Supplementary Materials for:

Microplastics exacerbate virus-mediated mortality in fish

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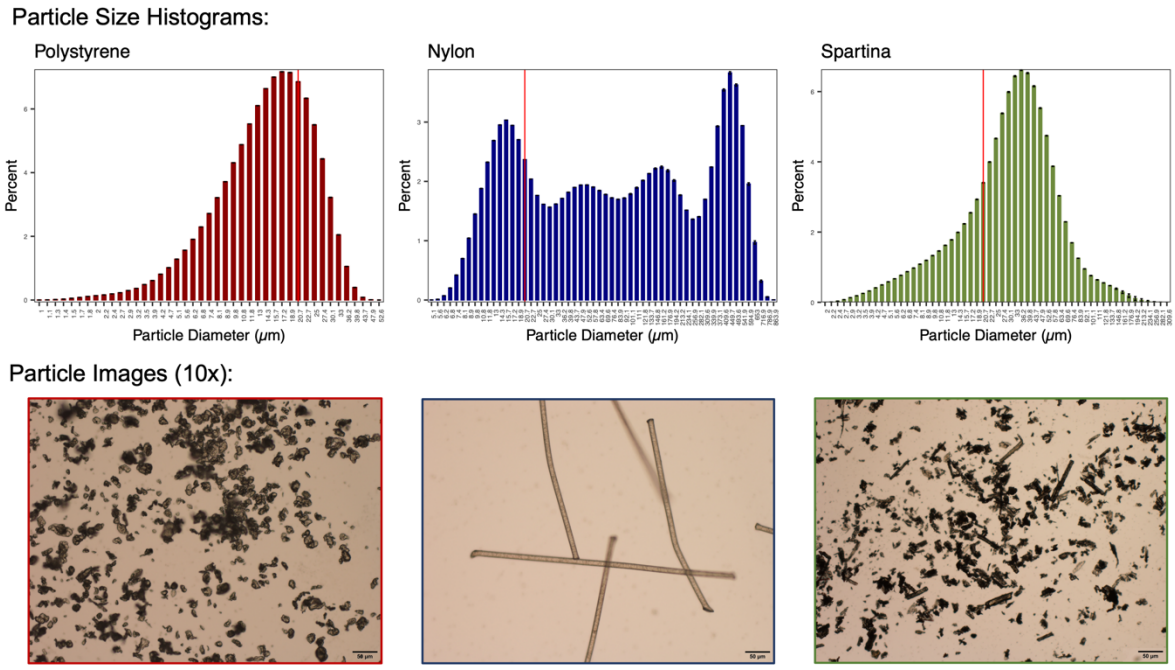

Fig. S1 Size characterization and microscopic images of polystyrene, nylon and spartina particles. Particle size histograms are illustrated in the first panel, with a red line at the 20 µm mark. Microscopic images of these particles at 4 and 10 times magnifications are shown below the histograms.

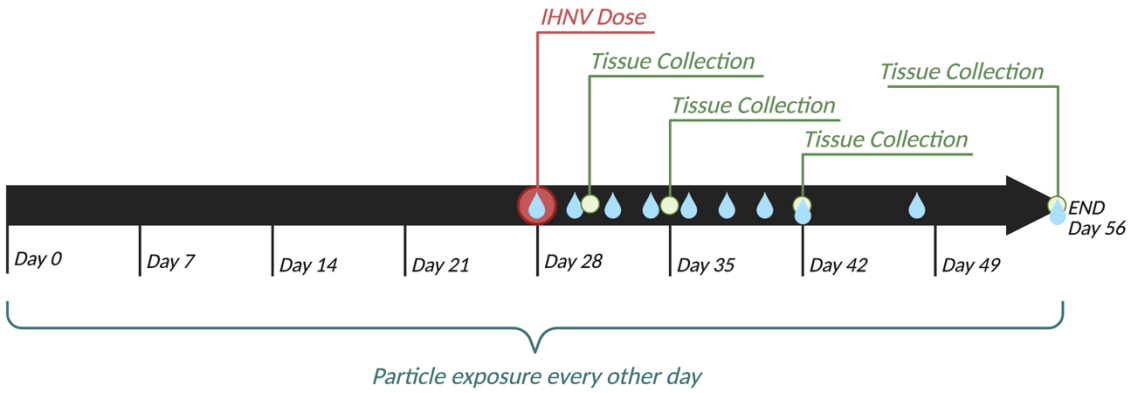

Fig. S2 Timeline of experimental activity. In total, the experiment lasted 56 days or 8 weeks. Fish were maintained with particles (except no particle controls) throughout all 8 weeks of the experiment. Fish were dosed with IHNV or a mock inoculate at the start of week 5 (day 28). Tissue samples from destructive sampling tanks were collected at days 31, 35, 42 and 56. Water sampled at 10 timepoints, on days 28, 30, 32, 34, 36, 38, 40, 42, 48, and 54. Image created with biorender.com.

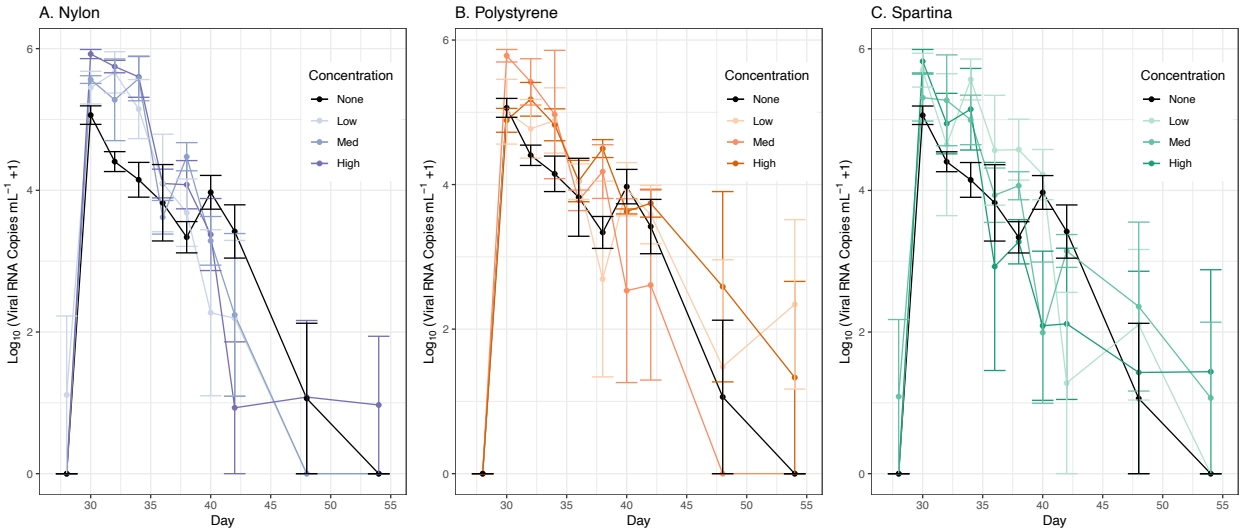

Fig. S3 Viral titer of RNA copies in water for all treatments (nylon, polystyrene and spartina) and microparticle concentrations (none/control, low, medium, and high) across day. Error bars are standard error across tanks (n=3). A linear fixed effects model of best fit included day, treatment (particle and concentration) and their interactions (respectively $F_{9,20} = 0.883$, p-value = 0.555; $F_{1,229} = 366.70$, p-value < 0.001; $F_{1,229} = 2.60$, p-value = 0.007).

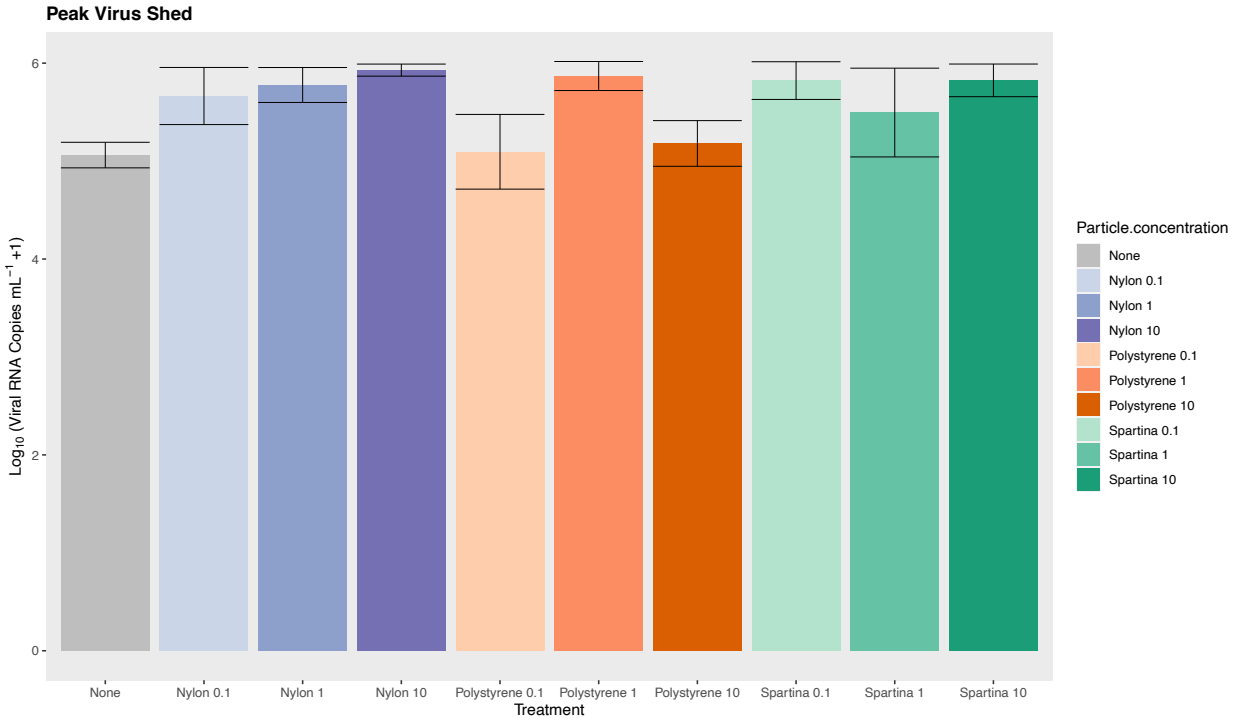

Fig. S4 Amount of virus shed at peak (highest) point of shed for each treatment with SEM (n = 3) for each treatment. A best fitting linear mixed effects model compared peak virus shed across treatment (particle and concentration) ($F_{9,20}$ = 1.834, p-value = 0.124). Treatments significantly different from the no particle control include High Nylon (t-value = 2.44, p-value = 0.024), medium polystyrene (t-value = 2.275, p-value = 0.034), low spartina (t-value = 2.143, p-value = 0.045) and high spartina (t-value = 2.149, p-value = 0.044).

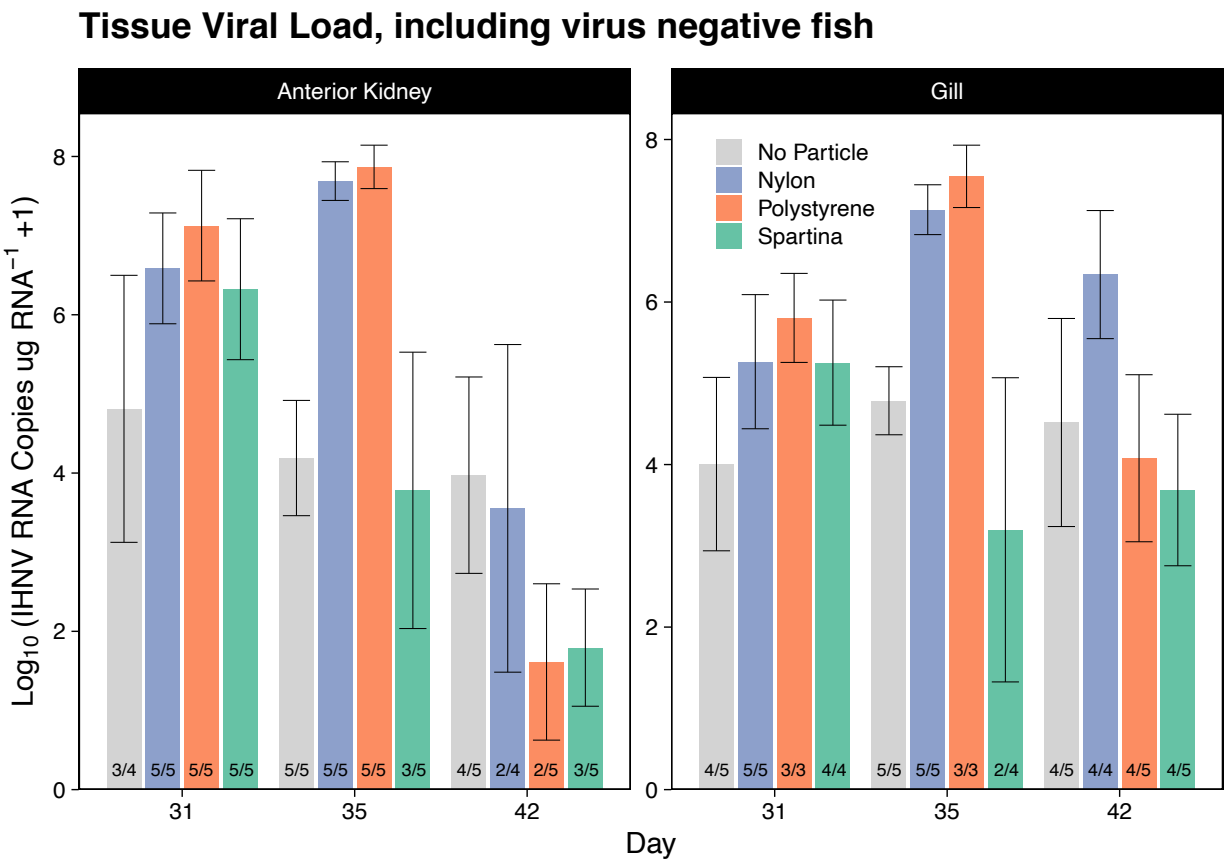

Fig. S5 IHNV loads in anterior kidney and gill tissues are presented for each collection day. Bars show mean viral RNA copies in one µg of RNA (+/- 1 SEM) for groups of fish terminally sampled on each day. Fish without virus detected are included in the means. The number of virus positive (numerator) out of total fish sampled (denominator) are shown as ratio at base of bars. In the anterior kidney, according to two-way ANOVA, viral load was significantly influenced by microparticle ($F_{3,46} = 2.07$, $p = 0.054$) and collection day ($F_{2,46} = 12.713$, p-value < 0.001) but not their interaction ($F_{6,46} = 2.03$, p-value = 0.081). In gill tissues, according to two-way ANOVA, viral load was significantly influenced by microparticle ($F_{3,46} = 13.82$, p-value = 0.034) and day ($F_{2,46} = 2.35$, p-value = 0.47), but not their interaction ($F_{6,46} = 5.00$, p-value = 0.35).

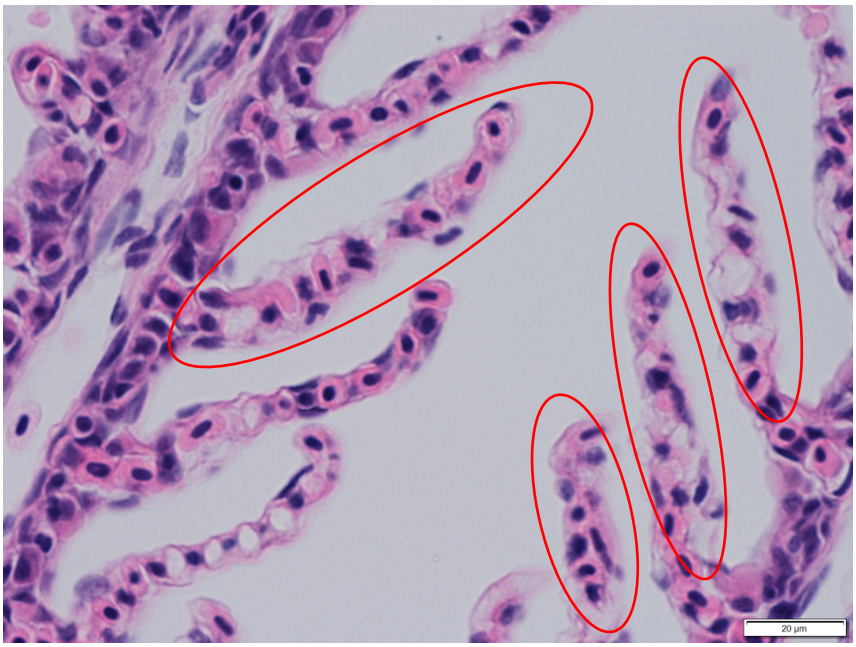

Fig. S6 Immune response in gill tissue of a fish exposed to only nylon (IHNV -). Appearance of mild damage to the respiratory epithelia and swelling of tissues is observed (circled in red), alongside regions with healthy gill lamellae.

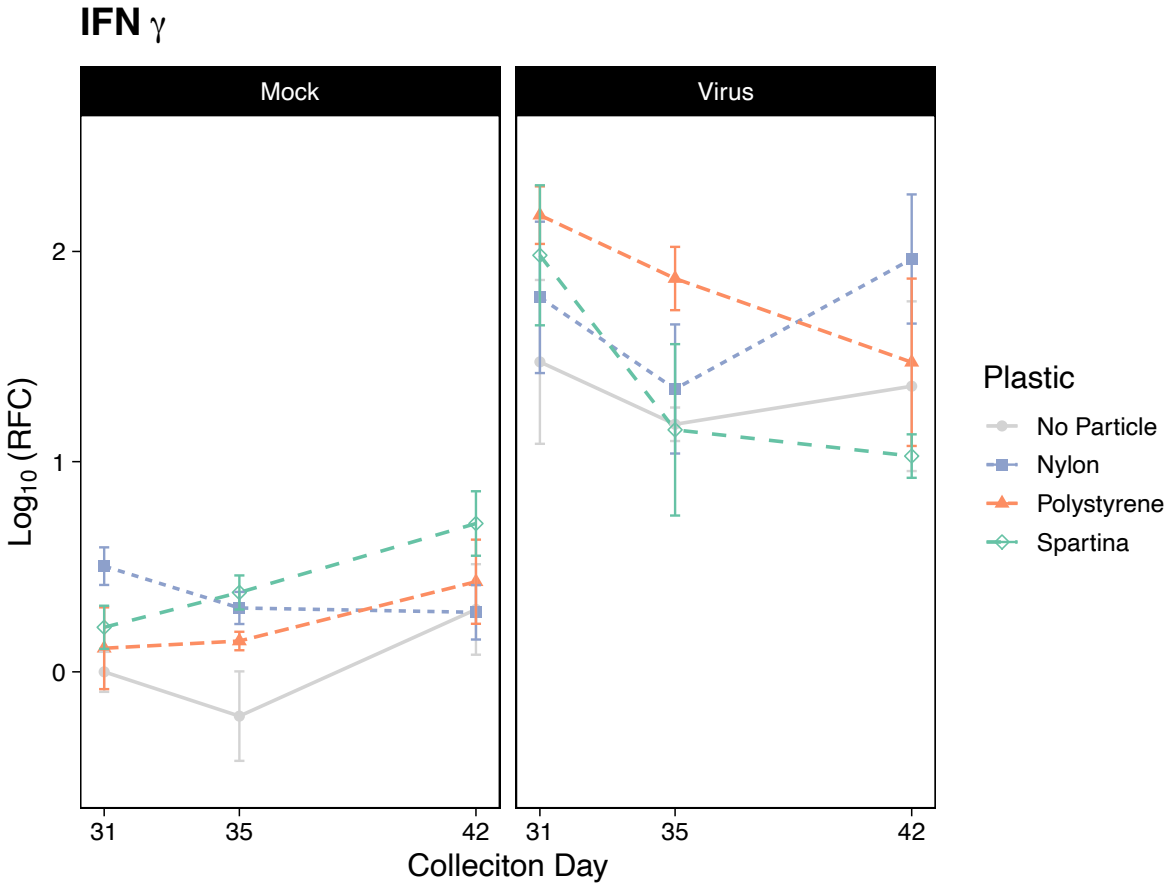

Fig. S7 Response of IFNγ to changes in microparticle exposure, among IHNV+ and – treatments, over time. The relative fold change (RFC; log_{10}-adjusted) compared to the control (no particle, IHNV-) on day 31 is plotted for each microparticle and virus treatment on each collection day, with +/- 1 SEM (n = 5, with exceptions: Table S2).

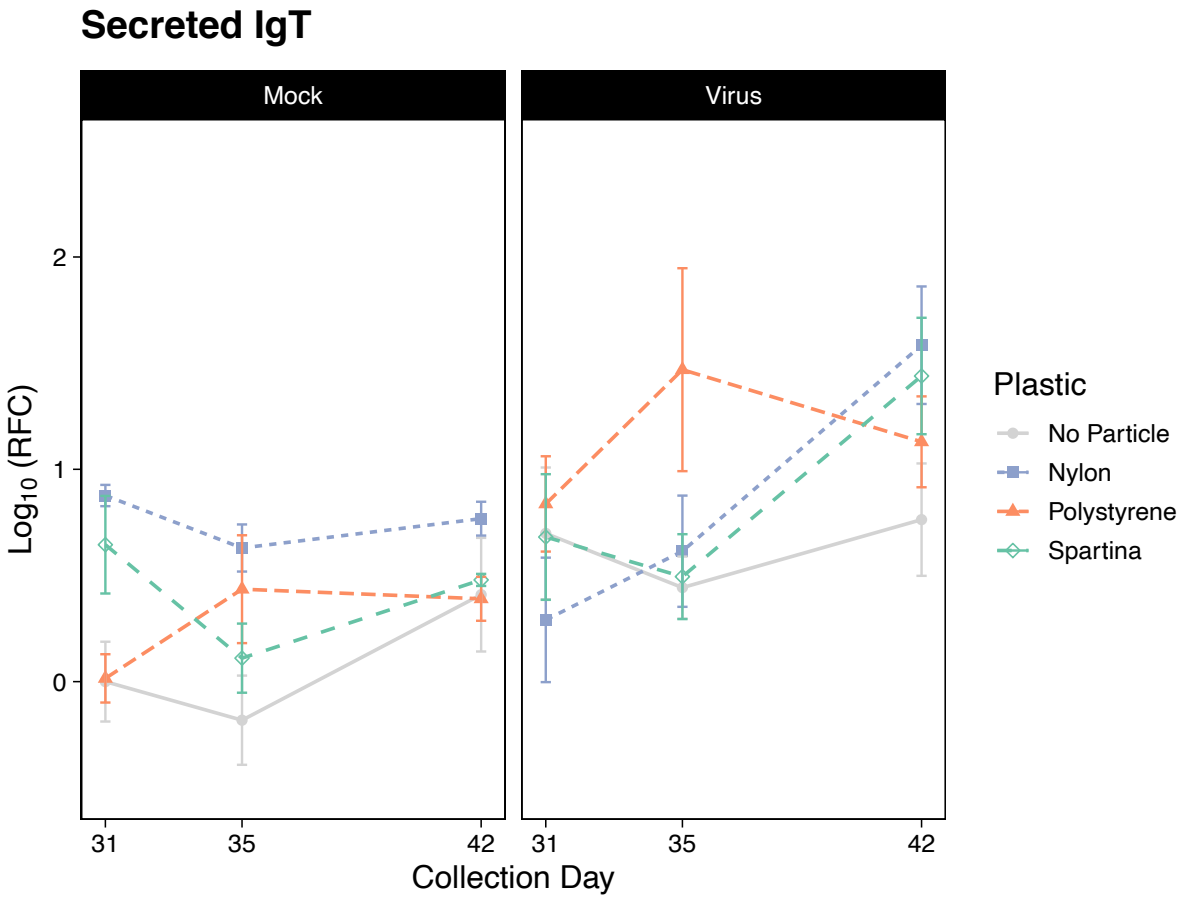

Fig. S8 Response of secreted IgT to changes in microparticle exposure, among IHNV+ and – treatments, over time. The relative fold change (RFC; log_{10}-adjusted) compared to the control (no particle, IHNV-) on day 31 is plotted for each microparticle and virus treatment on each collection day, with +/- 1 SEM (n = 5, with exceptions: Table S2).

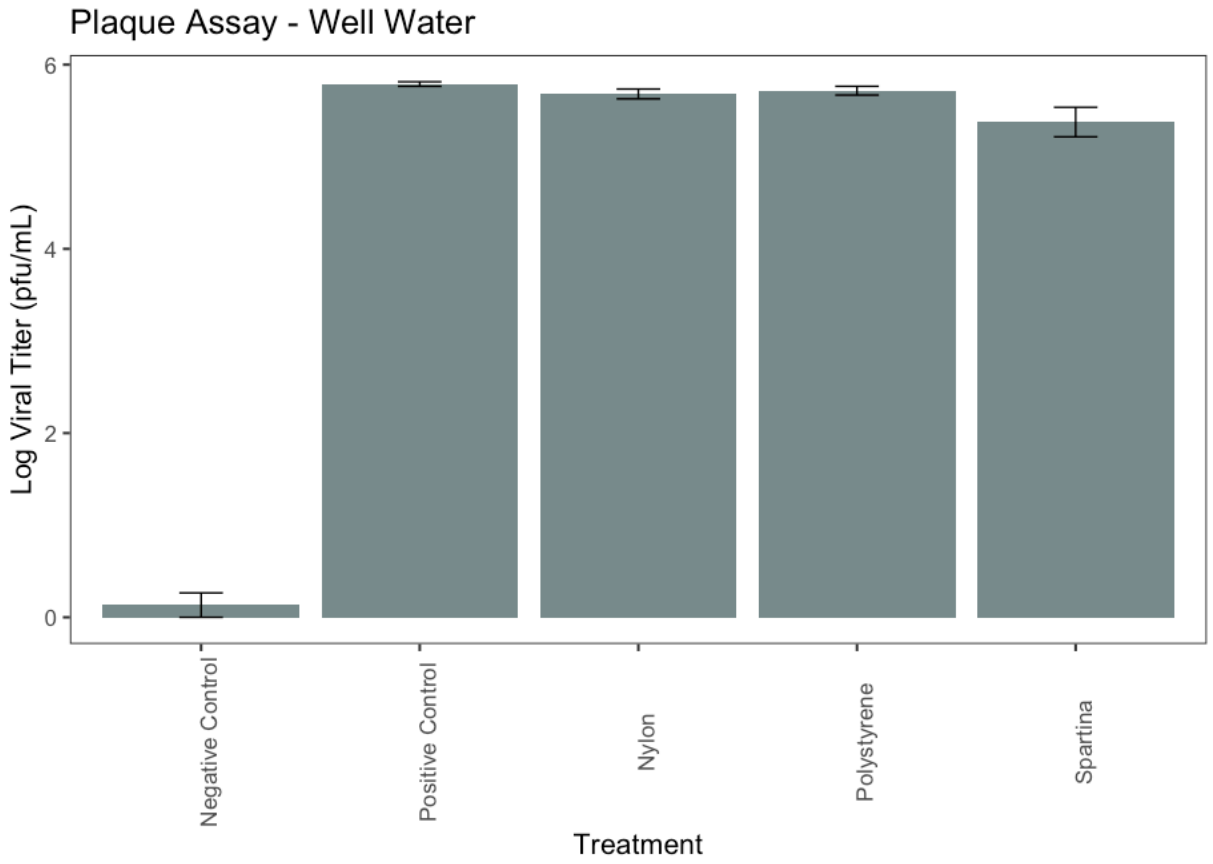

Fig. S9 Results of experiment investigating sorption of IHNV to microparticles. Particles were incubated with virus in fish water (i.e., water used for experiment) for 48 hours, then filtered (1 μm) and remaining virus in the water measured via plaque assay (*80*). Negative control was untreated water (no virus or microparticle added), while positive control was water with virus but without microparticles. The statistically similar results between positive control and all microparticles treatments suggests that plastics did not sorb IHNV virions (if virions sorbed, a portion of the virions would remain with plastics during filtration and viral titer would be significantly lower). Error bars are standard error (n=3).

Table S1 Experimental tank treatments, including particle type, concentration, viral status (IHNV+: virus present; IHNV-: virus absent) and number of tanks. Treatments with 4* tanks include 3 tanks for mortality monitoring and one tank for destructive tissue sampling.

Particle	**Concentration**	**IHNV +/-**	**Number of tanks**
No particle	N/A	IHNV +	4*
No particle	N/A	IHNV -	4*
Polystyrene	10 mg L^{-1}	IHNV +	4*
Polystyrene	10 mg L^{-1}	IHNV -	4*
Polystyrene	1 mg L^{-1}	IHNV +	3
Polystyrene	1 mg L^{-1}	IHNV -	3
Polystyrene	0.1 mg L^{-1}	IHNV +	3
Polystyrene	0.1 mg L^{-1}	IHNV -	3
Nylon	10 mg L^{-1}	IHNV +	4*
Nylon	10 mg L^{-1}	IHNV -	4*
Nylon	1 mg L^{-1}	IHNV +	3
Nylon	1 mg L^{-1}	IHNV -	3
Nylon	0.1 mg L^{-1}	IHNV +	3
Nylon	0.1 mg L^{-1}	IHNV -	3
Spartina	10 mg L^{-1}	IHNV +	4*
Spartina	10 mg L^{-1}	IHNV -	4*
Spartina	1 mg L^{-1}	IHNV +	3
Spartina	1 mg L^{-1}	IHNV -	3
Spartina	0.1 mg L^{-1}	IHNV +	3
Spartina	0.1 mg L^{-1}	IHNV -	3
		Total tanks	*68*

Table S2 The relative fold change (RFC) compared to the day 31 negative control (no particle, IHNV-) is provided (Livak and Schmittgen, 2001) for all immune markers, including the average RFC, number of fish (n), and standard error of the mean (se).

Treatment	Tissue	Day	Days post IHNV	n	memHCmu (mem-bound IgM)	se	secHCmu (secreted IgM)	se	secHCtau (secreted IgT)	se	IFNγ	se	MCSFR	se
No Particle	AK	31	3	5	4.1803	1.3841	4.0239	1.2673	3.8972	1.6036	0.9912	0.4106	2.8832	1.0134
No Particle	AK	35	7	5	4.1013	1.0118	1.5502	1.3126	17.7937	4.7333	1.9368	0.8562	2.3861	0.9295
No Particle	AK	42	14	5	15.7781	5.5076	3.3576	0.4904	8.8590	2.2420	1.0859	0.2553	10.4340	1.3343
No Particle	Gill	31	3	5	2.4977	0.6290	1.7870	0.7938	1.3422	0.4054	0.9189	0.3812	2.0576	0.8369
No Particle	Gill	35	7	5	0.0518	0.0294	3.6708	1.2253	0.9541	0.3311	0.9920	0.4942	0.3241	0.1633
No Particle	Gill	42	14	4	10.9264	3.8569	1.0131	0.3117	4.0430	1.9432	2.6699	0.9963	3.2589	0.8613
No Particle, IHNV+	AK	31	3	4	22.8061	8.6079	7.5216	1.6713	15.8409	6.5367	1658.0365	1072.0334	5.1573	1.8509
No Particle, IHNV+	AK	35	7	5	8.4069	5.6271	3.9794	1.9563	41.7656	23.5829	51.5515	13.5830	0.5208	0.1865
No Particle, IHNV+	AK	42	14	5	37.8353	17.0899	10.5185	2.6883	65.6960	23.0419	297.1495	273.1470	6.1987	1.4210
No Particle, IHNV+	Gill	31	3	5	13.7566	7.9240	7.0946	6.1624	15.0253	11.2335	79.4350	32.0292	2.1702	0.7044
No Particle, IHNV+	Gill	35	7	5	0.2785	0.1284	1.1401	0.3625	3.3420	0.8573	16.0116	2.5538	0.6364	0.2107
No Particle, IHNV+	Gill	42	14	5	6.0647	3.3030	3.7504	1.0411	9.1040	2.8377	88.8754	63.1862	2.3854	0.5738
Nylon	AK	31	3	5	28.9071	11.7578	4.5087	1.4244	14.2737	5.1847	1.2598	0.6422	5.1095	1.3916
Nylon	AK	35	7	5	9.3878	8.5482	13.4179	2.9707	23.0531	8.9608	2.2854	0.7153	2.0370	0.5034
Nylon	AK	42	14	5	23.9586	9.5642	3.0146	0.7182	18.4365	8.7425	2.0105	0.8705	3.0430	0.7398
Nylon	Gill	31	3	3	9.7609	2.7594	6.4553	2.6215	7.6290	0.9235	3.3127	0.6227	3.1480	0.7888
Nylon	Gill	35	7	5	0.3553	0.2489	0.6546	0.3222	4.8125	1.1095	2.1359	0.3543	0.7451	0.2471
Nylon	Gill	42	14	4	4.9616	1.4961	1.0972	0.5519	6.1594	1.1419	2.2016	0.6879	2.0434	0.3640
Nylon, IHNV+	AK	31	3	5	12.3628	4.4870	11.4801	5.6156	28.6993	10.3579	974.9283	330.0734	4.2861	2.0535
Nylon, IHNV+	AK	35	7	5	1.6446	0.4879	5.5577	1.6425	15.2658	6.6387	68.5139	31.6237	0.7258	0.3927
Nylon, IHNV+	AK	42	14	4	56.4690	42.5908	15.4498	6.1866	39.3101	20.2907	215.9877	93.1869	9.5340	2.7480
Nylon, IHNV+	Gill	31	3	5	3.7198	1.3009	4.0381	1.8846	3.1176	0.7840	139.7193	60.7723	1.4780	0.4718
Nylon, IHNV+	Gill	35	7	5	0.4138	0.1276	3.7867	2.8937	8.9784	6.0415	38.6800	13.3263	0.6980	0.2717

Nylon, IHNV+	Gill	42	14	4	17.7083	2.3810	24.3570	10.7162	66.7836	36.9781	188.5881	123.5041	16.9825	6.3162
Polystyrene	AK	31	3	5	15.7553	7.8113	3.2697	1.6097	8.2543	3.8410	1.1739	0.2202	3.1652	0.4804
Polystyrene	AK	35	7	3	0.3243	0.1694	8.0984	4.2431	2.7069	2.0547	0.8385	0.4342	0.2194	0.1032
Polystyrene	AK	42	14	4	41.4338	23.1899	4.5832	1.9222	5.1313	2.0014	2.2573	0.6594	6.9023	1.6008
Polystyrene	Gill	31	3	3	1.7059	1.0436	0.7949	0.4075	1.1085	0.2854	1.5350	0.5315	1.2070	0.0397
Polystyrene	Gill	35	7	5	0.1943	0.0669	20.6487	7.9037	6.2207	4.4601	1.4290	0.1323	0.5202	0.1119
Polystyrene	Gill	42	14	4	6.7134	4.5999	0.2964	0.0989	2.6482	0.5314	3.5706	1.4655	4.1556	1.5941
Polystyrene, IHNV+	AK	31	3	5	38.0394	9.8535	7.7027	1.0500	210.9475	118.9283	5385.0550	2272.5362	7.2981	2.6820
Polystyrene, IHNV+	AK	35	7	5	1.4743	0.7460	6.5257	3.2812	8.5328	1.3538	41.4649	6.5806	0.9828	0.5138
Polystyrene, IHNV+	AK	42	14	5	85.0914	49.1345	9.7286	3.7289	34.9815	17.5683	26.9142	15.9649	7.1402	3.6993
Polystyrene, IHNV+	Gill	31	3	3	2.3563	0.4454	1.8806	0.4678	8.9281	4.4395	163.8028	49.0376	2.1848	0.9855
Polystyrene, IHNV+	Gill	35	7	3	0.4711	0.2396	0.5460	0.1504	93.8551	83.6723	84.1242	30.1613	0.8649	0.1527
Polystyrene, IHNV+	Gill	42	14	5	8.0410	3.0670	8.8733	3.9849	20.2352	8.0074	90.8492	60.5818	5.1646	1.4636
Spartina	AK	31	3	5	34.0733	6.1127	5.2443	0.7447	15.4110	10.9727	0.9403	0.2514	3.7185	1.2312
Spartina	AK	35	7	5	1.2510	0.4256	1.5736	0.8507	5.2336	2.0166	1.1427	0.5749	0.6516	0.1935
Spartina	AK	42	14	5	26.5637	12.3750	3.9258	1.2417	17.8756	13.1105	2.2812	0.6275	4.1331	0.7763
Spartina	Gill	31	3	4	10.8012	3.2025	2.2225	1.1351	7.0991	4.2910	1.7679	0.4009	1.2354	0.2546
Spartina	Gill	35	7	5	0.3569	0.1147	50.8603	19.2745	1.7738	0.8017	2.5442	0.4297	0.5513	0.0745
Spartina	Gill	42	14	4	21.4343	10.6669	1.4062	0.7950	3.0322	0.1930	6.2673	2.5828	6.5706	1.3484
Spartina, IHNV+	AK	31	3	5	12.7538	3.7978	5.4053	2.2555	31.2131	15.6312	2386.9987	1035.7297	4.4345	2.0987
Spartina, IHNV+	AK	35	7	5	3.3392	1.1926	7.4540	4.7567	30.1077	8.6692	100.4033	53.8142	1.7768	0.6870
Spartina, IHNV+	AK	42	14	5	49.1304	16.5945	17.3233	7.3820	151.7579	54.8616	64.6861	46.6550	8.0868	1.8900
Spartina, IHNV+	Gill	31	3	4	2.6654	0.7048	1.8276	1.0869	8.3078	4.3078	193.0899	97.1257	1.7459	0.5649
Spartina, IHNV+	Gill	35	7	4	0.1874	0.0927	1.4448	0.4593	4.3295	2.0963	42.0480	29.1215	0.5221	0.0097
Spartina, IHNV+	Gill	42	14	5	7.5855	2.1936	5.0819	2.4851	55.5914	27.5335	11.9012	2.8586	4.5314	1.1874

Table S3 Markers used for genetic analyses, including forward and reverse primers and references for methodology.

Marker name	Forward Primer Sequence	Reverse Primer Sequence	Method	Reference
memHCmu (mem IgM)	5'-GCGCTGTAGAT CACATGGAA-3'	5'-TTTCACCTTGA TGGCAGTTG-3'	SYBR green	(Martins et al., 2015)
secHCmu (sec IgM)	5'-GCGCTGTAG ATCACATGGAA-3'	5'-GCAAGTCAG GGTCACCGTAT-3'	SYBR green	(Martins et al., 2015)
secHCtau (sec IgT)	5'-CGGGTAACTCAT GTGAAGACAAGT-3'	5'-AGTCAATAAGAA GACACAACGACACA-3'	TaqMan	(Zwollo et al., 2017)
	TaqMan Probe:	5'-CACACAGGTTAAA ATC-3'		
IFNγ	5'-CAAACTGAAAGTCC ACTATAAGATCTCCA-3'	5'-TTCTGAATTTTCCC CTTGACATATTT-3'	SYBR green	(Zou and Secombes, 2016)
MCSFR	5'-GAACTTTGCCCCTCC AGAGATATACAC-3'	5'-GATCACAATCCTCA GTAATCTTAGCTTGGC-3'	SYBR green	(Takizawa et al., 2016)
IHNV N-gene	5'-AGAGCCAAGGCA CTGTGCG-3'	5'-TTCTTTGCGGCT TGGTTGA-3'	TaqMan	(Purcell et al., 2013)
	TaqMan Probe:	5'-TGAGACTGAGCGGG ACA-3'		

Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262

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Takizawa, F., Magadan, S., Parra, D., Xu, Z., Korytář, T., Boudinot, P., Sunyer, J.O., 2016. Novel Teleost CD4-Bearing Cell Populations Provide Insights into the Evolutionary Origins and Primordial Roles of CD4+ Lymphocytes and CD4+ Macrophages. J. Immunol. 196, 4522–4535. https://doi.org/10.4049/jimmunol.1600222

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Zwollo, P., Hennessey, E., Moore, C., Marancik, D.P., Wiens, G.D., Epp, L., 2017. A BCWD-resistant line of rainbow trout exhibits higher abundance of IgT+ B cells and heavy chain tau transcripts compared to a susceptible line following challenge with Flavobacterium psychrophilum. Dev. Comp. Immunol. 74, 190–199. https://doi.org/10.1016/j.dci.2017.04.019

Statistics – Model Results

Mortality Model 1: Cox proportional hazard model including fixed (virus presence/absence, microparticle type and concentration, their interaction) and random tank effect. Model summary below.

Mortality Model 1: IHNV+ and IHNV-

	coef	se(coef)	se2	Chisq	DF	p
Nylon 0.1	0.8473	0.5678	0.3415	2.2271	1.0000	0.1356
Nylon 1	1.0075	0.5633	0.3291	3.1989	1.0000	0.0737
Nylon 10	1.8297	0.5514	0.2992	11.0102	1.0000	0.0009
Polystyrene 0.1	0.5630	0.5749	0.3559	0.9590	1.0000	0.3274
Polystyrene 1	1.2728	0.5740	0.3172	4.9173	1.0000	0.0266
Polystyrene 10	0.3935	0.5842	0.3768	0.4537	1.0000	0.5006
Spartina 0.1	0.8992	0.5652	0.3349	2.5313	1.0000	0.1116
Spartina 1	0.6098	0.5750	0.3558	1.1246	1.0000	0.2889
Spartina 10	0.7307	0.5772	0.3610	1.6028	1.0000	0.2055
virusVirus	5.8686	1.0105	1.0035	33.7298	1.0000	0.0000
frailty.tank.	NA	NA	NA	34.3286	13.9302	0.0018

Iterations: 7 outer, 110 Newton-Raphson

Variance of random effect= 0.2877886 I-likelihood = -1423.7

Degrees of freedom for terms= 2.2 1.0 13.9

Concordance= 0.876 (se = 0.008)

Likelihood ratio test= 515.4 on 17.16 df, p=<2e-16

Mortality Model 2: Cox proportional hazard model including fixed treatment (microparticle type and concentration) and random tank effect among IHNV+ treatments only. Model summary below.

Mortality Model 2: IHNV+ only

	coef	se(coef)	se2	Chisq	DF	p
Nylon 0.1	0.8529	0.5690	0.3392	2.2468	1.0000	0.1339
Nylon 1	1.0138	0.5648	0.3264	3.2223	1.0000	0.0726
Nylon 10	1.8494	0.5550	0.2932	11.1040	1.0000	0.0009
Polystyrene 0.1	0.5654	0.5756	0.3543	0.9651	1.0000	0.3259
Polystyrene 1	1.1492	0.5673	0.3209	4.1037	1.0000	0.0428
Polystyrene 10	0.3958	0.5848	0.3755	0.4580	1.0000	0.4985
Spartina 0.1	0.9049	0.5665	0.3325	2.5519	1.0000	0.1102
Spartina 1	0.6132	0.5759	0.3541	1.1337	1.0000	0.2870
Spartina 10	0.7348	0.5783	0.3592	1.6143	1.0000	0.2039
frailty.tank.	NA	NA	NA	33.8183	13.5238	0.0017

Iterations: 7 outer, 127 Newton-Raphson
Variance of random effect= 0.2841262 I-likelihood = -1411.3
Degrees of freedom for terms= 2.2 13.5
Concordance= 0.718 (se = 0.016)
Likelihood ratio test= 135.1 on 15.71 df, p=<2e-16

Virus shed in water, analysis of IHNV+ tanks. Results of linear mixed effects model including treatment, day, and their interaction (all fixed) and random effect of tank. Anova output in first table, followed by summary.

IHNV Shed (Fig. 3A)

	numDF	denDF	F-value	p-value
(Intercept)	1.0000	229.0000	4142.4030	<.0001
Particle.concentration	9.0000	20.0000	0.8830	0.5557
Day	1.0000	229.0000	366.6950	<.0001
Particle.concentration:Day	9.0000	229.0000	2.5960	0.0072

	Value	Std.Error	DF	t-value	p-value
(Intercept)	10.4930	1.3791	229.0000	7.6085	0.0000
Nylon 0.1	3.8166	1.9372	20.0000	1.9702	0.0628
Nylon 1	3.5316	1.9687	20.0000	1.7939	0.0880
Nylon 10	4.2174	1.9391	20.0000	2.1749	0.0418
Polystyrene 0.1	-1.2924	1.8488	20.0000	-0.6990	0.4926
Polystyrene 1	3.6971	1.9499	20.0000	1.8961	0.0725
Polystyrene 10	-0.7932	1.8030	20.0000	-0.4399	0.6647
Spartina 0.1	2.1588	1.9739	20.0000	1.0937	0.2871
Spartina 1	0.9815	1.9441	20.0000	0.5049	0.6192
Spartina 10	2.5037	1.9992	20.0000	1.2524	0.2249
Day		0.0373	229.0000	-4.8995	0.0000
Nylon 0.1:Day	-0.1002	0.0527	229.0000	-1.8994	0.0588
Nylon 1:Day	-0.0884	0.0537	229.0000	-1.6471	0.1009
Nylon 10:Day	-0.1039	0.0528	229.0000	-1.9660	0.0505
Polystyrene 0.1:Day	0.0416	0.0496	229.0000	0.8391	0.4023
Polystyrene 1:Day	-0.0952	0.0531	229.0000	-1.7913	0.0746
Polystyrene 10:Day	0.0345	0.0482	229.0000	0.7156	0.4750
Spartina 0.1:Day	-0.0460	0.0537	229.0000	-0.8574	0.3921
Spartina 1:Day	-0.0198	0.0527	229.0000	-0.3760	0.7073
Spartina 10:Day	-0.0697	0.0545	229.0000	-1.2779	0.2026

Viral load in gills, summary of linear model including microparticle type and collection day.

IHNV load in Gills (Fig. 3B)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.6804	0.4380	10.6860	0.0000	***
PlasticNylon	1.1528	0.4814	2.3950	0.0213	*
PlasticPolystyrene	1.0008	0.5268	1.9000	0.0645	.
PlasticSpartina	0.2837	0.5303	0.5350	0.5955	
Day35	1.0329	0.4522	2.2840	0.0276	*
Day42	0.1329	0.4427	0.3000	0.7656	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.249 on 41 degrees of freedom

Multiple R-squared: 0.2505, Adjusted R-squared: 0.1591

F-statistic: 2.741 on 5 and 41 DF, p-value: 0.03163

Viral load in anterior kidney, anova followed by summary of linear model including microparticle type, collection day and their interactions.

IHNV Load in Anterior Kidney (Fig. 3B)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Plastic	3.0000	40.4700	13.4900	6.0900	0.0019	**
Day	2.0000	21.4400	10.7210	4.8400	0.0139	*
Plastic:Day	6.0000	36.4000	6.0670	2.7390	0.0274	*
Residuals	35.0000	77.5300	2.2150			

$Plastic

	diff	lwr	upr	p adj
Nylon-No Particle	2.1276	0.4890	3.7663	0.0067
Polystyrene-No Particle	1.9150	0.2764	3.5536	0.0167
Spartina-No Particle	0.4034	-1.2721	2.0788	0.9151
Polystyrene-Nylon	-0.2126	-1.8513	1.4260	0.9850
Spartina-Nylon	-1.7243	-3.3997	-0.0488	0.0417
Spartina-Polystyrene	-1.5117	-3.1871	0.1638	0.0893

$Day

	diff	lwr	upr	p adj
35-31	-0.0542	-1.2683	1.1599	0.9934
42-31	-1.5977	-2.9916	-0.2037	0.0217
42-35	-1.5435	-2.9374	-0.1496	0.0273

$`Plastic:Day`

	diff	lwr	upr	p adj
Nylon:31-No Particle:31	0.1712	-3.6295	3.9719	1.0000
Polystyrene:31-No Particle:31	0.7112	-3.0895	4.5119	0.9999
Spartina:31-No Particle:31	-0.0923	-3.8930	3.7084	1.0000
No Particle:35-No Particle:31	-2.2256	-6.0263	1.5751	0.6600
Nylon:35-No Particle:31	1.2737	-2.5270	5.0744	0.9879
Polystyrene:35-No Particle:31	1.4533	-2.3474	5.2540	0.9678
Spartina:35-No Particle:31	-0.1131	-4.3624	4.1362	1.0000
No Particle:42-No Particle:31	-1.4487	-5.4236	2.5262	0.9772
Nylon:42-No Particle:31	0.6923	-4.0586	5.4432	1.0000
Polystyrene:42-No Particle:31	-2.3826	-7.1334	2.3683	0.8309
Spartina:42-No Particle:31	-3.4249	-7.6742	0.8244	0.2160
Polystyrene:31-Nylon:31	0.5400	-2.7515	3.8315	1.0000
Spartina:31-Nylon:31	-0.2635	-3.5550	3.0280	1.0000
No Particle:35-Nylon:31	-2.3968	-5.6883	0.8948	0.3465
Nylon:35-Nylon:31	1.1025	-2.1890	4.3940	0.9880
Polystyrene:35-Nylon:31	1.2822	-2.0093	4.5737	0.9634
Spartina:35-Nylon:31	-0.2843	-4.0850	3.5164	1.0000
No Particle:42-Nylon:31	-1.6199	-5.1111	1.8713	0.8890
Nylon:42-Nylon:31	0.5211	-3.8331	4.8754	1.0000
Polystyrene:42-Nylon:31	-2.5537	-6.9080	1.8005	0.6579

Spartina:42-Nylon:31	-3.5961	-7.3968	0.2046	0.0777
Spartina:31-Polystyrene:31	-0.8035	-4.0950	2.4880	0.9992
No Particle:35-Polystyrene:31	-2.9367	-6.2282	0.3548	0.1178
Nylon:35-Polystyrene:31	0.5625	-2.7290	3.8541	1.0000
Polystyrene:35-Polystyrene:31	0.7422	-2.5493	4.0337	0.9996
Spartina:35-Polystyrene:31	-0.8243	-4.6250	2.9764	0.9997
No Particle:42-Polystyrene:31	-2.1599	-5.6510	1.3313	0.5844
Nylon:42-Polystyrene:31	-0.0189	-4.3731	4.3354	1.0000
Polystyrene:42-Polystyrene:31	-3.0937	-7.4480	1.2605	0.3815
Spartina:42-Polystyrene:31	-4.1361	-7.9368	-0.3354	0.0232
No Particle:35-Spartina:31	-2.1333	-5.4248	1.1582	0.5168
Nylon:35-Spartina:31	1.3660	-1.9255	4.6575	0.9439
Polystyrene:35-Spartina:31	1.5457	-1.7458	4.8372	0.8812
Spartina:35-Spartina:31	-0.0208	-3.8215	3.7799	1.0000
No Particle:42-Spartina:31	-1.3564	-4.8476	2.1348	0.9640
Nylon:42-Spartina:31	0.7846	-3.5696	5.1389	1.0000
Polystyrene:42-Spartina:31	-2.2902	-6.6445	2.0640	0.7860
Spartina:42-Spartina:31	-3.3326	-7.1333	0.4681	0.1320
Nylon:35-No Particle:35	3.4993	0.2078	6.7908	0.0291
Polystyrene:35-No Particle:35	3.6789	0.3874	6.9704	0.0178
Spartina:35-No Particle:35	2.1125	-1.6882	5.9132	0.7253
No Particle:42-No Particle:35	0.7769	-2.7143	4.2680	0.9997
Nylon:42-No Particle:35	2.9179	-1.4364	7.2721	0.4674
Polystyrene:42-No Particle:35	-0.1570	-4.5112	4.1973	1.0000
Spartina:42-No Particle:35	-1.1993	-5.0000	2.6014	0.9925
Polystyrene:35-Nylon:35	0.1796	-3.1119	3.4711	1.0000
Spartina:35-Nylon:35	-1.3868	-5.1875	2.4139	0.9770
No Particle:42-Nylon:35	-2.7224	-6.2136	0.7687	0.2554
Nylon:42-Nylon:35	-0.5814	-4.9357	3.7728	1.0000
Polystyrene:42-Nylon:35	-3.6563	-8.0105	0.6980	0.1720
Spartina:42-Nylon:35	-4.6986	-8.4993	-0.8979	0.0058
Spartina:35-Polystyrene:35	-1.5664	-5.3671	2.2343	0.9464
No Particle:42-Polystyrene:35	-2.9021	-6.3932	0.5891	0.1823
Nylon:42-Polystyrene:35	-0.7610	-5.1153	3.5932	1.0000
Polystyrene:42-Polystyrene:35	-3.8359	-8.1902	0.5183	0.1280
Spartina:42-Polystyrene:35	-4.8783	-8.6790	-1.0776	0.0037
No Particle:42-Spartina:35	-1.3356	-5.3105	2.6393	0.9877
Nylon:42-Spartina:35	0.8054	-3.9455	5.5563	1.0000
Polystyrene:42-Spartina:35	-2.2695	-7.0204	2.4814	0.8694
Spartina:42-Spartina:35	-3.3118	-7.5611	0.9375	0.2560
Nylon:42-No Particle:42	2.1410	-2.3661	6.6481	0.8733
Polystyrene:42-No Particle:42	-0.9339	-5.4409	3.5732	0.9998
Spartina:42-No Particle:42	-1.9762	-5.9511	1.9987	0.8382
Polystyrene:42-Nylon:42	-3.0749	-8.2792	2.1295	0.6481
Spartina:42-Nylon:42	-4.1172	-8.8681	0.6337	0.1422
Spartina:42-Polystyrene:42	-1.0423	-5.7932	3.7085	0.9997

Results of three-way ANOVA gill histolopathological severity, with microparticle type, virus and day, as well as their interactions.

Histology (Fig. 4)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Plastic	3.0000	8.4359	2.8120	13.1579	0.0000	***
Virus	1.0000	29.5265	29.5265	138.1616	<2.20E-16	***
Day	3.0000	5.9834	1.9945	9.3325	0.0000	***
Plastic:Virus	3.0000	4.6951	1.5650	7.3231	0.0001	***
Plastic:Day	9.0000	8.1982	0.9109	4.2624	0.0001	***
Virus:Day	3.0000	4.3889	1.4630	6.8455	0.0003	***
Plastic:Virus:Day	9.0000	7.6311	0.8479	3.9676	0.0002	***
Residuals	124.0000	26.5000	0.2137			

Histology (Fig. 4) continued: Tukey's post-hoc three-way interactions (p-value of significant interactions only). Biologically significant interactions highlighted.

Treatments	diff	lwr	upr	p-value
No Particle:Mock:42-Nylon:Virus:35	-2.6000	-3.7323	-1.4677	0.0000
No Particle:Mock:42-Polystyrene:Virus:35	-2.0000	-3.1323	-0.8677	0.0000
No Particle:Mock:56-Nylon:Virus:35	-2.4000	-3.5323	-1.2677	0.0000
No Particle:Mock:56-Nylon:Virus:42	-1.6000	-2.7323	-0.4677	0.0001
No Particle:Mock:56-Polystyrene:Virus:35	-1.8000	-2.9323	-0.6677	0.0000
No Particle:Virus:42-No Particle:Mock:31	1.2000	0.0677	2.3323	0.0237
No Particle:Virus:42-No Particle:Mock:35	1.2000	0.0677	2.3323	0.0237
No Particle:Virus:42-No Particle:Mock:42	1.2000	0.0677	2.3323	0.0237
No Particle:Virus:42-Nylon:Virus:35	-1.4000	-2.5323	-0.2677	0.0019
No Particle:Virus:42-Polystyrene:Mock:35	1.2000	0.0677	2.3323	0.0237
No Particle:Virus:42-Spartina:Mock:35	1.2000	0.0677	2.3323	0.0237
No Particle:Virus:56-No Particle:Mock:31	1.5000	0.2990	2.7010	0.0016
No Particle:Virus:56-No Particle:Mock:35	1.5000	0.2990	2.7010	0.0016
No Particle:Virus:56-No Particle:Mock:42	1.5000	0.2990	2.7010	0.0016
No Particle:Virus:56-No Particle:Mock:56	1.3000	0.0990	2.5010	0.0176
No Particle:Virus:56-Nylon:Mock:31	1.3000	0.0990	2.5010	0.0176
No Particle:Virus:56-Nylon:Mock:56	1.3000	0.0990	2.5010	0.0176
No Particle:Virus:56-Nylon:Virus:31	1.3000	0.0990	2.5010	0.0176
No Particle:Virus:56-Polystyrene:Mock:31	1.3000	0.0990	2.5010	0.0176
No Particle:Virus:56-Polystyrene:Mock:35	1.5000	0.2990	2.7010	0.0016
No Particle:Virus:56-Polystyrene:Mock:56	1.3000	0.0990	2.5010	0.0176
No Particle:Virus:56-Spartina:Mock:31	1.5000	0.2340	2.7660	0.0042
No Particle:Virus:56-Spartina:Mock:35	1.5000	0.2990	2.7010	0.0016
No Particle:Virus:56-Spartina:Mock:42	1.3000	0.0990	2.5010	0.0176
Nylon:Mock:42-Nylon:Virus:35	-2.3500	-3.5510	-1.1490	0.0000
Nylon:Mock:42-Polystyrene:Virus:35	-1.7500	-2.9510	-0.5490	0.0000

Nylon:Mock:56-Nylon:Virus:35	-2.4000	-3.5323	-1.2677	0.0000
Nylon:Mock:56-Nylon:Virus:42	-1.6000	-2.7323	-0.4677	0.0001
Nylon:Mock:56-Polystyrene:Virus:35	-1.8000	-2.9323	-0.6677	0.0000
Nylon:Virus:35-No Particle:Mock:31	2.6000	1.4677	3.7323	0.0000
Nylon:Virus:35-No Particle:Mock:35	2.6000	1.4677	3.7323	0.0000
Nylon:Virus:35-No Particle:Virus:31	2.0000	0.8677	3.1323	0.0000
Nylon:Virus:35-No Particle:Virus:35	2.2000	1.0677	3.3323	0.0000
Nylon:Virus:35-Nylon:Mock:31	2.4000	1.2677	3.5323	0.0000
Nylon:Virus:35-Nylon:Mock:35	2.2000	1.0677	3.3323	0.0000
Nylon:Virus:35-Nylon:Virus:31	2.4000	1.2677	3.5323	0.0000
Nylon:Virus:35-Polystyrene:Mock:31	2.4000	1.2677	3.5323	0.0000
Nylon:Virus:35-Polystyrene:Mock:35	2.6000	1.4677	3.7323	0.0000
Nylon:Virus:35-Polystyrene:Virus:31	2.0000	0.8677	3.1323	0.0000
Nylon:Virus:35-Spartina:Mock:31	2.6000	1.3990	3.8010	0.0000
Nylon:Virus:35-Spartina:Mock:35	2.6000	1.4677	3.7323	0.0000
Nylon:Virus:35-Spartina:Virus:31	2.2000	1.0677	3.3323	0.0000
Nylon:Virus:42-No Particle:Mock:31	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:42-No Particle:Mock:35	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:42-No Particle:Mock:42	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:42-No Particle:Virus:31	1.2000	0.0677	2.3323	0.0237
Nylon:Virus:42-No Particle:Virus:35	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:42-Nylon:Mock:31	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:42-Nylon:Mock:35	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:42-Nylon:Mock:42	1.5500	0.3490	2.7510	0.0008
Nylon:Virus:42-Nylon:Virus:31	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:42-Polystyrene:Mock:31	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:42-Polystyrene:Mock:35	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:42-Polystyrene:Mock:42	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:42-Polystyrene:Virus:31	1.2000	0.0677	2.3323	0.0237
Nylon:Virus:42-Spartina:Mock:31	1.8000	0.5990	3.0010	0.0000
Nylon:Virus:42-Spartina:Mock:35	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:42-Spartina:Mock:42	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:42-Spartina:Virus:31	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:42-Spartina:Virus:35	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-No Particle:Mock:31	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:56-No Particle:Mock:35	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:56-No Particle:Mock:42	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:56-No Particle:Mock:56	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:56-No Particle:Virus:31	1.2000	0.0677	2.3323	0.0237
Nylon:Virus:56-No Particle:Virus:35	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-Nylon:Mock:31	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:56-Nylon:Mock:35	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-Nylon:Mock:42	1.5500	0.3490	2.7510	0.0008
Nylon:Virus:56-Nylon:Mock:56	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:56-Nylon:Virus:31	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:56-Polystyrene:Mock:31	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:56-Polystyrene:Mock:35	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:56-Polystyrene:Mock:42	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-Polystyrene:Mock:56	1.6000	0.4677	2.7323	0.0001

Nylon:Virus:56-Polystyrene:Virus:31	1.2000	0.0677	2.3323	0.0237
Nylon:Virus:56-Spartina:Mock:31	1.8000	0.5990	3.0010	0.0000
Nylon:Virus:56-Spartina:Mock:35	1.8000	0.6677	2.9323	0.0000
Nylon:Virus:56-Spartina:Mock:42	1.6000	0.4677	2.7323	0.0001
Nylon:Virus:56-Spartina:Mock:56	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-Spartina:Virus:31	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-Spartina:Virus:35	1.4000	0.2677	2.5323	0.0019
Nylon:Virus:56-Spartina:Virus:42	1.2000	0.0677	2.3323	0.0237
Polystyrene:Mock:42-Nylon:Virus:35	-2.2000	-3.3323	-1.0677	0.0000
Polystyrene:Mock:42-Polystyrene:Virus:35	-1.6000	-2.7323	-0.4677	0.0001
Polystyrene:Mock:56-Nylon:Virus:35	-2.4000	-3.5323	-1.2677	0.0000
Polystyrene:Mock:56-Nylon:Virus:42	-1.6000	-2.7323	-0.4677	0.0001
Polystyrene:Mock:56-Polystyrene:Virus:35	-1.8000	-2.9323	-0.6677	0.0000
Polystyrene:Virus:35-No Particle:Mock:31	2.0000	0.8677	3.1323	0.0000
Polystyrene:Virus:35-No Particle:Mock:35	2.0000	0.8677	3.1323	0.0000
Polystyrene:Virus:35-No Particle:Virus:31	1.4000	0.2677	2.5323	0.0019
Polystyrene:Virus:35-No Particle:Virus:35	1.6000	0.4677	2.7323	0.0001
Polystyrene:Virus:35-Nylon:Mock:31	1.8000	0.6677	2.9323	0.0000
Polystyrene:Virus:35-Nylon:Mock:35	1.6000	0.4677	2.7323	0.0001
Polystyrene:Virus:35-Nylon:Virus:31	1.8000	0.6677	2.9323	0.0000
Polystyrene:Virus:35-Polystyrene:Mock:31	1.8000	0.6677	2.9323	0.0000
Polystyrene:Virus:35-Polystyrene:Mock:35	2.0000	0.8677	3.1323	0.0000
Polystyrene:Virus:35-Polystyrene:Virus:31	1.4000	0.2677	2.5323	0.0019
Polystyrene:Virus:35-Spartina:Mock:31	2.0000	0.7990	3.2010	0.0000
Polystyrene:Virus:35-Spartina:Mock:35	2.0000	0.8677	3.1323	0.0000
Polystyrene:Virus:35-Spartina:Virus:31	1.6000	0.4677	2.7323	0.0001
Polystyrene:Virus:42-No Particle:Mock:31	1.2000	0.0677	2.3323	0.0237
Polystyrene:Virus:42-No Particle:Mock:35	1.2000	0.0677	2.3323	0.0237
Polystyrene:Virus:42-No Particle:Mock:42	1.2000	0.0677	2.3323	0.0237
Polystyrene:Virus:42-Nylon:Virus:35	-1.4000	-2.5323	-0.2677	0.0019
Polystyrene:Virus:42-Polystyrene:Mock:35	1.2000	0.0677	2.3323	0.0237
Polystyrene:Virus:42-Spartina:Mock:35	1.2000	0.0677	2.3323	0.0237
Polystyrene:Virus:56-Nylon:Virus:35	-1.8500	-3.0510	-0.6490	0.0000
Polystyrene:Virus:56-Polystyrene:Virus:35	-1.2500	-2.4510	-0.0490	0.0302
Spartina:Mock:42-Nylon:Virus:35	-2.4000	-3.5323	-1.2677	0.0000
Spartina:Mock:42-Polystyrene:Virus:35	-1.8000	-2.9323	-0.6677	0.0000
Spartina:Mock:56-Nylon:Virus:35	-2.2000	-3.3323	-1.0677	0.0000
Spartina:Mock:56-Nylon:Virus:42	-1.4000	-2.5323	-0.2677	0.0019
Spartina:Mock:56-Polystyrene:Virus:35	-1.6000	-2.7323	-0.4677	0.0001
Spartina:Virus:35-Nylon:Virus:35	-2.2000	-3.3323	-1.0677	0.0000
Spartina:Virus:35-Polystyrene:Virus:35	-1.6000	-2.7323	-0.4677	0.0001
Spartina:Virus:42-Nylon:Virus:35	-2.0000	-3.1323	-0.8677	0.0000
Spartina:Virus:42-Nylon:Virus:42	-1.2000	-2.3323	-0.0677	0.0237
Spartina:Virus:42-Polystyrene:Virus:35	-1.4000	-2.5323	-0.2677	0.0019

Spartina:Virus:56-Nylon:Virus:35	-2.0000	-3.1323	-0.8677	0.0000
Spartina:Virus:56-Nylon:Virus:42	-1.2000	-2.3323	-0.0677	0.0237
Spartina:Virus:56-Nylon:Virus:56	-1.2000	-2.3323	-0.0677	0.0237
Spartina:Virus:56-Polystyrene:Virus:35	-1.4000	-2.5323	-0.2677	0.0019

Anova and summary of linear effects model of IFNγ including microparticle type, day, and virus.

IFNγ in gill (Fig. 5A)

	Df	Sum Sq	Mean Sq	F value	p value	
Plastic	3.0000	2.0090	0.6700	2.3164	0.0805	.
Day	2.0000	2.4610	1.2310	4.2560	0.0169	*
Virus	1.0000	41.0560	41.0560	141.9993	0.0000	***
Residuals	97.0000	28.0450	0.2890			

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.1685	0.1372	1.2280	0.2224	
PlasticNylon	0.3510	0.1453	2.4150	0.0176	*
PlasticPolystyrene	0.3310	0.1506	2.1980	0.0303	*
PlasticSpartina	0.2228	0.1453	1.5330	0.1284	
Day35	-0.2682	0.1302	-2.0610	0.0420	*
Day42	-0.1045	0.1319	-0.7920	0.4301	
VirusVirus	1.2617	0.1059	11.9160	<2e-16	***

Anova and summary of linear effects model of secreted IgT including microparticle type, day, and virus.

Sec IgT Gill (Fig. 5B)

	Df	Sum Sq	Mean Sq	F value	p value	
Plastic	3.0000	2.7584	0.9195	3.3806	0.0213	*
Day	2.0000	4.0891	2.0446	7.5173	0.0009	***
Virus	1.0000	5.6450	5.6450	20.7549	0.0000	***
Residuals	97.0000	26.3823	0.2720			

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	-0.0031	0.1331	-0.0230	0.9816	
PlasticNylon	0.3963	0.1410	2.8120	0.0060	**
PlasticPolystyrene	0.3445	0.1461	2.3590	0.0204	*
PlasticSpartina	0.2927	0.1409	2.0770	0.0405	*
Day35	-0.0150	0.1262	-0.1190	0.9057	
Day42	0.3856	0.1279	3.0140	0.0033	**
VirusVirus	0.4679	0.1027	4.5560	0.0000	***