Seasonal occurrence of black flies (Diptera: Simuliidae) in a desert stream receiving trout farm effluent

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ABSTRACT: The distribution and abundance of black flies (Diptera: Simuliidae) in a small desert stream were influenced by environmental changes caused by recharge of water supply storage basins and an aquaculture operation. *Simulium virgatum* was the most abundant benthic insect collected in Whitewater Canyon (Riverside County, CA) after April; however, it was never found in trout farm effluent where the ammonium-nitrogen concentration was > 0.25 mg/liter. *S. virgatum* densities downstream of the input of water from the Colorado River aqueduct were lower than at other sampling sites in the Whitewater River. *Simulium tescorum*, an especially anthropophilic black fly, was most abundant during February and March, was not collected from late spring through early autumn, and was found only in the highly enriched, less variable flow of trout farm effluent. The mean concentrations of ammonium nitrogen and nitrate nitrogen in the trout farm effluent nearly 1 km from the fish holding ponds were ten and two times, respectively, the ambient levels in the Whitewater River upstream of the effluent discharge point. A combination of factors probably contributed to the presence of *S. tescorum* in the trout farm effluent including homogenization of the flow regime, enrichment of larval resources, and the development of riparian vegetation that provided oviposition and attachment sites. ***Journal of Vector Ecology* 36 (1): 187-196. 2011.**

Keyword Index: Black flies, enrichment, aquaculture, desert streams.

INTRODUCTION

Water use by humans has markedly changed flow regimes (Poff et al. 2007) and nutrient levels (Vitousek et al. 1997, Naylor et al. 1998, Grimm et al. 2008) in aquatic ecosystems. In order to meet the demands for potable water, for non-drinking domestic uses, for aquaculture and agricultural uses, and to process water-borne wastes from an ever-growing human population (Gleick 2009), water management strategies increasingly include the storage and recycling of water. Anthropogenic changes in water use have influenced not only the biodiversity of aquatic ecosystems (Lautenschläeger and Kiel 2005, Millennium Ecosystem Assessment 2005, Poff et al. 2007, Pramual and Kuvangkadilok 2009), they have the potential to influence significantly the abundance of important reservoirs and vectors of human pathogens (Malek 1975, Russell 1999, Walton 2002, Metzger 2004).

Discharges of nutrient-enriched water from municipal wastewater treatment plants, combined sewage-stormwater outflows, and agricultural operations including high-density farming operations such as feedlots and aquaculture can have a large impact on receiving waters. Increases in inorganic nutrient levels in streams receiving aquaculture effluent caused increased algal abundance (Carr and Goulder 1990, Boaventura et al. 1997). The diversity of benthic invertebrates downstream of effluent discharges or in agriculturally impacted streams often decreases as the abundance of pollution-tolerant organisms increases (Camargo 1992, Wright et al. 1995, Adamek and Sukop 1996, Loch et al. 1996, Strieder et al. 2006). The extent of the effects of nutrient-laden water on receiving waters is clearly related to the concentrations of particular nutrients and the magnitude of the discharge of enriched water relative to that of the receiving water. On average, salmonid rearing operations increase the concentrations of important limiting nutrients such as phosphorus and ammoniacal nitrogen by more than an order of magnitude in receiving waters (summarized by Pachón and Walton 2008).

Black fly larvae exploit increased levels of fine particulate organic matter associated with nutrient-enriched effluent (Camargo 1992, Adamek and Sukop 1996, Loch et al. 1996), but enrichment does not always change macroinvertebrate density, biomass or species diversity (Sabater et al. 2005, Pachón and Walton 2008). Allochthonous enrichment of lotic systems has been shown to have significant effects on simuliids, including changes in larval density, species composition, and developmental rate (Wotton 1979, Adler et al. 1982). Other environmental changes associated with enrichment, such as alteration of the flow regime and changes in streamside vegetation, can influence black flies (Kim and Merritt 1987). Environmental changes can influence the distribution and abundance of simuliid vectors of human pathogens which are typically sibling species, each with unique habitat preferences, dispersal capabilities, and vector competence (Adler et al. 2010).

This study examined the distribution and abundance of black flies in a small desert stream receiving effluent from a small-scale trout farm and hatchery in the eastern Banning Pass of southern California. Nutrient levels, potential resource levels in the water column and on the substrate for larval black flies, and physicochemical factors were quantified at one upstream and several downstream sites from the trout farm during a nine-month period.

MATERIALS AND METHODS

Study sites

The Whitewater River lies within a watershed of the San Bernardino Mountains and flows through Whitewater Canyon on the western edge of the Coachella Valley of southern California. Water was often diverted for human use in the surrounding areas and the diversion of water caused intermittent flow in the Whitewater River in the areas upstream of the Whitewater Trout Farm (33° 59′ 17.39″N, 116° 39′ 19.81″ W, 670 m above sea level [ASL]). Depending on the amount of snow pack and precipitation events, dry periods lasted for several months during some years[1]. The 118-ha trout farm had been in operation for more than 50 years prior to this study.

An 8-km section of the Whitewater River in the lower portion of Whitewater Canyon (Figure 1) was studied from February until October, 1998. The elevation at the highest point (trout farm: site TF) sampled was 655 m ASL and the lowest point (near Interstate 10: site I-10) was 375 m ASL, with a grade approximating 3.7% through Whitewater Canyon. The channel substrate consisted of granitic cobbles and boulders, with stretches of sandy bottom and small stones. Despite a narrow channel (< 10 m in most places), the width of the cobble- and bolder-strewn floodplain ranged between 175 and 300 m in most areas and was indicative of periodic spates. The watershed was subject to flash floods from rainfall.

Riparian vegetation was dominated by slender willow (*Salix exigua* Nutt.) and Fremont cottonwood (*Populus fremontii* S. Watson) and was well established downstream of the trout farm compared with upstream areas because water flow from the aquaculture operation into the Whitewater River was continuous during the summer. Dense riparian vegetation and vines covered the effluent stream from the trout farm to the Whitewater River.

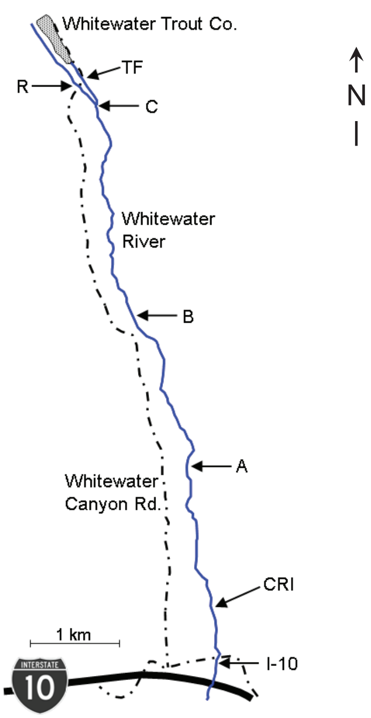

Figure 1. Location of sampling sites in Whitewater Canyon, Riverside County, CA.

Samples were taken at seven sites along the Whitewater River (Figure 1). Site I-10 was approximately 8 km from the trout farm rearing facility. Water flow at this site was dominated by Colorado River water that discharged from an aqueduct into the Whitewater River just below site CRI (Colorado River inflow). Site CRI was located approximately 6 km from the trout farm effluent discharge. Site A was approximately 2.4 km downstream of the trout farm discharge and was highly impacted by visitors during the summer because of its proximity to the road. Site B was approximately 1.4 km downstream of the trout farm discharge. Site C was at the confluence of the Whitewater River and the trout farm effluent stream. The reference site, site R, was upstream of the trout farm and was not affected by the trout farm effluent. Effluent from the trout hatchery entered a settling pond and was then released into a small stream which joined Whitewater River approximately 1 km downstream of the fish-rearing facility. Site TF was in the channel containing trout farm effluent before it joined with the Whitewater River.

Water for the fish-culturing operation was supplied from both artesian well water and diverted stream water from Whitewater Canyon (Jan Lawless, Whitewater Trout Co., Whitewater, CA; personal communication). The effluent discharge measured during this study was relatively constant at about 0.51 m^3/s (± 0.02, n = 4). The flow through Whitewater River varied greatly depending on precipitation and season, with an average flow of 0.84 m/s. However, this mean value for flow does not include flow measurements during short-term, sporadic flash floods. The flow at the confluence of Whitewater River and the trout farm effluent discharge was approximately 1.25 $m^{3/}s$, which was roughly a 1.5:1 dilution of stream water to effluent.

Seasonal trends in discharge of the Whitewater River were estimated using discharge data for a stream in an adjacent watershed. Discharge data were obtained from the United States Geological Service (USGS) on-line archives (http://waterdata.usgs.gov/nwis/rt). A 41-year (October, 1967 through June, 2009) data set collected at a gage station on Mission Creek (34° 00′ 40″N, 116° 37′ 38″ W, 717 m ASL) near Desert Hot Springs, CA was used to determine daily discharge during 1998 and to calculate daily long-term means for discharge using a 14-day running average of mean daily discharge. The Mission Creek gage station is associated with a 92.2 km^2 watershed and is located 3.5 km northeast of the trout farm.

[1]Pachón, R.T. 1999. Effects of a Small-scale Fish Farm on a Desert Stream: The Impact of Enrichment on Resources and Abundance of Aquatic Macroinvertebrates with an Emphasis on the Simuliidae. M.S. thesis, Department of Entomology, University of California, Riverside.

Invertebrate sampling

Aquatic insects were sampled approximately bi-weekly from February through July; thereafter, twice monthly in August and October. Cobbles were collected haphazardly from riffle areas observed to support black flies. Each cobble was carefully lifted from the stream, placed into a Ziploc™ bag (DowBrands L.P., Indianapolis, IN), numbered, and placed on ice for transport back to the laboratory. Three to six cobbles per site were collected on each date. All samples were kept on ice and processed within three days. Cobbles were scrubbed with a wire brush and organic matter was filtered through a screen (mesh size = 533 μm). Insects caught on the screen were preserved in 75% ethanol for later identification.

Insects were identified to order (non-abundant groups), to family (Diptera, Ephemeroptera and most Trichoptera), or genus (the most abundant caddisfly, *Hydropsyche* sp.) using the keys of Merritt and Cummins (1996) and Wiggins (1997). The impact of trout farm effluent on the non-black fly fauna is discussed elsewhere (Pachón and Walton 2008). The Simuliidae were identified to species using representative samples supplied by Peter Adler (Dept. of Entomology, Clemson University).

Simuliid densities were calculated by dividing raw insect counts by the colonizable area of the cobble. Cobble area was determined by wrapping each cobble with Saran Wrap™ (DowBrands L.P., Indianapolis, IN) and weighing the Saran Wrap to convert the weight to area (cm^2). Estimates for objects of known surface area were within 10% of the actual area. The colonizable area for black flies was estimated to be 1/2 of the total area of the cobble.

Nutrient concentrations and water column physicochemical variables

Nutrient concentrations were measured in duplicate 50-ml samples collected from the water column at each sampling site. Samples were kept on ice and processed within one day of collection. Concentrations of NH_4-N, NO_3-N, and PO_4-P and were determined colorimetrically using a Technicon Autoanalyzer 11 (Technicon Instruments Corporation, Tarrytown, NY).

On each sampling date, water temperature, dissolved oxygen concentration, conductivity, and pH were measured at each site with an electronic sensor array (ICM Aqua Check™, Perstorp Analytical, Wilsonville, OR). Physicochemical parameters were measured from 19 February until 19 October at sites, except sites A and B where measurements were discontinued after 10 May because of ongoing human recreational activities.

Water column resource levels

Water column chlorophyll concentration was measured in duplicate one-liter samples from the sampling site. Samples were placed on ice for transport back to the laboratory and until processed. Samples were filtered through Whatman GF-F glass fiber filters (diameter 47 mm; Whatman International Ltd., Maidstone, Kent, U.K.). Following freezing, pigments were extracted in 90% alkaline acetone and ground using a mortar and pestle. Extracts were held at 10° C for approximately one week. After centrifugation, the concentrations of phytopigments were determined using a BioSpec-1601 spectrophotometer (Shimadzu Corp., Tokyo, Japan) using the methods of Wetzel and Likens (1991). Biomass of chlorophyll and other phytopigments was estimated using the formulae given by Wetzel and Likens (1991).

Ash-free dry mass (AFDM) of resources in the water column for immature black flies and other herbivorous/detritivorous aquatic insects was measured in duplicate samples. Samples were agitated and filtered through pre-ashed (3 h at 500° C, cooled under desiccation), pre-weighed Whatman GF-F filters (diameter 47 mm). The material collected on each filter was dried for 24 h at 105° C, cooled under desiccation and weighed to assess the dry mass of total suspenoids/liter. The filtered material was then ashed at 500° C for 3 h and reweighed after cooling to room temperature under desiccation.

Cobble resource levels

Total matter (silt plus organic matter) and AFDM on each cobble was determined in a subsample (40-100 ml) of the material scrubbed and filtered from the surface of the cobble. The subsample was filtered through a pre-ashed, pre-weighed glass fiber filter (Whatman 934-AH). The material collected on the filter was dried for 24 h at 105° C, cooled under desiccation, and weighed to assess the dry mass, and then ashed for 3 h at 500° C. The ashed material was cooled under desiccation and reweighed to assess AFDM. Because the rationale of the sampling protocol was to establish baseline conditions in the Whitewater River before initiating an experiment along a transect in the river (sites C, R, and CRI), which did not include samples in the effluent stream from the trout farm, total matter on cobble surface and AFDM on cobble surface were not measured on cobbles retrieved from Site TF.

Chlorophyll biomass on each cobble was determined using a subsample (40-100 ml) of the material scrubbed and filtered from the cobble surface. Subsamples were collected as above and filtered through Whatman GF-F glass fiber filters. The filters were frozen for several days to several weeks before pigment extractions were made by grinding each filter using a mortar and pestle in 90% alkaline acetone. Extracts were held at 10° C for up to three weeks. Following centrifugation, the pigment concentrations in the extracts were read on a BioSpec-1601 spectrophotometer (Shimadzu Corp., Tokyo, Japan) using the methods of Wetzel and Likens (1991) and biomass of chlorophyll and other phytopigments was estimated using the formulae given in Wetzel and Likens (1991).

Statistical analyses

The statistical significance of differences in nutrients and chlorophyll in the water column and in chlorophyll and AFDM on cobbles among sites were tested using Kruskal-Wallis one-way ANOVA on ranks (Sigma Stat, ver. 2.03; Fox et al. 1995). Dunn's method was used as a post-hoc test to examine differences between sites using a control group (site R) for comparisons of unequal sample sizes. A one-way ANOVA was used to test for differences in total surface particulate matter on experimental cobbles at Sites C, CRI, and R. A post-hoc Tukey test was used to examine between site differences. A Kruskal-Wallis one-way ANOVA on ranks was used to test particulate total matter and AFDM differences in the water column at Sites C, CRI, and R; sites A and B did not have an adequate sample size for conducting statistical analyses of these variables.

RESULTS

Seasonal occurrence of Simuliidae

Simulium tescorum Stone and Boreham was the most abundant black fly in February and March. Larvae were not collected after April (Figure 2A). *Simulium tescorum* larvae were found in the trout farm effluent (Site TF) and were not found at other sampling locations in Whitewater Canyon (Figure 2B).

Simulium virgatum Zetterstedt larvae were the most common black fly species collected after April and reached highest abundance in late summer and early autumn (August and October; Figure 2A). *Simulium virgatum* was considered here to be a single morphospecies but probably was a species complex consisting of more than one species. This morphospecies was very rare between February and April. *Simulium virgatum* larvae were collected throughout Whitewater Canyon; however, the abundance of larvae was comparatively high at upstream locations (Sites R, C, and B). Larvae of *S. virgatum* were comparatively rare below site CRI where Colorado Aqueduct water entered the Whitewater River (Figure 2B). *Simulium tescorum*, *S. arcticum* Malloch, and *S. argus* Williston were considerably less abundant than were individuals in the *S. virgatum* complex.

Seasonal trends of discharge

Discharge in the Mission Creek watershed adjacent to the Whitewater River watershed increased appreciably during February, 1998 and fluctuated through mid-May as precipitation events added runoff to that caused by melting snow (Figure 3). Discharge declined about six-fold during June to low levels during July. Flow rates doubled during August, increased abruptly with thunderstorms during early September, and were comparatively constant throughout autumn beginning in mid-October, 1998.

The 41-year long-term mean for a 14-day running average of daily discharge increased during January, reached an annual peak flow in late February, and declined by mid-March to a comparatively constant vernal flow pattern (Figure 3). Snow melt probably contributed primarily to discharge after mid-March, declining in amount between late May and late summer to comparatively consistent levels across autumn. Slight increases in the long-term mean for discharge associated with convective rainfall from monsoonal air flow are evident in July and August.

Water column physicochemical variables and nutrient concentrations

Water temperature was less variable and the annual mean temperature of the trout farm effluent was about 2° C cooler than was water in the Whitewater River (Table 1). The trout farm effluent was about 1° C warmer than water in the Whitewater River in the first month of the study, but thereafter the water under the vegetation canopy over the effluent stream was always cooler than the water in the river channel that was exposed to sunlight.

Dissolved oxygen concentration in the trout farm effluent was always lower than that at the reference site and was below that observed in the Whitewater River on each sampling date (Table 1). Nevertheless, the oxygen demand in the effluent stream was never large enough to depress daytime dissolved oxygen levels below 7 mg/liter.

Specific conductance of the trout farm effluent did not differ appreciably from that elsewhere in the Whitewater River (Table 1). Specific conductance (mean: 609 mS/cm; range: 273-891 mS/cm) was highest at the I-10 site and was indicative of the input of water from the Colorado River

Table 1. Mean physicochemical variables (range in parentheses) and nutrient concentrations (± SE) in the trout farm effluent stream (TF), at the reference site (R), and downstream of the trout farm effluent discharge (WR) in the Whitewater River, Riverside County, CA.

Variable	Site		
	TF	R	WR[a]
Water temperature (°C)	16.5 (14.5-19.9)	18.8 (14.4-22.9)	18.5 (12.8-27.3)
pH	7.9 (7.8-8.0)	8.5 (8.2-8.6)	8.5 (8.0-8.6)
Specific conductance (μS/cm)	317 (274-353)	283 (234-352)	349 (233-891)
Dissolved oxygen (mg/liter)	7.3 (7.0-7.7)	8.4 (8.0-8.8)	8.7 (7.4-9.6)
NH_4-N (mg/liter)	0.30 ± 0.03	0.03 ± 0.01	0.05 ± 0.01
NO_3-N (mg/liter)	0.97 ± 0.15	0.48 ± 0.11	0.58 ± 0.18
PO_4-P (mg/liter)	0.107 ± 0.025	0.065 ± 0.021	0.073 ± 0.026

[a] WR is the composite of samples from sites C, B, A, CRI and I-10.

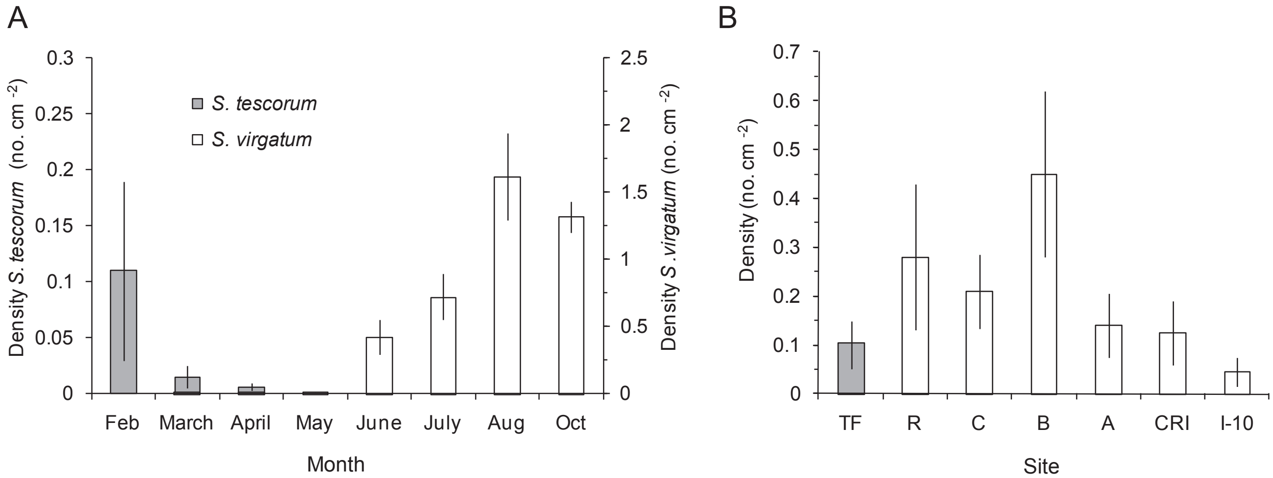

Figure 2. Density of *Simulium tescorum* and *S. virgatum* from February through October (A) and at sites in the Whitewater River (B). Error bars are standard errors.

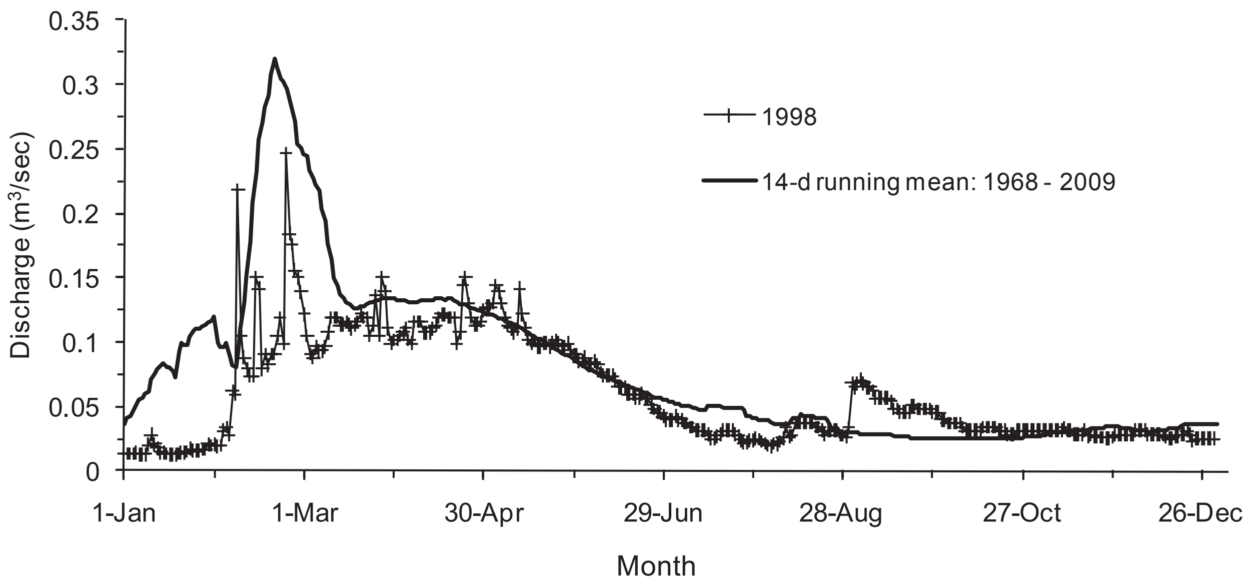

Figure 3. Daily discharge during 1998 and as a 14-day running mean for a 41-year data set for Mission Creek, Desert Hot Springs, CA.

Table 2. Mean (± SE) resource levels for larval black flies in the water column and on substrata in the trout farm effluent stream (TF), at the reference site (R), and downstream of the effluent discharge (WR) in the Whitewater River, Riverside County, CA.

Resource (unit of measurement)	Site		
	TF	R	WR[a]
Water column			
chlorophyll *a* (mg/liter)	120.0 ± 2.9	16.7 ± 2.0	104 ± 23.9
particulate matter (g/liter)	0.01 ± 0.001	0.13 ± 0.03	0.14 ± 0.07
AFDM[b] (g/liter)	< 0.01	< 0.01	< 0.01
Cobbles			
chlorophyll *a* (mg/cm^2)	5.1 ± 1.1	1.4 ± 0.4	5.0 ± 2.8
total organic matter (mg/cm^2)	–	0.45 ± 0.05	2.42 ± 0.46
AFDM[b] (mg/cm^2)	0.97 ± 0.11	0.10 ± 0.001	0.40 ± 0.11

[a]WR is the composite of samples from sites C, B, A, CRI and I-10. [b]Ash-free dry mass.

aqueduct. Mean specific conductance at the other sites from site C to site CRI was 284 μS/cm (range: 233-372 μS/cm). The pH of the effluent stream was ≤ 8.0 and was lower than the Whitewater River sites on every sampling date (Table 1).

Ammonium nitrogen (NH_4-N) concentration (Table 1) in the trout farm effluent was six to ten times higher than at the other sites, which did not differ significantly from one another (Kruskal-Wallis one-way ANOVA on ranks, H_6=35.94, P<0.001). Relative to the reference site, NH_4-N concentration was elevated nearly two-fold for 7 km downstream of the effluent discharge.

Mean nitrate-nitrogen (NO_3-N) levels (Table 1) in the trout farm effluent were two-fold higher than at the upstream reference site (Kruskal-Wallis one-way ANOVA on ranks, H_6=29.53, P<0.001; Dunn's method: TF ≠ R, I-10). NO_3-N concentration was elevated consistently for about 6 km from the confluence of the trout farm effluent discharge and the Whitewater River but did not differ significantly from that in the trout farm effluent. Nitrate-nitrogen concentration at the I-10 site was comparable to that at site R.

Phosphorus as orthophosphate (PO_4-P) levels in the trout farm effluent stream were 1.7 times higher than the mean PO_4-P concentration for the reference site (Table 1). However, orthophosphate concentration did not differ significantly among the sites (Kruskal-Wallis one-way ANOVA on ranks, H_6=3.02, P>0.5).

Water column resource levels

Water column chlorophyll levels in the trout farm effluent and downstream from site C were six to seven times the mean level at the reference site (Table 2; Kruskal-Wallis one-way ANOVA on ranks, H_6=16.73, P<0.05; Dunn's method: P<0.05). Chlorophyll *a* biomass in the Whitewater River downstream of the confluence of effluent stream with the river channel did not differ appreciably from levels in the trout farm effluent.

The mass of suspended particulate matter at site C was twice that at the reference site even though the particulate matter in the trout farm effluent was significantly lower than at the other sites (Kruskal-Wallis one-way ANOVA on ranks, H_6 = 19.09, P<0.01). The mean mass of water column suspenoids at the sites downstream of the trout farm effluent discharge did not differ markedly from site R (Table 2); however, suspended particulate matter declined downstream of site C. Suspended AFDM in the water column was consistently less than 0.01 g/liter and did not differ significantly among sites (Kruskal-Wallis one-way ANOVA on ranks, H_4=1.16, P>0.5).

Cobble resource levels

Chlorophyll biomass on cobbles at the upstream reference site was significantly lower than on cobble surfaces at sites receiving trout effluent (Kruskal-Wallis one-way ANOVA on ranks, H_4 = 10.66, P<0.05; Dunn's method). Mean chlorophyll biomass on cobble surfaces in the trout farm effluent and at all sites downstream of the trout farm was 3.6 times higher than at site R (Table 2).

Mean total particulate matter on cobble surfaces downstream of the trout farm was 5.4 times greater than at the reference site (Table 2). Total particulate matter at sites C and CRI was significantly higher than at site R (one-way ANOVA, $F_{2,16}$ = 3.74, P = 0.05; Tukey tests: R ≠ C, CRI). Surface AFDM on cobbles at sites downstream of the trout farm effluent discharge was four times that on cobbles at site R and also was significantly higher than at the reference site (Kruskal-Wallis one-way ANOVA on ranks, H_2=11.06, P<0.01).

The mean AFDM of particulate matter on cobbles at site TF was nearly an order of magnitude greater than at the reference site and more than twice that on cobbles downstream of the effluent discharge into the Whitewater River (Table 2). Based on the relationship of particulate matter mass to AFDM at the downstream sites and reference site (Table 2), about 5.4 g of particulate matter are estimated to have been present on cobbles at site TF.

DISCUSSION

The distribution and seasonal occurrence of black flies in Whitewater Canyon were influenced by environmental changes caused by effluent from a small-scale trout farm and hatchery. A combination of factors probably contributed to the presence of the anthropophilic black fly *S. tescorum* in the trout farm effluent including homogenization of the flow regime, enrichment of larval resources, and the development of riparian vegetation that provided oviposition and attachment sites. In addition to human-related changes in the Whitewater Canyon ecosystem caused by the discharge of nutrient-enriched wastewater that favored the production of an anthropophilic black fly species, addition of water for recharge of groundwater storage basins in the lower portion of the watershed reduced the abundance of a non-anthropophilic species, *S. virgatum*.

Simulium tescorum larvae were collected at an elevation of about 650 m in our study, which is nearly 200 m higher than for previous studies. Larvae of *S. tescorum* have been reported to be prevalent at low elevations in desert streams and in warm water in coastal southern California (Malibu Creek: Tietze and Mulla 1989); however, *S. tescorum* was only found in Whitewater Canyon from February until April when water temperatures were cool. In Thousand Palms Canyon, which is a desert stream located 31 km southeast of Whitewater Canyon, *S. tescorum* also was abundant from February through May and disappeared after June (Mohsen and Mulla 1982). *Simulium tescorum* was present in the lower portion of Malibu Creek from April through October and extended its distribution upstream (elevation of highest site sampled ~195 m) from August through October. Tietze and Mulla (1989) found this species was the predominant black fly at lower elevations (site M5: ~18 m ASL) and in slow flowing creeks (Corral Creek). Water temperatures in the trout farm effluent in February were about 1° C warmer than in the Whitewater River, but after February water in the Whitewater River was about 5° C warmer than the trout farm effluent. Water temperature in the Whitewater Canyon habitats never approached the maximum (32° C) observed for *S. tescorum* distribution (Lacey and Mulla 1980, Adler et al. 2004). *Simulium tescorum* larvae had not been collected above 458 m (Lacey and Mulla 1980, Tietze and Mulla 1989); however, adults have been found at more than twice this altitude (923 m) at the eastern edge of the species distribution near the Grand Canyon, Arizona (Lacey and Mulla 1980).

Simulium tescorum larvae were only found in the trout farm effluent. High levels of ammonium in polluted water have been associated with reduced emergence (Adler et al. 1982) and mortality (Grunewald 1978, Adamek and Sukop 1996) in some black flies. Grunewald (1978) found that concentrations above 0.6 mg ammonia/liter caused 100% mortality within 4 h in laboratory colonies of *Simulium erythrocephalum* de Geer. Tietze and Mulla (1989) noted reduced black fly numbers in a coastal canyon (Malibu Canyon) in southern California for ≤ 1.4 km below a discharge of reclaimed municipal wastewater with ammonia concentrations targeted at ≤ 0.3 mg/liter. Ammonium nitrogen levels in the trout farm effluent reached a maximum concentration of 0.59 mg/liter in February; the mean concentration across this study was 0.3 mg NH_4-N/liter. *Simulium tescorum* larvae may be more pollution-tolerant than other simuliid species; yet, the comparatively constant discharge of the trout farm effluent also might favor *S. tescorum* larvae.

Previous studies found that flow regime strongly affects the distribution of black flies, especially *S. tescorum*. Immature stages of *S. argus* and *S. tescorum* are found often in spring-fed desert creeks with relatively constant flow (Mohsen and Mulla 1982). *Simulium argus* is found on rocks and submerged plants across a broad range of elevations from the low desert (below sea level) to 1,646 m and in low (0.02-0.05 m/s) to moderate (0.3-0.8 m/s) flow (Lacey and Mulla 1980, Mohsen and Mulla 1982). *Simulium tescorum* larvae have been found in similar stream habitats, being prevalent on rocks and trailing vegetation in streams of low (0.02-0.06 m/s) to moderate flow (0.4-0.6 cm/s) within a temperature range of 10-32° C (Lacey and Mulla 1980, Mohsen and Mulla 1982). Mohsen and Mulla (1982) suggested that autumnal and winter flash floods eliminated *S. tescorum* larvae from Thousand Palms Canyon and, unlike *S. argus* which recolonized highly disturbed habitats after two to three months, *S. tescorum* larvae and pupae were not found again until one year later. Unlike Thousand Palms Canyon where *S. tescorum* co-occurred with but was at lower abundance than the year-round population of *S. argus* (Mohsen and Mulla 1982), *S. argus* was not found in the comparatively food-rich site TF in Whitewater Canyon.

Bottom-up enrichment of larval black fly resources from the nutrients in the trout farm effluent was evident considerable distances downstream from the confluence of the trout farm effluent stream and the Whitewater River. Ammonium-nitrogen and nitrate-nitrogen levels were enhanced 7 km (the maximum distance detectable in this study) and 6 km downstream, respectively. Orthophosphate levels at TF were nearly twice that at the reference site; but, PO_4-P concentration in the Whitewater River did not did not differ significantly among sites suggesting rapid utilization of this limiting nutrient. Algal resources in the water column of the Whitewater River increased six to seven-fold downstream of the reference site. Chlorophyll *a* biomass at all downstream sites was higher than at site R. Particulate matter at the confluence of the trout farm effluent stream and the Whitewater River (site C) was twice that at site R and declined downstream. Particulate matter concentration at sites CRI and I-10 was half that at site C (Pachón and Walton 2008).

Substrate resources were also enhanced by the nutrient-rich trout farm effluent. Epilithic phytoplankton biomass was lowest at site R, intermediate at other sites, and highest at site C. AFDM increased at all sites in the Whitewater River below the outflow from the trout farm effluent stream. The details of the longitudinal trends of resource abundance and the aquatic insect community are discussed by Pachón and Walton (2008). It was anticipated that black flies may have been negatively impacted by the trout farm because increased levels of periphyton on cobble surfaces inhibit the ability of black flies to colonize substrates (Mackay 1992, Zhang et al. 1998). However, simuliids were very abundant both upstream and downstream of the trout farm. Other workers have found simuliids to be more abundant downstream of trout farming facilities (Camargo 1992, Loch et al. 1996) and discharges of treated municipal effluent (Kramer and Mulla 1980). Wotton (1979) suggested that larvae of riverine black fly species often both graze on substrates and filter resources from the water column; whereas, black fly species associated with high food environments near outflows from lakes often are predominantly filter feeders that attain high densities by utilizing lacustrine-derived resources and recycling microbially enriched fecal material from conspecifics.

Enrichment effects of the aquaculture effluent were apparent in the Whitewater River, yet *S. tescorum* immatures were not found in the river channel. Based on this finding, enrichment may be of lesser influence on the distribution of *S. tescorum* immatures than are flow and possibly oviposition sites. *Simulium argus* and *S. tescorum* larvae have been associated with comparatively food-rich habitats, such as outflows from lentic habitats. *Simulium argus* larvae were abundant in the food-rich outlet of a montane lake (Mohsen and Mulla 1982). These same authors found the greatest densities of *S. tescorum* larvae below a spillway with flow of moderate velocity (0.6 m/s) draining a pond. Oviposition sites of *S. tescorum* are typically at the water line on emergent macrophytes trailing in the water current (Lacey and Mulla 1980). Tietze and Mulla (1989) suggested this species prefers slow moving waters with trailing cattails, bulrush, and smaller grasses. Black flies that utilize emergent vegetation as oviposition sites are especially susceptible to increased egg mortality from desiccation or loss of oviposition sites caused by variation of water levels, especially during spring (Rühm 1972, Golini and Davies 1987). In contrast to the Whitewater River where macrophytes and phreatophytes such as willows were rare, the trout farm effluent stream was surrounded by a thick growth of vegetation.

Simulium virgatum was the most abundant benthic organism present in the Whitewater River but was not found at site TF. Densities of *S. virgatum* larvae increased during the summer and were very high in the late summer and early autumn. Despite high larval numbers, adults were rarely seen and were not attracted to humans or companion animals (e.g., dogs) during the study. This distribution could be caused by *S. virgatum* females avoiding oviposition into the effluent or, if females oviposited in the effluent, then conditions such as flow or ammonia levels were not suitable for larvae. Because early instars were never detected in the TF samples, it is suspected that female *S. virgatum* avoided oviposition in the trout farm effluent stream. McCreadie et al. (1997) concluded that black fly assemblages in streams of similar size in northern Wyoming are the result of non-equilibrium processes; however, oviposition behavior is often more important than are processes influencing larval abundance such as larval survival, migration, and disturbance.

The increase of *S. virgatum* larval densities in the Whitewater River coincided with declining discharge from the melting snowpack in June and *S. virgatum* attained its greatest larval densities during the annual period of low discharge in late summer and autumn. Despite its prevalence during periods of comparatively low discharge, *S. virgatum* larvae have been found elsewhere in swifter currents than other co-occurring simuliid species (*S. argus*: Kramer and Mulla 1980; S. *aureum, S. tescorum, S. vittatum*: Tietze and Mulla 1989) which were found often on vegetation along creek edges. Mohsen and Mulla (1982) reported that *S. virgatum* eggs, larvae, and pupae were found in large numbers on rocks in the swiftest sections of Whitewater River where water velocity was 1.5-1.7 m/s.

Although *S. virgatum* larvae are found in swiftly flowing water, they apparently cannot maintain large larval aggregations during periods of very high flow. *Simulium virgatum* larvae were not found during the late winter when discharge in the Whitewater River is typically highest, and most variable, annually. This trend also might result from reduced adult activity during the coolest times of the year; however, the abundance of larvae was greatly reduced at site I-10 during the period that larval populations were largest at other sites. The I-10 site was subjected to high discharges caused by periodic input from the Colorado River aqueduct. Daily mean discharge rates were as high as 11.07 m^3/s during a two-week period of comparatively high flow after a gage station was established at Windy Point (33° 53' 56"N, 116° 37' 13" W; USGS Real-Time Water Data) downstream of the I-10 site in October 1998. Discharge across the historical record for this gauge station has been variable, ranging between 10 and 11 m^3/s during groundwater basin recharge events to undetectable discharge on other days. Besides the high but variable discharge and the comparatively high salt content of water during warm periods (as indicated by high specific conductance), nutrient and resource levels at the I-10 site were not significantly lower than at nearby sites on the Whitewater River. *Simulium vittatum* Zetterstedt larvae avoid scouring by moving to the hyporheic zone or onto vegetation along the periphery of the stream where flow rate is greatly reduced (Eymann and Friend 1986). The latter strategy is not possible for *S. virgatum* larvae in much of the study site, especially downstream of site I-10, because the wide, rocky floodplain is mostly devoid of streamside vegetation.

The trout effluent stream provided a habitat with a nearly constant flow and diminished disturbance regime, enriched larval black fly resources, and a comparatively thick growth of riparian vegetation that enhanced the abundance of the anthropophilic black fly, *S. tescorum*. Although enrichment effects on potential resources of larval black flies were evident across the entire distance covered by this study (7 km from the trout farm effluent discharge), natural disturbance factors and other environmental factors within the river floodplain were presumably strong enough to limit the distribution of *S. tescorum* to the trout farm effluent stream. Riparian vegetation was well-developed at a few other sites within the area of this study, but those sites were not sampled, so it is unknown whether *S. tescorum* larvae occurred within these isolated stands of streamside vegetation. In addition to human-related changes in the Whitewater Canyon ecosystem caused by the discharge of nutrient-enriched wastewater that favored the production of a nuisance black fly species, the addition of waters for recharge of groundwater storage basins in the lower portion of the watershed reduced the abundance of *S. virgatum*. McCreadie and Adler (2008) suggested that the species-specific causal factors responsible for rarity and commonness of black flies, such as dispersal, range limits, and stream conditions, might differ spatially as well as seasonally and that species distributions might be influenced strongly by source-sink population processes. It is uncertain whether *S. tescorum* would be present in the comparatively high elevations of the eastern Banning Pass in the absence of the homogenization of the flow regime, enhancement of oviposition and immature stage attachment sites, and enrichment of larval resources provided by the trout farm effluent.

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