Context-dependence in complex adaptive landscapes: frequency and trait-dependent selection surfaces within an adaptive radiation of Caribbean pupfishes

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Supplemental Methods

Field Experiment

After 1-2 months of rearing in a common laboratory environment on a shared diet of commercial pellet food and newly hatched brine shrimp, each juvenile F2 hybrid was anesthetized in buffered MS-222 (Finquel, Inc.), photographed laterally and dorsally using a digital SLR with 60 mm macro lens, and injected with a 1 x 0.1 mm stainless steel wire tag etched with a unique 5-digit identification number (Northwest Marine Technologies, Inc.) using a handheld single-shot injector to place each tag in the left epaxial musculature. Tagged hybrids were transported to San Salvador Island in oxygen-permeable fish bags (Kordon, Inc.) and released into semi-natural field enclosures (3 m diameter x 1.3 m height with 3.175 mm mesh-size forming a fully-enclosed mesh bag with added macroalgae and substrate) for three months from March through June, 2011. All surviving fish were recovered from enclosures by slowly removing substrate and systematically lifting up the bottom mesh and then transported to the Gerace Research Station on San Salvador.

Survivors were euthanized in an overdose of buffered MS-222, photographed for measurement of growth rate relative to the pre-release photographs, and immediately sampled for muscle tissue from the caudal peduncle region for analysis of stable isotope ratios. Tags were then removed from preserved specimens to determine survivorship of pre-release fish. F2 hybrid phenotypes were measured from pre-release photographs for 16 external traits and compared to measurements of laboratory-reared parental species from each lake population raised in the same common laboratory environment.

Stable Isotope Analyses of Survivors

Muscle samples from each hybrid survivor from high and low-density enclosures were dried at 60° C for at least 48 hours before storage. For comparison, stable isotope ratios of wild-caught individuals of all three pupfish species from both experimental lakes and dietary resources of gastropod tissue (*Cerithium* sp.), macroalgae (*Batophora oerstedii*), and wigeongrass (*Ruppia maritima*) were collected in the same month as the harvest of field enclosures. Tissues were subsampled to dry weights of 1 ± 0.2 mg and sent to the UC Davis Stable Isotope Facility for analysis of natural abundances of δ13C and δ15N stable isotope ratios on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Stable isotope ratios of hybrid survivor muscle tissue should approximately integrate diet over the previous three months in field enclosures; δ13C reflects dietary carbon source from four different food chains within coastal marine ecosystems: phytoplankton, benthic macroalgae, seagrasses (e.g. wigeongrass), and terrestrial inputs; δ15N reflects relative trophic position within each lake (Post 2002; Layman 2007; Layman et al. 2012). Different food chain lengths across different lakes, e.g. due to different proportions of benthic/limnetic habitat within the two focal lakes, prevent direct comparison of trophic position between lakes (Post 2002). All analyses of stable isotopes used only survivors from high-density enclosures due to the flat fitness surfaces observed in low-density enclosures.

Phenotype-diet associations were visualized by calculating thin-plate spline surfaces for carbon and nitrogen stable isotope ratios overlaid onto the discriminant morphospace in each lake to assess whether nonlinear interactions were present. Stable isotopic compositions of wild-caught pupfish species from both lakes and resources relative to isotopes of F2 hybrids in high-density and low-density enclosures were also visualized. The association between stable isotope ratios and each discriminant axis was then directly tested using linear regression. To test if dietary isotopic composition explained additional fitness variation in excess of the phenotypic fitness landscape, residual survival probabilities were compared to the nitrogen and carbon stable isotope ratios of hybrid survivors using smoothing splines as described above for competitor frequency. Generalized linear models were used to explore the relationships between stable isotope ratios, frequency-dependence, growth rates, and their interactions in both lakes.

Morphometric analyses – size-correction

A two-dimensional morphospace for each high-density field enclosure was estimated as described previously (Martin and Wainwright 2013a). In brief, all F2 hybrids and parental individuals of all three species in each lake were measured for 16 traits from their pre-release photographs (F2 hybrids) or photographs of juveniles of similar size (for each laboratory-reared parental population). These landmarks were chosen to capture the major divergent aspects of morphology within the radiation, including large differences in jaw length, body depth, and nasal protrusion distance and angle relative to the neurocranium (landmarks described in detail in supplementary material to Martin and Wainwright 2013a). No singular measurement best captured overall differences in size among the F2 hybrids; therefore, the first principal component axis of variation for seven strongly size-associated linear distances in all pooled F2 hybrids and parentals was calculated as a robust multivariate index of size. All linear distances were first log-transformed. Due to the frequent breakdown of trait-size relationships within the F2 hybrids, traits were size-corrected in three ways after visual inspection. First, traits which exhibited similar allometric scaling relationships in parentals and F2 hybrids were regressed against the multivariate size axis and standardized residuals were used for all downstream analyses. Second, if allometric scaling relationships broke down only within F2 hybrids, a linear regression calculated only from the trait values of the three parental species was used to calculate standardized residuals for all hybrid and parental individuals. Third, if no allometric scaling relationships existed in parentals or F2 hybrids, standardized trait values were used without size-correction. All size correction and statistical analyses were performed separately for each lake due to phenotypic differences in trophic morphology among lakes (Martin and Feinstein 2014). Alternative methods of size-correction produced qualitatively similar fitness landscapes (Martin and Wainwright 2013a). An R script for size-correction analyses and fitness landscape visualization is provided as supplementary material along with the raw trait data and size-corrected residual trait data available in the Dryad repository.

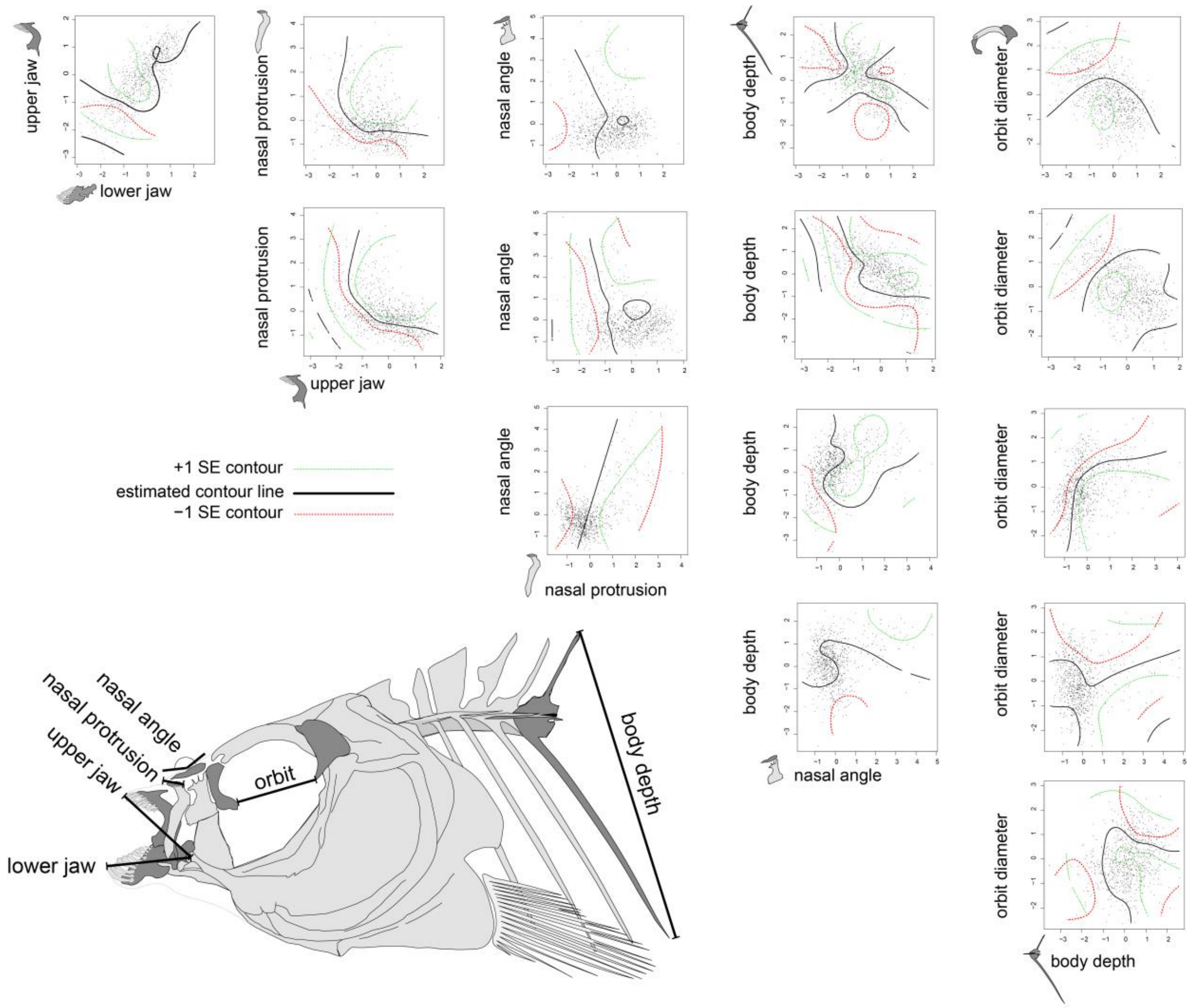
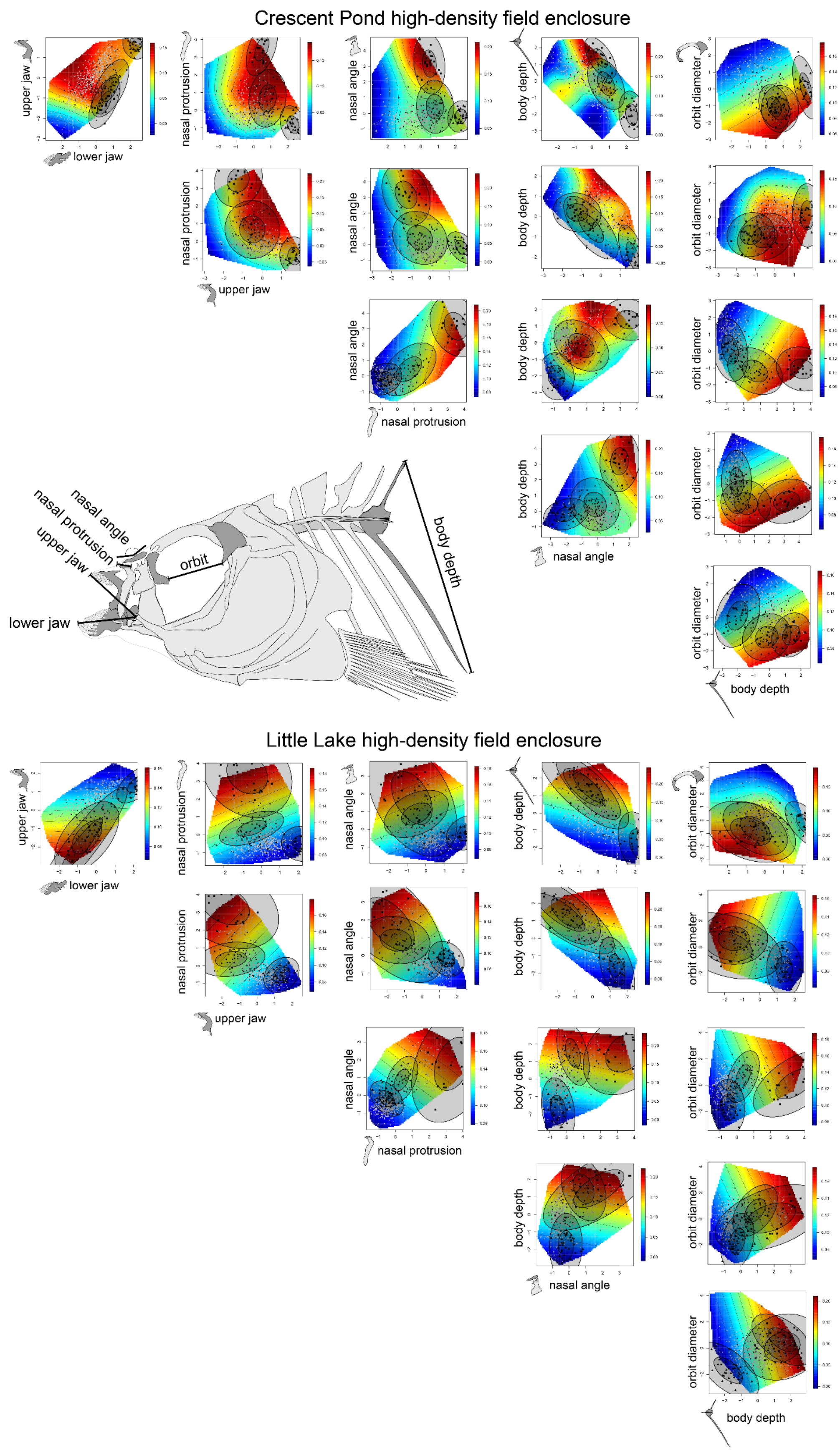


Fig. S1 Standard error contour lines (estimated: solid black; +1 SE: green dashed; -1 SE red dashed) for selection surfaces estimated for the six major functional traits best distinguishing among the three San Salvador species (schematic of linear distances measured from pre-release photographs of anesthetized hybrids is presented in lower left). Increasing statistical confidence in nonlinear contours is illustrated by concordant ±SE lines with narrower spacing as in the second column. The relationship between each pair of traits and survival in the high-density field enclosure in Crescent Pond was estimated with thin-plate splines fit using a generalized additive model. F2 hybrid individuals (n = 796) spanning the range of each two-dimensional morphospace are plotted as black points.



Crescent Pond high-density field enclosure
upper jaw
lower jaw
nasal protrusion
nasal angle
body depth
orbit diameter
nasal angle
nasal protrusion
upper jaw
orbit
lower jaw
body depth
Little Lake high-density field enclosure

Fig. S2 Pairwise interaction plot for all selection surfaces estimated for the six major functional traits best distinguishing among the three species within the San Salvador pupfish radiation (schematic of linear distances measured from pre-release photographs of anesthetized hybrids is presented in lower left). The relationship between each pair of traits and survival in high-density field enclosures in Crescent Pond (top, n = 796) and Little Lake (bottom, n = 875) was estimated with thin-plate splines. Shaded gray 95% (outer) and 50% (inner) confidence ellipses and bolded symbols represent phenotypic position of parental individuals (● generalist *C. variegatus*; ■molluscivore *C. brontotheroides*; ▲scale-eater *C. desquamator*). F2 hybrid individuals spanning the range of each two-dimensional morphospace are plotted as small black circles (survivors) or small gray circles (deaths).

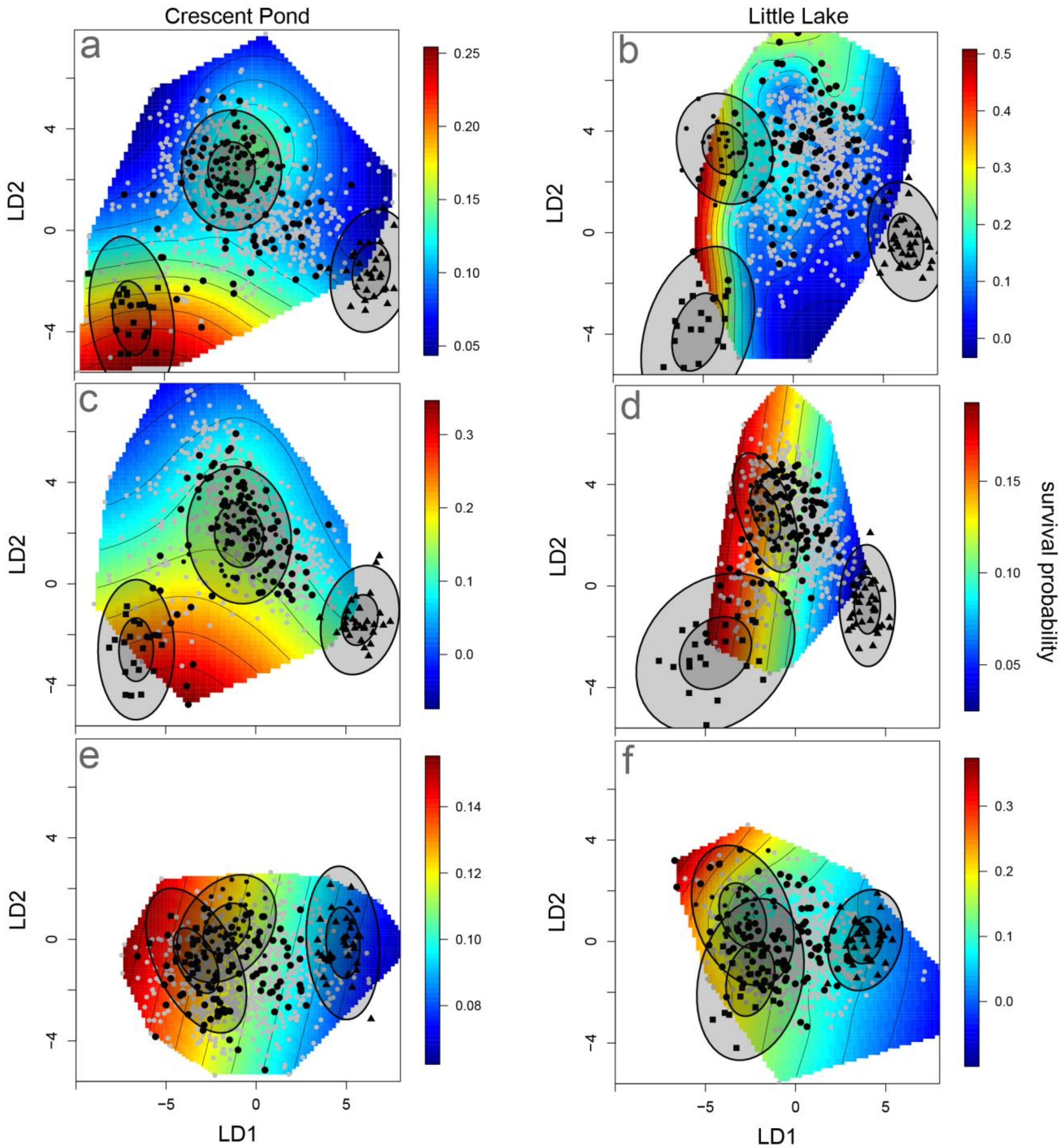


Fig. S3 Variation in survival fitness landscapes across different trait subsets for *Cyprinodon* pupfishes in high-density field enclosures on San Salvador Island, Bahamas. ***a-b*) Original discriminant morphospace estimated from 16 traits** (modified from (Martin and Wainwright 2013a)). ***c-d*) Discriminant morphospace estimated from the six functional traits** examined in Figs. 5-6, Table 3. ***e-f*) Discriminant morphospace estimated from an independent set of six**

traits providing minimal discrimination among the three San Salvador species: 1. eye roundness, 2. caudal peduncle height, 3. dorsal snouth length, 4. buccal width (dorsal view), 5. preopercular height, and 6. body length. Two-dimensional fitness surfaces (thin-plate splines) illustrate the probability of survival (heat color) relative to phenotypic position within the discriminant morphospace in high-density semi-natural field enclosures in Crescent Pond (first column) and Little Lake (second column). Shaded gray 50% (inner) and 95% (outer) confidence ellipses represent phenotypes for each of the three parental species in each lake (●generalist *C. variegatus*; ■molluscivore *C. brontotheroides*; ▲scale-eater *C. desquamator*). F2 hybrid survivors (small black circles) and non-survivors (small gray circles) after 3 months in the field enclosures are plotted within the discriminant morphospace.