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

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\textbf{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 H\textsubscript{2}S and CH\textsubscript{4} 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 mussels 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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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 endosymbions, 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 endosymbions. 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 endosymbions correspond to 4% of the gill wet weight of Bathymodiolus (Powell and Somero, 1986), the
of the model are mg C (carbon) d−1 and the energetic wastes of *B. azoricus* respectively, the filtering of particulate organic matter by *B.* 

3.2. Mathematical equations and parameters

The biomass of endosymbionts was also estimated. The model accounts for the uptake of sulphide (H2S) and methane (CH4), the oxidation of H2S and CH4 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 mg C (carbon) d−1. The considered average biomass of *B. azoricus* was 550 ind m−2 according to local observations by Colaço et al. (1998). The model assumes no limiting conditions of O2 or DIC.

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 to this, the uptake of sulphide (S) and methane (M) by endosymbionts (V_{S,M}) was described by a single Michaelis–Menten kinetics:

\[
V_{S,M} = V_{\text{max},S,M} \cdot \frac{[S, M]}{K_{S,M} + [S, M]}
\]

\( V_{\text{max},S,M} \) 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 (\( N = 39 \)), \( K_{S,M} \) 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 \( \mu \text{mol g}^{-1} \text{gill dry wt d}^{-1} \) (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 \( \mu \text{mol g}^{-1} \text{gill dry wt d}^{-1} \)).

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

- \( V_{\text{max},S} = 120 \mu \text{mol CH}_4 \text{ g}^{-1} \text{wet gill d}^{-1} \)
- 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 \( V_{\text{max},S} \), the 4% value acts as a constraint that limits the variation of \( V_{\text{max},S} \).

The value that fulfilled the above pre-requisites was 743 \( \mu \text{mol H}_2\text{S g}^{-1} \text{ wet gill d}^{-1} \).

3.2.2. Carbon gain from microbial oxidations

The carbon gained from chemosynthetic 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 CH4 consumed, mussels with functional symbionts produce about 0.3 mol CO2 (Kochevar et al., 1992) and, according to the proportion 0.3:0.55 for S: CH4, 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definition</th>
<th>Used value/expression</th>
<th>Lit. range</th>
<th>Obs.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR (l h(^{-1}) (g^{-1}))</td>
<td>Clearance rate</td>
<td>(CR = 7.45 \times W^{0.66})</td>
<td>–</td>
<td>Obt. for M. edulis</td>
<td>Jarnegren and Altin (2006)</td>
</tr>
<tr>
<td>(V_{\text{max}}) ((\mu)mol g(^{-1}) wet wt gill d(^{-1}))</td>
<td>Sulphide maximum uptake rate</td>
<td>743</td>
<td>14–96</td>
<td>Empirical + calibration</td>
<td>Dando et al. unpublished</td>
</tr>
<tr>
<td>(K_m) ((\mu)mol l(^{-1}))</td>
<td>Sulphide half-saturation constant</td>
<td>20</td>
<td>–</td>
<td>Calibration</td>
<td>–</td>
</tr>
<tr>
<td>(\gamma_S) (C-mol: S-mol)</td>
<td>Carbon gained from sulphide oxidation</td>
<td>0.16</td>
<td>0.013–0.3</td>
<td>Empirical + calibration</td>
<td>Tuttle (1985), Heijnen and Van Dijken (1992)</td>
</tr>
<tr>
<td>(V_{\text{maxCH}_4}) ((\mu)mol g(^{-1}) wet wt gill d(^{-1}))</td>
<td>Methane maximum uptake rate</td>
<td>120</td>
<td>96–240</td>
<td>Empirical; obt. for a cold seep mussel</td>
<td>Kochevar et al. (1992)</td>
</tr>
<tr>
<td>(K_{\text{mCH}_4}) ((\mu)mol l(^{-1}))</td>
<td>Methane half-saturation constant</td>
<td>1</td>
<td>–</td>
<td>Empirical; obt. for a cold seep mussel</td>
<td>Kochevar et al. (1992)</td>
</tr>
<tr>
<td>(\gamma_M) (C-mol:CH(_4)-mol)</td>
<td>Carbon gained from methane oxidation</td>
<td>0.3</td>
<td>0.3–0.55</td>
<td>Empirical; obt. for a cold seep mussel</td>
<td>Heijnen and Van Dijken (1992), Kochevar et al. (1992)</td>
</tr>
<tr>
<td>(\delta) (C symb:Host)</td>
<td>Carbon transferred from symbionts to host</td>
<td>0.425</td>
<td>0.25–0.65</td>
<td>Empirical + calibration</td>
<td>Fiala-Médioni and Felbeck (1990)</td>
</tr>
<tr>
<td>(R) ((\mu)mol C g(^{-1}) dry wt h(^{-1}))</td>
<td>Energetic losses due to respiration</td>
<td>(e^{2.69 \times W^{0.76}})</td>
<td>0.85–1</td>
<td>Experimental + calibration</td>
<td>Dando et al. unpublished</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiration coefficient</td>
<td>0.9</td>
<td>–</td>
<td>Experimental</td>
<td>Smith (1985), Conway et al. (1992)</td>
</tr>
<tr>
<td>(\mu) (mol cm(^{-3}))</td>
<td>Organic matter ingestion coefficient</td>
<td>(6.69 \times 10^{-5})</td>
<td>–</td>
<td>Obtained for P. canaliculus</td>
<td>Ren and Ross (2005) and references therein</td>
</tr>
<tr>
<td>DE (%)</td>
<td>Organic matter digestion efficiency</td>
<td>0.753</td>
<td>0.26–0.9</td>
<td>Obtained for M. edulis</td>
<td>Bayne et al. (1989)</td>
</tr>
<tr>
<td>POC(_{\text{sat}}) (mol l(^{-1}))</td>
<td>Half-saturation constant for organic matter</td>
<td>(1.63 \times 10^{-5})</td>
<td>–</td>
<td>Obtained for P. canaliculus</td>
<td>Ren and Ross (2005) and references therein</td>
</tr>
</tbody>
</table>

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), \(\mu\) and POC\(_{\text{sat}}\) were subsequently converted to mg C.
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 (\( N = 47 \))

<table>
<thead>
<tr>
<th>Shell length (mm)</th>
<th>10</th>
<th>30</th>
<th>50</th>
<th>70</th>
<th>90</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosymbionts (mg C m(^{-2}))</td>
<td>13</td>
<td>284</td>
<td>1184</td>
<td>3031</td>
<td>6118</td>
<td>10719</td>
</tr>
<tr>
<td>( B. ) azoricus (kg wet wt m(^{-2}))</td>
<td>0.01</td>
<td>0.23</td>
<td>1.01</td>
<td>2.67</td>
<td>5.51</td>
<td>9.84</td>
</tr>
</tbody>
</table>

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 (\( \delta \)) 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), \( \delta \) was set to 90%.

3.2.4. \( B. \) azoricus filter-feeding

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

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

\( \mu \) 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 CO\(_2\) produced to oxygen consumed (RQ) of 0.9:

\[
R = aW^b \cdot RQ \quad (9)
\]

\( W \) 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 (\( N = 47 \)).
is described by the following expression (Fig. 3B):

\[ \text{GillW} = 0.2754 \cdot \text{TotalW}^{0.9681} \] (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 ±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:

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

X is the state variable (endosymbiont and *B. azoricus* biomass, in the case of the present model), P is the parameter, \( \partial \) 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$_2$S and CH$_4$ used in the model are based on values estimated for the Menez Gwen or measured at MAR, and the corresponding endosymbiont biomass variation was estimated for the Menez Gwen: 60 \( \mu \)M of H$_2$S and 100 \( \mu \)M of CH$_4$ (Sarradin, unpublished) – ENDO-MG.

(1) Only endosymbiosis with H$_2$S and CH$_4$ concentrations estimated for Menez Gwen: 60 \( \mu \)M of H$_2$S and 100 \( \mu \)M of CH$_4$ (Sarradin, unpublished) – ENDO-MG.

(2) Only endosymbiosis with H$_2$S and CH$_4$ concentrations corresponding to maximal values measured at Menez Gwen, Lucky Strike and Rainbow vent fields: 303 \( \mu \)M of H$_2$S and 177 \( \mu \)M of CH$_4$ (Desbruyères et al., 2001) – ENDO-MAX.

(3) Endosymbiosis and symbiont digestion with H$_2$S 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$_2$S and CH$_4$ estimated for the Menez Gwen: ENDOFILTER-MG.

(5) Endosymbiosis and filter-feeding with maximal measured concentrations of H$_2$S and CH$_4$: ENDOFILTER-MAX.

(6) Endosymbiosis and filter-feeding with H$_2$S and CH$_4$ concentrations corresponding to minimal values measured at Menez Gwen, Lucky Strike and Rainbow vent fields: 0.3 \( \mu \)M of H$_2$S and 0.3 \( \mu \)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 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, $F_{1,4} = 2 \times 10^{10}$, $P < 0.001$, $r^2 = 1$ 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...
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).

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⁻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.
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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.

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).

Fig. 8 – Organic matter requirements (mg POC⁻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⁻¹ d⁻¹, respectively, to fulfil their carbon needs.

azoricus must filter between 0.04 and 1.4 mgPOC⁻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 mgPOC⁻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 (±10% to the estimated 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 dependency of small mussels 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 constant for organic matter uptake and clearance rate.
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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 H\(_2\)S and CH\(_4\) at Menez Gwen (ENDOFILTER-MG) and maximal concentrations of H\(_2\)S and CH\(_4\) 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 CH\(_4\). \(B.\) azoricus reacted more significantly to variations on \(S\)-related parameters than to CH\(_4\)-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 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

| Table 3 – Sensitivity of the endosymbionts (A) and \(B.\) azoricus (B) to variations of ±10% in the parameters |
| (A) |
| Endosymbionts | Sensitivity |
| \(V_{\text{maxS}} + 10\%\) | 0.7 |
| \(V_{\text{maxS}} - 10\%\) | 0.7 |
| \(V_{\text{maxCH}_4} + 10\%\) | 0.3 |
| \(V_{\text{maxCH}_4} - 10\%\) | 0.3 |
| \(K_{\text{mS}} + 10\%\) | -0.2 |
| \(K_{\text{mS}} - 10\%\) | -0.2 |
| \(K_{\text{mCH}_4} + 10\%\) | 0 |
| \(K_{\text{mCH}_4} - 10\%\) | 0 |
| \(\gamma S + 10\%\) | 0.7 |
| \(\gamma S - 10\%\) | 0.7 |
| \(\gamma CH_4 + 10\%\) | 0.3 |
| \(\gamma CH_4 - 10\%\) | 0.3 |
| \(\delta + 10\%\) | -0.9 |
| \(\delta - 10\%\) | -1.1 |

| (B) |
| \(B.\) azoricus |
| SL (mm) | 10 | 30 | 50 | 70 | 90 | 110 |
| \(V_{\text{maxS}} + 10\%\) | 2.5 | 2.3 | 2.2 | 2.1 | 2.1 | 2.0 |
| \(V_{\text{maxS}} - 10\%\) | 2.5 | 2.3 | 2.2 | 2.1 | 2.1 | 2.0 |
| \(V_{\text{maxCH}_4} + 10\%\) | 1.1 | 1.0 | 1.0 | 0.9 | 0.9 | 0.9 |
| \(V_{\text{maxCH}_4} - 10\%\) | 1.1 | 1.0 | 1.0 | 0.9 | 0.9 | 0.9 |
| \(K_{\text{mS}} + 10\%\) | -0.6 | -0.5 | -0.5 | -0.5 | -0.5 | -0.5 |
| \(K_{\text{mS}} - 10\%\) | -0.6 | -0.5 | -0.5 | -0.5 | -0.5 | -0.5 |
| \(K_{\text{mCH}_4} + 10\%\) | 0 | 0 | 0 | 0 | 0 | 0 |
| \(K_{\text{mCH}_4} - 10\%\) | 0 | 0 | 0 | 0 | 0 | 0 |
| \(\gamma S + 10\%\) | 2.5 | 2.3 | 2.2 | 2.1 | 2.1 | 2.0 |
| \(\gamma S - 10\%\) | 2.5 | 2.3 | 2.2 | 2.1 | 2.1 | 2.0 |
| \(\gamma CH_4 + 10\%\) | 1.1 | 1.0 | 1.0 | 0.9 | 0.9 | 0.9 |
| \(\gamma CH_4 - 10\%\) | 1.1 | 1.0 | 1.0 | 0.9 | 0.9 | 0.9 |
| \(\delta + 10\%\) | 0 | 0 | 0 | 0 | 0 | 0 |
| \(\delta - 10\%\) | 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 |
| \(POD_{\text{sat}} + 10\%\) | -8.2 | -5.4 | -3.0 | -1.9 | -1.2 | -0.8 |
| \(POD_{\text{sat}} - 10\%\) | -17.8 | -6.6 | -3.7 | -2.3 | -1.5 | -0.9 |
| \(\mu + 10\%\) | 16.3 | 6.0 | 3.4 | 2.1 | 1.4 | 0.9 |
| \(\mu - 10\%\) | 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.
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 µg C for the carbon content of marine bacteria, which follows within the literature range of 10⁻¹⁰–10⁻⁸ cell µg C (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-

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 (File 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 demonstrates a spatial transmission of endosymbionts in the genus Bathymodiolus (Won et al., 2003; Kádár et al., 2005).
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 $10^7$ to $10^9$ bacteria h$^{-1}$ (assuming $10^8$ cell µg 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 $10^9$ 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 $10^5$ 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 sp. is one of the last vent groups to survive after flow ceases at hydrothermal 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$_2$S, 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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