Whale songs lengthen in response to sonar

Male humpbacks modify their sexual displays when exposed to man-made noise.

There is growing concern about the effects of man-made noise on marine life. In particular, marine mammals that use sound to communicate, navigate, and detect predators and prey may try to avoid loud sound sources up to tens of kilometres away[1]. Here, in a study conducted in cooperation with the US Navy[2], we show that the singing behaviour of male humpback whales was altered when they were exposed to LFA (low-frequency active) sonar. As the song of these whales is associated with reproduction[3], widespread alteration of their singing behaviour might affect demographic parameters, or it could represent a strategy to compensate for interference from the sonar.

During the breeding season male humpback whales sing long, complex songs that are thought to be sexual displays[3]. Songs consist of a series of themes, progressing in a predictable order, that may repeat for several hours[4]. We used a small observation vessel to find singing humpbacks and conduct focal sampling[5], recording behaviour before, during and after playback. (Strictly speaking, we have evaluated the additional impact that LFA sounds have on a singing whale that is already being followed.)

We recorded the vocal behaviour of each focal singer continuously for several hours using a towed, calibrated hydrophone array[6]. When the whale was at the surface, observers sampled visible behaviour. Photographs of fluke and dorsal fin features confirmed the whale's identity throughout each follow[7]. At least two songs were recorded before the observation vessel requested the US Navy R/V *Cory Chouest* to transmit ten (in one case four) 42-s LFA signals at 6-min intervals. The sonar was broadcast at less than full strength, and no focal singer was exposed to a signal louder than 150 dB (with respect to 1 μPa).

Sixteen singers were followed during 18 playbacks. In nine follows, the whale sang continuously throughout the playback; in four the singer stopped when he joined other whales (typical of normal social interaction); and in five the singer stopped, presumably in response to the playback. We recorded at least one complete song in all conditions from six individuals, and pooled the songs of each of the two individuals that were subjects in two experiments. For these six whales, we measured the duration and theme structure of song spectrograms, comparing song duration in the three conditions using analysis of variance[8].

On average, humpback whales' songs were 29% longer during LFA playbacks (Fig. 1) — a particularly strong result, given the low power of the test and small sample size[9]. Song duration returned to normal after exposure, suggesting that this response has a limited duration. There was little difference in the likelihood of an aberrant theme transition across exposure conditions ($\chi^2 = 3.273$, $P = 0.195$), indicating that long songs resulted from longer themes within a normal song structure. Across the six singers, maximum received level of the sonar at the whale did not correlate positively with either the increase in mean song duration from pre-exposure to exposure condition ($r = -0.90$) or with the subsequent decrease from exposure to post-exposure condition ($r = -0.63$).

We suggest that humpbacks sang longer songs during LFA sonar transmissions to compensate for acoustic interference. Our study shows that it is possible to measure the behavioural responses of individual whales in controlled experiments at sea.

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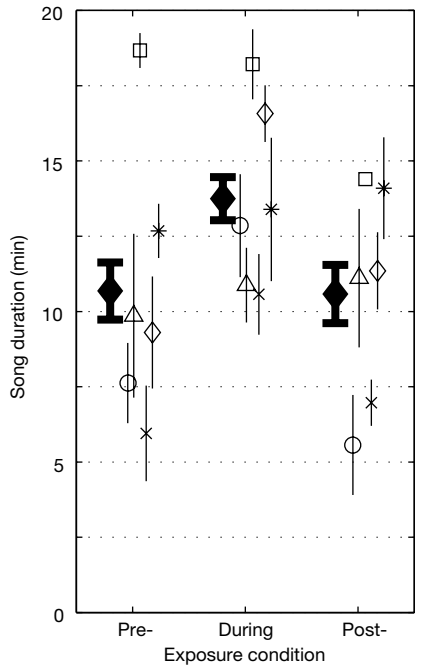

Figure 1 Duration of songs (±s.e.m.) produced by humpbacks before, during and after exposure to LFA sonar transmissions (bold, filled diamonds, mean of all six singers; other symbols, individual singers). The maximum received level of the sonar at the whale ranged from 130 to 150 dB re 1 μPa. Songs were grouped in the exposure condition if a sonar transmission occurred at any point during the song. The average number of songs per singer in the pre-exposure, exposure and post-exposure conditions was 3.2, 4.7 and 3.8, respectively. Differences were assessed using a mixed-model analysis of variance treating exposure condition as a fixed factor, whale identity as a random factor, and each song duration as an independent observation. The effect of exposure condition on song duration was statistically significant at $P = 0.047$ ($F_{2,10} = 4.200$, power = 0.50, $n = 6$).

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Nutrition

Antioxidant activity of fresh apples

Vitamin C is used as a dietary supplement because of its antioxidant activity, although a high dose (500 mg) may act as a pro-oxidant in the body[1,2]. Here we show that 100 g of fresh apples has an antioxidant activity equivalent to 1,500 mg of vitamin C, and that whole-apple extracts inhibit the growth of colon- and liver-cancer cells *in vitro* in a dose-dependent manner. Our results indicate that natural antioxidants from fresh fruit could be more effective than a dietary supplement.

Apples of the Red Delicious variety were extracted using 80% acetone and their content of phenolics and flavonoids determined[3,4]: the extracts contained 290.2 ± 4.2 and 219.8 ± 1.8 mg phenolics, and 142.7 ± 3.7 and 97.6 ± 3.9 mg flavonoids per 100 g apples with and without skin, respectively. There are known to be more phenolics in the skins of apples than in the flesh, and quercetin glycosides are found only in the skins[5].

We measured the total antioxidant activity of apples by using the total oxyradical-scavenging capacity (TOSC) assay[6] and found that apples with skin had a higher TOSC value than apples without skin (Fig. 1a). The total antioxidant activity of 1 g apples with skin was 83.3 ± 8.9 TOSC (μmol vitamin C equivalents) — that is, the

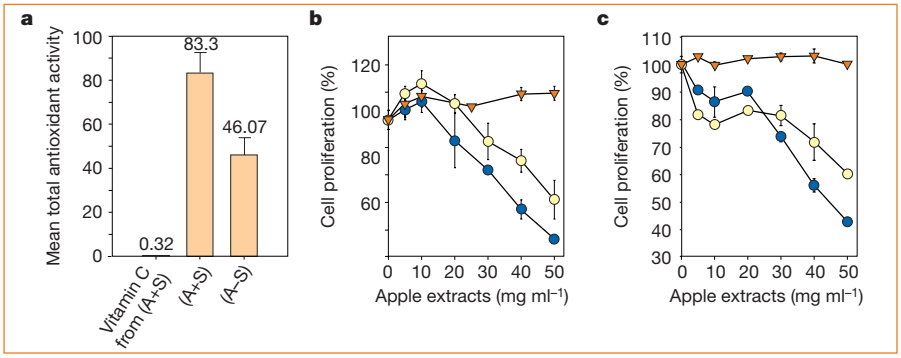

Figure 1 Antioxidant activity of apples and their effect on tumour-cell proliferation *in vitro*. **a,** Mean total antioxidant activity expressed by total oxyradical-scavenging capacity (TOSC; μmol vitamin C equivalents per g) assay for 1 g apple with skin (A + S), and for apple without skin (A − S). **b,** Inhibition of proliferation of Caco-2 colon-tumour cells by extracts of apple with and without skin. **c,** Inhibition of proliferation of HepG2 liver-tumour cells. Control samples were assayed as apple extracts, but they contained only vitamin C at the same concentration that exists in apples (0.057 mg g^{-1}). Cell proliferation was determined by using the MTS assay[10]. Blue circles, apple with skin; yellow circles, apple without skin; orange triangles, control extracts.

antioxidant value of 100 g apples is equivalent to 1,500 mg of vitamin C. Given that the average vitamin C content in fresh apples with skin is 5.7 mg per 100 g (ref. 7) and that the total antioxidant activity of 0.057 mg vitamin C (in 1 g of whole apples) is only 0.32 TOSC (Fig. 1a), then almost all of the antioxidant activity in apples must be due to phytochemicals.

We treated a colon-cancer cell line, Caco-2, with extracts equivalent to 0, 5, 10, 20, 30, 40 and 50 mg ml^{-1} apples for 96 hours (the treatment time for maximal response). Cell proliferation was inhibited in a dose-dependent manner after exposure to apple-extract concentrations above 20 mg ml^{-1} (Fig. 1b): at 50 mg ml^{-1}, inhibition was 43 ± 1% and 29 ± 4.1% for apples with skin and for apples without skin, respectively.

We also tested the effect of apple extracts on the proliferation of another cancer-cell line, HepG2 human liver-tumour cells. We found that apple extracts at 50 mg ml^{-1} inhibited the proliferation of these cells as well, by 57 ± 0.21% and 40 ± 0.64% for apples with and without skin, respectively (Fig. 1c). The extracts of apple with skin could thus significantly (*t*-test, $P < 0.031$) reduce tumour-cell proliferation compared with extracts of apples without skin. No cytotoxicity of the apple extracts was seen at any of the concentrations tested (data not shown).

We suggest that this strong inhibition of tumour-cell proliferation *in vitro* could be due to apples' combination of phytochemicals (phenolic acids and flavonoids), as these are natural antioxidants. It has been proposed that the consumption of whole fruits may provide the antioxidant balance needed to quench reactive oxygen species[8] which have been implicated in tumorigenesis[9]. Phytochemicals in apples other than ascorbic acid seem significantly to enhance their antioxidant properties and their capacity to inhibit the proliferation of tumour cells *in vitro*.

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Botany

Constraints to growth of boreal forests

Understanding how the growth of trees at high latitudes in boreal forest is controlled is important for projections of global carbon sequestration and timber production in relation to climate change. Is stem growth of boreal forest trees constrained by the length of the growing season when stem cambial cells divide[1], or by the length of the period when resources can be captured[2]? In both cases, the timing of the thaw in the spring is critical: neither cambial cell division nor uptake of nutrients and carbon dioxide can occur while the soil is frozen. Here we argue, on the basis of long-term observations made in northern Saskatchewan and Sweden, that the time between the spring thaw and the autumn freeze determines the amount of annual tree growth, mainly through temperature effects on carbon-dioxide uptake in spring and on nutrient availability and uptake during summer, rather than on cambial cell division.

From tree-ring analysis across several sites in the Siberian subarctic, Vaganov *et al.*[1] provide evidence that annual variability in mean ring width is determined by the date of the thaw through its influence on the date of cambial initiation, as well as by temperature during the subsequent early growing season. Variability in annual net ecosystem production (NEP) is also largely determined by the timing of the thaw[3], which enables the NEP to switch immediately and rapidly from a daily loss to a daily gain of CO_2.

Our own observations on black spruce trees at the Boreas Southern Study Area[4] show that when air temperature exceeds −1 °C and the overlying snow starts to melt, meltwater percolates down into the soil, the temperature of the upper soil horizons rises towards 0 °C, and the switch from a small net daily loss of carbon to a large net gain occurs over just a few days. In boreal conifers, the availability of soil water is a prerequisite for the recovery of photosynthetic capacity in spring and early summer[2].

The effect of frozen soils on annual CO_2 uptake by Norway spruce at 64° N is particularly dramatic because, before the thaw, daily solar radiation is already substantial and effectively being wasted from the perspective of CO_2 capture[5]. Thus, CO_2 uptake is synchronized and strongly stimulated by the thaw, and afterwards, once a critical temperature sum is reached, cambial activity and NEP increase together as the temperature rises.

Such observations related to the poor growth of trees in boreal forests have led to the presumption that their growth is constrained by temperature. By contrast with temperate and tropical forests[6], boreal forest trees are small in relation to their age and coniferous boreal forests have a very low net primary production of about 2.5 tonnes of carbon ha^{-1} yr^{-1} (refs 7,8). To investigate the extent to which low temperature is the primary controlling variable, a long-term nutrient-optimization and irrigation experiment on Norway spruce was set up at Flakaliden (64° N) in Sweden[9]. Since 1987, we have applied complete fertilizer daily through every growing season either in irrigation water or as a single solid dose at the start of the growing season. We found that growth on the fertilized plots (with or without irrigation) increased by a factor of four[10] (Fig. 1a), so air temperature cannot be the major direct constraint on tree growth.

However, temperature may be influencing tree growth indirectly through the length of the growing season and by its effects on decomposition of soil organic