ture for 60 min and then centrifuged at 100,000*g* for 30 min at 4°C. Supernatants and pellets were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) and the relative amounts of the proteins in each fraction were estimated by scanning densitometry of the stained gels. In the absence of actin, no SipA (at all concentrations used in the assay) was detected in pellets after centrifugation at 100,000*g*.

13. Samples were loaded onto carbon-coated grids, stained with 1% uranyl acetate, and visualized under the electron microscope.
14. Actin was isolated was from rabbit skeletal muscle [J. A. Spudich and S. Watt, *J. Biol. Chem.* **246**, 4866 (1971)] and labeled with pyrene [T. Kouyama and K. Mihashi, *Eur. J. Biochem.* **114**, 33 (1981)]. Pyrene–G-actin (4 μM) was polymerized at room temperature for 30 min in actin polymerization buffer (APB) [20 mM Pipes (pH 7.0), 75 mM KCl, 2 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM dithiothreitol, and 0.05 mM ATP] and then diluted to different concentrations in the presence or absence of equimolar concentrations of SipA. After a 4-hour incubation at room temperature, the fluorescence intensity was measured on a fluorescence spectrophotometer (Hitachi F-2000) with excitation wavelength set at 365 nm and emission wavelength at 407 nm. Alternatively, pyrene–G-actin was diluted to various concentrations in the presence and absence of SipA in APB without KCl and $MgCl_2$. Polymerization was initiated by adding 75 mM KCl and 2 mM $MgCl_2$. After a 4-hour incubation at room temperature, the fluorescence intensity was measured as described above. To examine the effect of SipA on F-actin stability, we diluted 1 μM of polymerized actin, in the presence or absence of SipA (1 μM), in APB (with or without KCl and $MgCl_2$) to 0.1 μM and measured the fluorescence intensity over time. To evaluate the effect of SipA on actin polymerization, we precleared pyrene–G-actin by centrifugation at 100,000*g* for 4 hours at 4°C after dilution in APB without KCl and $MgCl_2$. Actin concentration was adjusted to 1 μM in the presence or absence of SipA (1 μM). Polymerization was initiated by adjusting the buffer concentration to 75 mM KCl and 2 mM $MgCl_2$, and the fluorescence intensity was measured over time.
15. D. Zhou and J. E. Galán, unpublished results.
16. We thank E. Taylor for providing pyrene-labeled actin, and members of the Galán laboratory for critical reading of the manuscript. Supported by NIH grants AI30492 and GM52543 (J.E.G.) and DK25387 (M.S.M.). J.E.G. is an investigator of the American Heart Association.

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Regulation of Keystone Predation by Small Changes in Ocean Temperature

Eric Sanford

Key species interactions that are sensitive to temperature may act as leverage points through which small changes in climate could generate large changes in natural communities. Field and laboratory experiments showed that a slight decrease in water temperature dramatically reduced the effects of a keystone predator, the sea star *Pisaster ochraceus*, on its principal prey. Ongoing changes in patterns of cold water upwelling, associated with El Niño events and longer term geophysical changes, may thus have far-reaching impacts on the composition and diversity of these rocky intertidal communities.

It is predicted that increasing global temperatures will shift species' geographic ranges to higher latitudes or altitudes (*1*). On a local scale, communities may undergo gradual changes in composition as species with affinities for warmer temperatures become more abundant. However, temperature changes may have more immediate effects on local populations by altering the interaction between a species and its competitors, mutualists, predators, prey, or pathogens (*2*). Often a few key interactions contribute disproportionately to maintaining the composition and functioning of a community or ecosystem (*3*). If these interactions are sensitive to temperature, small climatic changes could generate system-wide ecological changes.

Here I report evidence from experiments in Oregon that slight fluctuations in water temperature regulate the impact of a keystone predator, the sea star *Pisaster ochraceus*, on its principal prey, the rocky intertidal mussels *Mytilus californianus* and *M. trossulus*. Paine's classic experiments in Washington state demonstrated that without predation on mussels by *Pisaster*, a diverse assemblage of low intertidal algae and invertebrates shifted to a monoculture of the competitively dominant mussel *M. californianus* (*4*). Densities of *Pisaster* and its effects on intertidal communities are highest during spring and summer in the Pacific Northwest (*5*, *6*). However, preliminary observations suggested that many sea stars became inactive in low zone channels or shallow subtidal waters during periods of upwelling (*6*). Water temperatures drop 3° to 5°C during these events, which generally last several days to three or more weeks (*7*). Upwelling is common along the Oregon coast from May to September.

I quantified sea star predation rates at three wave-exposed sites (*8*) within Neptune State Park (44°15′N, 124°07′W), Oregon, to test the hypothesis that the strength of the *Pisaster-Mytilus* interaction is reduced during periods of cold water upwelling. This 4-km stretch of coastline is composed of extensive rocky benches. The high intertidal zone is characterized by fucoid algae and barnacles, the mid zone by dense beds of *M. californianus*, and the low zone by a diverse mix of algae, sea grass, and invertebrates. At each site, I identified paired reefs (mean area ± SEM = 132.5 ± 49.7 m^2) isolated by surge channels. All sea stars were routinely removed from one reef in each pair and allowed to remain at natural densities on the other reef. In April and May 1997, I transplanted 20 clumps of 50 *M. californianus* (shell length, 4.5 to 5.5 cm) to the low intertidal zone on each reef (*9*).

From June through August 1997, I conducted five consecutive experiments to measure the intensity of sea star predation during periods lasting 14 days each. At the start of each period (*10*), I randomly selected and uncaged four mussel transplants per reef per site. I then recorded mussel survivorship and local sea star density (the number of sea stars in a 1-m radius around each transplant) on each of the first 6 or 7 days and again on day 14 (*11*). Temperature data-loggers installed in the low intertidal zone at each site recorded water temperatures when submerged, or air temperatures during low tide, every 30 min. Maximum air temperatures were used as a measure of potential heat stress during aerial exposure (*12*). Five maximum wave force dynamometers (*13*) at each site recorded variation in wave stress, a factor that can inhibit consumer activity.

The experiments encompassed periods with and without upwelling and thus tested whether per capita interaction strength [the difference in rates of mussel mortality on reefs with and without sea stars, divided by the local sea star density (*14*)] varied with fluctuations in water temperature. Per capita interaction strength was sharply reduced during a persistent upwelling event (Fig. 1, A and B). During upwelling, there was an even greater proportional drop in the collective impact of *Pisaster* (Fig. 1C) as a result of two effects: Individual sea stars consumed less (lower per capita effects), and the local density of sea stars was reduced (*15*), presumably as a result of more sea stars remaining inactive in channels or shallow subtidal waters. Both per capita and population interaction strength were significantly correlated with mean water temperature and were unrelated to variation in other environmental factors such as potential aerial heat stress or maximum wave forces (*16*).

I also examined *Pisaster* feeding rates in the laboratory (*17*) under three temperature regimes: constant 12°C, constant 9°C, and a

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treatment that simulated episodic upwelling by alternating between 12°C and 9°C every 14 days. Sea stars were fed *M. trossulus* (*18*) (shell length, 3.0 to 4.0 cm) ad libitum. The number of mussels consumed per tank was recorded every 14 days. Sea stars in 9°C tanks consumed, on average, 29% fewer mussels than those at 12°C (Fig. 2). As predicted, sea stars in the alternating treatment fed intensely at 12°C, more slowly at 9°C, then faster again at 12°C (*19*).

These results suggest that interannual variation in the frequency and intensity of coastal upwelling could alter the dynamics of these intertidal communities through strong effects on keystone predation. In recent decades, upwelling patterns in the California Current have changed substantially in response to more frequent and intense El Niño–Southern Oscillation (ENSO) events, interdecadal regime shifts in the North Pacific, and perhaps global warming (*20*). During ENSO years, warm water accumulates in the eastern Pacific, the thermocline is depressed, and upwelled waters are drawn from shallower, warmer layers. These effects were apparent at my field sites during the 1997–98 ENSO. Only 6.3% of the high tides during May through August 1997 had mean water temperatures below 9.5°C, whereas 36.7% of the high tides fell below this mark during the same period of 1996. It remains to be determined whether such variation is sufficient to alter community composition. Systematic changes in cold water upwelling, which may accompany global warming (*20*), would be expected to modify predation intensity by *Pisaster* during the summer, when sea star densities and effects are the highest. This change might alter both the vertical extent of mid zone mussel beds and the species composition of the low intertidal zone.

This study demonstrates that the local strength of a keystone interaction can be altered by slight temperature shifts and changes in the timing and intensity of seasonal events. At larger spatial scales, *Pisaster's* per capita impact on prey may decrease with increasing latitude (*21*), although this response could be modified by acclimatization or local adaptation. The sensitivity of *Pisaster* to temperatures well within its tolerance range suggests a need to consider physiological effects other than the acute stress associated with a species' thermal limits. The alteration of key species interactions by climatic change could have a more immediate and important impact on natural systems than slow shifts in the geographic distribution of species.

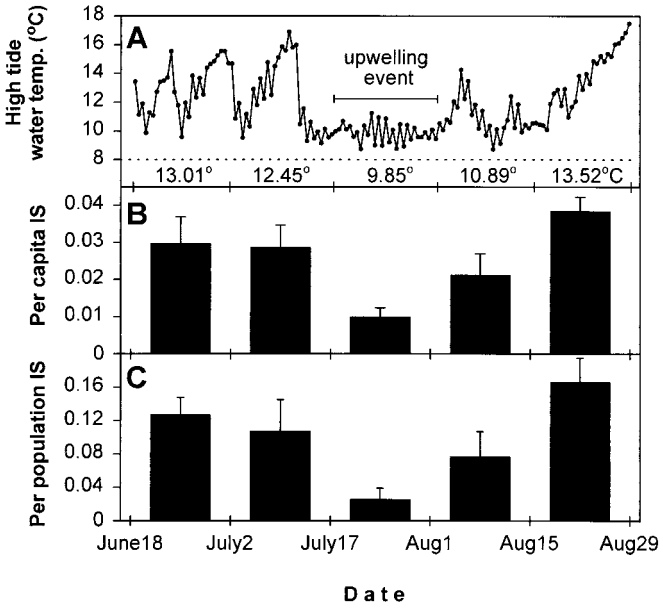

Fig. 1. Seawater temperature and sea star–mussel interaction strength (IS) during consecutive 14-day periods (*x* axis). All data are means (+SEM) of values recorded at three study sites. (**A**) High tide water temperatures (the mean from 2 hours before to 2 hours after each high tide). Temperatures above the *x* axis are the overall means for the 27 high tides of each period. (**B**) Mean per capita IS of sea stars on transplanted mussels during each 14-day period. (**C**) Mean per population IS of sea stars during each period. Both per capita and per population IS were significantly lower during upwelling than during the other four periods [ANOVA, contrast the third period (middle bars) with the other four; $F_{1,53} = 10.03$, $P = 0.003$ (per capita); and $F_{1,53} = 11.10$, $P = 0.002$ (per population)].

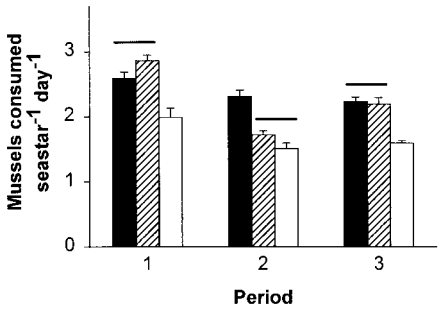

Fig. 2. Sea star feeding rates in three laboratory treatments: 12°C (solid bars), 9°C (open bars), and alternating (hatched bars) (for periods 1 through 3: 12°, 9°, and 12°C, respectively). Bars are mean consumption rates (+SEM) in treatments ($n = 4$ tanks per treatment) during three consecutive 14-day periods. Data were analyzed in a repeated measures ANOVA. Treatment ($F_{2,9} = 59.81$, $P < 0.001$), time ($F_{2,18} = 36.59$, $P < 0.001$), and time × treatment ($F_{4,18} = 5.70$, $P = 0.004$) were all significant. Within each time period, horizontal lines above bars indicate groups whose means do not differ (Tukey-Kramer, $P > 0.05$).

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8. Replicate sites (Strawberry Hill, Pigeon Reef, and Bob Creek Wayside) were several hundred meters apart. Water temperatures generally varied < 0.2°C over these distances.
9. Mussels from a common source were transplanted under plastic Vexar mesh cages that allowed mussels to reattach to the rock and shielded them from foraging sea stars until cages were removed. Mussels reattached securely. Mean percent survivorship (± SEM) 14 days after cages were removed was 97.4 ± 0.45% on reefs without sea stars ($n = 60$ transplants).
10. Starting dates were set as the first day of each spring tide series.
11. Sites were inaccessible during the neap tides, days 8 through 13.
12. Temperatures recorded by the data-loggers at low tide predicted *Pisaster* body temperature. On 20 dates, I used a digital thermometer and hypodermic probe to measure the body temperatures of 10 randomly selected sea stars at Strawberry Hill, 20 to 30 min before they were resubmerged by the incoming tide. Mean body temperature was significantly correlated with the maximum air temperature recorded by the data-logger during that low tide ($y = 0.76x + 2.75$, $R^2 = 0.85$, $P < 0.001$).
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14. Interaction strengths were calculated according to the method of S. A. Navarrete and B. A. Menge [*Ecol. Mon.* **66**, 409 (1996)]. I used the discrete time version of the Lotka-Volterra equation: $M_t = M_o e^{(\gamma - \alpha P)t}$, where M_t is the number of mussels at time t, M_o is the number of mussels when the transplant was first uncaged, e is the natural logarithm, γ describes the per capita rate of change for mussels in the absence of *Pisaster*, α is the per capita interaction strength of *Pisaster* on the per capita rate of change of mussels, and P is local sea star density. I estimated survival rates for each mussel transplant from the slope of the linear regression of $\ln(M_t/M_o)$ over time (days 1 through 14). Population interaction strengths (αP) were estimated by subtracting the mean mussel survival rate (the slope of the log regression) on reefs without *Pisaster* from the survival rate observed in mussel transplants on reefs with *Pisaster*. Dividing by the mean local sea star density for that transplant and time period gave per capita interaction strength (α). This procedure gave four independent estimates of interaction strength per site × time period combination.
15. Local sea star density differed among time periods and sites [two-way analysis of variance (ANOVA), $F_{4,53} = 2.80$, $P = 0.035$ (time period); and $F_{2,53} = 6.79$, $P = 0.002$ (site)]. Sea star density during the

upwelling period (17 July through 1 August) was 31.6% lower than during the other four periods (mean ± SEM = 2.48 ± 0.32 sea stars/m^2, n = 12 versus 3.63 ± 0.26 sea stars/m^2, n = 48) and was consistently higher at Pigeon Reef than at the other two sites (mean ± SEM = 4.36 ± 0.38 sea stars/m^2, n = 20 versus 2.92 ± 0.25 sea stars/m^2, n = 40).

16. I used multiple regressions to test whether variation in mean interaction strengths among time periods and sites was associated with (i) water temperature (the mean during 27 high tides per period), (ii) potential heat stress (the mean of maximum low tide air temperature on the five warmest days per period), or (iii) wave stress (the mean of maximum force per day on 5 to 7 days per period). Per capita interaction strength was associated with water temperature (P < 0.001) but was unrelated to potential heat stress (P = 0.18) or wave stress (P = 0.53). Similarly, population interaction strength was correlated with water temperature (P < 0.001) but not with potential heat stress (P = 0.13) or wave stress (P = 0.74). Site variables were significant in both models because both per capita and population interaction strength were consistently higher at Pigeon Reef, the site with higher sea star density (*15*). Together, water temperature and site explained 80.9% of the variation in mean per capita interaction strength and 82.4% of the variation in mean population interaction strength.

17. In early June 1996, 48 sea stars (wet weight, 118 to 138 g) were collected from Neptune State Park. Four individuals were randomly assigned to each of 12 closed 110-liter tanks held in a cold room, and heaters with controllers self-regulated treatments to ±0.1°C. Water was circulated by two pumps in each tank, and water quality was maintained by filters and weekly water changes. Salinity was maintained at 36 ± 1 parts per thousand, and the experiments were conducted under a schedule of 12 hours of light and 12 hours of darkness. All sea stars were initially acclimated without food at 11°C for 10 days, and then treatments were randomly assigned (n = 4 tanks per treatment).

18. *Mytilus trossulus* was used because this species is the most common prey item in *Pisaster's* diet at these sites. I quantified the diet of actively feeding sea stars (n = 1664) on 14 dates during the summer of 1995 and 1996. The percents of individuals feeding on a given prey species were as follows: mussels (*M. trossulus*, 56.0%; *M. californianus*, 5.0%), barnacles (*Pollicipes polymerus*, 41.8%; *Balanus glandula*, 6.0%; *Semibalanus cariousus*, 3.2%; *Chthamalus dalli*, 1.4%; *B. nubilus*, 0.7%), whelks (*Nucella* spp., 1.5%), and limpets (*Lottia* spp., 0.5%). The total exceeds 100% because *Pisaster* often feeds on several prey species at a time.

19. Results are presented for the first three periods of the experiment. Thereafter, sea stars became temporarily satiated on the ad libitum diet, and feeding rates declined sharply in all treatments. After 4.5 months, sea stars used in the experiment had energy stores (pyloric ceca) much larger than those of field animals. Thus, the initial phase of the experiment, with sea stars recently collected from the field, best reflects the effects of temperature on *Pisaster* with natural levels of energy reserves.

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21. *Pisaster ochraceus* ranges from at least Punta Baja in Baja California to Prince William Sound in Alaska, and populations near these geographic limits regularly experience water temperatures >20°C and <4°C, respectively.

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A Cytotoxic Ribonuclease Targeting Specific Transfer RNA Anticodons

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The carboxyl-terminal domain of colicin E5 was shown to inhibit protein synthesis of *Escherichia coli*. Its target, as revealed through in vivo and in vitro experiments, was not ribosomes as in the case of E3, but the transfer RNAs (tRNAs) for Tyr, His, Asn, and Asp, which contain a modified base, queuine, at the wobble position of each anticodon. The E5 carboxyl-terminal domain hydrolyzed these tRNAs just on the 3′ side of this nucleotide. Tight correlation was observed between the toxicity of E5 and the cleavage of intracellular tRNAs of this group, implying that these tRNAs are the primary targets of colicin E5.

A variety of proteinaceous toxins inhibit protein synthesis; they have been used to elucidate complicated cell mechanisms. Ribosomes are one of the most sophisticated targets and are susceptible to many toxins, including plant-derived ricin, bacterial Shiga toxin and Shiga-like toxins, and a fungal α-sarcin (*1*). Colicin E3 and cloacin DF13 are special ribonucleases (RNases) that cleave 16*S* ribosomal RNA (rRNA) at the 49th phosphodiester bond from the 3′ end (*2*). Colicins comprise a treasury of cytotoxins with well-defined structures and modes of action.

Among the E-group colicins, which share receptor BtuB for the initial step of killing, E3 to E6 quickly stop amino acid incorporation into treated cells, suggesting inhibition of protein synthesis. On the basis of analogy with E3, E4 to E6 have been thought to be RNases (*3*). The nuclease type colicins exhibit high conservation in their NH_2-terminal large regions required for receptor binding and membrane transfer, and their nuclease activities are exclusively due to their small COOH-terminal domains, where sequence variations are concentrated (*4*, *5*). In this respect, E4 and E6 are in fact E3 homologs (*6*); however, the COOH-terminal region of E5 exhibits no sequence similarity to E3 (*7*). Moreover, these colicins are accompanied by specific inhibitors, Imm proteins, which account for the immunity of colicinogenic cells. Here again ImmE5 is excluded from the homology shared by ImmE3, ImmE4, and ImmE6. We thus suspected that E5 has a different target site on ribosomes, or even outside of ribosomes, for possible interference with protein synthesis.

To examine E5 activity in vitro, we focused on its COOH-terminal nonhomologous domain; a plasmid ColE5-099 DNA segment encoding both the COOH-terminal 115 amino acids of E5 (E5-CRD) and ImmE5 was cloned under the colicin E3 promoter (*8*). The NH_2-terminal sequencing of the purified ImmE5 revealed that the *imm* gene starts 78 base pairs upstream of the location previously speculated (*7*) and produces a 108–amino acid protein (*9*).

E5-CRD in fact caused a substantial decrease of the MS2 RNA-dependent amino acid incorporation in a cytoplasmic fraction separated at 30,000*g* (S-30) of *E. coli* (*10*) (Fig. 1B). This decrease was not due to any contamination by nucleases because the decrease was completely prevented by preincubation with ImmE5, as in the case of E3-CRD and ImmE3 (Fig. 1A). Curiously, however, the inhibitory effect of E5-CRD, unlike that of E3-CRD, was not observed when the incorporation of [^{14}C]Phe was measured with polyuridylate [poly(U)] as the template (Fig. 1, C and D), suggesting different modes of action of E3 and E5.

The action of E5-CRD on RNA was examined (Fig. 2A). E5-CRD degraded protein-free *E. coli* rRNAs, and this degradation was effectively inhibited by ImmE5, excluding the pos-

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