the amount of carbon transferred to *B. azoricus* (δ). *B. azoricus* showed significant sensitivity to imposed variations on respiration, ingestion efficiency, half-saturation constant for organic matter uptake and clearance rate. Interestingly, for all these parameters, the sensitivity decreased as the mytilid size increased. This indicates the stronger depen-

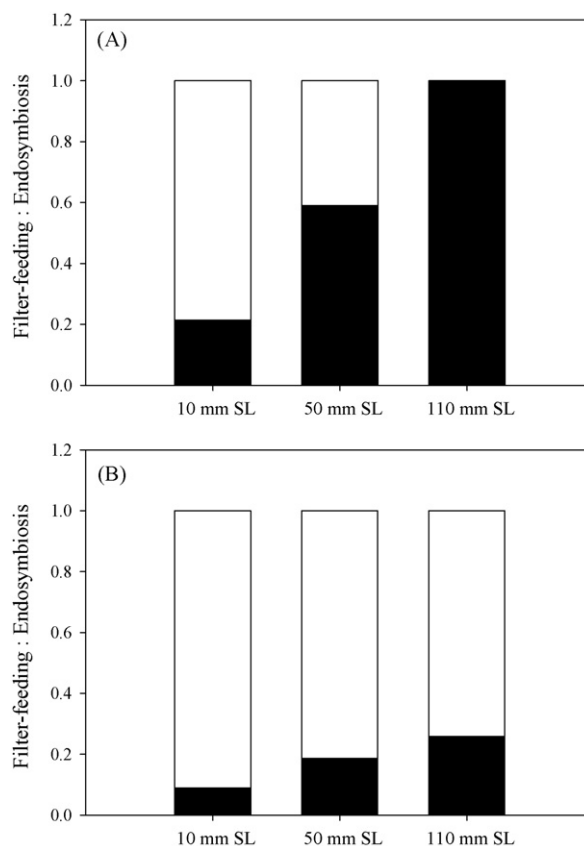


Fig. 7 – Relative contribution of filter-feeding (□) and endosymbiosis (■) (%) to the total energy budget of *B. azoricus* of different sizes (SL—shell length) under maximal concentrations of H₂S and CH₄ estimated for Menez Gwen (ENDOFILTER-MAX) (A) and minimal concentrations of H₂S and CH₄ measured at MAR (ENDOFILTER-MIN) (B).

dency of small mytilids on parameters related to filter-feeding compared to larger mussels. Contrarily to endosymbionts, *B. azoricus* did not react to variations on the amount of carbon transferred from the symbionts or the half-saturation

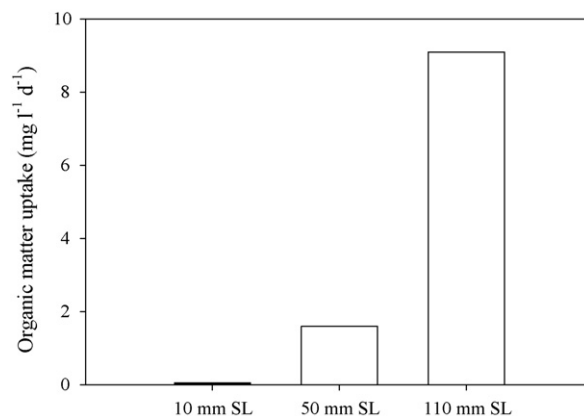


Fig. 8 – Organic matter requirements (mg POC l⁻¹ d⁻¹) of *B. azoricus* of different sizes, when filter-feeding is the only carbon source (FILTER). Mytilids with 10-, 50- and 110-mm SL require 0.05, 0.52 and 9.1 mg POC l⁻¹ d⁻¹, respectively, to fulfil their carbon needs.

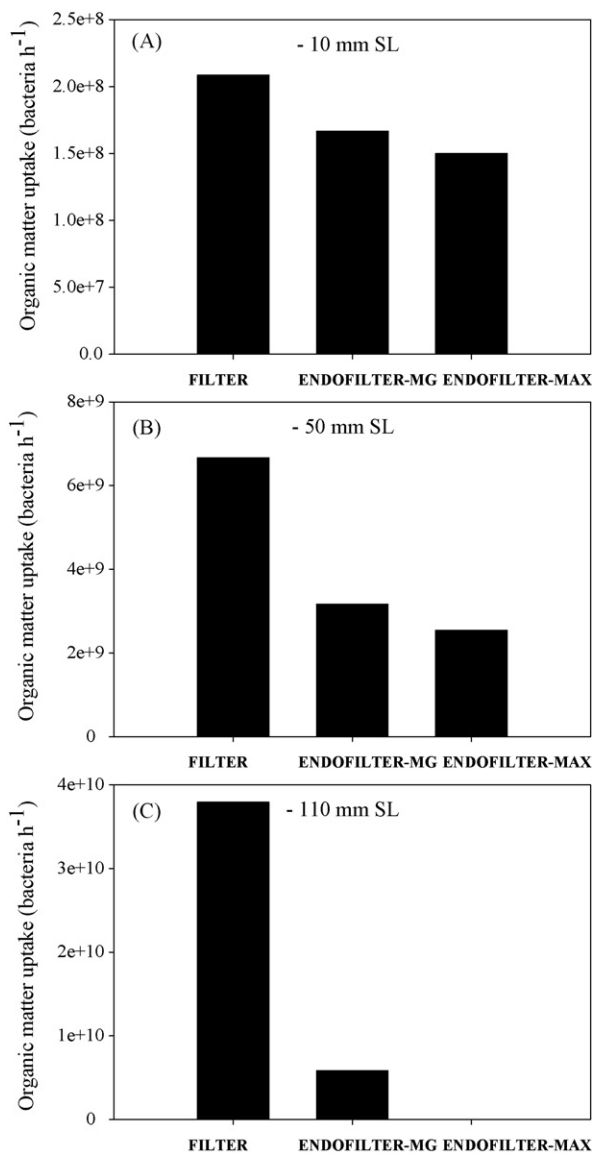


Fig. 9 – Organic matter needs (number bacteria h⁻¹) of *B. azoricus* of different sizes (A) 10 mm SL, (B) 50 mm SL and (C) 110 mm SL) and under different scenarios: only with filter-feeding (FILTER), with filter-feeding and endosymbiosis with external concentrations of H₂S and CH₄ at Menez Gwen (ENDOFILTER-MG) and maximal concentrations of H₂S and CH₄ at MAR (ENDOFILTER-MAX). *B. azoricus* with 110 mm SL and under ENDOFILTER-MAX scenario can rely completely on endosymbiosis for nutrition.

constant for the uptake of CH₄. *B. azoricus* reacted more significantly to variations on S-related parameters than to CH₄-related parameters (Table 3).

5. Discussion

5.1. Biomass of *B. azoricus* and endosymbionts at the Menez Gwen

Considering the size range 10–110 mm shell length, the estimated biomass of *B. azoricus* at the Menez Gwen varied

Table 3 – Sensitivity of the endosymbionts (A) and *B. azoricus* (B) to variations of ±10% in the parameters

(A)						
Endosymbionts	Sensitivity					
V _{max_S} + 10%	0.7					
V _{max_S} - 10%	0.7					
V _{max_{CH₄}} + 10%	0.3					
V _{max_{CH₄}} - 10%	0.3					
K _{m_S} + 10%	-0.2					
K _{m_S} - 10%	-0.2					
K _{m_{CH₄}} + 10%	0					
K _{m_{CH₄}} - 10%	0					
γ _S + 10%	0.7					
γ _S - 10%	0.7					
γ _{CH₄} + 10%	0.3					
γ _{CH₄} - 10%	0.3					
δ + 10%	-0.9					
δ - 10%	-1.1					
(B)						
<i>B. azoricus</i>	Sensitivity					
SL (mm)	10	30	50	70	90	110
V _{max_S} + 10%	2.5	2.3	2.2	2.1	2.1	2.0
V _{max_S} - 10%	2.5	2.3	2.2	2.1	2.1	2.0
V _{max_{CH₄}} + 10%	1.1	1.0	1.0	0.9	0.9	0.9
V _{max_{CH₄}} - 10%	1.1	1.0	1.0	0.9	0.9	0.9
K _{m_S} + 10%	-0.6	-0.5	-0.5	-0.5	-0.5	-0.5
K _{m_S} - 10%	-0.6	-0.5	-0.5	-0.5	-0.5	-0.5
K _{m_{CH₄}} + 10%	0	0	0	0	0	0
K _{m_{CH₄}} - 10%	0	0	0	0	0	0
γ _S + 10%	2.5	2.3	2.2	2.1	2.1	2.0
γ _S - 10%	2.5	2.3	2.2	2.1	2.1	2.0
γ _{CH₄} + 10%	1.1	1.0	1.0	0.9	0.9	0.9
γ _{CH₄} - 10%	1.1	1.0	1.0	0.9	0.9	0.9
δ + 10%	0	0	0	0	0	0
δ - 10%	0	0	0	0	0	0
CR + 10%	12	4.6	3.2	2.2	1.7	1.0
CR - 10%	8.6	6.5	3.5	2.1	1.1	0.7
R + 10%	-8.7	-7.5	-6.2	-5.2	-4.4	-3.8
R - 10%	-18.5	-8.6	-6.6	-5.1	-4.4	-3.8
POC _{sat} + 10%	-8.2	-5.4	-3.0	-1.9	-1.2	-0.8
POC _{sat} - 10%	-17.8	-6.6	-3.7	-2.3	-1.5	-0.9
μ + 10%	16.3	6.0	3.4	2.1	1.4	0.9
μ - 10%	8.4	5.8	3.4	2.1	1.4	0.8

The result is a positive or a negative number. The absolute value represents the distance to the initial value of the state variable. The negative and the positive sign indicate that the state variable and the parameters vary inversely or in the same way, respectively.

between 0.01 and 10 kg wet wt m⁻² (mean = 3.2 kg wet wt m⁻²), while the corresponding endosymbiont biomass ranged from 13 to 10719 mg C m⁻², which corresponds to 3.8 mg microbial carbon g⁻¹ wet wt gill. Our estimations of *B. azoricus* biomass are very similar to mussel biomasses reported for other

hydrothermal vents and cold seeps (e.g. 3.5 kg wet wt m⁻² at Lucky Strike – Van Dover et al., 1996; 2.2 kg wet wt m⁻² at vents in Galápagos Ridge – Fustec et al., 1988; 5.4–9 kg wet wt m⁻² at Barbados prism cold seeps – Olu et al., 1996). This reinforces the reliability of the present estimations, which were obtained from a significant shell length–weight regression and an average density of 550 ind m⁻² according to *in situ* observations (Colaço et al., 1998).

Assuming a value of 10⁸ cell μgC for the carbon content of marine bacteria, which follows within the literature range of 10⁷–10¹⁰ cell μgC (Page et al., 1990 and references therein; Schippers et al., 2005), our estimations of endosymbiont biomass correspond to 3.8 × 10¹¹ endosymbionts g⁻¹ wet wt gill, which is in agreement with reported values of endosymbiont abundance for *B. thermophilus* (1.70–1.81 × 10¹¹ g⁻¹ wet wt – Powell and Somero, 1986) and a mytilid of the Mariana Back-arc basin (0.8–2 × 10¹¹ g⁻¹ wet wt gill – Yamamoto et al., 2002).

The fact that model predictions show a very high fitting level with estimated biomass values for both *B. azoricus* and its endosymbionts reflects a general correct incorporation and description of processes in the model, as well as a consistent calibration. In practical terms, this confers robustness to model results and predictions for the tested scenarios.

5.2. Flexibility of *B. azoricus*'s nutritional strategy throughout life

Model results suggest that the dominant nutritional strategy of *B. azoricus* varies with body size and external conditions. Small and, presumably, young mytilids cannot derive enough energy from endosymbiosis to account for their energetic needs and, thus, filter-feeding must play an important role in their nutrition. Gradually, as the mussel increases size, the amount of energy derived from microbial oxidations also increases and, potentially, under non-limiting concentrations of H₂S and CH₄, *B. azoricus* is able to increase the ratio endosymbiosis: filter-feeding. At very high availability of H₂S and CH₄ (>300 μM H₂S and >150 μM CH₄), the largest and presumably, older mytilids (≥110 mm SL) can derive all their energy from endosymbiosis. However, if the concentrations of external H₂S and CH₄ decrease, larger *B. azoricus* can increase the contribution of filter-feeding to meet their energetic demands, as long as the external availability of organic matter allows it. The highest nutritional flexibility of larger mussels compared to smaller ones is related with the type of allometric relationship between gill weight and the uptake of H₂S and CH₄ by *B. azoricus* (Fig. 10). Based on 153 individuals collected in different years (2001, 2006 and 2007) and different seasons (summer, autumn and winter), the average size of *B. azoricus* at Menez Gwen was 60 mm SL. According to the present model and for concentrations of 60 μM H₂S and 100 CH₄, mytilids of 60 mm SL obtain about 58% of their energy from endosymbiosis and the rest from filter-feeding (42%), if the available POC is ~0.0067 mg l⁻¹.

The present results are also in agreement with the ontogenetic development of *B. azoricus* from planktotrophic larvae (Dixon et al., 2006) to mixotrophic adults. In addition, the gradual increasing contribution of endosymbiosis with *B. azoricus* size seems to be in accordance with the possible environmen-

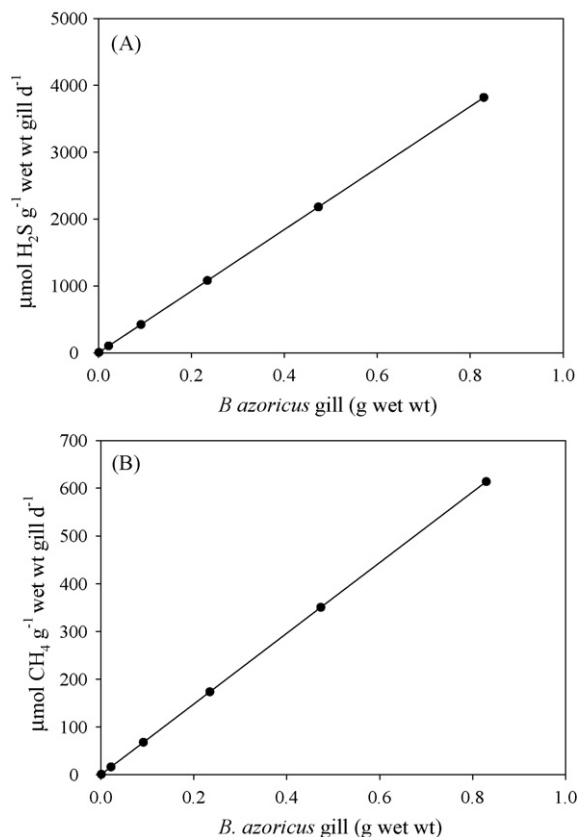


Fig. 10 – Relationship between sulphide uptake (A) and methane uptake (B) (μmol g⁻¹ wet wt gill d⁻¹) with gill weight (g wet wt) in *B. azoricus*.

tal transmission of endosymbionts in the genus *Bathymodiolus* (Won et al., 2003; Kádár et al., 2005).

5.3. Spatial distribution versus nutritional strategy?

Data from video observations and temperature time-series obtained at Menez Gwen and Lucky Strike vent fields indicate that *B. azoricus* exhibits a spatial segregation of sizes, with largest individuals living at the warmest areas with higher sulphide concentration and lower pH (Comtet and Desbruyères, 1998 and references therein). According to our results, we hypothesise that the observed spatial segregation may reflect the higher dependency of larger mytilids on endosymbiosis and, consequently, their location closer to the sources of reduced substrates. Small mytilids, which depend more on filter-feeding are located further way from the vent flow but within the mussel's bed, where particulate organic matter limitation is not likely to occur due to the existence of a biogenic flow generated by mussel pumping (Pile and Young, 1999).

5.4. POC requirements of *B. azoricus*

There is a general lack of information concerning POC concentrations at vent fields. The exception is some values reported for vents at the Galapagos Rift, ranging between 106 and 207 μg l⁻¹ (Smith, 1985). If values of POC at MAR are similar to these ones, according to model results, *B. azoricus*

will not experience any kind of organic carbon limitation. If the predicted organic matter requirements of *B. azoricus* are transformed to numbers of bacteria – assuming that free-living bacteria are one of the components of POC at vents (Levesque et al., 2005) and can, thus, be filtered by mytilids (e.g. Fiala-Médioni et al., 1986; Giere et al., 2003) – the values range from 10^8 to 10^9 bacteria h^{-1} (assuming 10^8 cell $\mu g C$) for the tested scenarios accounting for endosymbiosis coupled to filter-feeding. These values are coincident with the estimated amounts of bacteria required by a seep mussel: $\sim 10^8$ to $\sim 10^9$ bacteria h^{-1} (Page et al., 1990). If *B. azoricus* has to rely exclusively on filter-feeding, the number of required bacteria increases to 10^{10} bacteria h^{-1} for larger mussels (≥ 70 mm SL) but, even in this situation, the abundance of free-living bacteria at vents seems large enough to supply the energetic needs of *B. azoricus* ($\sim 10^4$ and $\sim 10^9$ cells ml^{-1} according to Giere et al., 2003). Recent findings indicate that, although free-living primary productivity is considered to be very high at vents, the bacterial biomass may be kept at low levels due to bacterial mortality and grazing by micro- and macroinvertebrates (Levesque et al., 2005).

Dissolved organic matter is another possible source of carbon and nutrients, if *B. azoricus*, like other marine invertebrates, is able to transport amino acids and other organic solutes across its body surface (e.g. Wendt and Johnson, 2006). In this case, vent mytilids can benefit from the potential surplus of DOM existing at mussel's beds as suggested by dissolved organic carbon (DOC) values measured in the vicinity of *Bathymodiolus* beds in the Lucky Strike and Menez Gwen vent fields (range 95–647 μM DOC – Sarradin et al., 1999).

Additionally, it seems that occasional peaks of surface-water primary production may act as potential food sources for both the adults and larvae of *B. azoricus* (Dixon et al., 2006).

5.5. Surviving after the cessation of vent flows

The predicted plasticity of nutritional pathways exhibited by *B. azoricus* may explain the fact that *Bathymodiolus* sp. is one of the last vent groups to survive after flow ceases at hydrothermal vents (Shank et al., 1998). Nevertheless, if the major source of particulate organic carbon, at vents, is provided by microbial autotrophic fixation of vent fluid DIC (Levesque et al., 2005), the ability of *B. azoricus* to survive in these circumstances will always be temporary and, most likely, related to external concentrations (H_2S , CH_4 , POC) at the moment flow ceases.

5.6. Limitations of our model

The model exhibits long-term stability and robustness to variations of parameters, initial- and external-conditions. Nevertheless, the model can be further improved, particularly, by incorporating experimental data, specifically obtained for *B. azoricus* (e.g. clearance rate, digestion efficiency and the ratio endosymbionts: gill weight). More insight into the processes involved in the uptake of S and CH_4 by endosymbionts will also benefit model's accuracy. For instance, the dual symbiosis of *B. azoricus* is contemplated in the model but not linked to the environmental availability of reduced compounds, whereas experimental evidence indicates that the relative number and activity of thio- and methanotrophs in

B. azoricus may be related to external sulphide and methane concentrations (Fiala-Médioni et al., 2002). In the future, when this process is better understood, it can be incorporated in the model. This is valid for any other process related with the use of resources and energy by *B. azoricus* and its endosymbionts.

6. Conclusions

The present results indicate that, under scenarios of external supply of sulphide, methane and POC, the predominant nutritional pathway of *B. azoricus* varies with the mytilid size, from a strong dependency on filter-feeding in small mussels until deriving the majority of its energy from endosymbiosis as exhibited by the largest mytilids. This variation is related with the relation between gill weight and mytilid size. Depending on external conditions, the present results also suggest that *B. azoricus* is able to regulate the endosymbiosis: filter-feeding-ratio, with large animals showing a higher nutritional flexibility than small animals.

Overall this work shows that, as a complement to empirical approaches, modelling can represent a valuable tool in the study and understanding of extreme ecosystems such as deep-sea hydrothermal vents.

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