species such as *D. serrata*[18], *D. immigrans*[19] and *D. mojavensis*[20]. Our results may not seem surprising, however time may not be such an important factor in the divergence of mate recognition systems[21]. However it will be interesting to see if New Zealand *D. pseudoobscura* have diverged in, for example five years time. Arita and Kaneshiro[22] and Ahearn[23] have both demonstrated nonrandom mating in laboratory maintained stocks of Hawaiian *Drosophila*. These authors argue that this is the result of known population crashes in the culturing history of the flies. Powell's[21] experiment, which was designed to test Carson's[24] flush-crash model of speciation, is supporting evidence for this claim. Isofemale lines (a culture derived from a single wild caught female) have been used commonly in crossing experiments. Population crashes can also be the result of stochastic events in culturing such as parasitism by mites, poor media and microorganism growth[25]. Hence it appears possible that at least some previous experiments which reported nonrandom mating are in fact, the result of various culturing procedures. Our results reinforce previous studies indicating that the male-female communication system of some *Drosophila* species at least, shows a remarkable stability. Hypotheses for the stability of the mate recognition include, the action of strong stabilizing selection[26–29], a lack of time for divergence, a lack of directional selection and that the *structure* of the male-female communication system simply results in stability[28,29].

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Ascorbate content of foliage of eucalypts and conifers utilized by some Australian and North American mammals

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Summary. Ascorbate content of foliage of five species of Australian eucalypts and 10 species of North American conifers exhibited similar seasonal variation within the range of 100–350 mg per 100 g fresh weight.
Key words. Ascorbate; foliage; eucalypts; conifers; plant-herbivore interactions; mammalian herbivores; nutrient content.

Most mammals are able to synthesize ascorbic acid (vitamin C), but some groups such as anthropoid primates, bats and guinea pigs, have been found to lack the enzyme gulonolactone oxidase (GLO, EC 1.1.3.8) which catalyzes the final step in the biosynthetic pathway of ascorbic acid from glucuronic acid. These animals are therefore dependent on a dietary source of the vitamin, chiefly fruits and vegetables; the nutritionally available ascorbic acid of these foods is well documented. Much less is known about the wider distribution of ascorbic acid in the flowers and leaves of plants[2].

Several species of Australian arboreal marsupials subsist almost exclusively on eucalyptus foliage. Two of these, the greater glider, *Petauroides volans* (maintained on *Eucalyptus radiata*) and the ringtail possum, *Pseudocheirus peregrinus* (maintained on *E. andrewsii* and she-oak, *Casuarina torulosa*) have much higher blood levels of L-ascorbic acid than is found in most mammals[3]. On the other hand, another arboreal marsupial, the brushtail possum, *Trichosurus vulpecula*, consumes some *Eucalyptus* foliage (e.g. *E. meliodora*) as well as fruits, vegetables and flower buds but does not maintain high blood levels of ascorbate. All of these species appear to have the ability to synthesize L-ascorbic acid as evidenced by the presence of GLO in liver[4,5].

In North America one of the most obligate folivorous mammals is the red tree vole, *Aborimus longicaudus*, which subsists almost entirely on needles of Douglas fir, *Pseudotsuga menziesii*[6]. The showshoe hare, *Lepus americanus*, in some localities feeds heavily on foliage of white cedar, *Thuja occidentalis* or spruce, *Picea* spp. in winter[7]. This hare has very low levels of GLO and is unable to maintain normal levels of ascorbate in blood and tissues when fed on a diet devoid of ascorbate[8].

Our investigations of the ascorbate metabolism and economy of several of these mammals[5,8], indicated the need for data on

the ascorbate content of foliage consumed. We know of no data on ascorbate content of foliage of *Eucalyptus* or *Casuarina*. Several reports on ascorbate content of needles of conifers in Europe[9–12] do not include *Thuja occidentalis* but one of them does give an ascorbate content for *Pseudotsuga menziesii* of 381 mg/100 g fresh weight[12]. The most extensively studied conifer is Norway spruce, *Picea abies*, in which pronounced seasonal variations in ascorbate content have been demonstrated[13,14]. We report here ascorbate contents of foliage of some eucalypts and conifers sampled throughout the year.

Materials and methods. Foliage from the eucalypts and some other Australian trees was collected in or near Armidale, NSW (31°S, 152°E, elev. ca 1000 m) and that from the conifers near St Paul, MN (45°N, 93°W, elev. ca 300 m). All of the conifers except eastern hemlock were growing on a single plot of approximately 0.5 ha. Almost all samples for a species were taken from the same single tree. Mature leaves were collected from branches easily reached from the ground and either analyzed immediately or sealed in plastic sacs and kept frozen until analyzed.

Ascorbate was determined with a 2,4-dinitrophenylhydrazine (DNPH) method[8,15,16]. Weighed samples were homogenized in a kitchen blender with 100 ml 4% trichloroacetic acid – 4,5% metaphosphoric acid per g of leaves. The mixture was filtered through cheesecloth and the filtrate treated with charcoal (Norit) to oxidize ascorbate to dehydroascorbate. Charcoal was centrifuged off and, if necessary, the supernatant was further clarified by filtration through Whatmann No. 1 paper. The DNPH derivative was formed by incubation of an aliquot of filtrate with a solution of 2% DNPH and 0.5% thiourea in 9 N H_2SO_4 for 90 min at 47°C. An equal volume of H_2SO_4 (9 vol. conc. H_2SO_4 + 1 vol. H_2O) was added and the absorbance read at 520 nm.

For at least one specimen of each species a qualitative identification of ascorbate was made. For this purpose the DNPH derivative was prepared as above and collected on glass fiber paper by filtration. It was washed successively with 0.2 M H_2SO_4, 0.2 M $NaHCO_3$ and H_2O, dried, dissolved in ethyl acetate and chromatographed on silica gel thin layer sheets (Eastman Chromogram No. 13181) with the solvent mixture of

Table 1. Ascorbic acid content of eucalypt leaves. Ascorbic acid (mg/100 g) on fresh (F) and dry (D) weight basis

Species	28 Mar 1982		23 Apr 1982		5 Jun 1982		20 Aug 1982		22 Oct 1982		15 Nov 1982		13 Jan 1983		27 Jan 1983		17 Mar 1983		18 Apr 1983		8 Jun 1983		14 Jul 1983		4 Aug 1983	
	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D	F	D
Manna gum *Eucalyptus viminalis*	152	373	–		–		165	377	266	556	356	727	186	425	168	365	192	430	216	434	234	549	119	249	222	448
Narrow-leaved black peppermint *E. nicholii*	158	360	–		–		267	527	305	589	301	589	114	253	168	390	172	394	216	445	255	514	173	347	267	460
Snow gum *E. pauciflora*	146	324	–		–		313	721	328	657	361	716	120	265	163	341	244	527	284	563	204	533	162	344	214	462
New England blackbutt *E. andrewsii*	124	331	112	265	93	236	(8 Sep) 296	636	285	612	216	576	108	371	127	339	184	413	264	538	(20 Jun) 135	328	202	470	235	545
Narrow-leaved peppermint *E. radiata*	120	230	100	222	87	191	(8 Sep) 254	540	285	604	268	544	142	319	135	338	132	340	228	485	(20 Jun) 83	178	97	222	196	414

Table 2. Ascorbic acid content of conifer needles. Ascorbic acid (mg/100 g) on fresh (F) and dry (D) weight basis

Species	27 Dec 1982		26 Feb 1983		25 Apr 1983		8 July 1983		14 Sept 1983		9 Nov 1983		25 Dec 1983	
	F	D	F	D	F	D	F	D	F	D	F	D	F	D
White cedar *Thuja occidentalis*	200	465	223	530	227	515	158	336	81	202	108	284	204	464
Red cedar *Juniperus virginiana*	200	408	181	393	113	227	97	231	85	202	122	340	212	424
White pine *Pinus strobus*	250	581	330	734	193	403	77	175	85	202	160	400	245	557
Red pine *Pinus resinosa*	305	693	303	688	220	458	125	284	125	261	220	523	237	515
Jack pine *Pinus banksiana*	305	726	365	830	300	612	182	414	129	294	216	540	261	593
Norway spruce *Picea abies*	190	–	223	495	283	567	141	336	125	329	172	453	187	425
Red spruce *Picea rubens*	350	795	355	771	270	500	214	486	113	283	240	571	310	704
White spruce *Picea glauca*	250	595	292	609	233	467	–	–	97	255	132	347	228	496
Eastern hemlock *Tsuga canadansis*	212	506	184	419	123	252	107	238	–	–	159	378	135	331
Douglas fir *Pseudotsuga menziesii*	172	392	348	773	267	523	194	422	226	565	182	445	253	550

Navon and Levinson[17]. Colored bands corresponding to the ascorbate derivative ($R_f = 0.57$) were scraped off, taken up in 65% acetic acid, centrifuged and their spectra scanned between 320 and 600 nm.

Dry matter contents of leaves were determined by oven drying of weighed samples at 80 °C overnight.

Results. At least one leaf specimen from each species was checked qualitatively for ascorbate. In every case only one red DNPH band was observed and it always had R_f close to 0.57. Absorbance spectra of these bands in 65% acetic acid corresponded closely to those of authentic DNPH derivative prepared from L-ascorbic acid (maxima at 360 and 505, and a minimum at 435 nm (A_{360}:A_{435}:A_{505} = 1:0.55:0.97). Ascorbate contents of the leaves calculated from A_{505} of the isolated DNPH ascorbate derivative in 65% acetic acid were approximately equal to those calculated from A_{520} of the entire mixture of derivatives in H_2SO_4.

Douglas fir needles contain 2-oxopropanal (methyl glyoxal)[18] but the molar concentration of this compound is only about one-thousandth that of ascorbate and its DNPH derivative is a yellow compound ($R_f = 1$) that does not interfere in the ascorbate analysis. Various other bands are present in the TLC patterns of some species but none of them exhibits appreciable absorbance in the 500–520 nm range.

The concentrations of ascorbate in the foliage of both the eucalypts and conifers studied are in the range 100–350 mg/100 g on a fresh weight basis and 200–750 mg/100 g on a dry basis (tables 1 and 2). The marked seasonal variation in ascorbate content is similar in both hemispheres; high values occur in late winter and early spring and low values in autumn (fig.). Data on foliage of a few other species, not sampled seasonally, are included in table 3.

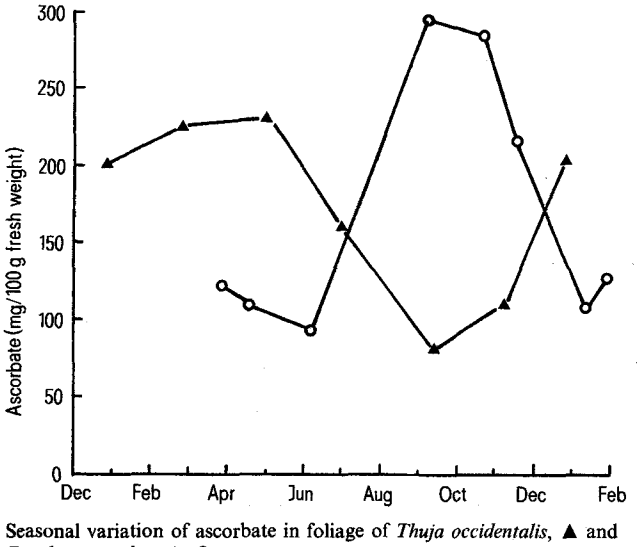
Discussion. The ranges and seasonal variations in ascorbate contents of foliage of most of the evergreen species reported here are similar to those found for Norway spruce (*Picea abies*) from Bonn[13] (50 °N, 7 °E, 100 m) and Graz[14] (47 °N, 15.5 °E, 500–1000 m). Two of our species, eastern hemlock (*Tsuga canadensis*) and red cedar (*Juniperus virginianus*) exhibited somewhat different patterns of variation from the others (table 2). The data from the *Eucalyptus* species indicates that the pattern may vary slightly from year to year. Seasonal variations in ascorbate content no doubt reflect changes in the balance between rate of synthesis and rate of utilization but the specific mechanisms affecting this balance have not been elucidated.

The foliage utilized by the animals in which we are interested furnish large supplies of ascorbate even in the season when their ascorbate content is at a minimum. Thus a ringtail possum consuming 100 g of leaves of *Eucalyptus andrewsii* per day would obtain 100 mg of ascorbate in autumn and up to 300 mg in spring. Similarly a snowshoe hare subsisting on 150 g of *Thuja occidentalis* foliage would get 120 mg of ascorbate in autumn and over 300 mg in spring. High intake of ascorbate is not of itself, however, a sufficient cause for the high blood levels observed in ringtail possums and greater gliders. Several species have not been shown to have high blood levels even when fed great excesses of ascorbate. Ringtails metabolize ascorbate rapidly[5] even while maintaining high blood levels; apparently the metabolic mechanisms have a high 'threshold' of some sort.

In man and guinea pigs a deficiency of ascorbate leads to reduced ability to detoxify foreign chemicals[19]. On this basis, one may speculate that high ascorbate intakes by animals feeding on evergreen foliage may facilitate the metabolism or excretion of undesirable compounds (e.g. phenols, terpenes) that are also in the foliage[20–22].

Table 3. Ascorbic acid content of foliage

Species	Date sampled	Locality	Ascorbate (mg/100 g) F	D
Grey gum *Eucalyptus punctata*	May 1982	Sydney, NSW	113	257
Forest red gum *Eucalyptus tereticornis*	May 1982	Sydney, NSW	107	230
Yellow box *Eucalyptus meliodora*	November 1983	Styx River, NSW	238	556
Angophora *Angophora floribunda*	May 1982	Armidale, NSW	102	214
Sally wattle *Acacia floribunda*	May 1982	Styx River, NSW	307	731
Fern leaf wattle *Acacia ficifolia*	May 1982	Styx River, NSW	290	653
Cootamundra wattle *Acacia baileyana*	May 1982	Armidale, NSW	269	637
She-oak *Casuarina torulosa*	May 1982	Styx River, NSW	129	279
She-oak *Casuarina torulosa*	May 1982	Armidale, NSW	255	565
Mistletoe *Amyema pendulum*	May 1982	Bendemeer, NSW	173	350
American holly *Ilex opaca*	May 1983	Elkins, WVA	187	354
Rhododendron *Rhododendron maximum*	May 1983	Elkins, WVA	284	522



Seasonal variation of ascorbate in foliage of *Thuja occidentalis*, ▲ and *Eucalyptus andrewsii*, ○.

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Catfish blood chemistry under environmental stress

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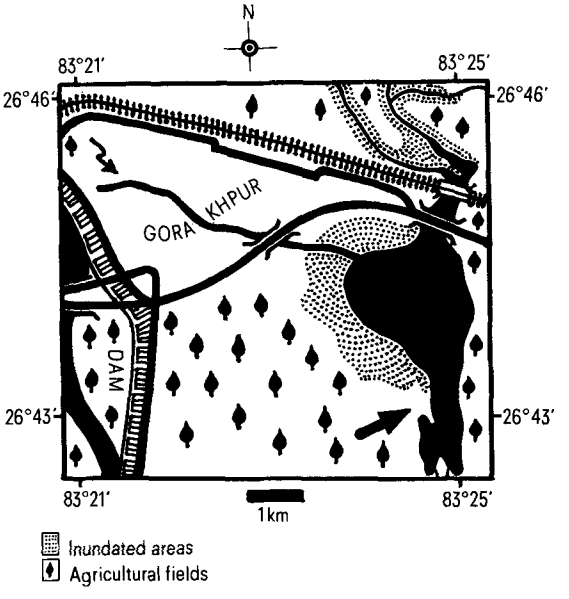
Summary. Blood chemistry of *Heteropneustes fossilis* exposed to sewage, fertilizers and inseticides showed signs of anemia, dehydration and disturbance in the pituitary-interrenal endocrine axis and the excretory function of gills. Hepatic and renal tissue damage was also indicated.
Key words. Heteropneustes fossilis; environmental stress; blood chemistry; sewage; fertilizer; insecticides; pollutants.

The chief body of fresh water in Gorakhpur is Ramgarh Lake, which lies at 26°42′–26°46′N and 83°23′–83°25′E and occupies approximately 15 km^2 (fig). It serves as a major source of fish and shellfish for this region which possesses a tropical climate with temperatures of 24 (18–40) °C and occupies an approximate area of 60 km^2 (fig.). The lake is heavily polluted; untreated sewage refuse from most of the city, including some of the most thickly populated areas, is discharged into the lake through a mostly open sewer covering a distance of approximately 6 km along its course. Moreover, Gorakhpur is primarily an agricultural zone; Ramgarh Lake is surrounded by agricultural fields, and even the areas inundated by it are used for cultivation when they are not immersed (fig.), so fertilizers and insecticides from surrounding agricultural fields also drain off into the lake. Organic enrichment through sewage, and contamination by agrochemicals, have progressively added to the toxicity of the water of the lake, which is evident from the fact that the fishery catch of the lake has dwindled by more than half during the past decade; whereas fish and shellfish from the lake used to be transported to other parts of the country, they hardly suffice for local requirements now. A toxicity assessment of the pollutants is therefore necessary. The pollutants have been found to have noticeable effects on the hematohistological, leucocytic and hemostatic features of feshwater fish and shellfish[1–4]. The effects of these pollutants on some chemical parameters of the blood of a common Indian freshwater catfish, *Heteropneustes fossilis*, are described here.

Material and methods. The experimental design was the same as that used earlier[1,2]. Fish were exposed up to 40 days to the following pollutants at the highest concentrations which permitted survival of 50% of the fish population for 30–40 days:
A) Sewage. Sewage was collected just before its entry to the lake and used in a concentration of 25%.
B) Sewage factors. Some selected chemical constituents of sewage (sewage factors) were determined. The yearly average values (in ppt) were found to be as follows:
Total nitrogen (N), 0.3; ammonia nitrogen (NH_3-H), 1.8; phosphate (PO_4), 43.6; sulphate (SO_4), 0.2; total alkalinity (HCO_3), 0.5; calcium (Ca), 0.1. These values were respectively 150, 850, 44, 45, 4 and 2 times higher than those in control.
The sewage factors were individually reproduced by adding KNO_3, NH_4Cl, $Na_2HPO_4 \cdot 12\,H_2O$, Na_2SO_4, $NaHCO_3$ and $CaCl_2$ to unpolluted water so as to produce the values listed above.
C) Fertilizers. Urea and potash (potassium oxide), 2 g/l each.
D) Insecticides. Chlorinated: BHC, 0.2 mg/l; endrin, 0.02 mg/l. Organophosphorus: Nuvacron (monocrotophos), 2 mg/l; Dimecron (phosphamidon), 20 mg/l.
Blood samples were collected every 10 days from both the treated and untreated fish. The samples were used for determining glucose level in whole blood, cholesterol level in serum, total protein level in serum, urea level in whole blood, and acid and alkaline phosphatase activity in serum. For every determination, 20–24 fish were used.
Experimental values differing significantly ($p < 0.05$) from corresponding controls have been considered as representative of change under stress, the rest being deemed normal.
Results. The normal values of the specified selected chemical parameters of the blood of *H. fossilis* are as follows:



Ramgarh Lake (thick arrow) and its surroundings. Thin arrow indicates the course of the sewer discharging refuse of Gorakhpur into the lake.