bound to particular SH2-containing proteins: Tyr1800 binding to a phospholipase C-γ (PLC-γ) or SHP-2 homologs, and Tyr245 to SEM-5 (Table 2) (24).


26. Nonstarved animals were fixed in 1% paraformaldehyde in phosphate-buffered saline, frozen at −70°C and washed, and then incubated overnight in sudan black.
other reefs, thereby maintaining our predator manipulations and the statistical independence of experimental replicates. The Chromis were free to emigrate, but this is a highly reef-attached species, and there was no evidence that any tagged fish successfully left one reef and colonized another (19). With this system in place, we ran experiments manipulating predator and prey densities to examine patterns and causes of mortality occurring shortly after the larvae had settled to the reef (20).

From 1992 to 1994, field observations and preliminary experiments showed that loss (mortality and emigration) from groups of new settlers shortly after settlement was often density-dependent on both continuous fore-reefs and patch reefs, that resident predatory fishes significantly affected survivorship during this period, and that there was substantial mortality even in the absence of resident predators (10). It was still unknown whether predators induced density dependence, and what role was played by transient predators.

In 1995, we determined the relative magnitude of mortality caused by resident and transient predators. We examined the effects of all four orthogonal combinations of predators (residents and transients, present and absent) on a single density of Chromis (20 settlers per reef, approximately the natural average (13)) in a randomized-block analysis of variance (ANOVA) design (n = 6 reefs per treatment) of 32-day duration. A fifth treatment, cage control, was added to explore any secondary effects of the reef enclosures we used to exclude transient predators (21), but it proved to be ineffective because partial cages clearly attracted these piscivores to reefs. However, mortality rates from the four cross-factored treatments were additive, suggesting independently that the complete cages had no substantial secondary effects on Chromis survival beyond the primary effect of excluding predators (Fig. 1). Specifically, resident predators accounted for a 0.17 difference in average mortality regardless of the presence (on uncaged reefs) or absence (on caged reefs) of transient predators. Transient predators accounted for a 0.32 difference in average mortality regardless of the presence or absence of resident predators. Although only the results on day 32 are shown, this additivity was characteristic of the entire experiment from day 12 onward. We concluded that transient piscivores had nearly twice the effect of resident piscivores on the survival of Chromis soon after settlement, that these effects were additive, and that cages were an effective and reasonably unbiased means of manipulating the presence of transient predators.

In 1996, we ran our main experiment by cross-factoring all four predator combinations (residents and transients, present and absent) each with eight densities of newly settled Chromis manipulated within natural limits (13)) in a regime design of 30-day duration (22). This experiment demonstrated that strong density-dependent mortality occurred unequivocally only when both kinds of predators were present (Fig. 2). In the absence of either or both suites of predators, mortality was statistically density-independent. However, there was a trend toward density dependence when only transient predators were present, and although nonsignificant, the power of this regression was only 0.20. Nonetheless, there was no indication of density dependence in the complete absence of predators, and strong density dependence was evident only when both kinds of predators were present. We concluded that density dependence soon after settlement in this system was induced by a combination of resident and transient predators (23).

These results raise two mechanistic questions. First, how did predators cause density-dependent mortality? Predators can induce density dependence in their prey over small temporal and spatial scales by either or both of the two mechanisms (24). We have no data regarding the first mechanism: predators may increase their per capita consumption rates disproportionately as a function of increasing prey density. Known as a type III functional response, this mechanism appears to be uncommon in fish. A second mechanism is that predators may alter their dispersion, thereby increasing their local density disproportionately as a function of increasing prey density (26). In the system we studied, resident predators are incapable of such an aggregative response because their movements among reefs are negligible over a period of weeks, even when reefs are much closer together than in our experiments (27). Accordingly, resident predators in the absence of transient predators did not cause detectable density dependence of newly settled Chromis. However, there was evidence of an aggregative response by transient predators, although the shape of the response function is presently unknown. Remote time-lapse video observations (28) of experimental reefs from dawn to dusk in 1996 revealed that reefs with high densities of Chromis settlers were visited more frequently by transient Caranx ruber jacks (mean ± SEM = 4.4 ± 2.1 jacks/min) than reefs with low prey densities (1.3 ± 0.4 jacks/min, Wilcoxon signed ranks test, P = 0.048, n = 13 paired day-long observations).

Second, what is the mechanism by which both resident and transient predators were necessary to induce strong density dependence in newly settled Chromis? Although the average effects of these predators were additive, their overall effect was synergistic. High variance in the relation between prey density and subsequent per capita mortality rendered the regression nonsignificant when only one suite of predators was present (Fig. 2, B and C). Behavioral observations explain this discrepancy (29). Apparently, prey fish lack substantial...
spatial refuges only when both suites of predators are present. As transient predators rapidly approach a reef, Chromis and other fish invariably dive for cover in the reef structure (15, 30). When resident predators are absent, this sheltering response often allows prey fish to escape from passing transient predators. When transient predators are absent, prey fish often escape from resident predators by staying in midwater above the reef, where many (like Chromis) feed on passing plankton (12, 31). Under both these circumstances, successful predatory attacks would be patchy in space and time because of variable shelter availability (27) and the vicissitudes of prey vigilance and predator stealth. Thus, mortality was highly variable and statistically density-dependent when only one kind of predator was present (Fig. 2, B and C). Only in the presence of both suites of predators were prey fish left with relatively little refuge, sandwiched between the attacks of resident piscivores below and transient piscivores above in a way that induced strong density dependence (Fig. 2A).

Despite the exact mechanisms, these field experiments demonstrate that predators are responsible for within-generation spatial density dependence (6) in the mortality of recently settled reef fish. Our future field experiments demonstrate that predators Induced strong density dependence (6) above the reef, where many (like Chromis) feed on passing plankton (12, 31). Under both these circumstances, successful predatory attacks would be patchy in space and time because of variable shelter availability (27) and the vicissitudes of prey vigilance and predator stealth. Thus, mortality was highly variable and statistically density-dependent when only one kind of predator was present (Fig. 2, B and C). Only in the presence of both suites of predators were prey fish left with relatively little refuge, sandwiched between the attacks of resident piscivores below and transient piscivores above in a way that induced strong density dependence (Fig. 2A).

Despite the exact mechanisms, these field experiments demonstrate that predators are responsible for within-generation spatial density dependence translates to temporal density dependence necessary to regulate populations (3, 6), and whether there are other sources of regulation. Overall, these results lend credence to the hypothesis that predation on juveniles may naturally regulate the abundance of commercially valuable species (8, 9).

These findings have cautionary ramifications for studies of population dynamics of marine fishes. First, the common practice of measuring larval survival at a site by sampling juveniles weeks to months after they have settled to the bottom (or by back-calculating settlement densities by using otoliths) may miss density dependence in the period shortly after settlement, thereby leading to the false conclusion that mortality after settlement is density-independent (5, 32). Second, if synergistic predation is a major source of density dependence in exploited fishes, then simple estimates of natural mortality in multispecies fisheries models are likely to yield erroneous results.

Third, overfishing stocks of piscivorous species, as widely occurs to grouper and jack in tropical regions (33), may have ramifications for the dynamics of other populations in the food web (34). Understanding the systemwide effects of fishing clearly requires knowledge of interactions between target species and the remainder of the exploited community.

REFERENCES AND NOTES


11. Resident predators in the Bahamian system, some of which forage at night, include moray eels (especially Gymnothorax moringa), large squirrelfishes (especially Holocentrus ascensionis), and some snapper (Lutjanus spp.), but mostly grouper (especially Epinephelus striatus). The larger predators (especially Lutjanus spp.) and a snapper (Lutjanus synagris), but by far, mostly jacks (particularly Caranx ruber). Unlike its predators, C. cyanus is not fished by humans.


13. H. P. A. Sweatman [Ecot. Monogr. 55, 469 (1985)] showed that settlement in the Pacific Chromis caerulea is facilitated by the presence of conspecifics. When we compared large continuous fore-reefs with patch reefs located behind the fore-reefs at our Bahamian site, group sizes of C. cyanus that had newly settled were statistically indistinguishable, averaging (±SEM) 16.5 (± 1.6) fish per group (51 maximum) on fore-reefs and 16.7 (± 3.0) fish per group (40 maximum) on patch reefs, to 1.4 m² in area (Mann-Whitney U-test: 0.44, n = 4 groups each). Therefore, we focused our experiments on patch reefs of manipulable size, approximately 9 m² in area, certainly a realistic spatial scale for this system (see [3] and [6] for discussion).


16. Translocated reefs, each averaging 11 coral heads 30 to >100 cm in diameter, grew on loose rubble in 3 to 4 m deep. They were attached to the bottom by earth anchors, sealed by heavy chain, and supported by floats. Although impermeable to larger fish, the cages were transparent to movements of newly settled Chromis and other small fishes. Partial control cages consisted of four net panels interdigitated with four open panels so that transient predators could enter.

17. Natural isolation distances of patch reefs in this region range broadly, up to several kilometers.

18. Drifter studies by B. Hickey and E. Elliott (University of Washington) showed that plastic bags of seawater in situ for transport, and released on experimental reefs to attain desired densities for at least 24 hours before the start of an experiment. To test whether this transplant method artificially increased Chromis mortality, we compared, in separate but complementary experiments each year, per capita mortality among three treatments: (i) groups of uncaptured fish, (ii) groups of fish captured and returned to the same location after being transported halfway to experimental reefs and back, and (iii) groups of fish experimentally transplanted to new reefs. Analysis of variance indicated that this did not indicate any deleterious effects of handling or transplanting (10).

19. Circular reef-enclosure cages, constructed of 1.5- cm nylon mesh, were 6 m in diameter and extended from the surface to the bottom (3 to 4 m deep). They were attached to the bottom by earth anchors, sealed by heavy chain, and supported by floats. Although impermeable to larger fish, the cages were transparent to movements of newly settled Chromis and other small fishes. Partial control cages consisted of four net panels interdigitated with four open panels so that transient predators could enter.


21. The fact that the intercepts of the mortality curves (an estimate of the underlying density-independent mortality rate) were nearly identical for caged reefs lacking all predators (0.027 vs. 0.026) and cage-exposed reefs exposed to all predators (0.309, Fig. 2A) is further evidence that the reef enclosures did not artificially affect the background mortality of the study fish. Note also that mortality rates from the experiment in 1995 (as well as in previous experiments) were similar to those in 1996 (Fig. 2), lending credence to the temporal generality of our results. Analysis of instantaneous per capita mortality rates revealed that the major period of density dependence occurred during the first 5 days of the experiment.


23. W. W. Murdoch and J. Bence, in Predation: Direct and Indirect Impacts on Aquatic Communities, W. C.
Conversion by Peyer’s Patch Lymphocytes of Human Enterocytes into M Cells that Transport Bacteria

Sophie Kernéis, Anna Bogdanova, Jean-Pierre Kraehenbuhl, Eric Pringault

The epithelium that lines the gut is impermeable to macromolecules and microorganisms, except in Peyer’s patches (PPs), where the lymphoid follicle-associated epithelium (FAE) contains M cells that transport antigens and microorganisms. A cultured system that reproduces the main characteristics of FAE and M cells was established by cultivation of PP lymphocytes with the differentiated human intestinal cell line Caco-2. Lymphocytes settled into the epithelial monolayer, inducing reorganization of the brush border and a temperature-dependent transport of particles and Vibrio cholerae. This model system could prove useful for intestinal physiology, vaccine research, and drug delivery studies.

Most microorganisms must cross epithelial barriers to exert their physiopathological effects and to interact with mucosa-associated lymphoid tissue (MALT). In the intestine, PPs are the major sites of antigen and microorganism sampling, which leads to immune responses or tolerance (1, 2). MALT is separated from the lumen by the FAE, which contains M cells. These epithelial cells transport foreign material to MALT and display large intraepithelial pockets filled with B and CD4 T lymphocytes, macrophages, and dendritic cells. Many pathogenic microorganisms exploit M cells to cross the digestive epithelial barrier (3). Thus, passage of antigens and microorganisms through M cells is an essential step for the development of mucosal immune responses and the pathology of many infectious diseases. Because M cells are a minor population in the FAE, they are difficult to characterize biochemically; hence, little is known about their cell biology. The ultrastructure and the transport capacity of M cells have been documented by morphological analysis and immunohistochemistry. However, the study of molecular mechanisms of microorganism–M cell interactions and signal transduction pathways that control translocation and cytoskeletal reorganization has been hampered by the lack of in vitro M cell models. Here we report the establishment of an in vitro model in which specific properties of FAE and M cells can be analyzed by biochemical and quantitative methods.

The analysis of MALT and FAE in immunodeficient mice (4) suggests that FAE and M cell formation could be regulated by the presence of immune cells. A subclone of the human, differentiated, absorptive, enterocyte cell line Caco-2 clone 1 (5, 6) was cultured with freshly isolated murine PP lymphocytes. We seeded Caco-2 cells by adding 5 × 10^5 cells on the lower face of 6.5-mm filters (3-μm pore Transwell filters, COSTAR, Cambridge, MA) and culturing them overnight. The filters were then transferred in the Transwell device with the epithelial cells facing the lower chamber of the cluster plates, as adapted from Kaoutzani et al. (7). Epithelial cells were cultured until they were fully differentiated (14 days). Lymphocytes were isolated from PPs of BALB/c mice (8). Dissociated cells were analyzed by FACSscan (fluorescent analyzer cell sorter) flow cytometry (Becton-Dickinson). Sixty percent of the cells were B cells [detected with a monoclonal antibody (mAb) to mouse B20 (CD45R), and the remaining 40% of cells were CD3 T cells (detected with a mAb to mouse CD3). This ratio corresponds to that described for PP follicle cells (9). Macrophages and dendritic cells, representing about 0.4% of follicle cells in such preparations, were not detected. The lymphocytes survived in culture for up to 7 days. Lymphoid cells (10^6) were added in the upper chamber facing the basolateral side of the Caco-2 cells. The cultures were maintained for 1 to 7 days. Lymphoid cells migrated through the pores of the filter and settled into the epithelial monolayer without altering the polarity of Caco-2 cells, as reflected by the maintenance of cell polarity markers (Fig. 1A through C) and transepithelial electric resistance of the monolayers (about 300 Ω cm^2) during the 7 days of culture. Within 2 days, the lymphocytes accumulated in intraepithelial pockets, as already described.

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Ocean plumbing
A key control on Earth's climate is the pattern of flow and mixing in the oceans; understanding how mixing varied in the past is critical for interpreting causes of past climate change. Christensen et al. (p. 913; see the Perspective by Alberède, p. 908) present detailed records of lead isotopes in the ocean extending back to about 50 million years ago obtained from two iron-manganese crusts in the Pacific Ocean. Changes in the lead isotope values seem to correspond to broad changes in the oxygen isotope composition of foraminifera (which reflect climate changes) and may reflect the pattern or intensity of ocean mixing.

Tiny gas cylinders
Fullerenes can be synthesized so that they trap rare gas atoms. Now carbon fibers, when subjected to high pressures and temperatures, can also irreversibly trap inert gases. Gadd et al. (p. 933) show that when carbon fibers are exposed to argon gas at high pressure and appropriate temperature conditions, the tubes seal after the gas has entered. The gas remains trapped in the tubes at high pressures, even after the outside pressure is released to atmospheric pressure. These fibers, which have an outer diameter of about 0.1 micrometer, thus form very small, high-pressure gas cylinders.

Readily resolving chiral compounds
Pure enantiomeric compounds (all right- or left-handed versions of the same molecule) are important building blocks for organic synthesis. Synthetic methods, however, often produce both diastereomeric and enantiomeric building blocks for organic synthesis. Synthetic methods, however, often produce both diastereomeric and enantiomeric building blocks for organic synthesis. Therefore, strategies for resolving enantiomers of an important class of compounds, terminal epoxides. A recyclable chiral cobalt catalyst was used to resolve racemic mixtures of these molecules into epoxides and 1,2-diols of nearly a single handedness (usually greater than 98 percent resolution). The only reagent consumed was water.

Insulin-like signals in worms
In Caenorhabditis elegans, harsh conditions can cause the worms to halt the normal reproductive development and enter a "resting" or dauer state. This process is controlled by a neuroendocrine pathway consisting of genes from the DAF family. Mutations in these genes causes abnormal dauer formation or, in some cases, longevity. Kinoshita et al. (p. 942; see the news story by Roush, p. 897) have now shown that one of the daf genes, daf-2, is an insulin receptor-like molecule. Such homology has not been seen before in worms and indicates that this insulin-like signaling pathway cooperates with the otherdaf pathway (which is a transforming growth factor-β-like signaling pathway) to control this fundamental developmental process.

No place to hide
Identifying factors that regulate fish populations has proven difficult, in part because of problems in obtaining accurate catch data. Hixon and Carr (p. 946) have approached the question experimentally: They translocated live fish populations to new locations to construct 32 similar habitats. These were populated with different densities of damselfish, exposed to two types of predators, reef-resident and transient piscivores, and the mortality rates of the damselfish assessed. In the presence of either one of the predator types, damselfish mortality was not density-dependent but both predators together caused density-dependent mortality. Transient predators attack in the waters above the reef, while resident predators attack within the reef structure.

Connect the quantum dots
Semiconductor devices use transistors to switch the flow of electrons, but difficulties with leakage currents limit the ultimate size of even field-effect transistors. A quantum-mechanical approach to encode logic states by the position of individual electrons on interconnected quantum dots (quantum-dot cellular automata, or QCAs). Orlov et al. (p. 928; see the news story by Glanz, p. 900) experimentally constructed a basic QCA cell from four aluminum quantum dots. Each pair is connected by tunneling barriers, and the two pairs are capacitively coupled. Transfer of an electron between the input pair of dots switches the position of an electron on the output pair. Hixon and Carr (p. 946) have approached the question experimentally: They translocated live fish populations to new locations to construct 32 similar habitats. These were populated with different densities of damselfish, exposed to two types of predators, reef-resident and transient piscivores, and the mortality rates of the damselfish assessed. In the presence of either one of the predator types, damselfish mortality was not density-dependent but both predators together caused density-dependent mortality. Transient predators attack in the waters above the reef, while resident predators attack within the reef structure.

Making M cells
Most of a mammal's immune system is not in the bloodstream and its associated lymph nodes, but rather it is intimately associated with the gut. Regions of the gut that contact this tissue contain specialized transport cells called M cells. Kernéis et al. (p. 949; see the cover and the Perspective by Madara, p. 910) established an in vitro culture system to convert gut epithelium into M cells by coculture with lymphocytes, particularly B cells.

Genetics and AIDS
A major endeavor in HIV (human immunodeficiency virus) research has been to discover the underlying mechanisms that allow some "long-term survivors" to resist disease 10 to 20 years after HIV-1 infection or to allow some highly exposed individuals to remain uninfected. Although a deletion in the chemokine receptor molecule CCR5 and certain human leukocyte antigen haplotypes have been associated with survival, they can only explain a small proportion of the cases. Smith et al. (p. 959) have found a mutation in another chemokine receptor, CCR2, that, when considered with the deletion in CCR5, explains the ability of about 25 percent of long-term survivors to avoid AIDS.

Pinpointing pain
How do we feel pain? Rainville et al. (p. 968) dissociate and monitor the physical aspects of exposure to hot water from the affective response by functional brain imaging. Under conditions where the somatosensory cortex is unaltered, activation in the anterior cingulate cortex is modulated by hypnotic suggestion, linking this frontal lobe pathway to the emotional reaction to pain.

Telomerase family tree
Telomerase, a ribonucleoprotein enzyme, replicates chromosome ends, or telomeres. The catalytic protein subunit, recently identified in a ciliate and in bakers' yeast, contains reverse transcriptase (RT) motifs. Nakamura et al. (p. 955; see the Perspective by Eickbush, p. 911) show that this subunit is conserved in fission yeast and humans. From building evolutionary trees, they conclude that telomerase form a discrete subgroup of RTs, probably of ancient origin.