Freshwater tributaries provide refuge and recolonization opportunities for mussels following salinity reversal

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HIGHLIGHTS

- Salinity trends and mussel demographics were assessed in a salinized river.
- Mussels were abundant in two large freshwater tributaries.
- Mainstem distribution was limited to sites around the lower tributary.
- Salinity has recently begun to decline in the mainstem of the river.
- Mussel recolonisation was detected at two mainstem sites.

GRAPHICAL ABSTRACT
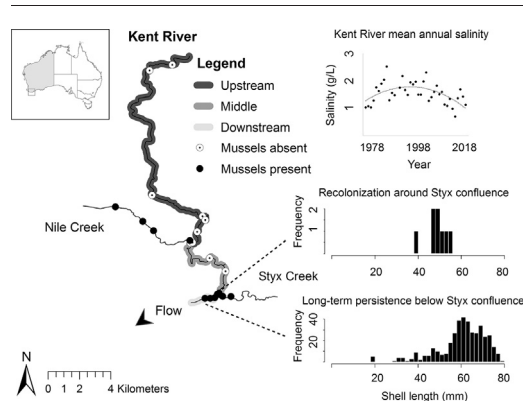
ABSTRACT

Reversing the effects of secondary salinization, and its impacts on aquatic biodiversity, is a growing global challenge, and particularly prevalent in Mediterranean-climate regions. Remnant freshwater tributaries in salinized landscapes provide significant biodiversity values, including discrete areas of refuge, dilution of salinized reaches, and potential source populations for recolonisation. The importance of these areas for aquatic fauna is widely accepted but rarely evaluated in the field. This study explored how spatial distribution of southwestern Australia's only freshwater mussel species, *Westralunio carteri*, has responded to the ongoing salinity trend in the Kent River catchment. Our results showed that salinity in the river has begun to reverse following improved catchment management, and also detected the first evidence of an associated recovery of the freshwater mussel population. Mussels in the mainstem were limited to sites around and downstream of a permanently flowing freshwater tributary, suggesting that dilution from this source provides a refuge in the lower reach. At two of those sites, all individuals were <15 years of age, indicative of recolonisation coinciding with salinity reversal around the turn of the century. Interestingly, mussels clearly persisted in other parts of the lower reach throughout the peak salinity period, when salinities regularly exceeded laboratory derived toxicity thresholds for the species. Mussels were not found in the majority of the mainstem or in highly acidic parts of the freshwater tributaries. The presence of old shells at those sites shows that the species was once widespread, and that the current distribution probably reflects a contraction due to historical salinization as well as acidification. Overall, our results show that the *W. carteri* population in the catchment has taken a first step towards recovery, and highlights

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the importance of freshwater tributaries in providing both refuge from disturbance and a source of new recruits.

1. Introduction

Secondary salinization is a growing threat to freshwater biodiversity globally, significantly modifying ecosystem structure and function, and it is not known whether its impacts can be reversed (Cañedo-Argüelles et al., 2013; Herbert et al., 2015; Cañedo-Argüelles et al., 2019). Mediterranean-climate (med-) regions are heavily impacted by secondary salinity, and the issue is exacerbated by coexisting stressors, such as acidification, water abstraction, and climate change (Degens et al., 2012; Robson et al., 2013; Estévez et al., 2019). In the med-region of southwestern Australia, widespread clearing of native vegetation has caused approximately 56% of the region's large rivers to turn brackish or saline (Mayer et al., 2005; Beatty et al., 2011), and much of the regions highly endemic aquatic fauna is now restricted to remnant freshwater tributaries closer to the coast (Beatty et al., 2011; Pettit et al., 2015). In recent years, legislated control on land clearing, and revegetation of the agricultural inland area has helped to slow secondary salinization, and salinity has even began to reverse in some rivers (Mayer et al., 2005). There is the potential for salt-sensitive fauna that have persisted within freshwater refuges to recolonize these formerly salinized areas, but there is as yet no evidence for this having occurred (Robson et al., 2013).

Freshwater mussels (Mollusca; Bivalvia; Unionida) are one of the worlds most threatened faunal groups (Hastie et al., 2000; Strayer et al., 2004; Lopes-Lima et al., 2017), and their ongoing decline has severely impacted ecosystem function (Vaughn, 2018). Mussels are highly vulnerable to salt pollution (e.g. Todd and Kaltenecker, 2012; Patnode et al., 2015), particularly at the juvenile and larval (glochidial) stages (Blakeslee et al., 2013; Prosser et al., 2017). As an obligate parasite, glochidia are further impacted because elevated salinities can reduce attachment success to host-fish (Beggel and Geist, 2015), and many freshwater fish may be excluded from salinized reaches (Beatty et al., 2011). While the salt-sensitivity of early life stages would be expected to impact new recruits in extant populations, field studies rarely assess recruitment (however see Johnson et al., 2014), and most studies report only mussel presence-absence (e.g. Klunzinger et al., 2015), or species abundance (e.g. Zipper et al., 2016). Demographic studies may be particularly important because many mussel species are long lived (sometimes >100 years), meaning that populations can persist for years without recruitment, resulting in an extinction debt (Haag, 2012; Ferreira-Rodríguez et al., 2019). In combination, these factors make mussel populations unlikely to recover quickly from disturbances (Strayer et al., 2004), and the potential for natural recolonization after extirpation is still mostly unstudied (for examples of recovery from other disturbances see: Riccardi et al., 2016, Gillis et al., 2017, Benson et al., 2018). To our knowledge, there have been no studies that have investigated mussel population recovery following salinity reversal, and only one has addressed the effect of salinity on mussel distribution in salinized med-climate rivers (Klunzinger et al., 2015).

Carter's freshwater mussel (*Westralunio carteri*, Iredale 1934; IUCN Redlist: Vulnerable (VU A2c)) is the only mussel species in southwestern Australia (Walker et al., 2014). While *W. carteri* was once widespread and sometimes highly abundant, its extent of occurrence has declined by 49% in the last three generations largely due to secondary salinization (Klunzinger et al., 2015). The species is almost never found in rivers where mean annual salinity exceeds 1.6 g/L (Klunzinger et al., 2015). The first acute salinity tolerance trials for the species found that it had an LD_{50} of 1.6–3.0 g/L, and a LD_{95} of 3.6–4.25 g/L, and no individuals survived for longer than eight days at

>5 g/L (Klunzinger et al., 2015). Subsequent trials, however, found that *W. carteri* may actually be more robust (LD_{50} = 5.87–5.96 g/L; LD_{95} = 6.01–6.12 g/L), and that survival was enhanced when using (1) gradual rather than acute salinity increases, (2) more natural feeding conditions, and (3) individuals collected from higher salinity habitats (Ma, 2018). These findings suggest that *W. carteri* has the capacity to respond to increasing salinity through both physiological acclimatization and genetic adaptation (Ma, 2018). In addition, the natural environment may provide spatial and temporal refuges that allow species to persist in otherwise unsuitable habitats (Robson et al., 2013). Conversely, natural environments may present additional stressors that exclude species from apparently suitable habitats (Beermann et al., 2018). While acute salinity trials are valuable for determining tolerances and showing short-term responses, the effect of life-long (>50 years) exposure at sub-lethal levels on population structure in natural settings is not known, and requires validation in the field.

An opportunity existed within the Kent River catchment in southwestern Australia to assess mussel distribution and population structure at a range of salinities in a highly salt-impacted landscape. Rapidly rising salinity in the Kent River was combated in the 1970s and 80s through clearing control and revegetation in the upper catchment. By 2002, these strategies had slowed the rising rate of salinity and it was expected that it would eventually begin to decline towards pre-disturbance levels (Mayer et al., 2005; De Silva et al., 2007). It is likely that *W. carteri* was once widespread through the river, but there are no pre-salinization distribution data available. Current salinity levels are among the highest where the species is known to occur, and mussels have only been detected in two fresh tributaries, as well as in the lower reach of the mainstem below the lower tributary (Klunzinger et al., 2012a, 2015).

The objective of the current study was to assess the ongoing salinity trend in the Kent River, and to explore how mussel spatial distribution and abundance has responded to that trend. Our hypotheses were that:

1. Long term salinity data would show that salinity has begun to decline since 2002.
2. This decline has facilitated increased mussel recruitment and recolonisation in the mainstem of the river.
3. Mussels would be more abundant in freshwater tributaries than in brackish sites in the mainstem, while being entirely absent in higher salinity areas (>3–4.25 g/L).
4. Although we expected mussels to be absent from high salinity sites, we predicted that we would find evidence (i.e. old shells) of previous occupation at those sites.

2. Methods

2.1. Study area

The effect of salinity on *W. carteri* distribution was assessed in the lower catchment of the Kent River (between 34°45′05″S and 34°53′20″S; Fig. 1a) on the south coast of southwestern Australia. The Kent River originates 80 km inland and drains a total area of c. 2500 km². Two hydrographic stations continuously monitor conductivity and discharge on the mainstem of the River; one north of the study area, ~45 km inland at Rocky Glen, and the other within the study area, ~15 km inland, below the confluences of two freshwater tributaries (Nile Creek and Styx Creek) (Fig. 1c). The Kent River is 'moderately saline' to 'saline' at the upper station (mean annual salinity ~4.17 g/L) and 'marginal' to 'brackish' at the lower station for most of the year
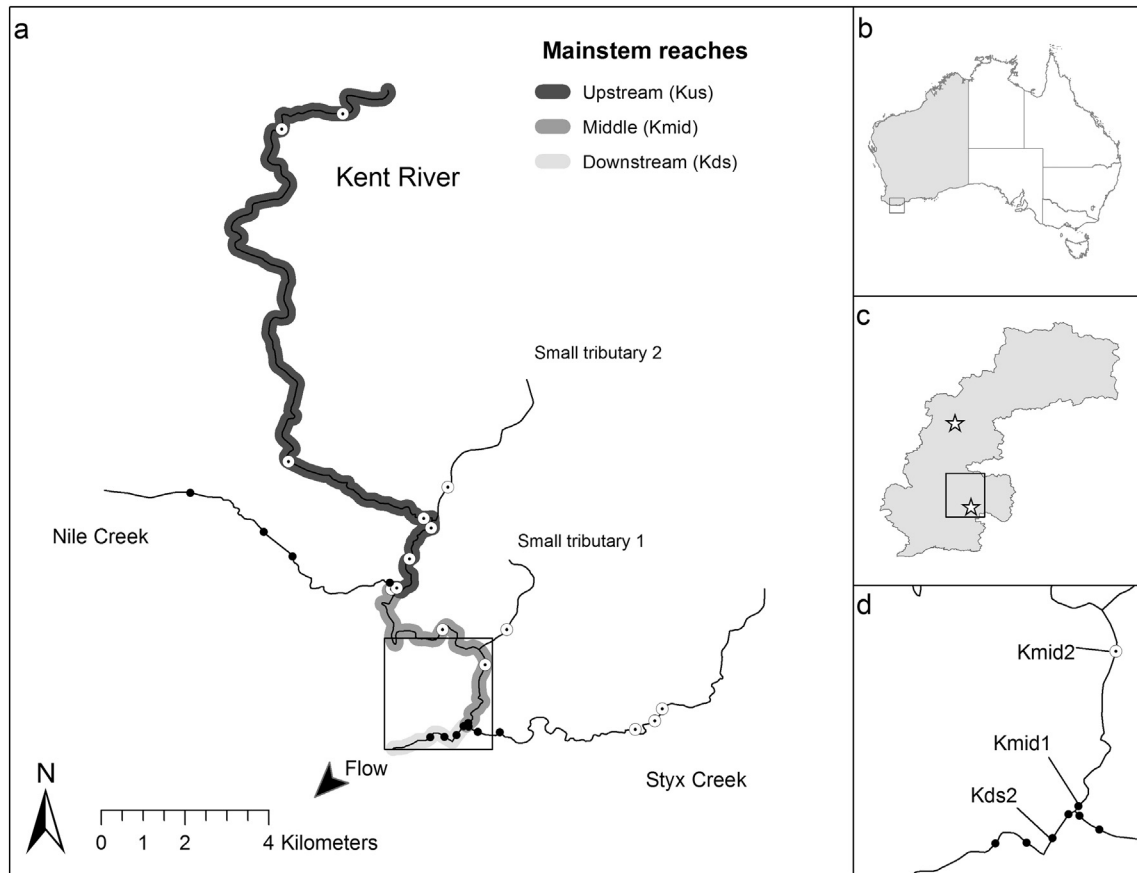
Fig. 1. (a) Site locations in the Kent River catchment showing *Westralunio carteri* presence and absence (full and open circles respectively). The mainstem of the river is in bold and is divided into three reaches (see legend). Inset maps show the locations of (b) the catchment in southwest Western Australia, (c) the study area within the catchment including the locations of the upper and lower hydrographic gauging stations, and (d) the three sites around the Styx confluence where continuous salinity data were collected. The location of panel (d) at the Styx confluence is outlined on panel (a). Note: site Kds2 was located at the lower gauging station.

(mean annual salinity ~1.65 g/L) (Mayer et al., 2005). At the onset of winter rainfall, flushing of the salinized upper catchment results in a pulse of saline water down the river, and mean monthly salinity reaches as high as ~8.88 g/L and ~3.05 g/L at the upper and lower stations respectively (Mayer et al., 2005). Nile Creek is located within an almost entirely uncleared catchment, with pristine natural vegetation. Styx Creek enters the Kent River ~10 km downstream of Nile Creek, and drains a mostly uncleared catchment with farmland bordering the lower reach.

Twenty-seven sites in the lower catchment that contained physical habitat likely to support *W. carteri* were sampled from November to January in 2016–17 and 2017–18 (Fig. 1a). We defined sites as being 'suitable' for *W. carteri* if the salinity level in a single surface water grab sample was below the acute tolerances for the species derived from the Collie River (LC50 = 3 g/L; LC95 = 4.25 g/L; Klunzinger et al., 2015). The uppermost site in the mainstem (Kus7) was 'unsuitable' as it exceeded these threshold values (>5 g/L), while all sites downstream of that point were <2.9 g/L. Four sites were sampled on Nile Creek (N1-N4), six on Styx Creek (S1-S6), and two small perennial creeks in the upstream reach (UStrib1 and UStrib2) were sampled at one location each. The Kent River was sampled at four locations downstream of Styx Creek (Kds1-Kds4), at four locations between Styx Creek and Nile Creek (Kmid1–4), and at seven locations upstream of Nile Creek (Kus1-Kus7). Three sites were surveyed at the Nile-Kent and Styx-Kent confluences (within 25–50 m of the confluence upstream, downstream, and in the tributary). Each sampling site consisted of a river segment 50 m long.

2.2. Physiochemical parameters

This study incorporated salinity records from field measurements, hydrographic gauging stations, government reports, and peer reviewed literature. These sources variably reported salinity as conductivity compensated to 25 °C (μs/cm), total dissolved solid (TDS mg/L), and salinity (g/L). To enable comparison, all values were converted to salinity (g/L) as follows: conductivity (μS/cm) measurements were converted using the formula $S = 0.4665x^{1.0878}$ (Williams, 1986); TDS mg/L measurements (Mayer et al., 2005; De Silva et al., 2007) were converted to conductivity[25] by dividing by 0.5333 (the specific TDS-conductivity relationship for the Kent River, obtained from those reports) before being converted to salinity using Williams (1986).

In situ measurements of pH, oxidation reduction potential (ORP), dissolved oxygen (%), turbidity (FNU), temperature (°C), and salinity (g/L) for each site were recorded using a Hannah multi-parameter probe between the 3rd and 9th January 2018 between the hours of 10 am and 2 pm in order to minimize temporal variability. Long term salinity data spanning the period January 1979 to September 2018 were obtained from the lower hydrographic station below the confluence with Styx Creek. The reliability of each salinity record from the hydrographic stations was assessed using quality codes provided with the data. The quality of the data for 1991 was listed as "not reviewed/quality unknown" and so that year was excluded entirely from analysis. Mean salinity measurements for each day were used to calculate an annual mean for each complete year of data, as well as determine how often stream salinity exceeded acute tolerance thresholds for *W. carteri*

(LC$_{50}$, LC$_{95}$, and 5 g/L as determined by Klunzinger et al., 2015) and how long these events lasted. An event was defined as a series of consecutive days above a threshold level. Additionally, Odyssey conductivity loggers were deployed at two sites in the Kent River above the confluence with Styx Creek (Kmid1 and Kmid2) (Fig. 1d). Combined with the hydrographic station at Kds2, this provided high spatial resolution regarding variability in salinity in this section of the river. Odyssey loggers were deployed on 4th December 2017 and collected on 1st October 2018. Hydrographic data for that same period was obtained from the upper and lower hydrographic gauging stations to investigate the relationship between salinity and flow.

2.3. Mussel sampling

Each site was sampled for mussels by one worker for 1 h using tactile searches combined with visual searching where possible (i.e. visibility c. 1–2 m). Within each site, an effort was made to search habitat likely to contain juvenile mussels (e.g. flow refuges and deeper sediment), however complete sediment excavations would be needed to fully assess abundance of young of the year and smaller individuals. As such, the methods employed are considered to be semi-quantitative. All live mussels collected were measured for longest shell length (SL) with digital Vernier calipers to the nearest 0.1 mm and returned to the water unharmed. The abundance of live mussels at each site was recorded as the number of individuals collected per hour and reported as catch per unit effort (CPU).

2.4. Data analysis

Salinity data from the lower hydrographic station were analyzed in River Analysis Package (RAP) to assess mean annual salinity in the Kent River over the period 1979–2017, as well as the frequency and duration of high salinity events in 15 year periods pre- and post-2002 (i.e. the endpoint of the previous assessment of salinity in the river; Mayer et al., 2005). Mann-Whitney U tests were used to compare the mean frequency and duration of recent annual salinity events (2003–2017) to historical levels (1987–2002 excluding 1991). Salinity data for 2018 from the lower hydrographic station were combined with data from Odyssey loggers to describe salinity dynamics around the confluence with Styx Creek.

The effect of all measured water quality parameters on mussel presence was tested using a generalized linear model, with a binomial distribution and a logit link function. Preliminary models indicated that temperature and turbidity explained very little of the variance in mussel presence, so they were removed from the final model, which included salinity, pH, dissolved oxygen and oxidation reduction potential. As our goal was to determine the relative importance of environmental factors, and not to develop a predictive model (Bumham and Anderson, 2002), we also used a multimodel inference approach to determine variable importance. The global model (including all four predictor variables) was used to generate a set of all possible models using the R package MuMIn (Barton, 2013). Models were ranked by the Akaike Information Criterion, and the importance of each variable was determined by the number of times it occurred within the top-ranked set (those within 4 AIC values of the best model).

Mean CPU, with bootstrap 95% confidence intervals, were calculated for sites where mussels were found. A Kruskal-Wallis test was used to compare mean CPU of mussels in sites on the Kent, Styx and Nile where mussels were found. Length-frequency data for Kent River sites were used to assess recruitment in the mainstem of the river using the proportion of individuals <54 mm SL (approximately 10 years of age (Klunzinger et al., 2014)) at each site as an indicator of recent recruitment. Differences in recruitment among sites were compared using a Kruskal-Wallis test. Post-hoc comparison was performed with pairwise Wilcoxon tests using Holm's adjustment. All statistical analyses were performed in R (R Core Team, 2018).

3. Results

3.1. Long term salinity trend in the mainstem

Salinity in the Kent River peaked near the turn of the century and has now begun to decline (Fig. 2a). In the 15 year period since 2002 the number of events exceeding the LC$_{50}$, LC$_{95}$, and 5 g/L thresholds for W. carteri has decreased significantly compared to the previous 15 year period (45.8%, 70.0%, and 65.0% decreases respectively; P < 0.05 for all, Fig. 2b). There was also a significant decrease in the number of days per year above those thresholds (70.1%, 88.1%, and 92.5% decreases respectively; P < 0.05, Fig. 2c). The longest event exceeding the LC$_{50}$ threshold lasted for 40 or more consecutive days in 1987, 1998 and 2002 (Fig. 2d). In that last event, the LC$_{95}$ and 5 g/L levels were also exceeded for 23 and 13 consecutive days respectively (Fig. 2e and f). No LC$_{95}$ or 5 g/L event has lasted longer than 5 and 2 days respectively since 2002. In 2010 and 2013 there were no events exceeding any threshold level for the first time since 1982.

3.2. 2017/18 salinity data in the mainstem

Mean daily salinity at Kds2 was below the LC$_{50}$ level (3 g/L) for all but two days of the sampling period peaking at ~3.92 g/L (Fig. 3a). Mean daily salinity at Kmid1 showed a similar temporal trend to Kds2, but was generally 0.5–1 g/L higher despite being <500 m upstream, peaking at 4.24 g/L (Fig. 3a). Mean daily salinity at Kmid2 was distinctly higher than at Kmid1 despite being <2 km upstream and there being no obvious freshwater inputs between them, and exceeded the LC$_{50}$ and LC$_{95}$ thresholds for the majority of the sampling period, peaking at 5.2 g/L.

The peak in salinity at all mainstem sites coincided with increased flow from the saline upper catchment at the onset of winter (Fig. 3b). Although flow stopped at the upper gauging station, in the lower reach it was maintained throughout the study period by freshwater input from Styx Creek (Fig. 3c). It was noted during field work that Nile Creek stopped flowing near the confluence by January (pers. obs. JB).

3.3. Mussel distribution and abundance in the mainstem and tributaries

No mussels were found at sites with salinity >1.48 g/L or with pH <4.5 (highly acidic; Degens et al. (2012)), and both factors had a significant effect on mussel distribution (GLM, P < 0.001; Table 1). Mussels were common at all sites in Nile Creek (82-465 CPU) as well as in the three lower sites in Styx Creek (115-194 CPU; Table 2). All other tributary sites lacked mussels despite being fresh (salinity <0.69 g/L); those sites were highly acidic and >20 degraded shells and various shell fragments were found in the sediment at S5. Mussels were only detected at five mainstem sites, four of which were downstream of Styx Creek while the fifth was 50 m upstream of the confluence. Kmid1 and Kds1 had the lowest CPU of all sites (8 and 38 mussels respectively) while Kds3 and Kds4 had among the highest CPU (404 and 505 mussels respectively). Numerous old shells were collected while snorkeling in deep pools (1–4 m deep) at the uppermost sites, Kus6 and Kus7, indicating that mussels were historically present at least that far upstream (~20 km and 30 km upstream of Nile and Styx Creeks respectively). There was no significant difference in mean CPU among reaches (Kent, Styx and Nile) using only sites where mussels were detected (Kruscal-Wallis: P > 0.05; Table 2).

3.4. Mussel recruitment at mainstem sites

Length-frequency distributions show that mussel recruitment has varied among mainstem sites over the past few decades, being historically limited at the three upper sites compared to the two lower-most sites. The proportions of larger individuals (54+ mm SL, equal to 10+
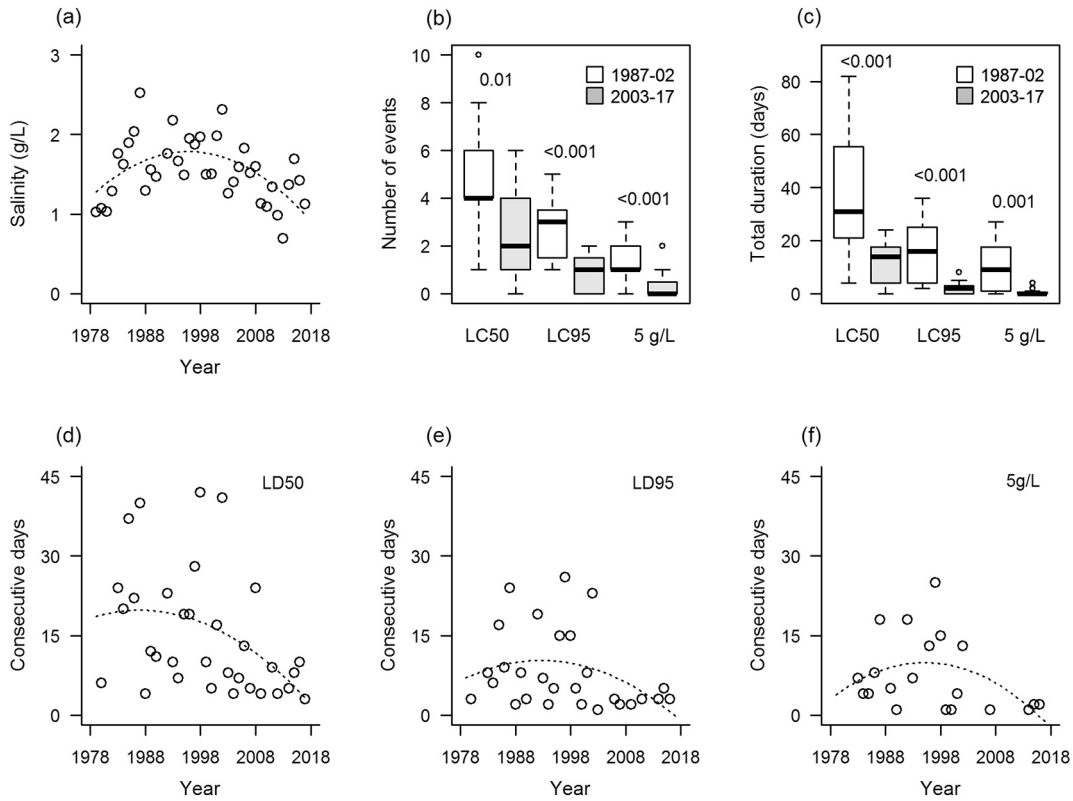
Fig. 2. (a) Mean annual stream salinity in the Kent River from 1979 to 2017. (b) Number of salinity events per year that exceeded various toxicity thresholds for *W. carteri* in the 15 year period up to 2002 (white boxplots) compared to the 15 year period after 2002 (grey boxplots), and (c) number of days per year exceeding those same thresholds. Probabilities refer to comparisons of numbers of salinity events or days exceeding threshold levels before and after 2002. (d–f) Longest salinity event each year that exceeded toxicity thresholds. Data were obtained from the Department of Water gauging station in the mainstem of the Kent River below the confluence with Styx Creek. Note, 1991 was excluded from all analysis due to uncertain quality of the data.

years of age) contributing to populations at Kmid1 (12.5%), Kds1 (21.1%), and Kds2 (25%) were not significantly different but were all significantly less than at Kds3 (69.9%) and Kds4 (80.4%) (pairwise-Wilcoxon test, P < 0.05 for all) (Fig. 4). Small *W. carteri* (<54 mm SL,

equivalent to <10 years of age) were present at all mainstem sites where mussels were detected, indicating that recent recruitment has occurred. No young-of-the-year were recorded at any of the sites sampled. All mussels found at Kmid1 were <54.8 mm SL, and are all likely
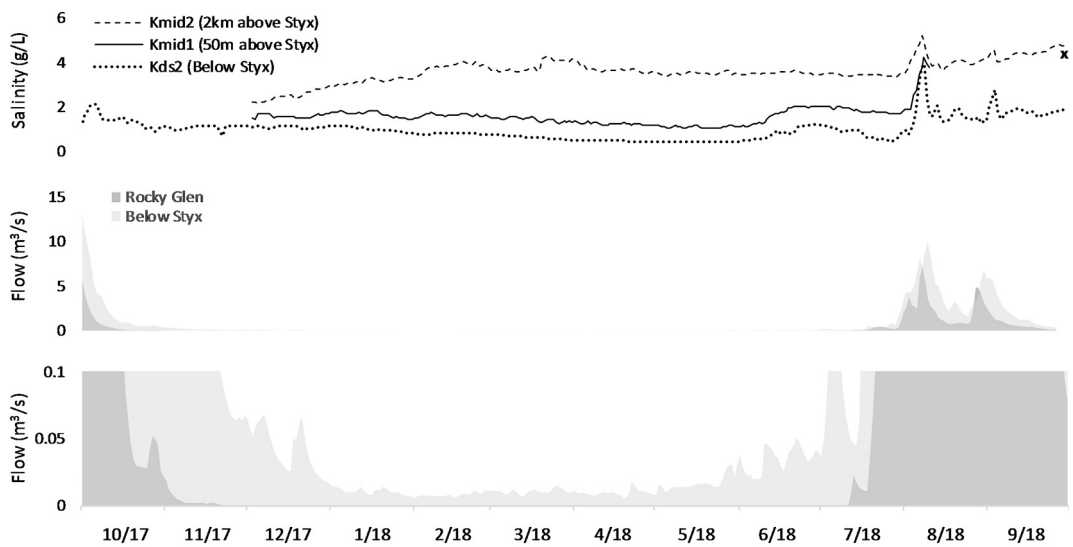

Fig. 3. The relationship between mean daily flow and salinity in the Kent River. (a) Mean daily salinity at three sites around the confluence with Styx Creek. Note, the logger at Kmid1 stopped working in August, so that series includes a spot measurement (x) taken on the final day during logger retrieval. (b) Mean daily flow at the upper (Rocky Glen) and lower (below Styx Creek) hydrographic stations on the mainstem of the river. (c) The same flow data magnified to show that flow stopped at the upper gauging station from late-2017 to mid-2018, while in the lower reach in was maintained by freshwater input from Styx Creek.
(Data obtained from: http://kumina.water.wa.gov.au/waterinformation/telem/stage.cfm).

Table 1

Association between water quality variables and *Westralunio carteri* presence ranked by importance (probability of being selected in the Akaike Information Criterion best-fit model), with coefficient estimates and standard errors (SE). Significant predictors (P < 0.05) are shown in bold.

Variable	Importance	Coefficient estimate (SE)	χ^2 (prob)
Salinity	**1.00**	**6.10 (2.64)**	**24.74 (<0.0001)**
pH	**1.00**	**−2.83 (1.32)**	**15.33 (<0.0001)**
ORP	0.55	0.02 (0.02)	2.23 (0.14)
DO	0.55	0	1.00

to be post-2002 recruits based on SL and examination of external annuli. Similarly, all mussels at Kds1 were <65 mm SL, and therefore likely to be <15 years old (based on Klunzinger et al., 2014; Benson et al., 2018).

4. Discussion

Reversing the effects of secondary salinization, and its impacts on aquatic biodiversity, is a growing global challenge (Cañedo-Argüelles et al., 2013; Herbert et al., 2015; Cañedo-Argüelles et al., 2019). This study adds to only a small number of examples of salinity caused by landscape clearing being successfully reversed through improved catchment management (Mayer et al., 2005), and we believe it is the first to detect the associated recovery of a freshwater mussel population. While *W. carteri* was not found in the majority of the Kent River, the presence of old shells at salinized sites shows that the species was once widespread, and that the current distribution likely reflects a contraction due to historical salinization. Mussels in the mainstem were limited to sites around and downstream of a permanently flowing tributary, suggesting that dilution from this source provides a freshwater refuge in the lower reach. At two of those sites, all individuals were likely <15 years of age, indicative of recolonisation coinciding with salinity reversal around the turn of the century. Interestingly, mussels clearly persisted in other parts of the lower reach throughout the peak salinity period, and those sites had among the highest densities recorded in this study. Overall, our results show that biodiversity in the catchment has taken a first step towards recovery from salinization, and highlights the importance of freshwater tributaries in providing both refuge from disturbance and a source of new recruits.

4.1. Spatial variability in mussel decline and persistence

Secondary salinization has dramatically altered the structure and function of freshwater ecosystems globally (Cañedo-Argüelles et al., 2013, Herbert et al., 2015, Cañedo-Argüelles et al., 2019), and is the primary driver of *W. carteri* decline in southwestern Australia (Klunzinger

et al., 2015). In this study, *W. carteri* was not found in the mainstem >50 m upstream of Styx Creek confluence, and the presence of old shells at our upper-most sites indicates that the species has been lost from at least 30 km of the river. Additionally, until the recent salinity reversal there was no recruitment occurring at sites immediately surrounding Styx Creek confluence, highlighting the precarious position of mussels in the mainstem. Without legislated control on land clearing, and revegetation of the upper catchment, salinity would have continued to rise and *W. carteri* could have been extirpated from the downstream reach as well.

In salinized catchments, freshwater tributaries serve as discrete areas of refuge and also dilute mainstem reaches (Beatty et al., 2011; Robson et al., 2013). This study found that *W. carteri* was abundant in the two largest tributaries of the Kent catchment, often being found within metres of the mainstem. Despite this proximity, mussels in the mainstem were limited to sites around and downstream of the confluence with Styx Creek. Interestingly, Styx Creek flowed continually throughout the study period, while Nile Creek stopped flowing by mid-summer, suggesting that permanent dilution from the Styx provides a flow-on effect for the lower reach that the Nile does not. This underscores the importance of conserving the integrity and permanence of freshwater flows in drying climates, which presents a significant challenge given the growing pressure on these resources from human population growth (Cañedo-Argüelles et al., 2013).

While the persistence of *W. carteri* in the Kent catchment can largely be attributed to the refuge provided by fresh tributaries, the species is also clearly more robust than first reported. Mussels were already known to occur in the lower reach (Klunzinger et al., 2012a), which is surprising given that mean annual salinities often exceeded 1.6 g/L in the 1980s and 90s, reaching >2.5 g/L in 1987 and surpassing 2.3 g/L as recently as 2002. Similarly, the frequency and duration of extreme events regularly exceeded laboratory derived toxicity thresholds for the species. Despite this, the largest mussels (up to 80 mm SL) collected at the lower-most sites are likely to be 35–80 years of age (based on Klunzinger et al., 2014), suggesting that they persisted there throughout the salinity peak at the turn of the century. Laboratory derived salinity thresholds for invertebrates have been shown to be strong predictors of their distribution in the field (Kefford et al., 2004; Horrigan et al., 2007), nonetheless, some species in those studies were still collected at salinities substantially higher than their LC_{50}. The findings of the present study add support to recent findings of Ma (2018) that *W. carteri*'s LC_{50} and LC_{95} thresholds may be substantially higher than previously reported when mussels are exposed to more natural high salinity conditions. Similarly, research in North America has shown that the salinity tolerance of *Lampsilis fasciola* glochidia is higher in natural surface water rather than reconstituted water used in many experiments (Gillis, 2011). The fact that the two lower-most sites had regular

Table 2

Catch per unit effort for *Westralunio carteri* (CPU; 95% Confidence Intervals) and water quality parameters (mean and SD) from sites in the Kent River catchment.

|	Reach	CPU 95% CI	pH	ORP	DO (%)	Salinity (g/L)	Turbidity (FNU)	Temp (°C)
Mainstem	Upstream of Nile	0	7.28 (0.63)	202.43 (58.25)	93.11 (13.71)	2.64 (1.17)	4.74 (7.88)	22.59 (3.59)
	Between Nile & Styx excluding Kmid1	0	7.39 (0.56)	172.02 (68.43)	88.34 (14.41)	2.57 (0.96)	4.62 (6.63)	22.0 (3.1)
	Downstream of Styx including Kmid1	43.6–393	6.93 (0.16)	156.54 (29.68)	66.52 (18.05)	1.35 (0.15)	2.7 (1.04)	22.2 (1.0)
Tributary	Small perennial tributaries 1 & 2	0	4.20 (0.39)	253.45 (63.43)	67.55 (5.59)	0.33 (0.03)	11.65 (1.77)	18.11 (4.08)
	Nile Creek	90–377	6.79 (0.76)	156.05 (52.87)	71.85 (17.5)	0.42 (0.07)	8.5 (3.41)	21.9 (4.5)
	Styx Creek: sites 4–6	0	4.09 (0.19)	229.63 (100.89)	84.9 (17.53)	0.61 (0.07)	15.13 (4.32)	24.2 (3.2)
	Styx Creek: sites 1–3	115–177	6.02 (0.59)	155.33 (9.4)	50.83 (39.54)	0.44 (0.08)	16.67 (3.98)	9.5 (1.6)
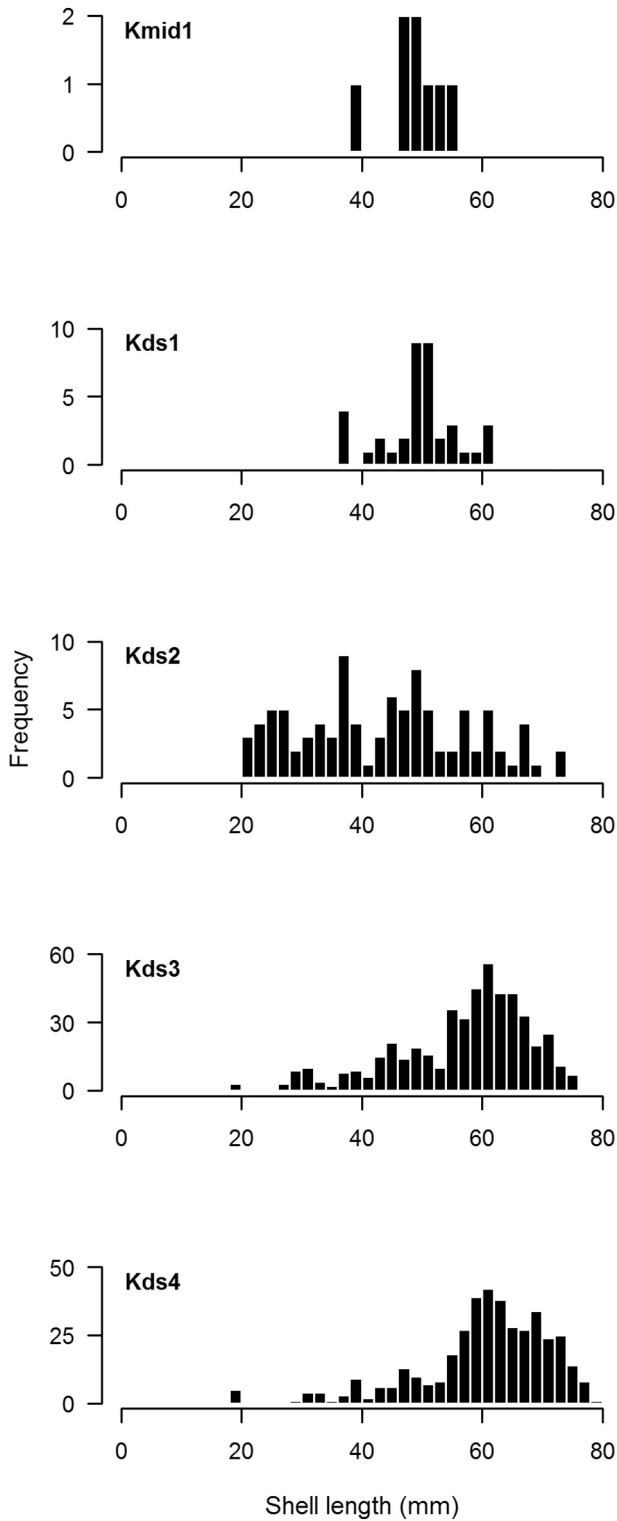
Fig. 4. Length frequency (SL) distributions for *Westralunio carteri* at five sites on the Kent River. Histograms are arranged from upstream (top) to downstream (bottom). The Styx confluence is intermediate to Kmid1 and Kds1. Note difference in y-axis values.

recruitment and among the highest densities in this study, while mussels were lost from other nearby sites in the lower reach, highlights the small-scale 'patchiness' that refuges may exhibit, and that mussel populations may appear robust even when they are in decline, depending on the spatial extent of the sites sampled.

While salinity appears to be the primary driver of *W. carteri* decline their distribution is also limited by a range of other natural and anthropogenic factors (Klunzinger et al., 2015). In this study, mussels were common at freshwater tributary sites ranging in pH from 5.4 to 7.5, however they were not detected at tributary sites where conditions were highly acidic (pH <4.5). Mollusks are often vulnerable to acidic conditions, in part because a high concentration of H^+ ions inhibits the uptake of calcium for shell building (Økland and Økland, 1986). Young mussels may be particularly susceptible, and laboratory experiments in North America have shown that one-week old juveniles of *Utterbackia imbecillis* and *Pyganodon cataracta* had a 96 h LC_{50} of pH 4.5, and that >50% died within 72 h at pH 4 (Dimock and Wright, 1993). It is not known whether the high levels of acidity at some sites in the present study occur naturally, or indicate some form of disturbance. Southwestern Australia is highly impacted by surface water acidification, a phenomena which is linked to rising saline groundwater, and point-source discharges caused by soil excavation (Halse et al., 2003; Degens et al., 2012). Numerous old mussel shells were detected at some acidic sites in this study, indicating their presence there in the past. Based on our data, if acidification of the upper part of Styx Creek is occurring, then the refuge provided by that tributary may have been reduced to <6 km of habitat.

4.2. Salinity reversal and mussel recruitment

Mussels are long-lived, slow-growing, and late to mature, so are unlikely to recover quickly from disturbance (Strayer et al., 2004), and there are very few studies on the recovery of mussel populations where they have been lost (Riccardi et al., 2016). In one of the few examples of recovery, Sietman et al. (2001) found that after mussels were extirpated from the Illinois River by urban waste pollution in the early 20th century, they did not begin recolonizing until the 1980s. Similarly, Riccardi et al. (2016) found that *Unio elongatulus* was not detected in Lake Orta for almost 90 years after an industrial pollution event wiped out most of the lakes biota in 1926. While other studies have found some evidence for invertebrate recovery following reductions in point-source salt pollution (Bäthe and Coring, 2011), species diversity and other biological metrics remain diminished in those rivers, and the time to full recovery is not known (Arle and Wagner, 2013). Additionally, point-source salt discharges represent a different mechanism from the landscape scale salinity studied here (sensu Zipper et al., 2016). In this study, we found that *W. carteri* was extirpated from, and has recently begun to recolonize, two of the five mainstem sites where mussels were detected. Based on age-at-length measurements for other populations of this species, lengths of >65 mm SL are not reached for >15 years (Klunzinger et al., 2014; Benson et al., 2018). No mussels of that size were found at the two recolonisation sites, while individuals of 65–80 mm SL dominated the population structure further downstream. The inferred age of the largest mussels from the two recolonisation sites suggests that they recruited post-2002, coinciding with salinity reversal in the river (Fig. 2). In combination, these results suggest that: (1) mussels in the lower reach of the river were approaching a tipping-point for extirpation before salinity began to reverse; and (2) recolonisation may begin relatively quickly when salinity is reversed.

While we believe that our results constitute the first evidence of recolonisation by freshwater mussels following salinity reversal, it is worth noting that recruitment may remain sporadic for some time. Young mussels (<54 mm SL) were common in the lower reach, however the two sites where recolonisation has occurred lacked a cohort in the 18–35 mm SL range, and no young-of-the-year were detected at any site. Although this could be due to difficulties in detecting smaller individuals or naturally sporadic recruitment (Hastie et al., 2000), it could also reflect annual fluctuations in salinity levels that have deviated from the overall trend of decline. For example, since 2012 the annual mean salinity has ranged from 0.69 to 1.69 g/L, with brief 5 g/L events each year from 2014 to 16 (Fig. 2).

4.3. Limits to mussel re-expansion

Despite increased recruitment in the lower reach, *W. carteri* distribution in the Kent River is still limited, and it remains to be seen just how far upstream they can recolonize. One factor that may be particularly limiting to mussel recovery is evapoconcentration, which is a growing concern in med-regions due to increasing aridity and longer periods of no-flow (Robson et al., 2013). Evapoconcentration has been implicated in the total loss of salt-sensitive fish species in other parts of southwestern Australia (Beatty et al., 2011). As with many med-rivers, the Kent is experiencing reduced flows and it is expected that no-flow periods will increase by >4-months per year (Barron et al., 2012). During the study period, the mainstem stopped flowing by mid-autumn, causing the lower reach (including Kmid1) to become fresher as it came under increasing influence of flow from Styx Creek. Above that point, salinity steadily increased (exceeding the LC_{50} for the majority of the study period), and no mussels were detected. Although our data show that mussels have survived at higher salinities at lower-reach sites in the past, the durations were never so prolonged. These extended periods of elevated salinity constitute a chronic exposure scenario that has not been evaluated in laboratory trials thus far. Interestingly, salinity at sites above Kmid1 never exceeded the higher, more recent laboratory-derived LC_{50} and LC_{95} values for the species, which were conducted over 30 days (Ma, 2018). Future laboratory experiments should consider chronic exposure scenarios, using lower doses over longer durations, and incorporate natural surface water and environmentally relevant mixtures of ions (Gillis, 2011; Kunz et al., 2013). Alternatively, rather than single contaminant laboratory trials, an in-field mesocosm approach might be effective, although salinity will be confounded with other stressors such as temperature.

Another issue potentially limiting mussel re-expansion is the interruption of *W. carteri*'s lifecycle by intra-annual variability in salinity levels. In many of southwestern Australia's rivers, salinity is elevated in winter-spring due to the increase in saline runoff from the catchment, which coincides with the release of glochidia by *W. carteri* (Klunzinger et al., 2012b). While salinity is known to be particularly toxic to the younger life-stages of some mussels (Prosser et al., 2017), and to reduce glochidial attachment to host-fish (Blakeslee et al., 2013; Beggel and Geist, 2015), this remains a key knowledge gap for the conservation of many species. Similarly, saline reaches may present a barrier to dispersal of salt-sensitive host-fish as some species, such as the critically endangered *Galaxias truttaceus*, are restricted to freshwater refuges in saline rivers (Beatty et al., 2011; Morgan et al., 2016). This fish species is believed to have driven *W. carteri* recolonisation via a fishladder in a reach of the nearby Goodga River (Benson et al., 2018), and is one of the few highly mobile fish in the region that would be capable of long-distance dispersal in the Kent River. There has been some research on the effect of salinity on fish species distribution in southwestern Australia (Beatty et al., 2011), however the impact this has on the connectivity of mussel populations is not well understood.

It is worth noting that while projected low rainfall scenarios will have negative effects on aquatic fauna by reducing freshwater inputs during summer base-flow (Beatty et al., 2011; Cañedo-Argüelles et al., 2013), the opposite may have also occurred here. Reduced saline-flow from the upper Kent River during summer-autumn has likely increased the freshening effect of Styx Creek on the lower reach in recent years. This may be in part responsible for the recent increase in mussel recruitment, and highlights the unpredictability of outcomes arising from interactions between large-scale stressors such as salinity and climate change (Cañedo-Argüelles et al., 2013; Estévez et al., 2019).

5. Conclusions

As one of the world's most heavily salinized landscapes (Halse et al., 2003; Pettit et al., 2015), southwestern Australia is providing a growing number of examples of salinity reversal through improved catchment management (Mayer et al., 2005), however, reversing the impacts on aquatic biodiversity represents an additional challenge (Arle and Wagner, 2013; Cañedo-Argüelles et al., 2013; Herbert et al., 2015). Mussels are important ecosystem engineers and their global decline is having measurable effects on ecosystem processes (Vaughn et al., 2015; Vaughn, 2018). The effects of mussel population recovery would be expected to be similarly detectable, however this has not been assessed. Similarly, the outcomes of these engineering activities may change with the altered environmental context (sensu Spooner and Vaughn, 2012), as pre-disturbance salinity levels may not be reached for many years, if at all.

While mussel populations are expected to recover slowly from disturbance (Strayer et al., 2004), this study has found some evidence of rapid recolonisation following salinity reversal. Despite this, the extent of recovery is very limited, and further re-expansion may not be possible due to other unknown factors. More research into the capacity for natural recovery is necessary. Secondary salinity will overlap and interact with other stressors such as acidification and climate change, making the outcomes of management hard to predict (Degens et al., 2012; Cañedo-Argüelles et al., 2013). In light of this unpredictability, freshwater tributaries globally, and particularly those in med-regions, will become increasingly valuable as refuges for aquatic fauna. Discussion of refuges in highly seasonal ecosystems generally focuses on areas of permanent water during drought (e.g. Magoulick and Kobza, 2003; Hermoso et al., 2013), however, more frequent and severe storms events could increase the regularity of hyper-saline runoff events, so refuge at high rather than low flow should also be considered (Robson et al., 2013). Maintaining the integrity and permanence of these refuges is essential, so appropriate conservation and restoration actions should be a priority for the management of freshwater biodiversity.

Declaration of Competing Interest

Acknowledgements

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