

flexibility it allows in optimizing bilayer composition and aggregate size, at each step of the self-assembly process. □

Methods

Interior vesicle aggregates. Unilamellar vesicles were prepared by mixing dilaurylphosphatidylcholine (DLPC; Avanti Polar Lipids, Alabaster, Alabama) and dipalmitoylphosphatidylethanolamine-conjugated biotin (DPPE-biotin; Molecular Probes, Eugene, Oregon) at 0.16 mol% total lipid in chloroform, evaporating the solvent, then hydrating the lipid film with aqueous buffer (100 mM NaCl, 50 mM TES, and 0.02 wt% NaN₃, balanced to pH 7.2) for 48 h at 37 °C to form a 30 mg ml⁻¹ dispersion of multilamellar vesicles. The solution was put through five freeze-thaw (liquid nitrogen-50 °C water bath) cycles followed by ten high-pressure extrusions through two stacked 100-nm-pore polycarbonate Nucleopore filters (Corning Costar, Cambridge, Massachusetts) to produce a relatively monodisperse dispersion of 100-nm unilamellar vesicles (Fig. 1a). A 100-nm vesicle of this composition contains ~80 DPPE-biotins protruding from the monolayer.

To aggregate the vesicles, sufficient (0.63 mg ml⁻¹) streptavidin (Molecular Probes, Eugene, Oregon) in the same buffer was added to produce an overall biotin-streptavidin ratio of 15:1; however, the ratio of biotin on the outside of the vesicle available for binding to streptavidin was 8:1. As streptavidin has four distinct binding sites for biotin, the ratio of exposed biotins to binding sites was 2:1, meaning there are excess surface ligands (Fig. 1). The addition of streptavidin solution diluted the dispersion of unilamellar vesicles to 20 mg per ml total lipid. Within an hour, the 20 mg per ml ULV/streptavidin suspension changed from clear and bluish to opaque and cloudy-white, indicating that vesicle aggregates were forming⁹. The aggregates were filtered under pressure through 1.0-µm Nucleopore filters to produce the sized aggregates shown in Fig. 2.

Cochleate cylinders. Cochleate cylinders were prepared by first making a dispersion of 100-nm unilamellar vesicles containing 10 mg ml⁻¹ of 1,2-dioleoylphosphatidylserine (DOPS; Avanti Polar Lipids, Alabaster, Alabama) with 0.16 mol% DPPE-biotin as previously described⁹. Equal (1 ml) volumes of the DOPS/DPPE-biotin vesicle solution (10 mg ml⁻¹) and a 6 mM CaCl₂ (Sigma, St Louis, Missouri) buffer solution were mixed. Immediately after mixing, the solution turbidity increased, indicating that cochleate cylinders had formed. Freeze-fracture transmission electron microscopy (not shown) confirmed that cochleate cylinders had formed, indicating the added DPPE-biotin did not alter the cochleate structure. 35 µl of 0.63 mg ml⁻¹ streptavidin solution was injected into 1 ml of the cochleate cylinder solution and allowed to incubate for one day to fully saturate the biotin-lipids at the cylinder surface.

Vesosome assembly. The sized vesicle aggregates and the cylinders were mixed at a 1:1 DLPC:DOPS mole ratio: 1.0 ml of the 5 mg ml⁻¹ DOPS cylinders (6.2 µmol of DOPS) was added to 0.19 ml of the 20 mg ml⁻¹ DLPC vesicles (6.2 µmol of DLPC). To remove calcium ions, 0.44 ml of 5 mM EDTA was added to 0.5 ml of the mixture, resulting in a solution containing 4.2 mg ml⁻¹ DOPS lipid and 3.2 mg ml⁻¹ DLPC lipid. Freeze-fracture TEM samples were prepared by standard techniques²² after one day of incubation before adding EDTA (Fig. 3a), and 5 h of incubation after adding EDTA (Fig. 3b).

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Temperature effects on the acidity of remote alpine lakes

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Climate variations and changes in sulphur and nitrogen deposition from the atmosphere influence the acid-base balance of sensitive lakes in a complex and site-specific way^{1–3}. For example, although lakes in several regions have shown a decline in sulphate concentration following reductions in atmospheric sulphate deposition^{4–6}, the expected recovery of pH and alkalinity has not always taken place, implicating an additional response to changes in the local climate. Here we report a study of 57 remote alpine lakes which shows that, between 1985 and 1995, lake pH and the concentration of sulphate, base cations and silica have increased, whereas inorganic nitrogen concentrations have decreased. This contrasts with atmospheric input trends, which have led to a decrease in sulphate and a slight increase in nitrogen deposition over the same period^{7,8}. We propose that the changes in lake chemistry are therefore likely to be caused by enhanced weathering and increased biological activity resulting from an increase in air temperature of about 1 °C since 1985. Our analysis of an alpine lake core covering a 200-year period provides further evidence for a strong positive correlation between pH and mean air temperatures, and thus for the high sensitivity of lakes at high altitudes and high latitudes to climate warming. In these remote locations, temperature effects, rather than acid deposition, appear to dominate changes in lake acidity.

We studied 57 low-alkalinity high-mountain lakes in glaciated and non-glaciated catchments, situated between 2,000 and 2,900 m above sea level (m.a.s.l.) on the northern (North Tyrol) and southern slope (East Tyrol, Carinthia) of the eastern Alps. The area is characterized by granites and gneisses of high sensitivity to acid deposition⁹. Soils are poorly developed with sparse vegetation, especially at very high altitudes where large portions (70–90%) of the catchments consist of bare rock. Samples were collected during the autumn overturn in 1985 and 1995 and analysed for pH,

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conductivity, alkalinity, silica, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), major anions and cations, and phosphorus. In Schwarzsee ob Sölden (SOS, 2,796 m.a.s.l.), a sediment core, covering the period from 1778 to 1991, was collected for analyses of fossil diatoms and chrysophytes, fossil pollen, and geochemistry. Monitoring¹⁰ of SOS began in 1984 and is still going on.

Since the minimum in 1885, air temperatures have warmed by ~2°C at mountain stations in the central Alps^{11,12}; 1°C of this change has occurred since 1985. The overall increase is more than twice that reported for the Northern Hemisphere average (Fig. 1a; compare also Fig. 4 for running averages) but comparable to the mean calculated from 20 stations in Austria, located predominantly at lower altitudes¹³. As in the Swiss Alps¹¹, total annual precipitation in the central Alps has not changed significantly since 1980 (Fig. 1b). Sulphate deposition has decreased since 1985, but inorganic nitrogen (dissolved inorganic nitrogen DIN = nitrate + nitrite + ammonium nitrogen) increased slightly during this period (Fig. 1c).

In contrast to deposition trends, base cations and SO₄²⁻ increased between 1985 and 1995 in most lakes, while inorganic nitrogen (DIN) decreased (Fig. 2). DON concentrations increased in all but four lakes. The pH (Fig. 2a) increased by an average of 0.25 units, calculated from differences in H⁺ concentrations. Alkalinity (Fig. 2b) increased in 63%, but decreased in 28%, of the lakes, with an average increase of

~10 μequiv.l⁻¹. The mean increase in silica of 10 ± 20 μmol l⁻¹ between 1985 and 1995 compares fairly well with an annual increase of 1.4 μmol l⁻¹ in the SOS (Fig. 3) from 1984 to the end of 1996. The SOS has undergone a gradual increase in sulphate (4.2 μequiv.l⁻¹ yr⁻¹) and base ion concentrations (4.4 μequiv.l⁻¹ yr⁻¹), whereas nitrogen decreased by 0.4 μequiv.l⁻¹ yr⁻¹ and chloride showed no significant change.

One of the most obvious effects of higher mean air temperatures on high mountain lakes is a shorter period of snow and ice cover, which improves exchange of gases and nutrients, wind-driven circulation and light conditions^{14,15}. Around 1900, for example, and during some years in the cooler period around 1970, the slopes in the catchment area of the SOS were permanently covered by snow. In 1995 these slopes were snow-free in summer, and the resultant increase in temperature exceeded by far the average difference of 1 or 2°C in air temperature (Fig. 1a), especially on south-facing slopes. Since 1980, glaciers in the eastern Alps have retreated continuously and 90% of the glaciers examined (n = 96) in 1995 have suffered a substantial loss in area¹⁶. This has created relatively large surfaces covered with fine glacial flour which provide, in combination with higher temperatures and liquid water, a reactive environment for intense chemical weathering and mineral dissolution¹⁷ (Fig. 2c).

In the last 10 years, SO₄²⁻ concentration has increased in 80% of

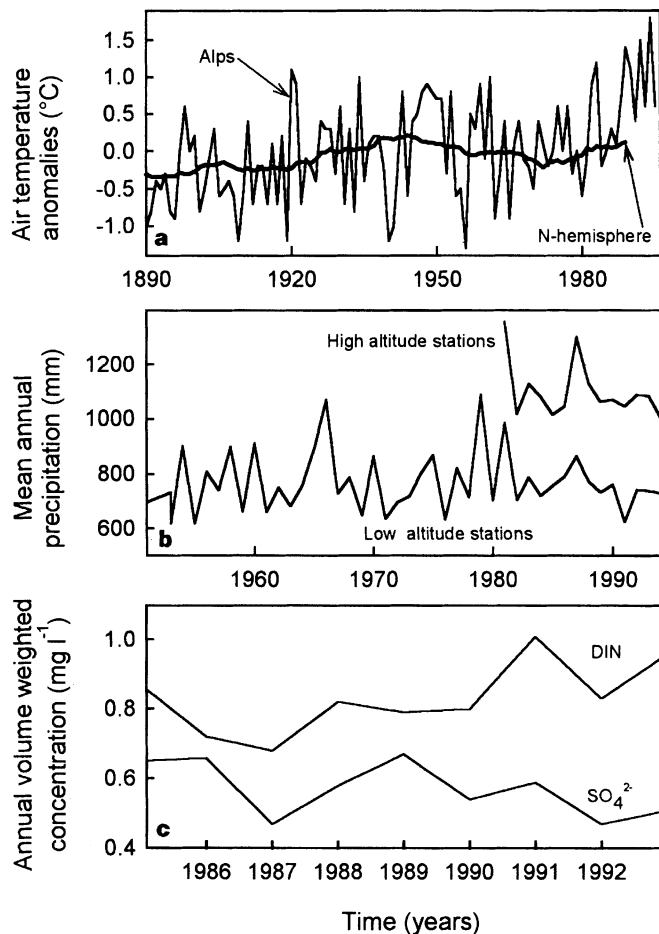


Figure 1 Temperature, precipitation and deposition trends in Alpine stations. **a**, Annual mean air temperature anomalies from five stations of the eastern Alps, and the Northern Hemisphere average; calibration period, 1901–50. **b**, Mean annual precipitation from five mountain stations. **c**, Trends in annual volume weighted sulphate and DIN (dissolved inorganic nitrogen: nitrate, nitrite and ammonium) concentrations in precipitation.

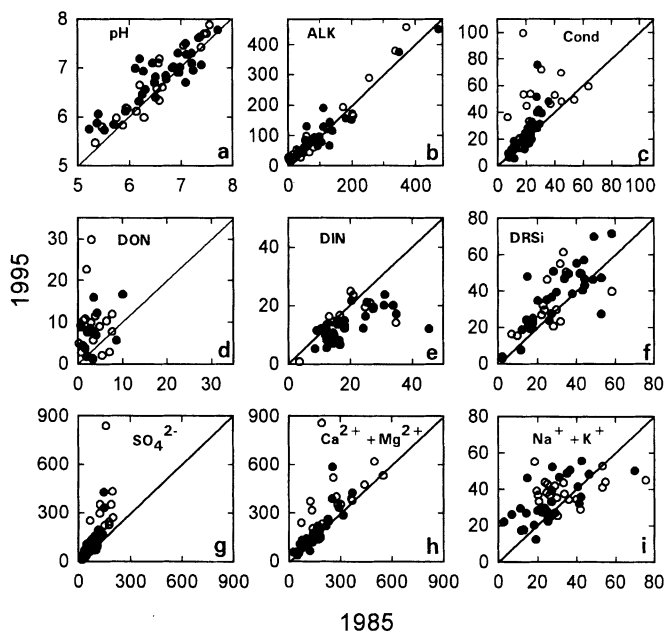


Figure 2 Comparison of chemical parameters from 57 high mountain lakes measured in 1985 and 1995. Open and closed circles represent lakes with glaciated and non-glaciated catchments, respectively. The solid lines represent the 1:1 ratio. **a**, pH; **b**, alkalinity (μequiv.l⁻¹); **c**, conductivity (μS cm⁻¹); **d**, dissolved organic nitrogen, DON (total dissolved nitrogen – DIN, μmol l⁻¹); **e**, dissolved inorganic nitrogen, DIN (nitrate + nitrite + ammonium, μequiv.l⁻¹); **f**, dissolved reactive silica, DRSi (μmol l⁻¹); **g**, sulphate (μequiv.l⁻¹); **h**, calcium + magnesium (μequiv.l⁻¹); **i**, sodium + potassium (μequiv.l⁻¹).

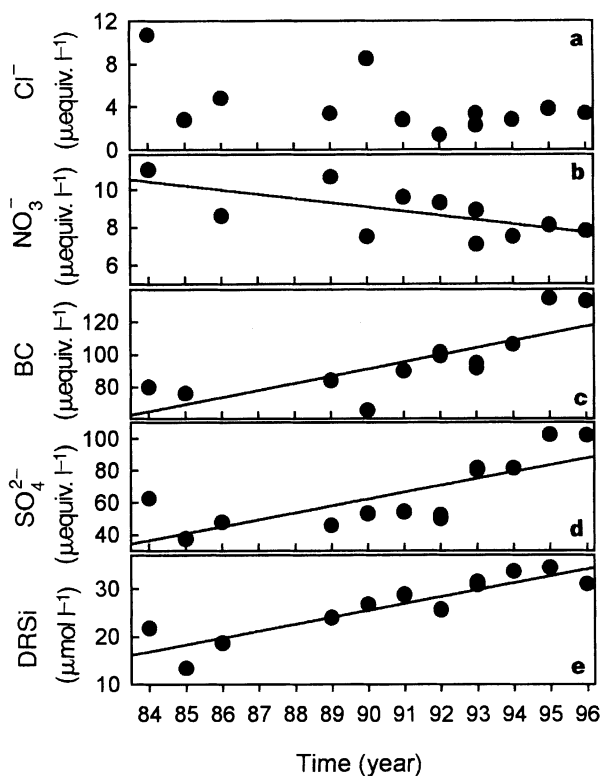


Figure 3 Changes in ion and dissolved reactive silica concentration in Schwarzsee ob Sölden under open lake conditions (summer) from 1984 to the end of 1996, and annual trends calculated from the slope of linear regressions. **a**, chloride. **b**, Nitrate, $-0.4 \mu\text{equiv. l}^{-1} \text{yr}^{-1}$ ($r^2 = 0.48$, $P < 0.01$). **c**, Base cations (BC), $4.4 \mu\text{equiv. l}^{-1} \text{yr}^{-1}$ ($r^2 = 0.60$, $P < 0.01$). **d**, Sulphate, $4.2 \mu\text{equiv. l}^{-1} \text{yr}^{-1}$ ($r^2 = 0.56$, $P < 0.01$). **e**, Dissolved reactive silica (DRSi), $1.4 \mu\text{equiv. l}^{-1} \text{yr}^{-1}$ ($r^2 = 0.79$, $P < 0.0001$).

the investigated lakes; in lakes surrounded by glaciers, concentrations were up to 400% higher than in 1985 (Fig. 2g). Pulses of sulphate-rich water low in alkalinity following drought or due to desorption from mineral surfaces have been documented at several acidified sites in North America^{18–21}. However, as annual precipitation was fairly constant in the eastern Alps during the last decade (Fig. 1b) and lakewater alkalinity increased slightly (Fig. 2b), we assume that dissolution of easily soluble calcium and magnesium sulphates in the catchment is the main factor responsible for the SO_4^{2-} increase. This is supported by the fact that the increase in SO_4^{2-} was generally well balanced by Ca^{2+} and Mg^{2+} both in non-glaciated ($r^2 = 0.81$, $P < 0.05$) and glaciated catchments ($r^2 = 0.97$, $P < 0.05$). Pyrite oxidation, which produces sulphuric acid and thus enhances the weathering of silicate or carbonate minerals, could also explain increased concentrations of SO_4^{2-} and base cations, but the comparably small increase in silica and alkalinity (Fig. 2f, b) suggests that most alkaline-earth and SO_4^{2-} ions are dissolved concomitantly (Fig. 2h). Monitoring data from the SOS (Fig. 3) showed similar rates of base-cation and sulphate increase (4.4 and $4.2 \mu\text{equiv. l}^{-1} \text{yr}^{-1}$, respectively), but a much lower rate for silica ($1.4 \mu\text{equiv. l}^{-1} \text{yr}^{-1}$) increase. The increase in silica, paralleled by equimolar amounts of sodium and potassium (Fig. 2f, i), indicates enhanced silicate weathering in $\sim 80\%$ of the lakes. Studies in granite and gneiss catchments have shown that weathering rates may intensify by an order of magnitude when temperature is increased from 0 to 25 °C while precipitation is kept constant²². Such temperature increases are possible in summer on sunny slopes which were previously snow-covered throughout the year.

Contrary to precipitation trends, DIN concentrations declined in 81% of the lakes. A shorter period of lake ice-cover, like that

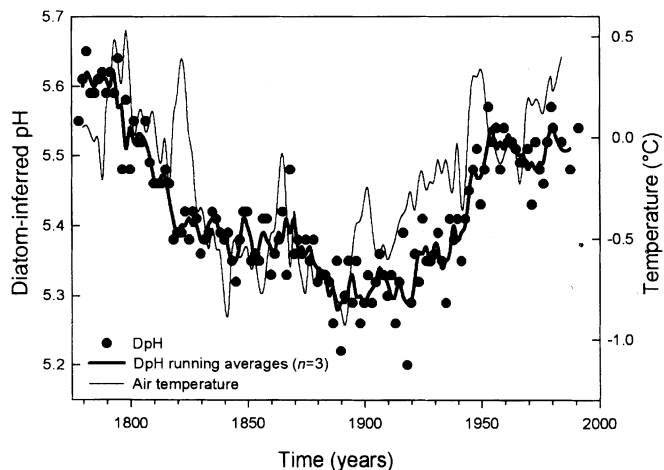


Figure 4 Diatom-inferred lakewater pH (DpH) from the sediment core of Schwarzsee ob Sölden and mean air temperature in Austria for the period 1778–1991. Air temperatures are the running averages ($n = 9$) of annual means from 20 stations¹³.

observed in the SOS, probably improved the conditions for phytoplankton growth by increasing light availability, temperature and nutrient input. The enhanced biological production would lead to faster recycling of nitrogen, which explains the lower DIN, and higher DON, concentrations found in almost all lakes (Fig. 2d, e). This assumption is supported by the relatively large increase in pH (0.25 units, compared to an alkalinity increase of only $10 \mu\text{equiv. l}^{-1}$) indicating higher productivity. The alternative explanation, that the above conditions promoted higher productivity of terrestrial plants, is less probable as the soils are poorly developed and low in organic content.

Further evidence for the dominant role of temperature on pH comes from palaeolimnological investigations in the SOS. In this lake, the diatom-inferred pH (DpH) has gradually increased since 1900 by 0.35 units (Fig. 4), following closely the mean air temperature of Austria. Statistical analyses of the mean air temperature and of the stratigraphical pollen, carbon, nitrogen and DpH data show a significant correlation between temperature and DpH, with 48% of the DpH variation explained by temperature and an additional 13% by carbon and nitrogen concentrations. A similar correlation was found for two lakes of the southern slope of the Alps, where acid precipitation has led to a decoupling of the pH–temperature relationship observed before 1900 (ref. 23). In the SOS, pH trends can be approximated by the formula $\text{pH} = 5.46 + 0.19 \text{ Temperature (}^\circ\text{C)}$ ($r = 0.68$, $P < 0.001$, $n = 128$). Notwithstanding the considerable differences in altitude and bedrock geology of the 57 lakes studied, the mean pH increase of 0.25 units between 1985 and 1995 corresponds surprisingly well with the predictions obtained from palaeolimnological data.

Our results show that climate warming is a determinant factor for the water chemistry of remote alpine lakes, and that it can mitigate or even compensate for the anticipated effects of acid deposition. A compensating effect on the expected recovery of pH and alkalinity following reduced sulphur deposition has previously been attributed to an increase in nitrogen loads²⁴, a decrease in base-cation deposition^{25–28} and reoxidation of reduced sulphur in lake sediments² or runoff from catchments^{18,19}. Changes in lakewater chemistry due to climate warming have been suggested before^{14,20}. But while these earlier studies identified climatic effects due to drought-induced changes in water retention times¹⁴ and groundwater flow²⁰, our data indicate that enhanced weathering and biological activity are the main mechanisms that cause the trends in ionic composition, nitrogen and silica concentration, and pH observed in the study area. This finding implies that aquatic ecosystems at both high altitudes and

high latitudes might respond with extreme sensitivity to climate warming. In addition, our results further illustrate that the quantity and direction of change due to climate warming depend on site-specific processes and are, for this reason, not readily predictable for aquatic systems in different climatic and geographical areas. □

Methods

Water analysis. Water samples were taken, during autumn overturn, from several depths with a Patalas-Schindler sampler. Analytical methods: alkalinity, Gran titration; major anions and cations, ion chromatography; total dissolved nitrogen, oxidation by potassium peroxodisulphate and reduction on a Cd column; dissolved reactive silica, oxalic acid/molybdate method. Internal quality controls of the data were made by ionic charge balance and by comparing the measured and calculated conductivity. External quality assurance was provided by regular participation in international ring tests.

Palaeolimnology. Recent sediments were obtained with a modified Kajak sampler (outer diameter = 6 cm) and cut in 0.25 cm slices. Fossil diatoms were extracted using standard techniques²⁹. DpH was calculated using weighted average regression and a regional alpine data set³⁰. The average annual sedimentation rate of the core, dated by ¹³⁷Cs and ²¹⁰Pb, was 1.5 mm ± 0.1 mm over the last 100 years. For sediment layers older than 100 years the dating error may be substantial.

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Structural features in a brittle-ductile wax model of continental extension

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Structural features produced during the rifting of continents depend on the layered rheological properties of the crust and lithosphere and, in particular, on the presence of any transitions between brittle and ductile behaviour¹. Here we use a wax model to explore the gross structural response of continental lithosphere under pure shear extension in the presence of a continuous brittle–ductile transition. The wax models were deformed under various boundary conditions to reflect a variety of different regions, most notably the Basin and Range province of North America. Our experiments show the development of listric normal faults, structures common to regions of continental extension. We also observe the formation of distributed and discrete rifting, and intrusion and occlusion of the upper brittle layer by the ductile lower layer. The factor controlling deformation style in each case appears to be the relative thickness of the brittle and ductile layers, although a relatively high rate of strain generally promotes discrete rifting.

Rheological layering in wax, including a continuous transition between brittle and ductile behaviour, may be made by generating a vertical temperature gradient giving a transition from brittle to ductile behaviour at some depth. Previous modelling of plate-boundary processes using wax involved a solid–fluid transition (rather than a brittle–ductile transition) and were able to demonstrate successfully the importance of the brittle layer in the production of ridge-transform structures at sites of ocean-floor spreading^{2,3}.

The first set of experiments was designed to test the influence of the relative thickness of brittle to ductile layers. A smoothly varying vertical thermal gradient was generated in the wax by heating from below (Fig. 1a), resulting in a relatively broad transition from a ductile to a brittle rheology (distinguishable by the relative opacity of the wax). The relative thickness of the brittle and ductile layers and the gradient of the transition was controlled by the time over which the upper surface was cooled (Fig. 1a). The upper portion of the slab remained brittle under all strain rates. Confining pressure is absent in these experiments and failure occurs by cracking rather than by faulting. If we adopt a coordinate system with *x* along the length of the slab, *y* perpendicular to *x* and within the slab, and *z* vertical, then the strain $e_{xx} > 0$ was imposed and both $\sigma_{yy} = \sigma_{zz} = 0$ and all shear stresses, $\sigma_{ij} (i \neq j)$, were equal to zero on model surfaces. Of course, complex local variations in e_{xx} , e_{yy} , and e_{zz} resulted from the brittle–ductile transition. These boundary conditions are similar to those adopted in various numerical models^{4–6} and are likely to be similar to those in actively extending continents. In particular, if the double-layered model is considered analogous to a continental crust, then the infinitely stiff base under the ductile layer represents the strong upper mantle beneath the Moho. Alternatively, the slab