Strong nonlinear selection against fluctuating asymmetry in wild populations of a marine fish

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Theoretical links between fluctuating asymmetry (FA) and fitness have led many to use FA as a proxy for average fitness. However, studies examining whether asymmetry actually correlates with individual fitness in wild populations are relatively rare and often use simple measures of association (e.g., correlation coefficients). Consequently, the pattern of selection on asymmetry in the wild is seldom clear. We examined selection on FA of pectoral fin morphology in two wild populations of a marine fish (the kelp perch; *Brachyistius frenatus*). As expected, variance in signed FA in each initial sample was significantly greater than that found in the surviving population, indicating selection against FA. Our estimate of the fitness surface confirmed perfect symmetry as the phenotypic optimum and indicated strong, nonlinear selection against asymmetry. No difference in the form of selection was detected between populations. However, the level of FA in the initial samples varied among populations, leading to an overall difference in the level of selective mortality. Our results suggest that selection on asymmetry in wild populations may be strongly nonlinear, and indicate that the demographic costs of asymmetry may play a substantial role in the dynamics of populations.

KEY WORDS: Curve, developmental instability, fitness, field research, fluctuating asymmetry, marine fish, natural selection.

Fitness, as defined by natural selection, depends on more than the genotype and environmental conditions that an individual is dealt. After fertilization, the organism must be able to develop accurately toward its "target" phenotype, but perturbations in the internal microenvironment during early development (i.e., developmental noise; Palmer 2004) ameliorated by the organism's inherent ability to buffer this noise (i.e., robustness; Mather 1953), combine to influence the final phenotype. The resultant mismatches between the target and realized phenotypes are said to arise from developmental instability (DI) with high levels of DI representing poor overall developmental accuracy (Hansen et al. 2006). Assuming most organisms are well-adapted (i.e., their target phenotypes match the fitness optima for their environments), DI within each individual will most often cause a shift of the phenotype away from the adaptive optimum. DI may be caused or exacerbated by external factors or maternal health, which in turn may be symptoms of larger phenomena of interest to conservation scientists. Such phenomena include toxin levels, pollution, climate change, and/or inbreeding depression (Lens et al. 2002b; Fessehaye et al. 2007; Lens and Eggermont 2008; Joubert and Bijlsma 2010; Schmeller et al. 2011; Sanchez-Chardi et al. 2013; Nuche et al. 2014).

Since the target phenotype of an organism is rarely knowable, DI is most often estimated by measuring fluctuating asymmetry (FA): the small, random differences between the right and left sides of a bilaterally symmetrical organism (Van Valen 1962). Both sides are generated by the same genetic blueprint, are exposed to virtually the same external environment, and the adaptive optimum for paired traits can in most cases be assumed to be perfect symmetry (Palmer 1996). Deviations from the optimum of symmetry can therefore be attributed to DI and its underlying causes, and are easily measured by subtracting the value of a structure (either metric or meristic) on the right side from the value of the same structure on the left. This is often referred to as the signed FA: FA = (L–R). Alternately, the unsigned FA can be calculated as the absolute value of the difference in trait values

(i.e., FA = |L–R|). Due to their simplicity, the variance of signed FA and/or the mean of the unsigned FA have been commonly used as indicators of developmental instability, and therefore fitness. Studies investigating and using FA as a proxy for fitness have compared captive versus wild populations, wild populations with or without a history of environmental stress, and captive populations that have been experimentally manipulated to simulate various selective pressures (Valentine et al. 1973; Lazic et al. 2013; Sanchez-Chardi et al. 2013; Mabrouk et al. 2014).

Many applications of this metric have been subject to methodological criticism however. Chief among these relate to the handling of measurement error and the possibility of confusing FA with other forms of asymmetry. The magnitude of FA is typically small and often difficult to measure, so even small amounts of measurement error can easily obscure true FA (Palmer 1996). It is therefore critical to estimate measurement error and remove it from the final FA estimate, a step rarely taken in early FA studies (Palmer and Strobeck 1986). In addition, other forms of asymmetry (namely directional asymmetry and antisymmetry) can inflate true FA estimates by the addition of nonrandom, heritable asymmetry, and so need to be subtracted from any FA estimate.

Another open question is whether there is in fact a biologically meaningful relationship between individual symmetry and fitness (Palmer 1996; Lens et al. 2002a). Given the intuitive links between DI, FA, and fitness, many studies have been published using FA as an indicator of average fitness or population health without dispute. In many of these studies, a comparison is made between mean FA estimates from two or more disjunct populations with differing levels of environmental stress (Eeva et al. 2000; Hendrickx et al. 2003; Lazic et al. 2013; Mabrouk et al. 2014) or before and after a natural disaster (Uetz et al. 2009; Brown and Brown 2011; Michaelsen et al. 2015). However, using FA as a direct indicator of population mean fitness assumes (1) that there is a negative relationship between FA and expected fitness of individuals, and (2) that this relationship is linear. The first of these assumptions appears to be supported, on average. Studies often (but do not always) find negative associations between asymmetry and reproductive success (Koshio et al. 2007; Vishalakshi and Singh 2008; Jaffe and Moritz 2010; Pavkovic-Luaic and Kekie 2011). Similarly, studies often (but do not always) find negative associations between asymmetry and measures of survival probability (Leung and Forbes 1996; Moller 1999; Vishalakshi and Singh 2008). Support for the second assumption–that the relationship is linear—is less clear. Many studies use simple measures of association (e.g., correlation coefficients) to describe the relationship between symmetry and fitness components. Such an approach is fine for verifying a relationship, but it may miss important information if the relationship between FA and fitness is nonlinear.
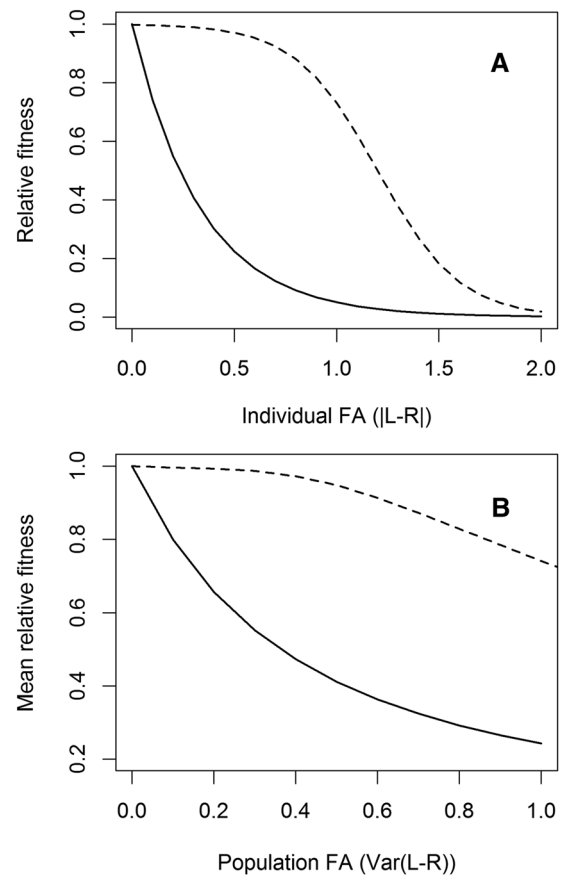

Figure 1. An illustration of nonlinear selection on individual asymmetry values and the consequences for the average fitness of populations: (A) Two hypothetical relationships between symmetry and relative fitness are illustrated; concave down (dashed line) and concave up (solid line). (B) The effects of these two distinct relationships on the mean fitness of the populations as overall FA increases. See main text for details.

If fitness surfaces are nonlinear, then the relationship between population FA and average fitness is not necessarily straightforward. Average fitness will depend on both the fitness function and the distribution of individual asymmetry values within the population. That is,

$$\overline{W} = \int p_{FA} w_{FA} .$$

Where $\bar{W}$ is average fitness, p_{FA} is the distribution of individual asymmetry values and w_{FA} is the function relating relative fitness to individual asymmetry value. In other words, average fitness will depend on how many individuals are in the high- and low-fitness regions of the fitness surface. For example, consider two fitness surfaces that have different shapes (one is concave-up and one is concave down) but converge on the same fitness for highly asymmetric individuals (Fig. 1A). For the concave-down surface, only the highly asymmetric individuals suffer a large loss of fitness. If asymmetry values are centered on zero

(and are assumed to follow a normal distribution in this example), then many individuals will be in high fitness regions and average fitness will be modestly sensitive to overall FA (Fig. 1B). In contrast, if the fitness surface is concave up, then even slightly asymmetric individuals suffer a large loss of fitness. In such cases, many individuals will be in a region where fitness changes rapidly with FA value. Changes in the variance of L–R values results in greater differences in relative fitness of individuals and average fitness is much more sensitive to overall levels of FA (Fig. 1B).

The purpose of this study was to measure natural selection on FA in a meristic trait in individuals from two populations of a marine fish and to quantify the functional form of the selection surface. In each population, we sampled (without replacement) the year's cohort of fish at two times–before and after a period of selective mortality- and measured asymmetry values within each sample. The first sample was taken from newborns and the second sample from the same cohort after reaching reproductive age. All collected fish were brought back to the lab to be photographed and measured. Although this was not a mark-recapture study, we believe that our samples were each a good representation of their respective populations. Marking and recapturing kelp perch is prohibitively difficult in practice, and taking fresh samples from each population still allowed us to make inferences about selection patterns by comparing changes in asymmetry during a period of selective mortality (see Brown and Brown 2011; Schmeller 2011; Michaelsen 2015; for similar study designs). Based on the assumption that higher asymmetry is an indicator of lower fitness, we hypothesized that the individuals from the first sample would have a higher mean FA and the second sample (from the surviving population) would have a lower mean FA. We were also able to estimate the shape of the fitness surface and compare the overall magnitude of selective mortality between the two study populations.

Methods
STUDY SPECIES
Kelp surfperch (*Brachyistius frenatus*) are found on nearshore reefs throughout the western coast of North America, ranging from British Columbia down to Baja California. They prefer dense kelp forest habitat and generally do not stray from the safety of the kelp bed (Carlisle 1960; Anderson 1994). Unlike most reef fishes (but like most surfperch species in the Northeast Pacific), kelp surfperch are livebearers. Young of the year are born on the same reef as their parents and field observations suggest they exhibit very little movement away from their home reef (Anderson 1994, 2001). Reproduction is seasonal, and parturition generally occurs from April to July (Hubbs and Hubbs 1954), though in a given year reproduction is more synchronous and during our study most young-of-the-year were born in May (D. W. Johnson, pers. obs.). Reproduction begins in the fall and courtship and mating have been observed from October to December (DeMartini 1988; Tootell and Steele 2012). Kelp surfperch grow rapidly and mature within their first year of age and their average generation time is just over one year (Baltz 1984).

Kelp surfperch (like most surfperch) rely heavily on their pectoral fins for both locomotion and balance. During burst locomotion, such as to evade a predator, the pectoral fins may be used for both added speed and rapid turning and braking (Webb 1973). More often, perch use their pectoral fins while foraging (Pink and Fulton 2014), using them for stabilization while feeding in the moving waters of their near-shore kelp forest habitat. These abilities may be influenced heavily by the size and shape of the fins and their consequent physical interactions with the water (Shoele and Zhu 2010), making differences in size between the left and right fins a potential impairment to the swimming ability of the fish. The likely importance of the pectoral fins for predator avoidance and foraging suggests that FA in fin size may be subject to natural selection via differential mortality.

We measured fluctuating asymmetry in pectoral fin ray number. The total number of fin rays varied substantially among individuals (Shubin 1995) and FA was readily detectable, even after accounting for measurement error. Due to similarities in developmental elements at work in tetrapod limb buds and the fin buds of fish (Grandel and Schulte-Merker 1998), the rays of teleost pectoral fins (such as those of the perches) are believed to be fixed in number for each individual from early development, similar to the digits of tetrapods (Shubin 1995; Lee et al. 2013). Therefore, the number of fin rays is not expected to change after birth, precluding the possibility that population FA changed due to individual changes in symmetry over time.

STUDY DESIGN
This study included the collection of data from two disjunct populations: one located on the mainland of Southern California, off the Palos Verdes Peninsula, and the other at Santa Catalina Island, at a site called Bird Rock (Fig. 2). Santa Catalina Island is located about 33 kilometers South of Palos Verdes—a large enough distance to preclude any relevant gene flow between the two populations of this live bearing, nonmigratory species (see, Bernardi 2000, 2005) for estimates of gene flow in closely related surfperches).

At each location, our approach to measuring selection was to take (and keep for analysis) an initial sample of the "young of the year" and then resample the same population later in the year, with collection again focused on members of that same cohort. Distributions of asymmetry values in the initial sample were compared to the distributions of asymmetry values in the sample of survivors to estimate selective mortality associated with FA
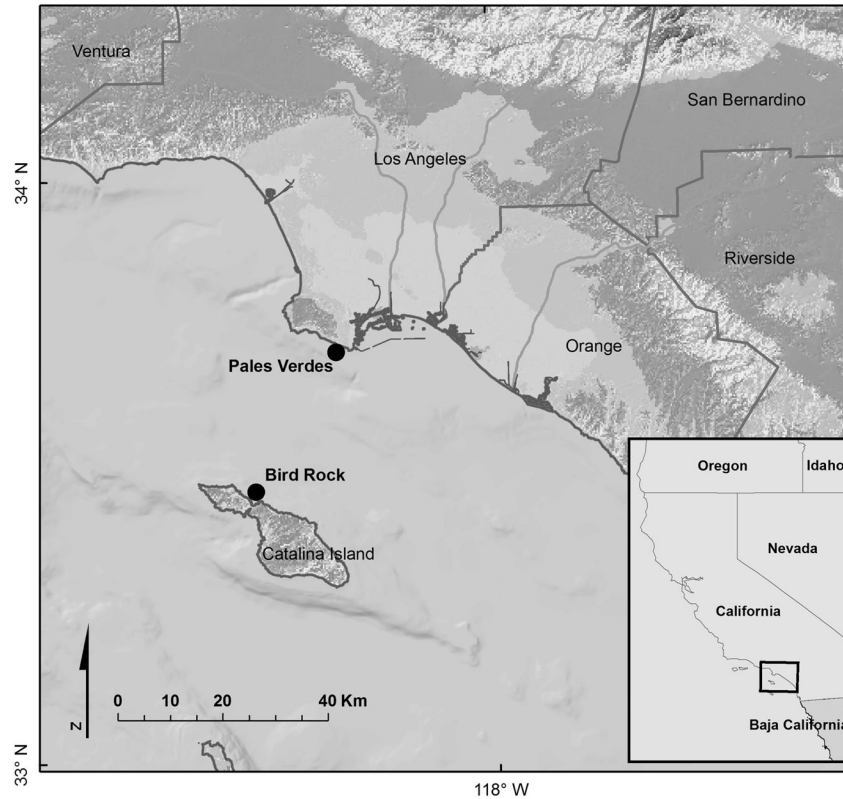
Figure 2. Map of the study region. The two populations studied are approximately 33km apart and separated by an expanse of deep water that prevents dispersal of live-bearing reef fish such as kelp perch.

(Lande and Arnold 1983; Johnson et al. 2012). The samples were collected by divers using hand-held nets in late May 2014 and again in the fall (Oct–Nov) of the same year for both populations. Our initial samples were collected shortly after the year's cohort had been born and our second sample represents those individuals that survived to the mating season. Although we measured a component of fitness (survival to maturity) rather than fitness itself, we believe that survival to maturity is a reasonable proxy for fitness in this short-lived species.

With our sampling technique we were able to capture $N_{initial}$ = 65 for Catalina, and $N_{initial}$ = 32 for Palos Verdes. Four months later, we collected $N_{survivors}$ = 36 from Catalina and $N_{survivors}$ = 37 from Palos Verdes. All collected fish were euthanized and brought back to the lab to be photographed three times: once to determine overall length, and close-up on the left and right pectoral fins using a dissecting scope-mounted camera (Fig. S1, Appendix S1). The images were saved and analyzed in the basic image processing software ImageJ (Rasband 2016).

Data Analysis

Each collected fish was photographed three times: once to show overall length, and one close-up of each pectoral fin. After all four samples were photographed, the images were randomized and dis-

tributed to two observers. Each observer independently recorded the number of fin rays that he or she counted in each photograph and two (signed) FA measures (i.e., number of left fin rays–number of right fin rays) were calculated based on each observer's fin ray count (our raw data is available at www.datadryad.org). FA measures calculated in this manner include ideal FA, but may also include measurement error and directional asymmetry (DA). Measurement error can be assumed to originate from differences between the two observers' counts, or possibly from the quality of the photographs themselves. To isolate ideal FA, we removed measurement error and DA via the following procedure.

We calculated a DA-corrected, signed FA value (FA_{obs}) for each individual as:

$$FA_{obs} = \left[(L - R) - \overline{(L - R)} \right],$$

where L and R represent the number of fin rays on the left and right sides (following Pelabon et al. 2004). We then corrected for measurement error. Because two different observers independently measured each fish, there are two FA measurements for each fish: FA_1 and FA_2. The variance of the difference between the two was calculated to estimate measurement error (δ_m^2):

$$\delta_m^2 = \text{Var} \left(FA_1 - FA_2 \right).$$

This value was then subtracted from the DA-corrected FA (FA$_{obs}$), which produced the final corrected FA (FA$_{corr}$), according to the following equation (Pelabon et al. 2004; Carter et al. 2009):

$$FA_{corr} = \sqrt{(FA_{obs})^2 - 2\delta_m^2/\pi}\ .$$

This procedure yielded measurements of corrected FA for each individual.

Note that the correction for measurement error results in an unsigned description of FA (i.e., the magnitude but not the direction of deviations from symmetry are reported). To test whether FA affected individual survival, we compared whether the mean value of FA$_{corr}$ was significantly different between the initial sample and the sample of survivors. If FA$_{corr}$ of survivors was on average less than the initial sample of the cohort, it would suggest greater mortality of asymmetric individuals. In contrast, if FA$_{corr}$ of survivors was greater than the initial sample, then it would suggest greater survival of asymmetric individuals. To compare the mean values of FA$_{corr}$ between the two groups (initial sample and survivors), we conducted a resampling test. In this procedure, we resampled individual fish from both the initial and survivor samples, calculated corrected FA, and compared the distribution of differences in corrected FA values pre and postselection. Resamples were made with replacement and the number of resamples matched the number in the original sample. To generate an approximate *P* value, we repeated this procedure 1000 times and compared how many of the resampled differences in corrected FA values (initial – final) were less than or equal to zero. This procedure allowed us to generate confidence intervals and to test (with 95% confidence) whether our observed differences in corrected FA were greater than zero. To illustrate the effect that correcting for measurement error and directional asymmetry had on the analyses, we also repeated this procedure using noncorrected FA values as our response variable. Resampling was done using the sample function embedded in a simple, computational loop in R (R Development Core Team 2015).

We were also interested in describing the selection surface (i.e., the relationship between individual asymmetry values (L–R) and expected values of relative fitness). When a cohort is sampled before and after a period of selective mortality, this can be accomplished based on the fact that selectively favored phenotypes will tend to commonly occur in both the before- and after-selection samples whereas those phenotypes that are selected against will tend to be represented to a much lower degree in the after selection sample. The conditional probability that a fish with an asymmetry value of *z* was captured in the sample of survivors, given that it was caught in either sample is:

$$h(z) = \frac{S_2(z)}{S_2(z) + S_1(z)}\ .$$

Where $S_1(z)$ and $S_2(z)$ are the number of individuals in the first and second samples that had an FA value of *z*. The value of *h(z)* ranges from 0 to 1 and *h(z)* can be estimated as a smooth function (Johnson et al. 2012). In this study, we used a generalized additive model with a logit link to describe *h(z)* by fitting a regression spline to the data.

Once *h(z)* has been estimated, it can be converted to a function that describes expected, relative fitness associated with values of asymmetry (in other words, a function that describes the selection surface). Following Anderson (1995), we calculated the relative fitness function, *f(z)*, as:

$$f(z) = \frac{S_1}{S_2}\ \frac{h(z)}{1 - h(z)},$$

where S_1 and S_2 are the number of fish sampled in the before- and after-selection samples, respectively. Note that although our measure of asymmetry is discrete, estimating *h(z)*, and therefore *f(z)* as a continuous function allows us to use the entire dataset and provides better estimates of conditional probabilities than evaluating *h(z)* individually for each value of *z* (particularly for values of *z* that were rarely observed and thus would have low sample sizes if considered in isolation). We fit *h(z)* with a continuous function, but present expected values of f(*z*) at discrete values of FA. All analyses were conducted in R (R Development Core Team 2015), and generalized additive models were fit using the package mgcv.

We were also interested in testing whether the selection surfaces differed between the two populations. After accounting for the fact that the ratio of sample sizes differed between the two populations (Appendix S2), we evaluated whether a model in which the fitness functions differed between the two populations fit the data significantly better than a model that described a single fitness function for both populations.

Results

The distribution of signed FA values in the before-selection samples at both Palos Verdes and Santa Catalina Island matched the shape of what would be expected if asymmetry in this metric character were generated by Gaussian developmental instability interacting with asymmetry thresholds (see Appendix S3 for a general description). Comparison between observed and expected distributions revealed no significant differences (Kolmogorov–Smirinov tests for Palos Verdes $P = 0.97$; for Santa Catalina Island $P = 0.461$), which rules out antisymmetry in fin ray counts and suggests that FA in this meristic character provides a suitable measure of underlying developmental instability (Palmer and Strobeck 1992). In addition, preliminary screening of the data [using a procedure outlined by Palmer and Strobeck (1986)] suggested that directional asymmetry was a small, non-significant component of asymmetry in our initial samples, and
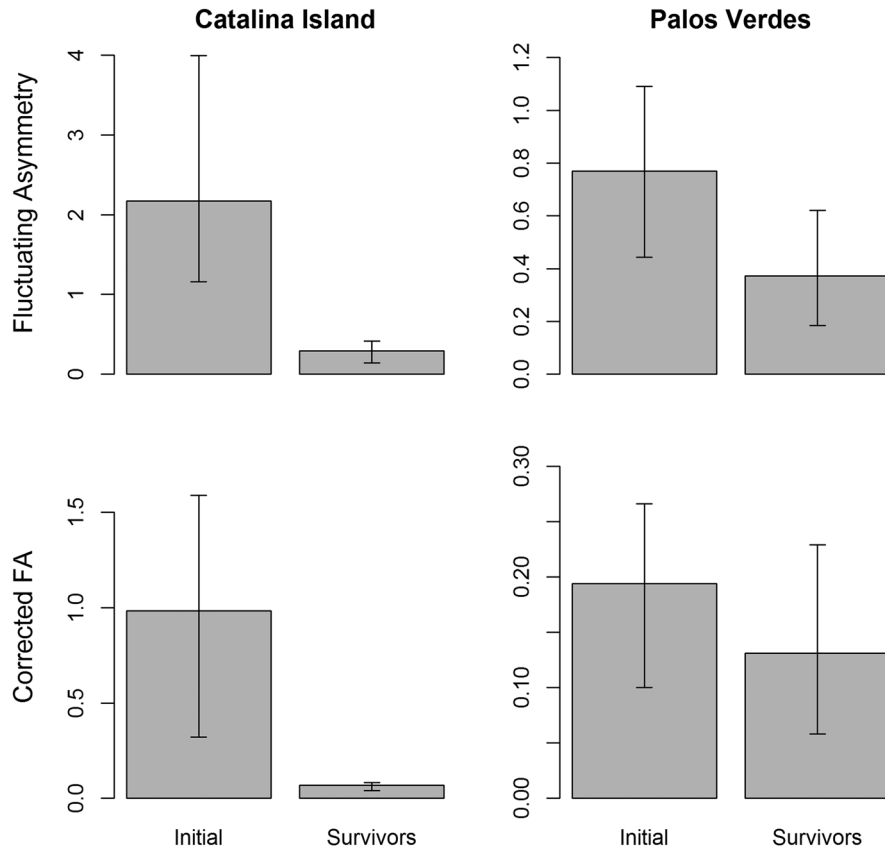
Catalina Island

Palos Verdes

Figure 3. Effects of selection against FA in both populations across time: Initial samples represent fish collected shortly after birth and survivor samples represent fish captured during the beginning of breeding season. Top panels illustrate changes in noncorrected FA and bottom panels illustrate changes in FA values that were corrected for measurement error and directional asymmetry. Error bars represent 95% confidence intervals (calculated by a resampling procedure).

measurement error was small compared to levels of fluctuating asymmetry (Appendix S4).

Average size of fish in the initial (before-selection) sample was 4.22 cm SL (range = 2.54–5.65) whereas the average size of fish in the sample of survivors (after selection) was 6.53 cm SL (range = 5.29–8.52). On average, fish at the Palos Verdes population were slightly smaller than those from Catalina Island in both the initial sample (3.97 vs 4.35 cm SL) and the sample of survivors (6.35 vs 6.87 cm SL). In the initial sample (before selective mortality occurred), asymmetry ($|L–R|$) was not correlated with standard length ($r = 0.039$, $P = 0.705$), suggesting no discernable link between asymmetry and growth rate.

As predicted, FA was measured at much higher levels in the initial samples for both populations (Fig. 3 top panels). At Catalina Island the FA value for the initial sample (2.17) was significantly higher than the FA value for the sample of survivors (0.293; resampling test: $P < 0.001$). The Palos Verdes population had an initial FA of 0.770 and the survivors had an FA value of 0.373 (resampling test $P = 0.022$). Correcting for measurement error and DA reduced our estimates of FA for both populations

and for both the initial and survivor samples (Fig. 3, bottom panels). However, selective loss of asymmetric individuals was still apparent and the reductions in FA_{corr} values were qualitatively similar to the reductions in FA values. The Catalina Island population had an initial FA_{corr} of 0.98 and the surviving population had an FA_{corr} of 0.07, which was significantly lower (resampling test, $P < 0.001$). In the Palos Verdes population, the initial sample had an initial FA_{corr} of 0.19 and the surviving population had an FA_{corr} of 0.13, which was not as dramatic a decrease as it was for Catalina Island (resampling test, $P = 0.16$).

In contrast to fluctuating asymmetry, the directional asymmetry component did not change appreciably during a period of selective mortality. DA values were slightly negative at Catalina Island, but the resampled confidence intervals overlapped zero for both the initial sample (mean = –0.146, 95%CI: –0.500, 0.178) and the sample of survivors (mean = –0.121, 95%CI: –0.266, 0.051). At Palos Verdes, estimated values of DA were very close to zero for both the initial sample (mean = 0.000, 95%CI: –0.300, 0.242) and the sample of survivors (mean = –0.013, 95%CI: –0.189, 0.135; see Appendix S5 for additional details).
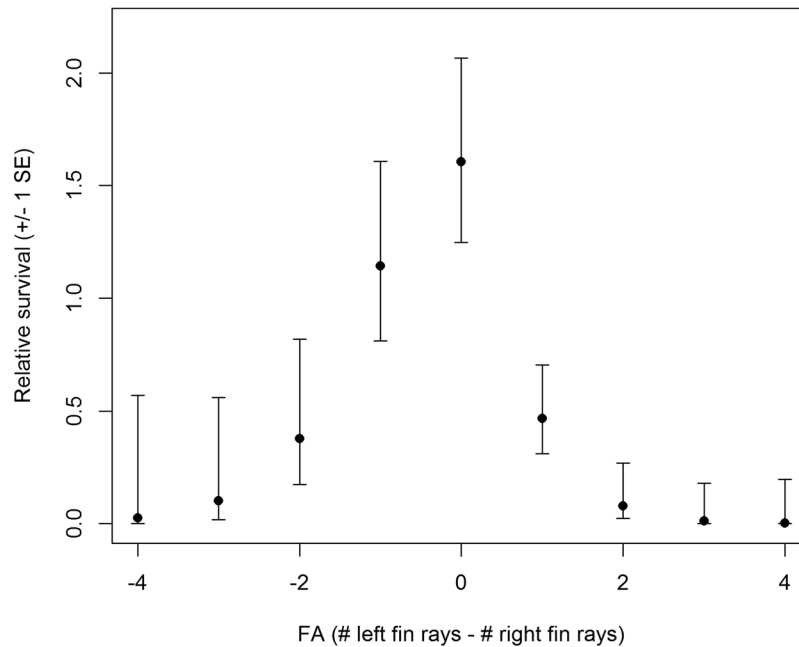
Figure 4. Selection surface relating asymmetry in fin ray counts to relative survival of kelp surfperch. Data points represent estimated values of relative survival at discrete values of asymmetry. Dashed line is included to illustrate the shape of the selection surface. The curve represents the pooled data from both populations.

The relative fitness functions appeared to be similar for the two populations. A model in which relative survival rate varied between populations did not provide a better fit to the data than a model in which relative survival rate was the same for both populations (drop in deviance = 0.454, df = 1.045, P = 0.518). The latter model indicated a complex fitness surface (Fig. 4 depicts a selection curve constructed out of the pooled data). The curve was normalized such that relative survival of an average individual within these populations is 1, and shows that those fish with zero FA had a relative survival rate ~1.6 × greater than the overall survival rate for all individuals.

Discussion

The decrease in mean FA and FA_{corr} indicated strong, selective mortality against FA in these populations. The selection curve constructed from our results (Fig. 4) indicates that fish with an asymmetry value of zero have a much higher (>1.6) relative survival rate compared to the overall survival rate of all individuals in the population. While individuals with a fin ray difference as great as four were encountered in the initial samples, such individuals were entirely absent from the samples from the surviving populations in both locations.

Given the importance of the pectoral fins for locomotion (Webb 1973; Lupandin 2005; Shoele and Zhu 2010), and the detrimental effects that asymmetry may have on their function, it is perhaps not surprising that our results show viability selection

against asymmetry. However, our study revealed that selection against FA is both large in magnitude and strongly nonlinear. For example, survival of fish with perfect fin ray symmetry was estimated to be, on average, 1.99 times greater than survival of fish with a difference of one fin ray, 7.03 times greater than survival of fish with a difference of two fin rays, and 28.24 times greater than survival of fish with a difference of three fin rays. These results underscore the functional importance of fin ray symmetry. A difference of even one fin ray corresponds to real world impairment, and fin ray differences of two or more result in an extremely low chance of surviving to maturity. Qualitatively similar results were recently published by Stringwell et al. (2014), who examined whether asymmetry (in either gill rakers, pectoral fin rays, or pelvic rays) affected survival of juvenile, hatchery-reared salmon. These authors found that when hatchery fish were released into the wild and recaptured, the proportion of asymmetric fish decreased dramatically. In contrast, the proportion of asymmetric fish in the hatchery environment and remained high throughout the experiment. Their results, like ours, suggest that asymmetry has a strong effect on the relative survival of young fish in the wild.

The variation in total fin ray numbers (12–17 per fin in our samples) led us to test for a possible correlation between fin ray number and asymmetry. We found that indeed, fish with fewer fin rays exhibited greater asymmetry. Moreover, we found that the population with the highest level of FA—Catalina Island—also had fish with fewer fin rays on average. If fin ray number decreases

with increased water temperature [as suggested by the results of (Ali and Lindsey, 1974)], then this may have been one of the many possible reasons for greater FA on Catalina Island. Whatever its cause, strong selection against FA will have an important effect on survival rates for the affected population(s).

Strong viability selection against FA suggests that FA must have important consequences for the dynamics of populations. Significant nonlinearity in the relationship between individual symmetry and survival indicates that the distribution of symmetry values in a population will play a substantial role in mediating survival, and therefore population dynamics. Average survival can be calculated as $\sum p_i w_i$, where p_i is the relative frequency of each phenotype (asymmetry value) and w_i is the relative fitness for each phenotype. For example, assuming that the selection surface is constant in space and time, then cohorts of fish with variation in asymmetry of 0.83 (our estimated value for the Palos Verdes population) would experience average survival rates that are 1.26 times greater than cohorts of fish with variation in asymmetry of 1.91 (our estimated value for the Catalina population). Other moments of the distribution (skewness, kurtosis) may also affect relative survival, but because the distributions in our samples were statistically indistinguishable from a Gaussian-derived distribution, differences in variances are sufficient to summarize differences in average survival rates.

Within these populations and within this generation of fishes, FA had a major influence on survival. And while studies have shown that the mechanisms of developmental instability (DI, of which FA is an indirect measure) are at least partially heritable (Carter and Houle 2011), it should be noted that the strong selection revealed here might not result in evolutionary adaptation. In other words, selection against FA may occur within each generation without resulting in the subsequent generation having lower DI and therefore, lower FA. That said, comparing changes in FA within and between populations, even at a generational level, will be a useful tool for ecologists and conservation scientists studying population dynamics.

Studies that directly examine the relationship between FA and fitness components are still relatively rare and often rely on a simple measure of association (e.g., correlation coefficients or regression slopes). By describing the shape of the fitness surface, we believe that investigators can get more detailed information on how FA affects the relative fitness of both individuals and populations. Such a shift in focus will allow investigators to test new hypotheses. For example, the functional form of selection on FA may depend on the traits measured, and how strongly those traits relate to performance. In this study, we observed that even small amounts of asymmetry in fin ray number (a trait that is strongly linked to swimming performance) resulted in a sharp, nonlinear decline in relative survival rates. However, FA does not necessarily result in sharp declines in relative survival. For example, Nosil

and Reimchen (2001) quantified patterns of selection on FA in the number and length of tarsal spines (traits related to feeding) in water boatmen (*Callicorixa vulnerata*) under resource-limited laboratory conditions. They found that relative survival declined slightly at low levels of FA and declined quickly at the highest levels of FA. Such results suggest that patterns of selection against FA may depend on trait type. In any case, understanding the functional form of the relationship between FA and relative fitness will be key to understanding the population-level consequences of FA. Quantifying the fitness surface is the first step in this process.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Example of image used to measure pectoral fin rays
Figure S1. The right pectoral fin of a kelp perch (Brachyistius frenatus) as photographed for analysis of fin ray meristics.
Appendix S2. Details regarding the among-population comparison of fitness surfaces
Appendix S3. The expected distribution of a meristic trait affected by developmental instability
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Appendix S4. An additional test for directional asymmetry and measurement error
Table S1. Results of a two-way ANOVA analyzing components of variation in pectoral fin ray counts.
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Figure S3. Estimates of directional asymmetry (DA) in pectoral fin ray counts.