ASYMMETRY ANALYSIS IN FISHES: A POSSIBLE STATISTICAL INDICATOR OF ENVIRONMENTAL STRESS

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ABSTRACT

One of the more difficult tasks in evaluating the possible deleterious effects of multiple toxicants on natural communities is in defining subtle sublethal effects before the onset of chronic morbidity. We reason that before detectable changes in either species diversity or species abundance occur subtle changes must take place in a number of important processes, ranging from molecular to behavioral changes. Unfortunately, changes in these parameters have proven most difficult to detect with current methodology. We, therefore, have been examining the possible use of fluctuating asymmetry as a possible measure of environmental stress.

Fluctuating asymmetry is simply the random deviation from perfect symmetry of any bilateral anatomical character. It is, therefore, a nonspecific measure of developmental perturbation.

Using asymmetry analysis on three species of marine teleost—barred sand bass, *Paralabrax nebulifer*; grunion, *Leuresthes tenuis*; and barred surfperch, *Amphistichus argenteus*—from southern California and Baja California, we have been able to define two possible asymmetry trends, historical and geographic. Asymmetry values are shown to increase as we approach highly populated areas (southern California) both from the north and south and also with time within southern California. Such increases in asymmetry correlate well with the known distribution of various toxicants from this same area.

Biological indices are urgently needed for efficiently assessing the effects of man-induced environmental stresses on the marine environment, a task widely acknowledged as both important and difficult (National Scientific Committee on Oceanography of the NAS-NRC Ocean Affairs Board, 1971). Several general approaches are now being used with mixed results: 1) the documentation of die-offs or sterility, such as results from anoxia, thermal shock, pesticides, industrial wastes, oil spills, etc., 2) the detection and description of anomalies ascribable to microorganisms associated with sewage, 3) the quantification of changes in species diversity and community organization, sometimes attributable to thermal, domestic, or industrial effluents, and 4) population changes in certain indicator species which are themselves either resistant or sensitive

to specific pollutants (Wilber, 1969). Many of the above methods have been criticized for their insensitivity, lack of standardization, and for being useful only after the occurrence of significant biotic changes. The method we propose may be a useful and sensitive alternative under certain conditions.

Our approach is morphological and is based on fluctuating asymmetry, the random signed differences between the right and left sides of a bilateral character (Van Valen, 1962). The extent to which deviations from perfect symmetry occur is thought to be an estimate of the regulatory capacity of the developmental system in a particular environment. Our working hypothesis is that asymmetry will be increased under certain kinds of environmental stress due to the failure of homeostatic regulatory mechanisms. Such developmental effects might occur before the concentration of toxicants in waters or food reach levels high enough to cause widespread morbidity. It would be slightly easier to use variation per se, such as estimated by the standard deviation or coefficient of variation of a unilateral char-

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acter, to measure such perturbations, but such variation is complex and probably of little use in measuring developmental homeostasis or stability of individuals within a population (Thoday, 1958; Soulé and Cuzin-Roudy, in press). The recent discovery that morphological variation may be highly correlated with mean heterozygosity based on electrophoretic analysis (Soulé and Yang, in press) reemphasizes this point.

Adams and Niswander (1967) suggested that, "It is possible that an overwhelming environmental stress (i.e., teratogen) could [cause] . . . increased asymmetry." We, therefore, decided to examine fluctuating asymmetry as a possible measure of environmental stress. Previous studies of asymmetry have not emphasized the environmental component (for references see Mather, 1953; Thoday, 1958; Reeve, 1960; Sakai and Shimamoto, 1965). The use of paired structures to analyze bilateral developmental differences in fish was first suggested by Hubbs and Hubbs (1945).

MATERIALS AND METHODS

To field test this hypothesis we chose three species of marine teleost fish—barred sand bass, *Paralabrax nebulifer*; grunion, *Leuresthes tenuis*; and barred surfperch, *Amphistichus argenteus*. These species belong to different families and differ greatly in their life histories. They are also abundant throughout the area of study, they are easily captured, and large collections were available from museums for comparative space and time series analyses.

Barred sand bass are generalized carnivores (Quast, 1968) and probably approach the longevity of kelp bass (*P. clathratus*), which can survive at least 32 years in the wild (Young, 1963). Barred sand bass are also of significant importance to the sport fishing industry in southern California (Pinkas, Oliphant, and Haugen, 1968) and are apparently nonmigratory (Young, 1969). Adults (those fish older than 1 year) live offshore in rock-sand ecotonal areas, except during the spawning season when they migrate short distances to sandy areas to spawn (Turner, Ebert, and Given, 1969). From examination of adults, barred sand bass

appear to be secondarily gonochoristic, as are kelp bass (Smith and Young, 1966), and are undoubtedly broadcast spawners.

Grunion are small atherinids that mature in about a year, are thought to be nonmigratory, live only 2 or 3 years, and are found in inshore waters (Walker, 1952). They spawn on sandy beaches during the high tides from late February or March through August or September (Walker, 1952). Grunion lack teeth and it is, therefore, assumed that they feed on small organisms (Frey, 1971).

Finally, barred surfperch are found almost exclusively in the surf zone and feed predominantly on sand crabs, *Emerita analoga*, which constitute roughly 90% of their diet (Carlisle, Schott, and Abramson, 1960). From tagging studies, barred surfperch appear to be nonmigratory (Carlisle et al., 1960). In contrast to barred sand bass and grunion, surfperch are viviparous (Eigenmann, 1894), with an apparent maximum life span of 6 years for males and 9 years for females (Carlisle et al., 1960).

All reference to length in this paper refers to the standard length in millimeters, defined as extending from the anterior notch between the premaxillaries to the end of the hypural plate. Variability is expressed as one standard error (SE) or one standard deviation (S) on either side of the mean.

Character Analysis

Seven characteristics were used for asymmetry analysis:

1. Number of pectoral fin rays: A count of the total number of pectoral fin rays, including the uppermost unbranched ray.

2. Total gill rakers: A total count of rakers and all raker rudiments that could be seen under a dissecting microscope. "Secondary" rakers, which frequently occur in barred sand bass with badly deformed rakers (Valentine and Bridges, 1969), were excluded from the count.

3. Scales above the lateral line: A count started approximately two scale rows in front of the first dorsal fin spine and extending ventral-posteriorly along a natural scale row to the pored lateral line scales.

4. Scales below the lateral line: A count

begun between the first anal fin spine and the vent, extending dorso-anteriorly along a natural scale row to the pored lateral line scales.

5. Pored lateral line scales: A count begun with the first anterior pored lateral line scale, usually located under or adjacent to the opercular series, and extending posteriorly to the end of the hypural plate. When the hypural plate is reached, a straight pin is inserted in the last pored scale counted and pushed through the caudal peduncle. When the scales are counted on the other side of the fish, the pin designates the point at which counting should cease.

6. Length of the dentaries: Measured from the symphysis to a small notch at the posterior edge of the dentary.

7. Length of the posterior lateral projection of the epiotic: Measured from the medial notch defining the projection to the tip of the projection.

Where practical, all characters were counted under a binocular microscope. For specimens too large to fit under a microscope, a magnifying glass was used.

Statistical Analysis

The procedure for meristic (countable and discrete) characters follows. For each individual in a sample, the count taken from the left side is subtracted from the corresponding count on the right to obtain the signed difference. Next, the standard deviation of the signed differences (S_{r-l}) is calculated for each sample. Then, the mean of the character $(\bar{x}_{r+l})$ is calculated for each sample by adding the absolute scores for both sides and dividing by the sample size. Finally, the squared coefficient of asymmetry variation (CV_a^2) for each population is defined as follows:

$$CV_a^2 = \left(\frac{S_{r-l} \times 100}{\bar{x}_{r+l}} \right)^2$$

F tests are employed to test for significant differences in asymmetry. We follow Lewontin (1966) in using the squared coefficient of variation as an analogue to the variance.

To obviate scaling problems associated with growth in morphometric characters (nondis-crete, measurable), each measurement (item) is divided by a suitable "general size" measurement (e.g., we utilize the length of the cranium as the standardizing measurement when studying bilateral bones in the cranial region). Each morphometric measurement is so treated before obtaining the signed difference. The variance of the resulting differences is then treated as if it were a CV_a^2 value.

After the above analyses had been performed on a series of barred sand bass from southern ($N = 74$) and Baja California ($N = 101$), we decided to restrict further asymmetry analyses to total gill raker and pectoral fin ray counts since asymmetry in all of the other characters showed a significant association with asymmetry in these (Table 1). Furthermore, pectoral fin rays and gill rakers are the easiest characters to quantify and appear to be the most accurate counts that can be taken on barred sand bass. For both grunion and barred surfperch only the pectoral fin ray and total gill raker counts were used for asymmetry analysis.

Locality Designations

We consider Point Fermin, the first major point north of the Los Angeles-Long Beach Harbors, to be our pivotal locality. Point Fermin is situated approximately equidistant between Point Conception and the California-Mexico border, the northern and southern boundaries for southern California. The distances between Point Fermin and the other localities mentioned in this paper are indicated in Table 2.

RESULTS

Barred Sand Bass

In our original analyses of barred sand bass asymmetry data, we used all characters previously listed. Results from these analyses are presented in Table 1. For all characters, both meristic and morphometric, asymmetry values for southern California barred sand bass are significantly higher than for Baja California or Mexican barred sand bass.

Of interest is the relation between asymmetry and length. Mexican barred sand bass were

TABLE 1.—Comparison of CV_a^2 values and character means ($\bar{x}_{r+l}$) of barred sand bass from southern California and Baja California.

Character	CV_a^2 values Southern California	N	CV_a^2 values Baja California	N	F ratios	F ratio probabilities	Character means Southern California	Baja California	t test probabilities
Dentaries / cranium[1]	[2]0.8056	66	[3]0.4868	66	1.621	<0.05	0.984	1.001	<0.01
LEP / cranium[1]	[2]10.3121	66	[4]5.4020	60	1.908	<0.01	0.546	0.484	<<0.001
Scales above lateral line	[5]8.5488	101	[6]3.8534	74	2.219	<0.001	37.485	37.595	<0.9
Scales below lateral line	[5]6.9209	101	[6]1.6268	74	4.254	<<0.001	74.465	75.189	<0.2
Pored lateral line scales	[5]2.2300	101	[6]0.5427	74	4.107	<<0.001	141.901	139.000	<0.001
Pectoral fin rays	[7]1.9604	295	[8]0.4101	210	4.780	<<0.001	35.068	35.448	<0.001
Total gill rakers	[7]8.3009	295	[8]1.9648	210	4.225	<<0.001	53.139	53.648	<0.1

[1] Variances for dentaries / cranium and lateral epiotic projections (LEP) / cranium $\times 10^4$.
[2] Mean standard length 288.4 ± 6.1 mm (SE).
[3] Mean standard length 293.7 ± 6.5 mm (SE).
[4] Mean standard length 292.9 ± 6.8 mm (SE).
[5] Mean standard length 287.0 ± 4.9 mm (SE).
[6] Mean standard length 293.6 ± 6.5 mm (SE).
[7] Mean standard length 193.6 ± 3.2 mm (SE).
[8] Mean standard length 193.3 ± 4.4 mm (SE).

TABLE 2.—Locality designation and approximate straight-line distance to Point Fermin.

Locality	Miles to Point Fermin
San Simeon	210
Santa Rosa Island[1]	100
Carpinteria	85
Point Fermin	0
Belmont Shore	9
San Clemente	53
San Diego	101
California-Mexico border	116
Bahia San Quintin (Mexico)	270
Bahia Sebastian Vizcaino (Mexico)	435
Turtle Bay (Mexico)	475
Southern Baja California (Mexico)	550-750

[1] Santa Rosa Island is 26 miles to sea from the nearest point of land, which is itself 100 miles from Point Fermin.

grouped into size classes (Table 3). From these data it would appear that in small individuals (< 100 mm) pectoral fin ray asymmetry may decrease as size increases, while gill raker asymmetry may decrease. For gill rakers the trend is probably an effect of incomplete development, character means are always lowest in smaller size classes (Tables 3, 4, 5).

Southern California barred sand bass were likewise grouped into size classes (Table 4). Comparing the data in these two tables (3 and 4) will illustrate three notable differences. First, small southern California fish (0.0-49.9 mm) have higher asymmetry levels than do their Mexican counterparts. Second, pectoral fin ray asymmetry increases with size in southern California barred sand bass, instead of decreasing and plateauing, as in Mexican barred sand bass. Third, the largest southern California fish have asymmetry levels four to five times higher than do Mexican fish. (In southern California barred sand bass the association of asymmetry in both characters with standard length is highly significant, $P < 0.01$, using Kendall's coefficient of concordance; Snedecor, 1956.)

Further analyses of these data suggest that these geographic differences in asymmetry are the consequence of both ontogenetic and historical processes that have been occurring in the southern California population. The possible ontogenetic change is an increase in asymmetry with size (age). The possible historical process is a secular increase in asymmetry. Both hypotheses are sufficient to account for the observed trends. Unfortunately, data are lacking to resolve this problem by straightforward regression analyses. Nevertheless, the following analyses suggest that both processes are operating.

First, the ontogenetic theory is examined by following cohorts (cohort, in this case, being defined as specimens spawned in the same year, regardless of the size or age of the fish at the time of capture). Our only collections containing enough specimens to perform such an analysis are those hatched in 1957, 1958, and 1959

TABLE 3.—CV_a^2 values and character means $(\bar{x}_{r+l})$ by size class of barred sand bass from Baja California.

Size class (mm)	N	Mean length ± SE	CV_a^2 values		Character means	
			Pectoral fin rays	Total gill rakers	Pectoral fin rays	Total gill rakers
0- 49.9	31	44.5 ± 0.8	1.0708	0.7428	35.290	51.613
50- 99.9	32	66.8 ± 2.5	0.7532	1.4556	35.656	53.094
100-149.9	33	115.7 ± 2.1	0.6724	2.3264	35.606	54.364
150-249.9	19	196.9 ± 7.4	0.4220	1.4668	35.316	54.316
250-299.9	36	279.8 ± 2.1	0.4592	2.6348	35.278	54.083
300 and up	59	329.5 ± 3.5	0.4088	2.3332	35.475	54.136

TABLE 4.—CV_a^2 values and character means $(\bar{x}_{r+l})$ by size class of barred sand bass from southern California.

Size class (mm)	N	Mean length ± SE	CV_a^2 values		Character means	
			Pectoral fin rays	Total gill rakers	Pectoral fin rays	Total gill rakers
0- 49.9	19	39.1 ± 1.4	1.3553	5.8338	34.789	50.842
50- 99.9	27	70.6 ± 2.8	0.9405	8.2111	34.852	52.556
100-149.9	80	130.1 ± 1.4	1.8461	6.0791	35.150	52.888
150-199.9	39	162.9 ± 1.6	2.1140	10.7590	35.077	54.846
200-249.9	28	228.5 ± 2.8	1.8782	11.5168	35.321	52.893
250-299.9	70	278.6 ± 1.6	2.8201	7.3304	35.014	53.186
300 and up	32	365.1 ± 7.7	2.3502	11.0300	35.094	53.656

TABLE 5.—CV_a^2 values and character means $(\bar{x}_{r+l})$ for southern California barred sand bass probably hatched in 1957, 1958, and 1959.

Size class (mm)	N	Mean length ± SE	CV_a^2 values		Character means	
			Pectoral fin rays	Total gill rakers	Pectoral fin rays	Total gill rakers
0- 99.9	21	49.1 ± 3.6	1.2116	4.4860	34.905	50.950
100-199.9	21	146.5 ± 3.8	1.1729	5.5034	35.476	54.952
200-299.9	14	278.9 ± 5.0	1.8465	9.3107	34.928	53.000
300 and up	21	357.7 ± 7.7	1.9322	13.0783	35.048	53.429

(Table 5). These three cohorts were pooled to increase sample size. From Table 5 it is evident that both pectoral fin ray and gill raker asymmetry increased as these particular cohorts aged. The specimens do not exist to perform a similar analysis on Mexican barred sand bass, although we might infer from Table 3 that significant asymmetry changes cease when fish exceed 100 mm.

An historical trend of increasing asymmetry is also indicated. In Figure 1 the appropriate specimens are grouped by their probable dates of hatch. There is evidence for plateauing of this trend since fish hatched after 1966 appear to be no more asymmetric than those hatched

in the late fifties. A sampling problem may exist because the collections differ in the average size of specimens examined. This could partially account for the apparent plateauing. To examine this, fish of similar size and age were analyzed (Table 6). The plateau is still apparent, so it is probably real.

To summarize the barred sand bass data, there is evidence for two asymmetry trends in southern California—an increase with size (age) and the other with time. An attempt to partition the asymmetry contributions of these two probable sources by using multiple regression produce inconclusive results due, we think, to the inadequate nature of the samples.

TABLE 6.— CV_a^2 values by year of hatch in the 100.0-199.9 mm size class of barred sand bass from southern California.

| Date of hatch[1] | N | Mean length ± SE | CV_a^2 values | |
			Pectoral fin rays	Total gill rakers
1956 and before	23	127.3 ± 3.1	0.6692	2.4805
1957, 1958, 1959	21	146.5 ± 3.8	1.1791	5.5033
1963, 1964, 1965	51	149.3 ± 2.4	2.7476	10.8313
1966 and later	20	133.4 ± 4.7	2.5353	7.7993

[1] The 1960, 1961, and 1962 year of hatch class of fish were not included in this analysis since N = 4.
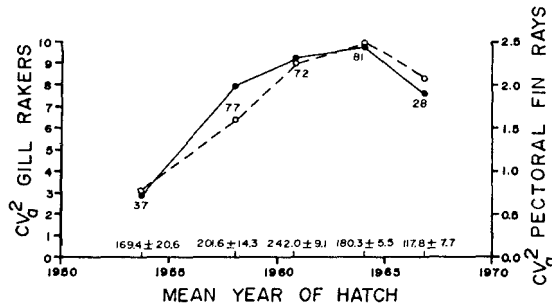
FIGURE 1.—CV_a^2 values for southern California barred sand bass grouped by probable year of hatch. Closed circles, gill rakers; open circles, pectoral fin rays. Numbers on upper abscissa indicate mean length of specimens in samples ± SE. To increase sample sizes fish from several consecutive years of hatch were pooled as follows: up to and including 1956; 1957 through 1959; 1960 through 1962; 1963 through 1965; 1966 and later.

Figure 2 graphically demonstrates how asymmetry values vary with increasing size, both in our Mexican fish and in fish from our southern California cohorts analysis.

Grunion

Only two bilateral meristic counts were made on grunion, the number of pectoral fin rays and total gill rakers. Most grunion were probably not over 2 years old and, hence, were not grouped into classes based on dates of hatch but rather only by dates of collection (Table 7). Our Turtle Bay sample is not large enough to determine asymmetry trends with body size.

The Turtle Bay collection has the lowest CV_a^2 values. Contemporary San Quintin and San Diego collections have statistically increas-

ing CV_a^2 values with time. We employed Kendalls' coefficient of concordance (Snedecor, 1956) on the data in Table 7 to test for asymmetry trends with date of capture for both characters in grunion from Belmont Shore. The results are significant, $0.05 < P < 0.01$, indicating a significant association between year of collection and asymmetry for both characters.
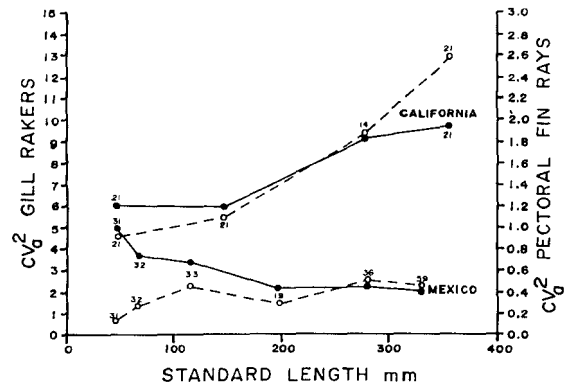

FIGURE 2.—CV_a^2 values for Mexican and California barred sand bass, by standard length. Mexican fish grouped by 50-mm size classes, all years of hatch combined. California fish grouped by 100-mm size classes, only fish hatched in the period 1957-59 included. Dots, gill rakers; open circles, pectoral fin rays. Numbers by data points indicate sample sizes.

Barred Surfperch

For this species, as for grunion, only pectoral fin rays and gill rakers were used in our analyses. From the data presented in Table 8 it can be seen that the barred surfperch data

TABLE 7.—CV_a^2 values and character means ($\bar{x}_{r+l}$) of grunion by locality.

Locality	Date	N	Mean length ± SE	CV_a^2 values		Character means	
				Pectoral fin rays	Total gill rakers	Pectoral fin rays	Total gill rakers
Turtle Bay	1948	49	109.5 ± 1.2	0.7340	0.8344	29.082	68.020
San Quintin	1970	57	126.8 ± 1.5	0.8376	1.5192	28.947	67.057
San Diego	1970	60	137.7 ± 1.6	1.0484	1.3832	28.383	68.000
Belmont Shore	1950-51	51	107.7 ± 1.2	.7364	2.6328	28.451	65.588
Belmont Shore	1963	30	77.8 ± 1.6	1.6376	2.9272	28.533	65.000
Belmont Shore	1966	22	81.9 ± 0.9	3.0388	2.8396	27.864	62.045
Belmont Shore	1968-69	27	97.3 ± 2.8	3.4248	4.2464	27.963	64.444

TABLE 8.—CV_a^2 values and character means ($\bar{x}_{r+l}$) of barred surfperch by locality.

Locality	Date	N	Mean length ± SE	CV_a^2 values		Character means	
				Pectoral fin rays	Total gill rakers	Pectoral fin rays	Total gill rakers
Bahia Sebastian Vizcaino	1952	61	126.1 ± 7.4	0.8028	0.7896	54.597	40.097
Bahia San Quintin	1970-71	44	196.5 ± 6.6	0.7484	1.6328	54.727	39.909
Belmont Shore	1958	73	108.1 ± 3.4	1.2204	2.3268	55.301	38.534
Belmont Shore	1969-70	44	98.2 ± 2.5	2.1660	4.6272	55.364	39.341
Carpinteria	1970	15	164.9 ± 0.5	0.5892	3.6308	53.933	38.667
Santa Rosa Island	1954	56	92.5 ± 7.4	1.5252	1.0700	52.339	38.125
San Simeon	1949	30	112.0 ± 5.4	.4736	1.1476	53.067	38.633

closely approximate the previously presented asymmetry trends for both barred sand bass and grunion. The two oldest collections, Bahia Sebastian Vizcaino and San Simeon, uniformly have low CV_a^2 values. The other relatively old collection, Santa Rosa Island, has moderately high pectoral fin ray asymmetry. The majority of specimens in this collection (37 of 56) are small, ranging between 46 and 77 mm. Barred surfperch, at parturition, are between 42 and 53 mm (Carlisle et al., 1960). A possible explanation for the higher Santa Rosa value is that these small individuals have not yet been exposed to prolonged stabilizing selection. The only other young barred surfperch available for comparison are 18 from Bahia Sebastian Vizcaino (50.1-77.5 mm). Of these 18 specimens, 5 are asymmetric for pectoral fin rays. For our Santa Rosa Island specimens 11 of 37 are asymmetric. Differences are not statistically significant.

To examine the association of asymmetry and size we grouped our three oldest collections (Bahia Sebastian Vizcaino, Santa Rosa Island, San Simeon) and analyzed these collections

by size class. Table 9 shows the results of this analysis. Neither gill raker nor pectoral fin ray asymmetry appear to increase with size (age). In Table 8, Figure 3, gill raker data points from Bahia Sebastian Vizcaino, Santa Rosa Island, and San Simeon are connected (solid line) because we feel that these three populations probably represent what might be termed "unperturbed" populations. All three collections are old, all were taken at least 100 miles from Los Angeles and all are probably little exploited by other than natural predators.

Also in Figure 3, all relatively recent collections (San Quintin, 1970-71, Belmont Shore, 1969-70, and Carpinteria, 1970) are connected by a dashed line. The extremely elevated CV_a^2 value for the Belmont Shore collection of 1969-70 should be noted, as well as the high value at Carpinteria.

DISCUSSION

In the discussion of these results we find it necessary to distinguish between two levels of causation. The first is the origin of the asym-

TABLE 9. —CV_a^2 values and character means ($\bar{x}_{r+l}$) by size class of barred surfperch.[1]

Size class (mm)	N	Mean length ± SE	CV_a^2 values		Character means	
			Pectoral fin rays	Total gill rakers	Pectoral fin rays	Total gill rakers
0- 74.9	52	58.6 ± 1.1	1.5348	0.8844	52.923	38.904
75-124.9	36	87.4 ± 1.2	0.6924	1.0908	53.639	39.278
125-174.9	34	144.8 ± 2.0	0.6400	1.0008	53.294	38.794
175 and up	26	201.8 ± 3.5	0.9012	0.8728	54.346	39.385

[1] Includes specimens from Bahia Sebastian Vizcaino, San Simeon, and Santa Rosa Island.
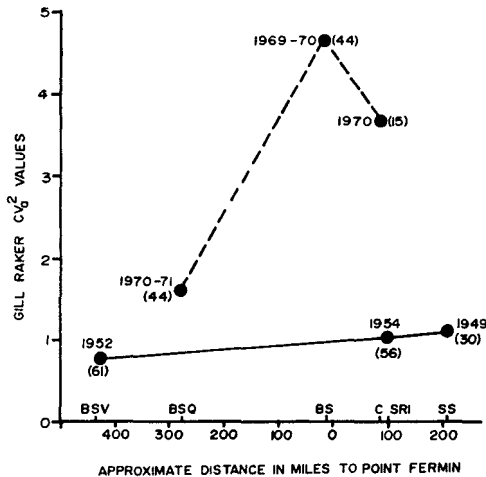
FIGURE 3.—Geographic and historic CV_a^2 trends in barred surfperch. Connected points are more or less "contemporary" samples. Letter codes are as follows: BSV, Bahia Sebastian Vizcaino; BSQ, Bahia San Quintin; BS, Belmont Shore; C, Carpinteria; SRI, Santa Rosa Island; SS, San Simeon. Dates of collection and sample sizes (N) for each collection are as indicated.

metry differences, and the second is the maintenance of these differences. We consider two possible origins: genetic and environmental. The parallel dichotomy with regard to maintenance is that the observed patterns are maintained by natural selection differentials in time and space, or the patterns are maintained by a geographically stable pattern in the distribution of the relevant environmental factor(s). (Other combinations of causative categories exist, but are rejected for lack of evidence and are ignored here.)

The hypotheses explaining our results are grouped into three categories: (A) hypotheses assuming a genetic origin, (B) hypotheses assuming an environmental origin stemming from natural causes, and (C) hypotheses assuming an environmental origin stemming from various pollutants.

Arguments for Category A Hypotheses

All genetic explanations assume the relaxation of stabilizing selection, thus allowing the accumulation of "abnormal" genotypes in the population resulting in an increase in the asymmetry variance of bilateral characters.

Various changes that could bring about such a decrease in the intensity of stabilizing selection in the populations studied are thought to have occurred in the recent past. These changes are of four types: (1) increased cropping of adults by fishermen, (2) a fish population explosion, (3) a decrease in the predator-prey ratio, and (4) an increase in the carrying capacity of the environment. A rapid increase in a population (type 2) depends on a high juvenile recruitment rate. In this respect, the effect of a population explosion is similar to that of increased fishing pressure on adults (type 1), although the former change is usually more transient than fishing pressure changes.

Fishing pressure and the number of fish being caught in southern California are increasing (Frey, 1971). Given that the adult populations of the three species in question have remained stable (there is no evidence to the contrary), then at some stage the removal of adults must be compensated for by increased survival of recruits. One possible consequence of this is an increase in the probability of survival

of abnormal or extreme juveniles. The geographical concordance of asymmetry in these fish and human population density in the area studied is certainly suggestive. Furthermore, asymmetry has apparently been increasing in southern California over the past two decades, as has the number of anglers.

There is evidence that a population explosion of southern California barred sand bass occurred some time during the time period 1957-59. Before this time barred sand bass constituted a minor fraction of the "bass" catch in southern California marine waters ("bass" refers to barred sand bass, kelp bass, and spotted sand bass, *P. maculatofasciatus*), whereas in 1964 they constituted over 50% of the bass catch from private sport fishing vessels (Pinkas et al., 1968). The rapid proliferation of one or two year classes of fish has been known to produce, at least temporarily, phenotypically aberrant cohorts (Whitney, 1961). Similar patterns may be postulated for asymmetry.

A decrease in the predator-prey ratio should have similar effects. One factor maintaining low variability and possibly low asymmetry variances is the elimination of phenotypically and, presumably, genetically deviant individuals via centripetal or stabilizing selection. With less predation there might be higher survival of deviants. Elimination of piscivorous predators may have resulted from increased fishing pressure and reduction in marine piscivorous birds and mammals.

Domestic sewage outfalls in closed bodies of water are known to stimulate productivity (MacKenthun, 1965, for a review). Similar stimulatory effects on marine phytoplankton are to be expected. Increasing primary productivity should lead to higher overall productivity and an increase in the carrying capacity of the environment, particularly near outfalls. An increasing carrying capacity should result in continual growth in the size of the populations of marine organisms associated with outfalls. Increases in asymmetry can easily be correlated with the increasing volume of sewage effluents in southern California. The apparent increase in size of barred sand bass populations can also be so correlated. The higher concentrations of fish near outfalls (reported by fishermen) as

opposed to adjacent areas would also be cited. These larger populations of fish may be a direct result of the increased availability of food in areas near outfalls.

Evidence Against
Category A Hypotheses

Increased cropping of adult fishes through angler success leading to an increase in asymmetry is a difficult hypothesis to test with the species we have chosen to study. Virtually no data exist on the numbers of grunion and barred surfperch taken yearly. Fisheries statistics for the three basses are pooled by the California Department of Fish and Game, making separation by species most difficult. Most of the bass catch is, however, composed of kelp and barred sand bass. Separating the bass catch into kelp and barred sand bass is difficult.

Prior to 1957 barred sand bass composed a very minor portion of the bass catch (Frey, 1971). From 1947 to 1959 the bass fishery experienced a general decline, reaching a 23-year low of 428,656 bass caught in 1959. Beginning somewhere around the early 1960's barred sand bass began constituting an increasing portion of the bass catch and by 1964, barred sand bass made up roughly half of the bass catch from private fishing vessels (Pinkas et al., 1968). It would, therefore, seem logical that the increase in the bass catch resulted as much from an increase in barred sand bass as from kelp bass. We have seen no information that the proportion of barred sand bass in the bass catch is declining. The total bass catch has been above 1 million for the past 7 years, averaging 1.2 ± 0.109 million individuals from 1963 to 1969. It would, therefore, appear that the barred sand bass population has stabilized.

Recall from Figure 1 that CV_a^2 values in barred sand bass have been increasing from at least 1954 through 1964. During this same period the bass catch passed through a declining phase and then a rapid increase in catch. If fishing pressure were to account partially for these data, we would expect to see relatively stable levels until sometime around 1960, when levels should begin to climb due, on one hand,

to the population explosion of barred sand bass and, on the other, to increased angler success. No such trends are to be found in our data. As previously mentioned, catch data for grunion and barred surfperch are lacking so that it is impossible to examine these species for the possible effects of fishing pressure.

The effects of a population explosion on asymmetry variance should be temporary and will cease when the population stabilizes. The highest asymmetry values should reflect periods of exceptionally high juvenile recruitment.

Our data on probable year of hatch and cohorts analyses show that asymmetry values of barred sand bass are lower in 1957-59 than in later periods. Recall that this is the time when a population explosion of barred sand bass is supposed to have begun. If the asymmetry is genetic and/or congenital, then the low asymmetry of these fish is inconsistent with the above prediction. For our data to support such an hypothesis it would have to be shown that the population of barred sand bass and, for that matter, grunion and barred surfperch, have been continually increasing. Our data do not support this premise. No data exist for either barred surfperch or grunion which can be used as a basis on which to test this premise.

The question of shifting predator-prey ratios is most difficult to analyze. There undoubtedly exist changes in such ratios not only from year to year, but also seasonally. Whether such changes can result in significant shifts in asymmetry variances is unknown. The fact that all three species occupy different habitats, all appear to be undergoing similar asymmetry changes, and all are probably preyed upon by different predators would seem to argue against such a simple cause and effect explanation.

Fish density around outfalls may be high, but this has never been shown to be an effect of the local productivity gradient. A logical alternative is that outfalls, being constructed over sand or muddy bottoms, provide an artificial habitat. Studies by the California Department of Fish and Game (Turner et al., 1969) have shown that artificial reefs in predominantly sandy areas of low relief rapidly attract large assemblages of fish. Barred sand bass were the most frequently observed species and were reported inhabiting artificial reefs within hours after construction terminated.

That larger populations of some species (e.g., barred sand bass) are apparently residing in southern California now than in the past may be ascribed to fluctuations in normal environmental conditions. Furthermore, of the three species studied, only barred sand bass and possibly grunion are likely to be found around sewer outfalls. Barred surfperch are predominantly inshore and seldom venture past the surfzone. They are, therefore, unlikely to be affected as much as are the other two species, unless an increase in primary productivity is widespread. Yet data on asymmetry for all three species are similar.

Arguments for Category B Hypotheses

Naturally occurring, clinally varying abiotic factors would also seem to be a logical source of induced meristic variation since asymmetry increases from Mexico northward. Experimentally, Schmidt (1919, 1920) with brown trout, *Salmo trutta*, and the blenny, *Zoarces viviparus*; Lindsey (1954, 1958) with the paradise fish, *Macropodus opercularis*; Blaxter (1957) with the herring, *Clupea harengus*; Tåning (1952) with the sea trout, *Salmo trutta trutta*; and others have clearly demonstrated that temperature plays a very important role in determining the ultimate meristic counts in a wide range of fishes. Barlow (1961) stated that a good correlation exists between cooler environmental temperatures and higher meristic scores. Other environmental factors such as light intensity (McHugh, 1954, with the grunion), light duration (Lindsey, 1958, with the kokanee, *Oncorhynchus nerka*), oxygen and carbon dioxide tensions (Tåning, 1944, 1952, with the sea trout), and salinity (Heuts, 1947, with the stickleback, *Gasterosteus aculeatus*) have been examined with similar results. About the only factors studied that have not been shown to significantly alter meristic counts are pH and phosphate and nitrate concentrations (Tåning, 1952).

Arguments Against
Category B Hypotheses

If asymmetry were to correlate well with mean annual water temperature, we would expect that it should continue to increase with latitude. Our data for barred surfperch are inconsistent with this hypothesis, as are those of Hubbs and Hubbs (1945), who have conducted the only large-scale study of asymmetry encompassing a large latitudinal transect. Hubbs and Hubbs (using a statistic differing from that used here) studied the asymmetry of pectoral fin rays in the Pacific staghorn sculpin, *Leptocottus armatus*, from Sitkalidak Island, Alaska, to Mission Bay, in southern California. Of the 12 populations examined, the highest asymmetry values were from their southernmost samples, Anaheim and Mission Bays in southern California (Table 10). The Tau coefficient (Snedecor, 1956), applied to these data, indicate a significant negative asymmetry correlation, $P < 0.05$, with increasing latitude.

Finally, not only are asymmetry values increasing clinally, they also appear to be increasing temporally. We know of no environmental variable showing these continuous trends. Most variables show cyclic fluctuations, whereas our asymmetry values apparently do not.

Arguments for
Category C Hypotheses

This hypothesis assumes only that as environmental stress increases so should asymmetry. At this point in time we must leave unspecified what type of stress may produce increases in asymmetry, although preliminary experiments with grunion would seem to indicate that p,ṕ-DDT may have this effect (Valentine and Soulé, 1972).[4] As a first approximation, we might assume that specific ubiquitous pollutants known to interfere with various metabolic processes might logically be suspect. Two such groups of agents are known to occur in the southern California environment (marine, terresterial, and atmospheric), chlorinated hydrocarbons (Risebrough et al., 1967; Schmidt,

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[4] Valentine, D. W., and M. Soulé. 1972. Asymmetry: The effect of p, p'-DDT on the development of fins in the grunion (*Leuresthes tenuis*). Unpublished manuscript.

TABLE 10.—Pectoral fin ray asymmetry in the Pacific staghorn sculpin, *Leptocottus armatus*.[1]

Locality	N	$\bar{x}_{r+l}$	$^2\dfrac{100(l+r)}{N}$
Alaska			
Sitkalidak Island	174	38.89	16.67
Admirality Island	104	38.49	22.12
Washington			
Puget Sound	245	38.06	20.41
Grays Harbor	103	37.82	15.53
Oregon			
Nestucca Bay	136	37.94	17.65
California			
Humboldt Bay	221	37.91	19.91
San Francisco Bay Region	223	—	21.97
Elkhorn Slough	145	—	22.75
Morro Bay	218	36.63	20.18
Santa Barbara	397	36.77	20.65
Anaheim Bay	109	37.01	29.36
Mission Bay	119	—	24.37

[1] Data recalculated from Hubbs and Hubbs (1945, Tables VII and VIII).

[2] Hubbs and Hubbs used this statistic to express "the amount of overall asymmetry . . . as a percentage figure in which l and r represent counts that are higher . . . on the left and right sides, respectively, and N is the total number of specimens, symmetrical plus asymmetrical, in the sample."

Risebrough, and Gress, 1971; Duke and Wilson, 1971; Burnett, 1971; Munson, 1972) and heavy metals (Tatsumoto and Patterson, 1963; Brooks, Presley, and Kaplan, 1967; Schroeder, 1971; Galloway, 1972). The distribution of pollutants in southern California waters is similar to the distribution of asymmetry values (Burnett, 1971; Galloway, 1972). This hypothesis would attribute the increases in asymmetry in the species studied to the presence of pollutants and possibly to duration of exposure.

Arguments Against
Category C Hypotheses

The only contraindicative evidence thus far is that if these asymmetries were the result of toxicants, we might expect to find behavioral and reproductive effects. None has as yet been observed, although our preliminary observations on the low viability of first spawned grunion eggs is suspicious (Valentine and Soulé, see footnote 4).

CONCLUSIONS

Several of the explanations presented assume a genetic origin of the asymmetries and involve altered demographic parameters: improved conditions leading to population growth; de-

creased predation resulting in decreased selection; increased fishing pressure. The potential interactions among these processes are manifold. Some are virtually mutually exclusive. For instance a population explosion of predators (including barred sand bass) is inconsistent with a model based on decreased predation. Some models have a superficial complementarity. For instance, a general increase in marine productivity, though resulting in population growth, would not necessarily alter selection against divergent phenotypes. Predator-prey ratios, for instance, might be expected to remain relatively constant.

Most theories that would appear to explain these data are based on a genetic hypothesis. It is not easy to sustain these hypotheses in view of the apparent age-dependency of the asymmetry. Skeletal abnormalities in these same fish (Valentine, in press) show similar temporal and spatial patterns. We tentatively conclude that the environmental stress hypothesis gives the best fit to the data. It is very possible that this approach may be limited in application to the detection of stress induced by agents affecting calcium metabolism, transport, or deposition. Recent experiments, conducted in our laboratory, with the fruit fly, *Drosophila melanogaster*, re-emphasizes this point. We attempted to increase the asymmetry of sternopleural chaeta by raising fruit flies from eggs to adults in culture medium treated with various toxicants (methylmercury chloride, cupric sulfate, tetraethyl lead, and p,p'-DDT). Toxicant concentrations ranged from 1 ppb (part per billion) to lethality and egg concentrations from 1 to 200 per vial. Results, in all instances, were negative.

Asymmetry analysis, if it proves fruitful for measuring environmental stress, has some important benefits. We think such an assay would:

1. permit continuous monitoring of the health of biological communities via periodic checks of specific sensitive organisms;

2. permit the detection of long-term trends (decades) by comparing preserved material with contemporary materials;

3. permit economies to be made in the monitoring of communities and;

4. increase the lead time between the detection of significant biological changes and community morbidity.

SUMMARY

The data presented show two trends, an increase in bilateral asymmetry with time and a consistent spatial pattern. Data for grunion and barred surfperch taken at one locality, Belmont Shore, near Los Angeles, over two decades clearly show increasing temporal asymmetry values. Data from barred sand bass and the other two species show increasing geographic trends from deep Baja California (an area virtually uninhabited) to southern California. Additionally, barred surfperch asymmetry values decrease north of Los Angeles, so that asymmetry is apparently not responding strictly to a latitudinal trend. Several hypotheses were examined, and it is tentatively concluded that environmental toxicant stress is the likeliest explanation.

We believe asymmetry analysis may be developed into a useful tool for examining the sublethal effects of multiple toxicants on natural or laboratory populations. Such analyses are relatively easy to perform and, thus, offer significant advantages over other commonly used assays.

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