

Climate-Related, Long-Term Faunal Changes in a California Rocky Intertidal Community

J. P. Barry,* C. H. Baxter, R. D. Sagarin, S. E. Gilman

Changes in the invertebrate fauna of a California rocky intertidal community between the period 1931 to 1933 and the period 1993 to 1994 indicate that species' ranges shifted northward, consistent with predictions of change associated with climate warming. Of 45 invertebrate species, the abundances of eight of nine southern species increased and the abundances of five of eight northern species decreased. No trend was evident for cosmopolitan species. Annual mean shoreline ocean temperatures at the site increased by 0.75°C during the past 60 years, and mean summer maximum temperatures from 1983 to 1993 were 2.2°C warmer than for the period 1921 to 1931.

Responses of natural communities to gradual climate change are expressed as latitudinal shifts in species' abundances and migration of geographic range boundaries. Such changes are best known for centuries or longer from terrestrial plant communities and pelagic marine systems (1, 2). Few studies exist concerning the effects of multidecadal climate change on coastal marine communities where fluctuations in the abundance and distribution of species are related principally to changes in recruitment and survival, rather than migration or transport of adults (1-3). Although coastal communities respond rapidly to environmental perturbations [for example, El Niño-Southern Oscillation (ENSO) events] (4), the protracted effects of climate change should be reflected in concomitant shifts by species with similar environmental requirements. During periods of warming, species' ranges should migrate poleward (5). Because species are generally most abundant in the center of their range and decline gradually toward range margins (6), range migration in response to climate warming should be evident by shifts in abundance at one location: Species adapted to cooler conditions should decline, while warm-adapted species should increase. Here we report changes in the abundances of 45 species of invertebrates in a central California intertidal community between the period 1931 to 1933 and the period 1993 to 1994. We also report continuous records of shoreline ocean temperatures spanning this period and consider several hypotheses to explain faunal shifts.

We replicated observations made by Hewatt (7) in 1931 to 1933 at Hopkins Marine Station (HMS) in Monterey Bay, Califor-

nia (36°37.3'N, 121°54.3'W), along a rocky intertidal transect 108 yards (98.8 m) long. Hewatt's transect crosses exposed granite bedrock and boulders from high to low intertidal zones at a site semiprotected from ocean swells. Brass bolts fixed to the bedrock in 1930 to position the transect were relocated, and the precise locations of 35 of Hewatt's original 1 yard (0.84 m²) plots were resurveyed. Plots were distributed from low to high intertidal zones (-0.2 to +1.2 m), although most (21 of 35) were in the middle zone (0.0 to +1.1 m). Our results are calculated from direct counts of all invertebrates identified in these plots during 1931 to 1933 and 1993 to 1994.

Hewatt's descriptions and photographs, and contemporary observations by Gislén (8), indicate that floral and faunal assemblages occupied distinct zones at HMS during the early 1930s. On rocks protected from wave shock, the uppermost or barren zone (+1.5 m or higher) was covered sparsely by microalgae and littorine snails. Acorn barnacles (*Balanus glandula*) and a few gastropod mollusks dominated the high zone (+1.1 to +1.5 m), and there was a sharp transition between this barnacle zone and the algal canopy bordering the middle zone. The upper middle zone was covered by a dense canopy of large seaweeds (*Pelvetia fastigata* and *Fucus distichus*) typical of cold temperate or boreal regions (8, 9) and inhabited by a suite of mobile invertebrates. The low zone (-0.5 to 0.0 m) lacked a conspicuous dominant species and was characterized as a diverse complex of red and brown algae and surfgrasses (*Phyllospadix* sp.).

We counted and identified over 58,000 individuals from 105 invertebrate taxa in 35 resurveyed plots. We chose 45 species from 105 taxa for further analysis (10). Each was assigned to a range category (northern, cosmopolitan, or southern) according to geographic distributional boundaries (11, 12).

Comparison of invertebrate abundances from the 1931 to 1933 and 1993 to 1994

surveys revealed two prominent patterns. First, shifts in population abundance (individuals per square meter) were widespread among species. Of the 45 species analyzed, 32 exhibited statistically significant relative changes in abundance (13), indicating profound change in community structure. Second, the pattern of community change was distinct among geographic range categories (Fig. 1A) and suggested a northward shift of species' ranges during this 61-year period: Abundances of southern species increased, northern species decreased, and cosmopolitan species showed no trend.

The abundances of eight of nine species in the southern geographic range group increased significantly. Gastropod mollusks, a faunal group common at sites with elevated thermal stress, showed the greatest changes. *Serpulorbis squamigerus*, a sessile aggregating gastropod, was not reported from HMS by Hewatt (7) and was rare in the mid-1960s (14), but it is now a common low to middle zone species. Two snails, *Ocenebra circumtexta* (not reported by Hewatt) and *Acanthina punctulata*, are now common middle to upper zone predators on barnacles. The limpet *Fissurella volcano*, a chiton (*Cyanoplax hartwegii*), and two southern anemones (*Corynactis californica* and solitary *Anthopleura elegantissima*) increased in the low to middle zones, where they may have benefited from warmer water. The southern rock barnacle, *Tetraclita rubescens*, was a common member of mussels beds and the *Pelvetia-Fucus* canopy during 1931 to 1933 and has increased considerably in abundance throughout its vertical range.

In contrast with southern species, the abundances of five of eight northern species decreased significantly. Two sea stars (*Lepidasterias hexactis* and *Pisaster ochraceus*), a small crab (*Petrolisthes cinctipes*), an anemone (*Anthopleura xanthogrammica*), and a limpet (*Tectura scutum*) became more scarce. *Anthopleura xanthogrammica* is a low zone anemone that thrives in cold water, particularly upwelling sites (12), and may have decreased owing to warmer summer water temperature (see below). *Petrolisthes cinctipes* occurs in cryptic habitats, such as under rocks and algal canopy or within mussel beds, and was not found in 1993 to 1994. *Tectura scutum* is an abundant limpet in the Pacific Northwest and the only limpet to decline in abundance. The abundances of two northern snails (*Crepidula adunca* and *Calliostoma ligatum*) increased. *Calliostoma ligatum* is primarily a subtidal species of kelp beds but also occurs in low to middle intertidal zones. Although the overall abundance of *C. ligatum* increased, its distribution shifted seaward in the intertidal, with increased abundance in the low zone and decreased abundance in the middle zone (15). *Crepidula adunca*, a common

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epibiont of *Tegula* sp. snails, increased in abundance overall ($P < 0.005$), but its frequency on host snails remained constant (approximately one *C. adunca* per seven *Tegula* sp.; $F = 0.07$, $P > 0.75$), because the abundance of its principal host (*Tegula funebralis*) also increased ($P < 0.01$).

In comparison with the southern and northern species, the pattern of change for cosmopolitan geographic range group was balanced. Abundances of more than half of the cosmopolitan species changed significantly (17 of 28 species), but the number of species that increased significantly

(9 of 28) was nearly equal to the number that decreased (8 of 28).

We evaluated the null hypothesis that the direction of change in species' abundance was unrelated to geographic range groups in two ways. Analyses of frequencies of significant increase or decrease in species' abundances indicated that the pattern of change was not independent of range categories (row by columns test; $G = 11.3$, $P < 0.005$). Analysis of variance (ANOVA) based on the use of mean ratios of abundance (1994 versus 1933) for each species indicated significant variation among range categories. Changes in southern species were significantly greater than changes in either cosmopolitan or northern groups (16) (Fig. 1B). Because geographic ranges for most species were derived from recent literature (11, 12), our range designations are conservative compared to those based on observed or known geographic ranges from 1931 to 1933, particularly if species' ranges have shifted northward.

Although Hewatt provided only qualitative descriptions of intertidal flora, several sources suggest that sweeping changes also occurred in the macrophyte assemblage over six decades. The upper zone remained dominated by barnacles but in 1993 to 1994 included moderate cover of algae (*Endocladia muricata*, *Mastocarpus papillata*) that graded into a dense middle zone algal turf (9, 17) rather than the distinct boundary between these zones re-

ported by Hewatt (7). The heavy *Pelvetia-Fucus* canopy decreased somewhat by 1947, when T. A. Stephenson and A. Stephenson (9) noted that these species were abundant but not ubiquitous in the middle zone. In 1994, *Pelvetia fastigata*, a cosmopolitan but principally cold-temperate species (8, 9), and *Fucus distichus*, a northern species, were still common, but low-growing turf algae [*E. muricata*, *M. papillata*, *Gelidium coulteri*, *Rhodoglossum affine*, *Gigartina canaliculata* (all cosmopolitan species)] characteristic of warm-temperate latitudes (8, 9) were the dominant space holders.

This 60-year period of faunal and floral change coincided with secular warming of ocean and air temperatures along the western North American coast. Annual mean shoreline temperature (ST) increased by 0.75°C during the past 60 years at HMS (18) (Fig. 2A). The annual maximum ST increased slightly faster than the annual minimum temperature (19), which probably remained low owing to seasonal wind-driven coastal upwelling (20). Comparison of average monthly ST for the decade preceding each intertidal survey shows that mean winter ST warmed by 0.5°C , and the average summer ST maximum was about 2.2°C higher before the recent survey than before the period 1921 to 1931 (Fig. 2B). Although continuous records of air temperature or fog and cloud cover at Monterey are not available for this 60-year period, atmospheric warming during this century is well documented, particularly for the Northern Hemisphere, including western North America (21).

ENSO events can also have important transient effects on the structure of marine communities. These events promote local shifts in community structure and extend geographic ranges, at least temporarily (4), with effects that can reverberate over a decade (22). Six of 10 years preceding Hewatt's study were characterized by moderate to strong ENSO events, compared to 5 of 10 years preceding the 1993 to 1994 study (23). Sea-surface temperature was anomalously high for 2 years preceding both surveys (considered weak to moderate events). However, the strong ENSO in 1925 to 1927 preceded Hewatt's study by 5 years, compared to a 10-year separation between our survey and the strong 1982 to 1983 event. Although the 1925 to 1927 ENSO event was not reflected in elevated ST at HMS, reports of southern species far north of their normal ranges along western North America were common (2), similar to reports during the 1982 to 1983 ENSO event.

Even though the reported histories of ENSO events preceding each study were similar, oceanic conditions influencing

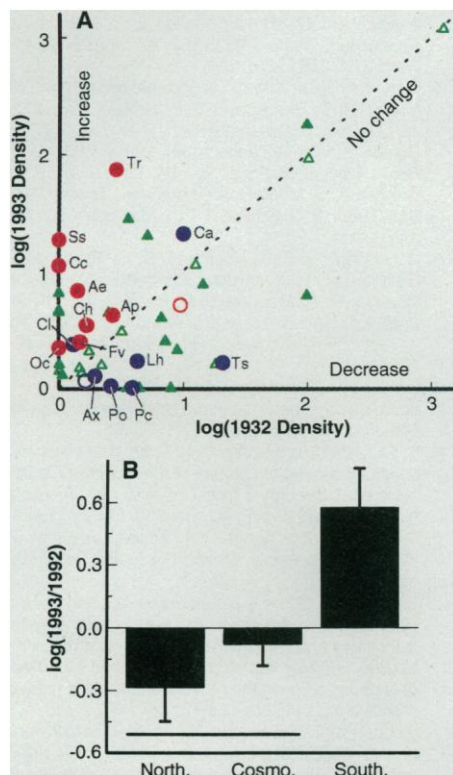


Fig. 1. Changes in the abundances of intertidal invertebrates between 1931 to 1933 and 1993 to 1994. **(A)** Mean abundance of individual species in 1993 to 1994 [$\log(\text{abundance} + 1)$] plotted against similar values from 1931 to 1933. Red circles, southern species; blue circles, northern species; green triangles, cosmopolitan species. Filled symbols indicate statistically significant changes; open symbols indicate nonsignificant values. Species name abbreviations: *Acanthina punctulata* (Ap), *Anthopleura elegantissima* (solitary) (Ae), *Anthopleura xanthogrammica* (Ax), *Tetraclita rubescens* (Tr), *Tectura scutum* (Ts), *Serpulorbis squamigerus* (Ss), *Corynactis californica* (Cc), *Cyanoplax hartwegii* (Ch), *Fissurella volcano* (Fv), *Ocenebra circumtexta* (Oc), *Calliostoma ligatum* (Cl), *Pisaster ochraceus* (Po), *Petrolisthes cinctipes* (Pc), *Crepidula adunca* (Ca), and *Leptasterias hexactis* (Lh). **(B)** Ratios of abundance [$\log[1993 \text{ abundance} + 1]/(1932 \text{ abundance} + 1)$]. Abundance is expressed as the number per square meter. Vertical lines indicate standard error bars. The horizontal bar overlaps statistically similar groups.

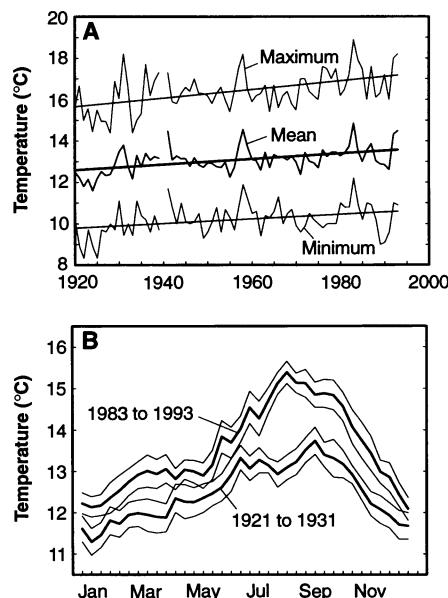


Fig. 2. Shoreline ocean temperature variation at Hopkins Marine Station during this century. **(A)** Annual mean, minimum, and maximum shore temperatures, including least squares linear regression lines for each parameter. **(B)** Seasonal variation in shore temperatures, based on 11-year averages preceding each survey period. Data are means \pm standard errors (SE).

larval transport and settlement may have differed considerably, making it difficult to evaluate their role in faunal change. The hypothesis that northward transport of larvae and other ENSO-associated effects were responsible for the observed pattern of community change is not supported, however, because changes in abundance were similar for species with or without pelagic dispersal phases. Two of five species having only local dispersal potential (no pelagic phase) increased, and one of five species decreased. Species with a short-lived pelagic phase (limited dispersal; 5 of 8 increased, 1 of 8 decreased) or long-lived pelagic larvae (wide dispersal; 10 of 27 increased, 9 of 27 decreased) had similar patterns of change (row by columns test; $G = 1.0$, $P > 0.5$). Thus, although ENSO effects may be important, the relation between the observed community shifts and ENSO events is unclear.

Changes in top predator populations clearly influenced some intertidal species. Sea otters (*Enhydra lutris*) can regulate intertidal and nearshore invertebrates (24) and were absent from Cabrillo Point in 1931 to 1933, which was known then as Mussel Point. Reoccupation of Cabrillo Point by sea otters in the 1950s (25) resulted in a reduction of mussel (*Mytilus californianus*) and urchin (*Strongylocentrotus purpuratus*) populations (26) and probably led indirectly to declines in species associated with mussel beds (*Halosydna insignis*, *Pachycheles rudis*, *Petrolisthes cinctipes*). Similar effects are expected to be minimal in this study because none of the plots surveyed were in mussel beds during either survey period (27). Otter-related effects on wave shock and availability of drift algae in the intertidal zone were also probably minor, compared to natural variation in kelp abundance caused by storms or other factors (28). Increase in the abundance of black oyster-catchers (*Haematopus bachmani*) may have affected some limpet populations (29), but changes in the principal prey species (*Lottia pelta*) were nonsignificant.

Anthropogenic impacts must also be considered but are expected to be minor. Before 1907, the site was adjacent to a Chinese fishing community that likely exerted heavy foraging pressure (30) on some invertebrates (for example, abalones, large limpets, and mussels). Since 1917, the site has been the property of HMS, with no public access, and has been fully protected as an ecological reserve since 1980.

The observed shift in community structure may also represent random variation. Because data exist only for the end points of this 61-year time series of community change, we cannot evaluate quantitatively

the pattern of temporal variation. However, reports of intertidal community patterns at HMS between 1931 and 1993 (7, 9, 14, 17, 25, 26) support a transition toward a more southern fauna. Furthermore, these data represent responses to environmental variability by 45 species from several invertebrate phyla, from which it is possible to evaluate the concordance of change in species' abundances among nearly the entire invertebrate community. Given the magnitude of change by intertidal populations and the constellation of processes influencing community structure, it is not surprising that shifts in abundance were nearly ubiquitous. It is compelling, however, that amid widespread community change, range-related patterns of change were prominent.

Although each mechanism proposed to explain faunal changes may have affected the HMS intertidal community, only two of five hypotheses considered (effects of ENSO events and long-term climate change) cannot be largely rejected. The observed changes in community structure reflect those predicted from long-term climate warming (5), and, indeed, ENSO events are an integral component of climate that probably act in conjunction with gradual warming to establish species in more northern sites. Climate-related parameters are also known to be linked closely to intertidal community patterns. Sea-surface temperature is correlated closely with the structure of intertidal communities (31), and thermal or desiccation stress caused by elevated air temperatures can have strong effects on intertidal biota, particularly in the upper intertidal (32). Furthermore, other studies at HMS between 1931 and 1993 (9, 14, 17) support the notion of a gradual transition toward a more southern flora and fauna, including reduction of the *Pelvetia-Fucus* canopy and replacement by algal turf, and northward expansion of southern species (*Chthamalus fissus*, *Serpulorbis squamigerus*).

In addition to documenting the pattern of long-term community change, our results underscore the importance of long-term studies of natural communities, particularly those including quantitative estimates of abundance and distribution of multispecies assemblages, in evaluating the effects of global climate change on natural communities. Shifts in community structure represent the integrated response of species assemblages to long-term climate change, superimposed on the effects of numerous shorter term factors. Because the tempo of coastal marine systems allows for rapid turnover and equilibration of species' distributions during multi-decadal periods of environmental variability, these systems are ide-

al for studies of climate-driven community change.

REFERENCES AND NOTES

1. D. H. Cushing, *Climate and Fisheries* (Academic Press, New York, 1982); C. B. Lange, S. K. Burke, W. H. Berger, *Clim. Change* **16**, 319 (1990); H. A. Mooney, E. R. Fuentes, B. I. Kronberg, *Earth System Responses to Global Change* (Academic Press, New York, 1993).
2. D. M. Gates, *Climate Change and Its Biological Consequences* (Sinauer, Sunderland, MA, 1993).
3. G. Shepherd, J. G. Pope, R. D. Cousens, *Rapp. P.-V. Reun. Cons. Int. Explor. Mer* **185**, 255 (1984); D. B. Chelton, P. A. Bernal, J. A. McGowan, *J. Mar. Res.* **40**, 1095 (1982); N. J. Aebischer, J. C. Coulson, J. M. Colebrook, *Nature* **337**, 753 (1990); P. K. Dayton, *Science* **245**, 1484 (1989); W. R. Coe, *J. Mar. Res.* **15**, 212 (1956); A. J. Southward, *J. Mar. Biol. Assoc. U.K.* **47**, 81 (1967); _____, E. I. Butler, L. Pennychuick, *Nature* **253**, 714 (1975); A. J. Southward, *ibid.* **285**, 361 (1980).
4. P. K. Dayton and M. J. Tegner, *Science* **224**, 283 (1984); R. T. Barber and F. P. Chavez, *ibid.* **222**, 1203 (1983); A. Schimmelmann, C. B. Lange, W. H. Berger, *Limnol. Oceanog.* **35**, 165 (1990); J. P. Barry, *Mar. Ecol. Prog. Ser.* **54**, 99 (1989); M. L. Dungan, T. E. Miller, D. A. Thompson, *Science* **216**, 987 (1982); F. C. Gunnill, *J. Exp. Mar. Biol. Ecol.* **85**, 149 (1985).
5. J. Lubchenco, S. A. Navarrete, B. N. Tissot, J. C. Castilla, in *Earth System Responses to Global Change*, H. A. Mooney, E. R. Fuentes, B. I. Kronberg, Eds. (Academic Press, New York, 1993), pp. 147-166; S. C. Keeley and H. A. Mooney, *ibid.*, pp. 209-238.
6. J. H. Brown, *Am. Nat.* **124**, 255 (1984).
7. W. G. Hewatt, thesis, Stanford University (1934); *Am. Mid. Nat.* **18**, 161 (1937).
8. T. Gislén, *Physiographical and Ecological Investigations Concerning the Littoral of the Northern Pacific*, sections 1 through 4 (Lunds Universitets Årsskrift, N.F. Avd. 2, **39** (5) and **40** (8), Lund, Leipzig, 1943).
9. T. A. Stephenson and A. Stephenson, *Life Between Tidemarks on Rocky Shores* (Freeman, San Francisco, 1972).
10. Of 105 taxa, 60 were rejected from analyses owing to (i) lack of quantitative abundance estimates for both time periods (9 taxa), (ii) taxonomic ambiguities (8 taxa), or (iii) low abundance [fewer than 10 individuals observed overall in plots or occurrence in fewer than five plots (43 species)].
11. Geographic range groups were defined as follows: (i) northern species group: southern range boundary near or north of Point Conception, California; (ii) southern species group: northern range boundary near or south of Cape Mendocino; (iii) cosmopolitan species group: all other species. Cape Mendocino and Point Conception are documented "faunal breaks," bracketing the central California region. R. F. Scagel, in *Marine Distributions*, M. J. Dunbar, Ed. (Univ. of Toronto Press, Toronto, Royal Society of Canada Special Publication 5, 1963), pp. 37-50; I. A. Abbott and G. J. Hollenberg, *Marine Algae of California* (Stanford Univ. Press, Stanford, CA, 1976); M. F. Foster, A. P. DeVogelaere, C. Harrold, J. S. Pearse, A. B. Thum, *Mem. Calif. Acad. Sci.* **1988**, 9 (1988); M. H. Horn and L. G. Allen, *J. Biogeogr.* **5**, 23 (1978); W. A. Newman, in *Historical Biogeography, Plate Tectonics, and the Changing Environment*, J. Gray and A. J. Boucot, Eds. (Oregon State Univ. Press, Corvallis, 1979), pp. 399-416. Range boundary assignments were based on geographic range information from several sources: R. H. Morris, D. P. Abbott, E. C. Haderlie, *Intertidal Invertebrates of California* (Stanford Univ. Press, Stanford, CA, 1980); R. C. Brusca, *Common Intertidal Invertebrates of the Gulf of California* (Univ. of Arizona Press, Tucson, ed. 2, 1980); E. F. Ricketts, J. Calvin, J. W. Hedgpeth, *Between Pacific Tides* (Stanford Univ. Press, Stanford, CA, ed. 4, 1968); E. N. Kozloff, *Marine Invertebrates of the Pacific Northwest* (Univ. of Washington Press, Seattle, 1987).
12. L. Francis, *Am. Zool.* **19**, 669 (1979).

13. Based on the *t* test or Wilcoxon signed ranks test ($\alpha < 0.05$).
14. M. G. Hadfield, thesis, Stanford University (1967). Hadfield studied *Serpulorbis squamigerous* at HMS during the early 1960s but relied on collections from southern California to obtain specimens.
15. Least squares linear regression of the change in abundance ($y = 1993$ abundance $- 1932$ abundance, $x =$ tidal height in feet) of *Calliostoma ligatum* indicated a significant shift toward lower tidal zones ($y = 3.4 - 3.7x$; $r^2 = 0.48$; $P < 0.0001$).
16. The logarithm of the ratio of mean abundance between 1993 and 1932 for species grouped by range categories differed significantly among range categories [one-way ANOVA; $F = 6.8$, $df = 2$, $P < 0.005$ (all species); $F = 6.2$, $df = 2$, $P < 0.01$ (only species with significant abundance changes)]. Results of multiple comparisons (with the Bonferroni probability level adjustment) indicated that the mean ratio of abundance for southern species was greater ($P < 0.025$) than that for cosmopolitan and northern species (Fig. 1B), either for comparisons with all species included or only for species exhibiting a significant change in abundance.
17. P. W. Glynn, *Beaufortia* **12**, 1 (1965); thesis, Stanford University (1963).
18. Shoreline temperatures were determined each morning (0600 to 0900 hours) at HMS from a bucket sample dipped from the shore.
19. Slopes for minimum, mean, and maximum ST for the period 1921 to 1993 are 0.011° , 0.013° , and 0.021°C per year, respectively. A test of homogeneity of slopes between minimum and maximum ST was marginally insignificant ($F = 2.59$, $P \approx 0.11$).
20. A. Bakun, *Science* **247**, 198 (1990); L. K. Rosenfeld, F. B. Schwing, N. Garfield, D. E. Tracy, *Cont. Shelf Res.* **14**, 931 (1994).
21. P. D. Jones, T. M. L. Widley, P. B. Wright, *Nature* **322**, 430 (1986); P. D. Jones *et al.*, *J. Climate Appl. Meteorol.* **25**, 161 (1986).
22. G. A. Jacobs *et al.*, *Nature* **370**, 363 (1994); M. J. McPhaden, *ibid.*, p. 326.
23. W. H. Quinn, V. T. Neal, S. E. Antunez de Mayolo, *J. Geophys. Res.* **92**, 14449 (1987). Strong to very strong ENSO events occurred in 1925 to 1927, 1933, and 1982 to 1983. Weak to moderate events occurred in 1930 to 1932 and 1986 to 1987. A moderate ENSO event also occurred in 1991 to 1993 (F. Chavez, personal communication).
24. J. A. Estes and J. F. Palmisano, *Science* **185**, 1058 (1974); P. K. Dayton, *U.S. Dep. Commer. Fish. Bull.* **73**, 230 (1975).
25. J. H. McLean, *Biol. Bull. (Woods Hole, Mass.)* **122**, 95 (1962).
26. L. F. Lowry and J. S. Pearse, *Mar. Biol.* **23**, 213 (1973).
27. The numerical density of *Mytilus californianus* in mussel beds exceeds 1900 m^{-2} (7) but averaged 6.2 ($SD = 11.7$) per plot in resurveyed plots during 1931 to 1933 and decreased to 1.5 ($SD = 6.2$) in 1993 to 1994 ($P < 0.02$). Thus, mussels were, and remain, a minor component of resurveyed plots.

28. A. W. Ebeling, D. R. Laur, R. J. Rowley, *Mar. Biol.* **84**, 287 (1985); H. L. Andrews, *Ecology* **26**, 24 (1945); P. K. Dayton, M. J. Tegner, P. E. Parnell, P. B. Edwards, *Ecol. Monogr.* **62**, 421 (1992); J. Watanabe, in preparation. Percentage cover of a 1-ha section of the HMS kelp bed varied from 4 to 78% over a 6-year period.
29. T. Hahn and M. Denny, *Mar. Ecol. Prog. Ser.* **53**, 1 (1989); D. R. Lindberg, K. I. Warheit, J. A. Estes, *ibid.* **39**, 105 (1987).
30. Human predation effects on intertidal biota are known from Chile; C. A. Moreno, J. P. Sutherland, H. F. Jara, *Oikos* **42**, 155 (1984).
31. M. M. Littler, in *The California Islands: Proceedings of a Multidisciplinary Symposium*, D. M. Power, Ed. (Santa Barbara Museum of Natural History, Santa Barbara, CA, 1980), pp. 265–306; R. R. Seapy and M. M. Littler, *ibid.*, pp. 307–324; S. N. Murray, M. M. Littler, I. A. Abbott, *ibid.*, pp. 325–340; C. D. McQuaid and G. M. Branch, *Mar. Ecol. Prog. Ser.* **19**, 145 (1984). See also R. F. Scagel in (11).
32. S. E. Emerson and J. B. Zedler, *Mar. Biol.* **44**, 315 (1978); F. C. Gunnill, *Mar. Ecol. Prog. Ser.* **3**, 231 (1980).
33. We thank J. T. Harvey, D. C. Reed, J. Watanabe, M. Yoklavich, and four reviewers for advice and comments on the manuscript.

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Minor Groove Recognition of the Conserved G·U Pair at the *Tetrahymena* Ribozyme Reaction Site

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The guanine-uracil (G·U) base pair that helps to define the 5'-splice site of group I introns is phylogenetically highly conserved. In such a wobble base pair, G makes two hydrogen bonds with U in a geometry shifted from that of a canonical Watson-Crick pair. The contribution made by individual functional groups of the G·U pair in the context of the *Tetrahymena* ribozyme was examined by replacement of the G·U pair with synthetic base pairs that maintain a wobble configuration, but that systematically alter functional groups in the major and minor grooves of the duplex. The substitutions demonstrate that the exocyclic amine of G, when presented on the minor groove surface by the wobble base pair conformation, contributes substantially ($2\text{ kilocalories}\cdot\text{mole}^{-1}$) to binding by making a tertiary interaction with the ribozyme active site. It contributes additionally to transition state stabilization. The ribozyme active site also makes tertiary contacts with a tripod of 2'-hydroxyls on the minor groove surface of the splice site helix. This suggests that the ribozyme binds the duplex primarily in the minor groove. The alanyl aminoacyl transfer RNA (tRNA) synthetase recognizes the exocyclic amine of an invariant G·U pair and contacts a similar array of 2'-hydroxyls when binding the tRNA^{Ala} acceptor stem, providing an unanticipated parallel between protein-RNA and RNA-RNA interactions.

Docking of the splice site helix of the *Tetrahymena* group I intron into the RNA active site is a model system for studying helix packing in the formation of RNA tertiary structure. The splice site helix (also called the P1 helix) contains the 5'-exon paired to the internal guide sequence (IGS) of the intron (1, 2) (Fig. 1). Following duplex formation, the helix is

docked into the ribozyme active site (3, 4) where the 5'-exon-intron boundary is cleaved by nucleophilic attack by exogenous guanosine (5). The 5'-splice site is defined by a U at the end of the 5'-exon; the U is paired with a G in the IGS (Fig. 1). While there is a general requirement for sequence complementarity between the 5'-exon and the IGS (1, 2, 6, 7), the only specific sequence requirement is at the 5'-splice site where the G·U pair is highly conserved (7). The evolutionary conservation of this pair provided the first indication that it plays an impor-

tant functional role for the ribozyme.

Replacement of the G·U pair with other combinations of natural bases has demonstrated that no other pair is as reactive (8, 9), though an A·C pair retains a portion of the wild-type activity (8). Because a protonated form of the A·C pair can be drawn in a wobble configuration, it was postulated that the shape rather than specific functional groups of the bases is important for ribozyme activity (8). To further address the contribution of the G·U pair to RNA folding, we have undertaken a systematic examination of the functional groups within the G·U pair. We substituted synthetic bases for the G·U pair (Fig. 2) in the context of the well-defined *Tetrahymena* L-21 *Scal* ribozyme (3, 10–13), a form of the group I intron that cleaves

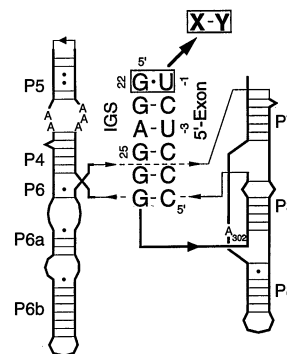


Fig. 1. Diagram of the splice site helix (capital letters) and catalytic core (solid lines) of the group I intron including the position numbers of specific bases within the helix (31). The phylogenetically conserved G·U base pair (box) is the site of base substitution, X·Y.

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