

Effects of Fish in River Food Webs

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Experimental manipulations of fish in a Northern California river during summer base flow reveal that they have large effects on predators, herbivores, and plants in river food webs. California roach and juvenile steelhead consume predatory insects and fish fry, which feed on algalivorous chironomid larvae. In the presence of fish, filamentous green algae are reduced to low, prostrate webs, infested with chironomids. When the absence of large fish releases smaller predators that suppress chironomids, algal biomass is higher, and tall upright algal turfs become covered with diatoms and cyanobacteria. These manipulations provide evidence that the Hairston, Smith, Slobodkin–Fretwell theory of trophic control, which predicts that plants will be alternately limited by resources or herbivores in food webs with odd and even numbers of trophic levels, has application to river communities.

THE ROLE OF FISH IN RIVER FOOD webs has been hotly debated. The earlier notion that physical factors play stronger roles than trophic interactions in structuring ecological communities in flowing waters (1) is being challenged by the view that both matter (2, 3). Although some field studies have shown that herbivorous fish can directly control algal standing crops in rivers (3, 4), and by implication must influence other parts of algal-based food webs, no studies in rivers have demonstrated that effects of predatory fish can cascade through food webs to alter primary producers, as has been shown in lakes (5, 6). In this report, I present experimental evidence of strong fish effects on both predatory and herbivorous insects, and on macro- and epiphytic algae in a river. These effects are direct and indirect, and propagate through four trophic levels in the river food web.

The study site was a 1-km reach of the South Fork of the Eel River (39°44'N, 123°39'W) in Mendocino County, California. The study reach is surrounded by an old-growth conifer forest dominated by Douglas fir (*Pseudotsuga menziesii*) and coastal redwood (*Sequoia sempervirens*). Nearly all precipitation falls between October and April. After winter floods, discharge drops steadily from a base flow of ~10 to 25 m³/s to a base flow of less than 1 m³/s during the summer, when large pools become nearly lentic. The active channel maintained by winter floods is considerably wider than the wetted channel during low-flow periods, so that much of the bed is sunlit. Filamentous green algae, dominated by *Cladophora glomerata*, proliferate on bedrock and boulders. *Cladophora* turfs attain lengths of several meters and cover much of the riverbed (Fig. 1A).

By midsummer, *Cladophora* turfs are much reduced, and remnants take on a ropy, pros-

trate, webbed appearance (Fig. 1B). This architecture results from dense infestations of chironomid larvae, dominated by *Pseudochironomus richardsoni*, which weave algae into retreats, or tufts, 0.5 to 1 cm long and reduce algal biomass. By midsummer, these chironomids are dominant components of the river arthropod assemblage (7). The three common fishes that summer in the study reach are juvenile steelhead (*Oncorhynchus mykiss* = *Salmo gairdneri*), California roach (*Hesperoleucas symmetricus*), and three-spined stickleback (*Gasterosteus aculeatus*). Steelhead and roach dominate the fish fauna

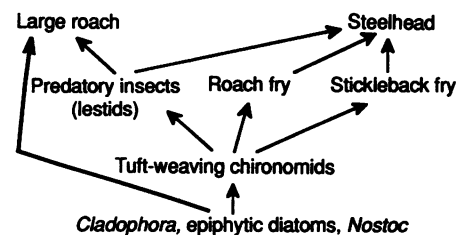
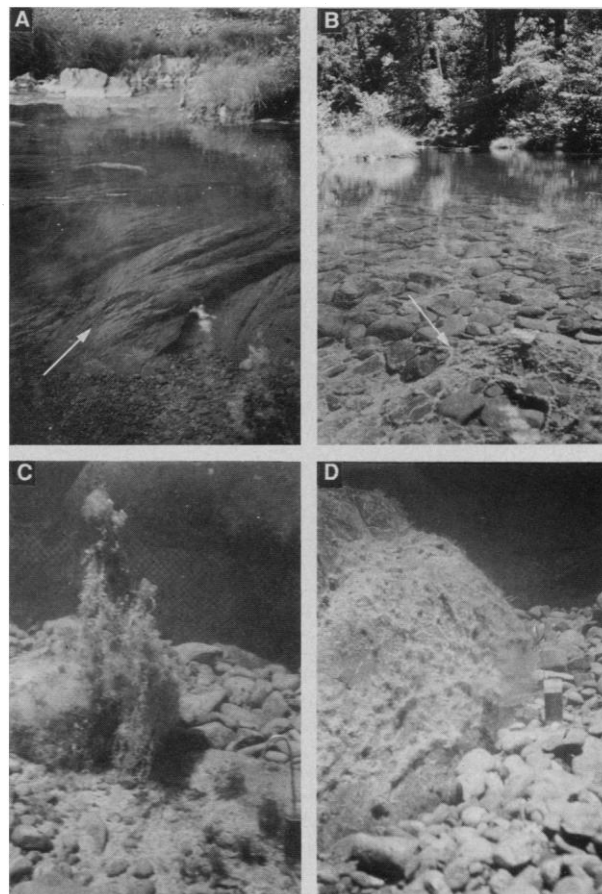


Fig. 2. Trophic relations of dominant biota in and around algal turfs during the summer low-flow period. Arrows point from prey to their consumers.

in spring and early summer. By midsummer, roach and the few overwintering stickleback both produce large numbers of fry. These fry, along with predatory aquatic insect larvae (Fig. 2), make up a guild of small, weakly swimming predators whose densities increase as water level drops during the summer low-flow period.

Food webs were allowed to develop in the presence or absence of fish in 12 large (6 m²) cages constructed around boulders or bedrock that supported large standing crops of *Cladophora* (8). The 3-mm mesh walls were transparent to most river insects but not to fish present in early June, when the experiment began. Three groups of four cages were distributed over a 1-km reach of river. In each group, two randomly selected cages

Fig. 1. (A) Study reach in early June. Arrow points to *Cladophora* turf 1.5 to 2 m long. Turfs are attached to boulder and bedrock substrates and do not occur on gravel (foreground). (B) Study reach in late July. Arrow points to remnants of *Cladophora* woven into a prostrate web by midges. (C) Underwater photograph taken in an enclosure in mid-July. *Cladophora* turfs 40 to 60 cm high are buoyed up by gas-filled *Nostoc* colonies. The sediment trap (lower right) is 11 cm high. (D) Underwater photograph taken in an enclosure in mid-July. *Cladophora* <2 cm high has the webbed appearance indicating midge infestation. The sediment trap is 11 cm high.



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were stocked with fish (enclosures); the other two were left unstocked (exlosures). Differences among groups were nonsignificant; therefore, the blocked design was dropped from the analysis.

Enclosures received 20 juvenile steelhead [28 to 50 mm SL (9)] and 40 roach (30 to 70 mm SL). These proportions reflected the proportions of fishes captured by trapping and electroshocking in early June (10). Fish were size-matched among enclosures, and the size distributions of stocked fish were representative of those of captured fish. Fish density in enclosures (ten individuals per square meter) was within the range of densities observed in the open river near large boulders with *Cladophora* cover but exceeded the density in larger river reaches (11).

Algal standing crops were quantified non-destructively along five cross-stream transects placed at even intervals along a longitudinal transect in each cage (12). In addition, cores of algae and of sediments were collected for measurements of algal standing crops (damp weights per unit area) and counts of associated invertebrates (13). Samples of *Cladophora* were preserved in 2% formalin for counts of epiphytes. During weeks 4, 5, and 6 of the experiment, I netted and weighed all algae floating in each cage.

Snorkeling, I counted fish fry (<30 mm SL) in each cage. At the end of the experiments, I sampled fish fry and larger, stocked fish from each cage and from the open river for examination of their gut contents.

After 5 weeks (mid-July), algal assemblages in enclosures were strikingly different from those in exlosures. In enclosures, *Cladophora* standing crops were much reduced (Fig. 3) and took on the prostrate, webbed architecture (Fig. 1D) produced by chironomid infestations (14). *Cladophora* standing crops in exlosures, remained higher than in stocked enclosures (Fig. 3) or in nearby sites sampled from the open river (15). *Cladophora* in exlosures became heavily overgrown with epiphytic diatoms and cyanobacteria dominated by nitrogen-fixing *Nostoc*. The weight of detached, floating algae (primarily *Nostoc*) skimmed from surfaces of enclosures exceeded that from enclosures by two orders of magnitude (Fig. 3D). Diatom epiphytes, dominated by the genus *Epithemia*, accrued more on *Cladophora* in enclosures than in enclosures ($P = 0.05$ from a Mann-Whitney U test comparing differences between weeks 0 and 5 of average epiphyte load per cell for *Cladophora* in enclosures and exlosures).

As suggested by the webbed appearance

of the algae, chironomid larvae were denser in enclosures (Fig. 4A). Exlosures, on the other hand, contained higher densities of predatory insects (Fig. 4B), 47% of which were lested damselfly nymphs, and of roach and stickleback fry, which did not colonize enclosures (Fig. 4C).

In a second experiment, I examined the potential impact of these small predators on colonization and establishment of tuft-weaving chironomid larvae. I stocked each of six buckets with four individuals of one of the three predator types. Each of these 18 enclosures, plus six predator-free enclosures, received a preweighed clump of *Cladophora* (6.8 to 7.3 g damp weight) devoid of chironomids (16). The buckets were interspersed in a wide, shallow area at the downstream end of a large pool and arranged so that flow through screened windows in each was similar (<5 cm/s). The 1-mm window screen mesh was transparent to chironomid larvae but not to the small predators enclosed.

After 20 days, the 24 buckets were collected, and the gut contents of predators were examined. *Cladophora* clumps were examined under $\times 10$ magnification for chironomids. About four times as many chironomids colonized *Cladophora* in exlosures as in enclosures with small predators (Fig. 5). Densities did not differ among enclosures, suggesting similar per capita effects for different small predator types. Chironomids were the dominant (by volume) item in the guts of 10 of 10 lestids; in 23 of 23 of the sticklebacks; and in 5 of 19 roach fry. Roach fry also ingested diatoms, which dominated (by volume) gut contents of 12 of 19 dissected individuals.

Adult roach stocked in enclosures, like roach fry, were omnivorous. Large roach fed on large aquatic arthropods, diatoms, and filamentous green algae (largely *Cladophora*). The numbers of individuals with gut contents dominated by these items were 9 of 28, 15 of 28, and 4 of 28, respectively. Juvenile steelhead, in contrast, were strictly carnivorous, feeding primarily on large aquatic (14 of 16) and terrestrial arthropods (1 of 16) and fish fry (1 of 16). The most common aquatic insects eaten by enclosed steelhead and roach were predatory lested, sialids, coenagrionids and naucoriids found in *Cladophora* turfs, and heptageniid, baetid, and siphonurid mayfly nymphs and elmids beetle larvae that consumed diatoms and detritus on gravel substrates. Diets of free-swimming steelhead and roach were similar to diets of their enclosed conspecifics, although animal food dominated in more of the guts of free-swimming roach, both in adults and in fry.

Large roach and juvenile steelhead, by suppressing small predators that fed on algi-

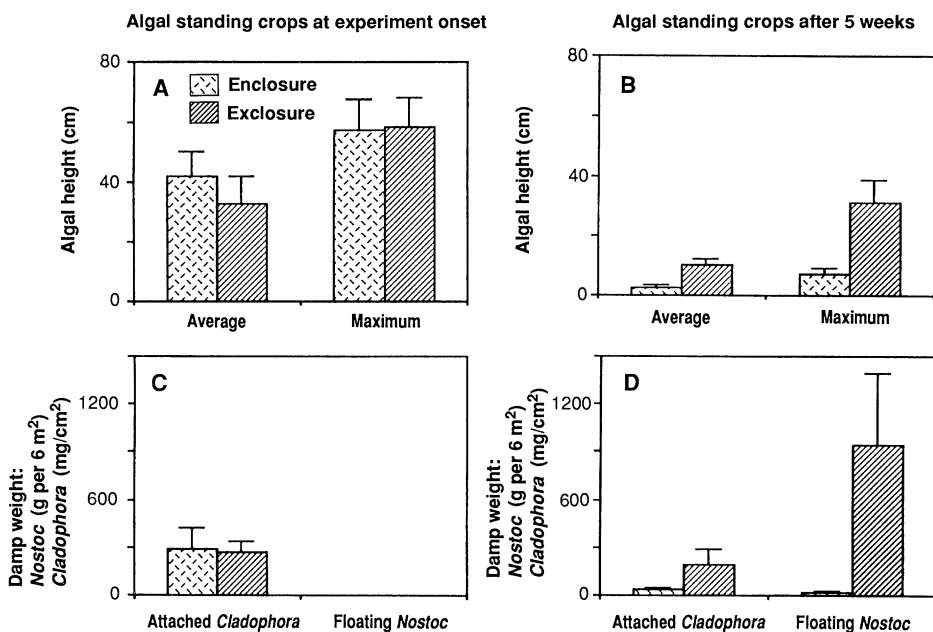


Fig. 3. (A) Algal heights (averages with 1 SE, $n = 6$, of means of 23 to 48 sites per cage, and of maximum heights) were initially similar in enclosures and exlosures [$t = 0.64$, $df = 10$, P (two-tailed) not significant for average height; $t = 0.07$, $df = 10$, P (two-tailed) not significant for maximum height]. (B) After 5 weeks, differences were significant for both average height [$t = 3.94$, $df = 10$, P (two-tailed) < 0.002] and maximum height [$t = 3.13$, $df = 10$, P (two-tailed) < 0.02]. Algae in enclosures declined with herbivory and fragmentation by chironomids and roach; in exlosures, smaller losses occurred with sloughing of *Nostoc* (D). (C) Initially, standing crops were similar in enclosures and exlosures ($t = 0.13$, $df = 10$, P (two-tailed) not significant), and no floating algae occurred. (D) After 5 weeks, standing crops of attached *Cladophora* and the mass of floating *Nostoc* harvested from 5 to 24 July differed in enclosures and exlosures [P (from Mann-Whitney U tests) = 0.004 for *Cladophora* and 0.001 for the total yield of floating *Nostoc*]. The damp weight of *Nostoc* collected from the water surface during 1 week (5 to 11 July) averaged (SE in parentheses) 3.4 (1.1) g in enclosures and 408.3 (275.5) g in exlosures ($P = 0.036$ from a Mann-Whitney U test).

vorous, tuft-weaving chironomids, exerted an indirect adverse effect on *Cladophora* mediated through four trophic levels. The occurrence of *Cladophora* in the guts of some large roach suggests that these fish also suppress the alga by direct herbivory. Although omnivory often obscures the roles played by consumers in food webs, in this case the macroscopic effects on plants exerted by large roach through two and four trophic levels are similar.

Fretwell, extending the argument of Hairston, Smith, and Slobodkin (HSS) (17), predicted that in food webs with even numbers of trophic levels, herbivores would deplete plants to produce habitats that look barren. In webs with odd numbers of trophic levels, plants released from herbivory would proliferate to produce green habitats, in which plants become limited by nutrients or other resources. This model, although developed primarily for terrestrial communities, can account for results reported here. In the absence of large fish, small predators suppress herbivorous chironomids, releasing *Cladophora*, its diatom epiphytes, and the associated cyanobacterium *Nostoc*. The proliferation of both *Nostoc*, a nitrogen fixer, and the dominant epiphytic diatom *Epihemia*, which contains a nitrogen-fixing cyanobacterial endosymbiont (18), supports the HSS-Fretwell prediction that algae in this three-trophic level web become nutrient-limited (in this case nitrogen-limited), giving nitrogen fixers a competitive advantage over other algae (19). In the presence of

large roach and juvenile steelhead, interactions mediated through two or four trophic levels curtail the proliferation of algal turfs and floating mats, and algal standing crops are reduced to relatively barren levels.

In an alternative model of trophic control, Menge and Sutherland (20) argued that the prevalence of omnivory in food webs should lead to increasing control by predation, and decreasing control by resource limitation, for populations at lower trophic levels. This model, developed for marine communities, would not predict that large fish would release chironomids from small predators but rather that large fish would also consume these small herbivores. Chironomids, however, were not heavily consumed by large roach or steelhead (21), possibly because larvae disperse as first instars (22, 23) too small to be pursued, or perhaps even perceived, by larger fish (5, 24). After they settle, tuft-weaving chironomids appear protected by their algal retreats from larger fish.

These life history features of the dominant herbivore in South Fork Eel *Cladophora* turfs, which thwart fish predation, are not idiosyncratic. Chironomids and other small omnivore-algivores that disperse as early instars and construct retreats after settling are dominant components of many freshwater biotas (22, 25). The unavailability of this important guild of small primary consumers to fish may be the key feature causing this river food web to follow the predictions of the HSS-Fretwell model rather than those of the Menge-Sutherland model, which requires more complete omnivory by top predators in food webs.

However, seasonal dynamics of the food web are consistent with another prediction from the Menge-Sutherland model: that food chains will lengthen, and the importance of predation will increase with decreasing environmental stress (20, 26). During winter, scouring floods (physical stress)

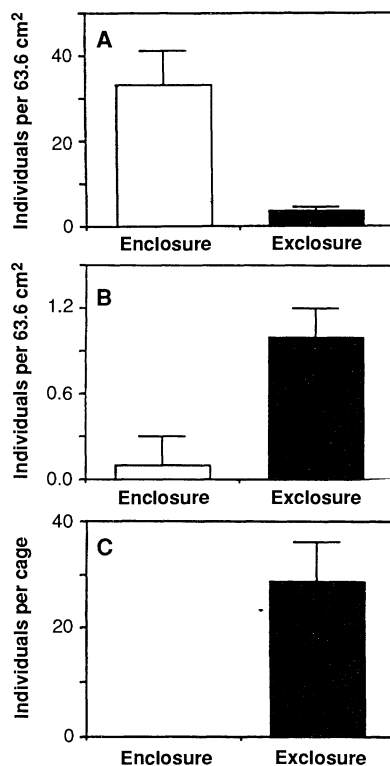


Fig. 4. (A) Densities (means of six enclosures with 1 SE) of chironomids (mostly *Pseudochironomus richardsoni*) differed in enclosures and exclosures after 5 weeks (P from a two-tailed Mann-Whitney U test = 0.002). At the onset of experiments, midge densities in enclosures and exclosures were similar (30.2 versus 24.8 individuals per 63.6 cm², respectively, $t = 0.41$, P not significant). (B) After 5 weeks, densities of predatory insects were higher in exclosures than in enclosures ($P < 0.02$ from a two-tailed Mann-Whitney U test). Initially, average densities of predatory insects were similar (0.78 versus 1.17 individuals per 63.6 cm², respectively, $t = 0.96$, P not significant). (C) Estimates (means of counts on three consecutive days) with 1 SE of fish fry counted in enclosures and exclosures after 5 weeks ($P = 0.001$ from a two-tailed Mann-Whitney U test). At the onset of the experiment, fish fry were not present in cages or in the open river.

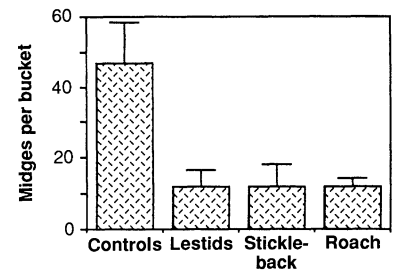


Fig. 5. More chironomids occurred in predator-free exclosures than in exclosures with one of three types of small predators ($F = 6.05$, $P < 0.01$, $df = 3,20$ from a one-way model I analysis of variance). A Scheffé's multiple contrast test [(14), p. 196] revealed that each predator treatment differed from the predator-free control, but that no differences existed between effects of different types of predators.

depress densities of algae and other river biota. When scouring flows subside in spring, *Cladophora* grows rapidly before animal densities build up (one trophic level), and green biomass accumulates. As insect and fish densities increase, trophic interactions of the types described here will prolong (three trophic levels) or shorten (two or four trophic levels) the persistence of green algal biomass. The high productivity of *Cladophora* in early summer may account for its support of a food web with dynamically significant interactions across as many as four trophic levels (27). *Cladophora* blooms occur in sunlit rivers worldwide (28), and fish-mediated effects on their accrual, architecture, and persistence will affect energy flow within these rivers and from rivers to watersheds (29).

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7. By July, tuft-weaving chironomids were the most numerous arthropod taxon in *Cladophora* samples, comprising 42% of 1021 individuals counted in 15

- samples. In July 1988, these chironomids comprised 79% of 2608 arthropods counted in seven larger samples of *Cladophora* turf (M. E. Power, *Oikos*, in press). Because invertebrates concentrate in *Cladophora* (densities, per area projected to the river surface, in algal samples were 29.2 to 41.1 times those in gravel samples) and because algal turfs cover 10 to 30% of the riverbed during summer, tuft-weaving midges are numerically dominant members of the river community at large. Their potential negative effect on *Cladophora* is indicated by results from an experiment in which 5 g (damp weight) of *Cladophora* were placed in small enclosures with 1-mm mesh walls, which are transparent to midge immigration. After 20 days of incubation, *Cladophora* weight (Y, grams damp weight) was negatively related to midge densities (individuals per enclosure): $Y = -0.11X + 4.81$, SE of slope = 0.048, $n = 48$, $P < 0.005$). In experimental outdoor channels, chironomid larvae enhanced export of algae [E. Eichenberger and A. Schlatter, *Verh. Int. Verein. Theoret. Angew. Limnol.* **20**, 1806 (1978)].
8. Cages were 3 m long by 2 m wide by 1.3 m high, and were constructed from plastic screen lined with black plastic shade cloth with a 3-mm mesh. Walls were flared outward at the top to reduce shading and to enhance stability. The shade cloth lining extended 60 cm below the bottom edge of the wall. This "skirt" was buried under gravel in order to anchor the cage and to preclude passage by large fish. Otherwise, the 6-m² enclosed riverbed was unmodified. Drift deflectors of aluminum flashing placed upstream and periodic cleaning prevented drifting detritus from clogging cage walls, which remained transparent to most stream invertebrates and fish fry throughout the 6-week experiment.
 9. Standard length (SL) measures from the anterior end of the head to the center of the crease formed by bending the caudal peduncle [K. F. Lagler, J. E. Bardach, R. R. Miller, D. R. M. Passino, *Ichthyology* (Wiley, New York, ed. 2, 1977), p. 403].
 10. Combined trapping and electroshocking efforts in the study reach from 4 to 6 June 1989 yielded 427 roach, 186 juvenile steelhead, 41 juvenile coho, and 1 gravid female stickleback.
 11. Estimates from snorkeling and shocking (4 to 10 June 1989, before appearance of roach and stickleback fry) ranged from 0.1 to 0.5 individual fish per square meter over an 800-m reach that included riffle and pool habitats. In a September shocking census of a nearby, 117-m² pool, combined densities of roach and steelhead were estimated at 1.26 individuals per square meter [L. R. Brown and P. B. Moyle, *Eel River Survey: Third Year Studies* (Report to the California Department of Fish and Game, Sacramento, 1989)].
 12. Where cross-stream transects intercepted boulder or bedrock, the only substrates supporting macroscopically conspicuous attached algae, I sampled the algae by recording at 10-cm intervals the dominant and subdominant taxa, algal height, condition, and density. Algal height was measured as the modal height of filaments at the sampled site. See M. E. Power and A. J. Stewart [*Am. Midl. Nat.* **117**, 333 (1987)] for methodological details.
 13. Algae and associated biota on bedrock were sampled at 1.0-m intervals where longitudinal transects intercepted boulder or bedrock substrates.
 14. Proportions (SE in parentheses) in six enclosures of 23 to 48 sites with webbed *Cladophora*, uncolonized *Cladophora* turf, and deposited *Nostoc* were 0.72 (0.08), 0.27 (0.08), and 0.01 (0.004), respectively. In the six enclosures, these proportions were 0.07 (0.03), 0.79 (0.05), and 0.15 (0.04), respectively. Average proportions of sites with webbed *Cladophora* differed between enclosures and enclosures [$F = 7.15$, $df = 10$, P (two-tailed) $<< 0.001$]. Before the t test was performed, proportions were arcsin log root-transformed [J. Zar, *Biostatistical Analysis* (Prentice-Hall, Englewood Cliffs, NJ, 1984), p. 241].
 15. Standing crops of attached *Cladophora* sampled from nearby open river sites were similar to standing crops inside all cages at the onset of experiments, and intermediate between standing crops in enclosures and enclosures after 5 weeks. On 5 June, standing crops (milligrams per square centimeter damp weight) from open sites, enclosures, and enclosures were $[\bar{x}$ (SE, n): 298.92 (54.43, 9), 286.37 (141.55, 6), and 265.57 (70.56, 6), respectively. On 16 July, these standing crops were 98.13 (18.08, 8), 33.6 (10.76, 6), and 195.49 (79.81, 6), respectively.
 16. *Cladophora* was brought into the laboratory, where filaments were gently teased apart and all invertebrates >1 mm long were removed, with the exception of cryptic ceratopogonid larvae, which had the diameter and color of *Cladophora* filaments. Cleaned samples were inspected under $\times 10$ magnification, then damp-weighted, and stocked in the stream in 12.7-liter plastic buckets.
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 21. Of 21 steelhead sampled, only 7 had any chironomids in their guts: 4 had one chironomid, 1 had two, 1 had four, and 1 had five. Of 46 large roach sampled, only 5 contained any chironomids: 3 had one, 1 had three, and 1 had seven.
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 30. I thank B. Harvey and W. Dietrich for discussion; J. Nielsen, P. Steel, T. Steel, H. Kifle, S. Grimm, S. Kupferberg, B. Rainey, S. Kely, P. Whiting, B. Harvey, and W. Dietrich for help with fieldwork; and W. Getz, T. Schoener, A. Worthington, J. Marks, S. Kupferberg, W. Dietrich, R. B. Hanson, and an anonymous reviewer for comments on the manuscript. This research was supported by NSF grant RII-8600411 and the California State Water Resource Center (W-726).

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Sequence-Specific Binding of Human Ets-1 to the T Cell Receptor α Gene Enhancer

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Expression of the human T cell receptor (TCR) α gene is regulated by a T cell-specific transcriptional enhancer that is located 4.5 kilobases (kb) 3' to the $C\alpha$ gene segment. The core enhancer contains two nuclear protein binding sites, $T\alpha 1$ and $T\alpha 2$, which are essential for full enhancer activity. $T\alpha 1$ contains a consensus cyclic adenosine monophosphate (cAMP) response element (CRE) and binds a set of ubiquitously expressed CRE binding proteins. In contrast, the transcription factors that interact with the $T\alpha 2$ site have not been defined. In this report, a λ gt11 expression protocol was used to isolate a complementary DNA (cDNA) that programs the expression of a $T\alpha 2$ binding protein. DNA sequence analysis demonstrated that this clone encodes the human *ets-1* proto-oncogene. Lysogen extracts produced with this cDNA clone contained a β -galactosidase-Ets-1 fusion protein that bound specifically to a synthetic $T\alpha 2$ oligonucleotide. The Ets-1 binding site was localized to a 17-base pair (bp) region from the 3' end of $T\alpha 2$. Mutation of five nucleotides within this sequence abolished both Ets-1 binding and the activity of the TCR α enhancer in T cells. These results demonstrate that Ets-1 binds in a sequence-specific fashion to the human TCR α enhancer and suggest that this developmentally regulated proto-oncogene functions in regulating TCR α gene expression.

MAMMALIAN T LYMPHOCYTES CAN be divided into two subsets on the basis of their expression of distinct heterodimeric cell-surface antigen receptor molecules (1). Greater than 90% of peripheral blood T cells, including cells of the helper and cytotoxic phenotypes, express the disulfide-linked α/β T cell receptor (TCR). In contrast, 2 to 10% of circulating

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