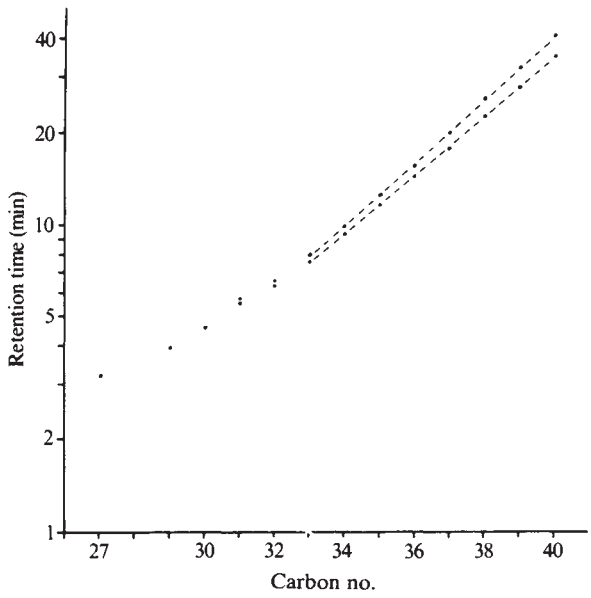

Fig. 3 Log/normal plot of retention times versus carbon number for C_{27}–C_{40} hopanes obtained from isothermal gas chromatography at 260 °C (other conditions as given in Fig. 1). The C_{33}–C_{40} hopanes show linear increase of log retention time, different for the C-22 diastereomers, indicating the presence of homologous series.

point to an advanced maturity level of the organic matter well within the oil window, as indicated by the dominance of 14β(H), 17β(H)-steranes[8]; this can easily be seen in Fig. 1 for the C_{29} steranes.

Extended 17α(H)-hopanes in Thornton bitumen are pairs of C-22 diastereomers[9] (Fig. 1). The carbon number maximum within this series at C_{35} either indicates a particular stability of this species or, more likely, a C_{35} precursor for most of the C_{31}–C_{35} extended hopanes as mentioned above. In contrast to hopane series reported so far, the extended hopanes in Thornton bitumen range up to C_{40}. Diastereomeric pairs are observed as in the C_{31}–C_{35} range together with a slight predominance of the first-eluting diastereomer (Fig. 1; the second C_{36} isomer coelutes with a dicyclic tetraterpane, probably perhydro-β-carotene). The mass spectrum of the first-eluting C_{40} hopane in Fig. 2 shows a fragmentation pattern consistent with a hopane structure with an extended side-chain. From the log/normal plot of retention times obtained at isothermal conditions against carbon number (Fig. 3), which shows a linear increase for the C_{33}–C_{40} hopanes, the conclusion may be drawn that the side-chain extension is linear, as found for the C_{31}–C_{35} hopanes[6], and no branching occurs.

The question now is whether the C_{40} hopanes represent another end member in this series related to a specific precursor or whether this series extends beyond C_{40}? This cannot be unequivocally answered from the analysis of this distillation cut. No hopanes above the C_{40} members marked in Fig. 1 could be detected in this sample, which may be due to the limiting experimental conditions (temperature) or the way the distillation cut was taken (exact temperature range unknown). If there were a C_{40} precursor, the latter argument could explain the lower abundance of the C_{40} hopanes relative to the C_{36}–C_{39} compounds.

As there is no precursor yet known for the C_{40} hopanes, another mechanism for the formation of the C_{36}–C_{40} extended hopanes is suggested. It has recently been found[10] that highly alkylated porphyrins in petroleum contain alkyl-chains up to C_{11}. These porphyrins seem to be catagenetic products originating from porphyrin moieties bound to the kerogen matrix and released only at higher maturation levels by thermal cracking[11]. A similar mechanism[3] for the extended hopanes seems possible if one assumes that bacteriohopanetetrol (C_{35}) is specifically bound into the kerogen matrix during early diagenesis through a covalent carbon–carbon bond.

In addition to the extended 17α(H)-hopanes, a series of moretanes (17β(H),21α(H)-hopanes) has been tentatively identified in Thornton bitumen (Fig. 1). The series seems to include C_{31}–C_{35} moretanes although only the C_{31} compound in the mass spectrum clearly showed the slight dominance of the D/E-ring fragment (m/z 205) over the A/B-ring fragment (m/z 191) (ref. 12). Trace amounts of these compounds have been reported[13] for a Toarcian shale from the Paris Basin. Unlike the 17α(H)-hopanes, however, the extended moretanes were not detected as diastereomeric pairs (R and S at C-22) in Thornton bitumen; it seems possible that they are not separable in the given conditions[2].

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Nitrous oxide cycling in Lake Vanda, Antarctica

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Nitrous oxide (N_2O) is a key intermediate for several steps of the aquatic nitrogen cycle. In oxygenated oceanic waters, bacterial oxidation of amino- and ammonium-N is a dominant source of N_2O (ref. 1); in regions of intense nitrification ΔN_2O values (difference between observed N_2O and air-equilibrium concentration) typically rise to 40–100 nmol l^{-1} (ref. 2). By contrast, anoxic waters are often undersaturated in N_2O because of respiratory consumption by denitrifying bacteria[3]. We describe here an extreme accumulation of nitrous oxide (ΔN_2O of >2,000 nmol l^{-1}) in the saline bottom waters of Lake Vanda (77°35′S, 161°40′E), a warm meromictic water body in the Dry Valley region of Antarctica. From *in situ* experiments assaying various components of the nitrogen cycle, we conclude that this N_2O is produced by a narrow band of nitrifiers lying well above the oxycline. Nitrous oxide is lost from this zone at slow rates by diffusion, ultimately to the atmosphere above, or below to the upper anoxic zone where it is consumed by a finely stratified population of denitrifying bacteria.

Lake Vanda was sampled at a mid-lake deep-water site (maximum depth 68 m) during the austral summer of 1980–81. Holes were bored through the 3–3.5-m permanent ice-cap and 1-l samples removed with a discrete-depth water sampler. The unusual temperature and salinity profiles of Lake Vanda have been described elsewhere[4,5]. Temperatures rise with increasing depth to a warm maximum (23.5 °C) in the region of high salinity (up to three times the conductivity of seawater) at the bottom of the lake (Table 1). Over the season of study the lake was well oxygenated to 59 m (~0.7 mmol l^{-1} throughout, S. Nakaya, unpublished data) but anoxic at 60 m and below.

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Table 1 Distribution of nitrogen and other characteristics of the mid-lake water column of Lake Vanda

Depth (m)	Forms of nitrogen (μg-atom l^{-1})					$N_2O:NO_3$ (molar ratio × 10^3)	Dissolved reactive P (μg-atom l^{-1})	Temperature (°C)	Conductivity (10^3 μS cm^{-1})
	NO_3-N	NO_2-N	N_2O-N	NH_4-N	Particulate-N				
3.25	3.5	0.10	0.05	0.9	0.7	7.1	0.02	4.5	0.6
5	4.1	0.11	—	0.6	—	—	0.01	5.0	0.7
15	2.2	0.08	0.15	0.2	0.3	34.1	0.03	6.5	1.1
25	2.1	0.04	0.18	0.1	0.7	42.8	0.01	7.0	1.2
35	2.3	0.05	0.19	0.1	0.5	41.3	0.01	7.0	1.2
45	4.4	0.02	0.51	0.7	0.4	57.9	0.02	9.0	2.3
47.5	7.3	0.07	0.72	—	—	49.3	0.02	9.5	—
50	47.0	0.17	1.96	16.9	0.6	20.9	0.01	12.5	8.0
51	89.7	0.27	2.18	19.1	—	12.1	<0.01	—	—
52	133.5	0.39	2.78	27.8	—	10.4	0.02	—	—
53	203.9	0.79	3.94	71.8	—	9.7	—	—	—
54	223.8	0.96	4.29	119.3	—	9.6	—	—	—
55	232.7	1.14	3.80	144.1	0.8	8.2	<0.01	18.5	39.5
56	165.4	1.90	3.51	269.2	—	10.6	0.02	—	—
57	106.3	0.46	2.91	363.3	—	13.7	0.04	—	—
58	56.6	0.27	2.53	458.2	1.3	22.3	0.17	20.0	56.0
59	13.2	0.33	1.04	599.4	—	39.4	0.10	—	—
60	3.1	0.10	0.05	686.9	1.9	8.1	0.15	22.0	60.9
62.5	<1.0	<0.1	<0.003	1005.6	—	—	2.21	22.5	79.5
65	<1.0	<0.1	<0.003	1346.5	—	—	5.24	23.5	80.6
67.5	<1.0	<0.1	<0.003	1747.4	—	—	8.15	23.5	80.8

Samples were collected 21 and 27 December 1980. All depths are relative to the water table, ~0.2 m below the ice surface. Water samples for nutrient analysis were filtered immediately on collection through acid-washed glass-fibre GF/C filters and stored frozen. Analyses were performed on a Technicon AutoAnalyzer II. Lakewater controls (sample without colour reagents) were run at all depths to correct for changes in refractive index. Further control samples were run with standard nutrient additions to correct for variable recoveries at increasing salt concentrations. Nitrite was measured by automated diazotization[18]. Nitrate was reduced to nitrite through a cadmium wire column[19] at *p*H 8 (ref. 20) and then analysed by standard diazotization[18]. NH_4 was determined by an automated phenol–hypochlorite method[21]; high values in the bottom waters of Vanda were checked manually, after 100-fold dilution, by oxidation to nitrite and diazotization[22]. Dissolved reactive phosphorus was by an automated molybdenum blue method[23]. Particulate nitrogen was filtered on to acid washed GF/C filters and stored frozen. Material was Kjeldahl-digested and the ammonium determined colorimetrically[24]. For gas analysis, 15-ml lakewater samples were removed from the water sampler by hypodermic syringe and injected into 30-ml Hypovials that had been sealed with neoprene[25] stoppers and flushed with O_2-free, N_2O-free nitrogen. These samples were immediately preserved with glutaraldehyde (2% v/v final concentration) and stored at 0 °C for up to 5 weeks. Before analysis, the samples were shaken vigorously for 1 h to ensure equilibration between liquid and headspace. 1-ml subsamples of the gas were injected into a Perkin-Elmer Sigma 4 electron-capture gas chromatograph fitted with a 2-m (3 mm o.d.) stainless steel column of Chromosorb 102. The GC was operated at column temperature of 55 °C with a carrier gas (95% Ar 5% CH_4) flow of 24 ml min^{-1}. The ^{63}Ni detector was run at 375 °C with a standing current of 3.5×10^{-9} A and calibrated with standard gas mixtures. Distribution coefficients were determined later on a range of samples by multiple equilibration[26] to allow for the effect of increasing salinity on N_2O solubility. The original lakewater concentrations of dissolved N_2O were back-calculated[26] from head-space levels and the empirically determined distribution coefficients. Conductivity was measured at 2 °C. The transition from oxic to anoxic water (oxycline) occurred between 59 and 60 m depth.

N_2O concentrations were well above air-equilibrium values at all depths down the oxygenated portion of the water column (Table 1). Air-equilibrium concentrations of N_2O, the saturation value for water in equilibrium with atmospheric nitrous oxide, range from 17 nmol l^{-1} at 4.5 °C to 9 nmol l^{-1} at 23 °C (calculated from the solubility data of Markham and Kobe[6] and measured values for N_2O in Antarctic air[7]). Immediately beneath the ice N_2O levels were 48% above air-saturation ($\Delta N_2O = 8.2$ nmol l^{-1}). Concentrations rose steadily with increasing depth, but sharply increased below 47.5 m to a maximum at 54 m (>20,000% air-saturation; ΔN_2O >2,000 nmol l^{-1}). N_2O tensions rapidly declined below the peak, and the gas was undetectable at 62.5 m and below. The deep N_2O maximum closely followed a nitrate peak previously reported by Torii *et al.*[8] and confirmed in the present study (Table 1). NO_3 and N_2O profiles are strongly correlated ($r^2 = 0.909$, $P > 0.001$) but an accumulation of nitrite in the lower depths was displaced 1–2 m downwards relative to the other forms of oxidized nitrogen (Table 1; N_2O compared with NO_2^- $r^2 = 0.606$).

At least four biological processes are thought to involve nitrous oxide[9]. N_2O is an intermediate in the oxidation of ammonia to nitrate by nitrifying bacteria:

$$NH_3 \rightarrow NH_2OH \rightarrow N_2O \rightarrow NO_2^- \rightarrow NO_3^-$$

It is also an intermediate in the reduction of nitrate to nitrogen gas by denitrifying bacteria:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Nitrous oxide has been considered a possible intermediate in assimilatory nitrate reduction by algae (reverse of the nitrifier sequence). Finally, N_2O is an alternative substrate for nitrogenase, the enzyme of N_2 fixation. This fourth process cannot be an important control on nitrous oxide levels in Lake Vanda. Acetylene reduction assays[10] on lakewater samples throughout the profile failed to demonstrate measurable nitrogenase activity at any depth.

The well-defined and coincident maxima of NO_3 and N_2O provide circumstantial evidence of a deep band of nitrifying bacteria. More conclusive evidence comes from experiments conducted *in situ* from 3.25 m to 57.5 m, to measure nitrypyrin-sensitive CO_2 fixation (Fig. 1). Significant differences ($P < 0.05$) between duplicates with and without this nitrification inhibitor were recorded only from 50 to 57.5 m. Rates were highest at 52.5 m and 55 m, in the region of abundant oxidized nitrogen.

There is some evidence from marine systems[11,12] that nitrous oxide and nitrite may accumulate as byproducts of nitrate assimilation by algae. In Lake Vanda, as in other Dry Valley lakes[13], algal production is low throughout most of the water column but rises to a sharp maximum just above the oxycline (Fig. 1). This photosynthetic peak lies well below the zone of N_2O accumulation, and algal assimilation is therefore unlikely to be a large source of nitrous oxide.

Denitrification has been invoked[2] to explain accumulation of N_2O in low-oxygen regions of the sea. Denitrification in Lake Vanda was assayed by *in situ* acetylene blockage[14] experiments at depths of 55, 58, 59, 59.5, 60, 61, 62.5 and 64 m. Significant denitrifier activity was recorded only in the upper anoxic depths of 59.5–62.5 m (Fig. 1). Nitrous oxide was very low or undetectable in this region, and therefore the upper anoxic zone must be a region of N_2O consumption rather than a net source.

A comparison of the molar ratio of N_2O to NO_3 provides a further guide to the processes controlling nitrous oxide production and loss. Lowest ratios were recorded in the region 52–56 m, the zone of peak nitrifier activity. The minimum (8.2×10^{-3}) falls within the range reported for nitrifying bacteria in culture[15], although it is high relative to typical accumulation ratios in the field—Elkins *et al.*[1] report an average ratio of 1.4×10^{-3} mol of N_2O produced per mol of NH_4 oxidized by aquatic nitrifiers. At higher and lower depths in Vanda the ratio

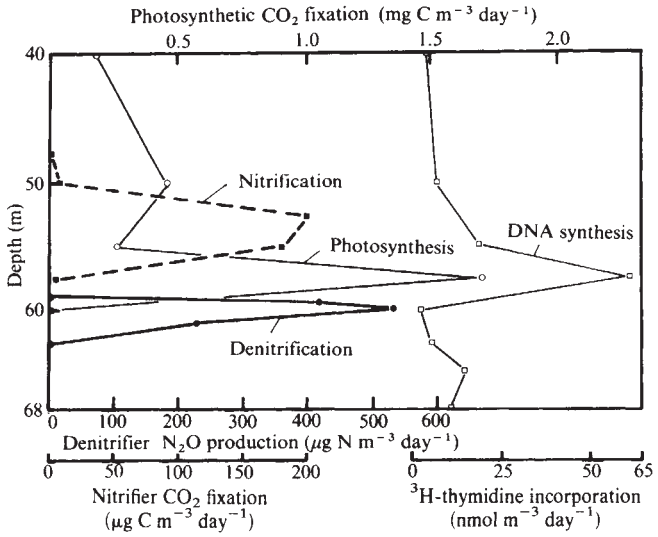

Fig. 1 Distribution of microbial activity at 40–68 m in Lake Vanda: nitrifier CO_2 fixation (■), photosynthetic CO_2 fixation (○), denitrifier N_2O production (●), ^{3}H-thymidine uptake (□). All rates were determined by *in situ* incubations. Nitrifier activity (18 December 1980) was estimated by the difference between dark CO_2 fixation with and without the nitrification inhibitor nitrypyrin[27]. Samples were preincubated with inhibitor (10 mg l^{-1} final concentration) for 1 h at the depth of collection, and then incubated for a further 5 h with ^{14}C-HCO_3 (0.6 μCi ml^{-1}). Dissolved inorganic carbon was determined by IR gas analyser. Photosynthetic rates (21 December 1980) were measured by 24-h *in situ* ^{14}C-HCO_3 (0.6 μCi ml^{-1}) incubations in light and dark bottles. Denitrifier activity (13 January 1981) was measured by the accumulation of nitrous oxide in samples of lakewater sealed in Hypovials and incubated at the depth of collection for 24 h with 10% acetylene[14], an inhibitor of nitrous oxide reductase. Samples were incubated with ^{3}H-thymidine (20 nmol l^{-1}, 16 December 1980) to estimate microbial rates of DNA synthesis[16]. At the end of the 5 h incubation, samples were filtered on to 0.22 μm Millipore membranes which were washed several times with 10% trichloracetic acid and air-dried. The filters were later counted by liquid scintillation spectrometry and corrected for adsorption of label (glutaraldehyde-killed controls) and self-absorption of radiation.

of N_2O to NO_3 increased, probably reflecting the selective loss of nitrate with time by algal uptake and sedimentation. Particulate nitrogen, and by inference seasonal NO_3 removal by the plankton, is low throughout the water column (Table 1). Movement of N_2O and NO_3 by turbulent diffusion away from the peak must therefore proceed very slowly for algal uptake to affect significantly the molar ratio of the two forms of oxidized N. This ratio decreased again from 25 m towards the ice, which would be consistent with selective loss of N_2O to the atmosphere by diffusion through the ice-cap, and at the lake edge where the ice melts away each summer to produce a moat of open water 1–10 m wide.

Denitrifier activity was not detected at depths greater than 62.5 m, where N_2O and NO_3 were also absent: denitrification rates in Vanda may ultimately be limited by the rate of transfer of oxidized N to the upper anoxic zone. Ammonium substrate is relatively abundant in the region of nitrifier activity, and it is possible that phosphorus (Table 1) rather than nitrogen availability exerts an overall control on this component of the N_2O cycle. Phosphate values increased with depth towards the oxycline (Table 1), and at these lower aerobic depths total microbial activity (as estimated by ^{3}H-thymidine incorporation into DNA[16]) increased to a water column maximum (Fig. 1). It remains unclear why the nitrifiers do not similarly peak in this superficially favourable environment at 57–59 m. A possible explanation is the steep ammonium gradient observed through this zone (Table 1). Nitrifying bacteria are inhibited by high levels of ammonia. *Nitrobacter* (nitrite oxidizer) is more sensitive than *Nitrosomonas* (ammonium oxidizer)[17], and it is of interest that nitrite accumulates towards the bottom of the nitrification layer where ammonia becomes increasingly concentrated.

The relative importance of denitrifier and nitrifier activity for nitrous oxide accumulation in oceanic environments has caused considerable debate[2]. In Lake Vanda, N_2O accumulated in the region of highest nitrifier activity where denitrification was undetectable. Conversely, N_2O concentrations were greatly reduced in the region where *in situ* denitrification rates were maximal. In this body of water nitrification would seem to be a net source and denitrification a net sink for nitrous oxide. These different components of the N_2O cycle occupy discrete bands of the stratified water column. This layered community may thereby prove to be a useful test of future models describing nitrous oxide production and loss in natural aquatic environments.

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An opiate system in the goldfish retina

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Recently, in addition to conventional neurotransmitters such as acetylcholine, dopamine, glycine and γ-aminobutyric acid (GABA), putative neuroactive peptide transmitters have been localized to specific retinal amacrine cells[1]. In particular, opiate receptors[2,3], assayable enkephalin immunoreactivity[4] and enkephalin-immunoreactive neurones[1,5] have been described in avian and mammalian retinae. However, little physiological evidence has been obtained for the involvement of neuropeptides in retinal function. Here we report that exogenous opiates affect both the release of GABA from GABAergic amacrine cells and the firing patterns of ganglion cells in the goldfish retina[6]. Our results show that the output of the retina is modulated by an opiate system whose neural organization and pharmacological properties resemble those described elsewhere in the vertebrate central nervous system.

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