Supporting Information

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S1 Methods

A cell viability assay was used to examine whether the ingestion of microplastic led to reductions in the ability of mussel haemocytes to absorb neutral red dye [24]. A 50 μL aliquot of haemolymph was pipetted into microtitre plates and agitated using a plate shaker (1400 rpm, 60 sec). The plate was left (50 min) to allow haemocytes to adhere to the bottom of the wells. After this excess cells were discarded by rinsing the plate with phosphate buffered solution. Neutral red dye (0.4 %) was added, and cells incubated in the dark (3 h, 25 ^{o}C) to prevent photolysis. Wells were washed with phosphate buffer solution before a solution of 1 % acetic acid and 20 % ethanol was added to resolubilise the dye. Dye retention and total protein was determined using a spectrophotometer (550 nm). Results were presented as optical density per mg protein.

The phagocytosis assay was used to determine whether the immune system of mussels had been compromised by ingesting microplastic. This assay quantifies the ability of mussel haemocytes to engulf yeast (zymosan) particles [24]. A 50 μL sample of haemocytes was transferred into a microtitre plate and agitated using a plate shaker (1400 rpm, 60 seconds). The plate was covered with a plate-sealer and incubated (10 °C, 50 min). Aliquots of 50 μL of neutral-red-stained and heat-stabilised zymosan suspension ($1x10^5$ particles mL^{-1} phosphate buffer) were added to each well, and the plate was incubated (10°C, 3 h). The cells were washed to remove residual haemocytes using 100 μL phosphate buffer (pH 7.4) and a series of zymosan standards were added. The dye was resolubilised via addition of 100 μL of 1% acetic-acid/20% ethanol. Microtitre plates were covered with a plate-sealer and incubated (10 min, 25°C), and then read at 550 nm.

Total protein was determined and the results presented as the number of zymosan particles phagocytosed per mg protein.

To determine whether ingestion and translocation of microplastic caused any measurable reductions in the capacity of the haemolymph to deal with oxidative stress, the Ferric Reducing Antioxidant Potential (FRAP) assay was used [25]. Haemolymph (30 µL) was transferred in triplicate into microtitreplate wells. Aqueous solutions of known Fe^{II} concentrations in the range of 0-100 µmol l^{-1} were used for calibration and 200 µL of reagent (300 mM acetate buffer, TBTZ (2,4,6-tripyridyl-*s*-triazine)) was placed into each well. The absorbance (595 nm) within each sample well was read immediately (time 0), incubated (10 min, 25 ºC) and read again. Results were presented as the change in absorbance at 595 nm.

Feeding behaviour was assessed by examining the rate of clearance of algal food particles from a stock solution [26]. A solution of *I. galbana* was prepared by diluting a concentrate with 100 mL of seawater to produce an initial concentration of algal-cells in the feeding vessels at ca. 15,000 algal cells 0.5 mL^{-1}. Beakers were filled with 350 mL of seawater (15 ^{o}C) and stirring bars. Individual mussels were carefully placed into beakers using forceps and positioned away from the moving bars. At any one time, the clearance rate of individual mussels (3 treatments; control, 3.0 and 9.6 µm polystyrene, each with 5 mussels) were independently measured, together with 3 'no mussel controls' to check that the particles of algae were continually in suspension. The mussels were left *in-situ* for at least 10 min to acclimatise and to ensure all the mussels had opened their valves. The algal solution was added to all the vessels and 20 mL of seawater-algal mixture was immediately removed from all vessels (t_0) and placed into pre-labelled plastic vials for

Coulter Counter analysis. The procedure was repeated on water samples taken 30 min (t_2) after the initial feeding. The abundance of *I. galbana* within samples was quantified using the Z2 Coulter Particle Analyser. The clearance rate of the individual mussels was then calculated using the equation below. Clearance Rate (hr^{-1}) = (v x 60/t) (In t_0 – In t_2), where: v = volume of water in feeding rate beaker (litres), t = duration of assay (hours), t_0 = initial cell count and t_2 = final cell count.

S2 Results

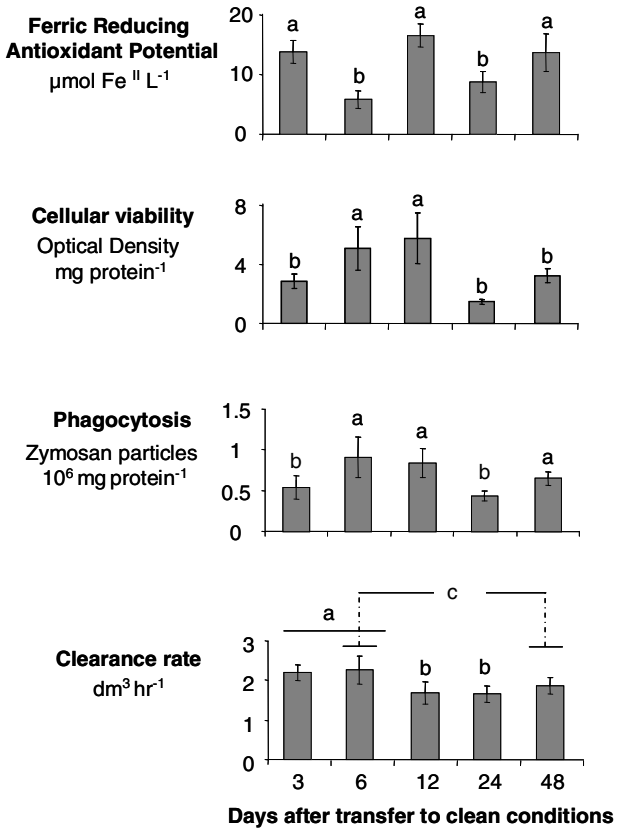

Figure S1-Sn. Temporal changes in health at different levels of biological organisation within *Mytilus edulis.* Values are expressed as mean±S.E. Differences between times marked with differing letters (a,b,c) were significant at $P<0.001$.