bound to particular SH2-containing proteins: Tyr$^{1800}$ binding to a phospholipase C–γ (PLC-γ) or SHP-2 homologs, and Tyr$^{1808}$ to SEM-5 (Table 2) (*24*).
14. M. J. Tauber, C. A. Tauber, S. Masaki, *Seasonal Adaptation of Insects* (Oxford Univ. Press, New York, 1986); V. B. O'Riordan and A. M. Burnell, *Comp. Biochem. Physiol.* **92B**, 233 (1989); *ibid.* **95B**, 125 (1990); J. D. Popham and J. M. Webster, *Can. J. Zool.* **57**, 794 (1978); W. G. Wadsworth and D. L. Riddle, *Dev. Biol.* **132**, 167 (1989).
15. E. Roovers *et al*., *Gene* **162**, 181 (1995); C. Hetru, K. W. Li, P. Bulet, M. Lagueux, J. A. Hoffmann, *Eur. J. Biochem.* **201**, 495 (1991); A. Kawakami *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6843 (1989); M. Arpagaus, *Roux's Arch. Dev. Biol.* **196**, 527 (1987).
16. B. A. Hemmings, *Science* **275**, 628 (1997).
17. P. Ren *et al*., *ibid.* **274**, 1389 (1996); W. S. Schackwitz, T. Inoue, J. H. Thomas, *Neuron* **17**, 719 (1996).
18. L. L. Georgi, P. S. Albert, D. L. Riddle, *Cell* **61**, 635 (1990); M. Estevez *et al*., *Nature* **365**, 644 (1993); G. I. Patterson, A. R. Koweek, A. Wang, Y. Liu, G. Ruvkun, unpublished data.
19. C. R. Kahn, D. Vicent, A. Doria, *Annu. Rev. Med.* **47**, 509 (1996).
20. H. Nagasawa *et al*., *Science* **226**, 1344 (1984); E. A. Jonas *et al*., *Nature* **385**, 343 (1997).
21. L. S. Mathews, G. Norstedt, R. D. Palmiter, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9343 (1986); H. M. Brown-Borg, K. E. Borg, C. J. Meliska, A. Bartke, *Nature* **384**, 33 (1996).
22. C. Finch, *Longevity, Senescence and the Genome* (Univ. of Chicago Press, Chicago, 1990).
23. D. L. Coleman, *Science* **203**, 663 (1979).
24. Z. Songyang *et al*., *Cell* **72**, 767 (1993).
25. M. Krause, in Caenorhabditis elegans: *Modern Biological Analysis of an Organism*, vol. 48 of *Methods in Cell Biology*, H. F. Epstein and D. C. Shakes, Eds. (Academic Press, San Diego, CA, 1995), p. 483.
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.
27. E. A. Malone and J. H. Thomas, *Genetics* **136**, 879 (1994).
28. We thank A. Coulson, S. Chissoe, B. Waterston, T. Barnes, E. Zachgo, E. Malone, T. Inoue, J. Thomas, E. Lambie, J. Avruch, J. Kaplan, the Massachusetts General Hospital sequencing facility, the *C. elegans* Genetics Center, and the Ruvkun and Kaplan labs for assistance. K.D.K. is supported by the Japanese Society for the Promotion of Science. This work was supported by NIH grant RO1AG14161.

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# Synergistic Predation, Density Dependence, and Population Regulation in Marine Fish

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Understanding natural causes of density dependence is essential for identifying possible sources of population regulation. Field experiments on a model system of coral reef fishes showed that small juveniles of *Chromis cyanea* suffer heavy mortality that is spatially density-dependent only in the presence of two suites of predators: transient piscivores attacking from above, and reef-resident piscivores attacking from below. In the absence of either kind of predator, early mortality of *Chromis* is virtually density-independent. Because piscivores may have regulatory roles in this and similar marine systems, overfishing these predators may have ramifications for the remainder of the exploited community.

Marine fish populations are notoriously dynamic (*1*). This is because of strong year-to-year variation in the recruitment of juveniles generated largely during the pelagic larval stage, when developing larvae are subject to various mortality sources that appear to be mostly density-independent (*2*). Despite these fluctuations, for any population neither to go extinct nor to increase without limit, at least one demographic rate must be density-dependent at some time and place (*3*–*6*). Here, we focus on the per capita (proportional) natural mortality rate, which is density-dependent if it increases with population size. Understanding such sources of population regulation is particularly timely as marine fisheries worldwide reach a state of crisis due mostly to overexploitation (*7*).

Detecting natural mechanisms of density dependence in exploited marine fishes has proven difficult because of a necessary reliance on in direct approaches based on often imprecise catch data, typically with high variance (*1*, *4*). Nonetheless, correlative analyses of demersal (bottom-oriented) species indicate that juveniles that have recently settled to the bottom may undergo density-dependent mortality (*8*), which often disappears in larger juvenile and adult stages (*1*, *9*). Such analyses have been supplemented by recent small-scale field experiments with reef fishes suggesting that density-dependent mortality may occur within several weeks after settlement to a reef (*5*). However, the sources of density dependence remain largely unknown, although it is often hypothesized that predators cause density-dependent mortality of early juveniles of commercially valuable species (*8*, *9*). Here, we provide experimental corroboration of this hypothesis by demonstrating spatial density dependence (*6*) in a coral reef fish.

Coral reef species provide excellent model systems for exploring density dependence after settlement in demersal fishes because they can be directly observed and experimentally manipulated in situ. New settlers and predators of our study species are particularly well-suited study subjects because they are easily counted, captured, and manipulated (*10*). We conducted our experiments on the Great Bahama Bank near the Caribbean Marine Research Center at Lee Stocking Island, Bahamas (*10*). Here, as elsewhere in this region, the planktivorous damselfish *Chromis cyanea* (Pomacentridae) and other species are attacked by two suites of predators, which themselves are often the targets of fisheries: resident piscivores, mostly grouper (family Serranidae), that inhabit the same reef as their prey, and transient piscivores, mostly jack (family Carangidae), that regularly swim between reefs (*11*). Typical of elsewhere in their range, *Chromis* at this site occur in distinct aggregations of tens of individuals, each group centered on a prominent coral head, although fish within each aggregation are only loosely social relative to other group-living damselfish (*12*). Each summer, these aggregations are replenished by groups of several to tens of settling larvae that appear near each new moon (*13*).

The ideal experimental design to test for density-dependent mortality and any role of predation (as well as competition and recruitment limitation) is to manipulate orthogonally the density of the prey species and the presence of predators, then compare the subsequent survivorship of the prey among different treatments for a sufficient time (*3*, *5*, *14*). In the past, this design had proven extremely difficult to implement in studies of marine fishes because movement of both predators and prey between closely neighboring replicate sites rapidly swamped manipulations of local abundance (*15*). We overcame this problem by standardizing the isolation of coral patch reefs, which involved literally translocating live reefs, coral head by coral head, to a large sand flat behind the fore-reef from 1991 to 1993 (*16*). This effort produced a matrix of 32 similar natural reefs isolated by 200 m from each other and at least 1 km from the nearest nonexperimental reef (*17*). The location of this matrix was an area that received relatively few settling larvae (*18*). This allowed us to control the density of newly settled *Chromis* without the confounding effects of heavy natural settlement (*10*). The isolation of the reefs also effectively inhibited resident (not transient) piscivores from any tendency to emigrate to

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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 regression 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 fishes (*25*). The 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

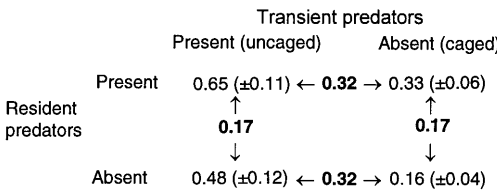


**Fig. 1.** Additive effects of resident and transient predators on per capita mortality rates of juvenile *C. cyanea* for the first 32 days after settlement in 1995. Listed are the four means (± SEM) of the cross-factored predator treatments ($n$ = 6 reefs each). The arrows connecting each pair of mortality rates give the mean between-treatment mortality differences. Note that reefs with transient predators removed were enclosed within cages.

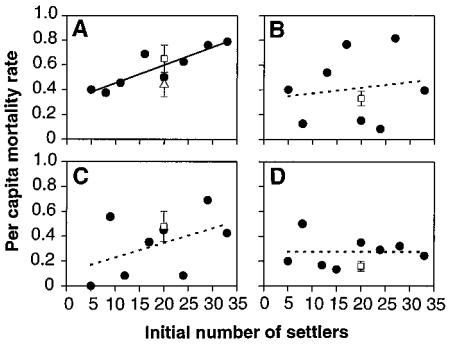


**Fig. 2.** The 30-day per capita mortality rates of newly settled *C. cyanea* on experimental reefs under four different predation regimes in 1996. Regression statistics by treatment ($n$ = 8 reefs each, solid circles): (**A**) all predators present—the unmanipulated control ($r^2$ = 0.804, $P$ = 0.003, $m$ = 0.015, $b$ = 0.309); (**B**) only resident predators present ($r^2$ = 0.024, $P$ = 0.717, $m$ = 0.005, $b$ = 0.325); (**C**) only transient predators present ($r^2$ = 0.211, $P$ = 0.252, $m$ = 0.012, $b$ = 0.112); and (**D**) all predators absent ($r^2$ 0.001, $P$ = 0.996, $m \ll$ 0.001, $b$ = 0.275). Note that the $y$ intercepts ($b$) of the four regressions are similar and that the sum of the regression slopes ($m$) from (B) and (C) (0.017) nearly equals that from (A) (0.015), indicating additive average effects of the different predators (see Fig. 1). Plotted for comparison are results (mean ± SEM) of the 1995 predator-manipulation experiment (open squares, $n$ = 6 reefs each) and the 1996 microtagging experiment (open triangle, $n$ = 4) (*19*).

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-independent 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 task is to determine whether this 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 supply 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


1. B. J. Rothschild, *Dynamics of Marine Fish Populations* (Harvard Univ. Press, Cambridge, MA, 1986); D. Cushing, *Population Production and Regulation in the Sea: A Fisheries Perspective* (Cambridge Univ. Press, Cambridge, UK, 1995).
2. E. D. Houde, *Am. Fish. Soc. Symp. Ser.* **2**, 17 (1987); K. M. Bailey and E. D. Houde, *Adv. Mar. Biol.* **25**, 1 (1989); M. R. Heath, *ibid.* **28**, 1 (1992).
3. M. P. Hassell, *Trends Ecol. Evol.* **1**, 90 (1986); W. W. Murdoch, *Ecology* **75**, 271 (1994); N. Cappuccino and P. W. Price, *Population Dynamics: New Approaches and Synthesis* (Academic Press, San Diego, CA, 1995).
4. R. J. H. Beverton *et al.*, in *Exploitation of Marine Communities*, R. M. May, Ed. (Springer-Verlag, Berlin, Germany, 1984), pp. 13–58; R. A. Myers, N. J. Barrowman, J. A. Hutchings, A. A. Rosenberg, *Science* **269**, 1106 (1995).
5. M. J. Caley *et al.*, *Annu. Rev. Ecol. Syst.* **27**, 477 (1996); M. A. Hixon, *Aust. J. Ecol.,* in press.
6. M. P. Hassell, T. R. E. Southwood, P. M. Reader, *J. Anim. Ecol.* **56**, 283 (1987); A. Stewart-Oaten and W. W. Murdoch, *ibid.* **59**, 1027 (1990).
7. J. R. McGoodwin, *Crisis in the World's Fisheries: People, Problems, and Policies* (Stanford Univ. Press, Stanford, CA, 1990); K. Crean and D. Symes, Eds., *Fisheries Management in Crisis* (Blackwell Scientific, Cambridge, MA, 1996).
8. H. W. van der Veer, *Mar. Ecol. Prog. Ser.* **29**, 223 (1986); S. Sundby, H. Bjorke, A. V. Soldal, S. Olsen, *Rapp. P-V. Reun. Cons. Int. Explor. Mer.* **191**, 351 (1989); R. A. Myers and N. G. Cadigan, *Can. J. Fish. Aquat. Sci.* **50**, 1576 (1993); K. M. Bailey, *Neth. J. Sea Res.* **32**, 175 (1994). Note that "births" in local populations of demersal marine fish are usually by the process of dispersive pelagic larvae settling to the bottom. By manipulating the density of settling larvae, one could essentially control local births to test for density dependence in subsequent mortality.
9. M. P. Sissenwine, in *Exploitation of Marine Communities*, R. M. May, Ed. (Springer-Verlag, Berlin, Germany, 1984), pp. 59–94.
10. M. H. Carr and M. A. Hixon, *Mar. Ecol. Prog. Ser.* **124**, 31 (1995); unpublished data.
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*). Transient predators include rare lizardfishes (*Synodus* spp.) and a snapper (*Lutjanus synagris*), but by far, mostly jacks (particularly *Caranx ruber*). Unlike its predators, *C. cyanea* is not fished by humans.
12. B. A. de Boer, *Bull. Mar. Sci.* **28**, 550 (1978).
13. H. P. A. Sweatman [*Ecol. 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. cyanea* that had newly settled were statistically indistinguishable, averaging (±SEM) 16.5 (± 1.6) fish per group (31 maximum) on fore-reefs and 16.7 (± 3.0) fish per group (40 maximum) on patch reefs 1 to 14 $m^2$ in area (Mann-Whitney *U*-test, $P = 0.48$, $n = 15$ groups each). Therefore, we focused our experiments on patch reefs of manipulable size, approximately 9 $m^2$ in area, certainly a realistic spatial scale for this system [see (*3*) and (*6*) for discussion].
14. A. Sih *et al.*, *Annu. Rev. Ecol. Syst.* **16**, 269 (1985).
15. M. A. Hixon, in *The Ecology of Fishes on Coral Reefs*, P. F. Sale, Ed. (Academic Press, San Diego, CA, 1991), pp. 475–508.
16. Translocated reefs, each averaging 11 coral heads 30 to >100 cm in diameter, grew on loose rubble and so did not have to be broken off the bottom. The coral heads were moved unharmed underwater on the central elevator platform of a specially designed motorized catamaran (*10*). Through the summer of 1996, the corals had survived well in their new locations. Translocated reefs used in each experiment had been recolonized by fishes to natural densities.
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 tidal-current water that brings settlement-competent larvae to our experimental reefs first passes over several kilometers of shallow fore-reef, giving larvae ample opportunity to settle before arriving at our experimental reefs.
19. In 1996, we microtagged and transplanted 20 new settlers of *Chromis* to each of four translocated reefs similar to the experimental reefs, but isolated by only 100 m from each other and nearby unmanipulated reefs. Over the next month, these fish disappeared at a per capita rate (mean ± SEM = 0.44 ± 0.10) comparable with that on experimental reefs exposed to predation (Fig. 2A). Importantly, despite 10 exhaustive searches over the month of all reefs within 200 m of the release sites, none of the missing tagged fish appeared on any neighboring reef, substantiating that disappearance in this system is tantamount to mortality. Moreover, even on continuous fore-reefs, of 95 new settlers microtagged in seven distinct social groups and followed for a month, only one fish emigrated successfully (to a group only 5 m away). For microtagging methods, see R. M. Buckley, J. E. West, D. C. Doty, *Bull. Mar. Sci.* **55**, 848 (1994); J. S. Beukers, G. P. Jones, R. M. Buckley, *Mar. Ecol. Prog. Ser.* **125**, 61 (1995).
20. For piscivore manipulations, resident predators were captured unharmed by divers with hand nets, tagged, and released at least 2 km from the study site. Transient predators were excluded from reefs by large cages (*21*). For *Chromis* manipulations, numbers of larger fish on experimental reefs were first adjusted to a narrow natural range (two to four fish per reef). New settlers, identified by their small size (<2 cm total length) and partial pigmentation (mostly gray in appearance), were then captured on the fore-reef with BINCKE nets (T. W. Anderson and M. H. Carr, *Env. Biol. Fish.*, in press), placed in 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 of these treatments indicated no deleterious effects of handling or transplanting (*10*).
21. 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.
22. For related examples of the regression approach, see G. E. Forrester, *Oecologia* **103**, 275 (1995); M. A. Steele, *Ecology* **78**, 129 (1997).
23. The fact that the *y* intercepts of the mortality curves (an estimate of the underlying density-independent mortality rate) were nearly identical for caged reefs lacking all predators (0.275, Fig. 2D) and uncaged 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 previous years) 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.
24. W. W. Murdoch and A. Oaten, *Adv. Ecol. Res.* **9**, 1 (1975).
25. W. W. Murdoch and J. Bence, in *Predation: Direct and Indirect Impacts on Aquatic Communities*, W. C.

Kerfoot and A. Sih, Eds. (Univ. Press of New England, Hannover, NH, 1987), pp. 17–30.
26. M. P. Hassell and R. M. May, *J. Anim. Ecol.* **43**, 567 (1974).
27. M. A. Hixon and J. P. Beets, *Ecol. Monogr.* **63**, 77 (1993).
28. As detailed elsewhere (*10*), we placed two Hi-8 video camera units underwater such that each viewed an entire experimental reef from the side, recording 15 s out of every 90 s from dawn to dusk. On 13 dates during the 1996 experiment, we conducted paired video samples of uncaged reefs with low (mean ± SEM = 5.4 ± 0.4 fish per reef) versus high (20.4 ± 1.7 fish per reef) experimental densities of newly settled *Chromis*.
29. We do not believe that this discrepancy could be due to nonbehavioral mechanisms, such as variable resident predator density. The densities of resident piscivores were unmanipulated in the two treatments where this suite of predators was present, so there was indeed natural variation among reefs in resident predator density. However, there was no significant difference in resident predator density between the treatment where all predators were present (mean ± SEM = 9.8 ± 1.3 fish per reef) and the treatment where only resident predators were present (11.0 ± 2.7 fish per reef, Mann-Whitney *U*-test, $P = 0.88$, $n = 8$ reefs each). Also, there was no correlation among reefs within either treatment between the density of resident piscivores and per capita settler mortality (correlation coefficient, $r$, $= -0.21$, $P = 0.61$ and $r = -0.42$, $P = 0.31$, respectively).
30. A. C. Hurley and P. H. Hartline, *Anim. Behav.* **22**, 430 (1974); E. S. Hobson and J. R. Chess, *Fish. Bull.* **76**, 133 (1978); M. Hixon and M. Carr, personal observation.
31. W. P. Davis and R. S. Birdsong, *Helgo. Wiss. Meeresunters.* **24**, 292 (1973); P. G. Sackley and L. S. Kaufman, *Copeia* **1996**, 726 (1996). Midwater is safe for small fishes because resident predators tend to stay in cover, being prey of very large transient predators, especially great barracuda (*Sphyraena barracuda*) at our study site. We often observed barracuda attacking and capturing resident piscivores, and barracuda tooth scars were common on these fish. Barracuda ignored new settlers.
32. P. J. Doherty and T. Fowler, *Science* **263**, 935 (1994).
33. W. J. Richards and J. A. Bohnsack, in *Large Marine Ecosystems: Patterns, Processes, and Yields*, K. Sherman, L. M. Alexander, B. D. Gold, Eds. (American Association for the Advancement of Science, Washington, DC, 1990), pp. 44–53; G. R. Russ, in *The Ecology of Fishes on Coral Reefs*, P. F. Sale, Ed. (Academic Press, San Diego, CA, 1991), pp. 601–635; S. Jennings and J. M. Lock, in *Reef Fisheries*, N. V. C. Polunin and C. M. Roberts, Eds. (Chapman & Hall, London, UK, 1996), pp. 193–218.
34. S. L. Pimm and J. B. Hyman, *Can. J. Fish. Aquat. Sci.* **44**, 84 (1987).
35. For backbreaking work, we thank G. Almany, T. Anderson, J. Beets, R. Buckley. M. Buckley, B. Byrne, D. Canestro, G. Forrester, R. Gomez, A. Kaltenberg, A. Menge, G. Savage, S. Thompson, C. Tinus, E. Tynes, K. Vicknair, and M. Webster, and for general support, the staff of the Caribbean Marine Research Center, especially C. Cooper, G. Dennis, W. Head, W. Keith-Hardy, R. Wicklund, and T. Wolcott. Thanks to G. Almany, T. Anderson, K. Bailey, P. Bailey, J. Beets, C. Briggs, B. Byrne, J. Lubchenco, D. Reed, W. Pearcy, S. Sogard, and M. Webster for constructive reviews. Supported by NSF (OCE-92-17163 and OCE-96-17483) and National Oceanic and Atmospheric Administration's National Undersea Research Program (CMRC-92-46, 93-12, 94-15, 95-3042, and 97-3109).

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# Conversion by Peyer's Patch Lymphocytes of Human Enterocytes into M Cells that Transport Bacteria

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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 form 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 $3 \times 10^5$ 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 FACScan (fluorescent analyzer cell sorter) flow cytometry (Becton-Dickinson). Sixty percent of the cells were B cells [detected with a monoclonal antibody (mAb) to mouse B220 (CD45)], 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. 1, A through C) and of transepithelial electric resistance of the monolayers (about 300 ohm · $cm^2$) during the 7 days of culture. Within 2 days, the lymphocytes accumulated in intraepithelial pockets, as already de-

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