

Fig. S1. Increased gill stiffness (calculated as a spring constant from force/length curves) after terrestrial acclimation is reversible in *K. marmoratus*. Force relative to gill arch length required to deform all four gill arches from the left side of control fish in water ($n=15$), after 7d ($n=13$) or 14d in air ($n=13$), or 14d in air followed by 14d ($n=12$) or 12 weeks ($n=12$) recovery in water. Different letters above treatment groups denote significant effects of treatment (two-way repeated-measures ANOVA and Holm-Sidak *post hoc* tests, $P<0.001$).

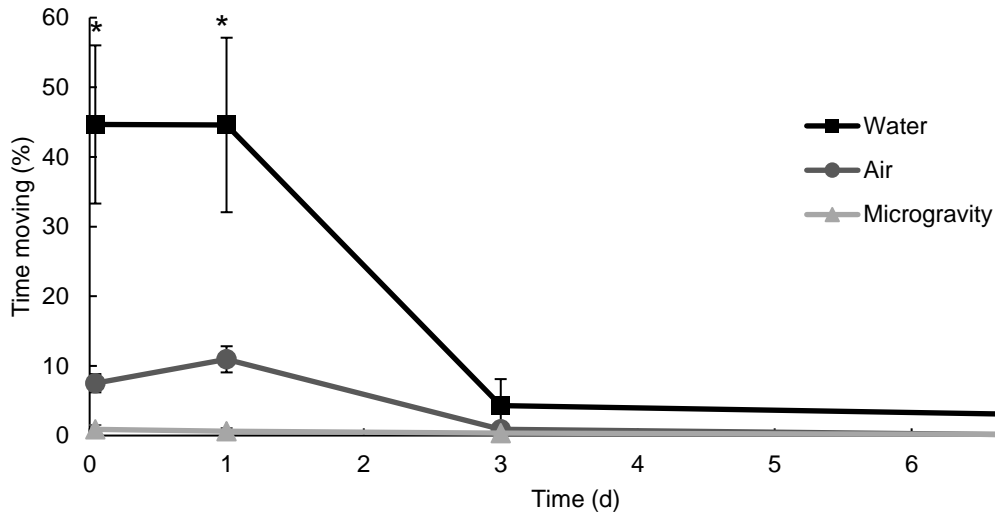


Fig. S2. Activity of mangrove rivulus in the simulated microgravity experiment. Control fish in water (black line; $n=6$) reduced activity to levels comparable to terrestrially-acclimated fish after 3 d of acclimation to a 6-well plate. Terrestrially-acclimated fish moved infrequently under both normal gravity conditions (dark grey line; $n=6$), and when exposed to simulated microgravity in the random positioning machine (light grey line; $n=6$). All fish were fasted for the duration of the experiment. Asterisks denote significant differences between water-acclimated and each terrestrially-acclimated group within a time point (repeated measures ANOVA and Holm-Sidak *post hoc* tests, treatment*time

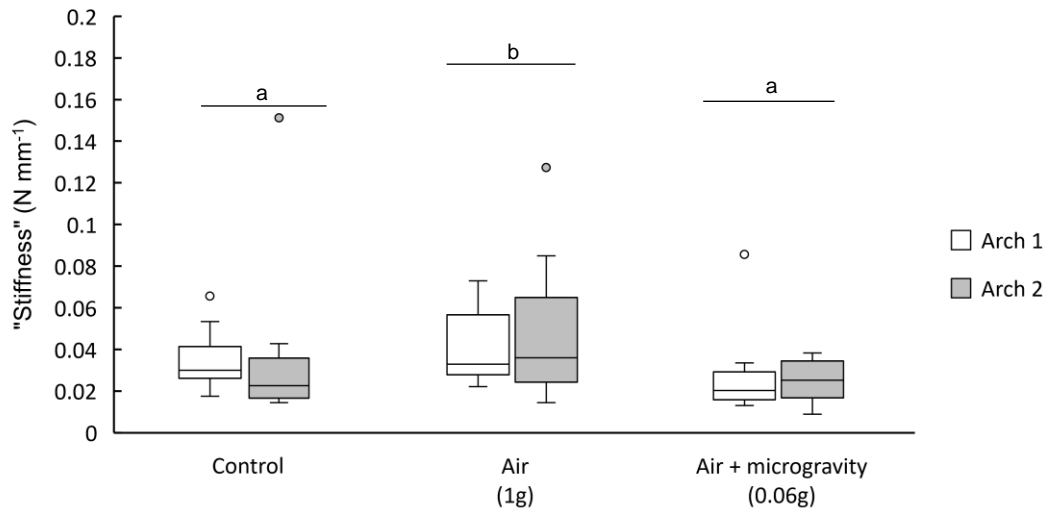


Fig. S3. Increased gill stiffness (calculated as a spring constant from force/length curves) after terrestrial acclimation in *K. marmoratus* does not occur in simulated microgravity. Force relative to gill arch length required to deform gill arches 1 and 2 in fish acclimated to water at 1g ($n=12$), 7d in air at 1g ($n=12$), or 7d in simulated microgravity (0.06g; $n=10$). Different letters above treatment groups denote significant effects of treatment (two-way repeated-measures ANOVA and Holm-Sidak *post hoc* tests, $P=0.025$).

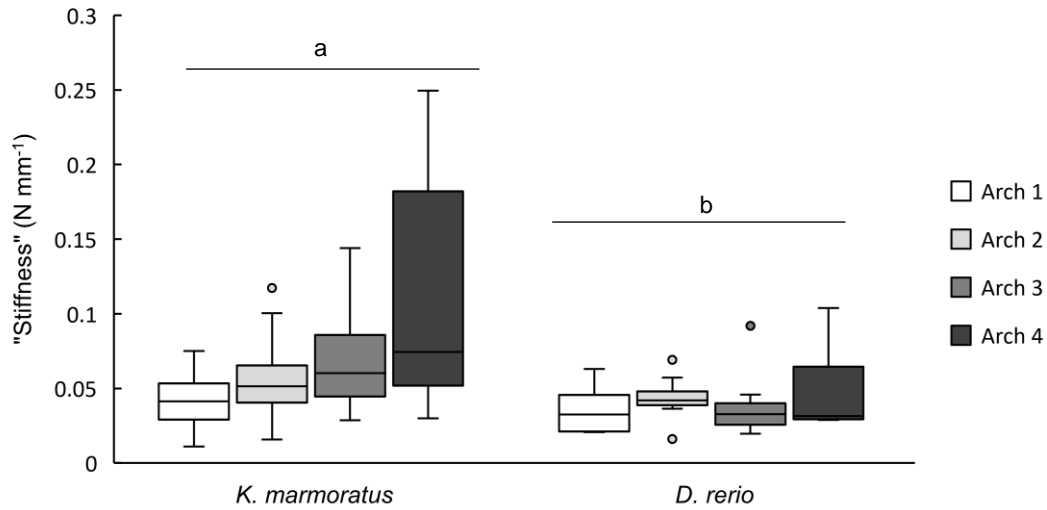


Fig. S4. Increased gill stiffness (calculated as a spring constant from force/length curves) of *K. marmoratus* versus *D. rerio* gill arches. Force relative to gill arch length required to deform gill arches from the left side of amphibious *K. marmoratus* acclimated to water ($n=15$) compared to those of the fully aquatic zebrafish ($n=9$). The *K. marmoratus* data presented here are the control values repeated from Fig. S1 for comparison. Different letters above treatment groups denote significant differences between species (two-way repeated-measures ANOVA and Holm-Sidak *post hoc* tests, $P=0.006$).

Table S1. Abundances of all collagen isoforms identified in the gills of *Kryptolebias marmoratus* acclimated to water or 14d in air.

NCBI Accession	Protein annotation	Coverage (%)	#Peptides	#Unique	Relative abundance (air/water)	P value
gi 1041063337	collagen alpha-2(I) chain	67	80	75	1.03	0.2041737945
gi 1041090756	collagen alpha-1(I) chain-like isoform X1	55	73	67	1.3	0.0000426580
gi 1041065690	collagen alpha-1(I) chain-like	50	70	66	1.33	0.0000012589
gi 1041073117	collagen alpha-1(II) chain isoform X2	12	13	10	1.09	0.0398107171
gi 1041074737	collagen alpha-1(II) chain-like isoform X2	4	5	2	0.89	0.0002398833
gi 1041075806	collagen alpha-2(IV) chain isoform X2	2	3	2	0.88	0.0028183829
gi 1041145053	collagen alpha-4(IV) chain-like	1	1	1	1.26	0.0000013804
gi 1041075808	collagen alpha-1(IV) chain	3	4	3	1.65	0.0001819701
gi 1041138366	collagen alpha-6(IV) chain-like	2	4	3	1.23	0.0034673685
gi 1041132849	collagen alpha-2(V) chain	8	12	9	0.99	0.7762471166
gi 1041125201	collagen alpha-1(V) chain-like isoform X2	5	9	6	1.2	0.0000012882
gi 1041106386	collagen alpha-1(VI) chain	33	33	32	1.24	0.0000676083
gi 1041145385	collagen alpha-3(VI) chain partial	27	56	55	1.2	0.0001096478
gi 1041106315	collagen alpha-2(VI) chain-like	33	37	33	1.18	0.4897788194
gi 1041145452	collagen alpha-3(VI) chain-like partial	23	39	37	1.1	0.0257039578
gi 1041113033	collagen alpha-2(VI) chain-like isoform X2	20	19	18	1.01	0.4677351413
gi 1041138699	collagen alpha-3(VI) chain-like partial	17	25	25	1.05	0.1348962883
gi 1041145951	collagen alpha-4(VI) chain-like partial	23	15	15	1.19	0.1621810097
gi 1041141506	collagen alpha-2(V) chain-like	12	12	11	1.13	0.0000933254
gi 1041063832	collagen alpha-2(IX) chain	5	3	1	0.61	0.0263026799
gi 1041119925	collagen alpha-1(IX) chain-like isoform X3	2	1	1	2.37	0.0000000000
gi 1041126807	collagen alpha-1(IX) chain-like	2	1	1	0.77	0.3801893963
gi 1041074889	collagen alpha-3(IX) chain isoform X1	2	2	1	0.89	0.0000000000
gi 1041062211	collagen alpha-1(X) chain-like	2	1	1	8.84	0.0000000000
gi 1041144154	collagen alpha-1(X) chain-like	3	1	1	1.69	0.0000000000
gi 1041118682	collagen alpha-1(XI) chain-like isoform X2	6	9	5	0.95	0.2398832919
gi 1041088438	collagen alpha-1(XI) chain-like	8	13	8	1.26	0.0011748976
gi 1041059509	collagen alpha-1(XI) chain-like	3	5	1	1.02	0.0000000000
gi 1041061459	collagen alpha-1(XII) chain isoform X2	25	72	65	0.74	0.0004365158
gi 1041140211	collagen alpha-1(XII) chain-like partial	17	19	18	0.98	0.1778279410
gi 1041146700	collagen alpha-1(XII) chain-like isoform X1	8	2	1	2.35	0.0000000000
gi 1041147096	collagen alpha-1(XII) chain-like	4	1	1	0.88	0.0056234133
gi 1041146058	collagen alpha-1(XII) chain-like partial	6	6	3	1.16	0.0025703958
gi 1041148375	collagen alpha-1(XII) chain-like partial	6	2	2	1.26	0.8511380382



Movie 1. Time lapse video of *K. marmoratus* during acclimation to microgravity in the random positioning machine. Images were captured every 2s and are played back at 60x speed.