

Supplementary material

Reproduction and embryo viability of a range-limited tropical freshwater fish exposed to fluctuating hypoxia

Nicole Flint^{A,B,E}, Richard G. Pearson^{B,C} and Michael R. Crossland^{C,D}

^ACentral Queensland University, School of Health, Medical and Applied Sciences, Bruce Highway, North Rockhampton, Qld 4702, Australia.

^BJames Cook University, College of Science and Engineering, Douglas, Townsville, Qld 4811, Australia.

^CJames Cook University, TropWater, Douglas, Townsville, Qld 4811, Australia.

^DUniversity of Sydney, School of Life and Environmental Sciences, Sydney, NSW 2006, Australia.

^ECorresponding author. Email: n.flint@cqu.edu.au

Table S1. Number of Utchee Creek rainbowfish eggs used to determine health of eggs and larvae produced by fish exposed to fluctuating hypoxia

The number of eggs in each nursery aquarium was governed by the number of eggs laid by each brood group on the final day of oxygen cycling. As the 5% DO treatment was lethal to adult fish, no eggs were collected from aquaria 1A and 1B

Nursery aquarium number	Treatment for breeding adults (minimum DO saturation)	Number of eggs
2A	10%	48
2B	10%	0
3A	20%	47
3B	20%	22
4A	30%	55
4B	30%	43
5A	40%	2
5B	40%	28
6A	50%	7
6B	50%	14
7A	60%	32
7B	60%	38
8A	100%	62
8B	100%	58
8C	100%	45
8D	100%	1

Table S2. Linear regression coefficients of determination (r^2), $F_{d.f.}$ and probability (P) for DO treatment and egg size

Egg size was measured on the day of spawning (day 1) and then once per week through the 4-week duration of the experiment

Time of measurement	r^2	$F_{1,14}$	P
Day 1	0.161	2.696	0.123
Day 7	0.037	0.465	0.508
Day 14	0.040	0.579	0.459
Day 21	0.014	0.181	0.678
Day 28	0.000	0.002	0.963

Table S3. Linear regression coefficients of determination (r^2), $F_{d.f.}$ and probability (P) for DO treatment and egg types within histological sections of ovaries of female Utchee Creek rainbowfish (five sections per female fish, combined)

Egg type in histological sections	r^2	$F_{1,30}$	P
Total eggs	0.018	0.555	0.462
Late globule stage (fully yolked) eggs	0.066	2.105	0.157
Hydrated eggs	0.009	0.259	0.614
Atretic eggs	0.069	2.240	0.145
Scar tissue infiltration	0.003	0.085	0.773

Table S4. Linear regression coefficients of determination (r^2), $F_{d.f.}$ and probability (P) for DO treatment and cell types within histological sections of testes of male Utchee Creek rainbowfish (five transects per male fish, combined)

Egg type in histological sections	r^2	$F_{1,46}$	P
Interstitial tissue	0.004	0.180	0.674
Empty lumen	0.136	7.254	0.010
Spermatocytes and spermatids	0.017	0.779	0.382
Sperm	0.006	0.276	0.602
Germ cells and spermatogonia	0.034	1.634	0.208

Table S5. Number of Utchee Creek rainbowfish eggs used to determine health of eggs and larvae exposed to fluctuating hypoxia

The number of eggs in each experiment aquarium was governed by the number of eggs laid by the two contributing brood groups (which were held in normoxic conditions)

Experimental aquarium number	Treatment for eggs and larvae (minimum DO saturation)	Number of eggs
1A	5%	23
1B	5%	21
2A	10%	20
2B	10%	60
3A	20%	30
3B	20%	95
4A	30%	79
4B	30%	45
5A	40%	21
5B	40%	25
6A	50%	38
6B	50%	20
7A	60%	31
7B	60%	36
8A	100%	47
8B	100%	29
8C	100%	20
8D	100%	24