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Seasonal and Longitudinal Water Quality Dynamics in Three Effluent-Dependent Rivers in Arizona

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Abstract

The seasonal and longitudinal water quality dynamics across different geographic and climatic factors were investigated across six reaches of three effluent-dependent rivers in Arizona. We observed water quality deterioration (e.g. elevated temperature and low dissolved oxygen) in some reaches during the hottest summer months and significantly greater natural remediation of water quality in longer reaches for several factors (temperature, dissolved oxygen and ammonia). Nearly all sites met or exceeded water quality conditions needed to support robust assemblages of native species (except Tres Rios reach during elevated ammonia in summer and fall measurement). However, this study also indicates that conditions based on temperature, oxygen level and ammonia may be stressful for the most sensitive taxa at sites closest to effluent outfalls, especially in summer. Overall, effluent-dependent streams have the capacity to serve as refuges for native biota, and they may become the only aquatic habitat available in many urbanizing arid and semi-arid regions.

Keywords: wastewater; in-stream natural purification; wastewater treatment plant; aquatic organism; urban arid region.

1. Introduction

In recent years, various studies from across the globe have reported overuse of surface and groundwater resources (Gleeson et al., 2012; Voss et al., 2013; Schewe et al., 2014; Wada and Bierkens, 2014). As a consequence, many rivers and streams have experienced flow regime alterations, including the loss of perennial flow and prolonged dry periods (Logan, 2006; Goodrich et al., 2018). New wastewater treatment plant construction is being located in urban and suburban areas where stream flow has become increasingly ephemeral (Tchobanoglous et al., 2003). These effluent inputs are especially noticeable in streams that have dried up due to water extraction, and now depend on effluent to sustain baseflow during dry seasons (Luthy et al., 2015). Such streams are known as being *effluent-dependent* (100% effluent during baseflow: Du et al., 2015) and are increasingly common across the globe (Hamdhani et al., 2020).

Effluent-dependent streams are considered as an altered system, thus the water quality could be dissimilar from that in natural streams (Brooks et al., 2006; Hamdhani et al., 2020). Differences noted in effluent-dependent or effluent-dominated streams include elevated temperatures (Boyle and Fraleigh, 2003; Canobbio et al., 2009) and nutrient levels, such as nitrate (Hur et al., 2007; Chen et al., 2009), ammonium/ammonia (Gafny et al., 2000; Boyle and Fraleigh, 2003), and phosphate (Birge et al., 1989; Chen et al., 2009). Furthermore, low dissolved oxygen levels are frequently observed in stream reaches closest to effluent outfalls (Birge et al., 1989; Matamoros and Rodríguez, 2017).

However, as effluent flows through a stream channel, natural remediation processes (e.g. aeration, nutrient uptake) can occur and lead to improved water quality conditions (Boyle and Fraleigh, 2003). Previous studies demonstrated that longitudinal distance from the effluent source is an important factor in these processes (Growns et al., 2009; Hughes et al., 2014), which suggests that the degree of remediation might differ depending on the length of effluent-dependent reaches. In natural stream systems, other factors such as seasonality and climate also play important roles in influencing water quality (e.g. Pionke et al., 1999; Morrill et al., 2005), so such factors likely are important in effluent-dependent streams as well. However, few studies have examined how water quality changes with varying longitudinal distances from effluent inputs and how those changes may differ by season or climate zone.

This study examined the dynamics of basic water quality parameters (e.g. temperature, dissolved oxygen, pH, nutrients) in six effluent-dependent reaches of differing lengths in three river systems across a climate gradient in Arizona (USA). Our goals were to investigate: (1) how water quality changes with distance from effluent outfalls, (2) how water quality and longitudinal patterns change with season and climate, and (3) whether water quality conditions in these systems are sufficient to support native aquatic species. We hypothesized that: (1) water quality would improve with increasing distance downstream from effluent outfalls due to natural remediation processes, (2) water quality conditions would deteriorate in summer, especially in reaches with hotter climates, and (3) water quality conditions would provide suitable habitat for native fishes and other aquatic organisms adapted to life in natural streams of the region.

2. Methodology

2.1. Site description

We sampled six effluent-dependent river reaches in Arizona: three reaches in the Santa Cruz River basin, two reaches in the Rio de Flag basin, and one reach in the Salt River basin (Fig. 1). Groundwater levels are at least 40 meters below the streambed surface for all six reaches, so each reach would dry completely during baseflow if effluents were not being added to the channel (Carlson et al., 2011; Arizona Department of Environmental Quality, n.d.). Study reach lengths ranged from 3 to >24 km. The reach in the Salt River and both reaches of the Rio de Flag were short (~3-5 km). In the Santa Cruz River, one reach was medium in length (~9 km) and two others were long (>24 km) (Table 1). Downstream of all six study reaches, the channels are dry except during periods of flooding.

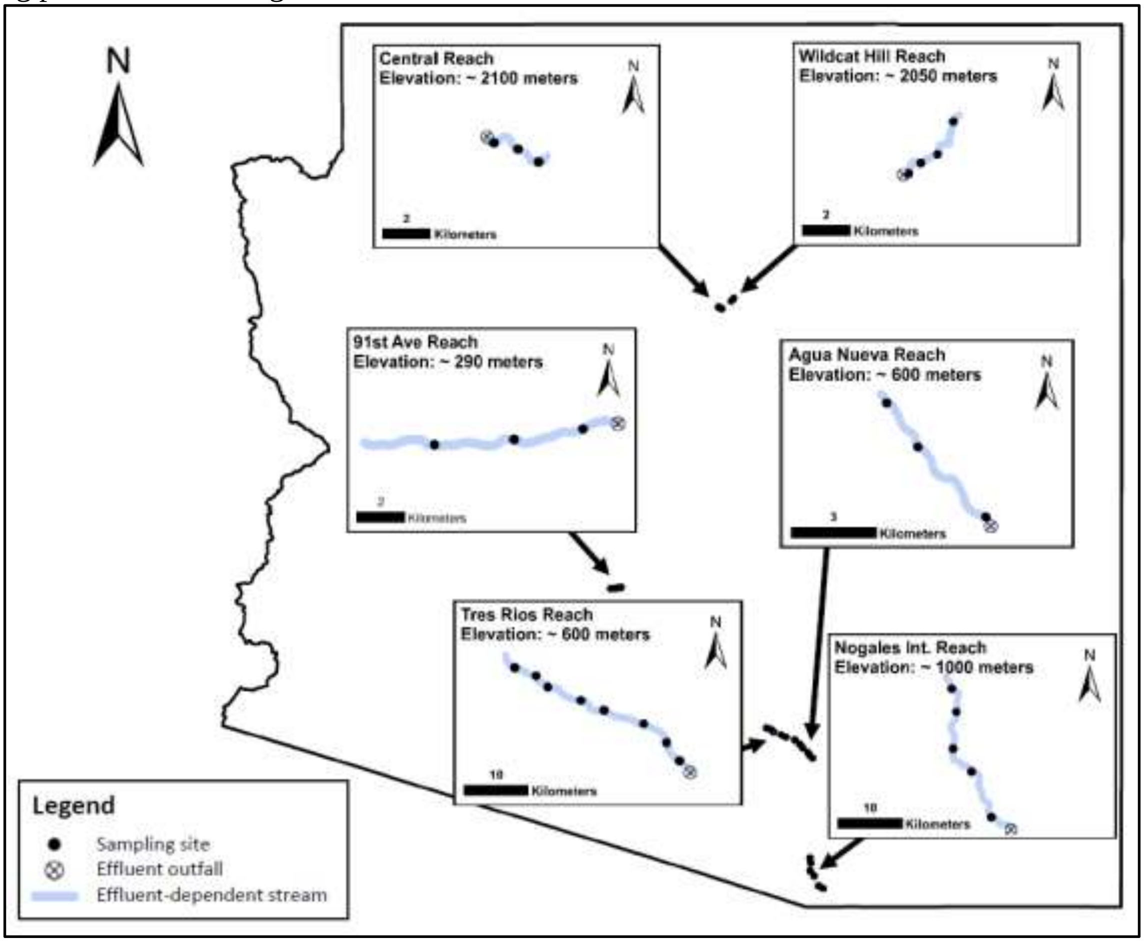

Fig. 1. Map of the study area showing effluent-dependent stream reaches (solid blue lines), effluent outfalls (circle with x), and sampling sites (solid black dot) in the state of Arizona. Each reach has been magnified to show more detail and each inset map has a corresponding scale bar.

The Santa Cruz River basin (22,000 km^2) is located in southern Arizona and northern Mexico (Webb et al., 2014). The climate is characterized by hot summers (average high July temp: 38°C) and moderately cool winters (average low January temp: 5°C). Annual average precipitation is ~300 mm and rainfall is bimodal, with the months of January and August being the wettest and May and November being the driest (data provided by NOAA ESRL Global Monitoring Division, Boulder, Colorado, USA [http://esrl.noaa.gov/]). The uppermost study reach in the Santa Cruz Basin (~25 km long) is supported by the Nogales International Wastewater Treatment Plant. This plant was constructed in 1943, upgraded in 2009, and discharges approximately 57 million liters of tertiary-treated effluent into the river each day (Santa Cruz County, 2019).

Fig. 2. Photos showing the six sampling reaches: A) Tres Rios (32.435°, -111.233°), B) Nogales (31.562°, -111.045°, and C) Agua Nueva (32.287°, -111.030°) reaches of the Santa Cruz River; D) 91st Ave reach (33.381°, -112.322°) of the Salt River; and the E) Central (35.183°, -111.631°) and F) Wildcat Hill (35.234°, -111.543°) reaches of the Rio de Flag.

The second Santa Cruz reach is shorter (~9 km) and is supported by the Agua Nueva Water Reclamation Facility (WRF), which was constructed in 1951, upgraded and modernized in 2014, and discharges approximately 30 million liters of tertiary-treated effluent into the river each day (Sonoran Institute, 2017). The third and longest (~31 km) reach is supported by the Tres Rios WRF, which was built in 1978, upgraded and modernized in 2014, and discharges approximately 115 million liters of tertiary-treated effluent each day (Sonoran Institute, 2017). In addition to baseflow from the treatment plants, all three study reaches in the Santa Cruz River experience seasonal flood events from precipitation runoff, which can surpass 280 m^3/s. The three perennial study reaches are separated by dry reaches (Fig. 1) and are only briefly connected during large flood events.

The Salt River basin (35,000 km^2) is located in central Arizona, with its headwaters in the White Mountains of eastern Arizona. This location has hot summers (average high July temp: 41°C) and mild winters (average low December temp: 7°C). Annual average precipitation is ~204 mm, and rainfall is bimodal. The months of March and July are the wettest, and June and October the driest (data provided by NOAA ESRL Global Monitoring Division, Boulder, Colorado, USA [http://esrl.noaa.gov/]). Our study reach (~6 km long) is supported by the 91st Avenue Wastewater Treatment Plant near Phoenix, which began discharging into the river in 1958, was upgraded in 2011, and processes an average of 530 million liters of tertiary treated effluent per day (US EPA, 2016).

The Rio de Flag basin (518 km^2) is located in the conifer forest-dominated, high elevation mountains (>2000 m) of north-central Arizona (Fig. 1 and 2). The climate is characterized by moderate to warm temperatures in summer (average high July temp: 27°C) and cold winters (average low January temp: -12°C). Annual average precipitation is ~588 mm, and rainfall is bimodal. The months of February and August are wettest, and June and November are driest (data provided by NOAA ESRL Global Monitoring Division, Boulder, Colorado, USA [http://esrl.noaa.gov/]). The Rio de Flag basin receives the highest average annual precipitation among our study basins. However, because of underlying volcanic geology, only 0.25 cm per year becomes stream flow within the Rio de Flag, making it one of the lowest percentage runoff systems in Arizona (Bills & Enyedy, 2015). Our upper 'Central' reach (~3 km long) is supported by the Rio de Flag Water Reclamation Plant. This plant began discharging into the river in 1993, was upgraded in 2009, and has a capacity of 15 million liters per day. Our lower 'Wildcat' study reach (~4 km long), located approximately 10 km downstream of the Central reach, is supported by the Wildcat Hill Water Reclamation Plant. This plant began discharging into the river in 1971 and has a capacity of 23 million liters per day (City of Flagstaff, 2020). Information on the actual daily discharge from these two plants is not publicly available, but is likely less than their total treatment capacity.

2.2.Data collection

To understand how water quality changes downstream from effluent outfalls, at least three sites were sampled for water quality within each of the 6 study reaches. The exact location and number of sampling sites for each reach were selected based on the availability of public access points and the length of reach, resulting in a range of 3 to 8 sampling sites per reach. A minimum of 0.8 km separated each progressive downstream sampling site within a reach, with an average distance of 3.5 km between sites (Fig. 1). To examine seasonal variation in water quality, we took measurements (described below) at each site at least once per season (winter = January to March, spring = April to June, summer = July to September, fall = October to December) in the year of 2018. To provide additional temporal resolution, one reach was sampled bi-monthly (i.e. 6x per year) and one reach was sampled monthly (i.e. 12x per year) (Table 1).

During each sampling event, we measured basic water quality parameters (dissolved oxygen, temperature, pH, specific conductance, and alkalinity) and nutrients (total phosphorus, ammonia, and nitrate). Specific conductance is hereafter referred to as conductivity. Basic water quality parameters were measured using in situ water quality probes: dissolved oxygen (Apera Instruments AI480 DO850 probe), pH, temperature, and conductivity (Apera Instruments SX823-B multiprobe). For nutrient concentrations (total phosphorus, total ammonia nitrogen, and nitrate), we collected 500 mL water samples from each site, immediately transported them to the laboratory at University of Arizona, and stored at 4°C until analyzed using a YSI EcoSense Model 9500 Photometer (within 48 hours). When nutrient analysis results exceeded the test's calibration range, samples were diluted and rerun, and the appropriate dilution factor correction applied.

2.3.Data Analysis

Each of the eight water quality parameters was modeled with LMEs (Pinheiro & Bates 2000) using distance from effluent outfall (Distance), Season, and the interaction between distance and season (Distance*Season) as predictors with WWTP (Site) as a random factor: [water quality] ~ Distance * Season, random = Site. Models were run in the statistical program R (version 3.5.1: R Core Team 2019) with the package 'glmmTMB' (version 1.0.2.1; Brooks et al. 2017). Histogram analysis indicated that there were no outliers in the dataset and all variables were approximately normally distributed, except for ammonia, which was log-transformed prior to analysis (data not shown). Finally, to visualize how overall water quality varied across the year at all sites, we included all 8 water quality parameters in a principal component analysis (PCA). The resulting plot allowed us to identify data clustering across river basins, reaches, and seasons. PCA was run with PC-ORD version 7.08 (McCune and Mefford, 2018).

3. Results

The effects of distance, season, and their interaction varied across specific water quality parameters. Some water quality parameters, such as temperature, dissolved oxygen, and ammonia showed consistent effects of distance from outfalls. However, other studied water quality parameters did not exhibit consistent patterns with distance from outfalls across. The Linear Mixed-Effect model indicated that temperature generally decreased with increasing distance from effluent outfalls. There was a significant interaction between distance and season in, where temperatures actually increased with distance from outfall in spring and summer (Fig. 3A). Across all study reaches, water temperature was usually elevated in spring and summer, and reduced in fall and winter. However, this pattern of increasing temperatures with distance from outfall in warmer months was not observed in the higher elevation Nogales reach, located 70 km upstream of the Tres Rios reach.

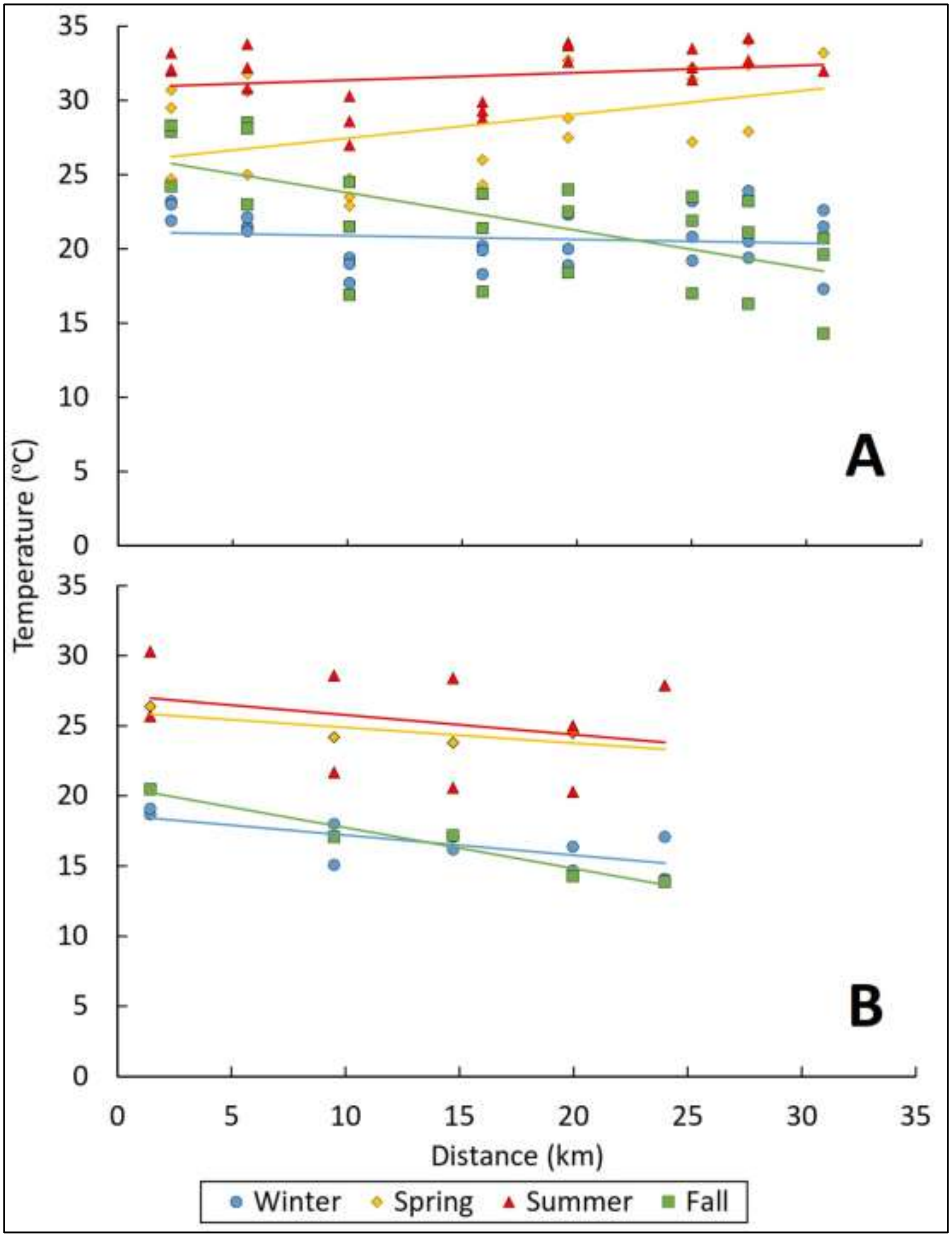

Fig. 3. Graph showing relationship between temperature (°C) on the y axis and distance (km) from outfall on the x axis at the Tres Rios Reach (A) and Nogales (B) reaches of the Santa Cruz River. Each point represents a sampling unit, with points coded by season. Solid lines represent the best fit regression corresponding to each season as denoted by color.

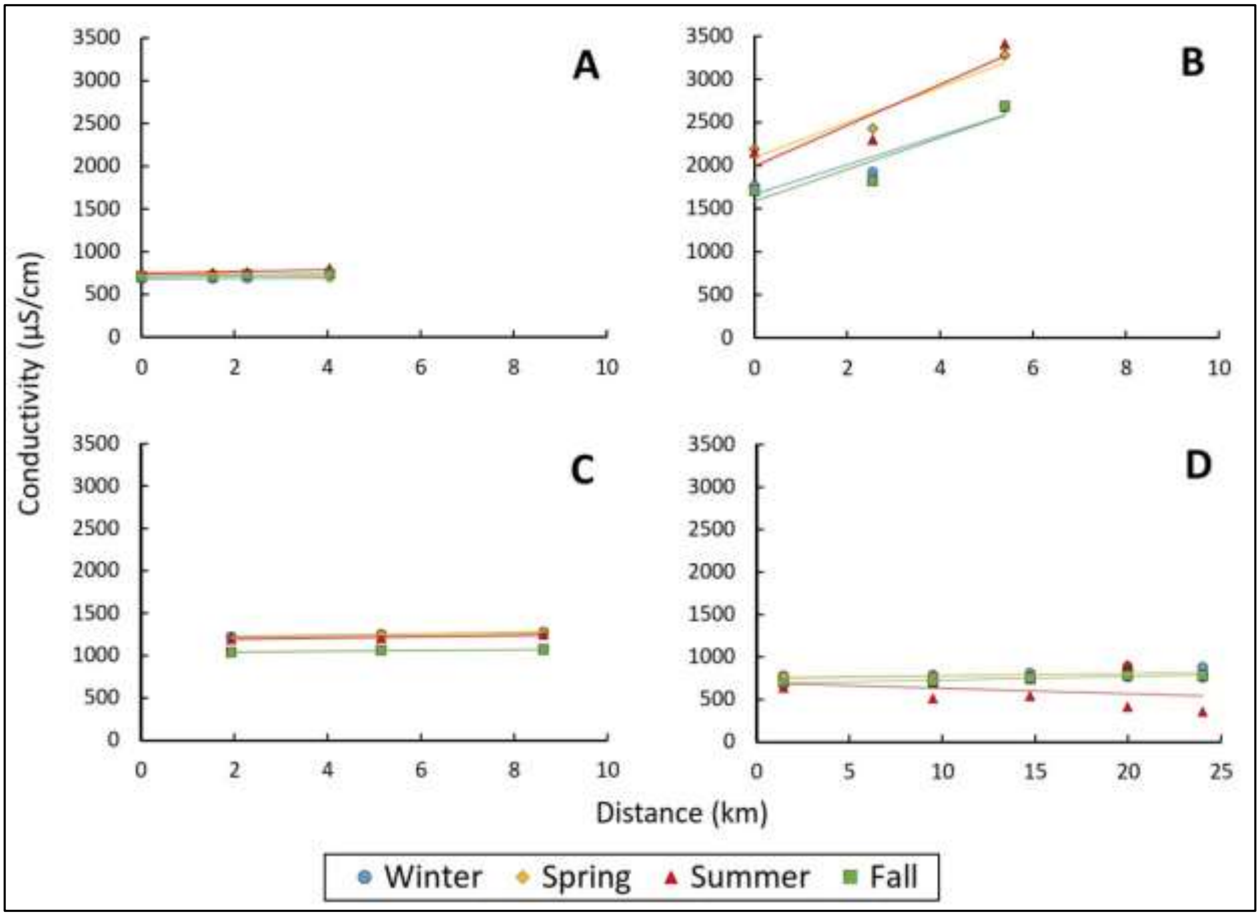

Fig. 4. Relationship between conductivity (µS/cm) and distance (km) from outfalls at the Wildcat Hill reach of the Rio de Flag (A), the 91st Avenue reach of the Salt (B), and the Agua Nueva (C) and Nogales (D) reaches of the Santa Cruz River. Each point represents a sampling unit, with points coded by season. Solid lines represent the best fit regression corresponding to each season.

Similar to temperature, our model indicated that dissolved oxygen also responded significantly to both season and distance from outfall. Concentrations of dissolved oxygen were low during summer and spring and high during winter and fall. Furthermore, dissolved oxygen concentrations were consistently lowest at sampling sites closest to effluent outfalls, and then increased with the distance downstream in each season. The lowest dissolved oxygen concentration at the Tres Rios reach in the summer dropped as low as ~3 mg/L.

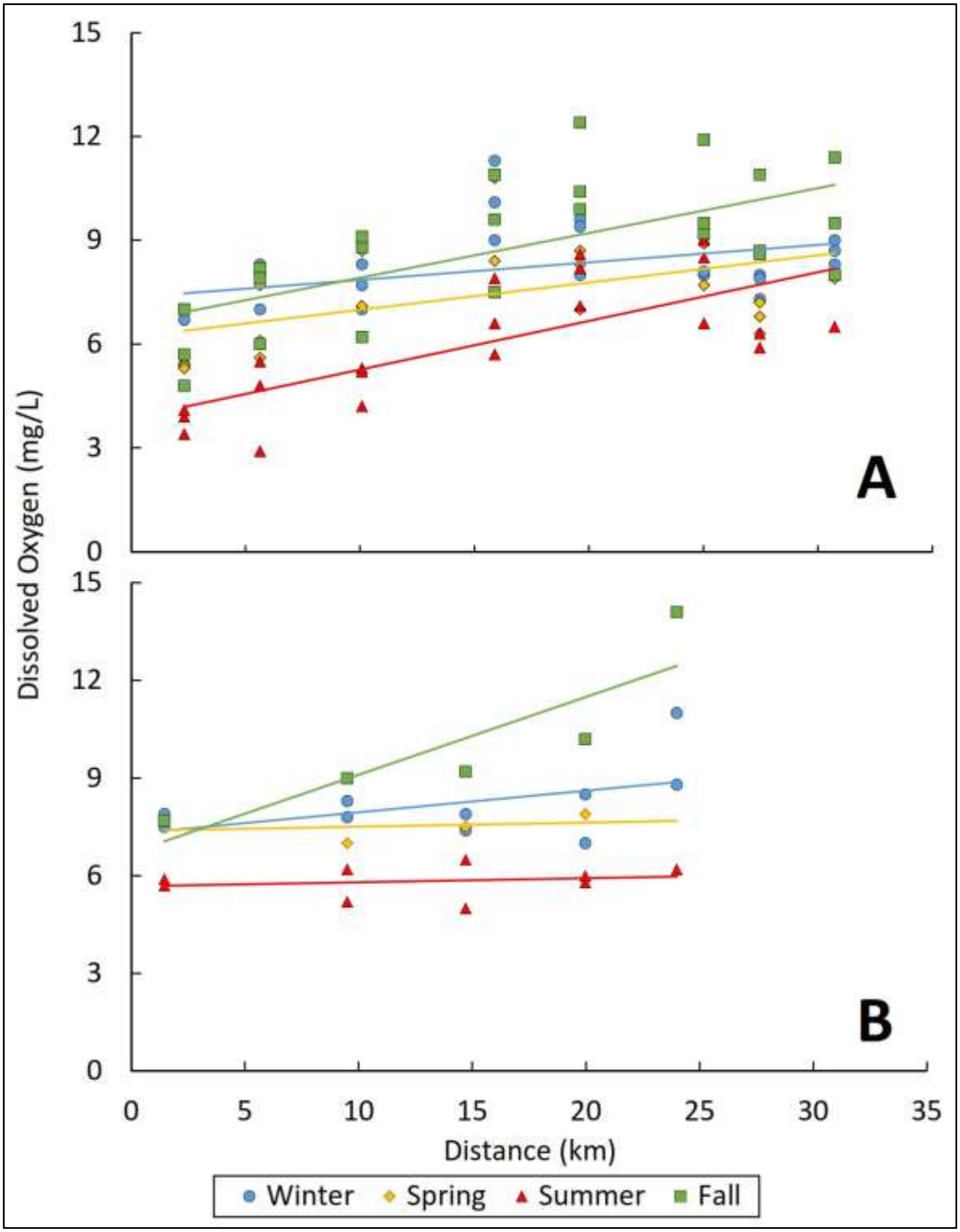

Fig. 5. Relationships between dissolved oxygen (mg/L) and distance (km) from outfalls at the Tres Rios Reach (A) and Nogales (B) reaches of the Santa Cruz River. Each point represents a sampling unit, with points coded by season. Solid lines represent the best fit regression corresponding to each season as denoted by color.

In general, conductivity values were stable across seasons and with increasing distance from effluent outfalls across most reaches. However, the Salt River at 91st Avenue reach had much higher initial conductivity values, which then increased by about 70% within 6 km from the effluent outfall.

The highest ammonia concentrations observed across all sites were at the long Tres Rios reach of the Santa Cruz River, with values as high as 5.36 mg/L N during the fall season. However, these high ammonia concentrations decreased with the distance downstream and reached undetectable levels at the end of the reach, ~30km downstream from the outfall. In other seasons, ammonia concentrations remained very low or undetectable across all sampling sites. Nitrate and phosphorus exhibited only minor changes with distance, with nitrate increasing slightly and phosphorus decreasing slightly.

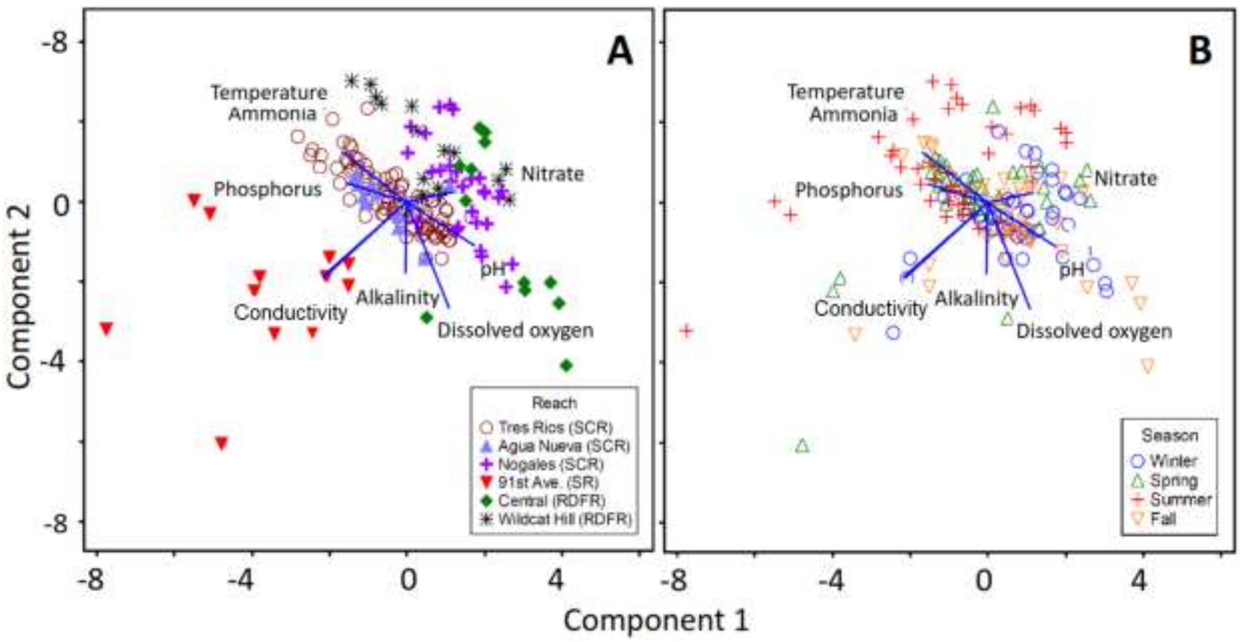

Fig. 6. PCA ordinations of all 8 water quality factors across all 6 samples reaches and seasons. The same ordination is coded here by reach (A) and season (B) to illustrate clusters. The loadings of the 8 water quality factors on the two primary axes are visualized with the blue vectors.

PCA ordination revealed at least three noticeable clusters of data points when considering all water quality parameters together (Fig. 6A). First, data points from the short 91st Ave reach of the Salt River formed a scattered open cluster apart from all other points, with the vector highlighting increased conductivity as being the primary cause of this distinction (Fig. 6A). Second, observations from the long Tres Rios reach and medium-length Agua Nueva reach of the Santa Cruz River overlapped in the center of the plot, indicating no single water quality parameter was responsible for that cluster. Third, data points from the higher elevation Nogales reach of the Santa Cruz River and Central and Wildcat Hill reaches of the Rio de Flag formed a partly separate cluster that was associated with higher nitrate values. Data points did not form any distinct clusters by season. However, a few patterns were evident from examining the vectors. Observations from the summer tended to have higher temperatures and ammonia levels, and lower pH levels, than those from other seasons (Fig. 6 B).

4. Discussion

4.1. Distance effect

The effect of longitudinal distance from effluent outfalls on water quality varied widely among measured factors. Effluent is generally warmer than natural baseflow temperatures, so heat exchange between the atmosphere and stream likely led to cooling trends (Boyle and Fraleigh, 2003). As temperatures cooled, dissolved oxygen levels rose. Increased oxygen levels were likely due to a combination of cooler temperatures, photosynthesis by macrophytes and algae, and atmospheric reaeration (O'Connor, 1967; Correa-González et al., 2014).

In contrast, longitudinal variation in pH was not significant, the magnitude of change was small and values remained circumneutral to slightly basic. These results are in line with other studies of streams receiving effluent, which reported relatively stable pH values downstream of effluent outfalls (Chen et al., 2009; Prat et al., 2013; Matamoros and Rodríguez, 2017). Relatedly, we also found insignificant longitudinal variation in alkalinity, which indicates the buffering capacity to neutralize acids and bases of a water body. Some previous studies have reported a decrease in alkalinity with distance downstream (Birge et al. 1989; Boyle and Fraleigh, 2003), a result likely due to dilution by other water sources. However, our effluent-dependent study reaches are not fed by any tributaries that can dilute the effluent during baseflow.

In natural streams, conductivity tends to be low in headwater reaches and then increase in the downstream reaches, especially when streams pass through urban areas (Chusov et al., 2014). However, previous studies of effluent-fed streams have reported elevated conductivities near outfalls and then relatively stable values downstream (Chen et al., 2009; Prat et al., 2013; Matamoros and Rodríguez, 2017). In general, our results show stable longitudinal conductivity values, with two exceptions. The short reaches of the Santa Cruz River and Salt River exhibited conductivity increases with distance downstream, especially on the Salt River. These patterns could be due to urban inputs (i.e. non-point sources) that were not mapped as part of our research, or due to underlying soil conditions. For example, the Salt River Valley has many areas that are high in soluble mineral salt (Harper, 1931).

Our model across different reach lengths on the nutrient levels shows that phosphorus and nitrate variations were not significantly affected by longitudinal distance from the outfalls. Previous phosphorus studies in the other effluent-fed streams (Birge et al. 1989; Chen et al., 2009) indicated concentration decreased with distance downstream. When we look at our long reaches separately we observed similar decreasing trends. This reduction is likely due to biotic and abiotic nutrient uptake along the reaches (Davis and Minshall, 1999; Stutter et al., 2010).

For nitrogen species, we found that ammonia levels decreased longitudinally as nitrate levels were relatively stable. High levels of ammonia have commonly been reported near effluent outfalls, with decreasing levels downstream (Sebenik et al., 1972; Hamdhani et al., 2020). In the long reach of Tres Rios, the average rate of ammonia loss in the fall season was about ten times greater than nitrate gain (-0.11mg/L/km of N ammonia; 0.01 mg/L/km of N nitrate). In summer, the average rate of ammonia loss was double that of nitrate gain (-0.08mg/L/km of N ammonia; 0.04 mg/L/km of N nitrate) (Fig. 6). The increased nitrate levels are likely due to nitrification occurring in the stream channel (Sebenik et al., 1972) but do not fully account for ammonia removal, suggesting mechanisms besides oxidation of ammonia to nitrate contributed to ammonia loss during river transport. These other mechanisms include volatilization, adsorption by cation exchange complex, fixation by clays, and fixation by organic matter (Van Schreven, 1968; Weiler, 1979; Freney et al., 1981; Simon and Kennedy, 1987; Kowalchuk et al., 1998; Chen et al., 2007; Lin et al., 2019). Since pH at all sites was always <9 (see Supplementary Material), total ammonia nitrogen was present mainly as ammonium ion ($NH4^+$), thus volatilization is not expected to have contributed significantly to ammonia loss in any of the study reaches.

4.2. Seasonal effects

Seasonal atmospheric and climatic factors, such as air temperature, solar radiation, relative humidity, cloud cover, and wind speed, work together to shape water temperatures through heat exchange between the atmosphere and the stream (Sinokrot and Stefan, 1994). This effect is usually stronger in unregulated stream systems (Bowles et al., 1977) and relatively weak in effluent-dependent stream systems, especially near effluent outfalls (Hamdhani et al., 2020). However, we did observe a significant effect of season on water temperatures across all six study reaches, with warmer water observed in spring and summer than in fall and winter, regardless of reach length. This pattern demonstrates the strong influence of atmospheric and climatic conditions on water temperature despite the stable, warm temperature of effluent at its point of release. Trends in dissolved oxygen levels across seasons were generally opposite to those of temperature, with the lowest values observed in summer; however this pattern was only significant in three of the six reaches (Table 2; Fig. 4). In other reaches, dissolved oxygen levels could be more strongly driven by non-temperature factors, such as variation in biochemical oxygen demand, water volume, and photosynthetic activity (Guasch and Subater, 1995; Mulholland et al., 2005; Mandal et al., 2010).

Conductivity and pH differed significantly by season across five of the six study reaches, but we could not detect any consistent patterns (Table 2; Fig. 5). For example, conductivity was often highest in the summer, but in some cases it was lowest in the summer (Fig. 5), and similar inconsistencies were observed with pH (data shown in supplementary material). Both factors can be influenced by precipitation and runoff, especially in urban areas where runoff may carry pollutants (Vega et al.,1998; Laudon et al., 2000). However, we avoided sampling during periods of precipitation runoff, thus all water quality measurements reflect conditions when flow was 100% effluent. Further study is needed to understand why these factors vary inconsistently by season in the six study reaches.

We observed higher variation and inconsistency in nutrient levels than in other measured parameters that we attribute mainly to seasonal variation in wastewater treatment plant influent and performance. The most substantial seasonal variations in ammonia, nitrate, and phosphorus occurred at the Tres Rios WRF, which discharges to our longest study reach of the Santa Cruz River (Fig. 6). In summer and fall, ammonia levels were much higher and nitrate levels were at their lowest, relative to concentrations observed in winter and spring, especially nearer to the effluent outfall. This finding was unexpected because conversion of ammonia to nitrate during wastewater (Siripong and Rittmann, 2007) and drinking water treatment (Liu et al., 2017) is usually highest in warmer seasons. We suspect that manipulation in treatment operations at Tres Rios WRF contributed to lessened nitrification capacity in summer (Hegg et al., 1979; Liu et al., 2012). Finally, phosphorus levels discharged in effluent from the Tres Rios WRF were higher during summer and spring, and lower during winter and fall. We speculate that this may be related to the type of *Accumulibacter* involved in phosphorus removal processes during wastewater treatment. Some types of *Accumulibacter* alter their activity patterns with seasonal-induced temperature changes (Flowers et al., 2013). Clearly, more research is needed to disentangle the complex seasonal changes in nutrient levels in effluent-dependent streams.

4.3. Geography and climate

Geographic and climatic settings strongly influence the structural and functional features of natural rivers and streams (Gasith and Resh, 1999; Shi et al., 2019), but it is unclear how strongly effluent-dependent systems might be influenced. For example, we expected that water temperature would be fairly consistent across all reaches, regardless of climate zone, given the warm temperature of effluent resulting from the treatment process (Brooks et al., 2006; Hamdhani et al., 2020). However, when considering all measured factors across all reaches, sampling sites, and sampling dates together, distinct clusters were observed for high elevation sites (>1,000 m), the medium elevation sites (~700 m), and the low elevation site (<300 m) (Fig. 7). As previously mentioned, the lowest elevation reach (91st Ave, Salt River) occurs in a stream basin with natural salt deposits (Harper, 1931). The high mineral content in the water likely contributed to the distinctness of that reach (Fig. 6A). Higher nitrate levels were observed in the higher elevation reaches. Because rates of biochemical reactions, including denitrification, are temperature-dependent (Dawson and Murphy, 1972; Xu et al., 2019), lower air temperatures may reduce nitrate removal capacity of the treatment plants in the high elevation sites.

In contrast to the distinct geographic clusters in the PCA ordination, we observed broad overlap of water quality conditions by season (Fig. 6B). This lack of pattern is likely due to idiosyncratic seasonal changes across the factors and reaches, as described in the previous section and observed in previous studies (Hamdhani et al., 2020). Thus, although individual reaches may exhibit clear seasonal trends in water quality, the variation in the significance of those trends across reaches in different locations obscures detection of any overall seasonal patterns (Gardner and McGlynn, 2009; Pratt and Chang, 2012).

4.4. Potential for effluent to support native aquatic species

The ability of effluent to enhance or re-create habitat for native aquatic species is still actively debated, but some conservation successes have been noted in recent years (Halaburka et al., 2013; Luthy et al., 2015). In our six study reaches, effluent has restored flow to rivers that had been dry for decades due to extraction of groundwater and surface water (e.g. Webb et al. 2014). However, prior to wastewater treatment plant upgrades, water quality was generally poor and aquatic biodiversity was low (Cordy et al., 2000; Walker et al., 2005; Sonoran Institute, 2017). In particular, high water temperatures, low dissolved oxygen levels, and elevated concentrations of ammonia are frequently cited as the cause of low biodiversity in effluent-dependent streams (e.g. Monda et al., 1995; Hamdhani et al., 2020; Eppehimer et al., 2020).

Many aquatic animals are adapted to specific temperature ranges (Carveth et al., 2006; Eliason et al., 2011) and warmer water can affect their growth, behavior and survial (Crawshaw, 1977; Schneider and Connors, 1982; Marine and Cech, 2004). Among 11 native Arizona fish species tested by Carveth et al. (2006), the most sensitive to high water temperatures was the Speckled dace (*Rhinichthy osculus*), which becomes disoriented at 34ºC and perishes at 36ºC. One of the most tolerant fishes identified was the Gila topminnow (*Poeciliopsis occidentalis*), which can withstand temperatures as high as 38-39ºC for short periods of time (Carveth et al., 2006). The highest temperature we recorded across all of our sites and seasons was 34.2ºC, but in our study not all instantaneous measurements were taken at the hottest times of the day for each reach, so it is possible that temperatures might increase a further 1 to 3ºC (Lowney, 2000) above our measured values. Taking that into account, the maximum temperatures that organisms might experience in some of these reaches could reach 37.2 during summer, which would be higher than Speckled dace could tolerate (Carveth et al. 2006). However, since the Lethal Thermal Method study conducted by Carveth et al. (2006) was generated with faster temperature rate of change in the laboratory (0.3ºC/min) than typically occur in the stream system, so fish in stream system should better escape potentially lethal conditions, unless temperature increases at a similar rate. This result suggests that studied effluent-dependent streams in the state are hypothetically thermally suitable for native fish, but sensitive species could be negatively impacted in the warmest reaches. In these cases, a gradient in fish composition could occur, where thermally sensitive species are only found further downstream from the outfalls. A similar pattern has been reported for the thermally-sensitive Greenside Darter (*Etheostoma blennioides*) in a Canadian effluent-fed stream (Brown et al., 2011).

Dissolved oxygen concentration also can be a direct indicator of the ability of a waterbody to support aquatic life. We occasionally observed low dissolved oxygen levels near effluent outfalls in summer, with concentrations as low as 2.9 mg/L, as has been observed in similar systems (Birge et al. 1989; Boyle and Fraleigh, 2003; Matamoros and Rodríguez, 2017). As well as temperature, in our study not all instantaneous measurements were taken at the lowest times of the day for each reach, so it is possible that dissolved oxygen might decrease 2 to 3ºC (Wang et al., 2003). Mortality or loss of equilibrium for fishes and other aquatic organisms can occur at concentrations between 1 and 3 mg/L (US EPA, 1986), and chronic exposure can cause behavioral changes that make individuals vulnerable to predation or other risk factors (Dean and Richardson, 1999). For native Arizona fishes, dissolved oxygen levels ranging from 0.22 to 1.47 mg/L have been reported as being lethal (Lowe et al., 1967). These findings suggest that oxygen levels were probably stressful for native fishes across all of our study sites near outfall and warm seasons. A gradient in fish composition could occur, where low oxygen sensitive species are only found further downstream from the outfalls.

Aquatic invertebrates can also be affected by low oxygen levels, with tolerant worms (Oligochaeta: Martins et al., 2008) and fly larvae (e.g. *Chironomus*, Chironomidae: Lencioni et al., 2008) replacing sensitive mayflies (Ephemeroptera) and stoneflies (Plecoptera) near effluent outfalls (Hamdhani et al., 2020). In fact, a previous study from the Santa Cruz and Salt Rivers reported low oxygen levels and only a few tolerant invertebrate taxa near effluent outfalls (Cordy et al., 2000). However, wastewater treatment plants supplying these reaches have since been upgraded, and recent findings from the Santa Cruz River demonstrate a robust aquatic invertebrate community with few apparent dissolved oxygen limitations (Eppehimer et al., 2020). Although invertebrate studies are not available for the other two rivers (Salt and Rio de Flag), dissolved oxygen measurements suggest that diverse aquatic invertebrate communities could be found there too.

Ammonia has direct toxic effects on aquatic species (e.g., Richardson, 1997; Hickey and Vickers, 1994), with concentrations greater than 2 mg/L as N (pH 7.0 and temperature 20°C) causing impairment of aquatic life (Constable et al. 2003; Yeom et al., 2007; US EPA, 2013). The vast majority of our measurements in Arizona were below this threshold, but at one site on the Santa Cruz River we did find concentrations as high as 3-5 mg/L in summer and fall. In a prior study of the Santa Cruz River, before treatment plants were upgraded, the absence of sensitive mayfly taxa was likely caused by elevated ammonia concentrations (Boyle and Fraleigh, 2003). However, recent work has shown that both mayflies and fish can even be found at locations with occasional high ammonia levels (e.g. lower Santa Cruz River: Sonoran Institute, 2017; Eppehimer et al., 2020). Together, these findings suggest that ammonia is no longer a primary concern for aquatic species in effluent-dependent streams of Arizona.

5. Conclusion

Results from this study suggest that distance from effluent outfalls, season, and climatic and geographic factors all play important roles in the water quality dynamics of effluent-dependent streams. Water quality conditions deteriorated somewhat in some reaches during the hottest months of the summer and, for several factors, we observed natural remediation of water quality in longer reaches. Our study also shows that effluent-dependent streams supported by high quality tertiary effluent meet or exceed water quality conditions needed to support robust assemblages of native species. However, conditions may be stressful for the most sensitive taxa at sites closest to effluent outfalls, especially in summer. Natural streams across arid and semi-arid regions are continuing to dry up due to climate change and water abstraction (Seager et al., 2007; de Graaf et al., 2019), but effluent-dependent streams are becoming more common (Luthy et al., 2015; Hamdhani et al., 2020). Our findings suggest that these systems have the capacity to serve as refuges for native biota, and they may become the only aquatic habitat available in many urbanizing arid regions.

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