TNA PROJECT REPORT

1. Project Information

Proposal reference number	JN_CALL_2_2
Project Acronym (ID)	MicroPlastox
Title of the project	Microplastics in the marine environment: estimation and ecotoxicological assessment
Host Research Infrastructure	Galway Bay Cabled Observatory (CPO) MS Color Fantasy (FA) Heraklion Coastal Buoy (HCB)
Starting date - End date	September 2017– May 2019
Name of Principal Investigator **Home Laboratory** **Address** **E-mail address**	João Pinto da Costa University of Aveiro CESAM & Department of Chemistry 3810-193 Aveiro, Portugal joao.pinto.da.costa@gmail.com jpintocosta@ua.pt

2. Project objectives

The main goal of the submitted research project is the assessment of the presence and prevalence of microplastic particles (MP) in European waters. From the collected particles, the creation of an online repository, detailing the findings, is expected (a temporary website has already been created: **https://themprepository.wixsite.com/themprepository**, as well as a social media page, **https://www.facebook.com/mprepository/**). The collected samples will be used in future studies assessing the potential ecotoxicological effects of these particles. The collected samples are presently being thoroughly analysed (optical microscopy, FTIR spectroscopy) and their effects currently under study, using bioluminescence inhibition test with *Vibrio fischeri*, algae growth inhibition test with marine microalgae *Phaeodactylum tricornutum* and *Isochrysis galbana* and 24-48h acute toxicity test with the crustacean *Artemia franciscana*, although additional tests may be carried out, depending on the availability of MP samples.

When successful, this project will provide the basis for the development of monitoring approaches for plastics and subsequent recommendations to be included in water policies and management strategies. Hence, the collection and cataloguing of the existing MPs sampled in aquatic systems will concomitantly allow for the assessment of ecotoxicological risks and effects of microplastics using adapted standardized methodologies, and the dissemination of the gathered data will effectively contribute to the development and establishment of water policies and will, hopefully, constituted significant contributions to the development of potential MP-related pollution ecotoxicology evaluation and subsequent development of specific regulations.

The activity described in this report has received funding
from European Commission's H2020 Framework Programme
under JERICO-NEXT project, grant agreement No. 654410.

3. Main achievements and difficulties encountered

The main contribution of this work will be not only the geographical coverage of sampling, but also the use of different sample collection methodologies. In fact, the inclusion of the neuston net in the Heraklion Coastal Buoy (images available at **https://themprepository.wixsite.com/themprepository/blog/net_away**) involved conceiving a protection mechanism that allowed for prolonged sampling campaigns; the net was, unfortunately, lost, probably removed by fishermen and an alternative was devised, based on the surface collection of samples, according to outings performed by HCMR at Heraklion.

On board the MS Color Fantasy, the innovative process used (firstly tested within the framework of this project) will quite probably become a reference in the future: it allows for the filtrations of thousands of litters of (sea)water, and, considering its use in well-defined routes, it will certainly contribute towards the elimination of one of the major gaps in MP research: replication, as multiple samplings my take place, repeatedly and throughout the year, also permitting a window into potential seasonal variation on the presence of these materials. However, as a brand new system, modifications and optimizations were required, and, therefore, sampling was delayed until late January 2019.

Lastly, the use of a neuston net at the Galway cabled observatory was a yet unreported sampling method, as the net was placed on the seabed for a couple of weeks, thus allowing confirmation on the presence of these materials throughout the water column. The main constraints were the weather and availability of a highly trained diver to this location.

4. Dissemination of the results

Although analyses of the collected samples are still underway, as well as ecotoxicological tests, this Project has been divulged online, including on social media (please see above).

There has been a peer-reviewed work published in which acknowledgement to the Jerico Next Program was made (**https://www.tandfonline.com/doi/full/10.1080/10643389.2018.1548862**), although the grant agreement number was not included an oversight that the Project Leader apologizes for.

In the near future, another publication reporting the prevalence of these materials and another focusing on their (potential) effects are also planned, which will include the appropriate acknowledgment.

5. Technical and Scientific preliminary Outcomes

As noted in Box 4, analyses are still underway. However, visual inspection has resulted in the confirmation of the presence of plastic particles, as detailed in Table 1 and exemplified in Figure 1, in samples collected in Norway.

Table 1 *– Summary of the main findings pertaining to data collected on board MS Color Fantasy.*

Path	Start/endpoints	Approx. water volume (L)	Number particles	Number fibers	Total MP	Estimated MP.m^{-3}
Oslo - Kiel	Oslo - Drobak	900	1	2	3	3.33
	Drobak - Torbjornskaer	1000	1	1	2	2.00
	Torbjornskaer - Skagen/Goteborg	2400	2	1	3	1.25
	Skagen/Goteborg - Kiel	7800	4	4	8	1.03
Kiel - Oslo	Kiel - Skagen/Goteborg	7800	4	3	7	0.90
	Skagen/Goteborg - Torbjornskaer	2100	2	0	2	0.95
	Torbjornskaer -Drobak	1200	0	4	4	3.33
	Drobak - Oslo	900	1	3	4	4.44

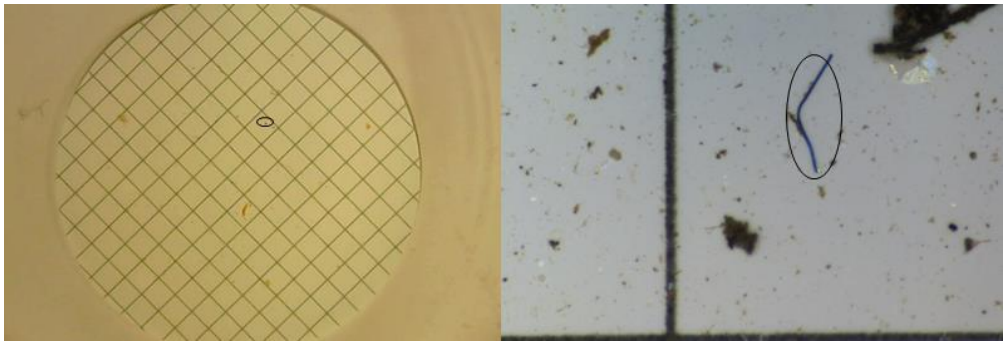

Figure 1 *– Some of the particles isolated from a sample collected on board the MS Color Fantasy filtration system (Norway). The highlighted particles are fibers.*

These are examples of the currently gathered data, although some of the collected samples are still under analysis; other particles, already characterized, are presently being used for initial ecotoxicological tests using the aforementioned species (Box 2), although some issues regarding the growth of the control individuals have been noted (thus affecting any inferences on the potential effects of these materials). These issues are presently being addressed, namely, by optimizing the growth conditions of these organisms.

In Greece, microplastics were also present in the collected samples, although their prevalence varied with dates of sampling, as exemplified in Table 2. However, it should be noted that additional samples than those listed in Table 2 were collected in Greece and these are still under analysis and that preliminary results suggest a considerably higher number of microplastics present in other samples, a variation that may stem not only from the sampling dates, but also locations.

Table 2 *– Preliminary findings pertaining to data collected from Heraklion Coastal Buoy.*

Date	Distance covered (km)	Approx. water volume (m^3)	Number particles	Number fibers	Total MP	Estimated $MP.m^{-3}$
22.10.2018	2.35	600.48	12	0	12	0.02
16.12.2018	3.58	913.73	11	1	12	0.01

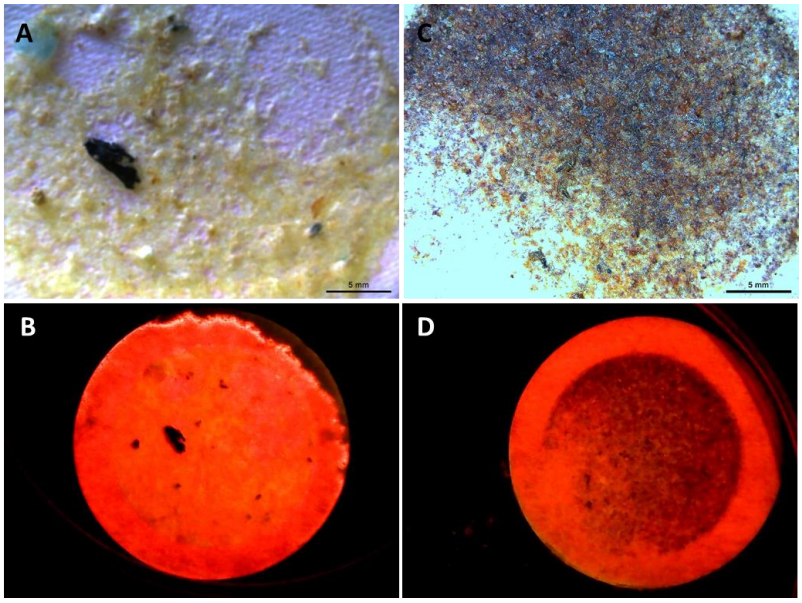

Figure 2 *– In A), detail of a 47 mm filter, detailing the presence of microplastics in samples collected on 16.12.2018. In B), an overview of the filter showed in A), following digestion (with H_2O_2) and Nile Red staining and under a 470 nm wavelength light. In C), detail of filtered samples collected on 22.10.2018, showing the high prevalence of organic matter. In D), overview of the filter described in C), following digestion (with H_2O_2) and Nile Red staining and under a 470 nm wavelength light.*

Meanwhile, samples from Galway and Heraklion are presently being processed, and, hopefully, a more detailed description of the identified particles will soon follow the present report. Briefly, however, it should be noted that almost all samples contained some MPs, whose chemical signature (assessed by FTIR) will expectantly contribute to the elucidation of the prevalence of these materials in European waters.

Partial ecotoxicological assays were carried out exposing *Danio rerio* embryos to different concentrations of microplastics. A fertilized egg was transferred into individual wells of a 24-well microtiter plate. Mortality was recorded at 96 h of exposure. Embryos were incubated at a temperature of 26.0 ± 1.0°C with a light/darkness cycle of 16:8h. Mortality, hatching, larvae length and abnormalities were evaluated using an inverted microscope coupled with camera, SMZ2800 with a Nikon Model LH-

M100C-1 camera. Some of these results are summarized in Figures 3 and 4. In the tested periods, no significant effects over the rate of hatching and percentage of survival were noted (Fig.3), as well as on larvae length (Fig. 4A). Nonetheless, some abnormalities were noted, following a period of 48h exposure to different concentrations of microplastics. Presently, additional studies are underway to assess the extent of these effects and their significance, as well as supplementary assays focusing on the use of lower, more environmentally relevant concentrations.

Hopefully, in the near future, the body of collected and obtained data will result in two publications stemming from the collaboration of the different partners in MicroPlastox: one, regarding the prevalence of these materials and a second one, detailing the effects (if any) of these collected particles and fragments in commonly used ecotoxicity monitoring species.

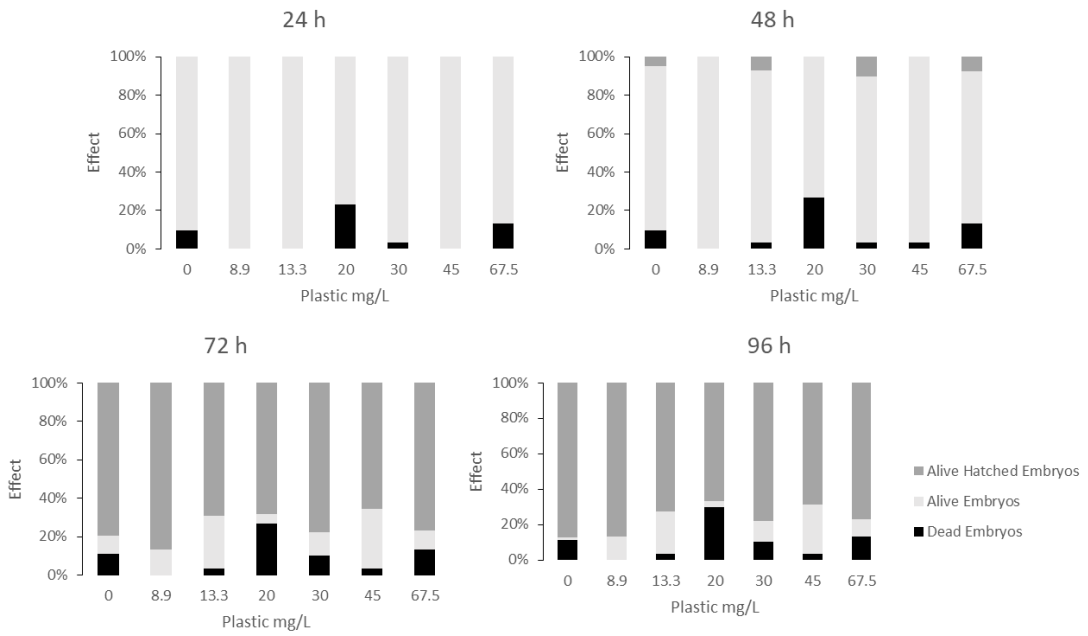

Figure 3 *– Effects of microplastics, in the described concentrations, on the proportion of dead, alive and alive hatched embryos, which are represented by the different colours of the bars, at 24, 48, 72 and 96h.*

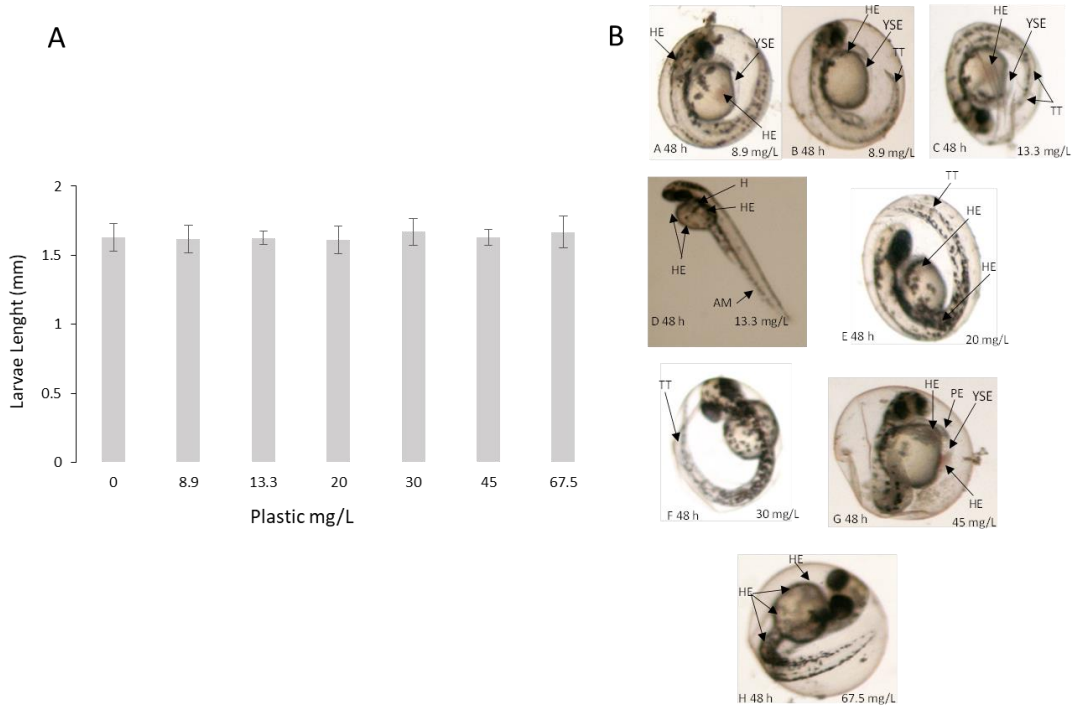

Figure 4 *- In A), the larvae length measured after 48h; In B), at the same period, Figure 4: Detected malformations in Danio rerio larvae. A) Embryo with yolk sac oedema (YSE), tail twisting (TT) and pericardial oedema (PE); B) Embryo with hemagglutination (HE) and TT; C) Embryo with TT, YSE, HE and PE; D) Embryo with axial HE, YSE and double tail; E) Embryo with TT, HE, PE and YSE; F) Larvae with HE and axial malformation (AM). Microplastic concentration is noted in each inset.*

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