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A tale of two clams: differing chemosynthetic life styles among vesicomyids in Monterey Bay cold seeps

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Introduction

Vesicomyid clams are a dominant and common component of the megafauna inhabiting most cold seep and hydrothermal vent communities. The bivalve family Vesicomyidae includes more than 50 species found nearly exclusively in sulphide-rich habitats such as cold seeps, hydrothermal vents, and accumulations of organic debris (e.g. whale carcasses), from 450 m to greater than 3000 m depth. All species investigated have been shown to rely nutritionally on sulphide-oxidizing endosymbiotic chemoautotrophic bacteria held in ctenidia (Fiala-Médioni et al., 1994). Thus, these bivalves require access to sulphide-rich pore fluids, but must also contend with the potentially extreme toxicity of sulphide.

Chemosynthetic bivalves in seep and vent habitats contend with extreme gradients in fluid chemistry and, unlike most heterotrophic species, must maintain some exposure to sulphide or methane to support chemoautotrophic productivity by bacterial endosymbionts. Sulphide concentrations in pore fluids can vary from micromolar to millimolar levels over cm scales both vertically and horizontally in seep sediments (Barry et al., 1997). Vesicomyid species are partially segregated along these sulphide gradients, suggesting species-specific ranges of sulphide tolerance, biological interactions, or other mechanisms to regulate species distributions. In view of the patchiness of sulphide in marine habitats, sulphide requirements to support endosymbiotic bacterial chemosynthesis, and toxicity of sulphide to metazoan physiological function, it is likely that considerable evolutionary divergence has occurred within the Vesicomyidae concerning sulphide physiology. Thus, we expect species inhabiting different sulphide concentrations to have differing physiological adaptations in order to support endosymbiont sulphide-based chemosynthesis, as well as mediate sulphide toxicity.

The species composition of vesicomyids can vary considerably both between and within seep sites. As many as five vesicomyid species inhabit individual fluid seeps in Monterey Bay, but their relative abundance varies greatly between seep locations (Barry et al., 1995, 1997). Species dominance among seep locations is related to the average sulphide concentration of interstitial fluids among sites. The distribution of vesicomyids within individual seeps is stratified along a sulphide gradient from the centre to the margins of seeps. Species that are apparently sulphide tolerant such as *Calyptogena kilmeri* (Bernard, 1974) dominate sites with-high sulphide levels and are present in central portions of low-sulphide seeps. In contrast, *Calyptogena pacifica* (Dall, 1891) is the principal species inhabiting low sulphide seeps and the margins of high-sulphide seeps.

In this paper, we investigate aspects of the physiology of *Calyptogena pacifica* and *C. kilmeri* that influence their habitat distribution, including results of growth rate studies that suggest potential metabolic constraints imposed by differences in sulphide physiology.

Methods

I. Sulphide Binding Affinity of Vesicomyid Blood

The sulphide binding affinity of blood samples from several individuals of *Calyptogena kilmeri* and *C. pacifica* was assayed in experiments using whole animals and dialysed blood serum. Specimens were collected from cold seeps in Monterey Bay using the ROV *Ventana* (operated by the Monterey Bay Aquarium Research Institute) and transferred to chilled seawater aquaria. Concentrated sulphide stock solution (1-10 mM NaS, pH 7.5) was pumped slowly into the aquarium and mixed thoroughly. Prior to the introduction of sulphide and following each 24 hour period, sulphide was measured from seawater samples drawn nearby the clams in the tank, and from blood samples from several individuals removed from the aquaria. Sulphide concentrations for all samples were analysed by gas chromatography (HP5890 modified for analysis of dissolved gases). Similar assays were performed to evaluate the sulphide binding affinity of blood serum separated from whole blood of each species (Kochevar & Barry, in prep.).

II. Vesicomyid Growth Rates

Growth rates of *Calyptogena kilmeri* and *C. pacifica* were measured in several field experiments at cold seeps in Monterey Bay. For each experiment, 50-100 individuals of either species were collected by ROV, measured to the nearest 0.1 mm, and labelled with a numbered tag. The clams were returned immediately (within 3 h) to the site of collection where they were held in bottomless enclosures to prevent emigration during the experiment. Enclosures were left in place for 8 months to 1.5 years, after which as many individuals as possible were recovered and remeasured. Von Bertalanffy growth curves were fitted to the growth interval data for each species.

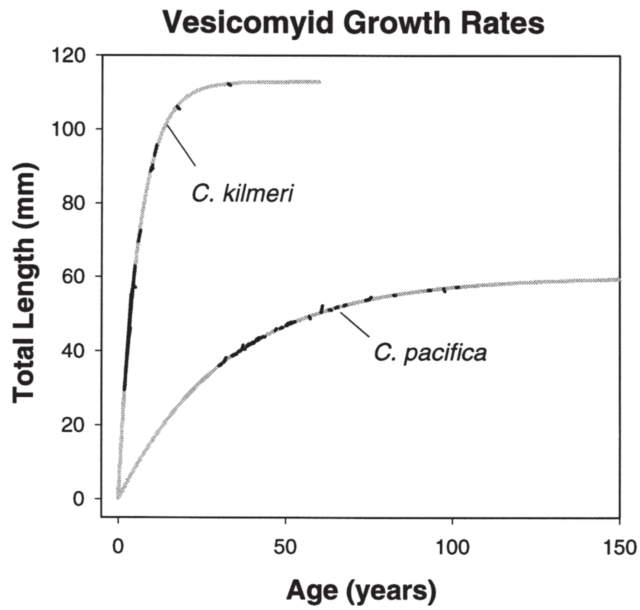

Figure 2. Growth rates of *Calyptogena kilmeri* and *C. pacifica*. Number of individuals used to fit von Bertalanffy growth curves are 29 and 43 for *C. kilmeri* and *C. pacifica,* respectively.

Results

I. Sulphide Binding Affinity

Blood or blood serum from both vesicomyid species exhibited a high binding affinity for sulphide, thereby elevating blood sulphide levels an order of magnitude or more above ambient levels. Similar binding affinity for whole blood or blood serum indicates that a serum-borne compound is responsible for sulphide binding affinity. *Calyptogena pacifica* exhibited a much higher capacity to bind sulphide than *C. kilmeri,* especially at low ambient sulphide concentrations (Fig. 1). Differing sulphide binding abilities for these species may influence their distribution among sulphide-rich habitats.

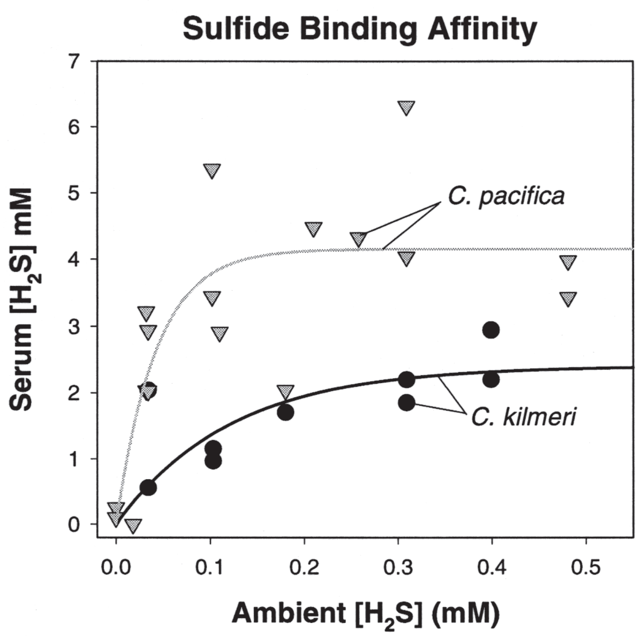

Figure 1. Sulphide binding affinity of blood from *Calyptogena kilmeri* and *C. pacifica,* based on assays of blood serum (exponential curve fits).

II. Individual Growth Rates

Growth rates of *Calyptogena kilmeri* were much greater than *C. pacifica* (Fig. 2). *C. kilmeri* individuals grew more than 20 mm per year at small sizes, and approached an asymptotic size near 120 mm after about 10-15 years. This rate of growth is comparable to that estimated for *C. magnifica* at hydrothermal vents. In contrast, *C. pacifica* grew very slowly. Nearly all individuals recovered had increased in total length by less than 1 mm. A von Bertalanffy growth curve fit to the growth intervals of the recovered individuals indicated that *C. pacifica* requires 60-150 years to approach a maximum size near 65 mm (slightly smaller for males).

Discussion

I. Differences in sulphide binding physiology

Differences between these vesicomyid species likely plays a large role in their segregation along environmental sulphide gradients by determining the habitability of the environment. Assuming that endosymbiont vitality and productivity is linked to sulphide availability in gill tissues, and that symbionts of both vesicomyids have similar sulphide requirements, then the shapes of their sulphide binding curves should reflect differences in environmental tolerance for sulphide. Species with steep binding curves (i.e. high sulphide affinity) such as *Calyptogena pacifica* should thrive in low-sulphide habitats that are probably intolerable to *C. kilmeri*. *C. pacifica* can elevate its blood sulphide concentration to quite high levels (ca. 2 mM) while inhabiting environments with sulphide levels 2 orders of magnitude lower (0.025 mM) (Fig. 1). In contrast, *C. kilmeri* requires 10 times greater ambient sulphide for the same internal sulphide concentration. Thus, *C. pacifica* can maintain high (and presumably sufficient) sulphide levels to support endosymbiont chemosynthesis, while inhabiting seeps with considerably lower sulphide concentrations than *C. kilmeri*.

The maximum sulphide levels habitable by *Calyptogena sp.* may also be linked to species-specific sulphide binding characteristics of their blood. Very rapid saturation of *C. pacifica* blood at ambient sulphide levels near ~0.15 mM may determine its upper boundary for environmental sulphide tolerance. Beyond saturation levels, additional sulphide entering the blood and tissues must be regulated by rates of sulphide degassing (to endosymbionts or the external environment), sulphide oxidation (symbiont-based or host-based), or membrane permeability. At intolerably high levels, sulphide toxicity will inhibit metabolic pathways and may have other negative impacts to host physiology. Although *C. kilmeri* has a lower overall sulphide affinity and saturates more slowly, it has a much broader dynamic range of sulphide reactivity than *C. pacifica*. Although it may be restricted from low-sulphide habitats, it can inhabit and thrive in sulphide-rich habitats that are prohibitively toxic to *C. pacifica*.

These divergent physiological ‘strategies’ also represent ecological trade-offs such that *Calyptogena pacifica* is well adapted for life in marginal habitats with low sulphide levels, while *C. kilmeri* is restricted to sulphide-rich sites. Each strategy may have important effects on the metabolism, growth, reproduction, and even the population demography of the species. For example, the degree to which sulphide binding affinity influences rates of symbiont productivity determines the strength of coupling between host nutrition and sulphide affinity. Because most host nutrition is thought to derive from its symbionts, host metabolism should be constrained by symbiont production, thereby limiting energy available for allocation to growth, reproduction, or other processes. Consequently, linkages between sulphide physiology and symbiont production very likely have strong, if indirect, effects on various aspects of host biology.

II. Consequences of Divergent Physiological Adaptations

Although many factors influence growth rates of individuals, sulphide physiology is involved, at least indirectly, in regulating rates of energy production to support growth in vesicomyids. The observed difference in growth between *C. kilmeri* and *C. pacifica* supports the hypothesis that sulphide binding affinity regulates, at least partially, rates of production by endosymbionts, and consequently, host productivity as well. If basal metabolic rates are similar, *C. pacifica* may have less energy to allocate towards growth than *C. kilmeri,* due to its presumably lower endosymbiont productivity. However, other factors, including rates of sulphide flux to symbionts, non-growth metabolic processes (e.g. reproduction, basal metabolism), potentially large differences in symbiont-specific productivity, and evolutionarily-derived allometric constraints on growth and size, may have even greater importance than sulphide binding affinity alone. It seems clear, however, that adaptations of vesicomyids have diverged among seep environments, leading to differences in habitat preference, but also resulting in constraints on growth and productivity. Unlike entirely heterotrophic metazoans, the evolution of bivalve-bacterial symbioses are linked to selective pressures influencing both the host and its symbionts. Because host metabolism, growth, and reproduction is coupled directly to symbiont production, diversification in this group has probably arisen in response to selection for maximal rates of symbiont productivity in a spatially heterogeneous environment.

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