



Energy acquisition and allocation in vesicomyid symbioses

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Introduction

Symbioses between sulphide-oxidizing bacteria and vesicomyid clams dominate invertebrate assemblages at cold seeps worldwide. This is true for Monterey Bay, CA as well. The vesicomyid clams *Calymene kilmeri* Bernard, 1974 and *C. pacifica* Dall, 1891 are the two predominant species at seep sites less than 1500 m depth in Monterey Canyon (Barry et al., 1997). These widely distributed clams serve as excellent models to explore the importance of energy balance in chemosynthetic symbioses. *C. kilmeri* and *C. pacifica*, like all vesicomyids, have reduced digestive systems and have developed symbiotic relationships with carbon-fixing, sulphide-oxidizing bacteria. These species, however, differ considerably in regards to metabolism and environmental preference, suggesting differing physiological strategies to thrive in the seep environment (Barry et al., 1997). These associations depend upon the productivity of endosymbionts, which is fueled by the oxidation of sulphide, and therefore upon sufficient sulphide flux from sea floor. For this reason, supply of sulphide and sulphide physiology are usually considered the most influential parameters limiting production by symbiont and host as well as macrofaunal distribution at seeps. We investigated the relationship between potential sulphide utilization of *C. kilmeri* and *C. pacifica*, two closely related vesicomyids within seeps in Monterey Bay, and differences in the overall production of these symbioses, incorporating recently published data as well as some unpublished results

concerning carbon and oxygen uptake, acid-base regulation, and reproduction.

Sulphide physiology

The distribution and abundance of *C. kilmeri* and *C. pacifica* are known to vary depending upon the chemical conditions of the sediment pore water in which they are found. *C. kilmeri* exploits areas with the highest sulphide exposure reported to date (4–18 mM), whereas *C. pacifica* dominates in areas of lower sulphide (0–4 mM, Barry et al., 1997; Goffredi & Barry, 2002). This difference in habitat sulphide suggests potential differences in the sulphide physiology of these two species. Sulphide physiology encompasses two important functions; (1) uptake of sulphide from the environment and transport from host to symbiont and (2) bacterial metabolism and conversion of sulphide to energy by the symbiont.

Although the anatomy of vesicomyids is generally similar to typical bivalve molluscs, several specializations enable their chemosynthetic life style. These clams have reduced digestive systems and rely on their symbionts for nutrition. They have greatly enlarged gills harbouring large numbers of endosymbionts, a large volume of haemoglobin-rich blood, and a highly extensible foot, used for movement and burrowing in sulphide-rich sediment. Vesicomyid clams accomplish the uptake of metabolites by spatially bridging the gap between sulphide-rich sediments and CO₂- and O₂-laden seawater. They take up sulphide from the reduced sediments through their highly vascularized foot, using a

unique extracellular sulphide-binding component. Although the exact nature of this component is unknown, researchers have suggested that zinc plays an important role in sulphide binding by this compound (Childress et al., 1993).

We recently examined a suite of physiological factors involving sulphide in these associations (Table 1; Goffredi & Barry, 2002). Our results indicate that *C. pacifica* is physiologically poised for the uptake of sulphide from low external concentrations while *C. kilmeri* possesses a less effective uptake mechanism, but has a higher tolerance for increased environmental sulphide levels (Barry & Kochevar, 1998; Goffredi & Barry, 2002). For example,

Table 1. Parameters involved in sulphide uptake and transport, and bacterial metabolism as measured in *C. pacifica* and *C. kilmeri*.

Parameter	<i>C. pacifica</i>	<i>C. kilmeri</i>
Sulphide Uptake and Transport		
H ₂ S Binding (above ambient levels)*†	10-60x ↑	5-10x
Zinc concentration (mM)	7.2 ± 0.8 ↑	4.8 ± 0.4
Foot volume (% of wet wt)	6.8 ± 0.8 ↑	6.0 ± 0.8
H ₂ S levels internally (mM)	2.7 ± 2.0 ↑	1.1 ± 0.8
H ₂ S consumption (mmoles g ⁻¹ gill h ⁻¹)	57.6 ± 9.6 ↑	7.8 ± 1.2
Bacterial metabolism		
Bacterial density (g bacteria g ⁻¹ animal)	110.4 × 10 ⁻⁴ ± 32.5 ↑	13.2 × 10 ⁻⁴ ± 2.3
Sulphide oxidase (I.U.)	6.7 ± 2.1 ↑	4.1 ± 1.2
APS reductase (I.U.)	38.9 ± 11.0 ↑	19.4 ± 6.5
Growth (per year)*†	0.001g (3%)	1.06g (15%) ↑

† = range (no SD available), all other values are $x \pm SD$, (up arrows = higher),

* Barry & Kochevar, 1998, all other values published in Goffredi & Barry, 2002

C. pacifica shows increased sulphide uptake ability via increases in surface area of the foot, carrying capacity of the blood, and concentration of sulphide-binding component in the extracellular fluid. It also appears that a greater potential for energy turnover and processing of sulphide by the endosymbionts correlates with this increased sulphide uptake ability in *C. pacifica*. This demonstrates that physiological variations do allow for the exploitation of separate micro-niches within Monterey Bay seeps, with the species most proficient at acquiring sulphide (*C. pacifica*) living in lower environmental sulphide levels, while *C. kilmeri* demonstrates a need for higher sulphide levels, but perhaps has a higher tolerance. Does this physiological variation between species, however, also influence host productivity (growth and reproduction)?

Growth and reproduction

Productivity includes the use of energy for the production of both somatic tissues (growth) and gametes (reproduction). Very little is known about the reproduction of vesicomid clams (Lutz et al., 1986; Karl et al., 1996). Preliminary measurements, conducted during one season, revealed equivocal measures of gonad biomass (both species show ~ 5-7% as a percentage of total biomass). Spawning has been successfully induced in *C. kilmeri* (~3000 eggs spawn⁻¹; J.P.B. unpublished data), however, the energetic cost of reproduction has not been determined for either *C. kilmeri* or *C. pacifica*. Individual growth rates, however, based on mark and recapture studies, were found to be considerably different. *C. pacifica* demonstrated a much slower annual growth rate (3% per year) than *C. kilmeri* (15% per year). These percentages are based on measures of growth in length, with *C. pacifica* growing 1 mm y⁻¹ and *C. kilmeri* growing 20 mm y⁻¹, and significant relationships between length and wet weight for these animals (*C. pacifica*, $y = 0.0002x^{2.9498}$ and *C. kilmeri*, $y = 0.0002x^{2.8615}$, where x is length in mm and y is weight in grams, Barry & Kochevar, 1998). A slower growth rate in *C. pacifica* is surprising given that this species possesses a seemingly greater potential for energy acquisition, as measured by sulphide consumption rates, binding ability and internal sulphide levels, as well as energy turnover, as measured by bacterial sulphide oxidation potential and bacterial densities (J.P.B. unpublished data; Goffredi & Barry, 2002). These results suggest that an increased ability to process sulphide may not result in an increased rate of host productivity. In contrast, the symbiosis with an apparent decreased capacity for sulphide throughput (*C. kilmeri*) has higher growth rates. Thus, productivity in *C. pacifica* must be limited by factors other than sulphide or there must be some undetermined physiological constraint on this species. An investigation of energy balance may aid in determining what these constraints or factors may be.

Energy balance

Studies of differences in energy flow and allocation in biological systems are important for understanding factors that guide physiological adaptations and community dynamics. All organisms allocate energy to physiological pathways critical for survival, however, there are very diverse strategies of energy acquisition and expenditure that an animal can employ. Energetic strategies and components of energy balance have been investigated in many organisms and, although some generalizations have been made regarding the relationship between environmental factors and energetic pathways, very little is known about energy balance in symbiotic associations.

Energy acquisition and expenditure is typically modeled by the following equation: $A = R + P_r + P_s + L$, where A is the energy absorbed (the difference between the amount of energy gained and that which is lost), R is a measure of the energy used in respiration (maintenance), P_r is the energy invested in reproductive tissue, P_s is the energy invested in somatic tissue, and L is a factor included to compensate for energy loss (Fig. 1) (Loo & Rosenberg,

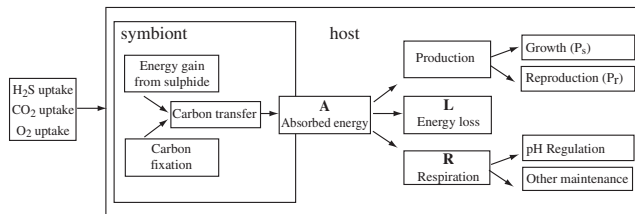


Figure 1. Model of energy acquisition and expenditure. Modified from Loo & Rosenberg, 1996. In most animals, increased productivity (growth and reproduction) is a direct consequence of increased carbon assimilation. For animal hosts of symbiotic bacteria, however, intermediate steps involving symbiont assimilation of inorganic carbon and production of organic carbon create complex interactions between carbon (or energy) uptake, assimilation, transfer, and eventual incorporation by the host. The energy absorbed by the host is allocated, as in non-hosts, to growth, reproduction, and maintenance.

1996; Plaut et al., 1996). In general, energetic resources are used primarily for maintenance (represented by respiration) and production (growth and reproduction). Larger size (growth = P_s) and higher fecundity (reproduction = P_r) vary in importance during different life stages of an organism. Typically, more energy is diverted to growth early in life, with more energy allocated toward reproduction later in life. Energy invested in growth can be substantial (up to 60% of the energy available, van Haren & Kooijman, 1993). Energy investment toward reproduction (fecundity, egg size, etc.), however, is highly variable among species, but can also be significant (10–90% of absorbed energy, Wells & Clarke, 1996). In many cases, when energy availability is compromised, growth is more strongly affected than reproduction (van Haren & Kooijman, 1993).

We have measured discrepancies between sulphide-related physiology (a component of A , energy absorbed) and productivity (P_s = growth and P_r = reproduction) in these two vesicomyid species. Energetically, differences in production can result from variations in the energy absorbed (including carbon acquisition rates and assimilation efficiencies), the proportion of energy allocated to maintenance, and energy lost through pathways such as mucous production and lipid storage (Cui & Liu, 1990). For *C. pacifica*, decreased growth may result from: (1) Decreased (or slower) carbon flux (energy absorbed = A) and/or (2) Increased energy allocated to maintenance pathways (respiration = R).

Option 1: Decreased energy absorbed (A).

The success of all symbiotic systems depends not only upon the exploitation of sulphide as an energy source, but also on the maintenance of adequate carbon flux from the environment to symbiont and, ultimately, to the host. Symbiotic associations depend upon the productivity of endo-symbionts and consequently have very different energy budgets and energy absorption than non-symbiotic animals. In general, increased productivity (growth and reproduction) in most animals is a direct consequence of increased carbon assimilation. For animal hosts of symbiotic bacteria, however, intermediate steps involving symbiont inorganic carbon (C_i) assimilation and organic carbon (C_o) production create complex interactions between carbon uptake, assimilation, transfer, and eventual incorporation by the host. Direct relationships between these processes may or may not exist.

When sulphide levels are sufficient, carbon flux from environment to symbiont to host may become more important in controlling productivity. The efficiency of carbon flux through symbiotic systems is influenced by three primary factors: (1) uptake (and source) of C_i , (2) rates of C_i fixation by the symbionts, and (3) transfer of C_o from symbiont to host. The first step in carbon flux through these systems is the uptake of inorganic carbon by the host. Preliminary results show that *C. pacifica* has significantly higher CO_2 uptake rates (10 mmol CO_2 g^{-1} h^{-1}) than *C. kilmeri* (6 mmol CO_2 g^{-1} h^{-1} ; Kochevar & Barry, unpublished data, $n = 2$), as well as significantly higher internal ΣCO_2 (4.7 ± 2.3 mM), than *C. kilmeri* (3.3 ± 0.8 mM; Table 2; Goffredi & Barry, 2002). This suggests

Table 2. Parameters involved in CO_2 uptake, O_2 uptake, acid-base balance, and hydrolysis as measured in *C. pacifica* and *C. kilmeri*.

Parameter	<i>C. pacifica</i>	<i>C. kilmeri</i>
CO_2 Uptake		
CO_2 levels internally (mM)	$4.7 \pm 2.3 \uparrow$	3.3 ± 0.8
CO_2 consumption (mmol g^{-1} gill h^{-1}) [†]	$\leq 10 \uparrow$	5 - 8
O_2 Uptake		
O_2 consumption (mmol g^{-1} gill h^{-1}) [†]	$14 \uparrow$	2
Hydrolytic enzymes		
Lysozyme (μg ml^{-1}) [‡]	$52.6 \pm 4.9 \uparrow$	29.9 ± 7.7
Acid phosphatase (mg ml^{-1} h^{-1}) [‡]	$49.3 \pm 23.7 \uparrow$	13.6 ± 4.5
Acid-base balance		
Hemolymph pH	$7.02 \pm 0.13 \uparrow$	6.91 ± 0.08
H ⁺ -ATPases (% of total)	$66.2 \pm 0.6 \neq$	29.3 ± 1.1

[†] rate measurements from R. Kochevar & J. Barry, unpublished data (range, no SD available), all other values are $x \pm SD$, (up arrows = higher),

[‡] Goffredi and Barry, in review; all other values published in Goffredi & Barry, 2002,

ATPase activity was measured via the methods found in Goffredi & Childress, 2001.

that *C. pacifica* is quite capable of removing C_i from the surrounding water, however, other factors involved in the second step in carbon flux through these symbiotic arrangements, such as rates of bacterial CO_2 fixation, affinity of the symbionts for CO_2 , and RuBPC/O activity, are currently unknown.

The third step in carbon flux through these systems involves C_o transfer (via translocation or digestion) from symbiont to host. Microscopic studies have provided evidence for potential digestion of symbionts in *C. pacifica*, including degrading bacteria, large heterogeneous vacuoles, mucus cells, and dividing symbionts (not seen in *C. kilmeri*, Goffredi et al., in review). Indirect microscopic evidence of digestion, including degenerative bacteria and abundant lysosomes and vacuoles, has also been demonstrated in the tubeworm *Riftia pachyptila* Jones, 1981 (de Burgh, 1986; Hand, 1987). In addition, increased activities of both lysozyme and acid phosphatase in *C. pacifica* also implicate digestion as a possible strategy for carbon transfer in this symbiosis (Goffredi et al., in review). The relative importance of digestion versus direct translocation, as well as the actual amount and efficiency of C_o transfer to the host, has not been established. Evidence gained from microscopy, along with biochemical indices, has suggested the potential for divergent strategies of carbon transfer from symbiont to host in *C. kilmeri* and *C. pacifica*.

Option 2: Increased energy allocation to maintenance pathways (respiration = R).

All organisms contend with tradeoffs between the use of energy for production versus energy for maintenance pathways. Production efficiency (the energy devoted to production as a function of energy absorbed) typically decreases with increasing maintenance costs. It is therefore expected that increases in maintenance (respiration) could significantly constrain growth and reproduction in organisms. For vesicomids, energy demands associated with sulphide acquisition and pH regulation may greatly influence the energy available for production. Respiratory energy consumption, or the oxygen required to support the ATP demands for all maintenance processes, can represent a large fraction of the total energy available (20–60%) depending upon the organism (Plaut et al., 1996). *C. pacifica* has demonstrated significantly higher respiration rates (O_2 uptake at $14 \text{ mmol O}_2 \text{ g}^{-1} \text{ h}^{-1}$), than *C. kilmeri* ($2 \text{ mmol O}_2 \text{ g}^{-1} \text{ h}^{-1}$; Table 2, Kochevar & Barry, unpublished data, $n = 2$). Increased respiration rates in *C. pacifica* may be necessary to supply oxygen to the symbionts or to meet the demands of increased maintenance processes, perhaps including the maintenance of sulphide uptake machinery (i.e. increased blood volume and increased concentration of the zinc-containing sulphide-binding component), digestion of symbionts (i.e. increased lysosome and hydrolytic enzyme production), and pH regulation.

pH regulation.

A large amount of energy (up to 30–50% of a total energy budget) can be required for the maintenance of ion homeostasis (Febry & Lutz, 1987; Gibbs & Somero, 1990). Proton pumping, in particular, is considered to be a significant contributor to metabolism in many organisms. For animal hosts of chemoautotrophic symbionts, sulphide exposure can result in extremely acidic conditions internally. In these systems, accumulation of protons and sulphate ions, resulting from bacterial sulphide oxidation, can occur at very high rates, up to 500 mmol h^{-1} in some chemoautotrophic associations (S.K.G., unpublished data, based on whole animal sulphide uptake rates and average animal biomass for *C. pacifica* and *C. kilmeri*). Previous results indicate that *C. pacifica* possesses more symbionts per gram animal than *C. kilmeri*, suggesting an even higher potential for H^+ production via symbiont autotrophy (Goffredi & Barry, 2002). This accumulation of protons, which can increase with increasing bacterial metabolism, creates a tremendous need for efficient proton elimination in these animals. Both species must work to maintain their internal pH despite internal processes acting to decrease it.

In addition, in laboratory experiments on vesicomids, increases in internal sulphide levels are typically associated with decreases in extracellular pH, postulated to arise from proton release during sulphide binding by the extracellular binding component (Childress et al., 1991, 1993). *C. kilmeri*, when freshly collected, demonstrates lower blood pH in relation to higher total internal sulphide (Goffredi & Barry, 2002). In contrast to these results, however, blood pH in *C. pacifica* was not found to be significantly correlated to internal sulphide levels. All vesicomids probably have a similar mechanism for sulphide binding by the specialized serum compound, thus, the lack of pH decrease in *C. pacifica* may suggest a greater capacity for pH regulation in this species. Therefore, protons released during binding of HS^- (from H_2S) are instantly compensated for by increased proton elimination, resulting in no net change in blood pH in *C. pacifica*.

Preliminary measurements for freshly collected animals show that *C. pacifica* has a slightly higher haemolymph pH (7.02 ± 0.13) than *C. kilmeri* (6.91 ± 0.08 ; Table 2). Thus, it is possible that *C. pacifica* expends more energy maintaining its alkaline internal pH than does *C. kilmeri*. It has been shown that the vent clam *C. magnifica* and the vent tubeworm *R. pachyptila*, both hosts of sulphide-oxidizing bacteria, use large concentrations of H^+ -ATPases, 2–3x higher than non-host animals (Goffredi & Childress, 2001). Likewise, *C. pacifica* appears to devote a larger percentage of its gill ATPases ($66.2 \pm 0.6\%$, $n = 2$) to the elimination of protons than does *C. kilmeri* ($29.3 \pm 1.1\%$ H^+ -ATPases, $n = 3$), suggesting a greater capacity for pH regulation in

C. pacifica (Table 2). This increase in proton elimination may be an important use of free energy in *C. pacifica*, and for other hosts of sulphide-utilizing bacteria as well. Some invertebrate hosts have evolved carbon-concentrating mechanisms, such as increased proton elimination, to create gradients favouring the internal movement of CO₂. Efficient pH regulation may prove equally important for *C. pacifica* in aiding the uptake of large amounts of CO₂ and H₂S from the environment, to support bacterial carbon fixation, justifying the trade-off between growth and acid-base balance.

Conclusion

Vesicomyids are important systems to study in that they are integral components of most seep communities, have diverse physiological capabilities and environmental requirements, and have wide geographical distribution. Interspecific comparative approaches may help us to identify and determine the adaptive significance of diverse physiological mechanisms. *C. pacifica* and *C. kilmeri*, in particular, display differences in microhabitat sulphide and oxygen levels, as well as species-specific physiological adaptations to both parameters. In addition, the overall productivity of these systems appears to rely upon factors other than sulphide-related physiology. The productivity of these chemosynthetic symbioses can be influenced by many factors. In some cases, the energy source can have an important role, as in the case of *Ridgeia piscesae* Jones, 1985, where differences in growth have been shown to be “somewhat” correlated with exposure to sulphide availability (Urcuyo, I. 2nd annual vent meeting). Other systems, including the clams in this study, have shown growth rates independent of energy availability. For example, bathymodiolid mussels (with methanotrophic symbionts) demonstrated decreased growth despite comparable methane (energy) levels to those in high mussel growth areas (Smith et al., 2000). The authors suggested that environmental oxygen levels also influenced growth rates as lower oxygen levels resulted in less overall growth. In our study, both species experience low oxygen levels (30–80 μM). Thus it is possible that growth in these systems is also influenced greatly by the presence of an electron acceptor. It is also possible that lower (transient or episodic) flux of sulphide around *C. pacifica* could be a factor limiting growth. We have, however, sampled these animals on a regular basis (up to 2–3 times per month) and have always found them (when healthy) to contain higher internal sulphide than *C. kilmeri*. Thus, if episodic sulphide availability is an issue for *C. pacifica* (as opposed to a reliable sulphide source for *C. kilmeri*), we have yet to measure it. It would be beneficial, in the future, to conduct

a series of whole-animal experiments in which changes in the internal milieu are measured in response to specifically controlled external conditions (such as ranges of both sulphide and oxygen). In addition, an investigation of how *C. kilmeri* is able to tolerate such high external levels of sulphide, i.e. peripheral sulfide defenses, would also be fruitful. This species is able to inhabit areas that are very under-utilized by other fauna, perhaps due to potentially higher sulphide tolerance and a faster growth rate.

Nonetheless, the present study demonstrates slower growth in *C. pacifica*, despite a seemingly greater potential for energy acquisition, and suggests that this species may allocate a significant amount of energy to maintenance pathways, perhaps most importantly pH regulation. There are still many unanswered questions concerning major factors governing the distribution, productivity, and overall physiology of these fascinating systems. The application of comparative approaches can provide unique perspectives that may answer some of the physiological and ecological questions that remain.

Acknowledgements

Funding for this project was provided by the Monterey Bay Aquarium Research Institute, supported by the Packard Foundation. The authors thank the captain and crew of the R.V.'s *Pt. Lobos* and *Western Flyer* and the R.O.V.'s *Ventana* and *Tiburón*. We also thank Josh Plant (MBARI) and Rob Frank (analytical lab, University of California, Santa Cruz) for logistical help with the FAA technique, and Randy Kochevar (Monterey Bay Aquarium) for previous work on *C. kilmeri* and *C. pacifica*.

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