Global spread of microorganisms by ships

Ballast water discharged from vessels harbours a cocktail of potential pathogens.

Commercial ships have spread many species around the world[1–3], but little is known of the extent and potential significance of ship-mediated transfer of microorganisms[3,4]. Here we show that the global movement of ballast water by ships creates a long-distance dispersal mechanism for human pathogens and may be important in the worldwide distribution of microorganisms, as well as for the epidemiology of waterborne diseases affecting plants and animals.

Ships have used ballast water for stability since the nineteenth century, discharging water at ports of call and en route[1]. Ports can receive relatively large volumes of ballast water — for example, the United States receives more than 79 million tonnes of ballast water from overseas each year[5]. Ballast tanks carry a diverse community of organisms, resulting in many biological invasions[2,3]. Pathogens, including those affecting humans, are common in coastal waters[6] and can also be transferred in ballast water[7,8].

We measured the concentrations of total bacteria, virus-like particles (VLPs) and the bacteria *Vibrio cholerae* O1 and O139, which cause human epidemic cholera, in the ballast water of vessels arriving to Chesapeake Bay (Fig. 1) from foreign ports. We collected water samples to estimate the abundance of total bacteria and VLPs, and also took both water and plankton samples to measure the concentration of *V. cholerae*, as the bacterium forms associations with plankton[9].

Our samples contained an average of 8.3×10^8 bacteria (s.e., 1.7×10^8; $n = 11$) and 7.4×10^9 VLPs (s.e., 2.3×10^9; $n = 7$) per litre. Given that Chesapeake Bay received an estimated 1.2×10^{10} litres of foreign ballast water in 1991 alone[5], our measures indicate that ballast water probably delivers large numbers of microbial species and potential pathogens to this estuary.

Vibrio cholerae was found in plankton samples from all ships, and both serotypes were detected in 93% of the ships (Fig. 2a). The concentration of *V. cholerae* O1 was significantly greater than that of O139 (Fig. 2a; Student's *t*-test, $t = 2.296$; d.f., 27; $P < 0.05$). Furthermore, there were 100 times more *V. cholerae* O1 and O139 in water samples than in plankton samples from the same ships (Fig. 2b; paired *t*-tests for serotype O1 and O139 respectively, $t = 8.10$; d.f., 6; $P < 0.001$ and $t = 10.05$; d.f., 6; $P < 0.001$), indicating that *V. cholerae* was not concentrated on zooplankton larger than 80 μm in diameter.

Vibrio cholerae is a useful model to examine the possible significance of ballast-mediated dispersal in transmission of pathogens. Our data indicate that *V. cholerae* can be delivered frequently by ships to estuaries with commercial ports, and our observations of dividing cells revealed that some bacteria are viable upon arrival. Although it remains difficult to estimate the concentration of viable cells[10], the transfer and release of *V. cholerae* by ships creates an opportunity for the colonization of coastal ecosystems. *V. cholerae* is a common component of freshwater and marine habitats, including Chesapeake Bay, where it persists without human contact[9,10]. Thus, should a novel genotype arrive in ballast water, local conditions may favour its establishment. The extent to which this has occurred, and the degree of geographic differences in the genetic structure of *V. cholerae* populations, is unknown.

We predict that coastal ecosystems are frequently invaded by microorganisms from ballast water. First, concentrations of bacteria and viruses exceed those reported for other taxonomic groups in ballast water by 6–8 orders of magnitude[2], and the probability of successful invasion should increase with inoculation concentration[11]. Second, the biology of many microorganisms may facilitate invasion, combining a high capacity for increase, asexual reproduction, and the ability to form dormant resting stages[3,12]. Such flexibility in life history can broaden the opportunity for successful colonization, allowing rapid population growth when suitable environmental conditions occur. Third, many microorganisms can tolerate a broad range of environmental conditions, such as in salinity or temperature, so many sites may be suitable for colonization[6,12]. This suite of factors may yield a high rate of invasion for microorganisms compared to invertebrates, which are already known to invade coastal habitats from ballast water[2,3].

Despite growing concern about biological invasions[7,11] and emergent diseases[9,13,14], the extent and effects of the transfer of microorganisms in ballast water are virtually unexplored[3]. We know of no published estimates of microbial genetic diversity in ballast water, and the fate of microorganisms discharged from ballast tanks remains unknown[3]. Given the magnitude of ongoing transfer and its potential consequences for ecological and disease processes, large-scale movement of microorganisms by ships merits attention from both invasion biologists and epidemiologists.

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Figure 1 Chesapeake Bay, on the US East Coast, receives some ten billion litres of foreign ballast water each year. Each litre typically contains about a billion bacteria and seven billion virus-like particles.

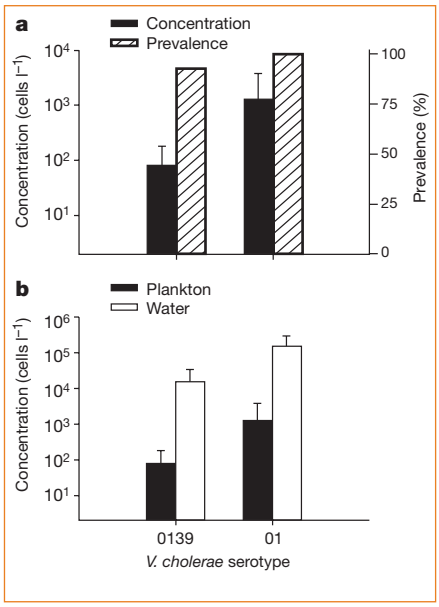

Figure 2 Prevalence and concentration of *Vibrio cholerae* serotype O1 and O139 in ships' ballast water. **a,** Prevalence and concentration associated with plankton samples. Prevalence shows the percentage of ships in which the respective serotypes were detected. Sample size differed between serotype O1 ($n = 15$ ships) and O139 ($n = 14$ ships). **b,** Comparison of concentration of each serotype between paired plankton and water samples from the same ballast tanks ($n = 7$ ships). Concentrations are shown as mean and standard error for respective serotypes and sample types as detected by direct count methods using fluorescent antibodies; further details are available from the authors.

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Materials science

The smallest carbon nanotube

We report here the discovery of the smallest possible carbon nanotube. This has a diameter of 4 Å, which is the narrowest attainable that can still remain energetically stable, as predicted by theory. These nanotubes are confined inside multiwalled carbon nanotubes and their diameter corresponds to that of a C_{20} dodecahedron with a single carbon atom at each of its twenty apices. Unlike larger carbon nanotubes, which, depending on their diameter and helicity, can be either metallic or semiconducting, these smallest nanotubes are always metallic.

Many of the extraordinary properties of carbon nanotubes[1] depend on their diameter. The smallest carbon nanotubes have been associated with the smallest fullerenes[2], with nanotube diameters of 7 or 5 Å corresponding to those of C_{60} and C_{36} structures, respectively. Carbon nanotubes with such small diameters have been observed experimentally[3–5].

The carbon nanotubes reported here were seen in cathodic deposits produced by arc-discharge of graphite rods in a hydrogen atmosphere without a metallic catalyst[6]. Under these conditions, more carbon nanotubes (all multiwalled) are kept open as hydrogen etches away the capping atoms, a unique feature that helps maintain a favourable environment for smaller carbon nanotubes to form inside already-grown multiwalled carbon nanotubes.

Figure 1 shows a high-resolution transmission electron micrograph of an 18-shell carbon nanotube produced in this way: each dark line represents the side wall of a cylindrical graphene shell in projection and the innermost shell has a diameter of 4 Å. The cylindrical structure of the nanotube is shown by the reduced contrast towards the centre of the nanotube, where there are fewer atoms in the smaller tubes. Multiwalled carbon nanotubes with innermost shells of diameter 5 or 7 Å were also detected in the same material.

Some of the smallest nanotubes seen were capped, and electron micrographs of the cap of a 4 Å nanotube indicate that the smallest nanotubes have an antichiral [3, 3] 'armchair' structure[7] (Fig. 1, inset). We propose that these 4 Å nanotubes grow out of half of a C_{20} dodecahedron, in which the C–C bonding angle is 108° — very close to the bonding angle (109.5°) in the sp^3 configuration of diamond. Growth into a full nanotube is realized through a step-by-step mechanism on both the outer and inner surfaces[8] (Fig. 1, inset). The hydrogen atmosphere facilitates the formation of the

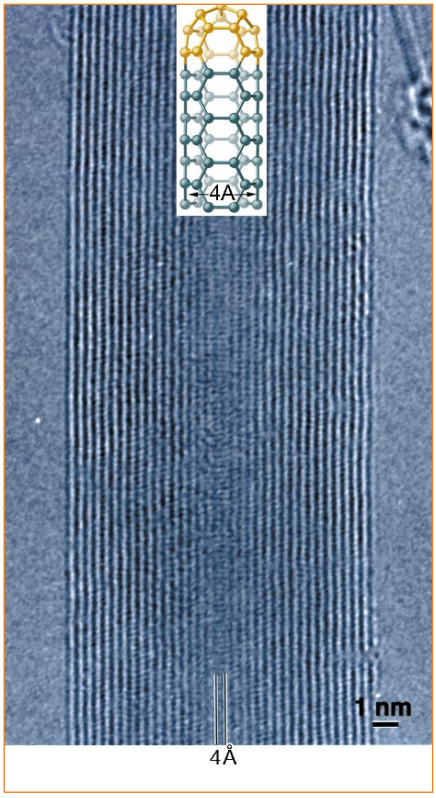

Figure 1 High-resolution electron microscope image of a 4 Å tubule (side walls are marked by lines) confined inside an 18-shell carbon nanotube. Dark lines correspond to the side walls of the single-wall shells. The blurring towards the centre is due to the smaller number of atoms in the smaller tubules. Inset, model for the formation and growth of a [3,3] armchair tubule of 4 Å diameter. Half of the C_{20} dodecahedron (yellow) serves as a nucleus and further growth into the tubule is realized by adding carbon atoms (blue).

halves of the C_{20} dodecahedron, stabilizing the structure by terminating certain dangling bonds with hydrogen, and keeps the inside of the nanotubes open so that, after the confining outer shells have been formed, carbon species can enter the core to form the innermost shell.

Although these tiny 4 Å carbon nanotubes should be energetically stable[9], the severe steric distortion resulting from the planar graphene changes the electronic structure significantly[10]. Electronic band-structure calculations indicate that all such tubules tend to be metallic, regardless of their helicity.

C_{20} fullerenes have recently been made using a gas-phase reaction method[11]. Our finding indicates that C_{20} fullerenes could also be produced by the arc-discharge method. It remains a challenge to produce single-walled carbon nanotubes of 4 Å diameter experimentally.

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Materials science

Single-walled 4 Å carbon nanotube arrays

Here we describe the smallest carbon nanotubes possible[1], prepared by the pyrolysis of tripropylamine molecules in the channels of porous zeolite $AlPO_4$-5 (AFI) single crystals[2]. These uniformly sized carbon nanotubes have a diameter of 0.4 nm and are the best example of one-dimensional quantum wires.

AFI is a type of transparent microporous crystal (Fig. 1) containing one-dimensional channels packed in hexagonal arrays, with an inner diameter of 0.73 ± 0.01 nm (ref. 3). The starting material we used for synthesizing single-walled carbon nanotubes (SWNTs) was tripropyl-