

Aerobic and anaerobic enzymatic activities of *Calyptogena gallardoi* (Vesicomidae): a clam associated with methane cold seeps off Chile

R.R. GONZÁLEZ^{1,2}, R.A. QUIÑONES^{1,2}, E. QUIROGA³ AND J. SELLANES^{1,4}

¹Centro de Investigación Oceanográfica en el Pacífico Sur-Oriental (COPAS), Universidad de Concepción, Casilla 160C, Concepción, Chile, ²Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160C, Concepción, Chile, ³Centro de Investigación en Ecosistemas de la Patagonia (CIEP), Bilbao 449, Coyhaique, Chile, ⁴Departamento de Biología Marina, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

Calyptogena gallardoi is a recently described species of vesicomid clam associated to the extensive gas-hydrate field reported for the Chilean margin along 35°S to 45°S. Enzymatic analysis in foot, gill and abductor muscle tissues of *C. gallardoi* collected in central-south Chile (~36°21'S 73°44'W), show high activities for malate dehydrogenase (MDH), strombine dehydrogenase (STRDH) and alanopine dehydrogenase (ALPDH) and a low activity of citrate synthase (CS) and ETS (electron transport system). Positive significant correlations (log–log scale) were found between enzymatic activities involved in anaerobic metabolism (MDH versus LDH and ALPDH versus STRDH), as well as between CS and opines dehydrogenases. The ratio MDH/LDH or any other opine dehydrogenase assayed was $\gg 1$ in all tissues analysed. These results indicate that *C. gallardoi* is highly adapted to the harsh anaerobic conditions of marine chemosynthesis-based communities inhabiting a reduced environment.

Keywords: aerobic, anaerobic, enzymes, *Calyptogena gallardoi*, methane cold seeps, Chile

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INTRODUCTION

Vesicomids are one of the most often mentioned taxa associated to megafaunal communities based on chemosynthesis. The presence of representatives of this family are used thus as indicators of benthic reducing environments in the world Oceans. *Calyptogena gallardoi* has been recently described by Sellanes & Krylova (2005), at the Concepción Methane Seep Area, in the eastern South Pacific. This new species extends the range of the genus *Calyptogena* to 36°22'S in the oriental margin of South America; more specifically, to the area of the Chilean margin, where the presence of an extensive gas-hydrate field between 35°S and 45°S has been inferred based on acoustic surveys (Grevemeyer *et al.*, 2003; Morales, 2003). Conservative estimates of the volume of gas stored as gas-hydrate in subsurface sediments of this area are in the order of $3.2 \times 10^{13} \text{ m}^3$, roughly 3% of the world total (Morales, 2003). Despite the extension of this area there is very little knowledge on the structure and composition of the benthic communities associated to these cold seeps and the metabolic rates of the species inhabiting the environment where the gas-hydrates are located are totally unknown. Here,

we report the enzymatic activities associated to energetic metabolism of *Calyptogena gallardoi*.

MATERIALS AND METHODS

Samples were obtained from two dredge hauls conducted off Concepción (36°21.46'S 73°44.08'W, water depth 934 m, and 36°16.40'S 73°40.70', 651 m) during the ONR/PUCV/UDEC Cruise (8–21 October 2004) onboard the AGOR 'Vidal Gormáz' (Oceanographic and Hydrographic Service of the Chilean Navy). The objective of the cruise was a survey of the hydrographic conditions, methane seepage and associated chemosynthetic communities in the bathyal zone off central Chile. Three specimens were analysed for their enzymatic activities present in foot, gill and abductor muscle tissues. Each tissue was analysed using three different sub-samples and each sub-sample was determined in triplicate. Enzymatic activities were measured at saturated concentrations of substrates and co-substrates. The homogenization buffer included 80 mM phosphate buffer (K_2HPO_4), pH 7.9, 1 mM dithiothreitol (DTT, except for ETS), 0.3% (wt/vol) polyvinyl pyrrolidone (PVP), 5 mM EDTA, 0.1% (vol/vol) Triton X-100 and 3% (wt/vol) bovine serum albumin (BSA). The homogenate was centrifuged at 5000 g during 5 minutes at 4°C. An aliquot of the supernatants was used for enzymatic assays. Pyruvate oxidoreductases (PORs: lactate dehydrogenase,

Corresponding author:
R.R. González
Email: rogonzal@udec.cl