**Supplementary materials to**

# First assessment of micro-litter ingested by dolphins, sea turtles and monk seals found stranded along the coasts of Samos Is-land, Greece

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**Table S1.** Number of MPs detected in the laboratory (mean ± Standard Deviation, S.D.) and assessed on GFF filters ($n$ = 10) before (pre-test) and during the manipulation (test).

| Test | Type | Size (mm) | Color | Number of MPs | |
|---|---|---|---|---|---|
| | | | | Mean | S.D. |
| pre-test | fibre | 0.20-0.50 | black | 1 | 0 |
| | | | pink | 1 | 0 |
| | | 1.01-2.50 | clear | 1 | 0 |
| | | | yellow | 1 | 0 |
| | fragment | 0.20-0.50 | white | 1 | 0 |
| test | fibre | 0.20-0.50 | black | 1 | 0 |
| | | | blue | 1 | 0 |
| | | | red | 2 | 0 |
| | | | yellow | 1.25 | 0.5 |
| | | 0.51-1.00 | black | 1 | 0 |
| | | | blue | 1 | 0 |
| | | | red | 1 | 0 |
| | | 1.01-2.50 | black | 1 | 0 |
| | | | blue | 1 | 0 |
| | | | red | 1 | 0 |
| | | 2.51-5.00 | black | 1 | 0 |
| | | | blue | 1 | 0 |
| | fragment | 0.20-0.50 | black | 1 | 0 |
| | | | blue | 1 | 0 |
| | | | green | 1 | 0 |
| | | | yellow | 1 | 0 |

**Table S2.** Summary of the most recent studies investigating microplastics (MPs) contamination in the gastrointestinal tracts (GIT) of stranded marine megafauna.

| Reference | Family | Species | N. of individuals analysed (with MPs) | Study location | Study date | Method of Quantification | Localization | Tot n. of particles | Particles type | Particle Size range (in mm) |
|---|---|---|---|---|---|---|---|---|---|---|
| ***(Bravo Rebolledo et al., 2013)*** | Phocidae | *Phoca vitulina* | 207 (13) | Texel, Netherlands | 2002 | Visual identification | stomach | 28 | | not stated |
| | | | | | | | intestines | 7 | | not stated |
| ***(Besseling et al., 2015)*** | Balaenopteridae | *Megaptera novaeangliae* | 1 (1) | Texel, Netherlands | 2012 | Visual identification and FT-IR | GIT | 45 | | 0.01-170 |
| ***(Lusher et al., 2015)*** | Ziphidae | *Mesoplodon mirus* | 3 (3) | Galway, Ireland | 2013 | Visual identification and FT-IR | GIT (oesophagus, stomachs and intestine) | 88 | Fibres (n = 69) | 0.3-7 (mean 2.16±1.39) |
| | | | | | | | | | Fragments (n = 19) | |
| ***(Ryan et al., 2016)*** | Cheloniidae | *Caretta caretta* (post-hatchling) | 230 (16) | Western Cape, South Africa | 2015 | Visual identification | GIT (stomach, intestine, cloaca and bladder) | 301 | Fragments (n = 229) | ND-17 (mean (4.7±2.4 × 3.0±1.4 × 1.0±0.5) |
| | | | | | | | | | Bags (n = 33) | |
| | | | | | | | | | Fibres (n =24) | ND-30 (mean 16.3±7.6) |
| | | | | | | | | | Pellets (n = 10) | |
| | | | | | | | | | Other (n = 5) | |
| ***(Pham et al., 2017)*** | Cheloniidae | *Caretta caretta* | 12 stranded (8) | Azores region, Portugal | 1996-2016 | Visual identification and FT-IR | GIT (oesophagus, stomachs and intestine) | 380 | Fragments (n = 213) | ND-310 (mean 20.3±1.6) |
| | | | | | | | | | Sheets (n = 98) | |
| | | | | | | | | | Foam (n = 4) | |

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| | | | | | | | | | Synthetic rope (n = 38) | |
| | | | | | | | | | Fishing line (n = 22) | |
| | | | | | | | | | Pellets (n = 5) | |
| ***(van Franeker et al., 2018)*** | Phocoenidae | *Phocoena phocoena* | 654 (44) | Texel, Netherlands | 2003-2013 | Visual identification and NIR | stomach | 71 | | not stated |
| ***(Hernandez-Gonzalez et al., 2018)*** | Delphinidae | *Delphinus delphis* | 35 (35) | Gacilian coast, Spain | not stated | Visual identification | stomach(s) | 411 | Fibres | 0.29-4.92 (mean 2.11±1.26) |
| | | | | | | | | | Fragments | 0.49-4.07 (mean 1.29±0.93) |
| | | | | | | | | | Beads | 0.95 (diameter) |
| ***(Lusher et al., 2018)*** | | NID Mysticeti | 45 (0) | Ireland | 1990-2015 | Visual identification | GIT (oesophagus, stomachs and intestine) | 500 | Fibres | 0.6-16.7 |
| | Balaenopteridae | NID Balaenopteridae | 2 (0) | | | | | | | |
| | Balaenopteridae | *Balaenoptera acutorostrata* | 90 (0) | | | | | | | |
| | Balaenopteridae | *Balaenoptera borealis* | 1 (0) | | | | | | | |
| | Balaenopteridae | *Balaenoptera physalus* | 24 (0) | | | | | | | |
| | Balaenopteridae | *Megaptera novaeangliae* | 5 (0) | | | | | | | |
| | Physeteridae | *Physeter macrocephalus* | 57 (0) | | | | | | | |
| | Physeteridae | *Kogia breviceps* | 9 (0) | | | | | | | |
| | | NID Cetacean | 50 (0) | | | | | | | |
| | | NID Odontoceti | 17 (0) | | | | | | | |
| | | NID beaked whale | 10 (0) | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|
| | Ziphidae | *Hyperoodon ampullatus* | 16 (0) | | | | | | | |
| | Ziphidae | *Mesoplodon bidens* | 11 (0) | | | | | | | |
| | Ziphidae | *Mesoplodon mirus* | 5 (0) | | | | | 98 | Fragments | 0.6-16.7 |
| | Ziphidae | *Ziphius cavirostris* | 38 (1) | | | | | | | |
| | Delphinidae | NID Delphinidae | 176 (0) | | | | | | | |
| | Delphinidae | *Delphinus delphis* | 755 (10) | | | | | | | |
| | Delphinidae | *Stenella coeruleoalba* | 219 (2) | | | | | | | |
| | Delphinidae | Delphinids | 17 (0) | | | | | | | |
| | | Delphinidae/Phocoenidae | 41 (0) | | | | | | | |
| | Phocoenidae | *Phocoena phocoena* | 608 (5) | | | | | | | |
| | Delphinidae | *Globicephala melas* | 338 (0) | | | | | | | |
| | Delphinidae | *Grampus griseus* | 68 (0) | | | | | | | |
| | Delphinidae | *Lagenorhynchus acutus* | 152 (0) | | | | | | | |
| | Delphinidae | *Lagenorhynchus albirostris* | 25 (0) | | | | | | | |
| | Delphinidae | *Orcinus orca* | 5 (1) | | | | | | | |
| | Delphinidae | *Tursiops truncatus* | 150 (2) | | | | | | | |
| ***(Nelms et al., 2019)*** | Delphinidae | *Lagenorhynchus acutus* | 1 (1) | British coast | 2014-2017 | Visual identification and FT-IR | GIT (stomachs and intestine) | 8 | Fibres (n = 229) | 0.10-200 (mean 2±2.3) |
| | Delphinidae | *Tursiops truncatus* | 1 (1) | | | | | 6 | | |
| | Delphinidae | *Delphinus delphis* | 16 (16) | | | | | 91 | | |
| | Phocidae | *Halichoerus grypus* | 3 (3) | | | | | 18 | | |
| | Phocoenidae | *Phocoena phocoena* | 21 (21) | | | | | 110 | | |
| | Phocidae | *Phoca vitulina* | 4 (4) | | | | | 17 | Fragments (n = 44) | 0.1-4 (mean 0.9±1.1) |
| | Physeteridae | *Kogia breviceps* | 1 (1) | | | | | 4 | | |
| | Delphinidae | *Grampus griseus* | 1 (1) | | | | | 9 | | |
| | Delphinidae | *Stenella coeruleoalba* | 1 (1) | | | | | 7 | | |
| | Delphinidae | *Lagenorhynchus albirostris* | 1 (1) | | | | | 3 | | |

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|---|---|---|---|---|---|---|---|---|---|---|
| **(Duncan et al., 2019)** | Cheloniidae | *Chelonia mydas* | 34 (34) | Cyprus Island, Greece | 2011-16 | Visual identification and FT-IR | GIT (entire) | 12 | Fibres | Mediterranean: ND-1.40±0.54; Atlantic: ND-2.87±0.20; Pacific: ND-2.85±0.23 |
| | Cheloniidae | *Caretta caretta* | 22 (22) | Cyprus Island, Greece | 2011-16 | | | 13 | | |
| | Cheloniidae | *Chelonia mydas* | 10 (10) | North Carolina, USA | 2016-17 | | | 4 | | |
| | Cheloniidae | *Caretta caretta* | 8 (8) | North Carolina, USA | 2016-17 | | | 1 | | |
| | Cheloniidae | *Lepidochelys kempii* | 10 (10) | North Carolina, USA | 2010-17 | | | 1 | | |
| | Dermochelyidae | *Dermochelys coriacea* | 2 (2) | North Carolina, USA | 2017 | | | 1 | Fragments and bead | Mediterranean: ND-0.07±0.01; Atlantic: ND-0.31±0.04; Pacific: ND-0.26±0.01 |
| | Cheloniidae | *Chelonia mydas* | 7 (7) | Queensland, Australia | 1993-2017 | | | 4 | | |
| | Cheloniidae | *Caretta caretta* | 3 (3) | Queensland, Australia | 2009-14 | | | 2 | | |
| | Cheloniidae | *Natator depressus* | 4 (4) | Queensland, Australia | 2006-14 | | | 2 | | |
| | Cheloniidae | *Lepidochelys olivacea* | 1 (1) | Queensland, Australia | 2016 | | | 0 | | |
| | Cheloniidae | *Eretmochelys imbricata* | 1 (1) | Queensland, Australia | 2016 | | | 0 | | |
| **(Zhu et al., 2019)** | Delphinidae | *Sousa chinensis* | 3 (3) | Guangxi Beibu Gulf, China | 2015 | Visual identification and FT-IR | Foregut (random samples) | 45 | Fibres, fragments and flakes | 0.1-4.8 (mean 2.2±0.4) |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Midgut (random samples) | 30 | | |
| | | | | | | | Mix-gut (random samples) | 2 | | |
| ***(Battaglia et al., 2020)*** | Delphinidae | *Tursiops truncatus* | 7 (7) | South Carolina, USA | 2017-2018 | Visual identification and FT-IR | GIT (stomachs and intestine) | 1964 | Fibres (n = 1495) | 0.125-5.00 |
| | | | | | | | | | Fragments (n = 103) | |
| | | | | | | | | | Film (n = 336) | |
| | | | | | | | | | Foam (N = 30) | |
| ***(Digka et al., 2020)*** | Cheloniidae | *Caretta caretta* | 28 stranded (26) | Greece | 2017-2019 | Visual identification and FT-IR | GIT (oesophagus, stomach, intestine) | 286 | Threads (n = 128) | 0.4-550 (mean 47±3.6) |
| | | | | | | | | | Sheets (n = 103 | |
| | | | | | | | | | Fragments (n = 46) | |
| | | | | | | | | | Foam (n = 6) | |
| | | | | | | | | | NC (n = 3) | |
| ***(Novillo et al., 2020)*** | Delphinidae | *Stenella coeruleoalba* | 43 (39) | Valencian Community, Spain | 1988-2017 | Visual identification and FT-IR | Digestive tract | 672 | Fibres (n = 494) | - |
| | | | | | | | | | Fragments (n = 160) | - |
| | | | | | | | | | Pellet (n = 17) | - |
| ***(Zhang et al., 2021)*** | Delphinidae | *Sousa chinensis* | 12 (12) | Pearl river Estuary, China | 2016-2018 | Visual identification and FT-IR | Stomach | 635 | Fibres (n = 443) | 1.69±1.04 |
| | | | | | | | | | Film (n = 30) | - |
| | | | | | | | | | Pellet (n = 19) | Ø 0.72 (SD = 0.24) |
| | | | | | | | | | Irregular particle (n = 143) | 0.78±0.71 |

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| ***(Philipp et al., 2021)*** | Phocoenidae | *Phocoena phocoena* | 30 (28) | Schleswig-Holstein, Germany | 2014-2018 | Visual identification and FT-IR | GIT (intestine) | 401 | Fibres (n = 202) | 0.1-2.0 |
| | | | | | | | | | Fragments (n = 199) | - |
| **This study** | Cheloniidae | *Caretta caretta* | 9 (9) | Samos Island, Greece | 2018-2019 | Visual identification | GIT (oesophagus, stomach, intestine) | 1969 | Fibres (n = 8560) | 0.21-5.00 (0.21-0.50, n = 4634; 0.51-1.00, n = 2061; 1.01-2.50 n = 2174; ≤0.20, n = 701; 2.51-5.00, n = 1069)¶ |
| | Cheloniidae | *Chelonia mydas* | 6 (6) | | | | | 1944 | | |
| | Phocidae | *Monachus monachus* | 2 (2) | | | | | 538 | | |
| | Delphinidae | *Delphinus delphis* | 3 (3) | | | | | 1442 | Fragments (n = 2079) | |
| | Delphinidae | *Stenella coeruleoalba* | 3 (3) | | | | | 3116 | | |
| | Delphinidae | *Tursiops truncatus* | 2 (2) | | | | | 1630 | | |

*NID: Not Identified; NC: Not Classified*

**References**


Battaglia, F.M., Beckingham, B.A., McFee, W.E., 2020. First report from North America of microplastics in the gastrointestinal tract of stranded bottlenose dolphins (Tursiops truncatus). Mar. Pollut. Bull. 160, 111677. https://doi.org/10.1016/j.marpolbul.2020.111677

Besseling, E., Foekema, E.M., Franeker, J.A. Van, Leopold, M.F., Kühn, S., Rebolledo, E.L.B., Heße, E., Mielke, L., Ijzer, J., Kamminga, P., Koelmans, A.A., 2015. Microplastic in a macro filter feeder : Humpback whale Megaptera novaeangliae. Mar. Pollut. Bull. 95, 248–252. https://doi.org/10.1016/j.marpolbul.2015.04.007

Bravo Rebolledo, E.L., Van Franeker, J.A., Jansen, O.E., Brasseur, S.M., 2013. Plastic ingestion by harbour seals ( Phoca vitulina ) in The Netherlands. Mar. Pollut. Bull. 67, 200–202. https://doi.org/10.1016/j.marpolbul.2012.11.035

Digka, N., Bray, L., Tsangaris, C., Andreanidou, K., Kasimati, E., Kofidou, E., Komnenou, A., Kaberi, H., 2020. Evidence of ingested plastics in stranded loggerhead sea turtles along the Greek coastline, East Mediterranean Sea. Environ. Pollut. 263. https://doi.org/10.1016/j.envpol.2020.114596

Duncan, E.M., Broderick, A.C., Fuller, W.J., Galloway, T.S., Godfrey, M.H., Hamann, M., Limpus, C.J., Lindeque, P.K., Mayes, A.G., Omeyer, L.C.M., Santillo, D., Snape, R.T.E., Godley, B.J., 2019. Microplastic ingestion ubiquitous in marine turtles. Glob. Chang. Biol. 25, 744–752. https://doi.org/10.1111/gcb.14519

Hernandez-Gonzalez, A., Saavedra, C., Gago, J., Covelo, P., Santos, M.B., Pierce, G.J., 2018. Microplastics in the stomach contents of common dolphin (Delphinus delphis) stranded on the Galician coasts (NW Spain, 2005–2010). Mar. Pollut. Bull. 137, 526–532. https://doi.org/10.1016/j.marpolbul.2018.10.026

Lusher, A.L., Hernandez-Milian, G., Berrow, S., Rogan, E., O'Connor, I., 2018. Incidence of marine debris in cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. Environ. Pollut. 232, 467–476. https://doi.org/10.1016/j.envpol.2017.09.070

Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: The True's beaked whale Mesoplodon mirus. Environ. Pollut. 199, 185–191. https://doi.org/10.1016/j.envpol.2015.01.023

Nelms, S.E., Barnett, J., Brownlow, A., Davison, N.J., Deaville, R., Galloway, T.S., Lindeque, P.K., Santillo, D., Godley, B.J., 2019. Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Sci. Rep. 9. https://doi.org/10.1038/s41598-018-37428-3

Novillo, O., Raga, J.A., Tomás, J., 2020. Evaluating the presence of microplastics in striped dolphins (Stenella coeruleoalba) stranded in the Western Mediterranean Sea. Mar. Pollut. Bull. 160, 111557. https://doi.org/10.1016/j.marpolbul.2020.111557

Pham, C.K., Rodríguez, Y., Dauphin, A., Carriço, R., Frias, J.P.G.L., Vandeperre, F., Otero, V., Santos, M.R., Martins, H.R., Bolten, A.B., Bjorndal, K.A., 2017. Plastic ingestion in oceanic-stage loggerhead sea turtles (Caretta caretta) off the North Atlantic subtropical gyre. Mar. Pollut. Bull. 121, 222–229. https://doi.org/10.1016/j.marpolbul.2017.06.008

Philipp, C., Unger, B., Ehlers, S.M., Koop, J.H.E., Siebert, U., 2021. First Evidence of Retrospective Findings of Microplastics in Harbour Porpoises (Phocoena phocoena) From German Waters. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.682532

Ryan, P.G., Cole, G., Spiby, K., Nel, R., Osborne, A., Perold, V., 2016. Impacts of plastic ingestion on post-hatchling loggerhead turtles off South Africa. Mar. Pollut. Bull. 107, 155–160. https://doi.org/10.1016/j.marpolbul.2016.04.005

van Franeker, J.A., Bravo Rebolledo, E.L., Hesse, E., IJsseldijk, L.L., Kühn, S., Leopold, M., Mielke, L., 2018. Plastic ingestion by harbour porpoises Phocoena phocoena in the Netherlands: Establishing a standardised method. Ambio 47, 387–397. https://doi.org/10.1007/s13280-017-1002-y

Zhang, X., Luo, D., Yu, R.Q., Xie, Z., He, L., Wu, Y., 2021. Microplastics in the endangered Indo-Pacific humpback dolphins (Sousa chinensis) from the Pearl River Estuary, China. Environ. Pollut. 270, 116057. https://doi.org/10.1016/j.envpol.2020.116057

Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., Wang, J., 2019. Cetaceans and microplastics : First report of microplastic ingestion by a coastal delphinid , Sousa chinensis. Sci. Total Environ. 659, 649–654. https://doi.org/10.1016/j.scitotenv.2018.12.389