Evaluating the potential of treated effluent as novel habitats

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PRIMARY RESEARCH PAPER

Evaluating the potential of treated effluent as novel habitats for aquatic invertebrates in arid regions

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Abstract Increasing anthropogenic demands for freshwater have altered many aquatic systems, including the drying of formerly perennial streams. The discharge of treated effluent has returned perennial flow in some of these streams, especially in arid and semi-arid regions, but the ability of treated effluent to support diverse aquatic communities is poorly understood. We examined the potential of treated effluent to create aquatic invertebrate habitat using the effluent-dependent Santa Cruz River in southern Arizona, USA as a case study. We identified 92 invertebrate taxa across our ten sampling sites and two sampling dates. Community composition was primarily shaped by water quality but also by stream drying (on daily time scales) and benthic substrate. Specifically, Linear Mixed-Effects models revealed a strong positive relationship between dissolved oxygen and taxonomic richness and a strong negative relationship between stream drying and invertebrate density. Although there are unique challenges to biota in effluent-dependent systems, our results suggest that treated wastewater could be managed to augment or recreate aquatic habitats that have been otherwise diminished or lost.

Keywords Wastewater · Stream ecology · Urban ecology · Stream drying · Water quality

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Introduction

As anthropogenic demand for freshwater continues to grow, alterations to natural lotic ecosystems will become more severe and environmental consequences more pronounced (Bischel et al., 2013; de Graaf et al., 2019). However, there are artificial sources of water that can support these important habitats. The discharge of treated effluent into stream systems is a common practice across the globe (Tchobanoglous et al., 2003) and has created or augmented base flow in many streams in arid and semi-arid regions (Brooks et al., 2006; Bischel et al., 2013; Luthy et al., 2015). However, the extent to which the physical and chemical properties of treated effluent can support aquatic communities is not well understood.

Previous research on water quality in effluent-fed streams has identified many challenges to ecological communities (Hamdhani et al., 2020). For example, nutrient loading from wastewater treatment plants can lead to toxic levels of ammonia (Monda et al., 1995; Aristi et al., 2015) and is a common cause of eutrophication (Martí et al., 2009; Bischel et al., 2013). In turn, eutrophication reduces dissolved oxygen and may affect aquatic communities more strongly than nonpoint sources of pollution (Carey & Migliaccio, 2009). Effluent discharge also can alter thermal regimes by increasing stream temperatures (Kinouchi et al., 2007; Plumlee et al., 2012), which could extirpate thermally sensitive taxa and result in decreased levels of dissolved oxygen.

In addition to these water quality issues, effluent inputs can change the natural flow regime (e.g., magnitude, frequency, and timing of flows) of a system (Bischel et al., 2013). Specifically, effluent discharge volume follows a cyclic pattern based on daily water use, which can result in artificial, diurnal pulses (Chesner & Pai, 1981; Butler & Graham, 1995; Enfinger & Stevens, 2006). However, it is not currently known how these impacts might translate to aquatic ecosystems. In streams that depend entirely on effluent for baseflow, diurnal discharge fluctuations can result in daily drying events in reaches downstream of wastewater treatment plants; such drying is likely to affect aquatic biodiversity in those reaches (Datry, 2012; Datry et al., 2014).

Without knowledge of the potential ecological benefits of discharging effluent into streams, water managers may choose one of many competing uses, resulting in reductions or elimination of "environmental" flows from effluent (Brooks et al., 2006; Bischel et al., 2013). Treated effluent is not only discharged into streams, but also has a wide variety of uses including agricultural irrigation and industrial cooling (Plumlee et al., 2012). As a result, there is a need to assess the biological and ecological value of effluent-dependent streams and to develop biological monitoring criteria for these unique systems (Boyle & Fraleigh, 2003).

In this case study, we examined aquatic invertebrate communities, and how they vary with water quality and flow conditions, in the effluent-dependent Santa Cruz River in southern Arizona, USA. Historically, portions of the Santa Cruz River had naturally perennial flow, but they became ephemeral in the 1930s due to diversions and extensive groundwater pumping (Logan, 2002). Discharge from wastewater treatment plants has provided a new source of perennial flow in two reaches of the river near the city of Tucson (Johnson et al., 2015). Our research aim was to identify the primary factors shaping aquatic invertebrate community structure in these effluent-dependent reaches to better understand the ability of effluent systems to support biota. We expected that richness, density, and composition of invertebrate communities would be driven by a combination of water quality and flow intermittence unique to effluent systems. Specifically, we hypothesized that taxonomic richness, invertebrate density, and relative abundances of sensitive taxa would be positively correlated with dissolved oxygen and negatively correlated with stream drying. We also hypothesized that the relative abundances of tolerant taxa would be positively correlated with stream drying, and negatively correlated with dissolved oxygen levels. As effluent-dependent streams become more common globally (Bischel et al., 2013), there is a need to understand their potential management as anthropogenic aquatic habitat.

Materials and Methods

Site description

The Santa Cruz River Basin comprises 22,000 km^2 of southern Arizona, USA and northern Sonora, Mexico (Webb et al., 2014). The climate of the basin is characterized by hot summers (mean July temperature ± SD: 30 ± 1.0°C) and moderately cool winters (mean January temperature ± SD: 11 ± 1.6°C). The annual mean precipitation for this region is low (~ 300 mm), and rainfall is bimodal: August and December are the wettest (mean ± SD: 54 ± 35.2 mm and 24 ± 26.3 mm, respectively), and May and June are the driest (mean ± SD: 4 ± 6.3 mm and 6 ± 9.6 mm, respectively) (data time range: 1948–2018, provided by NOAA NCDC Climate Data Online [http://ncdc.noaa.gov/]).

The present-day lower Santa Cruz River is now ephemeral, with groundwater levels approximately 80 m below the streambed (Carlson et al., 2011). However, perennial surface flow occurs at two reaches that are supported by effluent (Fig. 1). The shorter of the two perennial study reaches (~ 5 km long) is supported by the Agua Nueva Water Reclamation Facility (WRF), which was constructed in 1951, upgraded in 2013, and discharges ~ 30 million liters

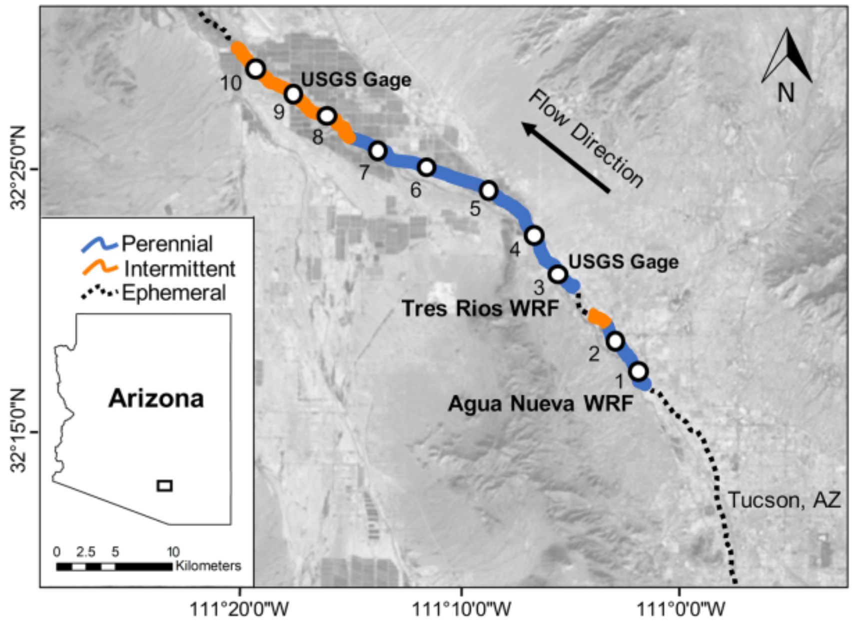

Fig. 1 Map of study area of the lower Santa Cruz River with the black dotted line indicating ephemeral reaches, the solid blue line indicating perennial effluent flow, and the solid orange indicating seasonally intermittent effluent flow. Sampling locations are denoted by white circles, and the locations of the water reclamation facilities (WRFs) and USGS gages are labeled

of effluent into the river each day (Sonoran Institute, 2017). The larger of the two reaches ($\sim$ 30 km long) is supported by the Tres Ríos WRF, which was built in 1978, upgraded in 2013, and discharges $\sim$ 115 million liters of effluent into the river each day (Sonoran Institute, 2017). Since 2014, both WRFs have discharged high-quality, tertiary-treated (as opposed to primary or secondary) effluent into the river (Dong et al., 2015; Johnson et al., 2015). Discharge from these WRFs exhibits strong diurnal fluctuations due to changes in water usage within the service area. As a result, flows increase and decrease twofold within a typical 24-h period (Fig. 2). In addition to flow from the WRFs, both study reaches experience seasonal floods, which can surpass 280 m^3/s, from precipitation runoff. Multiple large floods can occur during the summer monsoon season (July–September), but heavy winter rains (December–March) may also cause flooding (Fig. 2). Our two study reaches are separated by $\sim$ 1.5 km of ephemeral channel and are only connected during floods. Stream depth in these reaches typically varies from 0.1 to 1 m and width from 4 to 10 m; dominant substrates include sand, gravel, and cobble.

Data collection

We measured basic water quality and substrate parameters and collected aquatic invertebrates from ten sites along the two effluent-dependent reaches of the lower Santa Cruz River (Fig. 1). We sampled in two different months: December 2017 and April 2018. Sampling events occurred at least 44 days after high-flow events ($>$ 2.5 m^3/s) to reduce confounding influences from flood disturbances. Sampling sites were spaced an average of 3.89 km ($\pm$ 1.12 SD) apart and occurred where public land access allowed. While most sampling sites have perennial flow, our downstream-most sites in the Tres Ríos reach experience intermittent flow during dry or warm seasons (Fig. 1). We sampled all sites during the "low-flow" portion of each sampling date, as determined by the timing of the lowest effluent discharge volume from the WRFs.

We measured the following water quality parameters at each site: dissolved oxygen (DO) (mg/L) (Apera Instruments AI480 DO850 probe), pH, temperature (°C), total dissolved solids (TDS) (mg/L), conductivity (μS/cm) (Apera Instruments SX823-B multiprobe), salinity (ppt) (Apera Instruments

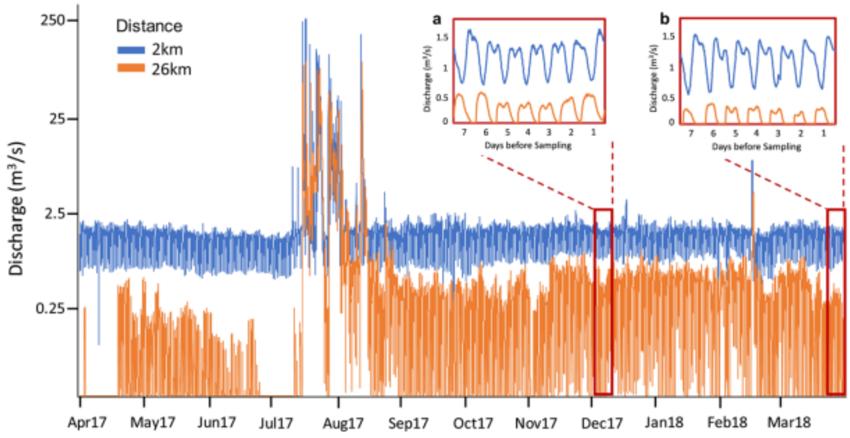

Fig. 2 Lower Santa Cruz River hydrographs from April 2017 to March 2018, with discharge measured in cubic meters per second (log transformed). The blue line is data from USGS gage #09486500 at Cortaro Road (Site 3), 2 km downstream from the effluent outfall, and the orange line is data from USGS gage #09486520 (Site 9), 27 km downstream from the effluent outfall. Summer monsoon precipitation events are responsible for peak flows observed in both reaches in July and August. Inset **a** is a detailed hydrograph of the week prior to our December sampling period, and inset **b** is the week prior to our April sampling period

Salt20 m), and ammonia (NH_3 mg/L), nitrate (NO_3 mg/L), and phosphate (PO_4 mg/L) measured in triplicate (YSI 9300 Photometer). At each site, we also sampled invertebrates along a 150 m reach following the reach-wide benthic sampling protocol (Ode et al., 2016). Briefly, this method creates a composite sample from 11 kicks (0.09 m^2 each, using 500 µm D-net) in various habitats (e.g., riffles, runs, pools) in proportion to their occurrence across the 150 m reach, for a total area sampled of 1.08 m^2 per site reach. We made visual estimates of substrate size and composition (e.g., silt, sand, gravel, pebble, cobble) in each area sampled. We also collected edge samples using 500 µm D-nets. Edge samples were a composite of five sweeps (covering $\sim$ 0.33 m^2 each) through submerged vegetation along the banks; the five sweeps were spaced roughly equally along the 150 m reach. Edge samples were qualitative in nature and were used to detect taxa that may have been missed in the reach-wide benthic samples. For richness analyses, we combined taxa lists from edge and benthic samples to determine total taxonomic richness for each reach. We preserved all samples in 95% ethanol and enumerated and identified individuals to the most practical taxonomic level, which was usually genus for insects and family or order for non-insects. Numerous keys were used for these identifications including Hungerford, (1948), Cook, (1974), Merritt et al., (2008), Westfall & May, (1996), Larson et al., (2000), Needham et al., (2000), Thorp & Covich, (2009), and Andersen et al., (2013), among others.

Finally, we used a combination of data from two US Geological Survey (USGS) stream gages (09486500 and 09486520, 15 min resolution) combined with time-lapse photography of staff gages (using trail cameras, 30 min resolution) to quantify drying regimes. Staff gages and trail cameras were placed at all sites along the Tres Ríos reach that did not have USGS gages. We ground-truthed gages to ensure zero discharge was accurate. Preliminary observations showed that the sites furthest from the Tres Ríos WRF experienced repeated drying, which might influence invertebrate communities. Although the downstream-most portion of the Agua Nueva reach experienced flow intermittence, our two sites on this reach were perennial.

Data analysis

We quantified the total richness and density of aquatic invertebrates at each reach, as well as relative abundances of individual taxa and of two broad categories of taxa: (1) Gastropods, Oligochaeta, and Diptera (GOLD), which include many tolerant species, and (2) Ephemeroptera, Plecoptera, and Trichoptera (EPT), which are generally sensitive

(Mandaville, 2002; Buffagni, et al., 2006; Chang et al., 2014). For each sampling date, we used linear regression to examine longitudinal changes with increasing distance from WRFs in these taxa, as well as in water quality parameters, including dissolved oxygen, temperature, pH, TDS, conductivity, and concentrations of ammonia, nitrate, and phosphate. Significance of linear regression was determined by *alpha* = 0.05. To test our hypotheses, we used Linear Mixed-Effects (LME) models to determine if six invertebrate metrics (total taxonomic richness, density, and relative abundances of GOLD, Ephemeroptera, Trichoptera, and combined EPT) could be predicted by two abiotic variables: dissolved oxygen and number of hours that the reach was dry in the week before sampling. To account for potential confounding influence of benthic substrate composition (Williams & Mundie, 1978; Quinn & Hickey, 1990), we also included proportion of fine sediment (silt and sand combined) as a third predictor in our models. Although we measured additional abiotic variables, overfitting concerns with our small sample size (n = 19) prevented us from using all variables as potential predictors in the models-we chose these three abiotic variables based on their known importance in shaping aquatic invertebrate communities.

LME models were run in the statistical program R (version 3.5.1: R Core Team, 2019) with the package 'nlme' (Linear and Nonlinear Mixed Effects Models, version 3.1: Pinheiro et al., 2019) (Pinheiro & Bates, 2000). Relative abundances of GOLD, Ephemeroptera, Trichoptera, and combined EPT were logit transformed as was fine sediment composition to improve normality. Each of the six invertebrate metrics was then modeled using dissolved oxygen concentration (DO), hours dry in the week before sampling (Drying), and logit transformed % fine sediment (Fine Sediment) as predictors with sampling site (Site) as a random factor: [invertebrate metric] ~ DO + Drying + Fine Sediment, random = Site. Using the 'dredge' function within R package 'MuMIn' (Multi-Model Inference, version 1.43: Bartoń, 2019), we used Akaike information criterion corrected for small sample size (AICc) to identify and rank the best model(s) to explain each invertebrate metric (Anderson & Burnham, 2002; Burnham & Anderson, 2004). An estimation of variance explained by each model was reported using pseudo, conditional R^2 values, hereafter referred to as pseudo R^2 (Nakagawa & Schielzeth, 2013; Nakagawa et al., 2017). Pseudo R^2 values were obtained by using the 'rsquared' function within R package 'piecewiseSEM' (Piecewise Structural Equation Modeling, version 2.1: Lefcheck & Freckleton, 2016).

Variation in community composition across the ten sites and two sampling dates was visualized with non-metric multidimensional scaling (NMDS) in PC-ORD version 5.0 (McCune & Mefford, 1999), with Bray-Curtis distance as the measure of community dissimilarity. Prior to NMDS analyses, we square-root transformed taxon abundances to reduce the influence of highly abundant taxa and removed taxa that only occurred in a single sample unit (McCune & Grace, 2002). Relationships between measured environmental variables and NMDS axes were assessed using Pearson's correlation coefficients. We also assessed Pearson's correlation coefficients between taxon abundances and NMDS axis values to describe the gradients in community composition visualized by the ordination. Finally, we tested for differences in community composition between the two sampling dates using multi-response permutation procedure (MRPP: Mielke & Berry, 2001).

Results

Environmental variables

In both the Agua Nueva and Tres Ríos reaches, water temperature was warm on both sampling dates (18–28°C), with temperatures at sites further from the WRFs usually higher in April and lower in December (Table 1). Dissolved oxygen was more variable in April (4.3–10.8 mg/L) than December (6.7–9.0 mg/L) but was often lowest near the effluent outfalls. The pH (7.6–8.8) was slightly basic and was consistently lowest near the WRFs regardless of sampling date. No clear longitudinal trends were apparent with TDS, conductivity, nitrate, or phosphate (Table 1), and salinity varied little among sites (range: 5–6 ppt). The only significant longitudinal model for water quality was for ammonia, which decreased with distance from effluent outfall in the Tres Ríos reach in December (R^2 = 0.59, P = 0.026). Finally, benthic substrate varied among sampling sites. Fine substrate composition (silt and sand) ranged from 30 to 50% in the Agua Nueva reach and from 10 to 70% in the Tres

Table 1 Water quality measurements from ten sites along the lower Santa Cruz River with December in shaded columns and April in unshaded columns

Site	WRF	Distance (km)	DO (mg/L)		Temp (°C)		pH		TDS (mg/L)		Conductivity (µS/cm)		Ammonia (mg/L)		Nitrate (mg/L)		Phosphate (mg/L)	
1	Agua	0.3	6.8	5.7	26	27	7.6	7.6	785	836	1105	1178	3.17	0.76	0.95	2.72	1.18	1.98
2	Agua	4	7.1	4.3	18	28	7.9	7.7	828	848	1167	1194	5.01	1.23	4.95	6.13	0.61	2.87
3	Tres	2	6.7	5.4	25	25	7.9	7.6	844	796	1188	1121	0.24	0.20	1.48	1.99	3.63	5.67
4	Tres	5	8.2	7.7	23	25	8.2	8.0	840	799	1183	1125	0.25	0.04	1.82	2.02	3.67	5.20
5	Tres	10	7.7	8.7	22	24	8.1	8.2	842	800	1185	1124	0.11	0.09	1.35	1.73	3.70	5.47
6	Tres	16	8.9	10.8	22	24	8.4	8.5	845	800	1189	1127	0.13	0.04	1.81	1.16	3.70	5.67
7	Tres	19	8.9	8.7	22	28	8.6	8.6	843	806	1187	1133	0.07	0.09	1.29	3.79	3.43	5.60
8	Tres	24	6.9	9.5	22	27	8.8	8.7	839	803	1182	1132	0.07	0.01	2.02	1.89	3.53	5.13
9	Tres	27	8.5	7.2	22	30	8.6	8.4	829	806	1182	1135	0.10	0.06	1.34	2.54	3.50	5.07
10	Tres	30	9.0	NA	21	NA	8.4	NA	836	NA	1168	NA	0.12	NA	1.46	NA	3.57	NA

These sites include effluent from two separate WRFs at varying distances downstream from the effluent outfall (Distance) and include dissolved oxygen (DO), temperature (Temp), pH, TDS, conductivity, ammonia concentration (Ammonia), nitrate concentration (Nitrate), and phosphate concentration (Phosphate). No values are available for Site 10 in April as it was dry during the sampling effort

Fig. 3 Paired photos illustrating the fluctuations in discharge in the lower Santa Cruz River within a 24-h period. **a**, **b** The daily high-flow and low-flow periods, respectively, of a perennial reach near the effluent outfall of the Tres Ríos WRF (Site 4). **c**, **d** The high- and low-flow periods, respectively, in an intermittent reach 24 km downstream of the effluent outfall (Site 8)

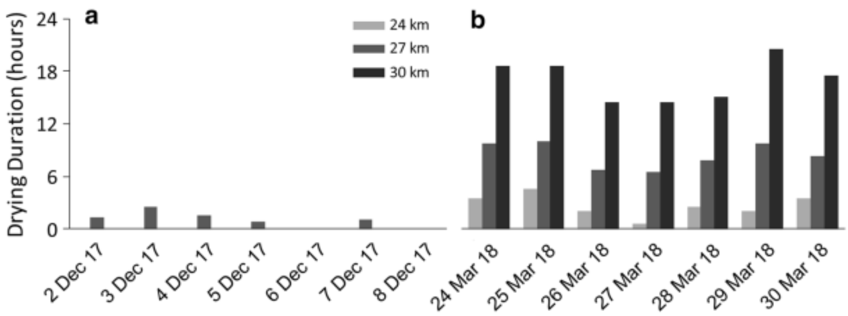

Fig. 4 Drying duration measured in hours per day at our three most downstream sites (8–10) with locations denoted by color, as measured in the week prior to **a** December sampling and **b** April sampling

Ríos reach. Sites 2, 3, and 9 had $\geq$ 50% fine substrate. The greatest proportion of cobble substrate occurred at sites 5 and 6 and ranged from 20 to 40%.

USGS gage records showed that baseflow in the Tres Ríos reach fluctuated daily from 0.7 to 1.6 m^3/s near the outfall and from 0 to 0.6 m^3/s 27 km downstream (Figs. 2, 3). As a result of daily fluctuations in discharge from the Tres Ríos WRF, the three farthest downstream sites were seasonally intermittent. Our time-lapse photography revealed that these three sites experienced drying events in the week prior to both December and April sampling. Drying (hours/day) was more severe in April than December, and was highest at the downstream-most site, 30 km from the Tres Ríos WRF (Fig. 4). Drying across these three intermittent sites ranged from a mean of 1.0 ($\pm$ 0.9 SD) hour/day in December to a mean of 9.3 ($\pm$ 6.3 SD) hours/day in April, with the downstream-most site drying for 17.0 h/day. Extended dry periods at the downstream-most site prevented us from measuring water quality or sampling aquatic invertebrates at that site in April.

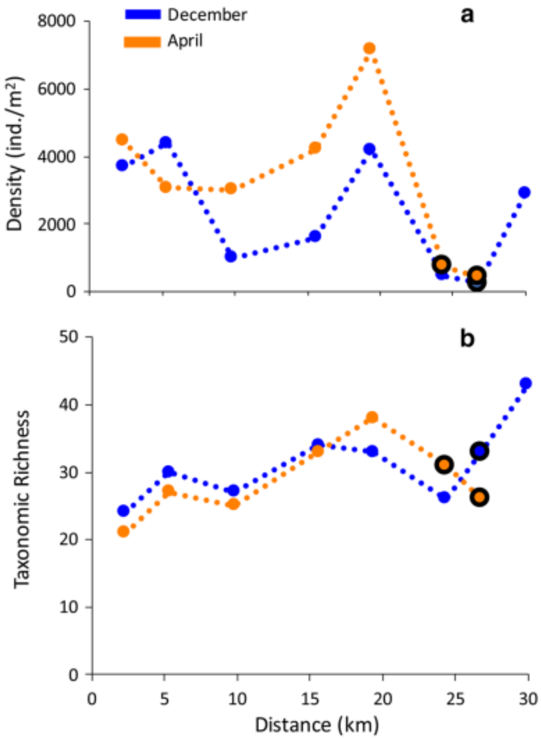

Fig. 5 **a** Invertebrate density (individuals/m^2) and **b** total taxonomic richness at sampling sites with increasing distance downstream from the Tres Ríos WRF outfall. December samples are denoted by the color blue and April samples with orange. The black outlines on some data points identify sites that experienced drying within the week prior to sampling

Invertebrate density and richness

In the longer Tres Ríos reach (sites 3–10), the lowest invertebrate densities for both sampling dates occurred at sites that experienced drying prior to sampling (Fig. 5a). No clear longitudinal trends were seen with densities in the Tres Ríos reach. At the two sites in the Agua Nueva WRF reach, densities were higher near the outfall. The LME model indicated that density was negatively related to DO, drying, and the proportion of fine sediment across our sites (pseudo R^2 = 0.68, weight = 1.000) (Table 2).

In both reaches, taxonomic richness was lowest at the sites closest to the WRF outfall, regardless of season (Fig. 5b). The highest richness observed overall (43 taxa) was at our farthest downstream site (30 km from Tres Ríos WRF) in December. The highest richness in April (38 taxa) was observed at 19 km, our farthest downstream site without flow intermittence (Fig. 5b). In the Agua Nueva reach, site richness values (25–34 taxa) were within the range of those found in the longer Tres Ríos reach. AICc selection revealed two models explaining taxonomic richness with AICc $\Delta < 2$. In the highest performing model (pseudo R^2 = 0.78, weight = 0.650), dissolved oxygen was positively related to richness, and in the second model (pseudo R^2 = 0.80, weight = 0.350) a positive relation to dissolved oxygen was selected again as well as a negative relationship with fine sediment composition (Table 2).

Invertebrate community composition

Eighty-seven invertebrate taxa were identified from the longer Tres Ríos reach, and 50 taxa were found in the shorter Agua Nueva reach (see Appendix 1). The most diverse orders were Diptera (41 taxa), Coleoptera (16 taxa), and Odonata (13 taxa). Chironomidae was the most diverse family with a total of 23 taxa. EPT taxa included the mayflies *Callibaetis*, *Camelobaetidius*, and *Fallceon*, and the caddisflies *Helicopsyche*, *Hydroptila*, *Nectopsyche*, *Protoptila*, and *Smicridea*; no stoneflies were found. All eight EPT taxa were observed in the Tres Ríos reach, but only *Callibaetis*, *Fallceon,* and *Hydroptila* were found in the shorter Agua Nueva reach. Across all sites and dates, the dominant taxa by relative abundances were *Polypedilum,* Chironomidae (29%), *Fallceon,* Baetidae (18%), *Rheotanytarsus*, Chironomidae (12%), and *Simulium*, Simuliidae (8%).

The combined relative abundances of EPT taxa varied from 2 to 67% across all samples, and values were generally lower in the Agua Nueva reach than in the longer Tres Ríos reach (Fig. 6c). With rankings by AICc $\Delta < 2$, our EPT relative abundance model was positively correlated with dissolved oxygen (pseudo R^2 = 0.22, weight = 0.365); however, the intercept performed best (weight = 0.635) suggesting that our measured environmental parameters included in the model were not strongly influential (Table 2). The relative abundance of Trichoptera varied between sampling dates, with an average of 15% (± 22% SD) in December and 4% (± 5% SD) in April. This relative abundance displayed a unimodal trend in December peaking at ~ 10 km downstream from the outfall (Fig. 6b). In our Trichoptera model rankings, relative abundance was negatively correlated with fine

Table 2 Summary of Akaike's Information Criterion, corrected for small sample size (AICc), for Linear Mixed-Effects models explaining variation in total taxonomic richness (Richness), invertebrate density (Density), Logit transformed Ephemeroptera relative abundance (Ephemeroptera), Logit transformed Trichoptera relative abundance (Trichoptera), Logit transformed EPT relative abundance (EPT), and Logit transformed GOLD relative abundance (GOLD) with explanatory variables dissolved oxygen (DO), total hours dry in the week before sampling (Drying), and Logit transformed fine sediment composition (Fine Sediment) with sampling site as a random factor in the model

Model	R^2	Rank	DO	Drying	Fine Sediment	df	logLik	AICc	ΔAICc	Weight
Richness	0.78	1	+			4	-51.105	113.067	0.000	0.650
	0.80	2	+		–	5	-49.847	114.309	1.242	0.350
Density	0.68	1	–	–	–	6	-143.214	305.428	0.000	1.000
Ephemeroptera		1				3	-34.215	76.030	0.000	1.000
Trichoptera		1				3	-38.989	85.577	0.000	0.505
	0.45	2			–	4	-37.380	85.617	0.040	0.495
EPT		1				3	-32.304	72.208	0.000	0.635
	0.22	2	+			4	-31.229	73.315	1.106	0.365
GOLD		1				3	-32.190	71.981	0.000	0.583
	0.21	2	–			4	-30.899	72.654	0.673	0.417

Only models with ΔAICc < 2 are summarized. Inclusion of a predictor variable and its direction are noted by ± . In some instances, the intercept performed best (no predictors were selected). As estimators of variance explained, pseudo, conditional R^2 values (R^2) are reported for models that included predictors. Weight is scaled from 0 to 1 and estimates the probability that a model is best among all others considered

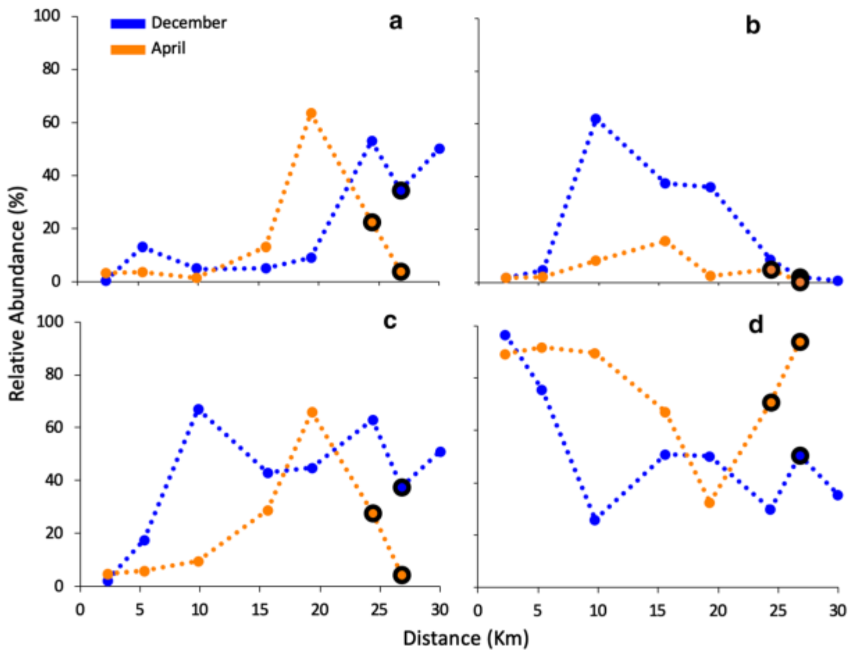

Fig. 6 Relative abundance (%) of **a** Ephemeroptera, **b** Trichoptera, **c** combined EPT taxa, and **d** combined GOLD taxa at sampling sites with distance downstream from Tres Ríos WRF outfall on the x axis. December denoted by the color blue and April with orange. The black outline indicates sites that experienced drying within the week prior to sampling

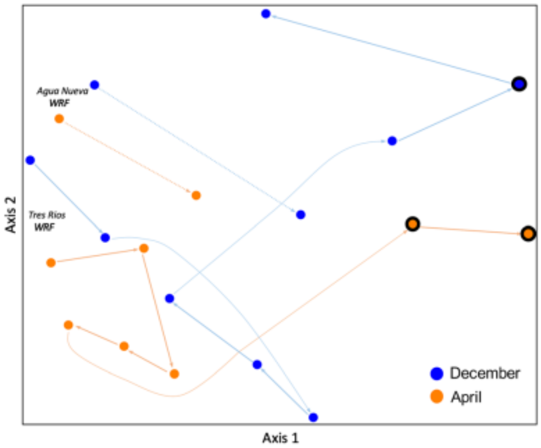

Fig. 7 NMDS plot of all invertebrate samples with blue identifying December and orange April and with dotted lines denoting the Agua Nueva reach and the Tres Ríos reach with solid lines. Locations of the outfalls are indicated with the corresponding WRF label, and arrows indicate the direction of flow along the longitudinal gradient. Black outlines on the three sample points on the right side of the plot indicate flow intermittence at those sites in the week prior to sampling

Table 3 Pearson's correlation coefficients between taxon abundances and NMDS ordination axis 1

Taxon	Axis 1 r
Polypedilum	– 0.83
Rhagovelia	– 0.68
Rheotanytarsus	– 0.66
Thienemanniella cf. *xena*	– 0.55
Oligochaeta	– 0.52
Tropisternus lateralis	0.50

Only taxa with $|r| \geq 0.5$ are reported

sediment (pseudo R^2 = 0.45, weight = 0.495), but the intercept performed best (weight = 0.505) indicating our model predictors were not strongly influential (Table 2). Relative abundances of Ephemeroptera generally increased with distance from the WRFs in December; however, they exhibited a unimodal trend with a peak of 63% at 19 km downstream of the Tres Ríos WRF in April (the last site before flow intermittence began) (Fig. 6a). Our Ephemeroptera model performed poorly: no predictors were selected (Table 2).

GOLD relative abundances were highest close to the outfalls and at sites that dried in the week prior to sampling. Two km downstream of the Tres Ríos WRF, GOLD relative abundances were 97% in December and 89% in April (Fig. 6d) and tended to decrease with increasing distance downstream, except in April when values sharply increased beginning at 24 km downstream of the WRF (at sites that experienced an average of 8.39 h dry per day in the week prior to sampling). The highly tolerant genus *Chironomus* (Chironomidae) was only present within 5 km of the WRF outfalls. Its relative abundances ranged from 6–20% in December and 0–11% in April. For the GOLD relative abundance model, dissolved oxygen was negatively correlated (pseudo R^2 = 0.21, weight = 0.417), but the intercept performed best (weight = 0.583) once again suggesting that our predictors were not strongly influential (Table 2).

Table 4 Pearson's correlation coefficients between taxon abundances and NMDS ordination axis 2

Taxon	Axis 2 r
Smicridea	– 0.77
Petrophila	– 0.70
Ischnura	– 0.58
Hemerodromia	– 0.56
Cladotanytarsus	– 0.55
Camelobaetidius	– 0.53
Ferrissia	– 0.51
Dolichopodidae	0.50
Ceratopogonidae	0.50
Forcipomyia	0.55
Trichocorixa calva	0.56
Physidae	0.57
Culicoides	0.57
Ostracoda	0.60

Only taxa with $|r| \geq 0.5$ are reported

NMDS ordination revealed overall community differences among all samples and sampling dates (Fig. 7; stress = 0.15, final instability = 0.00001, P = 0.004, R^2 = 0.80). Axis 1 described a gradient from samples with abundant Chironomidae (e.g., *Polypedilum*, *Rheotanytarsus*), oligochaetes, and broad-shouldered water striders (*Rhagovelia*) to samples with abundant water scavenger beetles (*Tropisternus*) (Table 3). Axis 2 described a gradient from samples with abundant caddisflies (*Smicridea*), moths (*Petrophila*), mayflies (*Camelobaetidius*), damselflies

(*Ischnura*), and true flies (e.g., *Hemerodromia*, *Cladotanytarsus*) to samples dominated by true flies (Dolichopodidae, Ceratopogonidae, *Forcipomyia*, *Culicoides*), snails (Physidae), water boatmen (*Trichocorixa*), and ostracods (Table 4). Axis 1 was positively correlated with the distance downstream from the outfalls ($r = 0.71$), while axis 2 was negatively correlated with the percent of cobble substrate and positively correlated with the percent of fine substrate ($r = -0.59$ and 0.58, respectively). Immediately below both WRFs, community composition shifted positively along axis 1 and negatively along axis 2, exhibiting lower abundances of some Chironomidae taxa and higher abundances of more sensitive taxa, such as Ephemeroptera and Trichoptera. However, this trend reversed itself ~ 8 km downstream of the Tres Ríos WRF, before shifting back far to the positive along axis 1 and 2, at sites > 16 km downstream of the WRF. Sites furthest from the Tres Ríos WRF experienced drying in the week preceding sampling (Fig. 7), and community samples from these reaches were characterized by higher abundances of lentic taxa (e.g., snails (Physidae), water boatmen (*Trichocorixa*): Table 4). MRPP results indicated that community composition did not vary significantly between the two sampling dates ($A = 0.03$, $P = 0.06$).

Discussion

Our case study builds on a growing but still relatively small body of literature (e.g., Boyle & Fraleigh, 2003; Halaburka et al., 2013; Arnon et al., 2015; Hamdhani et al. 2020) examining the ecological impacts of discharging treated effluent into streams. We documented a relatively high diversity of invertebrates in the lower Santa Cruz River, considering that 100% of its baseflow comes from effluent. Our results indicate that invertebrate communities in this river are primarily shaped by water quality (i.e., dissolved oxygen) but also by drying and sediment composition. Although there are unique challenges to biota in effluent-dependent systems, our results suggest that treated wastewater could be managed to augment or recreate aquatic habitats that have been otherwise diminished or lost.

Invertebrate taxa

We identified 92 invertebrate taxa from our sampling efforts, which was higher than anticipated given findings from previous studies in other effluent-dominated streams. When adjusted for taxonomic resolution, average taxonomic richness in the lower Santa Cruz River was 1.5–6 times higher than those observed from effluent-fed streams in California and Europe (Canobbio et al., 2009; Halaburka et al., 2013; Arce et al., 2014; Burdon et al., 2016). However, Ortiz & Puig (2007) identified a taxa richness range in a Spanish effluent-fed stream that was similar to what we observed in the lower Santa Cruz. Furthermore, Boyle & Fraleigh (2003) reported richness values in the effluent-dependent upper Santa Cruz River (70 km south of our study sites) similar to those we observed in the lower Santa Cruz. There are no naturally perennial reaches of the Santa Cruz that can serve as reference sites for these various effluent-dependent reaches, but comparisons with two perennial rivers (San Pedro River and Babocomari River) 100 km southeast of our study river may be informative. Bogan et al. (2013) found similar levels of reach-scale richness (35–45 taxa) when they sampled a similar benthic area (~ 1 m^2) in reaches of these rivers. These findings suggest that at least some reaches of the effluent-dependent Santa Cruz River approach the richness levels of natural rivers in the region.

Chironomidae were numerically dominant in the Santa Cruz River, and oligochaetes were ubiquitous, as has been observed in other arid effluent-fed streams (Boyle & Fraleigh, 2003; Ortiz & Puig, 2007; Canobbio et al., 2009). Effluent-fed streams may favor invertebrates with short lifespans and high reproductive rates, as is typical of species of Chironomidae and Oligochaeta (Ortiz & Puig, 2007). However, oligochaetes represented only 6% of the total abundance we observed in the Santa Cruz River. In contrast, oligochaetes were the most abundant taxon (55% relative abundance) in an Israeli effluent-dominated stream (Arnon et al., 2015) and are often among the most dominant taxa in effluent-fed systems globally (Hamdhani et al., 2020). Oligochaetes may be less dominant in the Santa Cruz River due to a combination of competition from abundant Chironomidae (Brinkhurst & Kennedy, 1965; Martin et al., 2007) and the relative lack of fine benthic sediment in some of our study sites (Marshall & Winterbourn, 1979; Lin & Yo, 2008).

We found eight EPT taxa in the lower Santa Cruz River, with at least some Ephemeroptera or Trichoptera occurring in all of our study reaches. Some previous studies reported zero EPT taxa in effluent-fed reaches (e.g., in a small, high-quality effluent stream in California: Halaburka et al., 2013). In an effluent-fed stream in Israel, Ephemeroptera only appeared after significant improvements to water quality were made (Plecoptera and Trichoptera were not present in the region: Arnon et al., 2015). In the Santa Cruz River, EPT were dominated by genera in the families Baetidae and Hydropsychidae, similar to observations from an effluent-fed stream in Italy (Canobbio et al., 2009). No Plecoptera were found in the Santa Cruz River, despite *Mesocapnia arizonensis* (Baumann & Gaufin) (Capniidae) being abundant in tributaries to the lower Santa Cruz and tolerant of stream drying (Bogan, 2017). The elevated temperatures of effluent may be inhospitable to Plecoptera (Boyle & Fraleigh, 2003; Plumlee et al., 2012).

Factors shaping community structure

Given taxonomic differences in pollution tolerance (Bonada et al., 2006), water quality dynamics play a large role in shaping invertebrate communities in effluent-dominated streams. Nutrient loading from treated wastewater (Carey & Migliaccio, 2009; Plumlee et al., 2012) can result in lower dissolved oxygen levels close to effluent outfalls, as we observed in the Santa Cruz River. However, nutrient cycling, photosynthesis, and greater air diffusion from turbulence work to increase dissolved oxygen levels downstream of outfalls (Boyle & Fraleigh, 2003). These gradients in dissolved oxygen can result in strong community gradients in effluent-fed systems. Although we did not observe a longitudinal dissolved oxygen gradient, we only found the low oxygen-tolerant midge *Chironomus* close to outfalls, where oxygen levels were lowest, as has been reported in similar studies (Boyle & Fraleigh, 2003; Arnon et al., 2015). Farther downstream from outfalls, taxa with higher oxygen needs (e.g., EPT) became more abundant. In fact, dissolved oxygen was a positive predictor of taxonomic richness in the Santa Cruz River, and it exhibited the strongest relationship of all our models (Table 2). Effluent discharge is known to be a source of oxygen stress and can shape invertebrate communities based on oxygen tolerance (Bunzel et al., 2013). By limiting a diversity of taxa and subsequently reducing competition, low dissolved oxygen levels could increase overall invertebrate density. For example, dissolved oxygen was a negative predictor of invertebrate density in our models (Table 2) and was likely influenced by high abundances of tolerant Chironomidae.

Although water quality improved downstream from effluent outfalls in the lower Santa Cruz, reaches furthest from outfalls experienced flow intermittence. Stream drying is known to strongly shape aquatic invertebrate community structure both regionally and globally (Bogan et al., 2013; Datry et al., 2014). In our study, communities at sites that experienced drying were characterized by higher abundances of water scavenger beetles (*Tropisternus*), which have some resistance to desiccation (Velasco & Millán, 1998) and are strong aerial dispersers (Bogan & Boersma, 2012). The relative abundances of GOLD taxa also increased in intermittent sites compared to perennial sites just upstream (Fig. 6d), most likely due to the ability of many dipterans (e.g., Chironomidae) to recolonize quickly each day via drift (Davies, 1976; Bruno et al., 2013). Relative abundances of EPT declined in drying reaches (Fig. 6b), which is unsurprising as many EPT taxa are not resistant to desiccation (del Rosario & Resh, 2000; Datry, 2012).

No previous studies have examined the effect of complete stream drying and rewetting on daily time scales, so we can only look to broader literature on stream drying to make comparisons with our data. In general, increased duration of stream drying results in decreases of both invertebrate density and taxonomic richness (Fritz & Dodds, 2004; Datry, 2012; Datry et al., 2014). Also, ephemeral wetlands have been shown to have lower taxonomic richness and higher proportions of taxa with short life cycles and high dispersal abilities than adjacent, permanent wetlands (Boda et al., 2018). In the Santa Cruz River, LME models revealed a negative influence of drying on invertebrate density, which is not surprising considering the limits of recolonization on sub-daily time scales. However, stream drying did not appear to affect the other five aquatic invertebrate metrics we tested. This unexpected result may be an artifact of our small sample size (n = 19) and methods, as our sites did not have a balanced drying gradient but rather were highly skewed towards perennial flow. Future studies in effluent-dependent streams should examine invertebrate community changes across a broad range of daily drying intensities.

Finally, benthic substrate has long been known to influence invertebrate community composition (Williams & Mundie, 1978; Quinn & Hickey, 1990). Our models revealed a negative relationship of both taxonomic richness and overall invertebrate density with fine sediment composition, as well as a negative relationship with Trichoptera relative abundance (Table 2). Additionally, NMDS ordination revealed that taxa such as caddisflies (*Smicridea*), mayflies (*Camelobaetidius*), and aquatic moths (*Petrophila*) were positively associated with larger cobble substrate, while taxa such as ostracods, biting midges (Ceratopogonidae, *Forcipomyia*), and water boatmen (*Trichocorixa*) were associated with finer sediment (Table 4). Our observations are in accordance with other studies that have noted how fine sediments may limit taxonomic and functional diversity in effluent-fed streams (Arnon et al., 2015; Mor et al., 2019).

Management implications and research needs

Effluent-fed streams are modified habitats that present challenges for environmental managers who want to support diverse ecosystems. First, the type of effluent and level of dilution in the receiving stream is an essential factor to consider, as the biotic impacts of effluent can change with composition, treatment standards, and dilution rates (Martí et al., 2009; Grantham et al., 2012; Mezzanotte et al., 2013; Burdon et al., 2016). Our study represents a scenario in which there is zero dilution of high-quality effluent for the majority of the year. Our limited results, and those of Boyle & Fraleigh (2003), suggest that the length of effluent-dependent streams in arid climates could be an important consideration for sensitive invertebrate taxa. Small-volume (i.e., shorter) effluent-dependent streams may be fundamentally limited in their ability to support sensitive taxa due to limited natural remediation of excess nutrients and low dissolved oxygen levels. Second, when effluent comprises 100% of baseflow in streams, diurnal patterns in effluent discharge result in daily stream drying in downstream-most reaches, making these portions less hospitable to many aquatic taxa. Engineering mechanisms are available to depress the magnitude of these diurnal fluctuations (e.g., constructed wetlands: Brown et al., 2011), but the cost may be prohibitive for many utilities. Although not a focus of this study, effluent can be a point source of contaminants of emerging concern, including pesticides (Bunzel et al., 2013), pharmaceutical byproducts (Dong et al., 2015; Grabicova et al., 2015), and microplastics (Ziajahromi et al., 2016; Foley et al., 2018), all of which can be detrimental to aquatic biota. These issues should be weighed when considering potential management options. Finally, biomonitoring programs need to be developed for these unique streams. Metrics developed to monitor natural streams may not work effectively in effluent-fed systems as water quality is often dramatically different and appropriate reference sites may be unavailable (Boyle & Fraleigh 2003; Brooks et al., 2006).

With population growth and climate change, natural streams face great uncertainty (Dudgeon et al., 2006; de Graaf et al., 2019). However, effluent supplies will increase with continued urban growth (Martí et al., 2009), and this additional treated wastewater could be managed to augment or recreate aquatic habitat where natural flow has diminished or been lost (Brooks et al., 2006; Martí et al., 2009; Bischel et al., 2013). Despite potential shortcomings (e.g., water quality and flow dynamics), effluent should receive more research attention as a potential tool of aquatic conservation in our era of global change.

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