# Appendix C from S. Fischer et al., "Rearing-Group Size Determines Social Competence and Brain Structure in a Cooperatively Breeding Cichlid"
(Am. Nat., vol. 186, no. 1, p. 000)

Do Different Time Lags between Isolation and Behavioral Observations Confound the Effect of Early Social Experience?

The social behavior of *Neolamprologus pulcher* can be observed only from an age of about 1 month (before that, they show almost no social behavior and are too small to classify these behaviors reliably). As a result of this experimental constraint, some isolation groups had longer lag times between isolation and first behavioral recording than most other groups (in most groups, the lag was 10 days, but in isolation-day groups 0 and 10, the lag was 30 and 20 days, respectively). In this appendix, we address whether the different time lags may have confounded our observed changes in social behavior in response to time juveniles spent with the family groups (see "Results"). If the different time lags affect the behavior of juveniles, there should be a significant interaction between isolation day and day of observation, which would indicate that, at a given observation day, juveniles isolated closer to this day would differ significantly in their behavioral frequencies from juveniles isolated earlier. To test for this potential effect, we analyzed a subset of the data set using only those groups of juveniles for which recordings of all four observation days (days 30, 40, 50, and 60) were available. This data set included the subgroups of isolation days 0, 10, 20, and 60. We used general linear mixed models with aggression or submission as the dependent variable, and, as in the models for the entire data set (table 2), we included observer identity and group identity in the random term and treatment, isolation day, observation day, and the interaction between treatment and isolation day in the fixed term of the model; to test for lag effects, the interaction between isolation day and observation day was also included in the fixed term. We log-transformed isolation and observation day to obtain the same scale for each predictor variable. The interaction between isolation day and observation day was not significant in the analyses of both aggression and submission (see table C1; fig. C1). Thus, we can conclude that the different time lags between isolation and behavioral recording did not affect our results. Other results of this model showed that older test fish showed more submission (see the factor "observation day" in table C1*b*) and that fish reared in large groups tended to showed more submission the longer they had stayed in their family groups (see the interaction term "treatment × isolation day" in table C1*b*). The effects of isolation day, rearing condition, and age (factor "observation day") on the frequencies of submission are in line with the analysis using the complete data set (see table 2*b*).
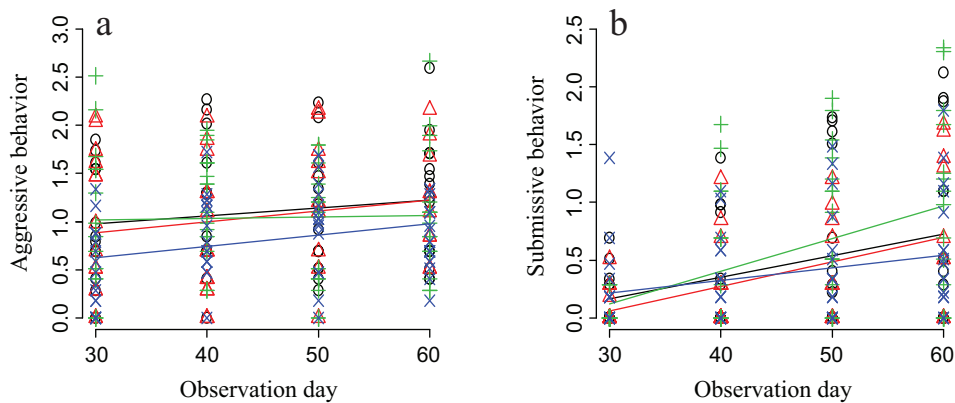
Figure C1: Aggressive (*a*) and submissive (*b*) behavior for juveniles isolated on day 0 (black circles and lines), day 10 (red triangles and lines), day 20 (green plus signs and lines), or day 60 (blue crosses and lines).

Table C1: Aggressive and submissive behavior of juveniles separated on days 0, 10, 20, or 60

Factors	Estimate ± SE	t	P
a, Aggressive behavior:			
Intercept	.602 ± .945	.636	.526
Treatment	.005 ± .177	.031	.975
Isolation day	−.044 ± .310	−1.433	.153
Observation day	.131 ± .240	.548	.548
Treatment × isolation day	.035 ± .044	.798	.426
Isolation day × observation day	.103 ± .082	1.254	.215
b, Submissive behavior:			
Intercept	−3.074 ± .869	−3.538	**<.001**
Treatment	−.066 ± .163	−.406	.686
Isolation day	.136 ± .294	.462	.644
Observation day	.941 ± .229	4.109	**<.001**
Treatment × isolation day	.080 ± .042	1.900	*.056*
Isolation day × observation day	−.047 ± .078	−.602	.543

Note: Treatment: small- or large-group rearing of juveniles; isolation day: the day juveniles were isolated from their family group; observation day: age of test fish when observations were performed. Reference category for the estimate treatment: small groups. $N = 18$ family groups and 821 observation (244 average values). Boldface indicates $P < .05$ and italics $.05 < P < .1$.