Materials and Methods

To establish the amount of microscopic plastic present in sedimentary marine habitats, sediment samples (n = 5 at each location, Fig. 1A) were collected from the strandline using a small trowel and from the subtidal using an Eckman grab. Plastics, and other low-density particles, were separated by adding 250ml of sediment to concentrated saline solution (1.2kg NaCl l⁻¹) and stirring for 30 seconds. After 2 minutes the supernatant was filtered (Whatman GF/A). Filters were dried at 20°C, sealed in petri-dishes to prevent contamination and examined using a microscope (x 30). Fragments were identified using a Perkin Elmer Spectrum 2000 FT-IR spectrometer with a narrow band MCT detector and Perkin Elmer Auto IMAGE FT-IR microscope. Spectra were corrected for background, then compared to spectra in a database of common polymers. The technique achieved a
high level of certainty (quality index >0.7) for most identifications. The abundance of microscopic fragments, that were identified as synthetic polymers using this approach, was then compared amongst sediments from replicate sandy intertidal (Kingsand, Cornwall and Bovisand, Devon), estuarine intertidal (St John’s Lake, Cornwall and Plym Estuary, Devon) and subtidal habitats (Coxside and Mill Bay, Devon) near Plymouth (Fig. 1A). Data were log10(x + 1) transformed and compared using ANOVA followed by post-hoc SNK tests. Sediment type was considered a fixed factor and site a random factor.

Accumulation of microscopic plastics in the marine environment was assessed using archived plankton samples. These were collected using continuous plankton recorders (CPR) towed behind vessels on standard shipping routes over the last 40 years and archived by the Sir Alister Hardy Foundation for Ocean Science (1). Samples were collected at 10m depth through a 127mm² aperture in the CPR on to a scrolling 280µm-mesh silkscreen. We examined two routes where regular samples were available, which had not been opened since collection. Four samples were examined from each decade since 1960. These were from a five-year period mid-decade (e.g. 1963-67 inclusive). Silk screens were viewed under a microscope and unusual fragments identified using FT-IR. Data were compared using ANOVA followed by post-hoc SNK tests. Decade was considered a fixed factor and route a random factor.

To determine the potential for microscopic plastics to be ingested we kept amphipods, *Orchestia gammarellus* (Pallas); lugworms, *Arenicola marina* L. and barnacles, *Semibalanus balanoides* (L.) in aquaria with small quantities of microscopic plastic. All
organisms were collected from intertidal habitats near Plymouth and transferred to aquaria where conditions were resembled those in the field, but with small amounts of microscopic plastic (range 20 - 2000µm diameter). *A. marina* (n=15) were kept at a density of one individual l⁻¹ in sediment containing 1.5g of microplastic l⁻¹. *O. gammarellus* (n=15) were kept amongst damp stones with 1g of microscopic plastic per individual. *S. balanoides* (n=150, ~ 10 individuals l⁻¹ seawater) were kept in seawater containing 1g of microplastic l⁻¹. Ingestion of microplastic plastic was quantified after several days from wormcasts (*A. marina*) or by dissection (Fig. S1).

Fig. S1. Microscopic plastics were ingested by polychaete worms, barnacles and amphipods during laboratory trials. Here a microscopic plastic fragment is pictured (arrowed) in the intestinal tract of the amphipod (*Orchestia gammarellus*).

References