 DOI: 10.1086/668597

Appendix from R. Riesch et al., "Predation's Role in Life-History Evolution of a Livebearing Fish and a Test of the Trexler-DeAngelis Model of Maternal Provisioning"

(Am. Nat., vol. 181, no. 1, p. 78)

Additional Methods for Classification of Aborted/Degenerative Embryos

Embryo development in poeciliid fishes follows a well-described path that allows one to clearly determine an embryo's developmental stage on a relative scale (e.g., Reznick 1981; Haynes 1995; Riesch et al. 2011*b*). During all of these stages embryonic tissue is distinctly demarcated from the rest of the oocyte, has a clearly defined structure (i.e., starting with stage 10, the head capsule is clearly differentiated from the rest of the body, and even at the earliest stages, most fins are clearly identifiable as distinct buds and later as actual fins (Reznick 1981; Haynes 1995; Riesch et al. 2011*b*; fig. A2). However, in degenerating embryos this clear demarcation and embryonic structure breaks down as the embryo enters advanced stages of decomposition: The first structures (if already present) to disappear are the body pigmentation and the fins, usually in combination with a general disintegration of the embryonic body as the degenerating embryo becomes more and more amorphous. Most of the time, only the remains of the black optic cups allow any identification as to what used to be the head of the regressed embryo (see fig. A3 for an example from *Poecilia mexicana*). For this study, we counted only those embryos as aborted that showed clear signs of this decomposition.

Additional Methods for the Mixed-Model MANOVA Procedure

To test significance of the "predation regime" term in our mixed-model MANOVA, we used the MIXED procedure in SAS (version 4.3, SAS, Cary, NC). Effectively, this procedure treats dependent variables as repeated measures on the individuals, with the interaction term of predation regime by index variable (see below) testing whether differences between predation regimes were observed for at least some dependent variables, while treating population as a random effect (Rencher 2002; see Hassell et al. 2012 for details). The optimal covariance structure for the mixed-model MANOVA was selected by comparing the performance of three commonly used structures (variance components, compound symmetry, unstructured) using the Akaike information criterion (Littell et al. 1996).

Example code for employing the Kenward-Roger degrees of freedom adjustment to test the significance of a fixed effect in a multivariate mixed-model:

```
PROC MIXED DATA=females_LH method=ml;

CLASS PR Pop ID var;

MODEL LH = var PR var*PR / noint DDFM=KENWARDROGER SOLUTION;

RANDOM Pop(PR);

REPEATED/SUBJECT=ID TYPE=VC GROUP=var;

RUN;
```

In this example, LH represents the life-history dependent variables, PR is the predation regime label, Pop is the population label, ID is a unique identifier for every specimen in the data set, and var is an index variable labeling each trait (i.e., dependent variable).

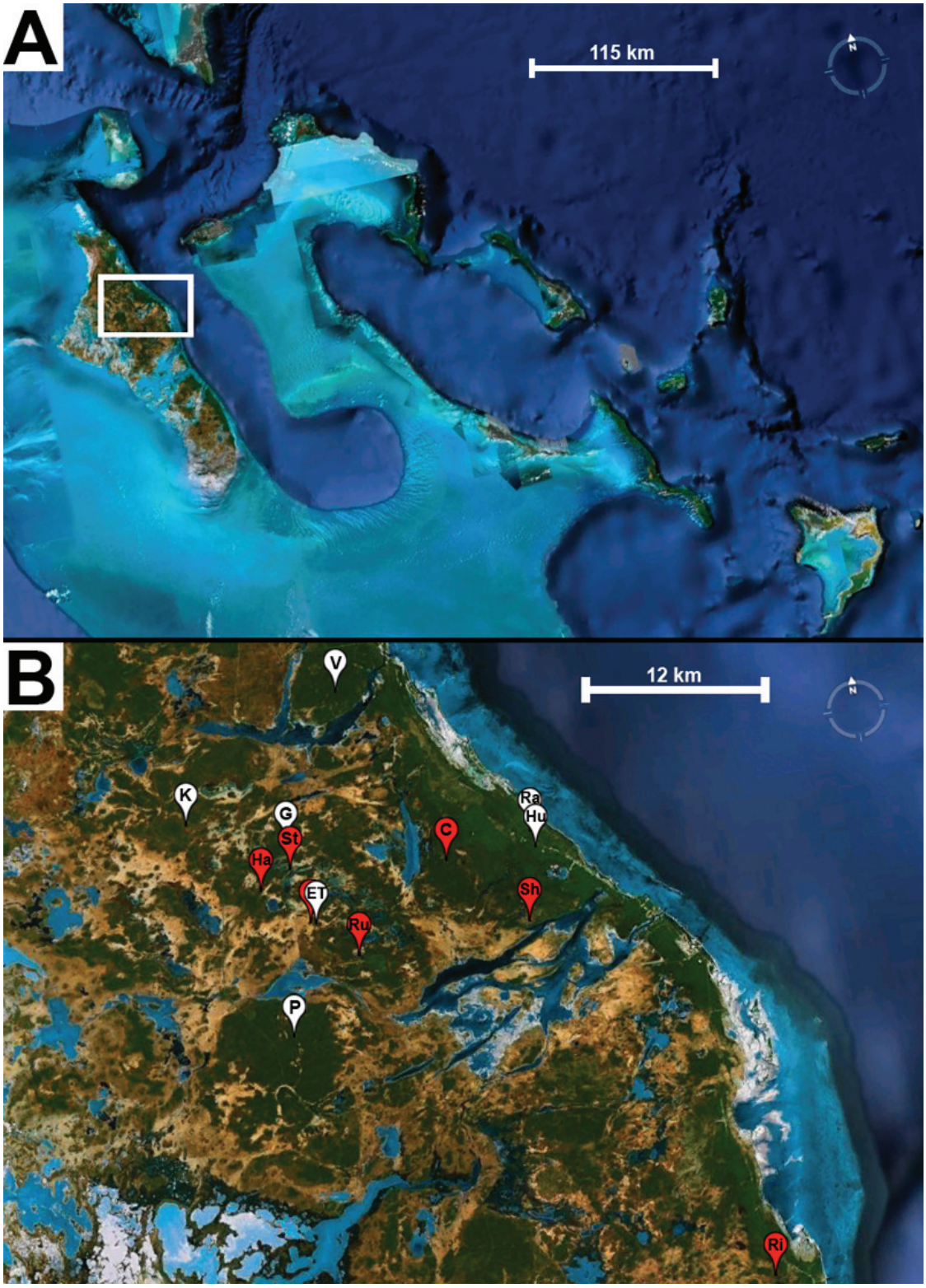

Figure A1: *A*, Overview of the general study area in the Bahamas. *B*, Magnification of the study area on northern Andros Island with locations of all sampled blue holes. Low-predation sites in white: East Twin (ET), Gollum's (G), Hubcap (Hu), Ken's (K), Pigskin (P),

Rainbow (Ra), Voy's (V). High-predation sites in red: Cousteau's (C), Hard Mile (Ha), Rivean's (Ri), Runway (Ru), Shawn's (Sh), Stalactite (St), West Twin (hidden behind white ET). Maps were created with Google Earth.

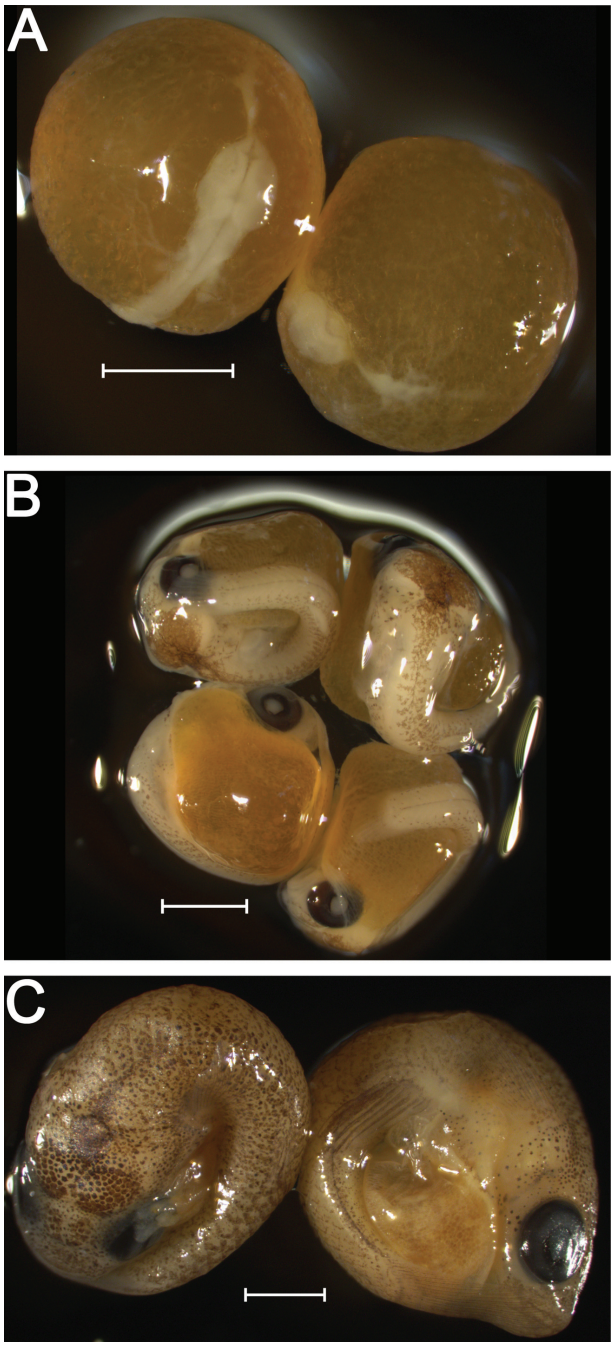

Figure A2: *Gambusia hubbsi* embryos at different representative developmental stages: *A*, stage 10; *B*, stage 30; and *C*, stage 50. Scale bar = 1 mm.

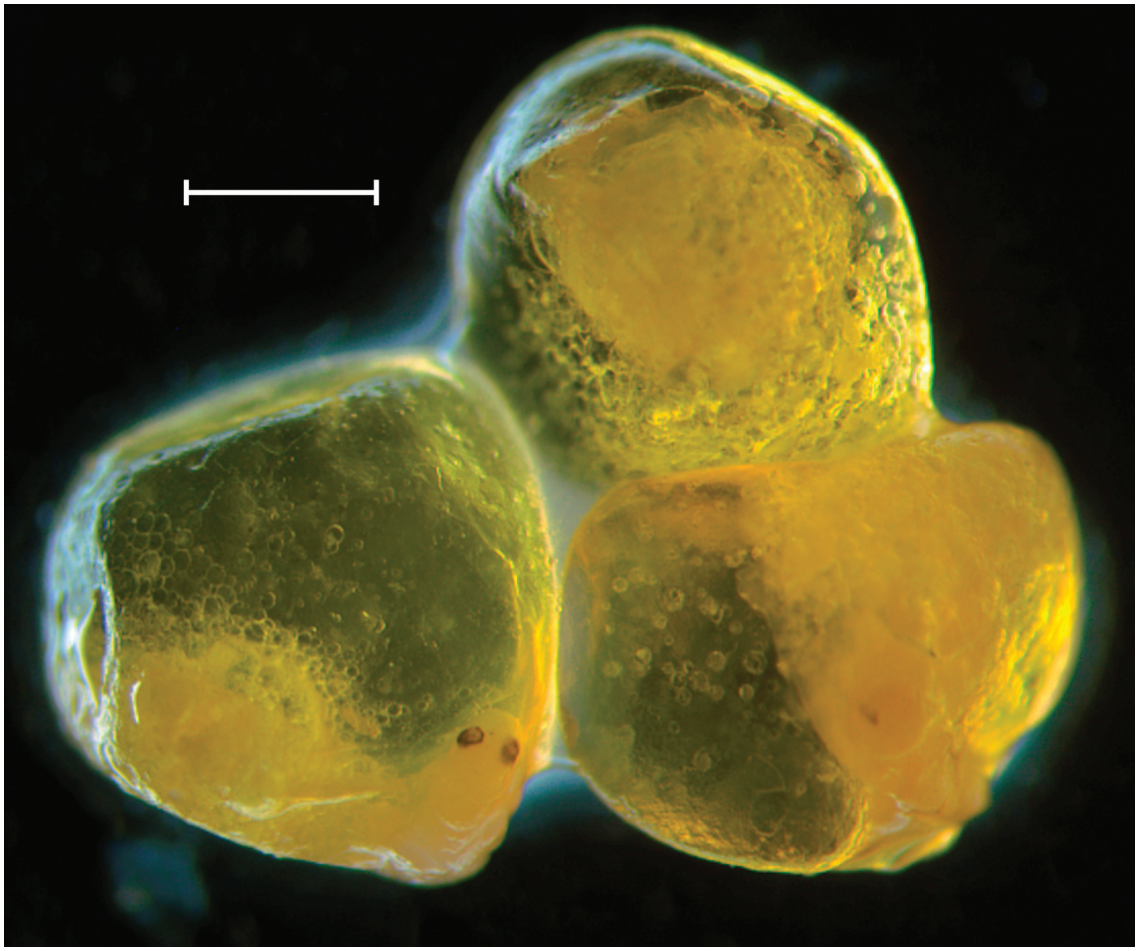

Figure A3: Regressing embryos of *Poecilia mexicana* in the advanced stages of decomposition; only these advanced stages were scored as aborted in this study. Scale bar = 1 mm.

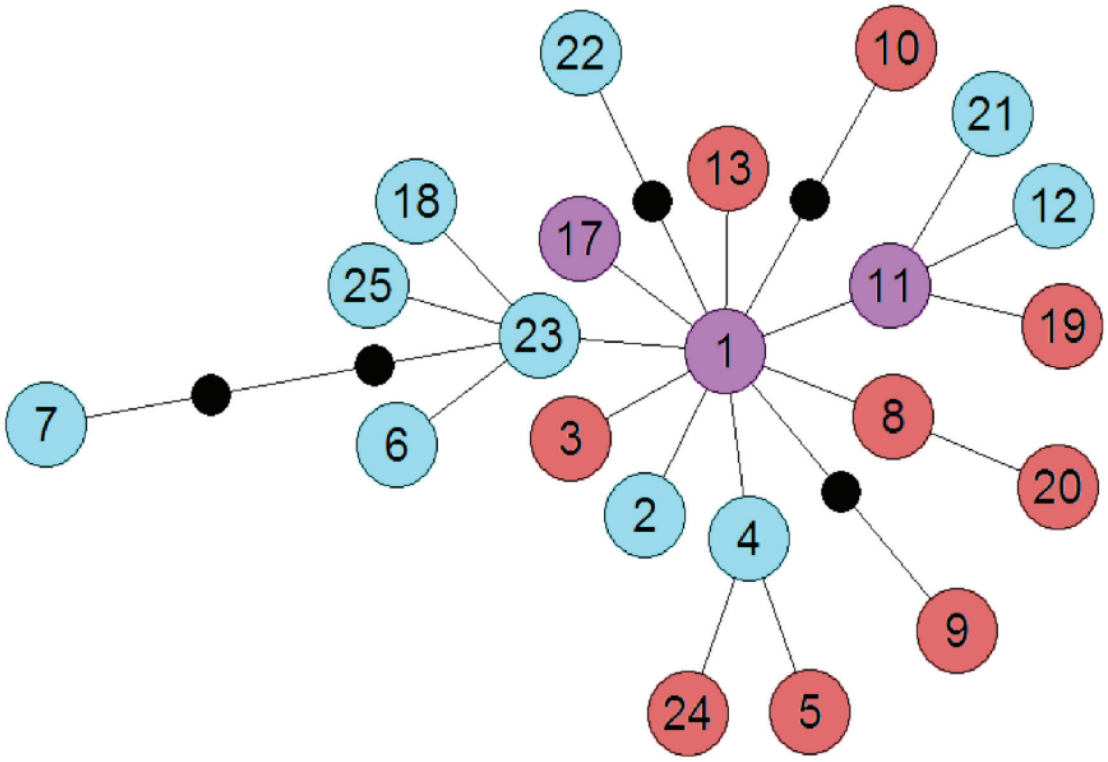

Figure A4: mtDNA haplotype network based on 72 sequences of an 886-bp fragment of the ND2 gene from 14 blue-hole populations of *Gambusia hubbsi*. Haplotype numbers refer to those in table A3. Haplotypes are colored according to the predation regime from which representative specimens were derived: blue = low predation, red = high predation, purple = both low- and high-predation populations. Small black circles indicate unobserved haplotypes and each solid line connecting haplotypes represents a single nucleotide substitution. Network was generated using Arlequin (Excoffier et al. 2005) and HapStar (Teacher and Griffiths 2011) software programs.

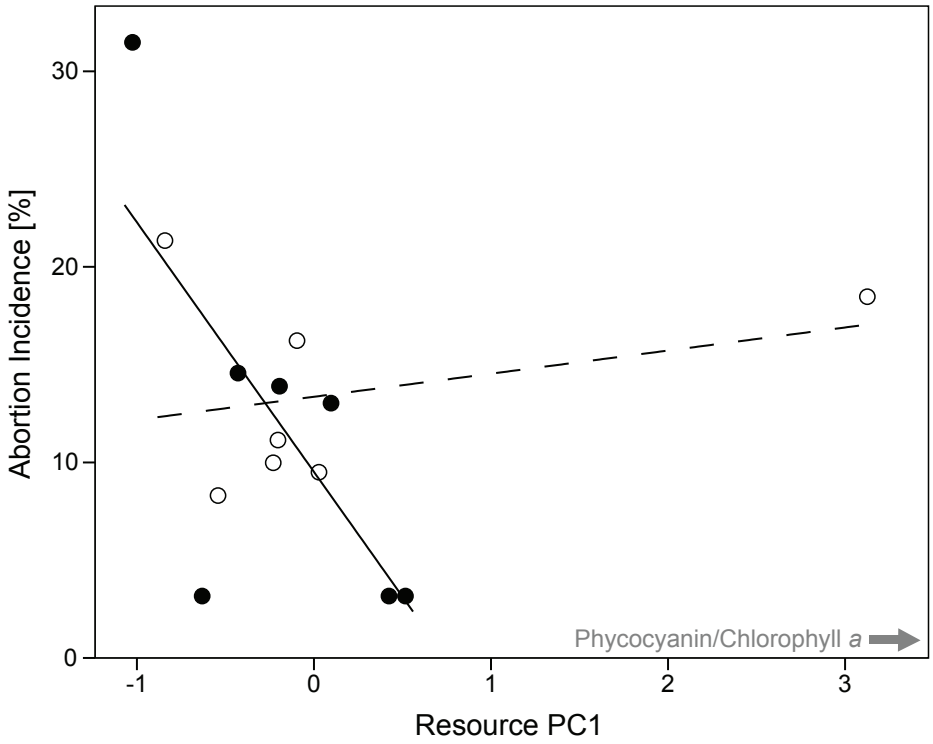

Figure A5: Regression of abortion incidence versus resource principal component 1, including all populations. Principal components were derived from a principal component analysis on five measures of resource availability. Open circles: low-predation blue holes; filled circles: high-predation blue holes.

Table A1. Loadings for the principal components analysis of resource availability

Factor	PC1	PC2	PC3	PC4	PC5
Phycocyanin	.937	−.091	−.035	−.165	.291
Chlorophyll *a*	.950	.040	−.071	.033	−.300
Zooplankton	−.129	.507	−.794	−.309	−.006
Small phytoplankton	−.008	.686	.569	−.452	−.030
Large phytoplankton	−.145	−.818	−.014	−.551	−.076
% variance	36.38	28.13	19.22	12.65	3.62
Eigenvalue	1.819	1.407	.961	.632	.181

Table A2. Canonical loadings derived from the predation regime term of the MANOVAs conducted separately for female and male *Gambusia hubbsi*

Trait	Females	Males
Standard length	−.04	−.10
Lean weight	**.66**	**.92**
Fat content	−.16	.33
Reproductive allocation (GSI for males)	−.15	**.68**
Fecundity	**.85**	
Embryo lean weight	**−.97**	
Embryo fat content	**−.53**	

Note: All variables were transformed as described in the text. GSI = gonadosomatic index.

Table A3. Canonical loadings derived from the predation regime term of the MANOVA conducted both sexes combined

Trait	Sex	Predation regime (PR)	PR × sex
Standard length	**.68**	.36	.17
Lean weight	.22	**.91**	.20
Fat content	−.12	.01	**.56**
Reproductive allocation (GSI for males)	**.73**	.38	**.75**

Note: All variables were transformed as described in the text. GSI = gonadosomatic index.

Table A4. Summary of sample sizes and mitochondrial haplotypes (ND2 gene, 886 bp) observed in Bahamas blue holes

Blue Hole	Predation	*N*	Haplotype(s)
East Twin	Low	5	18
Gollum's	Low	5	4, 6, 17
Hubcap	Low	5	1
Ken's	Low	5	2, 7, 11, 12
Pigskin	Low	5	23, 25
Rainbow	Low	6	1
Voy's	Low	5	1, 21, 22
Cousteau	High	5	8
Hard Mile	High	5	3
Rivean's	High	5	13
Runway	High	5	8, 11, 19, 20, 24
Shawn's	High	5	1, 5, 10
Stalactite	High	6	1, 9
West Twin	High	5	1, 11, 17

Note: Haplotype identification numbers refer to numbers given in GenBank accession information. All sequences were either retrieved from GenBank based on a prior study that included 9 of the 14 blue holes examined here (Langerhans et al. 2007; EF534741–EF534757) or were newly acquired and deposited in GenBank (JQ692089–Q692094, JX090201–JX090202).

Table A5. Analysis of molecular variance based on mtDNA

Source of variation	df	% of variation	*P*	*F*-statistic
Among predator regimes	1	2.55	.1760	$F_{CT} = .03$
Among populations within predation regimes	12	55.36	<.0001	$F_{SC} = .57$
Within populations	58	42.10	<.0001	$F_{ST} = .58$
Total	71			

Note: Percentage of variation, *P* values, and *F*-statistics were calculated according to Excoffier et al. (1992). Term F_{CT} is the correlation for random pairs of haplotypes within a predator regime, relative to random pairs drawn from the whole system; F_{SC} is correlation for random haplotype pairs within populations, relative to random pairs drawn from the same predator regime; F_{ST} is the correlation for random haplotype pairs within populations, relative to that of random pairs drawn from the whole system.

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