

Factors regulating productivity in chemoautotrophic symbioses; with emphasis on *Calyptogenia kilmeri* and *Calyptogenia pacifica*.

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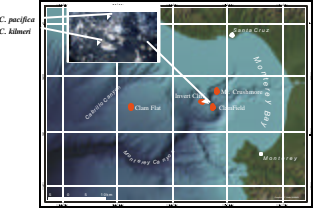
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Abstract

Symbioses involving sulfide-oxidizing bacteria and various metazoan phyla dominate megafaunal assemblages at cold seeps and hydrothermal vents worldwide. The predominant species found living at cold seeps in Monterey Bay are the vesicomyid clams *Calyptogenia kilmeri* and *C. pacifica*. The growth and survival of these clams depend directly upon the productivity of their chemoautotrophic endosymbionts, which is fueled by the oxidation of sulfide. For this reason, sulfide (energy) availability and sulfide physiology are thought to constrain symbiont and host production. Additional factors, however, are potentially equal in importance. Here we describe research concerning the productivity of two common clam species in relation to environmentally or physiologically limited processes. Although both species inhabit sulfide-rich sediments and depend nutritionally on their symbionts, many aspects of their life styles differ considerably. Our results indicate that *C. pacifica* is physiologically poised for the uptake of sulfide, as measured by increased sulfide consumption rates, sulfide binding ability, and internal sulfide levels, as well as energy turnover, as measured by sulfide oxidation potential, sulfur metabolism enzymes, and bacterial densities. In addition, *C. pacifica* demonstrates higher rates of oxygen consumption and aerobic metabolism, than *C. kilmeri*. Growth rates of *C. pacifica* (3% yr⁻¹), however, are considerably slower than *C. kilmeri* (15% yr⁻¹). This is surprising given that *C. pacifica* possesses a seemingly greater potential for processing sulfide. These results contradict the idea that sulfide limits the productivity of these two systems and, for this reason, we believe they are constrained by factors other than energy limitation.

Introduction

In the late 1970's scientists discovered novel deep-sea ecosystems based on chemosynthetic processes, fueled primarily by hydrogen sulfide, rather than photosynthetic carbon input (Crosier et al. 1979). Despite the poisonous nature of sulfide, numerous metazoans are known to inhabit areas of high sulfide. One such sulfide-rich ecosystem, teeming with chemosynthetically supported life, is Monterey Bay, California, USA.



Invertebrate communities living in association with cold seeps in Monterey Canyon were discovered in the 1980's. High faunal biomass in these areas is supported almost entirely by bacterial chemosynthesis, made possible by high concentrations of hydrogen sulfide, available for bacterial conversion into energy. In fact, many seep animals exist in symbiotic relationships with carbon-fixing, sulfide-oxidizing bacteria, relying upon these symbionts for nutrition (review Childress and Fisher, 1992). The two predominant clams living in such symbioses at the Monterey seeps are *Calyptogenia kilmeri* and *C. pacifica* (Barry et al. 1996).

Anatomy

Invertebrate-bacterial symbioses are extremely common among deep-sea environments of high sulfide. The host presumably benefits from nutritional integration with the symbionts which overcomes the problem of food limitation in the deep sea, whereas the symbionts benefit from an environment protected from fluctuations of necessary metabolites. These arrangements have proven extremely successful as estimates of biomass and growth rates of some phyla have surpassed those of most animal life on the planet.

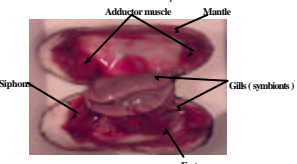
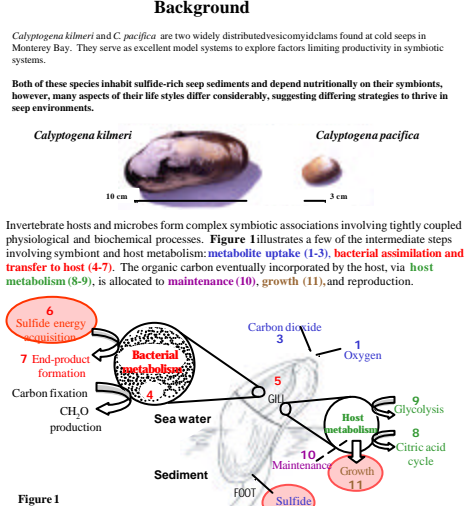



Figure 1 We recently examined physiological processes comparing *C. pacifica* to *C. kilmeri*. Our results indicate that there are notable differences between these two species.

Table 1: Physiological parameters observed in *C. pacifica* and *C. kilmeri*.

Parameter	<i>C. pacifica</i>	<i>C. kilmeri</i>	n	sig.
Respiration/energy				
1 O ₂ Uptake (μmole/g/h)	14.0	5	3.0	5 *
Hemoglobin (mM)	1.3 ± 0.1	14	1.8 ± 0.1	21 *
2 H ₂ S Uptake and Transport	See Table 3	See Table 3		
3 CO ₂ Uptake (μmole/g/h)	30.0	5	6.0	5 *
CO ₂ Internally (mM)	4.7 ± 2.3	16	3.3 ± 0.8	43 *
Bacterial metabolism				
4 Bacterial density (x 10 ⁶ g animal)	6.6 ± 0.7	8	3.9 ± 0.3	5 *
5 Sulfide consumption	See Table 3	See Table 3		
6 H ₂ S Oxidation (turnover by symbionts)	See Table 3	See Table 3		
7 Elemental sulfur (S ₀) level (% of gill)	1.3 ± 2.1	7	4.1 ± 2.4	11 *
Polysulfide (S _x ²⁻) level (% of gill)	0.3 ± 0.1	6	0.6 ± 0.1	4 *
Host metabolism				
8 Aerobic (Citrate synthase [IU/g ^{1/2} body)	1.0 ± 0.5	8	0.3 ± 0.2	7 *
9 Anaerobic (Dehydrogenase [IU/g ^{1/2} body)	4.9 ± 1.0	6	7.1 ± 0.8	5 *
% anaerobic dehydrogenase	08 ± 1.6		29.6 ± 4.2	
% acetate dehydrogenase	06 ± 2.3		55.7 ± 5.3	
Maintenance				
10 pH regulation (H ⁺ -ATPase (%))	66.2 ± 0.6	2	29.3 ± 1.1	3 *
Host production				
11 Growth rate	See Table 4	See Table 4		

↑ arrows = higher rates, ↓ arrows = lower rates, * = significant difference >95%, ** = significant difference >99%

* *C. pacifica* demonstrates higher CO₂ and O₂ uptake rates, higher internal CO₂ levels, more symbionts per gram of biomass, higher levels elemental sulfur, a higher aerobic potential, as measured by CS activity in both gill and adductor muscle, and a larger percentage of ATPase activity to the elimination of protons.

* *C. kilmeri* demonstrates a larger amount of polysulfides in the gill tissues, a higher anaerobic potential, and a higher percentage of dehydrogenase activity comprised of ODH activity.

We investigated sulfide-related processes in *C. kilmeri* and *C. pacifica* (noted in pink above) in order to determine if sulfide-limitation, at any step during sulfide movement, can act to constrain productivity in symbiotic systems.

References

Coffield et al. 1979. *Science* 203: 1073-1083.
 Childress and Fisher. 1992. *Oceanography and Marine Biology: Annual Review*, 30: 337-441.
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 Barry et al. 1996. *Deep-Sea Research* 43: 1739-1762.

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Hypothesis

H1: Sulfide availability and sulfide-related physiology regulate productivity in chemoautotrophic symbiotic systems.

In order for chemoautotrophic symbioses to thrive, symbionts must have access to an electron donor used as an energy source (sulfide), an electron acceptor (oxygen or nitrate), and inorganic carbon (CO₂ or HCO₃⁻). These associations depend upon metabolism of the endosymbionts which is fueled by the oxidation of sulfide, and, therefore, dependent upon sufficient sulfide flux from sea floor seepage or venting.

For this reason, the supply of sulfide and sulfide physiology are usually considered the most influential parameters limiting production by symbiont and host.

Table 2: Patterns expected for sulfide-limitation

Rate	Sulfide-limitation
H ₂ S Uptake	-
H ₂ S Transport to symbionts	-
H ₂ S Oxidation	-
Growth (or Reproduction)	-

Arrows indicated increased or decreased rates and capacities.

If the productivity of a symbiotic system is influenced primarily by the ability to process sulfide (energy), then growth rates of the host (a component of host production) should correlate positively with sulfide uptake rates, the ability to transport sulfide, and ability of the symbiont to extract energy from sulfide (Table 2).

- To support bacterial production of organic carbon using sulfide as an energy source:
- the host must achieve sufficient rates of sulfide uptake and internal sulfide levels via extremely effective sulfide binding mechanisms (Table 3).
 - the endosymbiont populations present in these hosts must harness energy from the oxidation of sulfur compounds, presumably via effective enzymatic pathways (Table 3).
 - the host uses organic carbon transferred from the symbiont to fuel host processes, such as growth and reproduction (Table 4)

Table 3 describes our measurements of parameters involved in acquiring sulfide energy, including sulfide uptake and binding, sulfide availability to symbionts, and other sulfide-related physiology.

Table 3: Sulfide-related parameters observed in *C. pacifica* and *C. kilmeri*

Rate	<i>C. pacifica</i>	<i>C. kilmeri</i>	n	sig.
H ₂ S Uptake (whole animal - nmol/d / g animal / h)	50	23	5	*
H ₂ S Consumption (gill - nmol/d / g animal / h)	11	4	2	5 *
H ₂ S levels internally (nM)	2.9 ± 2.0	16	1.1 ± 0.8	43 *
H ₂ S Binding (above ambient levels)	10-60x	10	5-10x	10 *
Zinc (nM)	6.3 ± 0.8	14	4.7 ± 0.3	36 *
Blood volume (% of total biomass)	36.8 ± 6.0	10	32.5 ± 4.6	10 *
H ₂ S Oxidation (S-Oxidase [IU])	6.7 ± 2.1	6	6.2 ± 1.0	8 *
APS reductase [IU]	41.0 ± 4.6	5	28.2 ± 1.0	6 *

↑ Up arrows = higher rates, Down arrows = lower rates
 * Indicates significant difference >95%, ** indicates significant difference >99%

* *C. pacifica* demonstrated higher rates of whole animal H₂S uptake as well as sulfide consumption by intact gills under physiologically relevant conditions than *C. kilmeri*

* *C. pacifica* demonstrated increased H₂S levels internally, resulting from increased sulfide binding ability.

* Increased sulfide binding ability in *C. pacifica* is presumably due to higher concentrations of zinc (which binds sulfide 1:1) and a larger % blood (as related to total body mass), than *C. kilmeri*.

* *C. pacifica* demonstrated significantly higher sulfideoxidase and APS reductase activity than *C. kilmeri*. Sulfide oxidase is involved in the oxidation of sulfide to sulfate (a pathway providing the most reducing power), and APS reductase catalyzes the reactions between AMP and sulfide.

Table 4 describes our measurements of productivity-related parameters.

Table 4: Production patterns observed in *C. pacifica* and *C. kilmeri*

Production	<i>C. pacifica</i>	<i>C. kilmeri</i>	n	sig.
Reproduction (gonad - as % of total weight)	6.2 ± 0.4	5.2 ± 0.2	13	13 *
Growth rate (% yr ⁻¹)	3	15	50	50 *

↑ Up arrows = higher rates, Down arrows = lower rates (* indicates significant difference >95%)

* Preliminary measurements, conducted during one season, reveal inconclusive evidence for differences in gonad biomass (both ~ 5-6% as a % of total biomass).

* Mark and recapture studies have provided an individual growth rate of 15% yr⁻¹ for *C. kilmeri*, whereas *C. pacifica* grows much slower (only 3% yr⁻¹; Barry and Kochaveev 1998).

These two symbiotic systems do not appear to be sulfide limited. *C. kilmeri* lives in higher sulfide levels and *C. pacifica* has a greater ability to take up sulfide. Data show that internal sulfide levels are sufficient for bacterial productivity in both species and that growth rates of the hosts do not correlate with sulfide-related physiology.

Conclusion

C. pacifica, the symbiotic system with an apparent increased capacity for energy throughput (Table 3) has considerably lower growth rates (Table 4), indicating a dependence of productivity upon parameters other than sulfide.

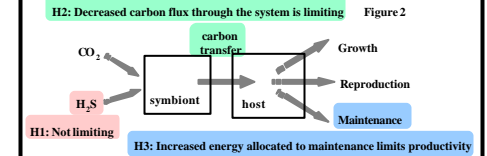
Our results suggest that an increased ability to acquire and process sulfide does not result in increased host productivity.

ALTERNATE HYPOTHESES

Energy acquisition and allocation

Studies of energy flow and allocation in biological systems are important for understanding key factors that guide physiological adaptations and community structure. All organisms allocate energy to physiological pathways critical for survival, however, some species possess diverse strategies of energy acquisition and expenditure.

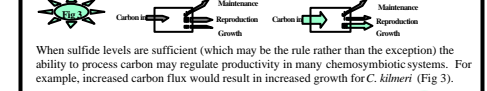
It is possible that *C. pacifica* and *C. kilmeri* experience differences in the amount of carbon flux through the systems as well as the proportion of energy used for maintenance (Figure 2), leading to differences in overall productivity.



Factors limiting productivity (AND Future Directions)

H2: Carbon flux from symbiont to host limits productivity in *C. pacifica*

An alternative, but largely unexplored, view of symbioses suggests that when sulfide is not limiting, carbon flux (and factors regulating flux) from environment to symbiont to host may become more important in controlling productivity.

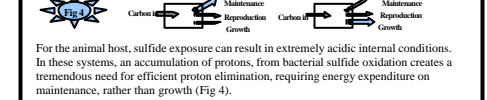


Preliminary results differences between *C. kilmeri* and *C. pacifica* relate to inorganic carbon (#1 and #2) and organic carbon transfer (#3), indicating the potential for carbon-limitation at some step in the movement of carbon from environment to symbiont to host.

We expect that an investigation into carbon cycling, rather than sulfide or oxygen, will resolve the discrepancy between growth rates and the potential for energy production observed in these vesicomyid species.

H3: Energy expenditure on maintenance limits productivity in *C. pacifica*.

All organisms contend with tradeoffs between the use of energy for growth and reproduction versus maintenance pathways. A primary use of maintenance energy is likely to be the elimination of bacterial waste products.



Preliminary results: *C. pacifica* demonstrates an increased ability for proton regulation, via high levels of H⁺-ATPase.

A large amount of energy (up to 30-50% of the total energy budget) can be required for the maintenance of ion homeostasis and it is expected that energy devoted to ion regulation constrains productivity in *C. pacifica*.