Supplementary Information

Multiple impacts of microplastics can threaten marine habitat-forming species

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49 **Supplementary Methods**

50 **Microplastic mixture, size class distribution and quality check**

51 Plastic particles were sieved onto 1000 µm and 20 µm meshes to obtain homogeneous size classes
52 among different polymers, and these were rinsed 3 times with 0.02 µm pre-filtered milliQ sterilized
53 seawater to exclude as much as possible the presence of nanoparticles. We weighted 1 g of each
54 polymer (stock amount) by using an analytical balance (AS, RADWAG, accuracy ± 0.001 g), then
55 we counted under stereomicroscope at 50× magnification (Zeiss Stami 2000), the number of
56 particles contained in each aliquot. The relative quantity of the different plastic polymers added to
57 the 12 L was estimated on the basis of the weight of the particles contained within the stock amount
58 for each experimental treatment (100, 500 and 1000 microplastic particles L^{-1}), and considering the
59 percentage of each polymer reported for the natural environment (Supplementary Table 5). Before
60 addition to the systems, the different polymers were combined and homogenized to obtain
61 microplastic mixtures.

62 A further analysis on the microplastic mixtures revealed a very similar abundance of particles for
63 the three size classes selected (20-200 µm, 200-500 µm and 500-1000 µm; variation coefficient <10
64 %).

65 The FT-IR spectroscopy analyses (Perkin Elmer, software Packages Spectrum 5.3.1) performed on
66 a subsample of the microplastic mixture showed that the chemical characteristics of the polymers
67 remained identical comparing the samples before and after milling.

68 Before adding microplastics to each tank, we also tested the potential contamination of the
69 polymers by prokaryotic DNA, by qPCR analyses using the TaqMan technology[1] targeting
70 prokaryotic 16S rRNA genes. The results of qPCR analyses revealed that the copy number of 16S
71 rRNA genes associated with the plastic mixture was always below detection limits.

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77 **DNA extraction and quantitative PCR analyses for assessing microplastics' contamination by**
78 **prokaryotic DNA**

79 DNA extraction from microplastics was performed using QIAGEN DNeasy Blood & Tissue Kit
80 with some modifications. In brief, microplastic mixture samples (≈ 25 mg) were digested in
81 proteinase K at 56 °C overnight. Then, samples were subjected to ultrasound treatment (three 1-min
82 treatments using a Branson Sonifier 2200; 60W), after that, they were processed according to the
83 manufacturer's protocol under laminar flow hood.

84 The prokaryotic 16S rDNA sequences were amplified using the forward primer, 5′-
85 TCCTACGGGAGGCAGCAGT-3′ and reverse (5′-GGACTACCAGGGTATCTAATCCTGTT-3′)[2].
86 The TaqMan probe was (5′-CGTATTACCGCGGCTGCTGGCAC-3′), which contained a
87 fluorescent reporter dye (6-carboxyfluorescein) covalently attached to the 5′-end and a fluorescent
88 quencher dye (6-carboxytetramethylrhodamine) attached downstream of the reporter dye. The
89 cycling conditions were: 3 minutes at 95°C, followed by 40 cycles of 15 sec 95°C, 1 minute, 60°C.
90 All of the qPCR reactions were performed in a volume of 15 µL with a CFXConnectTM Real-Time
91 PCR detection system (BioRad, Milan, Italy) using iQ Supermix (2×; Bio-Rad) containing 40 mM
92 Tris-HCl, pH 8.4, 100 mM KCl, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 50 U mL^{-1} hot-
93 start iTaq DNA polymerase and 6 mM $MgCl_2$. In addition, each reaction mix contained 0.1/0.1 µM
94 primers/probes, and 1 µl of template DNA.

95 To test for the absence of 16S rDNA gene copies, linear calibration curves were performed from a
96 standard solution of *E. coli* (from 0.2 to 200 pg μL^{-1}).

97 To test for possible inhibition of qPCR, using undiluted aliquots of extracted DNA, in addition to
98 running all sample extracts in serial 10-fold dilutions. All samples, standards, and negative controls
99 were analysed in triplicate qPCR reactions.

100 The iCycler software analysis programme was used to calculate the Ct values and generate standard
101 curves. The "Ct cut off value" of analysed samples was set at 35 cycles of the PCR above which
102 any measurement response was considered as amplification or fluorescence artefacts and not due to
103 contamination. Since no contamination was detected in the samples investigated, the procedure
104 utilised here allowed us to demonstrate that the microplastic mixture added to the mesocosms was
105 devoid of any external contamination by prokaryotic DNA.

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108 **Monitoring microplastic concentrations in the seawater during the time-course experiment**

109 Changes in microplastic concentrations were monitored in the CTRL MPs (i.e., control tanks
110 containing seawater added with 1000 microplastic particles L^{-1} without corals) at day 1, 7 and 14 of
111 the time course experiment. This system allowed us to quantify the potential loss of microplastic
112 particles from the system (e.g., due to the adsorption onto the tank walls) not attributable to the
113 ingestion by corals. To monitor particle concentration in the CTRL MPs, 1 litre of seawater was
114 filtered at each sampling time onto 20 µm pore size filters (Sefar Nitex, Agrinova) and
115 microplastics were counted under stereomicroscope as reported above. The number of microplastic
116 particles lost during the experiment was determined by difference between the concentration of
117 microplastic particles initially added to the treatments (considering 1000 microplastic particles L^{-1})
118 and the number of microplastic particles counted in the CTRL MPs at each sampling time.

119 The amount of microplastic mixture removed by water sampling for the different analyses carried
120 out in the experiment was estimated considering the water volumes collected for such analyses and
121 the abundance of microplastics contained therein (assuming a homogeneous distribution of
122 microplastic particles in the tanks).

123 The number of microplastic particles, which remained at the end of the experiment in the tanks
124 containing corals exposed to 1000 microplastics, was determined after filtering all seawater of the
125 tanks (onto 20 µm pore size filters) and counting microplastic particles under the microscope.

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128 **Supplementary results**

129 **Microplastic concentrations in sea water during the time-course experiment**

130 The monitoring of microplastic concentrations in the CTRL MPS (i.e., control tanks containing the
131 highest concentrations of microplastics without coral branches; 1000 microplastic particles L^{-1})
132 revealed a number of microplastic particles ranging from 608 to 633 L^{-1}, after 1, 7 and 14 days
133 respectively (equivalent to an average recovery of 62% of the amount added at the beginning of the
134 experiment). These data were used to correct the microplastic concentrations, to which corals were
135 exposed in the experimental systems.

136 At the end of the experiment, we recounted all the particles remaining in the control tanks (CTRL
137 MPS), and we confirmed that, on average, ca. 38% of the initial microplastic mixture was
138 potentially adsorbed to wall tanks. The tanks with the coral branches, at the end of the experiment,
139 contained on average ca. 5% of the total microplastic mixture initially added. We also estimated
140 that ca. 1% of the microplastic mixture was removed by water sampling used for all the analyses,
141 and that, based on the results of our study, most of the microplastics added to the systems were
142 taken by corals and Artemia nauplii used to feed them.

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144 **Supplementary Figures**

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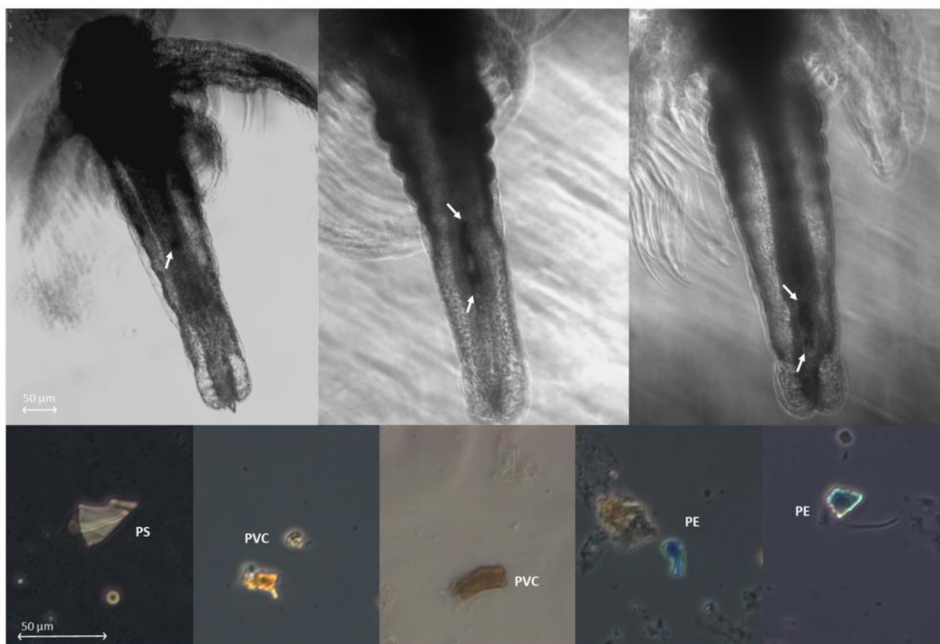

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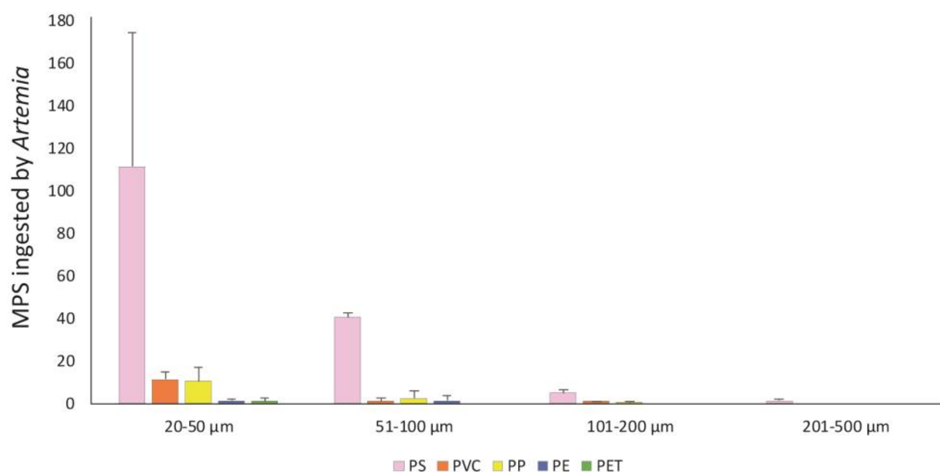

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153 **Supplementary Figure 1. Microplastic polymers ingested by *Artemia salina*: (a)** images of

154 naupliar stages of *Artemia salina* with microplastic fragments (see the white arrows) in digestive

155 tract; below images of microplastic polymers, after enzymatic digestion, ingested by nauplii during

156 the experiment; **(b)** number of different microplastic polymers ingested by *A. salina* (100 nauplii)

157 according to the different size ranges (20-50 µm, 51-100 µm, 101-200 µm and 201-500 µm) over

158 10 days of experiment exposed to 1000 microplastic particles L^{-1}. Polymers with size > 200 µm

159 were not ingested by *A. salina*. Plastic abbreviations: PS= polystyrene, PVC= polyvinylchloride,

160 PE= polyethylene, PP= polypropylene, PET= polyethylene terephthalate**.** Data are represented as
161 mean ± standard deviation.

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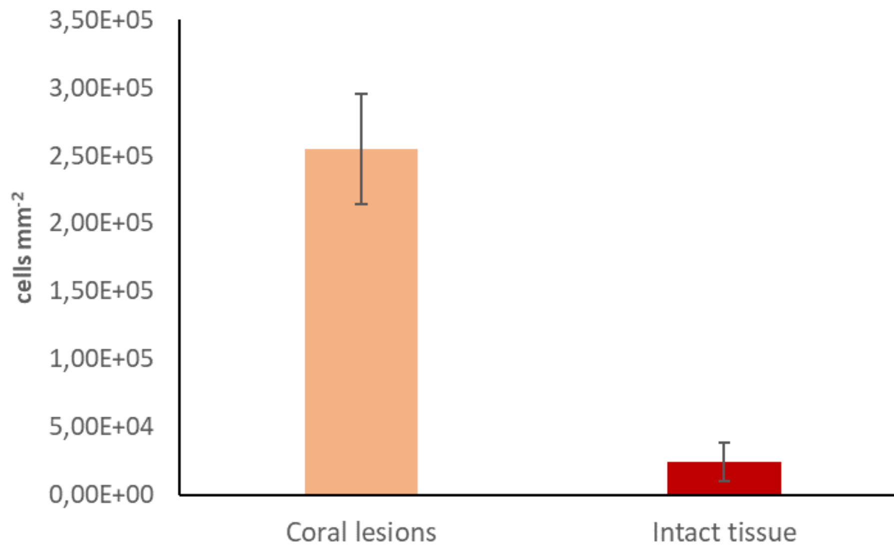

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173 **Supplementary Figure 2.** Prokaryotic abundance, determined by SEM analyses, around lesions of
174 the corals exposed to high concentration of microplastic particles L^{-1} and on the intact tissue. Data
175 are represented as mean ± standard deviation.

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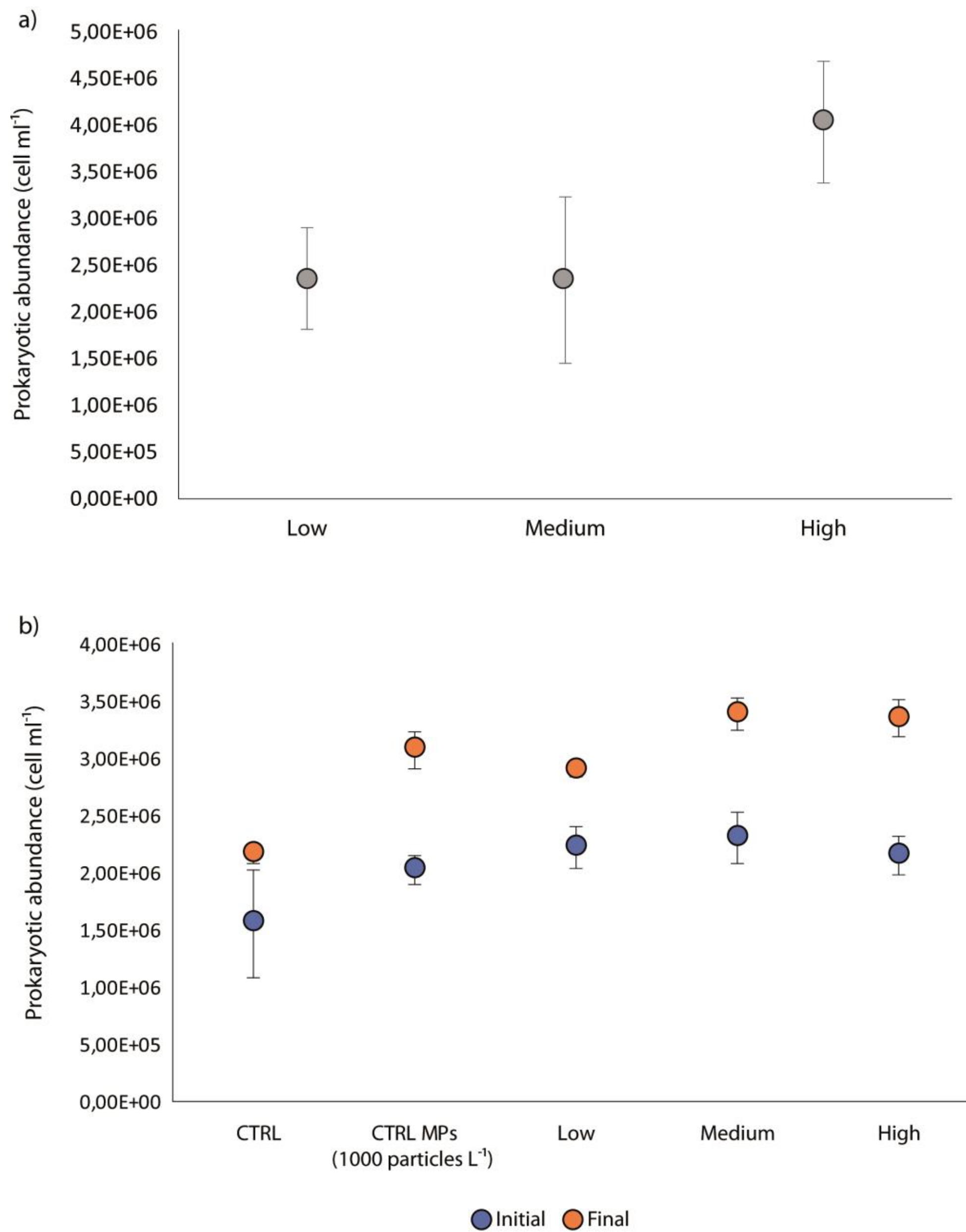

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189 **Supplementary Figure 3. Prokaryotic abundance in coral mucus and seawater. a)** Total
190 prokaryotic abundances in mucus after 14 days of the experiment at increasing microplastic
191 concentrations (low, medium and high concentrations of microplastic particles). In the controls,
192 coral mucus was not produced. **b)** Total prokaryotic abundance in seawater of the experimental
193 systems with and without microplastics (CTRL, control). The prokaryotic abundance in systems

194 containing seawater and microplastics (1000 microplastic particles L^{-1}) but without corals (Ctrl
195 MPs) is also shown. Data are represented as mean ± standard deviation.

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197 **Supplementary Table 1.** Time-course feeding experiments (at t_0 and after 2 and 4 hours) of *C.*
198 *rubrum* using *A. salina* nauplii in the different microplastic treatments (low, medium and high
199 concentrations of microplastic particles) and in the controls at 2nd and 10th days.

2nd day of experiment		Ctrl	Low concentration	Medium concentration	High concentration
Replicate	**Sampling time (h)**	**Nauplii per L of seawater**	**Nauplii per L of seawater**	**Nauplii per L of seawater**	**Nauplii per L of seawater**
r1	0	1033	1125	990	863
r2	0	1116	1387	1084	896
r3	0	950	863	896	829
r1	2	808	567	520	675
r2	2	929	675	691	761
r3	2	688	458	349	589
r1	4	675	292	263	475
r2	4	820	355	398	554
r3	4	530	228	127	396

10th day of experiment	Ctrl	Low concentration	Medium concentration	High concentration

Replicate	Nauplii per L of seawater	Nauplii per L of seawater	Nauplii per L of seawater	Nauplii per L of seawater	nauplii/L of seawater
r1	0	1210	1063	942	1250
r2	0	1286	1228	1039	1330
r3	0	1134	897	844	1170
r1	2	1000	750	858	1200
r2	2	1000	899	955	1250
r3	2	1000	601	761	1150
r1	4	825	525	708	1100
r2	4	850	671	816	1150
r3	4	800	379	601	1050

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203 **Supplementary Table 2.** Equation of the linear regression, regression coefficient (R), P value of
204 the linear regression model applied to the results of the time-course feeding experiments (see
205 Supplementary Table 1).

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2nd day of experiment			
Treatment	**Linear regression equation**	**R**	**P**
Ctrl	y=-89.58x+1018.05	0.82	0.0059
Low concentration	y=-208.33x+1077.77	0.91	0.0005
Medium concentration	y=-181.87x+954.58	0.92	0.0003
High concentration	y=-96.87x+864.58	0.93	0.0001

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10th day of experiment			
Treatment	**Linear regression model**	**R**	**F**
Ctrl	y=-96.25x+1204.16	0.97	1.35683E-05
Low concentration	y=-134.37x+1047.91	0.86	0.0025
Medium concentration	y=-58.33x+952.77	0.75	0.0198
High concentration	Y=-37.50x+1258.33	0.76	0.0165

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211 **Supplementary Table 3.** Results of Student's t-test comparing gene expression levels analysed from
212 the controls (i.e., samples not exposed to microplastic particles) at the beginning (T_0) and after 10
213 days (T_{10}).

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	cytb		*MtMutS*		*hsp70*		*hsp60*		*EF1*		*MnSOD*	
	p-value	***t-value***	***p-value***	***t-value***	***p-value***	***t-value***	***p-value***	***t-value***	***p-value***	***t-value***	***p-value***	***t-value***
T_0 vs T_{10}	0.1864	1.5930	0.1555	1.7470	0.1397	1.8390	0.0728	2.420	0.0212	3.6810	0.9529	0.0628

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Supplementary Table 4. Results of one-way ANOVA showing the effects of treatment (low, medium and high concentrations of microplastic particles) on the expression levels of *cytb, MtMutS, hsp70, hsp60, EF1, MnSOD* genes compared to control, and multiple comparisons test between control and different levels of treatment.

					Bonferroni-adjusted multiple comparisons					
Source	**df**	**SS**	**F**	**P**	**control vs low conc.**		**control vs medium conc.**		**control vs high conc.**	
cytb					*p-value*	*t-value*	*p-value*	*t-value*	*p-value*	*t-value*
Treatment	3	0.3161	12.78	P=0.0020	>0.9999	0.9199	0.0292	3.374	0.0015	5.609
Residual	8	0.06594								
MtMutS										
Treatment	3	0.0733	6.012	P=0.0190	>0.9999	0.4993	0.0307	3.340	0.0430	3.114
Residual	8	0.0325								
hsp70										
Treatment	3	1.57E-04	10.52	P=0.0038	>0.9999	0.938	0.0163	3.658	0.0026	5.156
Residual	8	3.99E-05								
hsp60										
Treatment	3	2.39E-05	15.62	P=0.0010	>0.2179	1.923	>0.9999	0.0235	0.0050	4.642
Residual	8	4.84E-06								
EF1										
Treatment	3	5.70E-08	378.1	P<0.0001	>0.9999	0.6218	<0.0001	28.04	>0.9769	1.047
Residual	8	4.02E-10								
MnSOD										
Treatment	3	3.55E-07	14.03	P<0.0001	>0.9999	0.3015	>0.9999	0.4338	0.0017	5.530
Residual	8	5.91E-08								

222 **Supplementary Table 5.** Plastic polymers added to the systems, density and contribution (%) of
223 each polymer in the mixture.

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Polymers	**Density** **(g cm^{-3})**	**%**
1. Polyethylene (PE)	0.91-0.97	76.6
2. Polypropylene (PP)	0.89-0.92	10.9
3. Polystyrene (PS)	1.04–1.1	7.3
4. Polyvinyl chloride (PVC)	1.16–1.58	3.3
5. Polyethylene terephthalate (PET)	1.37–1.45	1.8

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237 **Supplementary Table 6.** Gene names, acronyms, functions and primer sequences (5'=>3') are
238 reported for the reference gene and the genes analysed. *primer sequences retrieved from
239 Haguenauer et al. [3]

Gene and acronym	Primer	Sequence (5'=>3')	Function	References
Cytochrome oxidase I (COI)*	Cr_COI_For Cr_COI_Rev	GTTCCCACCGGGATTAAGAT CTCCGGTTAAACCACCAATG		
Heat shock protein 70 (*hsp70*)*	Cr_HSP70_For Cr_HSP70_Rev	TCGACCCAAAGTTGAAGTCC TGCATCTTTTGTCGCTTGAC	chaperonin involved in folding new polipeptide chains	4
Heat shock protein 60 (*hsp60*)*	Cr_HSP60_For Cr_HSP60_Rev	TATCGCCAAGGAAGGTTTTG TCTTCCGGGGTTGTTACTTG	chaperonin involved in folding new polipeptide chains	4
Manganese superoxide dismutase (*MnSOD*)*	Cr_MnSOD_For Cr_MnSOD_Rev	ATGGGGATGGCTTGGTTATT ATACCGAACAAAGGCACCAG	antioxidant activity, scavenger of free radicals	5
Mismatch repair protein (*mtMutS*)*	Cr_MtMus_For Cr_MtMus_Rev	ATAAGCCGGATGTTCCTAGTGTA CCATTTGAAGCAAGGATCTTTTA	DNA repair	6
Elongation factor-1 (*EF1*)*	Cr_EF1_For Cr_EF1_Rev	CTCCATCTGCCATTTCCACT GCTGCTTTTGGTGGATCATT	delivery of aminoacylated tRNA to the elongating ribosomes	7

Cytochrome b (*cytb*)	Cr_Cytb_F2 Cr_Cytb_R2	TGGGAGCTAGTATCTTGGTGC GGTTCCTCTACCGGGTTAGC	Component of the electron transport system	8

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