calculations and physical forcing models show that anthropogenic perturbations are of sufficient magnitude to influence chemocline fluctuations. Contrary to the claim of Sinninghe Damsté *et al.*, it is not generally assumed that the rise in the chemocline occurred only recently and is without historical precedent. It is widely recognized that the mixed-layer depth and hydrographic properties of the Black Sea are strongly influenced by interannual climate variations. Physical oceanographic models and sedimentological evidence suggest that the chemocline has undergone large vertical fluctuations in the recent past. For example, lithogenic accumulation rates vary by nearly an order of magnitude throughout Unit II sediments and are correlated with varve thickness in Lake Saki (Crimea), indicating that large, climatically forced changes in freshwater input and water mass balance were a feature of Black Sea hydrography throughout the Holocene[3]. The issue raised by Murray *et al.* was whether anthropogenic perturbations have accentuated the frequency and amplitude of these natural variations.

What is required are high-resolution sediment analyses[4]. If annual changes in pigment deposition could be measured, then the frequency and perhaps the amplitude of the most recent rise in the chemocline could be compared to historical chemocline depth fluctuations[4,5]. The sampling interval for this type of study needs to be as small as possible, and certainly less than 10 years. The few, widely spaced samples, each covering 100–1,000 years of deposition, in ref. 1 are not relevant to the issue of anthropogenic influences on the chemocline, which could only have occurred in this century. The historical presence of anoxygenic photosynthesis and a shallow chemocline in the Black Sea over longer timescales does not make an anthropogenic influence on the most recent shoaling of the chemocline any less likely. High-resolution analyses of sediments at a 5–7-year sampling interval covering the past 1,500 years have yielded only ambiguous results, and it is unlikely that anthropogenic effects on the chemocline can be monitored by such sedimentological analyses[4]. Intensive time-series hydrographic studies such as those currently being made as part of the HydroBlack programme are needed to clarify the issue.

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SINNINGHE DAMSTÉ *ET AL.* REPLY — Our paper[1] is about sulphur-bound biomarkers and their isotope compositions, a line of enquiry that we have been following for some time (see refs 6, 7). The Black Sea, with its high sulphide content and photosynthetic bacteria, was a logical site to continue this work. As we hoped, very significant quantities of isotopically and structurally distinctive materials were recovered when desulphurization techniques were applied. These techniques indicated that green photosynthetic bacteria had flourished in the past and, therefore, that anthropogenic effects are not a prerequisite for a shallow chemocline. This is what we said.

In fact, the strongest statement we can find about the timing of the shoaling of the chemocline is not in our paper but in Repeta's[4]: "Anoxygenic photosynthesis is most likely a recent phenomenon in the Black Sea initiated by a shallowing of the chemocline over the past decade and the subsequent development of a suboxic layer". Our results show this 'suggestion' to be incorrect. We offered these data not as an attack on Repeta's work, but as a refinement of available information.

Why has the chemocline risen? We show only that anthropogenic factors are not decisive, and this does not prove that they have no role in the present episode. Our samples were chosen to allow examination of sulphur-bound biomarkers, not to investigate short-term environmental changes. We fully agree that high-resolution studies are required for the latter purpose.

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Quantitative PCR

SIR — In their Product Review[1] on the competitive polymerase chain reaction (PCR), a technique that compares the accumulation of two products derived from a known amount of standard and the target, respectively, to quantitate the initial amount of target, Siebert and Larrick claim that "the initial ratio of target-to-competitor cDNA remains constant throughout the amplification", and that it is therefore "not necessary to obtain data during the exponential phase of the reaction".

On inspecting the available literature, it turns out that this point has rarely been investigated thoroughly[2]. Some authors who have tested this claim experimentally find that the two products accumulate with different amplification efficiencies[3,4], so that quantification of target would lead to erroneous results if these different kinetics were not taken into account. In many systems, products accumulate up to the same concentration, irrespective of the initial amount of target. This plateau concentration, which is approximately 10^{-7} M[5], is of course reached at later cycles if the initial amount of target is lower[6–10]. The plateau concentration is reached when the concentration of *Taq* polymerase becomes rate-limiting, when reaction by-products have accumulated or, most probably, when PCR products successfully compete with primers during the annealing step.

The reason for the discrepancy in the kinetics of product accumulation in different competitive PCR systems[2,4] is unclear. Therefore, when performing quantitative PCR protocols, we recommend that quantitative analysis is restricted to the exponential phase of product accumulation, as has been suggested[11,12], or at least that the time course of accumulation of both products is carefully established when setting up a new system.

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