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

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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 and 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_2S and CH_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 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 endosymbioisis 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.

Keywords: *Bathymodiolus azoricus*; Endosymbiosis; Filter-feeding; Size; Organic matter; Carbon-flux model

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] and [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] and [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] and [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] and [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, 1993; 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. Although, food web characterization is required as an initial step in understanding an ecosystem (Link, 2002), in vent mussel communities the complex balance of heterotrophy and autotrophy still remains to be explored (Pile and Young, 1999). 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. 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).

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. 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.

MATERIAL AND METHODS

Model conceptualization. A carbon (C) flux model was developed to describe the energy flow through *Bathymodiolus 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 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 (POC) by *B. azoricus*, the transfer of energy from the symbionts to the host and the energetic wastes of the *B. azoricus* (Fig. 2). The flow units of the model are mg C (carbon) d^{-1}. The considered average biomass of *B. azoricus* was 500 ind m^{-2} according to local observations by Colaço et al. (1998). The model assumes no limiting conditions of O_2 or DIC.

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

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

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

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

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

104 sulphide (S) and methane (M) by endosymbionts ($V_{S,M}$) was described by a single Michaelis-
105 Menten kinetics:

106 $$V_{S,M} = Vmax_{S,M} \cdot \frac{[S,M]}{Km_{S,M} + [S,M]} \quad (3)$$

107 $Vmax_{S,M}$ - Maximum uptake rate of sulphide (S) or methane (M) (μmol g^{-1} gill dry wt d^{-1} using a
108 gill dry wt: gill wet wt = 0.162 based on *B. azoricus* from the Menez Gwen (N=39)), $Km_{S,M}$ -
109 Half-saturation constant for the uptake of sulphide or methane (μmol l^{-1}), [S,M] - Concentration
110 of sulphide or methane (μmol l^{-1}).
111 In the available literature, we found no values for maximum uptake rates of sulphide or methane
112 by *B. azoricus*, therefore, we had to use values reported for similar species. Like vent mussels,
113 methane mussels also uptake reduced substrates, mostly, through their gills (e.g. Le Pennec at
114 al., 1990) at rates ranging from 96 – 240 μmol g^{-1} wet wt d^{-1} (Kochevar et al., 1992). Methane
115 mussels only have methanotrophs in their gills, while *B. azoricus* has both thiotrophs and
116 methanotrophs. Possibly, the uptake of methane by methane mussels is higher than the uptake of
117 methane by *B. azoricus*, which can also uptake sulphide. For this reason, we assumed that the
118 average rate of methane uptake by methane mussels corresponds to the maximum methane
119 uptake rate for *B. azoricus* (i.e. 120 μmol g^{-1} wet wt gill d^{-1}).
120 Model routine was used to obtain the maximum uptake of sulphide by *B. azoricus* ($Vmax_S$)
121 while assuming that:

122 - $Vmax_M$ = 120 μmol CH_4 g^{-1} wet wt gill d^{-1}
123 - A certain size mussel and the corresponding biomass assuming a density of 500 ind m^{-2}
124 - Endosymbiont biomass correspond 4% of the gill weight; because endosymbiont biomass
125 depends on $Vmax_S$, the 4% value acts as a constraint that limits the variation of $Vmax_S$

126 The value that fulfilled the above pre-requisites was 743 μmol H_2S g^{-1} wet wt gill d^{-1}.
127

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 the nature be 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: CH4, the biomass yield of sulphide was set at 0.16 mol CO_2. These processes were described by:

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

S- Carbon gain from sulphide oxidation, V_S – Sulphide uptake, γ_S – Biomass yield of sulphide and

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

M- Carbon gain from methane oxidation, V_M – Methane uptake, γ_M – Biomass yield of methane

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 to 65% of the carbon fixed by the symbionts (δ) is for the host nutrition. Thus, T in equations 1 and 2 is defined as:

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

The value 43% of carbon transferred from symbionts to *B. azoricus* was obtained through model calibration (see **Calibration**). For the scenario (see **Simulations**), 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%.

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

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

μ - Ingestion coefficient (mol cm^{-3} converted to mg l^{-1}), CR - Clearance rate (l d^{-1}), p - Functional response of particulate organic matter (POC), DE - Digestion efficiency (%).

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

POC - Organic matter concentration (mg l^{-1}), POCsat – Half- saturation constant for POC (mg l^{-1}).

Bathymodiolus azoricus energy wastes. In the present model, the energy wastes of *B. azoricus* were described by an allometric relation, which accounts for respiration assuming a molar ratio of CO_2 produced to oxygen consumed (RQ) of 0.9 :

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

W – Weight of *B. azoricus* (g dry wt), a and b - 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).

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. 3A) and on an average density value of *B. azoricus* observed, *in situ*, corresponding 550 individuals.m^{-2} (Colaço et al., 1998). To facilitate comparison with published data, *B. azoricus* biomass was converted to kg wet wt m^{-2}. The relation between the 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):

178 $$\mathrm{GillW} = 0.2754 \cdot \mathrm{TotalW}^{0.9681} \qquad (10)$$

179 Endosymbiont biomass was estimated assuming that endosymbionts correspond to 4% of the gill
180 wet weight of *B. azoricus* according with the value estimated for *B. thermophilus* (Powell and
181 Somero, 1986), using a gill wet wt:gill dry wt =6.2 based on *B. azoricus* from the Menez Gwen
182 (N=39) and a C: dry wt for endosymbionts of 0.5 (Bratbak 1985).
183
184 **Calibration.** The model was calibrated through the trial-error method, against the estimated
185 biomass of endosymbionts and *B. azoricus* at the Menez Gwen. As previously mentioned,
186 throughout the calibration process, the constraint that endosymbionts correspond to 4% of *B.*
187 *azoricus* gill wet weight was respected.
188
189 **Sensitivity analysis**. Sensitivity analysis was performed after imposing variations within the
190 range ± 10% to each parameter, while all the others were kept unchanged. Sensitivity to external
191 conditions was also tested by the series of performed simulation scenarios (see **Simulations**),
192 which incorporated ranges of variations for external concentrations based on real measurements:
193 0.3-303 μM H_2S, 0.3-177 μM CH_4 and 0-0.008 mg l^{-1} POC.
194 To estimate the sensitivity of parameters, the following expression (Jørgensen, 1994) was used:
195

196 $$S = \frac{\left[\partial X / X\right]}{\left[\partial P / P\right]} \qquad (11)$$

197 X- State variable (endosymbiont and *B. azoricus* biomass, in the case of the present model), P-
198 Parameter, ∂ - Variation between the final and the initial values
199
200 **Simulations.** In every simulation, a certain initial weight of *B. azoricus* (in mg C) and the
201 corresponding weight of endosymbionts (in mg C) were considered to initialize the model. At
202 each run, the model assesses if an individual mytilid, with a certain weight and harbouring the

corresponding biomass of endosymbionts, can sustain 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 could sustain 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 µM of H_2S and 100 µM 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 µM of H_2S and 177 µM 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 µM of H_2S and 0.3 µM 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).

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RESULTS

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 of 10 to 110 mm SL (shell length), 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, $F_{1, 4}=2x10^8$, $P<0.001$, $r^2=1$ and ANOVA, $F_{1, 4}=1x10^{11}$, $P<0.001$, $r^2=1$, respectively) (Fig. 4A and B).

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* can not 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 mussel's size. 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 H_2S and CH_4 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 H_2S and CH_4 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 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. azoricus* must filter between 0.04 to 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).

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

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 decreases as the mytilid size increases. This indicates the stronger dependency 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 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).

DISCUSSION

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

Considering the size range 10-110 mm shell length, estimated biomass of *B. azoricus* at the Menez Gwen varies between 0.01 and 10 kg wet wt m^{-2} (average = 3.2 kg wet wt m^{-2}), while the corresponding endosymbiont biomass ranges 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 500 ind m^{-2} according to *in situ* observations (Colaço et al., 1998).

Assuming a value of 10^8 cell μg C for the carbon content of marine bacteria, which follows within the literature range of 10^7-10^{10} cell μg C (Page et al., 1990 and references therein; Schippers et al., 2005), our estimations of endosymbiont biomass correspond to 3.8 x 10^{11} endosymbionts g^{-1} wet wt gill, which is in agreement with reported values of endosymbiont

306 abundance for *B. thermophilus* (1.70-1.81 x 10^{11} g^{-1} wet wt- Powell and Somero, 1986) and a
307 mytilid of the Mariana Back-arc basin (0.8 – 2 x 10^{11} g^{-1} wet wt gill- Yamamoto et al., 2002).
308 The fact that model predictions show a very high fitting level with estimated biomass values for
309 both *B. azoricus* and its endosymbionts reflects a general correct incorporation and description of
310 processes in the model, as well as a consistent calibration. In practical terms, this confers
311 robustness to model results and predictions for the tested scenarios.
312
313 **Flexibility of *B. azoricus*'s nutritional strategy throughout life**
314 Model results suggest that the dominant nutritional strategy of *B. azoricus* varies with body size
315 and external conditions. Small and, presumably, young mytilids can not derive enough energy
316 from endosymbiosis to account for their energetic needs and, thus, filter-feeding must play an
317 important role in their nutrition. Gradually, as the mussel increases size, the amount of energy
318 derived from microbial oxidations also increases and, potentially, under non-limiting
319 concentrations of H_2S and CH_4, *B. azoricus* is able to increase the ratio endosymbiosis: filter-
320 feeding. At very high availability of H_2S and CH_4 (> 300 μM H_2S and > 150 μM CH_4), the
321 largest and presumably older mytilids (≥ 110 mm SL) can derive all their energy from
322 endosymbiosis. However, if the concentrations of external H_2S and CH_4 decrease, larger *B.*
323 *azoricus* can increase the contribution of filter-feeding to meet their energetic demands, as long
324 as the external availability of organic matter allows it. The highest nutritional flexibility of larger
325 mussels compared to smaller ones is related with the type of allometric relationship between gill
326 weight and the uptake of H_2S and CH_4 by *B. azoricus* (Fig. 10). Based on 153 individuals
327 collected in different years (2001, 2006 and 2007) and different seasons (summer, fall and
328 winter), the average size of *B. azoricus* at Menez Gwen was 60 mm SL. According to the present
329 model and for concentrations of 60 μM H_2S and 100 CH_4, mytilids of 60 mm SL obtain about
330 58% of their energy from endosymbiois and the rest from filter-feeding (42%), if the available
331 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 environmental transmission of endosymbionts in the genus *Bathymodiolus* (Won et al., 2003; Kádár et al., 2005).

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 hypothise 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).

POC requirements of *B. azoricus*

In bibliography, we found a general lack of information concerning POC concentrations at vent fields. The exception was some values reported for vents at the Galapagos Rift, ranging between 106 – 207 $\mu g\ l^{-1}$ (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 – 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: ~ 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* (~ 10^4 and ~ 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 (DOM) 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* (Comtet et al., 2000; Dixon et al., 2006).

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.

. 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 regulation 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.

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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LITERATURE CITED

Bayne BL, Hawkins AJS, Navarro E, Iglesias IP (1989) Effects of seston concentration on feeding digestion and growth in the mussel *Mytilus edulis*. Mar Ecol Prog Ser 55:47-54.

Bratbak G (1985) Bacterial biovolume and biomass estimations. Appl Environ Microbiol 49(6):1488-1493

Cavanaugh CM, Wirsen CO, Jannasch HW (1992) Evidence for methylotrophic bacteria in a hydrothermal-vent mussel (Bivalvia: Mytilidae) from the Mid-Atlantic Ridge. Appl Environ Microbiol 58:3799-3803

Childress JJ, Fisher CR, Brooks JM, Kennicutt MC, Bidigare R, Anderson AE (1986) A methanotrophic marine molluscan (Bivalvia, Mytilidae) symbiosis: Mussels fuelled by gas. Science 233 (4770):1306-1308

Colaço A, Desbruyères D, Comtet T, Alayse AM (1998) Ecology of the Menez Gwen hydrothermal vent field (Mid-Atlantic Ridge/Azores Triple Junction). Cah Biol Mar 39:237-240

Comtet T, Desbruyères D (1998) Population structure and recruitment in mytilid bivalves from the Lucky Strike and Menez Gwen hydrothermal vent fields (37º17' N and 37º50' N on the Mid-Atlantic Ridge) Mar Ecol Prog Ser 163:165-177

436

437 Conway NM, Howes BL, Capuzzo JEM, Turner RD, Cavanaugh CM (1992) Characterization
438 and site description of *Solemya borealis* (Bivalvia; Solemyidae), another bivalve-bacteria
439 symbiosis. Mar Biol 112:601-613

440 Desbruyères D, Biscoito M, Caprais JC, Colaço A, Comtet T, Crassous P, Fouquet Y,
441 Khripounoff A, Le Bris N, Olu K, Riso R, Sarradin PM, Segonzac M, Vangriesheim A
442 (2001) Variations in deep-sea hydrothermal vent communities on the Mid-Atlantic Ridge
443 near the Azores plateau. Deep-Sea Res Part I 48:1325-1346

444 Distel DL, Lee HKW, Cavanaugh CM (1995) Intracellular coexistence of methano- and
445 thioautotrophic bacteria in a hydrothermal vent mussel. Proc. Natl. Acad. Sci. USA 92:9598-
446 9602

447 Dixon DR, Lowe DM, Miller PI, Villemin GR, Colaço A, Serrão-Santos R, Dixon LRJ (2006)
448 Evidence of seasonal reproduction in the Atlantic vent mussel *Bathymodiolus azoricus*, and
449 an apparent link with the timing of photosynthetic primary production. J Mar Biol Ass UK
450 86:1363-1371

451 Fiala-Médioni A, Métivier C, Herry A, Le Pennec M (1986) Ultrastructure of the gill of the
452 hydrothermal-vent mytilid *Bathymodiolus* sp. Mar Biol 92:65-72

453 Fiala-Médioni A, Felbeck H (1990) Autotrophic processes in invertebrate nutrition: Bacterial
454 symbiosis in bivalve molluscs. Mellinger J (ed): Animal Nutrition and Transport Processes.
455 1. Nutrition in Wild and Domestic Animals. Comp Physiol. Basel, Karger, vol 5, pp 49-69

456 Fiala-Médioni A, McKiness ZP, Dando P, Boulegue J, Mariotti A, Alayse-Danet AM, Robinson
457 JJ, Cavanaugh CM (2002) Ultrastructural, biochemical, and immunological characterization
458 of two populations of the mytilid mussel *Bathymodiolus azoricus* from the Mid-Atlantic
459 Ridge: evidence for a dual symbiosis. Mar Biol 141:1035-1043

460 Fisher CR, Childress JJ, Oremland RS, Bidigare RR (1987) The importance of methane and
461 thiosulfate in the metabolism of the bacterial symbionts of two deep-sea mussels. Mar Biol
462 96:59-71
463 Fustec A, Desbruyères D, Laubier L (1988) Estimation de la biomasse des peuplements associés
464 aux sources hydrothermales profondes de la dorsale du Pacifique oriental à 13ºN. Oceanol
465 Acta 8 :15-21
466 Giere O, Borowski C, Prieur D (2003) Biological productivity in hydrothermal systems, Chapter
467 10. Energy and Mass Transfer in Marine Hydrothermal Systems. Edited by Halbach PE,
468 Tunnicliffe V, Hein JR. Dahlem University Press, pp211-233
469 Goffredi SK, Barry P (2002) Energy acquisition and allocation in vesicomyid symbioses. Cah
470 Biol Mar 43:345-350
471 Heijnen JJ, Van Dijken JP (1992) In search of a thermodynamic description of biomass yields
472 for the chemotrophic growth of microorganisms. Biotechnol Bioeng 39:833-858
473 Ingvorsen K, Zehnder AJB, Jørgensen BB (1984) Kinetics of sulfate and acetate uptake by
474 *Desulfobacter postgatei*. Appl Environ Microbiol 47(2):403-408
475 Järnegren J, Altin D (2006) Filtration and respiration of the deep sea living bivalve *Acesta*
476 *excavata* (J.C. Fabricius, 1779) (Bivalvia; Limidae) J Exp Mar Biol Ecol 344:122-129
477 Jørgensen SE (1994). Fundamentals of Ecological Modelling, 2nd Edition, Elsevier, Amsterdam.
478 Kádár E, Bettencourt R, Costa V, Serrão Santos R, Lobo-da-Cunha A, Dando P (2005)
479 Experimentally induced endosymbiont loss and re-acquirement in the hydrothermal vent
480 bivalve *Bathymodiolus azoricus*. J Exp Mar Biol Ecol 318:99-110
481 Kochevar RE, Childress JJ, Fisher CR, Minnich E (1992) The methane mussel: roles of symbiont
482 and host in the metabolic utilization of methane. Mar Biol 112:389-401
483 Link J (2002) Does food web theory work for marine ecosystems? Mar Ecol Prog Ser 230:1-9
484 Le Pennec M, Donval A, Herry A (1990) Nutritional strategies of the hydrothermal ecosystem
485 bivalves. Prog Oceanogr 24:71-80

Levesque C, Limén H, Juniper SK (2005) Origin, composition and nutritional quality of particulate matter at deep-sea hydrothermal vents on Axial Volcano, NE Pacific. Mar Ecol Prog Ser 289:45-52

Lovley DR (1985) Minimum threshold for hydrogen metabolism in methanogenic bacteria. Appl Environ Microbiol 49(6):1530-1531

Manahan DT (1983) The uptake and metabolism of dissolved amino acids by bivalve larvae. Biol Bull 164:236-250

Megrey BA, Rose KA, Klumb RA, Hay DE, Werner FE, Eslinger DL, Smith SL (2007). A bioenergetics-based population dynamics model of Pacific herring (Clupea harengus pallasi) coupled to a lower trophic level nutrient-phytoplankton-zooplankton model: Description, calibration and sensitivity analysis. Ecol Model 202 (1-2): 144-164

Moloney CL, Field JG (1989) General allometric equations for rates of nutrient uptake, ingestion, and respiration in plankton organisms. Limnol Oceanogr 34(7):1290-1299

Needham HD (1996) Some features of the North America-Africa plate boundary. J Conf Abs 1:834-835

Olu K, Sibuet M, Harmegnies F, Foucher J-P, Fiala-Médioni A (1996) Spatial distribution of diverse cold seep communities living on various diapiric structures of the southern Barbados prism. Prog Oceanogr 38:347-376

Page HM, Fisher CR, Childress JJ (1990) Role of filter-feeding in the nutritional biology of a deep-sea mussel with methanotrophic symbionts. Mar Biol 104: 251-257

Pile AJ, Young CM (1999) Plankton availability and retention efficiencies of cold-seep symbiont mussels. Limnol Oceanogr 44(7):1833-1839

Pond DW, Bell MV, Dixon DR, Fallick AE, Segonzac M, Sargent JR (1998) Stable-carbon-isotope composition of fatty acids in hydrothermal vent mussels containing methanotrophic and thiotrophic bacterial endosymbionts. Appl Environ Microbiol 64:370-375

511 Powell MA, Somero GN (1986) Adaptations to sulphide by hydrothermal vent animals: sites and
512 mechanisms of detoxification and metabolism. Biol Bull 171:274-290
513 Raulfs EC, Macko SA, Van Dover CL (2004) Tissue and symbiont condition of mussels
514 (*Bathymodiolus thermophilus*) exposed to varying levels of hydrothermal activity. J Mar Biol
515 Ass U K 84:229-234
516 Ren JS, Ross AH (2005) Environmental influence on mussel growth: A dynamic energy budget
517 model and its application to the greenshell mussel *Perna canaliculus*. Ecol. Model.
518 189:347-362
519 Sarradin PM, Caprais JC, Riso R, Kerouel R, Aminot A (1999) Chemical environment of the
520 hydrothermal mussel communities in the Lucky Strike and Menez Gwen vent fields, Mid
521 Atlantic Ridge. Cah Biol Mar 40:93-104
522 Schippers A, Neretin LN, Kallmeyer J, Ferdelman TG, Cragg BA, Parkes RJ, Jørgensen BB
523 (2005) Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria.
524 Nature 433:861-864
525 Siebers D, Winkler A (1984) Amino-acid uptake by mussels, *Mytilus edulis*, from natural sea
526 water in a flow-through system. Helgol Mar Res 38(1):189-199
527 Smith, KL (1985) Deep-sea hydrothermal vent mussels: nutritional state and distribution at the
528 Galapagos Rift. Ecology 66(3):1067-1080
529 Tuttle JH (1985) The role of sulfur-oxidizing bacteria at deep-sea hydrothermal vents. Biol Soc
530 Wash Bull 6:335-343
531 Unanue M, Ayo B, Agis M, Slezak D, Herndl GJ, Iriberri J (1999) Ectoenzymatic activity and
532 uptake of monomers in marine bacterioplankton described by a biphasic kinetic model.
533 Microb Ecol 37:36-48
534 Van Dover CL, Desbruyères D, Segonzac M, Comtet T, Saldanha L, Fiala-Médioni A, Langmuir
535 C (1996) Biology of the Lucky Strike hydrothermal field. Deep-Sea Res Part I 43(9):1509-
536 1529

Wendt DE, Johnson CH (2006) Using latent effects to determine the ecological importance of dissolved organic matter to marine invertebrates Int Comp Biol 46(5):634-642

Won Y-J, Hallam SJ, O'Mullan GD, Pan IL, Buck KR, Vrijenhoek RC (2003) Environmental acquisition of thiotrophic endosymbionts by deep-sea mussels of the genus *Bathymodiolus*. Appl Environ Microbiol 69(11):6785-6792

Yamamoto H, Fujikura K, Hiraishi A, Kato K, Maki Y (2002) Phylogenetic characterization and biomass estimation of endosymbionts associated with invertebrates dwelling in chemosynthetic communities of hydrothermal vent and cold seep fields. Mar Ecol Prog Ser 245:61-67

563 Figure 1- The hydrothermal vent fields south-west the Azores Triple Junction at the Mid-Atlantic
564 Ridge (MAR).
565
566 Figure 2- Simplified conceptual diagram of the *Bathymodiolus azoricus*-endosymbiont C-flux
567 model. SL- Shell length, W- Weight, H_2Sext, CH_4ext and POCext- Environmental
568 concentrations of sulphide, methane and particulate organic matter, respectively. SulphideOxid
569 and MethaneOxid- Sulphide and methane oxidation by endosymbionts, respectively. TransferC-
570 Transfer of carbon from endosymbionts *to B. azoricus*. See Table 2 and text for parameter
571 definition.
572
573 Figure 3- Shell length (mm) *versus* weigh (g dry wt of soft tissue) (N=47) (**A**) and gill weight (g
574 dry wt) *versus* total weight (g dry wt) (N=153) (**B**) of *B. azoricus* collected at Menez Gwen.
575
576 Figure 4- Predicted *versus* observed biomass of *B. azoricus* (kg wet wt m^{-2}) (**A**) and
577 endosymbionts (mg C m^{-2}) (**B**). The two regressions are highly significant: ANOVA, $F_{1,}$
578 $_4=2x10^8$, $P<0.001$, $r^2=1$ and ANOVA, $F_{1,4}=1x10^{11}$, $P<0.001$, $r^2=1$, respectively.
579
580 Figure 5- Model predictions for biomass variation of *B. azoricus* of different sizes (shell length-
581 SL): 10, 50 and 110 mm (**A**, **B** and **C**, respectively), with endosymbiosis as the only carbon
582 source and under different conditions: H_2S and CH_4 concentrations estimated for Menez Gwen-
583 ENDO-MG (——), maximal H_2S and CH_4 concentrations measured at MAR- ENDO-MAX (----)
584 and maximal concentrations with digestion of symbionts- ENDODIGEST-MAX (**……**).
585
586 Figure 6- Model predictions for biomass variation of *B. azoricus* of different sizes: 0.01 kg wet
587 wt m^{-2} corresponding to 10 mm SL (——), 1.01 kg wet wt m^{-2} corresponding to 50 mm SL (----)

588 and 9.84 kg wet wt m^{-2} corresponding to 110 mm SL (**……**) with endosymbiosis and filter-
589 feeding as carbon sources and under external concentrations of H_2S and CH_4 estimated for
590 Menez Gwen (ENDOFILTER-MG) (**A**). The relative contribution of filter-feeding (□) and
591 endosymbiosis (■) varies with the size of *B. azoricus*.
592
593 Figure 7- Relative contribution of filter-feeding (□) and endosymbiosis (■) (%) to the total
594 energy budget of *B. azoricus* of different sizes (SL- shell length) under maximal concentrations
595 of H_2S and CH_4 estimated for Menez Gwen (ENDOFILTER-MAX) (**A**) and minimal
596 concentrations of H_2S and CH_4 measured at MAR (ENDOFILTER-MIN) (**B**).
597
598 Figure 8- Organic matter needs (mg POC l^{-1} d^{-1}) of *B. azoricus* of different sizes if filter-feeding
599 is the only carbon source (FILTER). Individuals with 10-, 50- and 110 mm SL require 0.05, 0.52
600 and 9.1 mg POC l^{-1} d^{-1}, respectively, to fulfil their nutritional needs.
601
602 Figure 9- Organic matter needs (number bacteria h^{-1}) of *B. azoricus* of different sizes (**A**- 10 mm
603 Sl, **B**- 50 mm SL and **C**- 110 mm SL) and under different scenarios: only with filter-feeding
604 (FILTER), with filter-feeding and endosymbiosis with external concentrations of H_2S and CH_4 at
605 Menez Gwen (ENDOFILTER-MG) and maximal concentrations of H_2S and CH_4 at MAR
606 (ENDOFILTER-MAX). *B. azoricus* with 110 mm SL and under ENDOFILTER-MAX scenario
607 can rely completely on endosymbiosis for nutrition.
608
609 Figure 10- Relationship between sulphide uptake (**A**) and methane uptake (**B**) (µmol g^{-1} wet wt
610 gill d^{-1}) with gill weight (g wet wt) in B. azoricus.
611
612

Table 1- Parameter definition, values and mathematical expressions used in the standard run and information about literature range and methods used to obtain the final values. 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 mg C.

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 *M. edulis*	Järnegren and Altin, 2006
VmaxS ($\mu mol\ g^{-1}$wet wt gill d^{-1})	Sulphide maximum uptake rate	743	14 – 96	Empirical+ calibration	Dando et al. unpublished
KmS ($\mu mol\ l^{-1}$)	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
$VmaxCH_4$ ($\mu mol\ g^{-1}$ wet wt gill d^{-1})	Methane maximum uptake rate	120	96 - 240	Empirical; obt. for a cold seep mussel	Kochevar et al. 1992
$KmCH_4$ ($\mu mol\ l^{-1}$)	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 (Csymb:Chost)	Carbon transferred from symbionts to host	0.425	0.25 - 0.65	Empirical + calibration	Fiala-Medioni and Felbeck 1990
R ($\mu mol\ C\ g^{-1}$ 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	$6.69x10^{-5}$	-	Obtained for *P. canaliculus*	Ren and Ross 2005 and references therein
DE (%)	Organic matter digestion efficiency	0.753	0.26 – 0.9	Obtained for *M. edulis*	Bayne et al. 1989
POC_{sat} ($mol\ l^{-1}$)	Half-saturation constant for organic matter	$1.63x10^{-5}$	-	Obtained for *P. canaliculus*	Ren and Ross 2005 and references therein

Table 2- Estimated biomass *B. azoricus* and corresponding endosymbionts at the Menez Gwen, assuming an average density of 500 ind m^{-2} (Colaço et al., 1998) and based on a significant shell length-dry weight regression for *B. azoricus* (N=47).

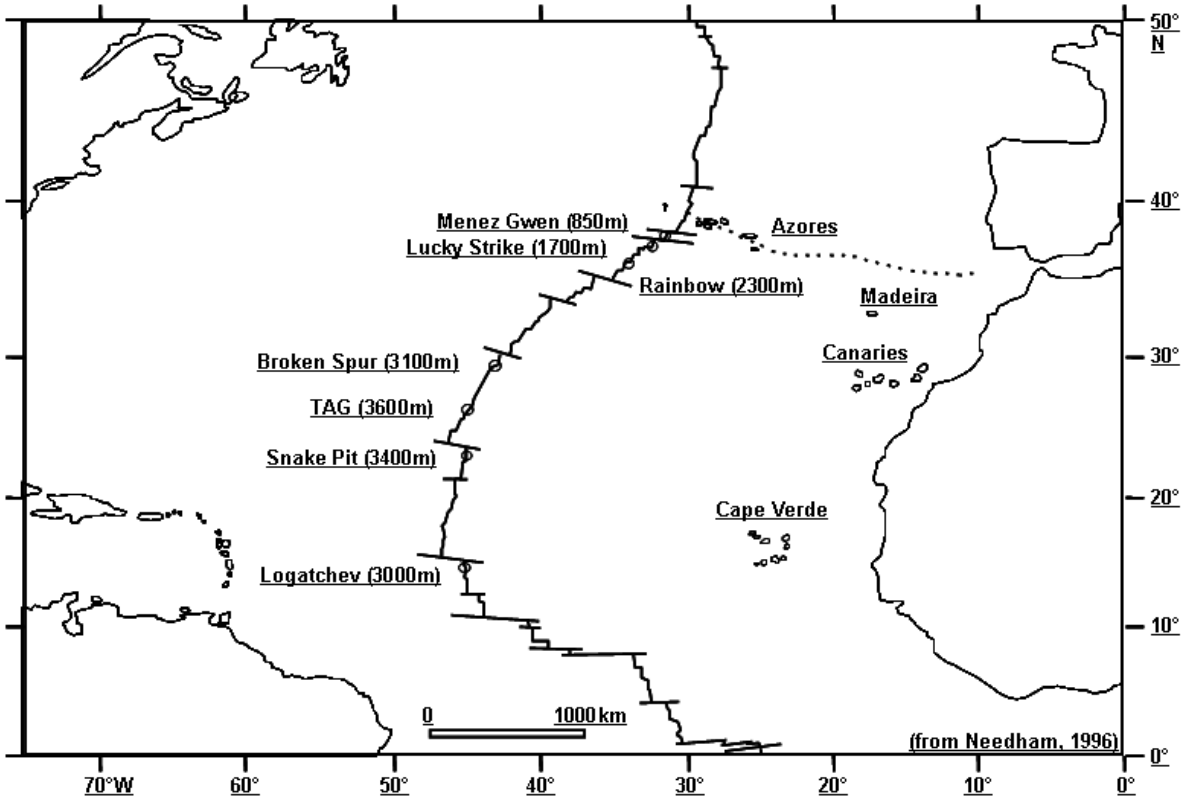
Shell length (mm)	10	30	50	70	90	110
Endosymbionts (mg C m^{-2})	13	284	1184	3031	6118	10719
***B. azoricus* (kg wet wt m^{-2})**	0.01	0.23	1.01	2.67	5.51	9.84

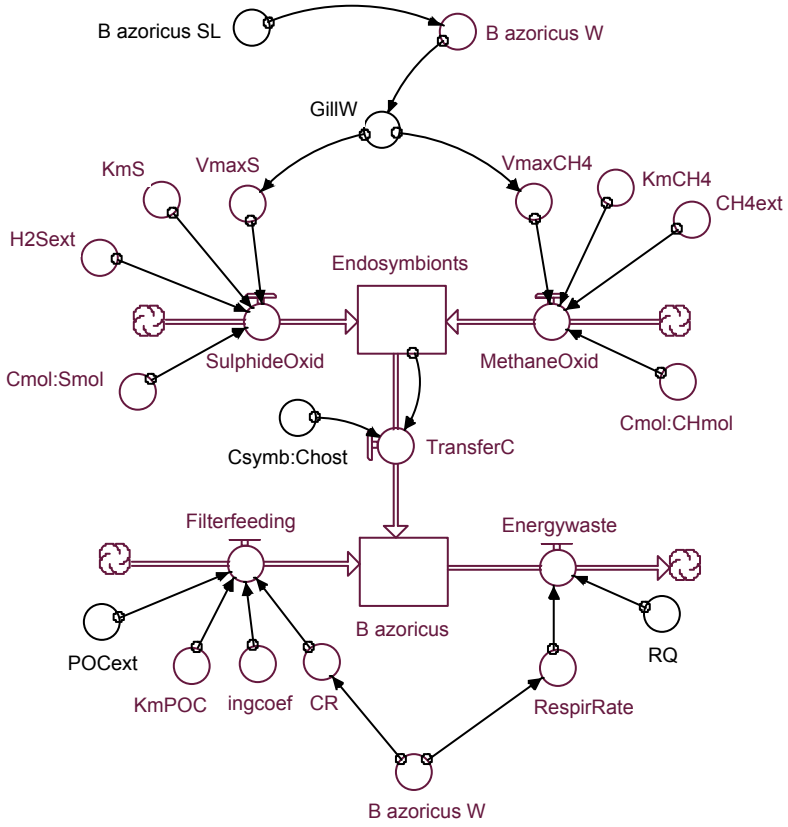
Table 3- Sensitivity of the endosymbionts (A) and *B. azoricus* (B) to variations of ± 10% in the parameters. The result is a positive or a negative number. The absolute value represents the distance to the initial value of the state variable. The negative and the positive sign indicate that the state variable and the parameters vary inversely or in the same way, respectively.

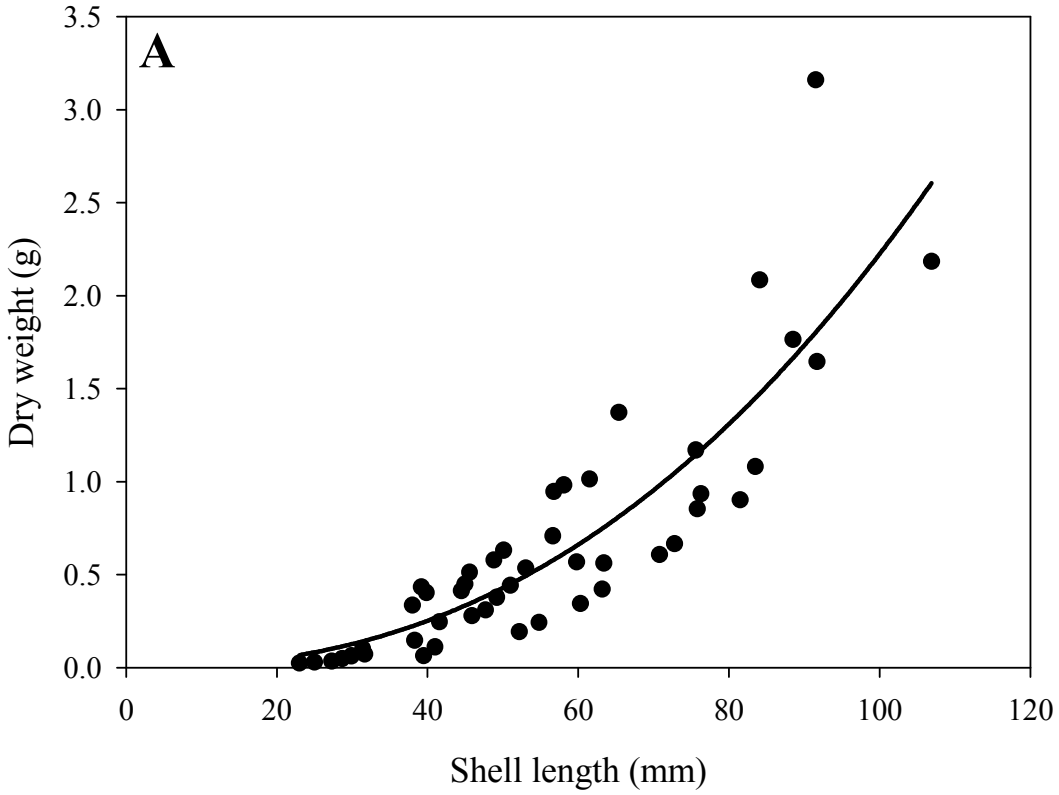
A- Endosymbionts	**Sensitivity**
$V_{max}S$ +10%	0.7
$V_{max}S$ -10%	0.7
$V_{max}CH_4$ +10%	0.3
$V_{max}CH_4$ -10%	0.3
K_mS +10%	-0.2
K_mS -10%	-0.2
K_mCH_4 +10%	0
K_mCH_4 -10%	0
γ_S +10%	0.7
γ_S -10%	0.7
γ_{CH4} +10%	0.3
γ_{CH4} -10%	0.3
δ +10%	-0.9
δ -10%	-1.1

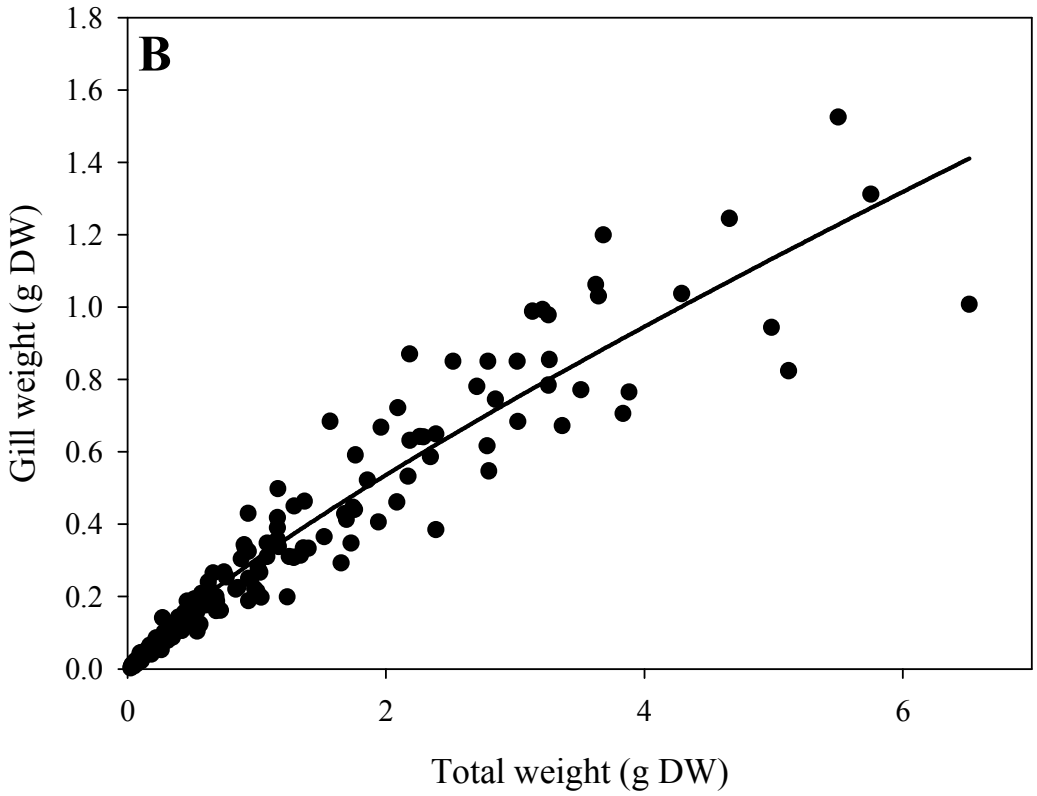
B- B. azoricus	**Sensitivity**					
SL (mm)	**10**	**30**	**50**	**70**	**90**	**110**
$V_{max}S$ +10%	2.5	2.3	2.2	2.1	2.1	2.0
$V_{max}S$ -10%	2.5	2.3	2.2	2.1	2.1	2.0
$V_{max}CH_4$ +10%	1.1	1.0	1.0	0.9	0.9	0.9
$V_{max}CH_4$ -10%	1.1	1.0	1.0	0.9	0.9	0.9
K_mS +10%	-0.6	-0.5	-0.5	-0.5	-0.5	-0.5
K_mS -10%	-0.6	-0.5	-0.5	-0.5	-0.5	-0.5
K_mCH_4 +10%	0	0	0	0	0	0
K_mCH_4 -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

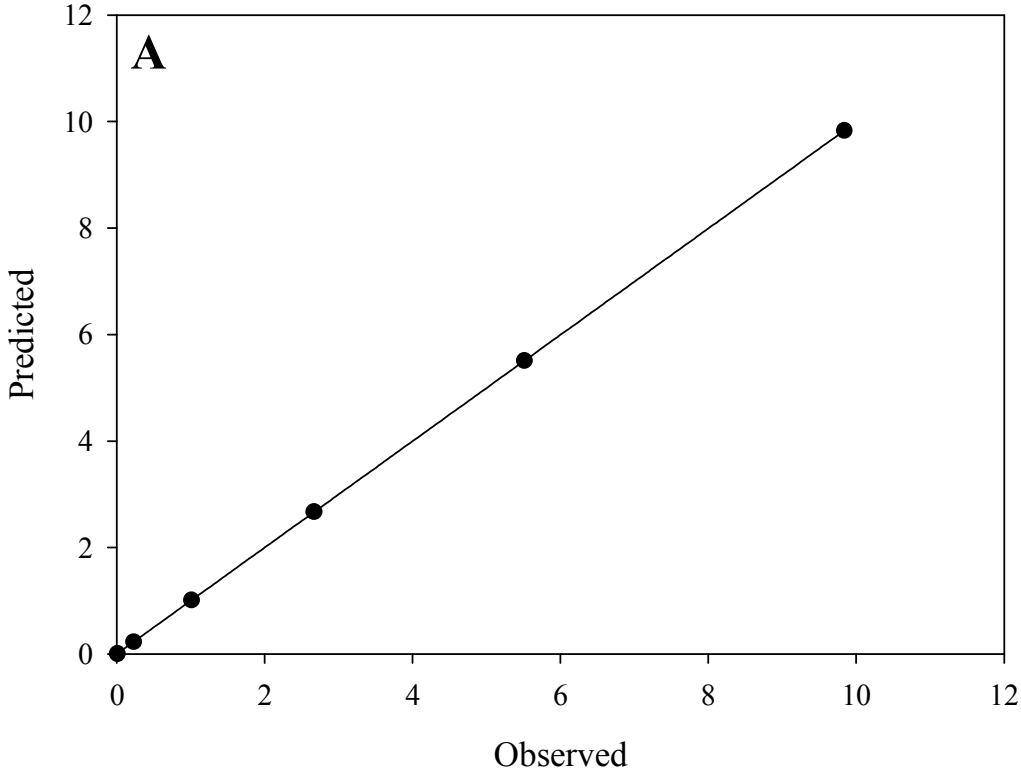
Figure 1

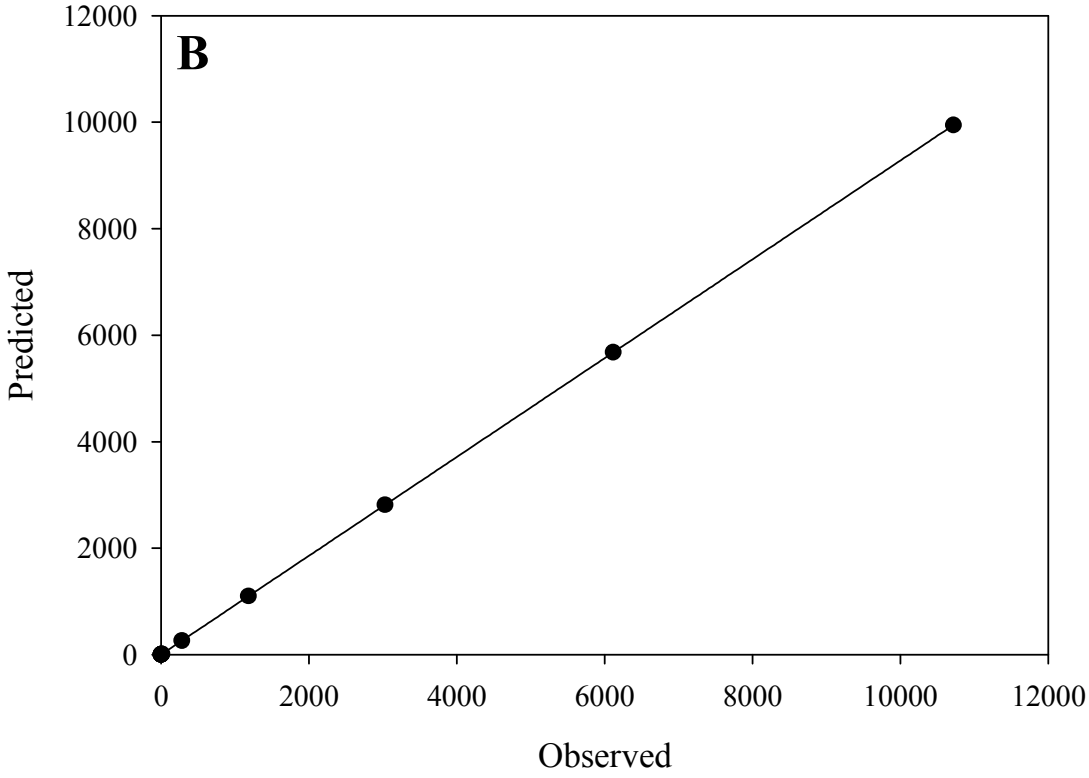

Menez Gwen (850m)
Lucky Strike (1700m)
Rainbow (2300m)
Azores
Madeira
Canaries
Broken Spur (3100m)
TAG (3600m)
Snake Pit (3400m)
Cape Verde
Logatchev (3000m)
0
1000 km
(from Needham, 1996)
50° N
40°
30°
20°
10°
0°
70°W
60°
50°
40°
30°
20°
10°
0°

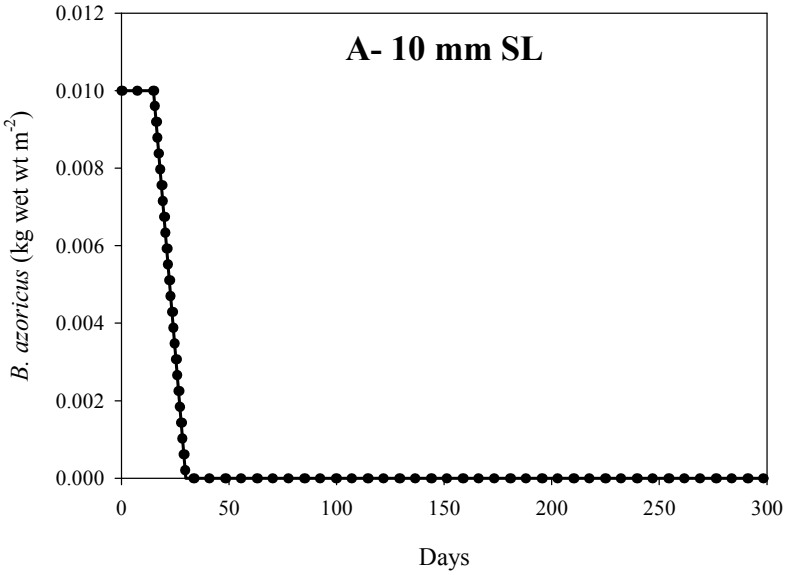

B azoricus SL
B azoricus W
GillW
KmS
VmaxS
VmaxCH4
KmCH4
CH4ext
H2Sext
Endosymbionts
SulphideOxid
MethaneOxid
Cmol:Smol
Cmol:CHmol
Csymb:Chost
TransferC
Filterfeeding
Energywaste
B azoricus
POCext
RQ
KmPOC
ingcoef
CR
RespirRate
B azoricus W

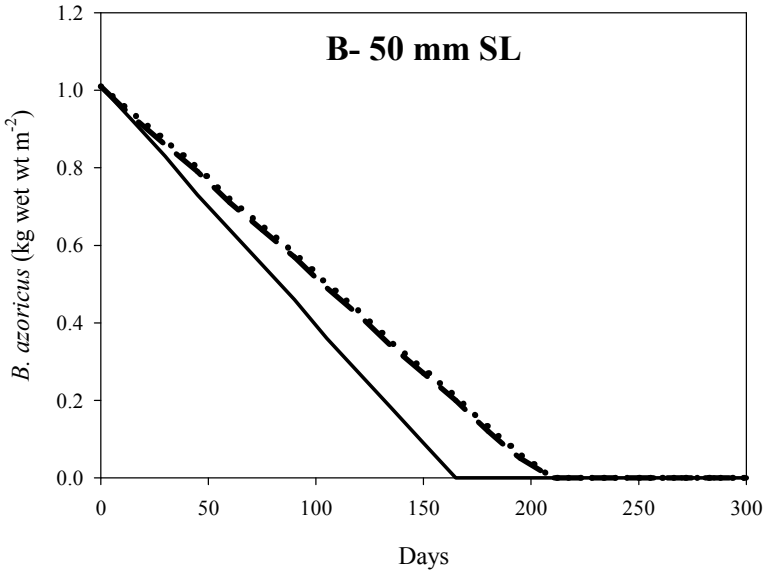

A
Dry weight (g)
Shell length (mm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
20
40
60
80
100
120

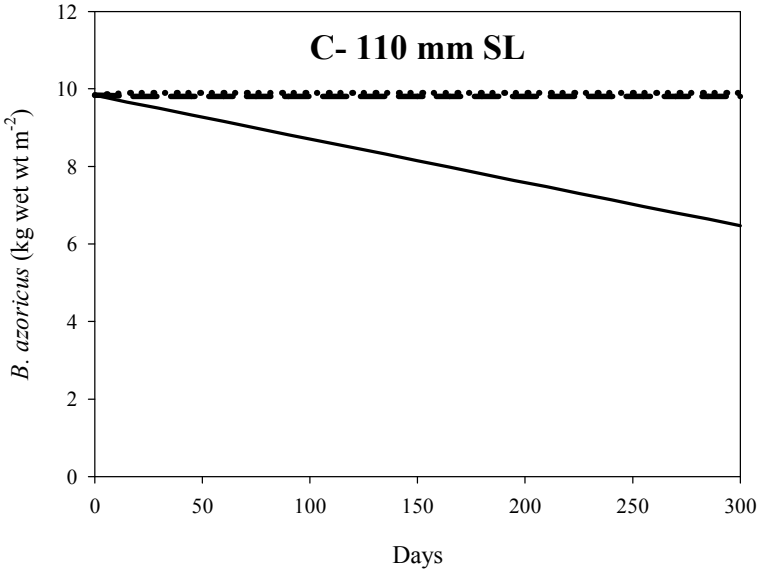

B
Gill weight (g DW)
Total weight (g DW)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0
2
4
6

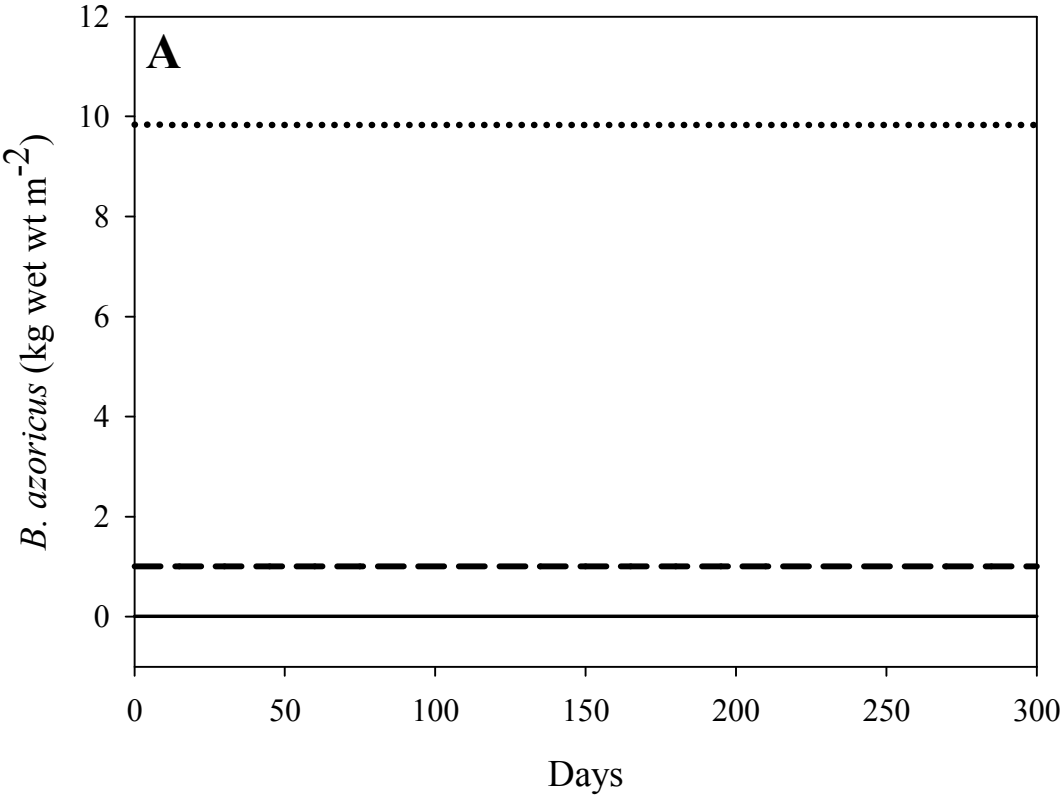

A
Predicted
Observed
0
2
4
6
8
10
12

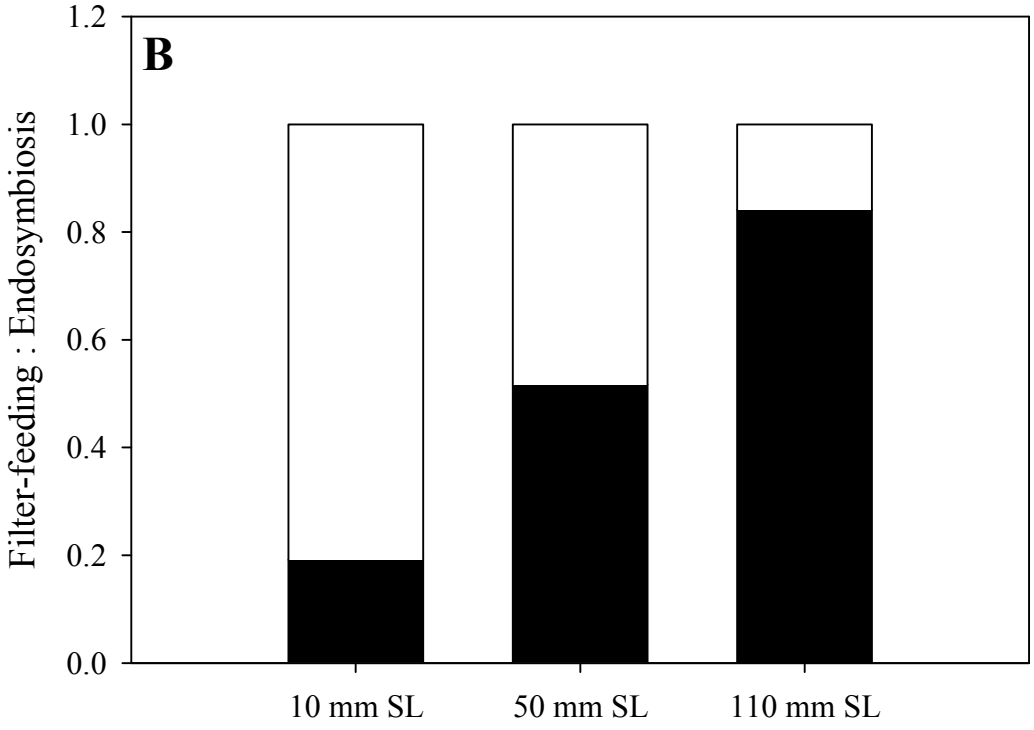

B
Predicted
Observed
0
2000
4000
6000
8000
10000
12000

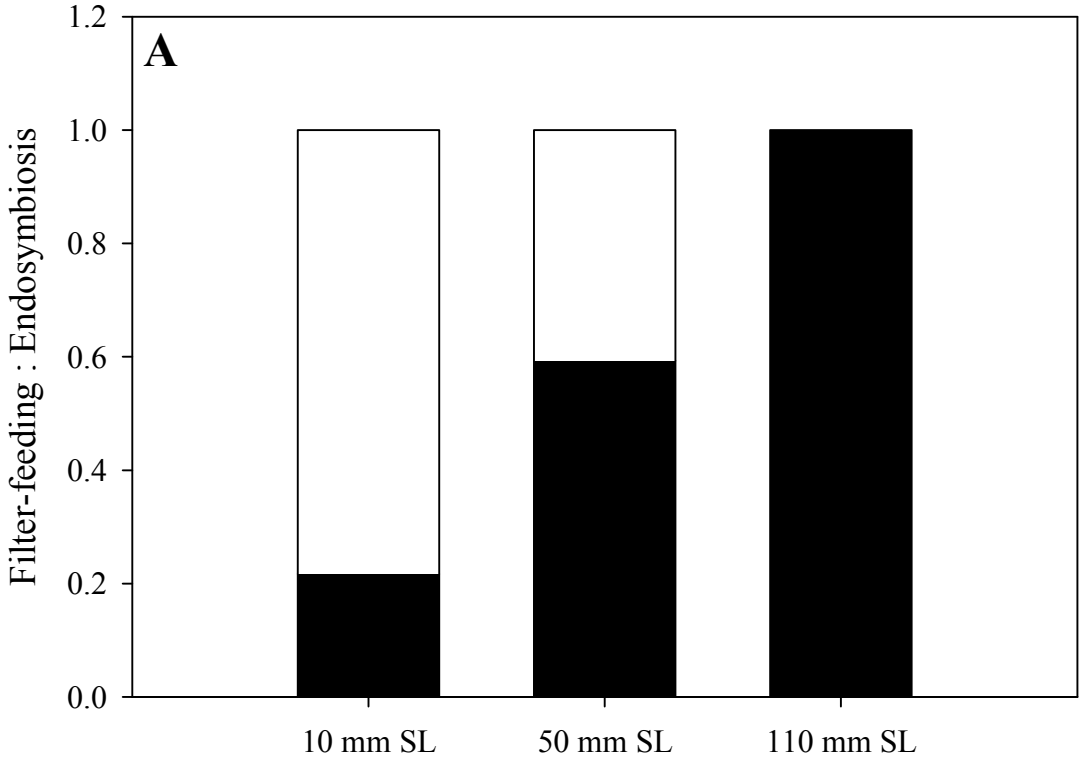

A- 10 mm SL
B. azoricus (kg wet wt m-2)
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0
50
100
150
200
250
300
Days

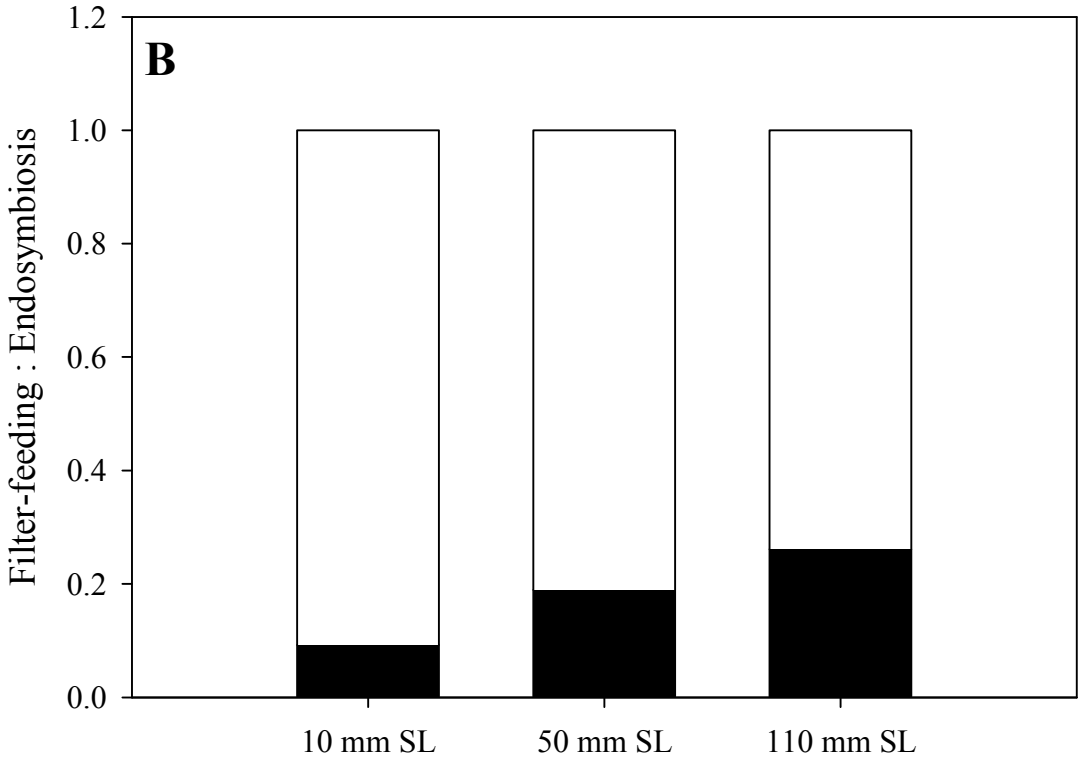

B- 50 mm SL
B. azoricus (kg wet wt m-2)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
Days

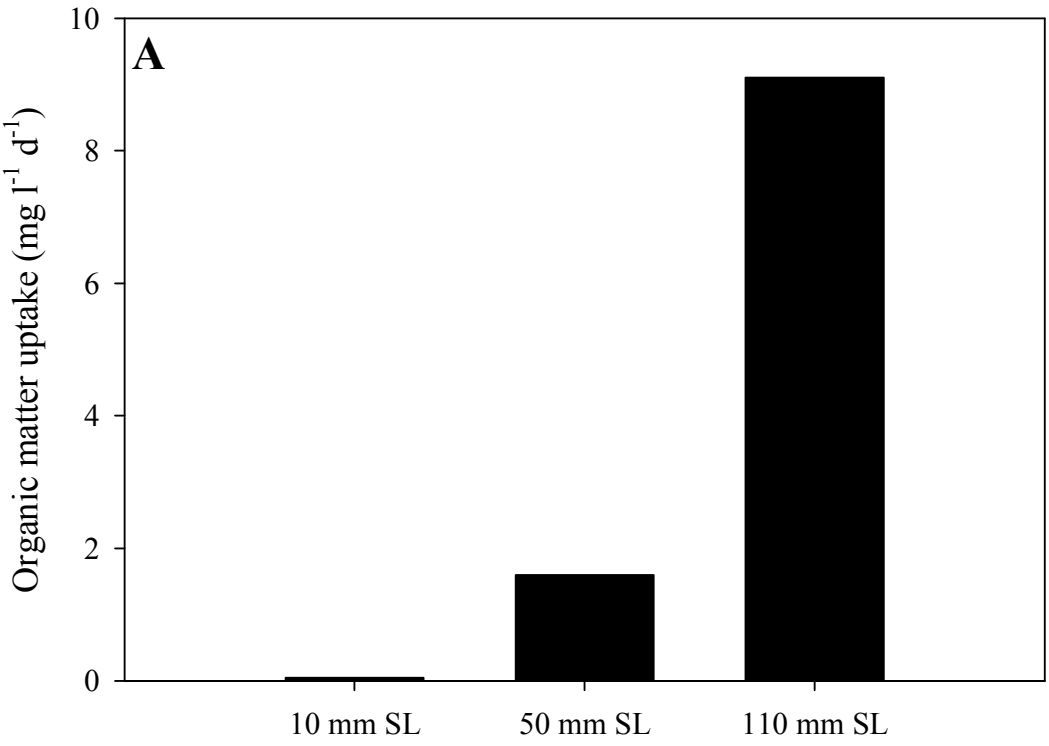

C- 110 mm SL
B. azoricus (kg wet wt m-2)
12
10
8
6
4
2
0
0
50
100
150
200
250
300
Days

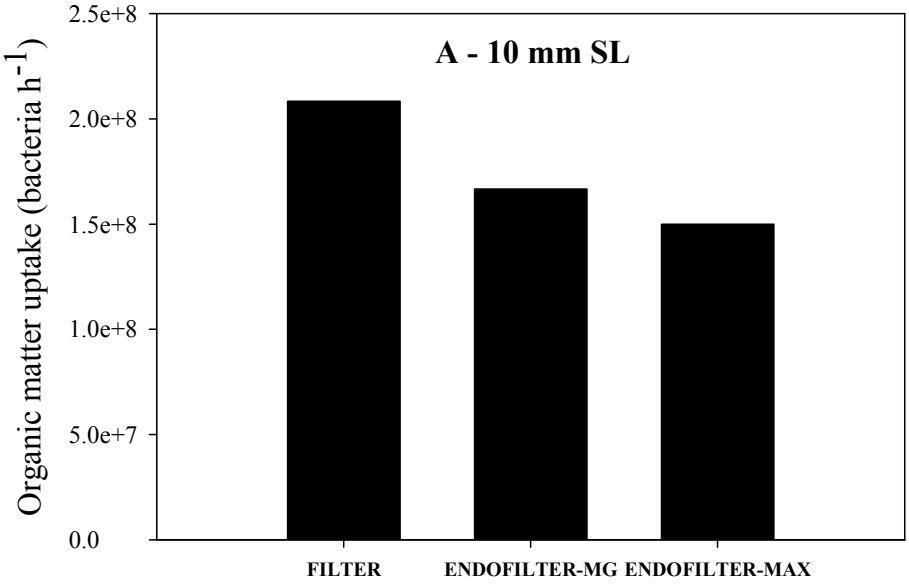

A
12
10
8
6
4
2
0
B. azoricus (kg wet wt m-2)
0
50
100
150
200
250
300
Days

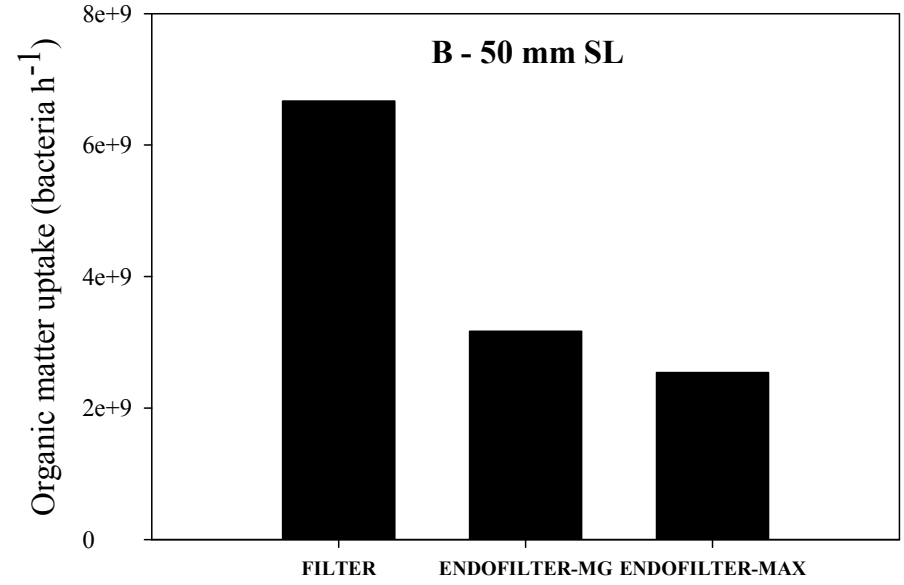

B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Filter-feeding : Endosymbiosis
10 mm SL
50 mm SL
110 mm SL

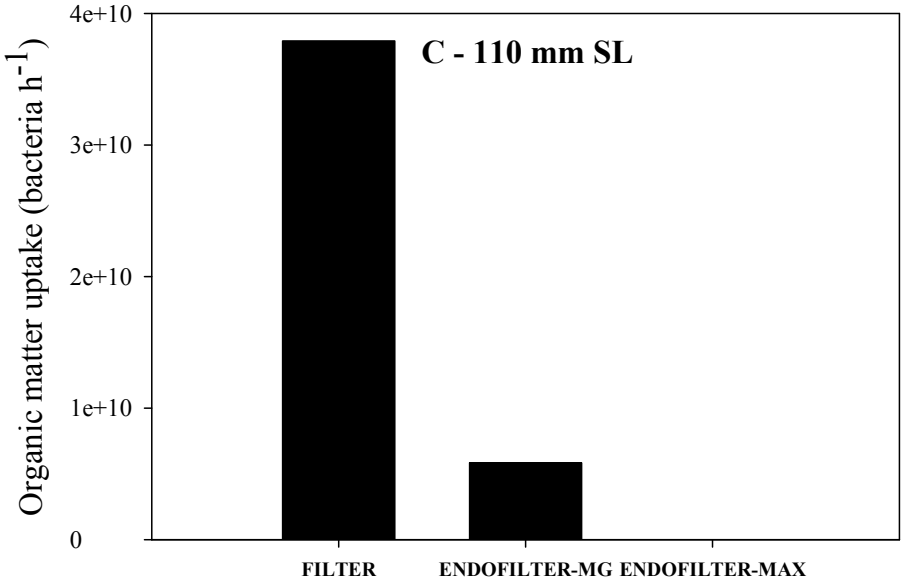

A
Filter-feeding : Endosymbiosis
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10 mm SL
50 mm SL
110 mm SL

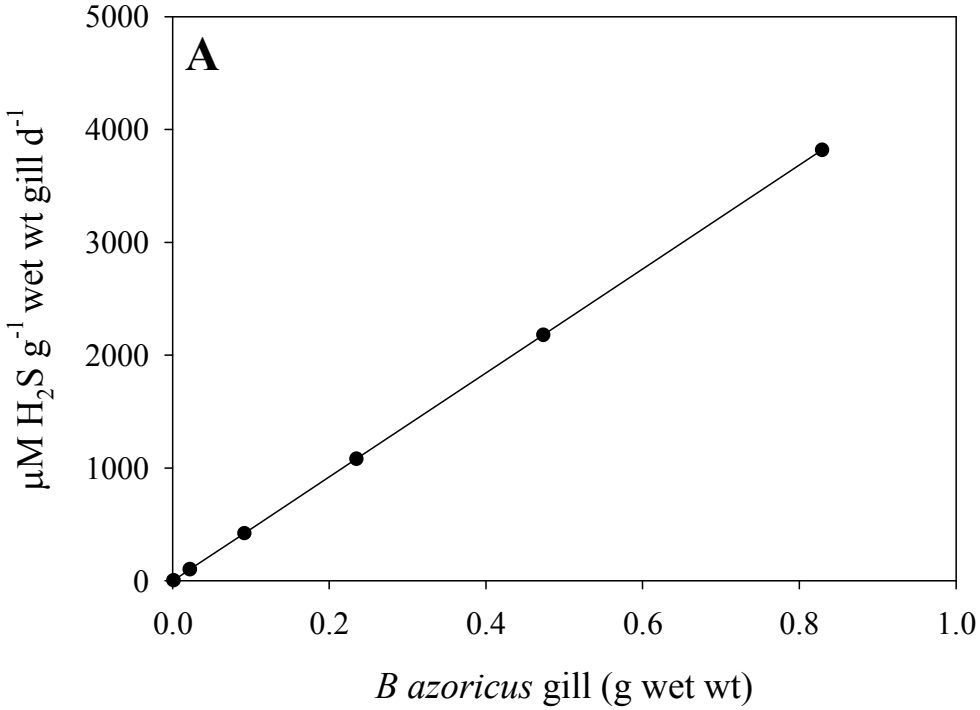

B
Filter-feeding : Endosymbiosis
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10 mm SL
50 mm SL
110 mm SL

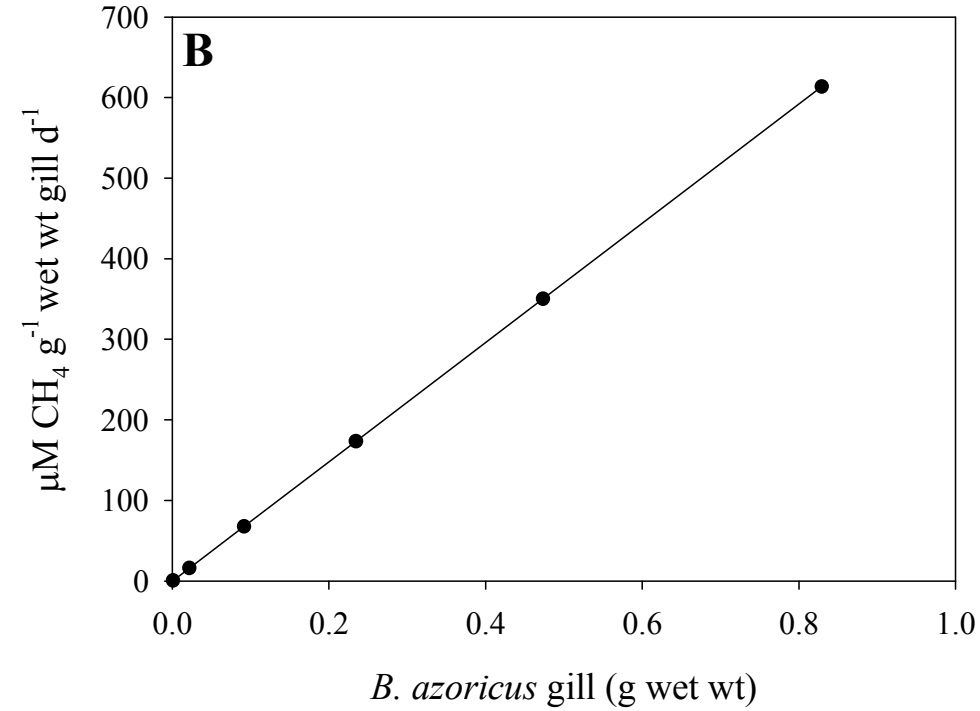

A
Organic matter uptake (mg l-1 d-1)
10
8
6
4
2
0
10 mm SL
50 mm SL
110 mm SL


A - 10 mm SL
Organic matter uptake (bacteria h-1)
2.5e+8
2.0e+8
1.5e+8
1.0e+8
5.0e+7
0.0
FILTER
ENDOFILTER-MG
ENDOFILTER-MAX


B - 50 mm SL
Organic matter uptake (bacteria h-1)
8e+9
6e+9
4e+9
2e+9
0
FILTER
ENDOFILTER-MG
ENDOFILTER-MAX


C - 110 mm SL
Organic matter uptake (bacteria h-1)
4e+10
3e+10
2e+10
1e+10
0
FILTER
ENDOFILTER-MG
ENDOFILTER-MAX


A
5000
4000
3000
2000
1000
0
0.0
0.2
0.4
0.6
0.8
1.0
µM H_2S g^{-1} wet wt gill d^{-1}
B azoricus gill (g wet wt)


B
700
600
500
400
300
200
100
0
0.0
0.2
0.4
0.6
0.8
1.0
µM CH_4 g^{-1} wet wt gill d^{-1}
B. azoricus gill (g wet wt)