ELECTRONIC APPENDIX

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Strategic growth decisions in helper cichlids

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Electronic Appendix A

Strategic growth decisions in helper cichlids

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We created 32 artificial breeding groups by introducing a focal large helper male (standard length SL = 40-45 mm) and a small helper male (SL = 30-35 mm) in each compartment. Two days later, we added a breeding female (SL = 55-65 mm) and either a large or a small breeder male (each in half of the cases, SL = 70-75 mm or 46-51 mm respectively). Large breeder males were 30.0 mm (range 25.3-33.5 mm) and small breeder males were 6.2 mm (range 3.5-9.3 mm) larger than the focal large helper males. Non-accepted group-members were replaced, until a stable group with a size-dependent dominance rank formed (*i.e*. individuals showed the submissive behaviours tail-quivering and zig-zag swimming towards larger group members). We measured body mass (1 mg accuracy) and SL (0.5 mm accuracy, measured by all three authors and averaged), and repeated the measurements after 30 days to determine growth (sequence 1). After 30 days, all breeder males were replaced: half of the groups with a large breeder male now received a new large breeder male of similar size (27.4 mm larger then the focal helper on day 30, range 24.5-30.2 mm), the other half received a new small breeder male (6.8 mm larger then the focal helper on day 30, range 5.3-8.7 mm). Similarly, half of the groups with a small breeder male now received a new large breeder male (27.1 mm larger than the focal helper on day 30, range 25.8-30.7 mm), the other half received a new small breeder male (6.3 mm larger than the focal helper on day 30, range 5.5-9.5 mm). Such breeder male replacements occur naturally (Taborsky & Limberger 1981; Balshine *et al.* 1998) and helpers were all accepted. Again, growth was measured after 30 days (sequence 2).

The four treatments were abbreviated as follows: SS, SL, LL, LS, where: S. or .S = small breeder male in sequence 1 or 2, L. or .L = large breeder male in sequence 1 or 2 (figure 1). Additionally, we created 8 single breeding pairs (SP, focal male and female breeder of SL = 40-45 mm, similar in size to ensure stable rank, pair building and breeding) and growth was determined on day 30 and 60 (sequence 1 and 2) to compare with the focal helpers and test for status-dependent growth. Each section included groups of all five treatments (figure 1), and no significant effects of section on the results were detected.

All statistical analyses were done with General Linear Models (GLM) in SPSS 11.0 (Lead Technologies Inc.). To assess the status-dependent growth hypothesis, male breeders from the SP treatment were compared with the focal male helpers in all other treatments (SS, SL, LL, LS) using Repeated Measures GLM. Two helper males were discarded from the analyses, since one helper died after the first sequence was completed and one helper changed status during the second sequence. As expected, male breeders grew faster than similar sized large helper males, in line with status-dependent growth (table A1).

To assess the strategic growth hypothesis (using ln[helper growth] as dependent variable, to account for exponential diminishing growth in fish), focal helper males breeding with differently sized breeding males were compared, controlling for random individual, fixed sequence and the ln-transformed helper size at the start of each sequence effects in a Mixed GLM. Since the focal growth rate was highly sensitive to both the initial focal helper size at each sequence (ln[helper

SL or mass]) and the exact difference in ln[SL or mass] between the focal helper and his breeder male, exact values or differences in values were fitted, instead of fixed treatment effects. All 32 helpers were measured at the start (initial size) and the end of the first and the second sequence. The only two exceptions were one male helper who died after the first sequence was completed and was replaced with a size-matched helper, and one helper who changed status during the second sequence; the results of this sequence were discarded in all analyses (hence sample size reduced from 64 to 63). The results are shown in table A2. The same analyses were performed on the small helper males (table A3). All 32 small helpers were measured at the start (initial size) and the end of the first and the second sequence, with one group discarded from the analyses as described above (n = 63). Since small helpers might adjust their growth rate not to the difference in initial size with the large helper or the breeding male, but rather to the growth rate of the large helper or the breeder male, we also constructed a model incorporating these two factors. However, growth of the large helper and breeder male did not affect growth of the small helper male (ln[growth in SL] of large helper: P=0.60, of breeder male: P=0.31; ln[growth in mass] of large helper male: P=0.84, of breeder male: P=0.66).

Table 1. Status-dependent growth in the cichlid *Neolamprologus pulcher*. Results of SPSS Repeated Measures GLMs on 30 day growth in SL and mass as dependent variables, showing breeder males (n = 8) that were initially the same size as large helper males (n = 30) grow faster. All interactions were non-significant.

	Mean Square	**d.f.**	***F***	***P***	***Coefficient* ± *s.e.*** *(Sequence 1 / 2)*
Dependent Variable: ***growth SL***					
Tests of Between-Subjects Effects					
Intercept	882.501	1	797.7	< 0.0001	Seq. 1: 3.317 ± 0.212 Seq. 2: 4.150 ± 0.187
Status	10.039	1	9.08	0.005	Seq. 1: 1.143 ± 0.462* Seq. 2: 0.640 ± 0.407*
Error	1.106	36			
Tests of Within-Subjects Effects					
Sequence	4.271	1	4.27	0.077	
Sequence * Status	0.799	1	0.62	0.436	
Error(Sequence)	1.288	36			
Dependent Variable: ***growth mass***					
Tests of Between-Subjects Effects					
Intercept	44626304.1	1	884.8	< 0.0001	Seq. 1: 737.99 ± 36.94 Seq. 2: 874.10 ± 42.07
Status	904002.2	1	17.92	<0.001	Seq. 1: 306.64 ± 80.50* Seq. 2: 228.40 ± 91.68*
Error	50.437.1	36			
Tests of Within-Subjects Effects					
Sequence	118837.764	1	2.73	0.107	
Sequence * Status	19330.839	1	0.44	0.51	
Error(Sequence)	43575.056	36			

* Large helper males are the reference category: coefficient is set to zero.

Box's M Test of Equality of Covariance Matrices, Growth SL: M = 3.561, d.f.1 = 3, d.f.2 = 2256.262, F = 1.057, P = 0.366. Growth mass: M = 4.368, d.f.1 = 3, d.f.2 = 2256.262, F = 1.297, P = 0.274.

Levene's Test of Equality of Error Variances, Growth SL: Sequence 1, $F_{1,36} = 1.261$, $P = 0.269$; Sequence 2, $F_{1,36} = 2.101$, $P = 0.156$. Growth mass: Sequence 1, $F_{1,36} = 0.005$, $P = 0.945$; Sequence 2, $F_{1,36} = 2.586$, $P = 0.117$.

Table A2. Strategic reduced growth in large male helpers of the cichlid *Neolamprologus pulcher*. Results of SPSS Mixed GLMs on 30 day growth in ln-transformed SL and mass as dependent variables, showing that large helper males grew more slowly when helping small breeder males (*n* = 63). 'Sequence' was fitted as a fixed factor, 'Individual' was fitted as a random factor, whereas the ln-transformed initial helper size at sequence 1 or 2 (abbreviated ln[helper size]) and the ln-transformed difference in size between the breeder male and helper male (abbreviated ln[difference size]) were both fitted as continuous factors. All interactions were non-significant.

	Mean Square	**d.f.**	***F***	***P***	***Coefficient ± s.e.*** *(Sequence 1 / 2)*
Dependent Variable: ***ln[growth SL]***					
Intercept	2.771	1	82.79	< 0.0001	56.914 ± 6.227
Sequence	3.487	1	104.2	< 0.0001	-1.442 ± 0.141*
Individual	0.191	31	5.7	< 0.001	
Ln[helper SL]	2.664	1	79.6	< 0.001	-14.702 ± 1.648
Ln[difference SL]	0.271	1	8.11	0.008	0.125 ± 0.044
Error	0.033	28			
Dependent Variable: ***ln[growth mass]***					
Intercept	1.701	1	32.95	< 0.001	30.746 ± 5.342
Sequence	1.3	1	25.18	< 0.001	-1.203 ± 0.240*
Individual	0.138	31	2.68	0.005	
Ln[helper mass]**	1.026	1	19.87	< 0.001	-3.093 ± 0.694
Ln[difference mass]**	0.011	1	0.22	0.643	0.019 ± 0.042
Error	0.052	28			

* Sequence 2 is the reference category: coefficient is set to zero.
** Fitting ln[helper SL] and ln[difference SL] instead of mass gave essentially the same results.

Table A3. No evidence for adjustments in growth were detected in the small male helpers of the cichlid *Neolamprologus pulcher*. Results of SPSS Mixed GLMs on 30 day growth in ln-transformed SL and mass as dependent variables (n = 63). 'Sequence' was fitted as a fixed factor, 'Individual' was fitted as a random factor, whereas the ln-transformed initial helper size at sequence 1 or 2 (abbreviated ln[helper size]) and the ln-transformed difference in size between the breeder male and small helper male (abbreviated ln[difference size bs]) or the difference in size between the large helper male and small helper male (abbreviated ln[difference size ls]) were all three fitted as continuous factors. All interactions were non-significant.

	Mean Square	**d.f.**	***F***	***P***	***Coefficient ± s.e.*** *(Sequence 1 / 2)*
Dependent Variable: ***ln[growth SL]***					
Intercept	1.351	1	16.21	< 0.001	42.060 ± 10.406
Sequence	1.326	1	15.9	< 0.001	-1.328 ± 0.333*
Individual	0.112	31	1.34	0.22	
Ln[helper SL]	1.278	1	15.33	0.001	-11.483 ± 2.933
Ln[difference SL bs]	0	1	0.02	0.897	0.016 ± 0.121
Error	0.083	28			
Dependent Variable: ***ln[growth SL]***					
Intercept	0.595	1	7.26	0.012	36.143 ± 13.344
Sequence	0.733	1	8.94	0.006	-1.177 ± 0.394*
Individual	0.105	31	1.29	0.25	
Ln[helper SL]	0.642	1	7.84	0.009	-10.019 ± 3.579
Ln[difference SL ls]	0.041	1	0.5	0.484	0.347 ± 0.489
Error	0.082	28			
Dependent Variable: ***ln[growth mass]***					
Intercept	0.712	1	10.47	0.003	19.223 ± 5.907
Sequence	0.424	1	6.23	0.019	-0.907 ± 0.363*
Individual	0.11	31	1.61	0.102	
Ln[helper mass]**	0.304	1	4.46	0.044	-1.755 ± 0.831
Ln[difference mass bs]**	0.084	1	1.23	0.276	-0.075 ± 0.068

Error	0.068	28			
Dependent Variable: ***ln[growth mass]***					
Intercept	0.375	1	5 .29	0.029	18.819 ± 8.152
Sequence	0.271	1	3.83	0.061	-0.902 ± 0.461*
Individual	0.108	31	1.52	0.134	
Ln[helper mass]**	0.231	1	3.25	0.082	-1.731 ± 0.960
Ln[difference mass ls]**	0	1	0.03	0.867	-0.057 ± 0.335
Error	0.071	28			

* Sequence 2 is the reference category: coefficient is set to zero.
** Fitting ln[helper SL] and ln[difference SL] instead of mass gave essentially the same results.