Lake ecosystems

Rapid evolution revealed by dormant eggs

Natural selection can lead to rapid changes in organisms, which can in turn influence ecosystem processes[1]. A key factor in the functioning of lake ecosystems is the rate at which primary producers are eaten, and major consumers, such as the zooplankton *Daphnia*[2], can be subject to strong selection pressures when phytoplankton assemblages change. Lake Constance in central Europe experienced a period of eutrophication (the biological effects of an input of plant nutrients) during the 1960s–70s[3], which caused an increase[4] in the abundance of nutritionally poor or even toxic[5] cyanobacteria. By hatching long-dormant eggs[6] of *Daphnia galeata* found in lake sediments, we show that the mean resistance of *Daphnia* genotypes to dietary cyanobacteria increased significantly during this eutrophication. This rapid evolution of resistance has implications for the ways that ecosystems respond to nutrient enrichment through the impact of grazers on primary production.

Genetically distinct[3] *Daphnia galeata* clones were collected as diapausing eggs from sediments of known age[3] obtained from a core taken from Lake Constance in 1997. Parthenogenetic lines were maintained for each clone for at least 40 generations to minimize maternal effects. We tested 32 clones from three sediment ages for their resistance to dietary cyanobacteria: 12 clones from 1962–64 and 1969–71 (before and just after the appearance of cyanobacteria; Fig. 1), 10 clones from 1978–80 (peak eutrophication), and 10 clones from 1992–94 and 1995–97 (when the period of eutrophication had passed).

Resistance to cyanobacteria was measured as the effect of diet on the somatic juvenile growth rate (g_j) of *Daphnia*[7]: $g_j = [\ln(W_m) - \ln(W_n)]/t$, where W_m and W_n are the masses of mature and neonate *Daphnia*, respectively, and t is the development period. For each clone, g_j was measured for animals fed two different diets (supplied at 1 mg carbon per litre): one diet ($g_{j,\,poor}$) contained a mixture of a toxic cyanobacterium (*Microcystis aeruginosa*, 20% by carbon content) and a high-quality algal resource (*Scenedesmus obliquus*, 80% by carbon content); the other diet ($g_{j,\,good}$) contained only *Scenedesmus*. *Microcystis* was originally isolated from Lake Constance in 1972, is toxic to *Daphnia pulicaria*[8] and contains high concentrations of microcystin-LR hepatotoxin, whereas *Scenedesmus* promotes growth and reproduction in *Daphnia*[6]. *Microcystis* and *Scenedesmus* were fed to *Daphnia* as single cells of similar size (4.2 μm).

For each clone, $g_{j,\,good}$ and $g_{j,\,poor}$ were measured separately by using three replicate flow-through vessels (5–17 individuals each) with food levels in excess of growth-limiting concentrations[9]. Because there was variation among clones in $g_{j,\,good}$ (likelihood ratio test, $\chi^2 = 120.1$, d.f. = 1, $P << 0.0001$), resistance to dietary cyanobacteria was standardized as the growth-rate reduction, R, which is the fractional reduction in g_j on poor food relative to that on good food: $R = (g_{j,\,good} - g_{j,\,poor})/g_{j,\,good}$.

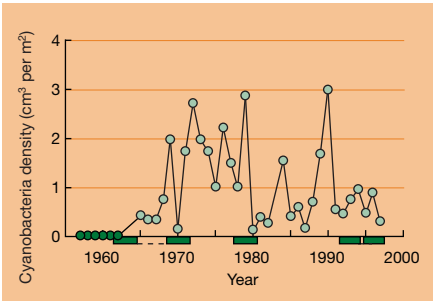

Figure 1 The summer density of planktonic cyanobacteria (total cell volume) in Lake Constance[4]. Filled circles, years when cyanobacteria were recorded as extremely rare; thick bars, sediment ages from which dormant *Daphnia* eggs were studied; the dashed line connects time periods analysed together.

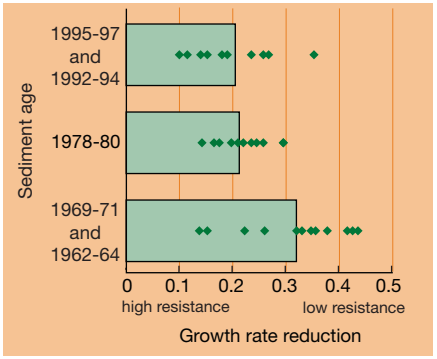

Figure 2 Resistance of *Daphnia* genotypes to dietary cyanobacteria. Data points represent estimates of growth-rate reduction for individual clones.

The *Daphnia* population evolved an increased ability to cope with a diet containing cyanobacteria (Fig. 2). Genotypes from both 1978–80 and the 1990s exhibit lower growth-rate reduction than those from 1962–64 and 1969–71 (ANOVA planned contrasts, $F = 10.3$, d.f. = 1, 29, $P = 0.003$, and $F = 11.9$, d.f. = 1, 29, $P = 0.002$, respectively). The rapid response observed during 1969–80 is attributable entirely to a reduction in genetic variance (likelihood ratio test, $\chi^2 = 8.24$, d.f. = 1, $P = 0.002$). There was a broad range of *Daphnia* genotypes of different growth-rate reductions in the lake during 1962–64 and 1969–71, but the genotypes that were most heavily affected by dietary cyanobacteria were virtually eliminated within ten years of continued summertime exposure to cyanobacteria.

The mean resistance of *Daphnia* to cyanobacteria was unchanged between 1978–80 and the 1990s (ANOVA planned contrast, $F = 0.06$, d.f. = 1, 29, $P = 0.82$). However, the variance of growth-rate reductions was greater in the 1990s than in 1978–80 (likelihood ratio test, $\chi^2 = 4.72$, d.f. = 1, $P = 0.015$), perhaps as a result of the water column being reinvaded by animals hatching from the dormant egg bank[6].

These short-term evolutionary changes may significantly affect the course of ecosystem change. Greater abundance of cyanobacteria during eutrophication is typically considered to be a response to increased nutrient inputs[10]. However, rapid adaptive evolution in grazing zooplankton populations may be an important feedback mechanism that is critical to understanding the net effect of eutrophication on primary producers in lakes.

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Correction

Food contamination by PCBs and dioxins

A. Bernard, C. Hermans, F. Broeckaert, G. De Poorter, A. De Cock, G. Houins
Nature **401,** 231–232 (1999)

The average ratio of polychlorinated biphenyls (PCBs) to polychlorodibenzodioxins (PCDDs) or polychlorodibenzofurans (PCDFs) in samples of contaminated feedstuff, chicken meat and eggs (Fig. 1b) was incorrect as published (see fourth paragraph): the average ratio of PCB:PCDD/F was about 50,000:1 (not 22,000: 1). The conclusions of our report are unchanged.

Food contamination by PCBs and dioxins

An isolated episode in Belgium is unlikely to have affected public health.

In February 1999, a poisoning episode broke out in several poultry farms in Belgium. The Belgian authorities took immediate safeguards to protect public health and implemented a large-scale food-monitoring programme. Here we analyse the scale of the contamination and assess the likelihood of its adversely affecting the health of the general population.

The first symptom of contamination was a sudden drop in egg production. A few weeks later, there was a marked reduction in egg hatchability, along with reduced weight gain and increased mortality of chicks. These birds presented with ascites, subcutaneous oedema of the neck and neurological disturbances (ataxia). Histology revealed degenerative changes of the skeletal and cardiac muscles. The lesions resembled 'chick-oedema disease', which was reported during the 1950s to 1970s in several outbreaks of poultry poisoned by polychlorinated biphenyls (PCBs) and dioxins[1], suggesting that dioxins might be responsible for the Belgian cases. High concentrations of dioxins in chicken meat and feedstuff were subsequently found and, at the end of April, the source of dioxins was traced to a stock of recycled fat that had been delivered to a manufacturer of animal feed in mid-January.

Analysis of contaminated feedstuff and poultry products showed a consistent pattern of dioxin congeners, dominated by the polychlorodibenzofurans (PCDFs) (Fig. 1a). Polychlorodibenzodioxins (PCDDs), in particular the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin involved in the 1976 Seveso accident in Italy and in contamination by the Agent Orange defoliant, were present only in minor proportions (less than 5%) of total congeners. This pattern of PCDD and PCDF congeners was virtually identical to that in the contaminated rice oil that poisoned 2,000 people in 1968 in Yusho, Japan[2].

All contaminated samples contained increased amounts of PCBs, which closely correlated with the concentrations of PCDDs and PCDFs (Fig. 1b), having an average ratio of PCB:PCDD/F of about 22,000:1. The PCB pattern in feedstuff was closely matched to a PCB mixture composed mainly of Aroclor 1260 (Fig. 1c). In contaminated poultry meat and eggs, the distribution of PCB congeners was altered because of the preferential biological transformation and clearance of the lower chlorinated congeners (PCB 52 and 101). These findings led us to use the PCB analysis test to identify contaminated food products.

By using this test to survey PCB contamination of food, we confirmed that it was mainly poultry that was affected, with PCB concentrations in eggs and chicken meat being up to 250 times the tolerance level of 0.2 μg per g fat (Fig. 1b). Some pigs were also contaminated, but to a lesser extent than chickens, with up to 75 times the tolerance level, and no symptoms of poisoning were observed. In contrast, bovine livestock were free of contamination: from a total of 1,320 milk samples, only two were slightly (40 and 60%) above the tolerance level for PCBs (0.1 μg per g fat) but were within the tolerance level for dioxins (5 pg I-TEQ per g fat).

These findings can be explained by the longer life cycles of pigs and cattle and their lesser dependence on manufactured food mixtures compared with chickens. They also reflect the total amount of PCBs and dioxins introduced into the animal feed. By extrapolating the concentrations in chicken feedstuff to the initial volume of contaminated fat (maximum, 80 tonnes), the total amount of dioxins inadvertently mixed in the recycled fat can be estimated at about 1 g and that of PCBs at about 50 kg. Theoretically, such quantities could contaminate millions of chickens but have no effect on other livestock on the 3,000 farms that may have taken delivery of contaminated feed.

The outbreak of dioxin contamination in Belgium is an almost exact replica of the outbreaks of poultry poisoning by polychlorinated compounds that occurred repeatedly in the United States and Japan in the 1950s to 1970s. These outbreaks produced the same effects on the avian reproductive system as the Belgian contamination. Two of these previous reports also involved PCB mixtures (Kanechlor 400 and Aroclor 1242)[3,4].

As in earlier accidents, it is very unlikely that the isolated episode of contamination in Belgium will cause adverse health effects on the general population. It would require the consumption of 30–40 meals of highly contaminated chicken or eggs (dioxin levels of around 1,000 pg TEQ per g fat) to double the body's PCB and dioxin burden. Even in such an extreme case, the maximum body burden would still be at least a factor of

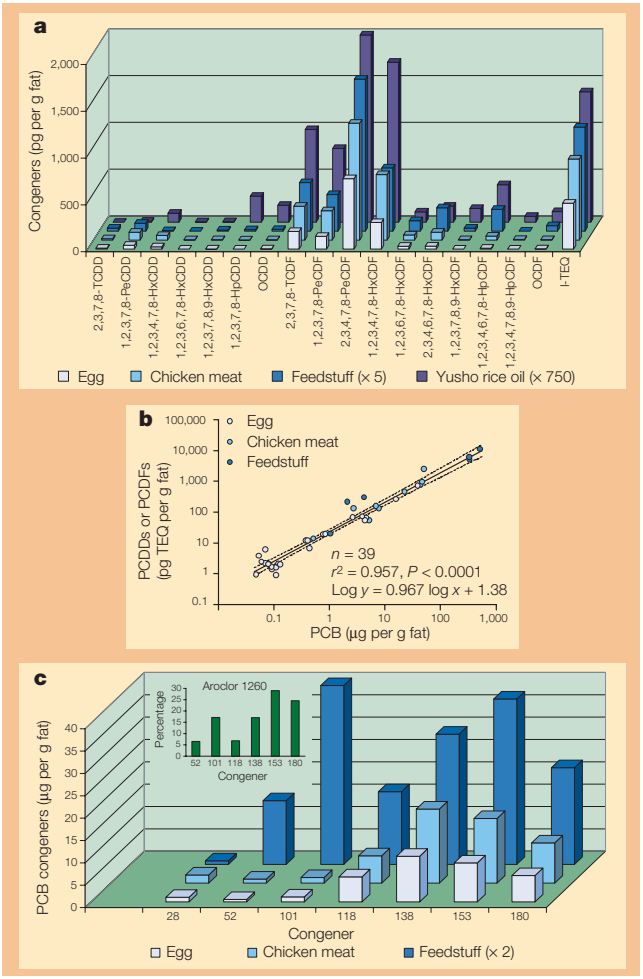

Figure 1 Dioxin and PCB congeners in contaminated Belgian poultry. **a,** Comparison of 2,3,7,8-substituted PCDD and PCDF congeners between Yusho rice oil and contaminated poultry. Each value is the mean of two (feedstuff and chicken) or five samples; egg samples are a mixture of 10 to 12 eggs. Total dioxin activity was calculated by using the 1998 international toxic equivalent factors (I-TEQ) of the World Health Organization. Values for feedstuff and Yusho oil have been divided by 5 and 750, respectively, for ease of representation. **b,** Correlation between PCDDs or PCDFs and PCBs in poultry feedstuff, chicken meat and eggs. PCB values correspond to the sum of the seven mono- and multi-*o*-PCB marker congeners (IUPAC 28, 52, 101, 118, 138, 153, 180) measured by high-resolution gas chromatography/mass spectrometry. **c,** Distribution of PCB congeners in contaminated poultry feedstuff, chicken meat and eggs (samples as in **a**). Values for feedstuff have been divided by 2.

one hundred less than in the victims of the Yusho accident and in the Seveso residents (referring to the mean value in the most contaminated zone), but would be similar to the two- to threefold increase in PCB and dioxin body burden of subjects affected in the 1980s[5] or of those regularly eating contaminated seafood[6].

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Reproductive biology

Pheromones and regulation of ovulation

There is controversy surrounding the issue of whether there is menstrual synchrony in women who live together[1-4], particularly in the case of the coupled-oscillator model developed[5] to explain similar data from rats. Stern and McClintock[6] have proposed that the rat model applies to women, with the effect being mediated by two opposing axillary 'pheromones' that could affect major reproductive events and have potential for "either contraception or treatment of infertility".

This claim[6] is based on data derived from four cycles from each of 20 subjects treated with axillary compounds to change the cycle length from that of each subject's baseline cycle. Subjects' upper lips were wiped daily with pads worn in the axillae of donors in follicular or ovulatory phases of their cycles. The cycles of subjects wiped with follicular pads appeared to be shortened by 1–14 days, whereas cycles of those wiped with ovulatory pads were longer by 1–12 days. One-third of cycles did not change or changed in the opposite direction.

My main criticism of the study is the use of the value of the single first cycles, receiving carrier-only treatment, to derive the data analysed. Such single observations have no within-subject variance and the irregular statistical manoeuvre of converting all 20 observations to zero masks any between-subject variance and provides an illusory zero baseline with indeterminate confidence limits. Carrier-only treatments should have been distributed throughout this long experiment to give a balanced crossover design with three treatments (carrier, follicular and ovulatory) and two or more complete replications to confer confidence limits to the baseline observations, thus making comparisons valid.

Each group has an apparent outlier favourable to the model: one of −14 comprises 25% of the total shortening, whereas that of +12 makes up 22% of the increase. Excluding these two outliers would abolish the claim of significance.

Reference to the first cycle throughout the long experiment may have introduced biases because of environmental, physiological and social drifts. McClintock[1] reported that women "who estimated seeing males less than three times per week" had cycles 1.5 days shorter than those who spent more time with males. Others claim that cycles are affected by sexual activity or the phase of the moon[7,8].

I am not convinced of the validity of the coupled-oscillator model derived from rat studies[5]. I also question the "definitive evidence" that pheromones regulate human ovarian function because, if these exist, their characterization will require large, carefully designed experiments, a controlled social and physical environment, and a clearly defined endpoint measured in hours.

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McClintock replies — We first reported menstrual synchrony almost three decades ago, and it has since been verified repeatedly. But synchrony does not always occur, and the circumstances in which it does not occur tell us a great deal about the social and physical conditions required[1,2]. Menstrual synchrony is but one manifestation of a more fundamental mechanism, the chemosensory regulation of ovulation, which occurs in other species not only as synchrony, but also as asynchrony, extreme cycle regularity, changes in the timing of puberty, birth cycles and reproductive ageing[1,2].

One of the studies[3] cited by Whitten to support his statement that synchrony is still controversial describes a non-random relationship between the cycles of lesbians living together, albeit not in synchrony. It is therefore consistent with our idea that ovarian-modulating chemosignals provide a basic mechanism that also gives rise to diverse forms of social regulation of ovulation.

Whitten implies that our data were poorly controlled, skewed and selected, but his criticisms do not hold with a careful reading of the experimental details[4]. The main criticism is based on a misreading of our procedure for within-experimental-subjects control. Each woman in the group was studied for five, not four, cycles and was exposed first to the carrier and then to follicular and ovulatory axillary compounds (in balanced randomly assigned order). We did not ignore the environmental, physiological and social factors that cause variation between women in the length of the menstrual cycle to be greater than the variation within women. Our within-subjects design controlled for this, and included the standard normalization procedure of expressing data as a change in cycle length from the baseline cycle preceding each condition. Statistical analyses were done only on these four change-scores (the baseline cycle lengths, all standardized to zero, were only depicted graphically).

Two other analyses also demonstrated the effect of human axillary compounds on ovarian function. A second control group of women who received only the carrier (frozen and thawed undercast pads with a few drops of alcohol) were significantly different from the experimental group, who also received both types of axillary compound.

Third, we analysed the absolute length of cycle phases, not change scores, by using log-survivor analysis developed for temporal data[5]. All temporal data are "skewed" and randomly have a Poisson, not a normal, distribution, and extreme values are not "outliers" but part of the temporal distribution. Survivor analysis indicated that axillary compounds change the timing of the preovulatory surge of luteinizing hormone, rather than the onset or duration of menses or the lifespan of the corpus luteum. So we did use a "clearly defined endpoint measured in hours": the timing of the preovulatory surge of luteinizing hormone[6], and not just the coarser measure of menstrual-cycle length. The concordance of our three independent statistical methods creates a robust foundation for concluding that axillary compounds significantly change the length of the menstrual cycle by altering the follicular phase.

Our log-survivor plot confirmed that the main effect is not driven just by the extreme cases, and their removal does not "negate the claim". Moreover, the extreme