

Geographic variability in eco-physiological traits of benthic calcifiers along natural temperature and pH gradients: the Chilean coast as a natural laboratory.

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Background: Chilean coast includes a nested hierarchy of environmental processes modulating the abundance and distribution patterns of the benthic species. At biogeographic scale there is an latitudinal gradient in sea surface temperature and pCO_2 fluxes, and this gradient interact with the regional influence of upwelling and river discharge dynamics (Fig. 1 A-H; compiled from Torres et al. 2011, Fuenzalida et al. 2010, Ramajo et al. 2013, Lardies et al. 2014, Navarrete et al 2015, Perez et al. 2015). In this study, we explore individual and population level responses of benthic calcifiers confronting this natural variability.

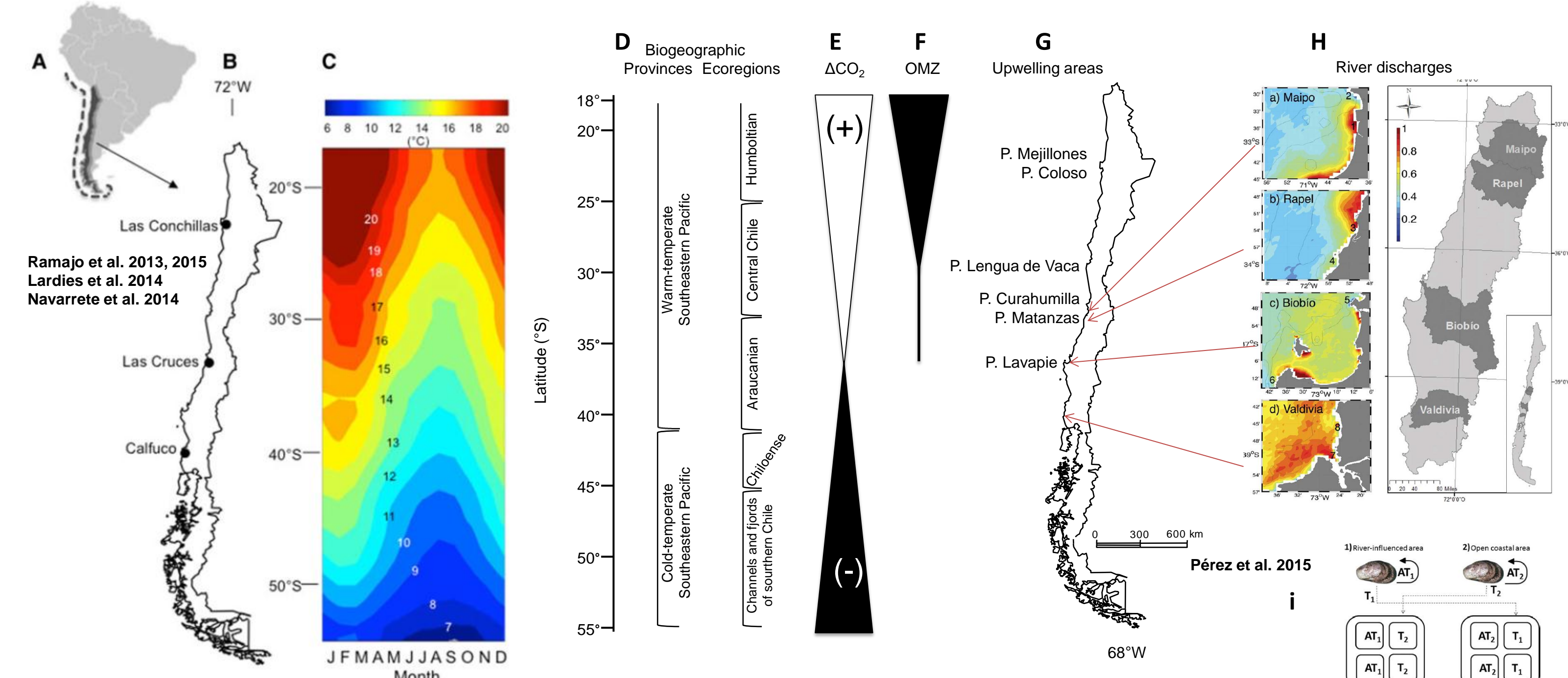
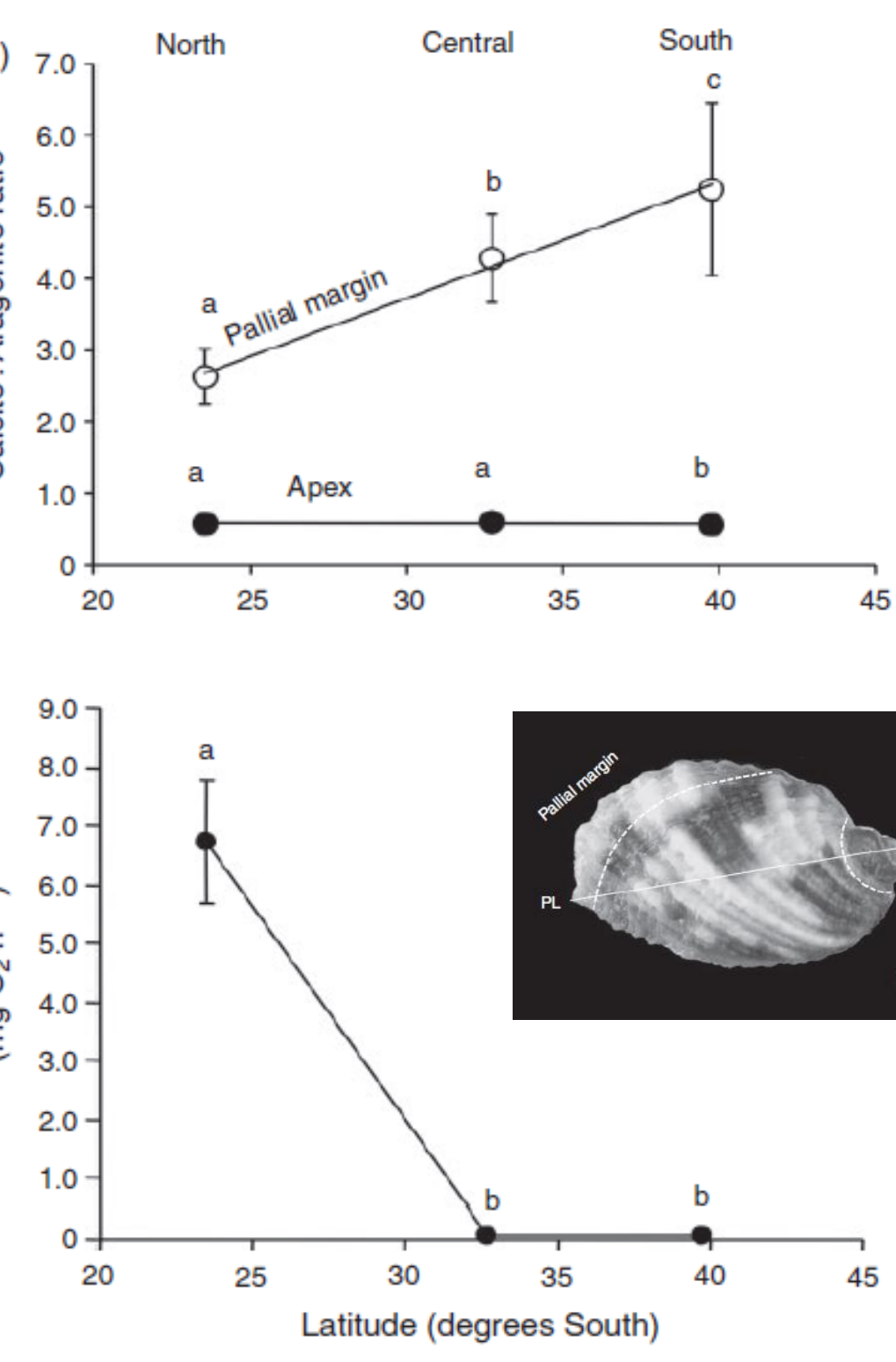


Fig. 1. Benthic realm of the southern Pacific Coastal Ecosystem along the Chilean coast (A-C, From Ramajo et al. 2014). Classification of the coastal ecosystem in: Provinces and ecoregions (D); Schematic latitudinal gradients in CO_2 fluxes (E), where (+) and (-) indicate CO_2 net outgassing and sink, respectively; Oxygen Minimum Zone (F); major upwelling areas in northern and central Chile (G); Horizontal arrows in SST and ΔCO_2 indicate the occurrence of significant anomalies associated to upwelling regions; major river influencing the nearshore areas of central-southern (H) and scheme of the reciprocal transplant experimental design (I, from Perez et al. 2015).

Methods: We use field survey along the Chilean coast and in situ reciprocal experiments (Fig. 1i, Perez et al. 2015, Ramajo et al. in press), to expose benthic calcifiers to natural changes in temperature and carbonate system parameters. We measured spatial variation in bio-mineralization, metabolism, abundance patterns in recruitment and carbonate production by benthic calcifiers (gastropods, mussels, barnacles).



Findings: Along the Chilean coast, we found changes in the proportion of calcium carbonate forms precipitated by the gastropods *C. concholepas* across their shells: the calcite:aragonite ratio in the pallial shell margin (i.e. newly deposited shell) increase significantly from northern to southern populations and this increase in calcite precipitation in the shell of juveniles snails was associated with a decrease in oxygen consumption rates in these populations (Fig. 1 from Ramajo et al. 2015).

In the case of mussels, the exposure to river-influenced conditions (Maipo river, Fig. 1H) increased metabolic rates and reduced growth rates, as compared to mussels experiencing marine conditions. While the energy investment strategies of the two local populations resulted in similar net calcification rates, these rates decreased significantly when mussels were transplanted to the river-influenced site. Stressful conditions at the river-influenced site were reflected evidenced by decreased survivorship across treatments (Fig. 3A-D). Freshwater inputs modify the organic composition of shell periostracum through a significant reduction in polysaccharides (Fig. 4).

Using intertidal barnacles, we tested the level of phenotypic plasticity in metabolism measured over two temperatures in the laboratory and we compared the slopes of the reaction norms among populations. Spatial variation in barnacle's metabolism showed non-clinal plasticity in the thermal reaction norm along the latitudinal gradient of the Chilean coast (Fig. 4 A-B). This non-clinal spatial patterns was also recorded for the Heat-comma of the individuals, but chill-comma showed a almost gradual decreasing toward highest latitudes (Fig. 5A-C). However, these cold resistance of southern population need more time for to recover from these sub-lethal conditions. These differences between local populations showed a biogeographic-scale dependence of the thermal sensitivity in the metabolism (Fig. 6).

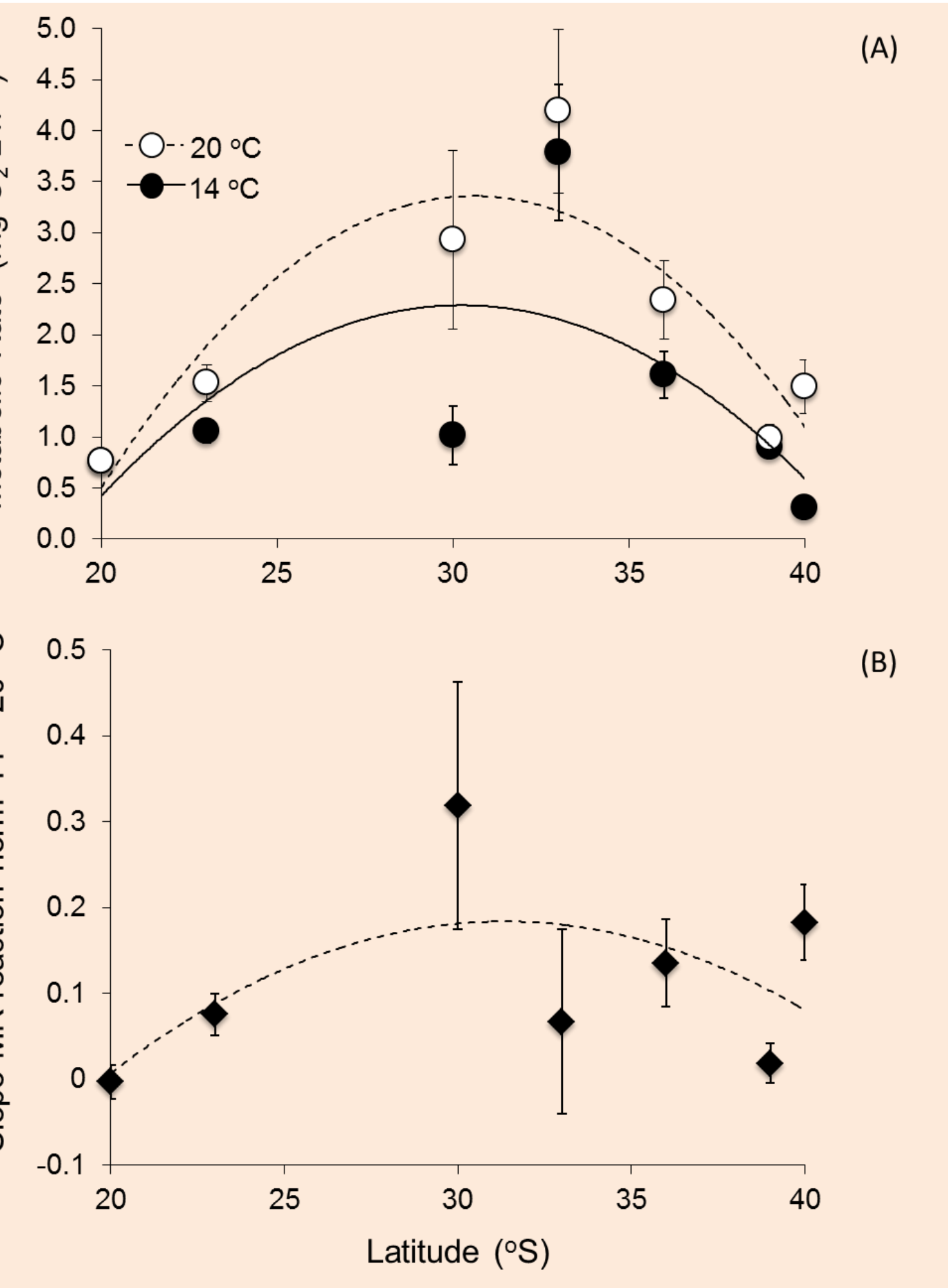


Fig. 4. Metabolic rates of the barnacles *Jehlius cirratus* (mean \pm SE) at 14 and 20 °C for each location across the latitudinal range; (b) The mean slopes of the metabolic rate (MR) reaction norm for 14–20°C across the latitudinal range. For each graph the lines represent the significant best fit using 2nd order polynomial regression.

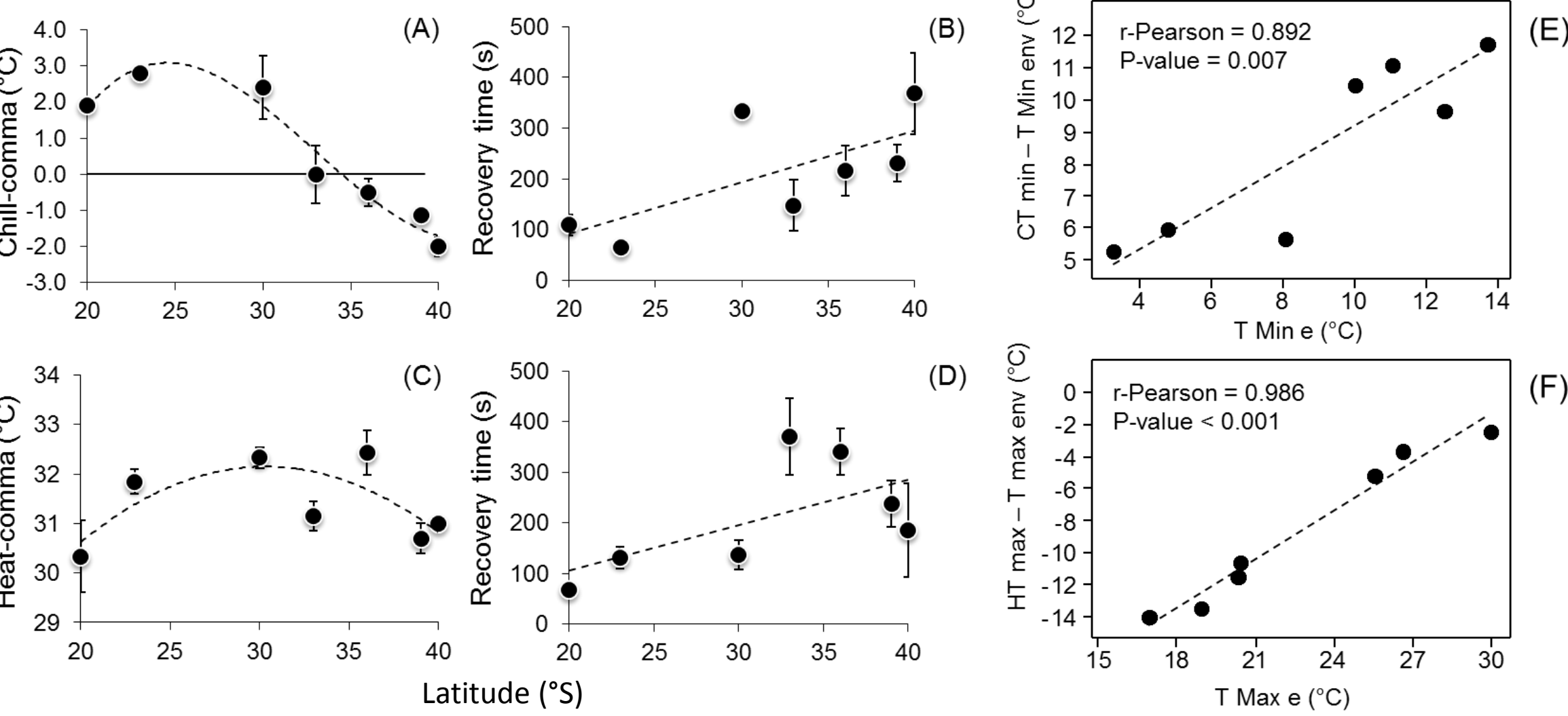


Fig. 5. *Jehlius cirratus*. Chill-comma (A) and recovery time from chill-comma (B); Heat-comma (C) and recovery time from heat-comma (D) recorded across the latitudinal range. For each graph the lines represent the significant best fit using polynomial regression; Relationship of cold (E) and heat (F) thermal safety margin with absolute environmental thermal minimum and maximum recorded at each site.

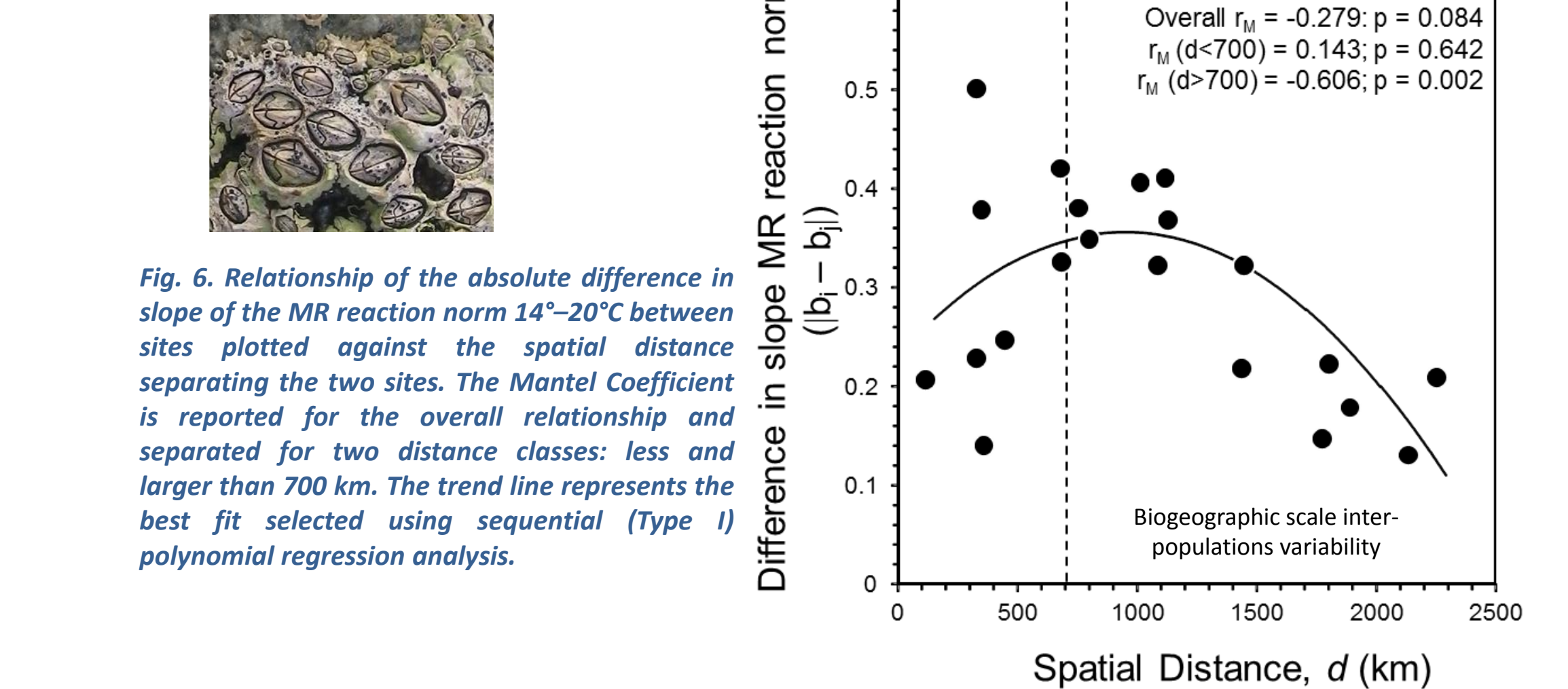


Fig. 6. Relationship of the absolute difference in slope of the MR reaction norm 14–20°C between sites plotted against the spatial distance separating the two sites. The Mantel Coefficient is reported for the overall relationship and separated for two distance classes: less and larger than 700 km. The trend line represents the best fit selected using sequential (Type I) polynomial regression analysis.

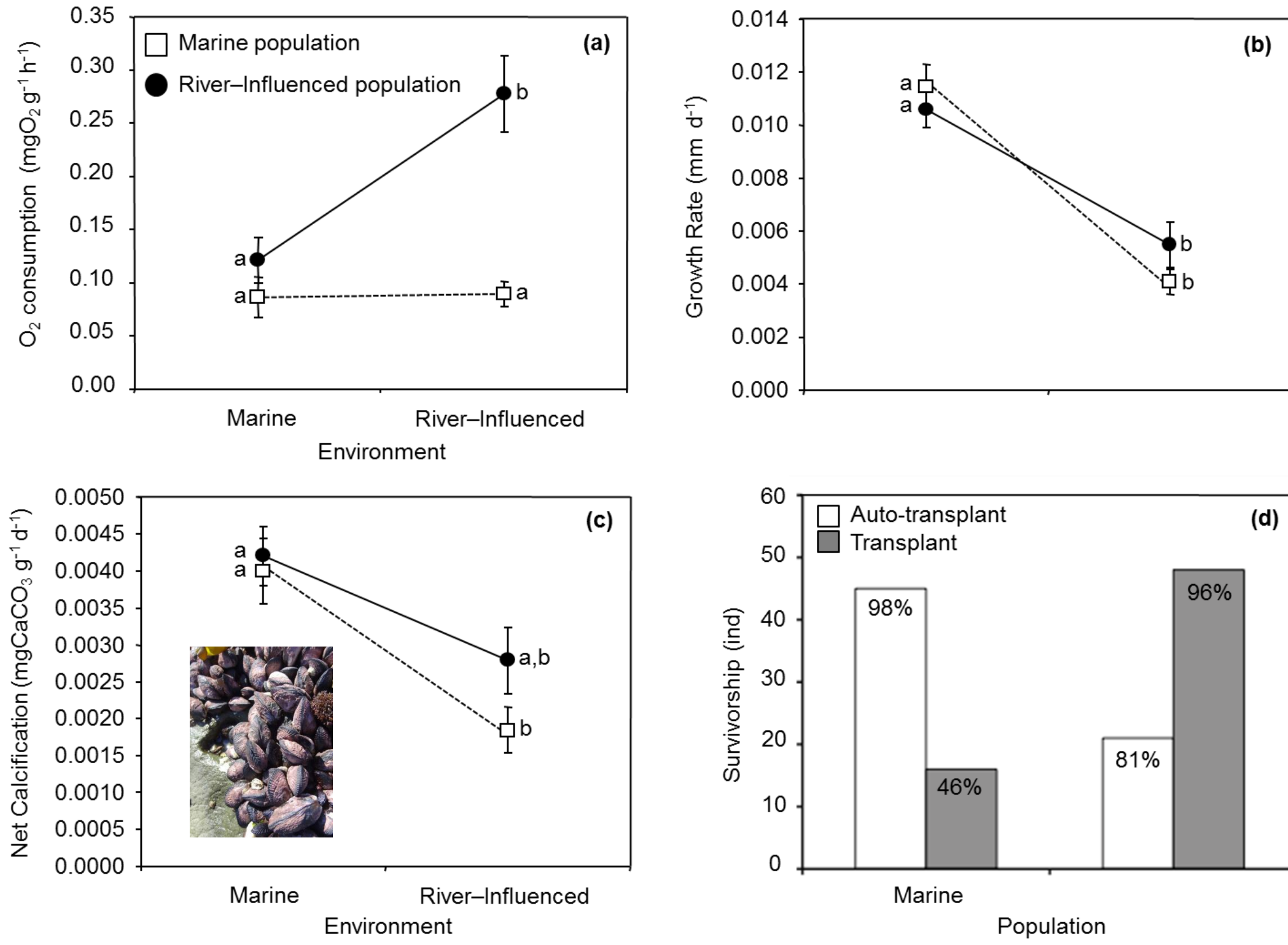
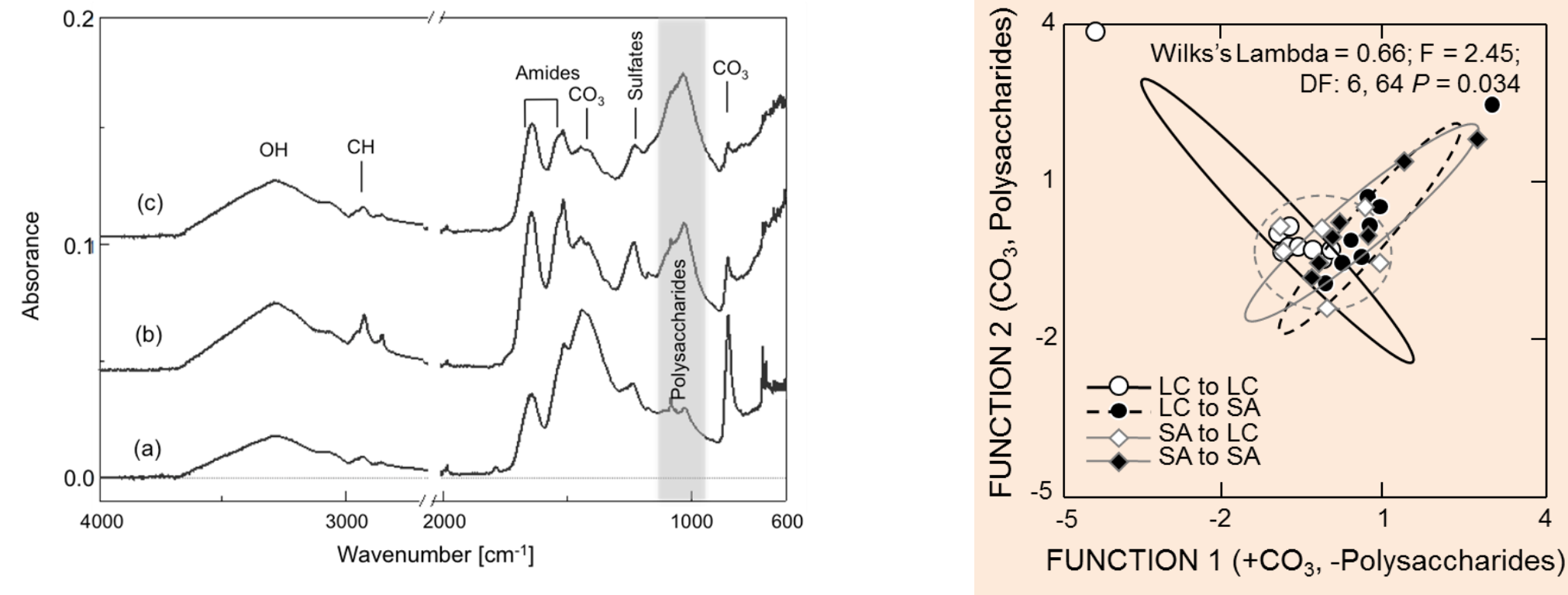


Fig. 3. *Perumytilus purpuratus*. (A) Metabolic rates measured as oxygen consumption ($mgO_2\ g^{-1}\ h^{-1}$), (B) growth rate ($mm\ d^{-1}$), (C) net calcification rate ($mg\ CaCO_3\ g^{-1}\ d^{-1}$) and (D) survivorship (individuals and %) of individuals exposed to environmental conditions of marine and river environmental under a reciprocal transplant experimental design

Fig 4. (left) ATR-FTIR spectra of the outer surface of the mussels *P. purpuratus* shell showing peaks associated to proteins, polysaccharides, sulfates, carbonates and lipids composing the periostracum and carbonate peaks from the underlying shell mineral. Individuals with a thin periostracum showing strong carbonate bands (A), with a normal periostracum (B) and with a periostracum with a high amount of polysaccharides (C). (Right) Discriminant functions analysis generated using relative levels (absorbance values from FTIR) of carbonate signals (CO_3) in combination with polysaccharides recorded on the shell periostracum of *P. purpuratus* for each treatment after 154 days of cross-reciprocal experiment. Inset is showing the summary of multivariate analysis of variance (MANOVA).



Conclusions: Our result suggests that along the Chilean coast calcite secretion may be favored when metabolic rates of *C. concholepas* are lowered, as this carbonate mineral phase might be less energetically costly for the organism to precipitate. In the case of mussels, although our field experiment did not identify specific environmental factors underlying these biological differences, the observed phenotypic changes imply that plasticity plays a strong role when *P. purpuratus* are exposed to river-induced environmental variability in their habitats, and suggests that the lack of this exposure may promote less tolerant mussels with potential for local adaptation. Barnacles evidenced plasticity in metabolism and intra-population variability in thermal responses, with a strong role of thermal safety margins in determining the extent of these population differences. These result suggest that the environmentally-variable latitudinal template imposed along the Chilean coast promote the expression of phenotypic plasticity to cope with these variations, but these expression may trade-offs and compromise other important organismal responses.