Supplemental Information

Transcriptomic and long-term behavioral deficits associated with developmental 3.5 GHz radiofrequency radiation exposures in zebrafish

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Number of figures: 2
Number of tables: 8

RFR exposure setup

Faraday cages (exposure chamber and sham control), that were fit to house a 6-well microtiter plate, were constructed with copper. The lid of the chamber was fitted with a Fractus (model FR05-107) ultra-wide band, omnidirectional antenna with a 3.1–5 GHz frequency range. The signal was generated using a transmitter (Analog Devices, model ADRV9364-Z7020) connected to a power amplifier (PA) (Mini-circuits ZHL-42+). Using a spectrum analyzer (Tektronix RSA3408A) with a 30 dBm measurement threshold, we performed preliminary assessments to measure the output from the PA. With a transmitter output of -9 dBm at 3.5 GHz, the PA output was 26.7 dBm. During our experiments, we set the transmitter to an output of -6 dBm at 3.5 GHz and expected a PA output of 30–32 dBm signal power. The amplified signal was administered to the zebrafish embryos via SMC cable connection from the PA to antenna attached to the RFR chamber.

Library preparation and sequencing

The first step in the workflow involves purifying the poly-A containing mRNA molecules using poly-T oligo- attached magnetic beads. Following purification, the mRNA is fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments are copied into first strand cDNA using reverse transcriptase and random primers. This is followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. These cDNA fragments then have the addition of a single 'A' base and subsequent ligation of the adapter. The products are then purified and enriched with PCR amplification. We then quantified the PCR yield by Qubit and pooled samples together to make a single strand DNA circle (ssDNA circle), which gave the final library. DNA nanoballs (DNBs) were generated with the ssDNA circle by rolling circle replication (RCR) to enlarge the fluorescent signals at the sequencing process. The DNBs were loaded into the patterned nanoarrays and pair-end reads of 100 bp were read through on the DNBseq platform for the following data analysis study. For this step, the DNBseq platform combines the DNA nanoball-based nanoarrays and stepwise sequencing using Combinational Probe-Anchor Synthesis Sequencing Method. Overall strategy is illustrated in figure S1.

qRT-PCR

Forward and reverse primers were designed within NCBI’s Primer-BLAST and synthesized at Integrated DNA Technologies (Coralville, IA). Primers were validated by observation of size and specificity of bands via gel electrophoresis and melt curve analysis following qPCR reactions.

For qPCR, total RNA was extracted as described previously[1]. 10 µL one-step qRT-PCR reactions were set up consisting of 5 µL SYBR Green master Mix and 0.08 µL reverse transcriptase enzyme mix (Power SYBR® Green RNA-to-CT™ 1-Step Kit; Applied Biosystems, Foster City, CA), 0.2 µL each of 10 µM forward and reverse primers, and 20 ng RNA per reaction. The QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) was used under the following cycling conditions; reverse transcription at 48 °C for 30 minutes, denaturation and activation of SYBR® polymerase at 95 °C for 10 minutes, followed by 40 cycles of amplification (95 °C for 15 seconds, 60 °C for 1 minute).

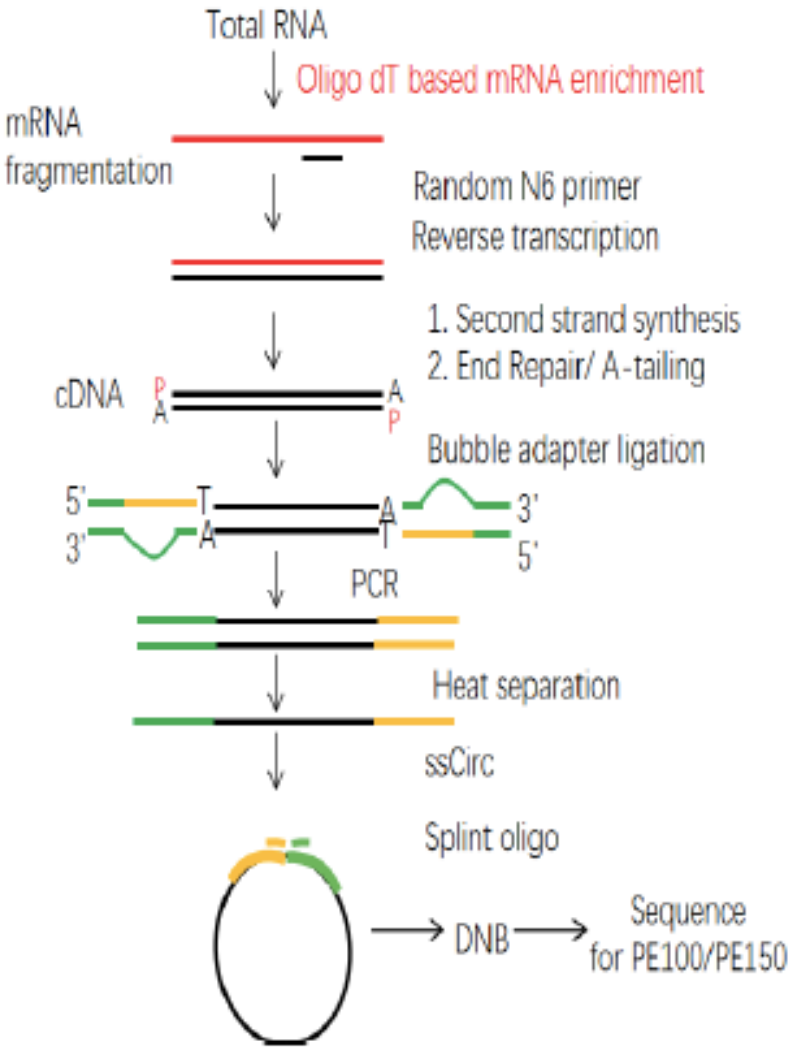
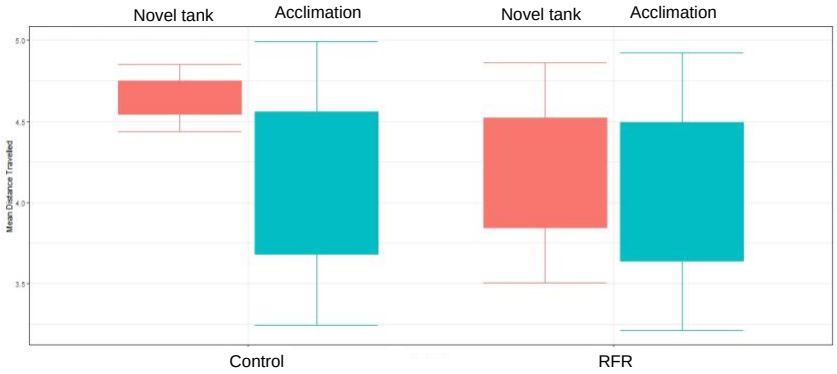
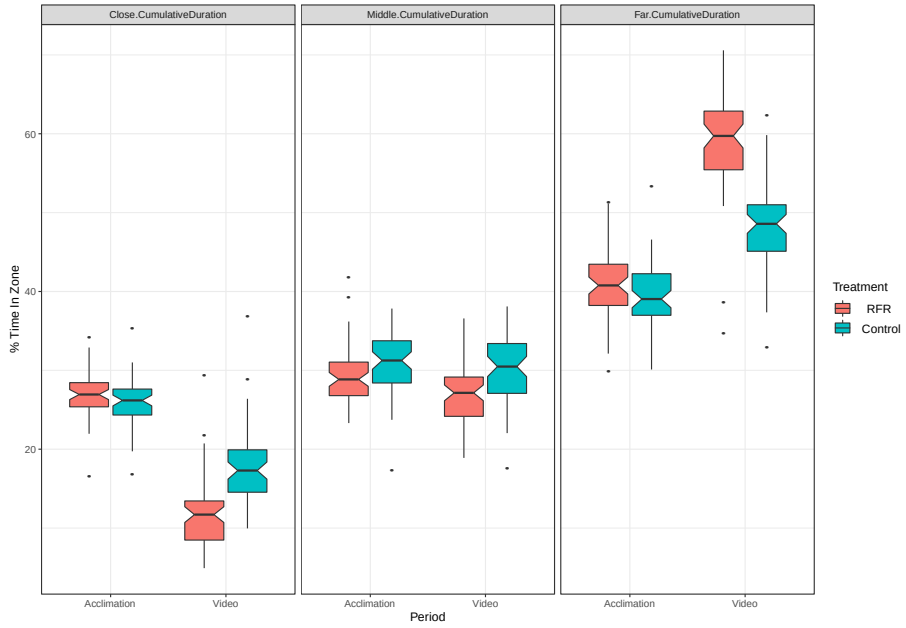


Figure S1: Library preparation for DNBSeq.

Novel tank



Predator



Schooling

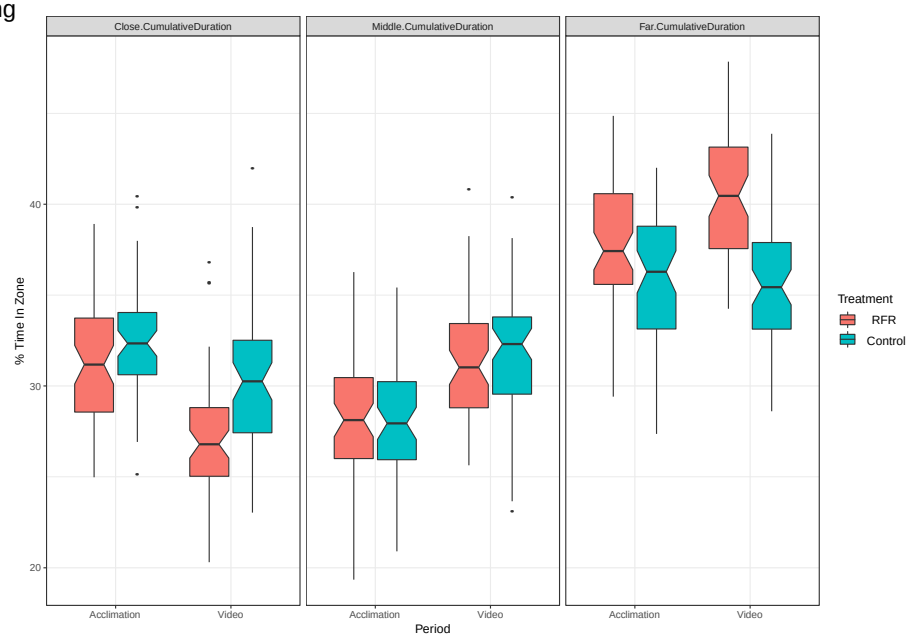


Figure S2: Mean distance travelled, and percent time spent in each zone during the novel tank, predator and schooling assays.

Sample	Reads(M)	Reads(M)	Bases(Gb)	Q20(%)	Q30(%)	Ratio(%)	Ratio(%)
5G_48hpf_1	47.48	47.03	4.7	97.63	89.73	99.06	0.15
5G_48hpf_2	47.48	46.87	4.69	97.88	90.71	98.73	0.15
5G_48hpf_3	47.48	46.66	4.67	97.8	90.42	98.28	0.18
5G_48hpf_4	40.18	39.6	3.96	97.86	90.83	98.55	0.3
Control_48hpf_1	47.48	47.1	4.71	97.78	90.23	99.21	0.15
Control_48hpf_2	47.48	46.69	4.67	97.8	90.35	98.35	0.15
Control_48hpf_3	47.48	46.74	4.67	97.77	90.23	98.44	0.19
Control_48hpf_4	49.97	48.93	4.89	97.91	91.08	97.92	0.27

Table S1: Sequencing reads and Quality scores for each replicate sample. 5G=RFR

Sample	Total CleanReads	Total MappingRatio	Uniquely MappingRatio
5G_48hpf_1	47,030,410	87.79%	55.71%
5G_48hpf_2	46,871,520	87.99%	56.19%
5G_48hpf_3	46,660,940	86.28%	54.81%
5G_48hpf_4	39,603,976	86.35%	55.10%
Control_48hpf_1	47,102,398	87.22%	55.05%
Control_48hpf_2	46,690,566	87.36%	55.54%
Control_48hpf_3	46,737,104	86.43%	54.64%
Control_48hpf_4	48,933,040	86.89%	55.27%

Table S2: Sequencing clean reads and mapping to genome. 5G=RFR

GeneID	ange(5G_48hpf/Cor	Padj	Symbol
XR_002459562.1	3.699549726	2.62E-11	*hnrnpdl*
XR_002459782.1	2.071611427	0.028531519	*LOC103911943*
170783	1.70235286	0.02568343	*mkrn2*
554157	-1.343386872	1.37E-06	*ctss2.1*
100537272	-1.138405297	0.003231089	*LOC100537272*
791612	-0.973093183	0.007407116	*mat2ab*
335651	-0.770299807	0.032964928	*rbp4l*
266640	-0.765009612	0.040819274	*unc45b*
353179	0.755534151	0.040644698	*otx5*
108179505	-0.750811315	0.01313212	*LOC108179505*
393665	0.672852337	0.013091304	*ndrg1b*
XR_663224.3	0.608441649	0.003942241	*NA*
394021	-0.608274497	0.000123723	*shmt1*
449685	-0.598107485	0.029812037	*zgc:136410*
321928	-0.586315538	0.007407116	*phgdh*
327512	-0.582518358	0.007407116	*psat1*
XR_222144.3	-0.523167806	0.007407116	*NA*
334393	-0.507966323	0.034116435	*iars*
30314	-0.46400709	0.011749663	*apoeb*
100333269	-0.439320064	0.028531519	*aldh1l2*
282673	-0.438069374	0.028531519	*npc2*
324940	-0.419354602	0.02568343	*aars*
406739	-0.38413495	0.040819274	*pla2g12b*
563195	-0.34812378	0.032964928	*rrp12*
570653	0.345418623	0.029661332	*fam117ba*
79375	0.309299407	0.032964928	*foxc1b*
436759	-0.292600352	0.007407116	*rpl38*
556556	-0.275435508	0.034116435	*rpl32*

Table S3: Differentially expressed genes (p_{adj}<0.05) at 48 hpf.

Target	Primer	sequence
hnrnpdl	UC4056_hnrpdl_tvx2_F1	CCTTGTTTGTACCTGCCAGC
	UC4057_hnrpdl_tvx2_R1	AAGTCAACCTGCCCACCACA
LOC103911943	UC4060_LOC103911943_tvx5_F1	TGGCAGTGACTGGAAACCAA
	UC4061_LOC103911943_tvx5_R1	CTGCCACAAGAATCCACTGC
mkrn2	UC4064_mkrn2_tvx1_F1	CAGGAAGGCGGAATCAACGA
	UC4065_mkrn2_tvx1_R1	ACACGCTCCTCTCTGGTAGT
β actin	UC3716_beta-actin_new_F	GATCAAGATCATTGCCCCACC
	UC3716_beta-actin_new_R	GAGTCGGCGTGAAGTGGTAA

Table S4: Primer sequences used for qPCR

Novel tank-statistics

```
                 Df Sum Sq Mean Sq F value Pr(>F)
Treatment         1  0.133  0.1330   0.133  0.734
Status            1  0.208  0.2082   0.208  0.672
Treatment:Status  1  0.084  0.0845   0.084  0.786
Residuals         4  4.004  1.0010
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mean.dist ~ Treatment * Status, data = (acc.sum))

$Treatment
              diff       lwr      upr     p adj
5G-Ctrl -0.2578953 -2.222095 1.706304 0.7339074

$Status
                              diff       lwr      upr     p adj
Acclimation-Novel Tank -0.3226562 -2.286856 1.641543 0.6719936

$`Treatment:Status`
                                          diff       lwr      upr     p adj
5G:Novel Tank-Ctrl:Novel Tank    -0.46341575 -4.536236 3.609405 0.9633755
Ctrl:Acclimation-Ctrl:Novel Tank -0.52817673 -4.600997 3.544644 0.9478282
5G:Acclimation-Ctrl:Novel Tank   -0.58055149 -4.653372 3.492269 0.9330468
Ctrl:Acclimation-5G:Novel Tank   -0.06476099 -4.137581 4.008059 0.9998859
5G:Acclimation-5G:Novel Tank     -0.11713574 -4.189956 3.955685 0.9993288
5G:Acclimation-Ctrl:Acclimation  -0.05237476 -4.125195 4.020446 0.9999396
```

Table S5: 2-way ANOVA for Novel tank data. *** statistically significant

Predator: Far zone-statistics

```
                          Df   Sum Sq Mean Sq F value   Pr(>F)
Treatment                  1   192957  192957 105.468  < 2e-16 ***
status                     1   960000  960000 524.725  < 2e-16 ***
Sex                      1      138     138   0.075   0.7838
Treatment:status           1   111744  111744  61.078 5.74e-15 ***
Treatment:Sex            1   261082  261082 142.704  < 2e-16 ***
status:Sex               1     8595    8595   4.698   0.0302 *
Treatment:status:Sex     1     1235    1235   0.675   0.4114
Residuals              21508 39349496    1830
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
324 observations deleted due to missingness
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = duration ~ Treatment * status * Sex, data = zone.only)

$Treatment
             diff       lwr       upr p adj
Ctrl-5G -5.989387 -7.132512 -4.846262     0

$status
                      diff      lwr      upr p adj
Video-Acclimation 13.36144 12.21814 14.50474     0

$Sex
         diff        lwr      upr     p adj
M-F 0.1598852 -0.9833151 1.303085 0.7839865

$`Treatment:status`
                                       diff        lwr        upr     p adj
Ctrl:Acclimation-5G:Acclimation  -1.324214  -3.462413  0.8139845 0.3836521
5G:Video-5G:Acclimation          17.904725  15.788918 20.0205318 0.0000000
Ctrl:Video-5G:Acclimation         7.463289   5.341967  9.5846114 0.0000000
5G:Video-Ctrl:Acclimation        19.228939  17.111496 21.3463814 0.0000000
```

Ctrl:Video-Ctrl:Acclimation	8.787503	6.664550	10.9104566	0.0000000
Ctrl:Video-5G:Video	-10.441435	-12.541835	-8.3410361	0.0000000

$`Treatment:Sex`

	diff	lwr	upr	p adj
Ctrl:F-5G:F	-13.0533072	-15.186978	-10.919637	0.0000000
5G:M-5G:F	-6.7818735	-8.897946	-4.665802	0.0000000
Ctrl:M-5G:F	-5.8905914	-7.971177	-3.810006	0.0000000
5G:M-Ctrl:F	6.2714337	4.111390	8.431478	0.0000000
Ctrl:M-Ctrl:F	7.1627158	5.037424	9.288007	0.0000000
Ctrl:M-5G:M	0.8912821	-1.216341	2.998905	0.6978247

$`status:Sex`

	diff	lwr	upr	p adj
Video:F-Acclimation:F	12.082654	9.9504953	14.214814	0.0000000
Acclimation:M-Acclimation:F	-1.125233	-3.2635629	1.013096	0.5297121
Video:M-Acclimation:F	13.484497	11.3656078	15.603385	0.0000000
Acclimation:M-Video:F	-13.207888	-15.3280383	-11.087737	0.0000000
Video:M-Video:F	1.401842	-0.6986994	3.502384	0.3159714
Video:M-Acclimation:M	14.609730	12.5029256	16.716535	0.0000000

$`Treatment:status:Sex`

	diff	lwr	upr	p adj	
Ctrl:Acclimation:F-5G:Acclimation:F	-8.8329331	-12.423054	-5.2428124	0.0000000	
5G:Video:F-5G:Acclimation:F	16.1970580	12.710581	19.6835351	0.0000000	
Ctrl:Video:F-5G:Acclimation:F	-0.8892627	-4.455854	2.6773290	0.9951819	
5G:Acclimation:M-5G:Acclimation:F	-8.5491231	-12.115007	-4.9832390	0.0000000	
Ctrl:Acclimation:M-5G:Acclimation:F	-2.4562803	-5.962797	1.0502362	0.3996935	
5G:Video:M-5G:Acclimation:F	11.1334700	7.600793	14.6661468	0.0000000	
Ctrl:Video:M-5G:Acclimation:F	7.0547294	3.580482	10.5289774	0.0000000	
5G:Video:F-Ctrl:Acclimation:F	25.0299911	21.475533	28.5844490	0.0000000	a
Ctrl:Video:F-Ctrl:Acclimation:F	7.9436704	4.310597	11.5767442	0.0000000	
5G:Acclimation:M-Ctrl:Acclimation:F	0.2838100	-3.348569	3.9161892	0.9999979	
Ctrl:Acclimation:M-Ctrl:Acclimation:F	6.3766528	2.802537	9.9507690	0.0000017	
5G:Video:M-Ctrl:Acclimation:F	19.9664031	16.366618	23.5661883	0.0000000	
Ctrl:Video:M-Ctrl:Acclimation:F	15.8876626	12.345199	19.4301260	0.0000000	
Ctrl:Video:F-5G:Video:F	-17.0863207	-20.617012	-13.5556295	0.0000000	

```
5G:Acclimation:M-5G:Video:F          -24.7461811 -28.276158 -21.2162047 0.0000000
Ctrl:Acclimation:M-5G:Video:F        -18.6533383 -22.123333 -15.1833438 0.0000000
5G:Video:M-5G:Video:F                 -5.0635880  -8.560016  -1.5671600 0.0003045
Ctrl:Video:M-5G:Video:F               -9.1423286 -12.579712  -5.7049455 0.0000000
5G:Acclimation:M-Ctrl:Video:F         -7.6598604 -11.268986  -4.0507348 0.0000000
Ctrl:Acclimation:M-Ctrl:Video:F       -1.5670176  -5.117499   1.9834635 0.8844842
5G:Video:M-Ctrl:Video:F               12.0227327   8.446413  15.5990525 0.0000000
Ctrl:Video:M-Ctrl:Video:F              7.9439921   4.425376  11.4626079 0.0000000
Ctrl:Acclimation:M-5G:Acclimation:M    6.0928428   2.543072   9.6426130 0.0000054
5G:Video:M-5G:Acclimation:M           19.6825931  16.106979  23.2582072 0.0000000
Ctrl:Video:M-5G:Acclimation:M         15.6038525  12.085954  19.1217510 0.0000000
5G:Video:M-Ctrl:Acclimation:M         13.5897503  10.073340  17.1061610 0.0000000
Ctrl:Video:M-Ctrl:Acclimation:M        9.5110097   6.053303  12.9687168 0.0000000
Ctrl:Video:M-5G:Video:M               -4.0787406  -7.562974  -0.5945067 0.0092652
```

Predator: middle zone- statistics

```
                          Df   Sum Sq Mean Sq F value   Pr(>F)
Treatment                  1    31525   31525  24.176 8.86e-07 ***
status                     1    15949   15949  12.231 0.000471 ***
Sex                        1     3550    3550   2.722 0.098988 .
Treatment:status           1     3788    3788   2.905 0.088314 .
Treatment:Sex              1    17931   17931  13.751 0.000209 ***
status:Sex                 1   311190  311190 238.643  < 2e-16 ***
Treatment:status:Sex       1     4035    4035   3.094 0.078585 .
Residuals              21508 28046445    1304
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
324 observations deleted due to missingness
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = duration ~ Treatment * status * Sex, data = zone.only)

$Treatment
```

```
            diff      lwr     upr p adj
Ctrl-5G 2.420931 1.455851 3.38601 8e-07

$status
                       diff       lwr        upr     p adj
Video-Acclimation -1.722218 -2.687445 -0.7569904 0.0004708

$Sex
          diff       lwr       upr     p adj
M-F -0.8118211 -1.776964 0.1533219 0.0992241

$`Treatment:status`
                                       diff        lwr        upr     p adj
Ctrl:Acclimation-5G:Acclimation  1.5633606 -0.2418063  3.3685275 0.1164775
5G:Video-5G:Acclimation         -2.5587386 -4.3450016 -0.7724756 0.0013344
Ctrl:Video-5G:Acclimation        0.6833116 -1.1076074  2.4742306 0.7607244
5G:Video-Ctrl:Acclimation       -4.1220992 -5.9097429 -2.3344555 0.0000000
Ctrl:Video-Ctrl:Acclimation     -0.8800490 -2.6723451  0.9122472 0.5875382
Ctrl:Video-5G:Video              3.2420502  1.4687952  5.0153052 0.0000156

$`Treatment:Sex`
                    diff        lwr        upr     p adj
Ctrl:F-5G:F    4.3010015  2.4996573  6.1023457 0.0000000
5G:M-5G:F      1.0063134 -0.7801732  2.7928000 0.4698075
Ctrl:M-5G:F    1.6528827 -0.1036443  3.4094097 0.0737964
5G:M-Ctrl:F   -3.2946881 -5.1182980 -1.4710782 0.0000205
Ctrl:M-Ctrl:F -2.6481188 -4.4423891 -0.8538486 0.0008629
Ctrl:M-5G:M    0.6465693 -1.1327843  2.4259229 0.7867903

$`status:Sex`
                                  diff         lwr        upr     p adj
Video:F-Acclimation:F       -9.415088 -11.2151557 -7.6150194 0.0000000
Acclimation:M-Acclimation:F -8.550064 -10.3553410 -6.7447861 0.0000000
Video:M-Acclimation:F       -2.759986  -4.5488505 -0.9711211 0.0004295
Acclimation:M-Video:F        0.865024  -0.9249058  2.6549538 0.6002731
Video:M-Video:F              6.655102   4.8817266  8.4284768 0.0000000
Video:M-Acclimation:M        5.790078   4.0114152  7.5687403 0.0000000
```

$`Treatment:status:Sex`

	diff	lwr	upr	p adj
Ctrl:Acclimation:F-5G:Acclimation:F	4.5914651	1.5605178	7.622412	0.0001195
5G:Video:F-5G:Acclimation:F	-9.1353678	-12.0788143	-6.191921	0.0000000
Ctrl:Video:F-5G:Acclimation:F	-5.1854970	-8.1965800	-2.174414	0.0000049
5G:Acclimation:M-5G:Acclimation:F	-5.8782503	-8.8887359	-2.867765	0.0000000
Ctrl:Acclimation:M-5G:Acclimation:F	-6.6750637	-9.6354283	-3.714699	0.0000000
5G:Video:M-5G:Acclimation:F	-1.5136066	-4.4960570	1.468844	0.7866550
Ctrl:Video:M-5G:Acclimation:F	0.5156186	-2.4175034	3.448741	0.9994885
5G:Video:F-Ctrl:Acclimation:F	-13.7268329	-16.7276720	-10.725994	0.0000000
Ctrl:Video:F-Ctrl:Acclimation:F	-9.7769621	-12.8441723	-6.709752	0.0000000
5G:Acclimation:M-Ctrl:Acclimation:F	-10.4697154	-13.5363392	-7.403092	0.0000000
Ctrl:Acclimation:M-Ctrl:Acclimation:F	-11.2665288	-14.2839642	-8.249093	0.0000000
5G:Video:M-Ctrl:Acclimation:F	-6.1050717	-9.1441782	-3.065965	0.0000000
Ctrl:Video:M-Ctrl:Acclimation:F	-4.0758465	-7.0665592	-1.085134	0.0009468
Ctrl:Video:F-5G:Video:F	3.9498708	0.9690967	6.930645	0.0015268
5G:Acclimation:M-5G:Video:F	3.2571175	0.2769469	6.237288	0.0208094
Ctrl:Acclimation:M-5G:Video:F	2.4603042	-0.4692269	5.389835	0.1764532
5G:Video:M-5G:Video:F	7.6217612	4.6699137	10.573609	0.0000000
Ctrl:Video:M-5G:Video:F	9.6509865	6.7489874	12.552986	0.0000000
5G:Acclimation:M-Ctrl:Video:F	-0.6927533	-3.7397453	2.354239	0.9972971
Ctrl:Acclimation:M-Ctrl:Video:F	-1.4895666	-4.4870482	1.507915	0.8043881
5G:Video:M-Ctrl:Video:F	3.6718904	0.6525945	6.691186	0.0055921
Ctrl:Video:M-Ctrl:Video:F	5.7011157	2.7305363	8.671695	0.0000000
Ctrl:Acclimation:M-5G:Acclimation:M	-0.7968134	-3.7936949	2.200068	0.9928481
5G:Video:M-5G:Acclimation:M	4.3646437	1.3459436	7.383344	0.0003145
Ctrl:Video:M-5G:Acclimation:M	6.3938689	3.4238951	9.363843	0.0000000
5G:Video:M-Ctrl:Acclimation:M	5.1614570	2.1927392	8.130175	0.0000037
Ctrl:Video:M-Ctrl:Acclimation:M	7.1906823	4.2715248	10.109840	0.0000000
Ctrl:Video:M-5G:Video:M	2.0292253	-0.9123273	4.970778	0.4206779

Predator: close zone-statistics

```
                          Df   Sum Sq Mean Sq F value   Pr(>F)
Treatment                  1    31525   31525  24.176 8.86e-07 ***
status                     1    15949   15949  12.231 0.000471 ***
Sex                        1     3550    3550   2.722 0.098988 .
Treatment:status           1     3788    3788   2.905 0.088314 .
Treatment:Sex              1    17931   17931  13.751 0.000209 ***
status:Sex                 1   311190  311190 238.643  < 2e-16 ***
Treatment:status:Sex       1     4035    4035   3.094 0.078585 .
Residuals              21508 28046445    1304
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
324 observations deleted due to missingness
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = duration ~ Treatment * status * Sex, data = zone.only)

$Treatment
            diff      lwr     upr p adj
Ctrl-5G 2.420931 1.455851 3.38601 8e-07

$status
                        diff       lwr        upr     p adj
Video-Acclimation -1.722218 -2.687445 -0.7569904 0.0004708

$Sex
          diff       lwr       upr     p adj
M-F -0.8118211 -1.776964 0.1533219 0.0992241

$`Treatment:status`
                                       diff        lwr        upr     p adj
Ctrl:Acclimation-5G:Acclimation  1.5633606 -0.2418063  3.3685275 0.1164775
5G:Video-5G:Acclimation         -2.5587386 -4.3450016 -0.7724756 0.0013344
Ctrl:Video-5G:Acclimation        0.6833116 -1.1076074  2.4742306 0.7607244
5G:Video-Ctrl:Acclimation       -4.1220992 -5.9097429 -2.3344555 0.0000000
Ctrl:Video-Ctrl:Acclimation     -0.8800490 -2.6723451  0.9122472 0.5875382
Ctrl:Video-5G:Video              3.2420502  1.4687952  5.0153052 0.0000156
```

$`Treatment:Sex`

	diff	lwr	upr	p adj
Ctrl:F-5G:F	4.3010015	2.4996573	6.1023457	0.0000000
5G:M-5G:F	1.0063134	-0.7801732	2.7928000	0.4698075
Ctrl:M-5G:F	1.6528827	-0.1036443	3.4094097	0.0737964
5G:M-Ctrl:F	-3.2946881	-5.1182980	-1.4710782	0.0000205
Ctrl:M-Ctrl:F	-2.6481188	-4.4423891	-0.8538486	0.0008629
Ctrl:M-5G:M	0.6465693	-1.1327843	2.4259229	0.7867903

$`status:Sex`

	diff	lwr	upr	p adj
Video:F-Acclimation:F	-9.415088	-11.2151557	-7.6150194	0.0000000
Acclimation:M-Acclimation:F	-8.550064	-10.3553410	-6.7447861	0.0000000
Video:M-Acclimation:F	-2.759986	-4.5488505	-0.9711211	0.0004295
Acclimation:M-Video:F	0.865024	-0.9249058	2.6549538	0.6002731
Video:M-Video:F	6.655102	4.8817266	8.4284768	0.0000000
Video:M-Acclimation:M	5.790078	4.0114152	7.5687403	0.0000000

$`Treatment:status:Sex`

	diff	lwr	upr	p adj
Ctrl:Acclimation:F-5G:Acclimation:F	4.5914651	1.5605178	7.622412	0.0001195
5G:Video:F-5G:Acclimation:F	-9.1353678	-12.0788143	-6.191921	0.0000000
Ctrl:Video:F-5G:Acclimation:F	-5.1854970	-8.1965800	-2.174414	0.0000049
5G:Acclimation:M-5G:Acclimation:F	-5.8782503	-8.8887359	-2.867765	0.0000000
Ctrl:Acclimation:M-5G:Acclimation:F	-6.6750637	-9.6354283	-3.714699	0.0000000
5G:Video:M-5G:Acclimation:F	-1.5136066	-4.4960570	1.468844	0.7866550
Ctrl:Video:M-5G:Acclimation:F	0.5156186	-2.4175034	3.448741	0.9994885
5G:Video:F-Ctrl:Acclimation:F	-13.7268329	-16.7276720	-10.725994	0.0000000
Ctrl:Video:F-Ctrl:Acclimation:F	-9.7769621	-12.8441723	-6.709752	0.0000000
5G:Acclimation:M-Ctrl:Acclimation:F	-10.4697154	-13.5363392	-7.403092	0.0000000
Ctrl:Acclimation:M-Ctrl:Acclimation:F	-11.2665288	-14.2839642	-8.249093	0.0000000
5G:Video:M-Ctrl:Acclimation:F	-6.1050717	-9.1441782	-3.065965	0.0000000
Ctrl:Video:M-Ctrl:Acclimation:F	-4.0758465	-7.0665592	-1.085134	0.0009468
Ctrl:Video:F-5G:Video:F	3.9498708	0.9690967	6.930645	0.0015268
5G:Acclimation:M-5G:Video:F	3.2571175	0.2769469	6.237288	0.0208094
Ctrl:Acclimation:M-5G:Video:F	2.4603042	-0.4692269	5.389835	0.1764532
5G:Video:M-5G:Video:F	7.6217612	4.6699137	10.573609	0.0000000

Ctrl:Video:M-5G:Video:F	9.6509865	6.7489874	12.552986	0.0000000
5G:Acclimation:M-Ctrl:Video:F	-0.6927533	-3.7397453	2.354239	0.9972971
Ctrl:Acclimation:M-Ctrl:Video:F	-1.4895666	-4.4870482	1.507915	0.8043881
5G:Video:M-Ctrl:Video:F	3.6718904	0.6525945	6.691186	0.0055921
Ctrl:Video:M-Ctrl:Video:F	5.7011157	2.7305363	8.671695	0.0000000
Ctrl:Acclimation:M-5G:Acclimation:M	-0.7968134	-3.7936949	2.200068	0.9928481
5G:Video:M-5G:Acclimation:M	4.3646437	1.3459436	7.383344	0.0003145
Ctrl:Video:M-5G:Acclimation:M	6.3938689	3.4238951	9.363843	0.0000000
5G:Video:M-Ctrl:Acclimation:M	5.1614570	2.1927392	8.130175	0.0000037
Ctrl:Video:M-Ctrl:Acclimation:M	7.1906823	4.2715248	10.109840	0.0000000
Ctrl:Video:M-5G:Video:M	2.0292253	-0.9123273	4.970778	0.4206779

Table S6: 3-way ANOVA and number of fish for predator response data.

Schooling: close zone-statistics

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	27285	27285	15.890	6.73e-05 ***
status	1	55438	55438	32.286	1.35e-08 ***
Sex	1	578543	578543	336.935	< 2e-16 ***
Treatment:status	1	8683	8683	5.057	0.0245 *
Treatment:Sex	1	42508	42508	24.756	6.56e-07 ***
status:Sex	1	107168	107168	62.413	2.92e-15 ***
Treatment:status:Sex	1	108213	108213	63.022	2.15e-15 ***
Residuals	21243	36475844	1717		

```
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
589 observations deleted due to missingness
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = duration ~ Treatment * status * Sex, data = zone.only)

$Treatment
```

```
            diff      lwr      upr    p adj
Ctrl-5G 2.266404 1.152002 3.380806 6.72e-05

$status
                       diff      lwr       upr p adj
Video-Acclimation -3.230784 -4.34527 -2.116298     0

$Sex
         diff       lwr      upr p adj
M-F -10.42832 -11.54272 -9.31392     0

$`Treatment:status`
                                       diff       lwr        upr     p adj
Ctrl:Acclimation-5G:Acclimation  0.9754015 -1.108865  3.0596681 0.6253357
5G:Video-5G:Acclimation         -4.4933540 -6.546348 -2.4403605 0.0000000
Ctrl:Video-5G:Acclimation       -0.9604588 -3.023065  1.1021476 0.6291417
5G:Video-Ctrl:Acclimation       -5.4687555 -7.538404 -3.3991065 0.0000000
Ctrl:Video-Ctrl:Acclimation     -1.9358603 -4.015045  0.1433246 0.0785885
Ctrl:Video-5G:Video              3.5328952  1.485061  5.5807294 0.0000552

$`Treatment:Sex`
                     diff        lwr        upr     p adj
Ctrl:F-5G:F     5.5412813   3.459745   7.622817 0.0000000
5G:M-5G:F      -7.6512547  -9.704716  -5.597793 0.0000000
Ctrl:M-5G:F    -7.7724366  -9.799291  -5.745582 0.0000000
5G:M-Ctrl:F   -13.1925359 -15.299988 -11.085084 0.0000000
Ctrl:M-Ctrl:F -13.3137179 -15.395254 -11.232182 0.0000000
Ctrl:M-5G:M    -0.1211819  -2.174643   1.932279 0.9987588

$`status:Sex`
                                    diff         lwr        upr     p adj
Video:F-Acclimation:F         1.293229  -0.7859074   3.372366 0.3796936
Acclimation:M-Acclimation:F  -5.860212  -7.9444418  -3.775981 0.0000000
Video:M-Acclimation:F       -13.544454 -15.6129292 -11.475978 0.0000000
Acclimation:M-Video:F        -7.153441  -9.2172097  -5.089672 0.0000000
Video:M-Video:F             -14.837683 -16.8855397 -12.789826 0.0000000
Video:M-Acclimation:M        -7.684242  -9.7372697  -5.631214 0.0000000
```

$`Treatment:status:Sex`

	diff	lwr	upr	p adj
Ctrl:Acclimation:F-5G:Acclimation:F	8.737266	5.2291198	12.2454115	0.0000000
5G:Video:F-5G:Acclimation:F	4.213589	0.8313144	7.5958637	0.0039771
Ctrl:Video:F-5G:Acclimation:F	6.685364	3.2181948	10.1525333	0.0000000
5G:Acclimation:M-5G:Acclimation:F	1.427724	-2.0231714	4.8786201	0.9153992
Ctrl:Acclimation:M-5G:Acclimation:F	-4.663099	-8.0759509	-1.2502468	0.0009051
5G:Video:M-5G:Acclimation:F	-12.262617	-15.6931446	-8.8320898	0.0000000
Ctrl:Video:M-5G:Acclimation:F	-6.546665	-9.9271658	-3.1661644	0.0000000
5G:Video:F-Ctrl:Acclimation:F	-4.523677	-8.0039365	-1.0434167	0.0020869
Ctrl:Video:F-Ctrl:Acclimation:F	-2.051902	-5.6147220	1.5109188	0.6569907
5G:Acclimation:M-Ctrl:Acclimation:F	-7.309541	-10.8565271	-3.7625555	0.0000000
Ctrl:Acclimation:M-Ctrl:Acclimation:F	-13.400364	-16.9103482	-9.8903807	0.0000000
5G:Video:M-Ctrl:Acclimation:F	-20.999883	-24.5270552	-17.4727104	0.0000000
Ctrl:Video:M-Ctrl:Acclimation:F	-15.283931	-18.7624666	-11.8053948	0.0000000
Ctrl:Video:F-5G:Video:F	2.471775	-0.9671760	5.9107260	0.3646973
5G:Acclimation:M-5G:Video:F	-2.785865	-6.2084080	0.6366786	0.2095827
Ctrl:Acclimation:M-5G:Video:F	-8.876688	-12.2608688	-5.4925069	0.0000000
5G:Video:M-5G:Video:F	-16.476206	-19.8782114	-13.0742009	0.0000000
Ctrl:Video:M-5G:Video:F	-10.760254	-14.1118070	-7.4087012	0.0000000
5G:Acclimation:M-Ctrl:Video:F	-5.257640	-8.7641028	-1.7511766	0.0001491
Ctrl:Acclimation:M-Ctrl:Video:F	-11.348463	-14.8174917	-7.8794340	0.0000000
5G:Video:M-Ctrl:Video:F	-18.947981	-22.4344006	-15.4615618	0.0000000
Ctrl:Video:M-Ctrl:Video:F	-13.232029	-16.6692354	-9.7948228	0.0000000
Ctrl:Acclimation:M-5G:Acclimation:M	-6.090823	-9.5435873	-2.6380590	0.0000024
5G:Video:M-5G:Acclimation:M	-13.690341	-17.1605777	-10.2201053	0.0000000
Ctrl:Video:M-5G:Acclimation:M	-7.974389	-11.3951797	-4.5535991	0.0000000
5G:Video:M-Ctrl:Acclimation:M	-7.599518	-11.0319252	-4.1671114	0.0000000
Ctrl:Video:M-Ctrl:Acclimation:M	-1.883566	-5.2659743	1.4988418	0.6952102
Ctrl:Video:M-5G:Video:M	5.715952	2.3157105	9.1161937	0.0000096

Schooling: middle zone-statistics

```
                          Df   Sum Sq Mean Sq F value   Pr(>F)
Treatment                  1       22      22   0.017    0.897
status                     1    61169   61169  45.540 1.53e-11 ***
Sex                        1    70806   70806  52.715 3.99e-13 ***
Treatment:status           1      864     864   0.643    0.422
Treatment:Sex              1     1785    1785   1.329    0.249
status:Sex                 1    49629   49629  36.949 1.23e-09 ***
Treatment:status:Sex       1       49      49   0.036    0.849
Residuals              21243 28533082    1343
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
589 observations deleted due to missingness
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = duration ~ Treatment * status * Sex, data = zone.only)

$Treatment
             diff        lwr      upr     p adj
Ctrl-5G 0.0649238 -0.9207056 1.050553 0.8972711

$status
                      diff     lwr      upr p adj
Video-Acclimation 3.393673 2.40797 4.379377     0

$Sex
        diff      lwr      upr p adj
M-F 3.648219 2.662589 4.633848     0

$`Treatment:status`
                                        diff       lwr      upr     p adj
Ctrl:Acclimation-5G:Acclimation -0.3564340 -2.199857 1.486989 0.9598212
5G:Video-5G:Acclimation          2.9953882  1.179625 4.811152 0.0001327
Ctrl:Video-5G:Acclimation        3.4458208  1.621555 5.270086 0.0000072
5G:Video-Ctrl:Acclimation        3.3518222  1.521328 5.182316 0.0000151
Ctrl:Video-Ctrl:Acclimation      3.8022547  1.963327 5.641183 0.0000006
Ctrl:Video-5G:Video              0.4504325 -1.360768 2.261633 0.9194032
```

$`Treatment:Sex`

	diff	lwr	upr	p adj
Ctrl:F-5G:F	0.5111452	-1.329862	2.352153	0.8918527
5G:M-5G:F	4.2259414	2.409764	6.042118	0.0000000
Ctrl:M-5G:F	3.5768875	1.784242	5.369532	0.0000017
5G:M-Ctrl:F	3.7147962	1.850867	5.578725	0.0000017
Ctrl:M-Ctrl:F	3.0657423	1.224735	4.906750	0.0001111
Ctrl:M-5G:M	-0.6490540	-2.465231	1.167123	0.7951454

$`status:Sex`

	diff	lwr	upr	p adj
Video:F-Acclimation:F	0.3078657	-1.531020	2.146751	0.9733167
Acclimation:M-Acclimation:F	0.5393319	-1.304059	2.382722	0.8759755
Video:M-Acclimation:F	6.9566137	5.127157	8.786070	0.0000000
Acclimation:M-Video:F	0.2314662	-1.593827	2.056760	0.9880607
Video:M-Video:F	6.6487480	4.837528	8.459968	0.0000000
Video:M-Acclimation:M	6.4172818	4.601488	8.233076	0.0000000

$`Treatment:status:Sex`

	diff	lwr	upr	p adj
Ctrl:Acclimation:F-5G:Acclimation:F	0.31997583	-2.782792	3.422744	0.9999860
5G:Video:F-5G:Acclimation:F	0.11639065	-2.875051	3.107832	1.0000000
Ctrl:Video:F-5G:Acclimation:F	0.80844521	-2.258081	3.874972	0.9932086
5G:Acclimation:M-5G:Acclimation:F	1.21885477	-1.833279	4.270988	0.9291249
Ctrl:Acclimation:M-5G:Acclimation:F	0.16250218	-2.855984	3.180988	0.9999998
5G:Video:M-5G:Acclimation:F	7.26445035	4.230332	10.298569	0.0000000
Ctrl:Video:M-5G:Acclimation:F	6.96473455	3.974862	9.954607	0.0000000
5G:Video:F-Ctrl:Acclimation:F	-0.20358518	-3.281690	2.874519	0.9999993
Ctrl:Video:F-Ctrl:Acclimation:F	0.48846938	-2.662655	3.639594	0.9997781
5G:Acclimation:M-Ctrl:Acclimation:F	0.89887894	-2.238241	4.035999	0.9887763
Ctrl:Acclimation:M-Ctrl:Acclimation:F	-0.15747365	-3.261867	2.946920	0.9999999
5G:Video:M-Ctrl:Acclimation:F	6.94447452	3.824878	10.064071	0.0000000
Ctrl:Video:M-Ctrl:Acclimation:F	6.64475871	3.568179	9.721338	0.0000000
Ctrl:Video:F-5G:Video:F	0.69205457	-2.349514	3.733623	0.9972838
5G:Acclimation:M-5G:Video:F	1.10246412	-1.924593	4.129521	0.9561744
Ctrl:Acclimation:M-5G:Video:F	0.04611154	-2.947016	3.039239	1.0000000
5G:Video:M-5G:Video:F	7.14805970	4.139167	10.156952	0.0000000

```
Ctrl:Video:M-5G:Video:F                6.84834390  3.884074  9.812614 0.0000000
5G:Acclimation:M-Ctrl:Video:F          0.41040956 -2.690870  3.511689 0.9999234
Ctrl:Acclimation:M-Ctrl:Video:F       -0.64594303 -3.714114  2.422228 0.9983446
5G:Video:M-Ctrl:Video:F                6.45600514  3.372453  9.539557 0.0000000
Ctrl:Video:M-Ctrl:Video:F              6.15628933  3.116264  9.196315 0.0000000
Ctrl:Acclimation:M-5G:Acclimation:M   -1.05635259 -4.110138  1.997433 0.9668830
5G:Video:M-5G:Acclimation:M            6.04559558  2.976357  9.114835 0.0000000
Ctrl:Video:M-5G:Acclimation:M          5.74587978  2.720373  8.771386 0.0000001
5G:Video:M-Ctrl:Acclimation:M          7.10194817  4.066167 10.137729 0.0000000
Ctrl:Video:M-Ctrl:Acclimation:M        6.80223236  3.810673  9.793792 0.0000000
Ctrl:Video:M-5G:Video:M               -0.29971580 -3.307048  2.707617 0.9999889
```

Schooling: far zone-statistics

```
                          Df   Sum Sq Mean Sq F value   Pr(>F)
Treatment                  1    59610   59610  32.613 1.14e-08 ***
status                     1     7366    7366   4.030 0.044715 *
Sex                        1   186440  186440 102.002  < 2e-16 ***
Treatment:status           1    11992   11992   6.561 0.010432 *
Treatment:Sex              1    82944   82944  45.379 1.67e-11 ***
status:Sex                 1    27140   27140  14.848 0.000117 ***
Treatment:status:Sex       1    95188   95188  52.078 5.51e-13 ***
Residuals              21243 38827998    1828
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1
589 observations deleted due to missingness
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = duration ~ Treatment * status * Sex, data = zone.only)

$Treatment
             diff       lwr      upr p adj
```

```
Ctrl-5G -3.349922 -4.499694 -2.20015     0

$status
                      diff      lwr      upr     p adj
Video-Acclimation 1.177644 0.027785 2.327502 0.0447156

$Sex
        diff      lwr      upr p adj
M-F 5.919924 4.770152 7.069697     0

$`Treatment:status`
                                       diff        lwr         upr     p adj
Ctrl:Acclimation-5G:Acclimation -1.8244355 -3.9748546  0.32598361 0.1288736
5G:Video-5G:Acclimation          2.6613472  0.5431938  4.77950069 0.0068417
Ctrl:Video-5G:Acclimation       -2.1685697 -4.2966411 -0.04049834 0.0438559
5G:Video-Ctrl:Acclimation        4.4857827  2.3504452  6.62112022 0.0000003
Ctrl:Video-Ctrl:Acclimation     -0.3441342 -2.4893102  1.80104175 0.9763927
Ctrl:Video-5G:Video             -4.8299169 -6.9427472 -2.71708670 0.0000000

$`Treatment:Sex`
                     diff         lwr       upr     p adj
Ctrl:F-5G:F   -7.5885223 -9.73612386 -5.440921 0.0000000
5G:M-5G:F      2.0276740 -0.09096193  4.146310 0.0665449
Ctrl:M-5G:F    2.3489027  0.25771767  4.440088 0.0204010
5G:M-Ctrl:F    9.6161963  7.44185600 11.790537 0.0000000
Ctrl:M-Ctrl:F  9.9374250  7.78982346 12.085027 0.0000000
Ctrl:M-5G:M    0.3212287 -1.79740723  2.439865 0.9799350

$`status:Sex`
                                    diff       lwr       upr     p adj
Video:F-Acclimation:F       -1.097618 -3.242744  1.047509 0.5535491
Acclimation:M-Acclimation:F  3.621148  1.470767  5.771530 0.0000896
Video:M-Acclimation:F        7.041311  4.907184  9.175438 0.0000000
Acclimation:M-Video:F        4.718766  2.589495  6.848036 0.0000000
Video:M-Video:F              8.138929  6.026075 10.251782 0.0000000
Video:M-Acclimation:M        3.420163  1.301974  5.538352 0.0001967

$`Treatment:status:Sex`
```

	diff	lwr	upr	p adj
Ctrl:Acclimation:F-5G:Acclimation:F	-10.3569096	-13.9764004	-6.7374189	0.0000000
5G:Video:F-5G:Acclimation:F	-3.6127493	-7.1023738	-0.1231248	0.0362779
Ctrl:Video:F-5G:Acclimation:F	-8.5429290	-12.1201426	-4.9657155	0.0000000
5G:Acclimation:M-5G:Acclimation:F	-4.5052990	-8.0657226	-0.9448755	0.0031587
Ctrl:Acclimation:M-5G:Acclimation:F	1.6569503	-1.8642221	5.1781228	0.8452745
5G:Video:M-5G:Acclimation:F	4.7656633	1.2262546	8.3050721	0.0011672
Ctrl:Video:M-5G:Acclimation:F	-0.5951182	-4.0829125	2.8926761	0.9995799
5G:Video:F-Ctrl:Acclimation:F	6.7441603	3.1534406	10.3348800	0.0000003
Ctrl:Video:F-Ctrl:Acclimation:F	1.8139806	-1.8619200	5.4898812	0.8100325
5G:Acclimation:M-Ctrl:Acclimation:F	5.8516106	2.1920472	9.5111740	0.0000345
Ctrl:Acclimation:M-Ctrl:Acclimation:F	12.0138600	8.3924730	15.6352470	0.0000000
5G:Video:M-Ctrl:Acclimation:F	15.1225730	11.4834518	18.7616941	0.0000000
Ctrl:Video:M-Ctrl:Acclimation:F	9.7617914	6.1728504	13.3507324	0.0000000
Ctrl:Video:F-5G:Video:F	-4.9301797	-8.4782794	-1.3820800	0.0006694
5G:Acclimation:M-5G:Video:F	-0.8925497	-4.4237210	2.6386216	0.9947566
Ctrl:Acclimation:M-5G:Video:F	5.2696996	1.7781083	8.7612910	0.0001296
5G:Video:M-5G:Video:F	8.3784126	4.8684313	11.8883940	0.0000000
Ctrl:Video:M-5G:Video:F	3.0176311	-0.4402966	6.4755588	0.1399828
5G:Acclimation:M-Ctrl:Video:F	4.0376300	0.4198754	7.6553845	0.0164714
Ctrl:Acclimation:M-Ctrl:Video:F	10.1998794	6.6207472	13.7790116	0.0000000
5G:Video:M-Ctrl:Video:F	13.3085923	9.7115176	16.9056671	0.0000000
Ctrl:Video:M-Ctrl:Video:F	7.9478108	4.4015112	11.4941105	0.0000000
Ctrl:Acclimation:M-5G:Acclimation:M	6.1622494	2.5998981	9.7246006	0.0000043
5G:Video:M-5G:Acclimation:M	9.2709624	5.6905845	12.8513402	0.0000000
Ctrl:Video:M-5G:Acclimation:M	3.9101808	0.3808182	7.4395434	0.0178999
5G:Video:M-Ctrl:Acclimation:M	3.1087130	-0.4326349	6.6500609	0.1348037
Ctrl:Video:M-Ctrl:Acclimation:M	-2.2520685	-5.7418306	1.2376936	0.5119493
Ctrl:Video:M-5G:Video:M	-5.3607815	-8.8689433	-1.8526198	0.0000988

Table S7: 3-way ANOVA and number of fish for schooling data.

Startle: Nested anova-statistics

Error: Tap

```
          Df Sum Sq Mean Sq F value Pr(>F)
Treatment  1  7.675   7.675   2.260  0.272
Sex        1  3.989   3.989   1.175  0.392
Residuals  2  6.791   3.396

Error: Tap:Treatment
          Df Sum Sq Mean Sq F value  Pr(>F)
Treatment  1 11.127  11.127  69.311 0.00363 **
Sex        1  0.083   0.083   0.519 0.52339
Residuals  3  0.482   0.161
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1

Error: Within
           Df Sum Sq Mean Sq F value   Pr(>F)
Sex         1    237  236.82   35.06 4.59e-09 ***
Residuals 875   5910    6.75
---
Signif. codes:  0 ë***í 0.001 ë**í 0.01 ë*í 0.05 ë.í 0.1 ë í 1


Startle: Posthoc
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mean.distance ~ Treatment + Tap + Sex, data = (sum))

$Treatment
             diff        lwr       upr     p adj
Ctrl-5G 0.2231004 -0.1188736 0.5650745 0.2007339

$Tap
           diff        lwr       upr     p adj
2-1 -0.19360249 -0.9449570 0.5577520 0.9555210
3-1 -0.30854178 -1.0620278 0.4449442 0.7963760
4-1 -0.24445474 -0.9979407 0.5090312 0.9018022
5-1 -0.43969612 -1.1910506 0.3116584 0.4979819
3-2 -0.11493929 -0.8684253 0.6385467 0.9936697
4-2 -0.05085226 -0.8043382 0.7026337 0.9997412
5-2 -0.24609363 -0.9974481 0.5052609 0.8986702
```

```
4-3  0.06408704 -0.6915244 0.8196985 0.9993603
5-3 -0.13115434 -0.8846403 0.6223317 0.9895252
5-4 -0.19524137 -0.9487274 0.5582446 0.9546285

$Sex
        diff       lwr      upr p adj
M-F 1.033826 0.6918128 1.375839     0
```

Table S8: 2-way nested ANOVA for startle tap data. **/*** statistically significant

Reference:

1. Dasgupta, S.; Dunham, C. L.; Truong, L.; Simonich, M. T.; Sullivan, C. M.; Tanguay, R. L., Phenotypically Anchored mRNA and miRNA Expression Profiling in Zebrafish Reveals Flame Retardant Chemical Toxicity Networks. *Frontiers in cell and developmental biology* **2021,** *9*, 654.