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ORIGINAL PAPER

The influence of abiotic and biotic conditions on lifecycle stages is critical for estuarine seagrass resilience

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Abstract

Abiotic and biotic factors influence seagrass resilience, but the strength and relative importance of the effects are rarely assessed over the complete lifecycle. This study examined the effects of abiotic (salinity, temperature, water depth) and biotic (grazing by black swans) factors on *Ruppia* spp. over the complete lifecycle. Structures were set up in two estuaries (−33.637020, 115.412608) that prevented and allowed natural swan grazing of the seagrasses in May 2019, before the start of the growing season. The density of life stage(s) was measured from June 2019 when germination commenced through to January 2020 when most of the seagrass senesced. Our results showed that swans impacted some but not all life stages. Seedling densities were significantly higher in the plots that allowed natural grazing compared to the exclusion plots (e.g. 697 versus 311 seedlings per m-2), revealing an apparent benefit of swans. Swans removed ≤ 10% of seagrass vegetation but a dormant seedbank was present and new propagules were also observed. We conclude that grazing by swans provides some benefit to seagrass resilience by enhancing seedling recruitment. We further investigated the drivers of the different lifecycle stages using general additive mixed models. Higher and more variable salinity led to increased seed germination whilst temperature explained variation in seedling density and adult plant abundance. Bet-hedging strategies of *R. polycarpa* were revealed by our lifecycle assessment including the presence of a dormant seedbank, germinated seeds and seedlings over the 8-month study period over variable conditions (salinity 2–42 ppt; temperatures 11–28 °C). These strategies may be key determinants of resilience to emerging salinity and temperature regimes from a changing climate.

Keywords Lifecycle · Herbivory · Salinity · Temperature · Colonising seagrass

Introduction

Identifying the drivers of resilience of foundation plant species, that have a key role in structuring ecosystems, is complex but projecting resilience to future conditions is even harder (Thrush et al. 2008; Duarte et al. 2009; Lefcheck et al. 2017). Understanding the conditions required for each life stage and for species to complete their lifecycles could improve these predictions (Radchuk et al. 2013). The increased emergence of extreme events is shifting the timing and duration of favourable conditions for the different stages of the lifecycle across global ecosystems (Wetz and Yoskowitz 2013; Hallett et al. 2018). Environmental changes are occurring more rapidly in shallow estuarine ecosystems with the potential to disrupt the lifecycles of foundation plant species (Oczkowski et al. 2015; Scanes et al. 2020). These disruptions can erode the resilience of foundation plant species and lower the amount and/or quality of ecosystem goods and services (Preen and Marsh 1995; Kendrick et al. 2019).

As foundation plant species play a significant role in determining ecosystem function, changes to their ecological resilience have major implications for the whole ecosystem (Kendrick et al. 2019). Ecological resilience is an emergent property that allows structure and function to be maintained following disturbances via two key processes: resistance and recovery (Levin and Lubchenco 2008). These processes of resistance and recovery manifest in different lifecycle stages, that enable populations to resist and/ or recover and ultimately, survive and reproduce (Brock 1982). Generally,

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more is known about the conditions that enable adult life stages to persist compared to earliest life stages (Kuiper-Linley et al. 2007). However, the conditions that enable the earliest life stages to transition are important to understand to clarify predictions about population dynamics and resilience with climate change (Duncan et al. 2019). Each life stage and/or transition can have distinct environmental requirements, meaning a suite of biotic and abiotic factors are likely to be important for completing the lifecycle and building resilience (Ren et al. 2012). Such knowledge can lead to more effective management actions such as optimisation of fire regimes for the longevity of plants in fire-prone regions (Bradstock and Auld 1995; Gordon et al. 2017). The combined performance of individual lifecycle stages in the context of the complete lifecycle will determine population persistence (Radchuk et al. 2013). This understanding for marine plant species, many of which depend on sexual reproduction for resilience, is presently lacking (Kendrick et al. 2022).

Colonising seagrass species commonly dominate estuaries and typically exhibit annual lifecycles, making substantial investment in sexual reproduction to produce dormant seeds (Kilminster et al. 2015). Seeds must transition through dormancy to germinate, and the seedlings go through multiple life history stages to reach maturity. Whether the plants complete their lifecycle and reproduce can be regulated by the differing responses of each life-history stage to abiotic and biotic factors (Strazisar et al. 2015), yet whether a factor is considered a key driver of resilience is commonly based on its effect on a single life stage (Rodríguez-Pérez and Green 2006; Tatu et al. 2007). However, the strength of effect of different stressors can differ (Mitchell and Wass 1996), they may interact (Bell et al. 2019) and positive effects on some life stages may compensate for negative effects on others. Thus, conclusions based on single life-history stages and stressors should be treated with caution; assessing effects across multiple life history stages is likely to be more accurate for determining whether these factors are key driver(s) of resilience (Hootsmans et al. 1987; Strydom et al. 2017).

In highly seasonal estuaries, the growing season for seagrasses can coincide with periods of intense fluctuations in both abiotic and biotic factors (Cho and Poirrier 2005). To cope with such variability, plant life cycles are generally annual, with rapid plant development to maximise sexual reproductive output and high densities of seeds (Brock 1983). Salinity and temperature changes are primary factors controlling seed germination, seedling establishment and growth (Xu et al. 2016; Cumming et al. 2017). Seagrasses are a major food source for herbivorous waterfowl so grazing might also influence the life cycle but often depends upon which part of the plant is consumed (Congdon and McComb 1981). For example, if leaves remain after grazing,

then plants can still photosynthesize, grow and reproduce but if all parts are removed, then recovery will proceed via germination of seeds (Jacobs et al. 1981; Nakaoka and Aioi 1999). If enough seeds germinate, complete their life cycle and reproduce, then the reductions of adults may not be negative for overall resilience. Most studies regarding grazing effects on seagrass have focussed on the response of adults, and so the impacts to earlier life stages, which could potentially compensate for the negative effects at the adult stage, are less well known (Lee et al. 2007; Rodríguez-Pérez and Green 2006; Bakker et al. 2016). Understanding the relative importance of plant–herbivore interactions and environmental conditions can inform conservation management actions for ecologically significant ecosystems supporting seagrasses and grazing waterfowl but is hampered by limited collection of in situ data (Rodríguez-Pérez and Green 2006; Tatu et al. 2007).

Southwestern Australia (SWA) has a Mediterranean climate and climate change effects are rapidly progressing in many of its estuaries (Hallett et al. 2018), raising concern for the ecosystems within them. Estuaries in the region are characterised by seasonal and inter-annual variation in environmental conditions which are expected to become more extreme under future conditions (Hallet et al. 2018). The estuaries are also habitat for herbivorous waterfowl (Choney et al. 2014) whose grazing pressure can be expected to interact with changes in abiotic conditions. There has even been speculation that drying of regional wetlands will force waterfowl to retreat to estuaries as refugia, intensifying grazing pressure (O'Dea et al. 2022). Whilst seagrasses are subjected to both abiotic and biotic pressures, phenological studies are relatively scarce and so the importance of these factors for the ability of species to compete their lifecycle remains unknown. In this study, we examined the response of several sequential life history stages of two seagrass species, *Ruppia megacarpa and Ruppia polycarpa*, to a range of abiotic and biotic factors in two seasonally hypersaline estuaries in Southwestern Australia. The goal was to understand the key drivers of resilience. Low biomass of *Ruppia* spp. in the estuaries in the past led to the proposal that swan grazing is a potential stressor of seagrasses (Chambers and Paice 2018). Using a manipulative field experiment, we quantified the effects of abiotic (surface and porewater salinity, water depth, temperature) and biotic (swan grazing) factors on the complete lifecycle of *Ruppia* spp. Plots were set up that excluded and allowed natural swan grazing and we collected data on the phenology of *Ruppia* spp. and environmental conditions over an annual cycle. We hypothesised that salinity and temperature would have the greatest impact on seeds and seedlings, and swan grazing would have the greatest negative impact on adults. Overall, we considered that the implications for resilience would depend on the successful completion of the life cycle.

Methods

The Vasse Wonnerup Wetland System (VWWS) comprises two seasonally hypersaline microtidal estuaries (i.e. the Vasse and the Wonnerup, Fig. 1) characterised by eutrophic and variable water quality conditions that are expected to worsen under future warming (Tweedley et al. 2019; McCallum et al. 2021). Both estuaries are shallow ($\leq$ 1.5 m depth) and characterised by dramatic fluctuations in water levels (0.2–1 m) driven primarily by inundation from rainfall and river flow in the wet season and evaporation rates in the dry but also influenced by flood gate operation which allows intermittent opening to the ocean (Chambers and Paice 2018, Fig. 1). In 1990, the VWWS was listed as a wetland of international significance under the RAMSAR convention due to the abundance and diversity of waterbirds that use the wetland to feed and breed including the largest breeding colony of black swans in SWA (Wetland Research and Management 2007). In the VWWS, *R. polycarpa* dominates and co-occurs with *R. megacarpa* and are preferred food sources for black swans (Kissane 2019). To assess the influence of environmental conditions and grazing on each lifecycle stage, a grazing exclusion experiment was conducted with regular sampling extending through the period from winter to summer at three sites in the Vasse estuary and three sites in the Wonnerup estuary (Fig. 1), to capture environmental variability between estuaries and amongst sites within each estuary. Sites within each estuary were selected to represent the range of seagrass distribution, where swans have been recorded feeding and to capture a range of water quality conditions; e.g. that vary with distance from flood gates (Chambers and Paice 2018). The estuaries are generally more dry and saline in summer and autumn, and more so in the upper sections that dry out over this period (March–May) resulting in salinity and temperature values of 90–132 ppt and 30 °C, respectively (Lane et al. 2011; Tweedley et al. 2014). There is usually broader variation in salinity within a year in the Vasse (e.g. annual range in 2016 was 0–99 ppt) compared to the Wonnerup (e.g. annual range in 2016 was 0–82 ppt) but Wonnerup generally has a higher median salinity (Online Resource Fig. 1). The variation in salinity may be due to a range of factors including differences in river flow with the Vasse estuary that is fed by three rivers (Sabina, Abba and Vasse) compared to the Wonnerup estuary which is fed by a single river, the Ludlow (Fig. 1).

In May 2019 at each site, two plots (7 × 5 m) separated by 1 m were established; one plot was 'grazing-excluded' to exclude swans from grazing based on the design of Tatu et al. (2007), and the other to act as a control

Fig. 1 Vasse Wonnerup estuaries located in Southwestern Australia within which seagrass monitoring sites (sites 1, 2, 3 in each estuary, $n=6$) and where sediment cores (to measure porewater salinity) were collected

Fig. 2 Photo of experimental plots set up at each site to evaluate the influence of access to grazing by black swans (grazing-allowed) or absence of grazing (grazing-excluded) on the abundance of seagrass lifecycle stages over time. Note that dashed lines have been overlaid on the image to make the boundaries of the 'grazing-allowed' plot more distinguishable
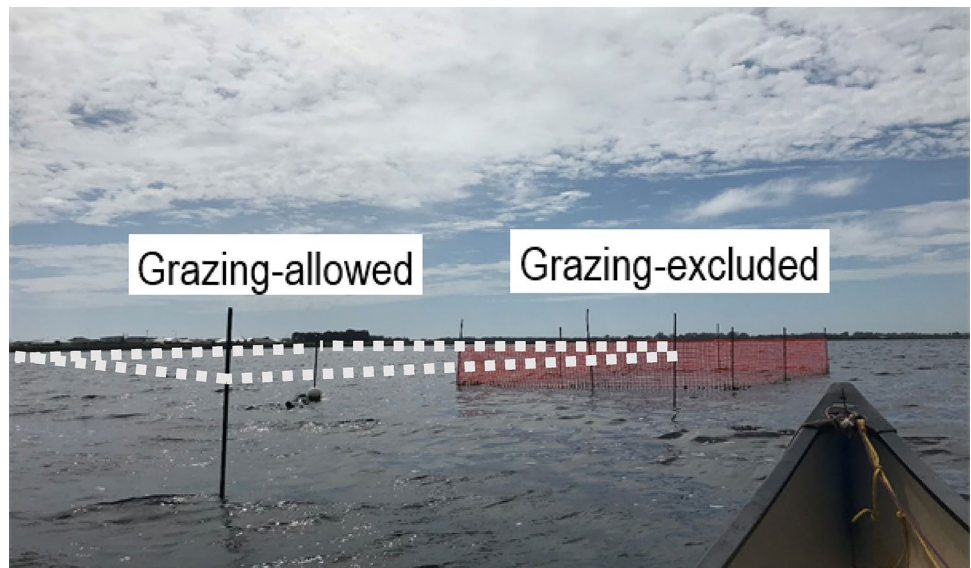
where grazing could occur, hereafter, 'grazing-allowed' (Fig. 2). The small addition of nutrients from swans would be unlikely to affect effect on the seagrass in the nearby plot as the estuaries are highly eutrophic (McCallum et al. 2021) and being shallow (< 1 m depth), highly susceptible to wind-driven forces such that inputs from birds would quickly become a dispersed input rather than localised. The plot perimeter was marked with ten metal reinforcing bars inserted into the sediment with barrier fencing composed of plastic barricade mesh secured onto the bars of the 'grazing-excluded' plot to prevent swans entering (Fig. 2). The fencing extended 1 m above the water surface to approximately 15 cm below the water surface when installed. Whilst black swans can feed on seagrass up to a depth of 1 m, 15 cm below the water surface was selected in favour of maintaining water flow and minimising the accumulation of plant matter that could break the fencing. Grazing scars were not observed within the plot areas suggesting that 15 cm below the water surface was an effective prevention. Plant debris accumulated on the barrier was removed during each monthly sampling trip to allow water flow but ensure swans could not enter. The height was modified when water levels changed. The 'grazing-allowed' plots lacked barrier mesh, allowing swans to enter and graze (Fig. 2). Sites were monitored monthly from June 2019, just prior to winter rainfall which was expected to be a trigger for germination (Vollebergh and Congdon 1986, pers comm Kath Lynch), to January 2020 when very little seagrass remained. During each monthly monitoring trip, measurements were taken in both plots at each site to estimate the density and state of different lifecycle stages: sediment seedbank, germinated seed and seedling density, canopy height, canopy cover, plant volume inhabited (PVI) and biomass. The presence

or absence of flowering and reproductive shoots was also recorded. Swans were observed entering and feeding in all grazing-allowed plots.

Seagrass cover, canopy height and percent volume inhabited (PVI)

Discriminating between *R. polycarpa and R. megacarpa* could only be done from seeds and seedlings so all measures relating to adult plants combine both species. Another macrophyte, *Stuckenia pectinata*, was also present and was included in the cover, canopy height and PVI measurements due to difficulties with distinguishing it from *Ruppia* in situ but was excluded for biomass. Snorkellers entered the plots on flotation boards to avoid disturbing or damaging the benthic habitat. Ten replicate measurements of cover and canopy height (20 × 30 cm) were taken haphazardly in each plot. Macrophyte cover (%) was estimated visually in 5% increments but on three monitoring trips, visual assessment of cover could not be used due to zero visibility, so the amount of seagrass was estimated by feeling the amount attached to the sediment within the 20 × 30 cm area. Maximum canopy height (cm) was measured on the longest leaf of an individual plant selected at random to the nearest 5 mm. Water depth was recorded in the middle of each plot to the nearest cm. Canopy height, cover and water depth measurements per 0.06 m² area were used to calculate 'plant volume inhabited' (PVI). PVI (m³) describes relative plant habitat to water volume, as per Canfield et al. (1984):

$$PVI = \frac{H \times C}{d} \times \frac{1}{A}$$

where H is canopy height (m), C is cover (%), d is the water depth (m) and A is the sample area (m²). When canopy

height was greater than the water depth, it was adjusted to the water depth measure so that PVI values never exceeded 100% of the water volume.

Seagrass seedbank and seedling density

Seedbank (the number of ungerminated seeds with intact seed coat), germinated seed and seedling densities were estimated from ten replicate cores (4.8 cm inside diameter, 15 cm depth) collected haphazardly in each plot at each time. Seagrass measurements in subsequent months were unlikely to be affected as the area sampled by the ten cores (0.06 m^2) amounted to 0.001% of the experimental plot area (35 m^2). Each sample was stored in a ziplock bag at 4 °C until processing, then flushed with freshwater through a 710 μm sieve to capture *Ruppia* spp. seeds that are typically > 1 mm in length (Brock 1982). Following Mason (1967), seeds and seedlings were identified to species. Germinated seeds were categorised as those that had an emerging cotyledon (> 1 mm but < 10 mm) whilst seedlings were those in which the leaf was longer than 1 cm and adventitious roots were present, indicative of successful establishment (Gu et al. 2018).

Seagrass biomass

Cores for seagrass biomass estimates were collected monthly from October 2019 to January 2020 to capture the pre-peak, peak biomass (generally November) and die-off periods (Chambers and Paice 2018). These cores were collected after the canopy height and cover measurements. To ensure the same area was never sampled more than once, each plot was divided into 35 × 1 m^2 squares, a unique set of five random numbers was generated using the RAND function in Excel and assigned for each month. Each core (9.6 cm Ø, 3 cm depth) was 'threaded' over the seagrass to capture all biomass originating from within the core area. Above ground (leaves, branches, flowers if present) and below ground (rhizomes, roots) material were stored in calico bags and, where possible, macroalgae was removed in situ. In the laboratory, epiphytic material was removed from the leaves by gently scraping with a microscope slide and any remaining macroalgae was also removed. *Ruppia* spp. was separated from other macrophytes and separated into above and below ground parts and dried at 60 °C for 48 h to achieve a constant weight. These dry weight (DW) values were standardised to m^{-2} for comparison with other studies.

Environmental parameters: benthic salinity and temperature, water depth and porewater salinity

At each site from June 2019 to January 2020, benthic salinity and temperature were measured continuously and discrete measures of water depth were taken at each site once per month. Discrete measures of porewater salinity were obtained but due to logistical reasons, measurements were commenced in July 2019 and measured once per month until January 2022.

To measure porewater salinity, one sediment core (9.6 cm Ø) was collected each month (June 2019 to January 2020) at each site just outside the plot area. The core was pushed into the sediment to a depth of 15 cm and then a bung was placed immediately at the bottom of the core, surface water was removed by siphoning and another bung was placed at the top of the core. Cores were secured upright and transported back to Edith Cowan University for processing. Within 48 h of collection, cores were sliced at 5 cm intervals and each slice was placed in a separate sealed bag and stored at 4 °C prior to processing. Porewater was extracted with a Wild-coTM sediment squeezer with 100 μm mesh and measuring salinity using a conductivity metre. Benthic salinity and temperature were measured at hourly intervals using loggers (HOBO™ U24-002-C) that were attached at the sediment water interface to a reinforcement bar reflecting the conditions seagrass was growing in at each site (hereafter, benthic salinity and temperature). The logging periods at Vasse site 2 (from July 2019) and Wonnerup site 2 (from August 2019) were shorter than at other sites due to logistical issues with initial deployment. Each logger was placed into a PVC pipe cover with holes to allow water flow but reduce biofouling according to the manufacturer's datasheet for logger deployment. Each month additional in situ water temperature and salinity measurements were taken with a Thermoscientific multimeter placed next to the logger for data calibration, and the loggers were cleaned and data retrieved. Each deployment's data set was calibrated with the in situ measurements from the beginning and the end of the deployment period using the non-linear, seawater compensation based on the PSS-78 scale via from the HOBOware® Pro Conductivity Assistant. The calibrated subsets of data were combined to create a dataset for each site for the available period and checked for quality by plotting the hourly salinity and temperature data. Additional information outlining the methods are given in the Online Resource (Tables 1 & 2).

Statistical analyses

For each lifecycle stage, there were ten measurements per plot in each of the eight time periods giving $N = 960$ for each variable, except biomass with five measurements per plot collected from October to January ($N = 240$). For each month, the measurements were averaged to create a value for each site within each estuary ($n = 3$ per estuary per month). Sites were considered the experimental units. To assess the effects of swan grazing on the density of each lifecycle stage, separate permutational analysis of variance was conducted

Table 1 Timing of lifecycle stages of *Ruppia* spp. observed (**1-** dormant seedbank, seed germination, seedlings, **2-** peak seedling density, **3-** flowering, **4-** peak biomass/ abundance and **5-** senescence) and associated minimum and maximum environmental conditions

Life stage	Months	Benthic salinity (ppt)		Porewater salinity (ppt)		Benthic temperature (ºC)		Water depth (m)	
		Min	Max	Min	Max	Min	Max	Min	Max
1	Jun to Jan	2	42	7	38	12	28	0.2	0.9
1	Jun to Jan	9	42	13	45	11	28	0	0.9
2	Jun	40	63	n.s.	n.s.	12	16	0.3	0.4
	Sep	2	4	5	10	14	23	0.7	0.7
	Jan	n.d.	n.d.	39	45	n.d.	n.d.	0.2	0.2
2	Jun	32	39	n.s.	n.s.	12	16	0.4	0.5
	Nov	12	22	15	24	17	28	0.6	0.7
3	Oct	16	24	2	14	16	24	0.7	0.7
3	Sep to Nov	10	22	13	17	14	28	0.6	0.9
4	Oct to Nov	2	8	7	11	16	28	0.6	0.7
4	Nov	12	22	15	24	17	28	0.6	0.7
5	Jan	21	46	33	45	20	25	0.2	0.2
5	Jan	34	44	36	67	23	27	0	0.1

Data shaded grey are for the Vasse estuary and data in white are for the Wonnerup estuary. Estuaries are located in Southwestern Australia. *n.s.* not sampled, *n.d.* no data. Timing was determined by either in situ observation during monthly sampling or from pairwise comparisons of 'Site x Month'.

Benthic salinity and temperature data collected by loggers deployed at three sites within each estuary ($n = 3$) whilst porewater salinity and water depth were discrete monthly measures taken at each site per estuary ($n = 3$)

with estuary, plot type (two levels: grazing-allowed, grazing-excluded) and month as fixed factors. Analyses for seed-bank, germinated seeds and seedlings were conducted for both species combined. The analyses for variables relating to adult plants (canopy height, cover, PVI, biomass) were for both species combined. For cover measures that were greater than 0% but less than 5% cover, a value of 2.5% was assigned for the statistical analysis. Prior to conducting the analyses, the assumption of homogeneity of dispersions was confirmed using PERMDISP (*p*-values < 0.05) on the raw data and if assumptions were not met, then transformations were attempted. If after transformation, the PERMDISP was still not met, the α-value was adjusted to 0.01. Following a significant main effect or interaction, post hoc pairwise tests were used to identify where those differences occurred. The aforementioned analyses were performed using PRIMER v7 multivariate software package with the PERMANOVA + add-on module (Clarke and Gorley 2006; Anderson et al. 2008).

To investigate which environmental variables best explained the spatial and temporal patterns in the different life history stages, generalised additive mixed models (GAMMs) were used. GAMMs offer the flexibility of being able to analyse a range of distribution and data types (e.g. continuous and categorical) and are useful for capturing non-linear relationships that frequently exist in ecology (Murase et al. 2009; Beatty 2019). Because each life history stage is a reflection of environmental conditions leading up to the time of sampling, we assessed the relationship between the abundance of the relevant life history stage and environmental variables integrated over different preceding time periods: long term ($\approx$ 32 days prior to the seagrass sampling) or short term (fortnight before sampling) at each sampling time (July–December 2019) (Online Resource 1, Table 2). Although seagrass sampling occurred in June 2019 and January 2020, no environmental data were collected in May and there was extensive logger malfunction in January so these periods were excluded from the analyses. Response variables were the densities of ungerminated and germinated seeds and seedlings, which were combined for both *Ruppia* spp., seagrass PVI and biomass. For each site, the response variable was represented by the mean of the ten measurements collected in each 'grazing-excluded' plots only, to remove the effects of grazing. This resulted in $N = 6$ for the short-term and long-term timesteps for each variable, matching the replication in the environmental data.

Water depth, salinity and temperature environmental data were selected for their influence on the distribution and abundance of *Ruppia* spp. (Brock 1986; Santamaría and Hootsmans 1998; Carruthers et al. 1999). Given the availability of continuous salinity and temperature data, we were able to calculate several metrics including: minimum, mean, maximum, variation (standard error) and rate of change. The mean of an environmental variable may not be the driver causing variability in the response variable but rather its variation, the rate of change or the maximum value (Ralph 1999; Steward et al. 2006; Fernández-Torquemada and Sánchez-Lizaso 2011; Griffin and Durako 2012). Of 22 possible variables considered, 17 were excluded due to strong collinearity ($r_s \geq 0.7$, Online Resource Table 3). The

Table 2 PERMANOVA Mains test for the effects of location, plot type and month on seedling density and percent volume inhabited of *Ruppia* spp in the Vasse and Wonnerup estuaries, Southwestern Australia

Seedling density	df	SS	P	PVI df	SS	P
Location	**1**	**599**	**0.0080**	1	0.14	0.3048
Plot type	**1**	**816**	**0.0024**	1	0.27	0.1588
Month	**7**	**7015**	**0.0001**	**7**	**67**	**0.0001**
Lo × Pl	**1**	**455**	**0.0206**	1	0.06	0.5205
Lo × Mo	**7**	**2123**	**0.0017**	**7**	**6**	**0.0001**
Plot type × Month	**7**	**2881**	**0.0002**	7	0.33	0.9232
Lo × Pl × Mo	7	736	0.2506	7	0.76	0.5798
Residual	64	5057		64	8	
Total	95	19,682		95	83	
Seedbank density				*Germinated seed density*		
Location	**1**	**5324**	**0.0321**	1	84	0.0940
Plot type	1	320	0.6050	1	82	0.0952
Month	7	6712	0.5549	**7**	**459**	**0.0413**
Lo × Pl	1	755	0.4280	1	8	0.5815
Lo × Mo	7	2801	0.9239	**7**	**461**	**0.0389**
Plot type × Month	7	4911	0.7395	7	72	0.9266
Lo × Pl × Mo	7	1174	0.9940	7	363	0.1043
Residual	64	72,863		64	1844	
Total	95	94,860		95	3372	
Canopy height				**Cover** ($\alpha = 0.01$)		
Location	**1**	**0.06**	**0.004**	**1**	**0.10**	**0.0008**
Plot type	1	0.02	0.1381	1	0.005	0.4332
Month	**7**	**4**	**0.0001**	**7**	**2**	**0.0001**
Lo × Pl	1	0.0002	0.5816	1	0.0006	0.7884
Lo × Mo	**7**	**0.1**	**0.0146**	**7**	**1**	**0.0001**
Plot type × Month	7	0.05	0.45	7	0.02	0.9069
Lo × Pl × Mo	7	0.08	0.1758	7	0.12	0.0456
Residual	64	0.5		64	0.5	
Total	95	4		95	4	
Biomass						
Location	**1**	**77**	**0.0158**			
Plot type	1	3	0.6812			
Month	**3**	**216**	**0.0021**			
Lo × Pl	1	14	0.3531			
Lo × Mo	**3**	**171**	**0.0050**			
Plot type × Month	3	6	0.9469			
Lo × Pl × Mo	3	13	0.8504			
Residual	32	436				
Total	47	935				

Statistically significant factors are indicated in bold (unless otherwise stated: $\alpha = 0.05$)

variables retained were water depth, long-term (monthly) average temperature, long-term rate of change in temperature, long-term average salinity and coefficient of variation in salinity which were considered surrogates for the collinear variables. To reduce model overfitting and aid in data interpretation, the maximum number of predictors was limited to three, pairwise correlation tests were run automatically and models with correlated predictors (> 0.28) were included in each model set but never in the same model (Graham 2003). The GAMMs analyses were run in the R Language for Statistical Computing (version 4.0.5 Core Team 2021). The full subsets 'FSSgam' package (Fisher et al. 2018) was used and is based on the 'mgcv' (Wood 2011) and 'gamm4' packages (Wood and Scheipl 2016). The Akaike Information Criteria corrected for small samples sizes (AICc) and adjusted r^2 were considered during model selection. Models within two

Table 3 Outputs of the generalised additive mixed model analyses for predicting the condition of seagrass variables each month (July 2019–January 2020)

Response variable	Models (sig. of predictor)	AICc	delta.AICc	wi.AICc	r^2	r^2-sq-adj	# Basis functions (k)	Deviance explained (%)
Germinated seeds	Average salinity (p=0.012)	233.70	0.00	0.41	0.18	0.147	3	17.20%
	Average salinity + coefficient of variation in salinity (0.3126)	234.64	0.93	0.26	0.20	0.10	3	19.70%
Seedlings	Average temperature (p=0.0003) + rate of change temperature (p=0.11)	257.23	0.00	0.47	0.42	0.329	3	42.10%
	Average temperature (p=7.7e-5)	257.61	0.38	0.39	0.37	0.282	3	37.30%
Percent volume inhabited	Average temperature (p=2e-16) + rate of change temperature (p=0.005)	119.05	0.00	0.87	0.81	0.78	3	82.50%

AICc Akaike Information Criterion corrected for small sample sizes, delta. *AICc* AIC differences, wi.AICc- model weights, and coefficient of determination (r^2). The most parsimonious model with the best predictive power is shaded and best supported models are within 2 delta. AICc. Family = Gaussian, link = identity

AICc of the lowest AICc (or best fit model) were considered (Burnham and Anderson 2002). The importance of each predictor was calculated by summing the AIC weights across all best-fitting models involving that variable (Burnham et al. 2011). In GAMMs, errors are assumed to be mutually independent; however, time series of environmental data may be autocorrelated. Therefore, models were also tested for temporal autocorrelations using the 'acf' and 'pacf' functions, which revealed low autocorrelation removing the need for an autocorrelation term in the models. Model quality was also assessed using the 'gam.check' function within the mgcv package.

Separate GAMMs models were tested for each lifecycle stage (seedbank, germinated seeds, seedlings and adult plants). The null model (null.terms) included site specified as a random effect. Model coefficients were estimated using restricted maximum likelihood (REML) method. All response variables were square root transformed except for biomass which was log(X+1) transformed. The distribution of the predictor variables was visually inspected, and square root transformations were applied to all of them except for the rates of change which were not transformed. Based on the distribution of the response variables, a Gaussian error distribution with an identity link function was selected for the GAMMs.

Results

Environmental conditions and phenology of *Ruppia* species

Environmental conditions varied over the annual cycle with distinct trends for each variable (Online Resource 1, Fig. 2). Average temperature increased progressively over the study period in both estuaries (15 °C in June to 27 °C in January). Benthic salinity was highest in June with values of 60 ppt and 40 ppt in the Vasse and Wonnerup and then declined reaching a minimum of 2 ppt in September in the Vasse and 5 ppt in the Wonnerup in October before increasing progressively for the remaining months (Fig. 3). Porewater salinity followed a similar pattern to benthic salinity but the range was less, from 6 to 48 ppt. Water depth followed an inverse pattern to benthic salinity. It increased from June, ~0.4 m to maximums in August, September (0.85 m) and then declined to January (0–0.9 m) and some sites in the Wonnerup had completely dried out (Fig. 4).

The phenology of *Ruppia* spp. was generally similar in the two estuaries. Seedlings were absent during a reconnaissance survey in autumn (May) but were observed in winter (June) when salinity was high and temperature was low (42 ppt, 12 °C, Table 1). Germinated seeds and seedlings were observed for all remaining time periods under a variety of conditions (Table 1) but the time of peak seedling densities differed slightly between estuaries: in the Wonnerup, this was winter (June) during high salinity–low temperature conditions and again in spring (November) in low salinity–high temperature conditions (Table 1); whilst in Vasse, this was also winter and spring (June, September) but additionally in January under high salinity–temperature conditions (Table 1). Thus, seedling emergence occurred under a wide range of environmental conditions. From September,
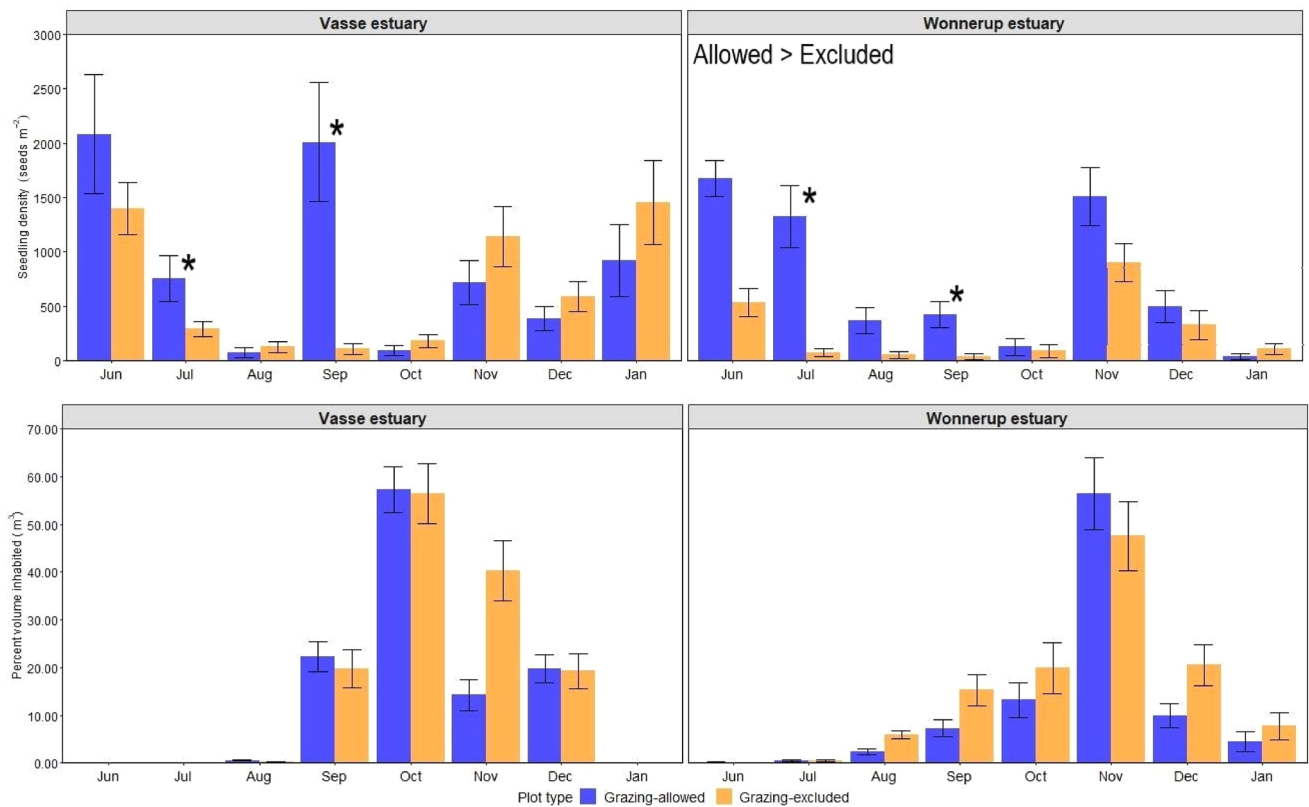
Fig. 3 Seedling density (top panels) and PVI (bottom panels) of *Ruppia* spp. measured in plots that allowed grazing ('Grazing-allowed') and where grazing was excluded ('Grazing-excluded') between June 2019–January 2020 in sites in the Vasse and Wonnerup estuaries, Southwestern Australia. Stars indicate the month that significant differences between plot types occurred (pairwise tests; $p < 0.05$). Values are average ($\pm$ SE)

extensive branching was observed amongst plants in both estuaries and in some cases, plant length reached or exceeded the top of the water column (0.6–0.7 m depth). Flowering and production of seed-bearing shoots began in September for the Wonnerup and in October for the Vasse, for a duration of 2–3 months. Flowers and seed-bearing shoots coincided with minimum benthic and porewater salinity (16 ppt; 1.5 ppt in the Vasse and 10 ppt; 13 ppt in the Wonnerup), maximum water depth (0.75–0.92 m) and temperatures between 18.5 and 20 °C (Table 1). Vegetation peaked mid-spring (October) in the Vasse and late-spring (November) in the Wonnerup when salinity had increased but was still low (~ 10 ppt) and temperature was still increasing (22.6 °C, Table 1). Early senescence began as water levels dropped at the start of summer (December) and was completed by mid-January when sites had either dried out or were very shallow (< 0.25 m, Table 15). By then, temperatures exceeded 27 °C and porewater salinities were 45 ppt and 67 ppt in the Vasse and Wonnerup, respectively (Table 1). The lifecycle duration of *Ruppia* spp. from seed germination to senescence was 7 months with a seed dormancy period of at least 5 months. These stated durations are approximate based on monthly

sampling from June to January and the observation that no seedlings were present in May when the initial site visit was conducted.

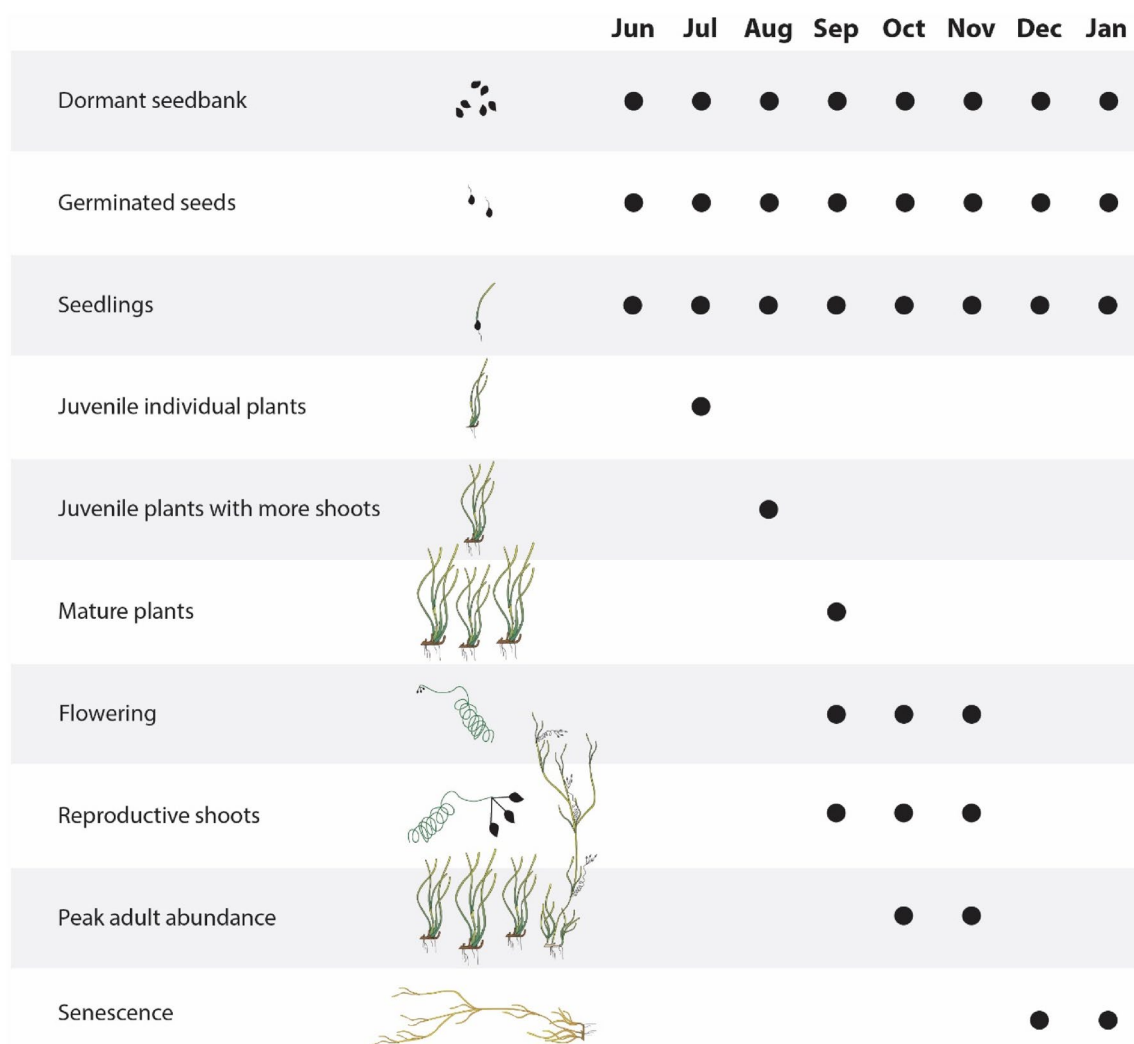## Effects of grazing on lifecycle stages of Ruppia

Grazing scars were observed at all sites in the 'grazing-allowed' plots from September to January, inclusive. The seedbank (17,574 total), germinated seeds (142 total) and seedlings (1110 total) were dominated by *R. polycarpa*, by 78%, 96% and 81% of the total, respectively. Of *R. megacarpa* found, all germinated seeds and > 60% of seeds and seedlings were found in the Wonnerup. Average seedling density ranged from 37 ± 26 to 2081 ± 547 seedlings m^{-2} (Fig. 5) with significant interactive effects of location and plot type (Lo x Pl, $p < 0.05$). For the Wonnerup estuary, seedling density was significantly higher in the grazing-allowed (746 ± 72) plot compared to the grazing-excluded plot (267 ± 38) (Fig. 3). There was also a significant interactive effect of plot type and month, with pairwise comparisons indicating significantly higher seedling densities in the grazing-allowed plot compared to the

Germinated seeds

Seedlings

PVI
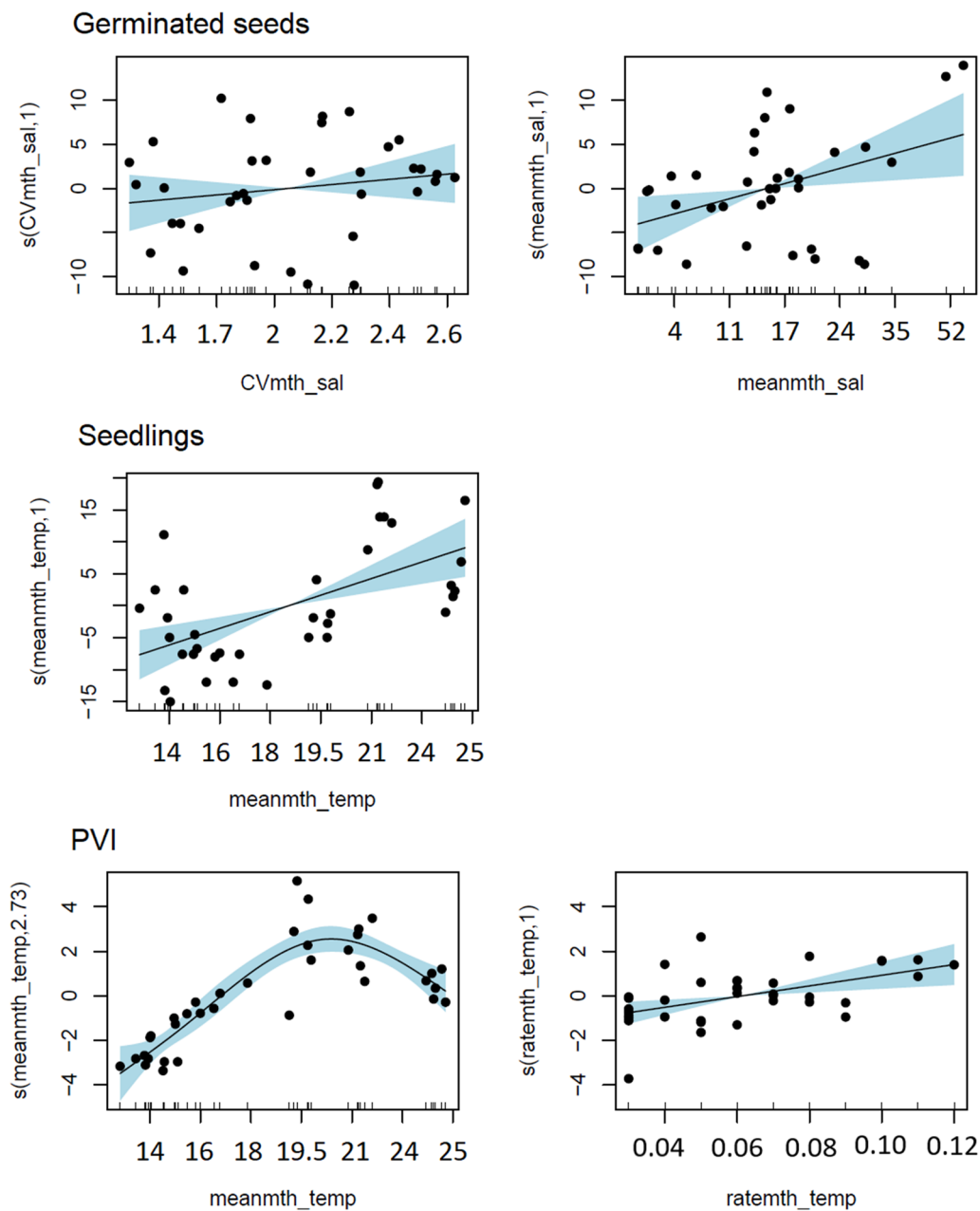
Fig. 4 Generalised additive mixed models (GAMMs) relating the smoothed effects of environmental variables on seagrass germinated seed density, seedling density and percent volume inhabited. Shaded regions indicate 95% confidence intervals

grazing-excluded plot in July (1041 ± 181 versus 184 ± 41) and September irrespective of Estuary (PTxMo, $p < 0.05$, Table 2; Fig. 3). PVI increased from June, peaked in October in the Vasse (57 ± 5 m³) and in November in the Wonnerup (~56 ± 8 m³) and then declined with little remaining in January (Fig. 3). Month had a significant effect on PVI ($p < 0.05$) and no other factors were significant (Table 2). Plot type, or the interaction with any other factors, was not significant for the remaining seagrass variables. There was no effect of grazing on seedbank density, germinated

seeds or biomass. Seedbank density varied significantly between estuaries (Table 2), with the density in the Vasse estuary being significantly greater than the Wonnerup (10,906 ± 336 versus 9322 ± 417 seeds m⁻²). Location and month interacted to affect germinated seed densities, cover, canopy and biomass; significant differences occurred between the estuaries in some months and not others (Table 2). For instance, biomass was significantly higher in the Vasse compared to the Wonnerup in October (36 ± 11 versus 19 ± 30 g DW m⁻²).

		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Dormant seedbank		●	●	●	●	●	●	●	●
Germinated seeds		●	●	●	●	●	●	●	●
Seedlings		●	●	●	●	●	●	●	●
Juvenile individual plants			●						
Juvenile plants with more shoots				●					
Mature plants					●				
Flowering					●	●	●		
Reproductive shoots					●	●	●		
Peak adult abundance						●	●		
Senescence								●	●

Fig. 5 Conceptual diagram with timing, presence and duration of the lifecycle stages of *Ruppia polycarpa* indicated by ● observed between June 2019 and January 2020 in the Vasse and Wonnerup estuaries, Southwestern Australia

Effects of environmental conditions on Ruppia lifecycle stages

Temperature and salinity metrics (average, rate of change, variation) best explained the spatial and temporal patterns in seagrass response variables apart from seedbank density and biomass where no models were supported (Table 3). Either one or two models were best supported.

Two models predicted the germinated seed density. The first model included average salinity which explained 17.2% of the total deviance ($p = 0.012$, r^2-sq-adj $= 0.147$, Table 3). The second model included variation in salinity and explained 19.7% of the deviance in germinated seeds ($p = 0.019$, r^2-sq-adj $= 0.10$, Table 3). There was a positive effect of increasing average salinity on the density of germinated seeds between 2 and 52 ppt (Fig. 4). Similarly, higher densities of germinated seeds were associated with more

variation in salinity with a change in salinity from $+0.2$ to 1.4 ppt day^{-1} (Fig. 4). Average salinity was a slightly stronger predictor (0.372) than the variation in salinity (0.325) with the remaining predictors assigned low importance (< 0.1, Table 3).

For seedling abundance, a model including the average temperature and rate of change in temperature was supported but the latter was not a significant predictor ($p = 0.11$, Table 3). Average temperature on its own was the next top model, explaining 37.3% of the deviance in seedling abundance (r^2-sq-adj $= 0.354$, Table 3). Increasing average temperatures between 13.6 and 24.7 °C was associated with greater seedling abundances (Fig. 4). Average temperature and rate of change in temperature were assigned equal importance (0.534, Table 4).

Average temperature and rate of change in temperature were significant predictors of average PVI explaining

Table 4 Variable importance of environmental predictors from the generalised additive mixed model (GAMMs) analyses for predicting various lifecycle stages of *Ruppia* spp. (*Ruppia polycarpa* and *Ruppia megacarpa*) in Southwestern Australia.

Response variable	Water depth	Average salinity	Variation in salinity	Average temperature	Rate of change temperature
Germinated seeds (sqrt)	0.075	0.674	0.293	0.104	0.053
Seedlings (sqrt)	0.001	0	0.135	0.861	0.474
PVI (sqrt)	0	0	0.027	0.973	0.871

Colour scale indicates relative importance: most important = purple to least important = light yellow

82.5% of the deviance (Table 3) but average temperature was more important (0.973 versus 0.871, Table 4). There was a positive effect of increasing average temperatures on PVI but only up to 21 °C after which further increases caused reductions in PVI (Fig. 4). Higher PVI was associated with increased changes in temperature between +0.03 and 0.12 °C day^{-1} (Fig. 4).

Discussion

This study represents the first complete lifecycle assessment of the key habitat former *Ruppia* for two variable estuaries, demonstrating that grazing, temperature and salinity influenced different stages of the lifecycle, but not always as expected. Swan grazing did not have a significant negative impact on adult plants. Seedling abundance increased in the presence of natural swan grazing in one estuary across time and only during some months in the other estuary. These results reveal a benefit for seagrass resilience since more seedlings can enhance population recovery (Olesen et al. 1994). Our approach that considered grazing effects at all life stages provided increased confidence that swan grazing was not a major stressor to seagrasses in this system and at this time. We also found evidence that *Ruppia* is likely to be resilient to the changes in salinity associated with climate change as germinated seeds and seedlings were present over a broad range of salinity, temperature and water depth conditions. This is indicative of bet hedging. Studies in variable terrestrial (Fan et al. 2018) and aquatic environments (Vollebergh and Congdon 1986) suggest these bet-hedging strategies are critical for the resilience of annual plant populations over multiple generations.

Swan grazing benefits seagrass resilience

The relative importance of herbivores in seagrass ecosystems can be difficult to predict due to the influence of multiple factors including grazing intensity (Dos Santos et al. 2013). We hypothesised that if swan grazing was a major stressor of seagrasses, then the abundance of each or more lifecycle stages would be significantly greater without grazing. Unexpectedly, plots grazed by swans had significantly higher seedling densities which could be related to grazing scars created by swans which form 'pits' in the sediment and

that were observed during most months (September–January). It is possible that more seeds became 'trapped' in the pits created by swans in the 'grazing-allowed' plot meaning there were simply more seeds available to germinate (Zipperle et al. 2010). It is also possible that swans may have moved seeds up the sediment profile but this could not be substantiated as we did not measure the change in burial depth of seeds. Ailstock et al. (2010) showed that seeds at shallower depths are more likely to germinate and become established seedlings but this was not evaluated in relation to grazing. Despite the grazing scars observed in the plots, there was no effects of grazing, and seagrasses would likely have been able to recover from grazing, given vegetative material was retained, a dormant seedbank was present and new seeds were produced (Jacobs et al. 1981). Overall, our results highlight that swan grazing is not a major stressor of seagrass in this system but rather, at least at the current intensities, is beneficial for seagrass resilience since seedling recruitment aids seagrass recovery (Olesen et al. 1994). It may be important to consider these results in the context of variability in swan abundance, both within and amongst estuaries. Swan numbers varied substantially over the sampling period and in all previous sampling seasons. During the study period (June 2019–January 2020), the average number of swans observed across the entire system was higher (342) than in most other seasons (158–232) (Online Resource Table 5). It is possible that the positive effects of swan grazing on seagrass may not be as strong when swan numbers are lower, or negative effects may be significant at higher swan numbers (Dos Santos et al. 2013).

Waterfowl abundance and grazing intensity could increase on permanent waterbodies as the extent and number of ephemeral wetlands decline under a drying climate (Roshier et al. 2001). Other studies have shown that increasing grazing intensity can reduce resilience (Dos Santos et al. 2013). To explore the potential for grazing to have negative effects in the future, we calculated the time it would take for swans to completely graze or remove 20% of seagrass biomass, a percentage loss associated with a decline in resistance (Dos Santos et al. 2013). An average seagrass consumption rate was not available for *Ruppia* spp. and so a rate of 394 g dry mass (DM) swan^{-1} d^{-1} was used based on data for grazing of *Zostera* which also exhibits colonising characteristics (Dos Santos et al. 2013). Based on seagrass biomass and swan abundance in November (2018–2019), the peak time for both

attributes, we estimated it would take 2,411 swans 39 days to graze 20% of the biomass or 197 days to graze out both estuaries entirely (Online Resource 1, Table 6). Swans migrate seasonally to the VWWS to breed, so it is unlikely that such a high number of swans remain in the system over an annual cycle and swan count data from 2015 to 2020 clearly supports this (Online Resource 1, Fig. 3). Alternatively, it would take 3,122 swans1 month to graze out 20% of the biomass (Online Resource 1, Table 6). The average densities of swans in December 2018 and 2019 was 3,204 suggesting this scenario may be more plausible. However, in this system, a 20% reduction in biomass may not necessarily equate to a reduction in resilience in the following season because the biomass of *Ruppia* spp. is reduced to zero over each annual cycle anyway and especially if seeds are produced and a seedbank is present. On the other hand, Lekammudiyanse et al. (2022) found that grazing which occurred prior to flowering caused significant reductions in flowering densities of *Z. muelleri* and concluded that this likely reflects the re-allocation of resources away from reproduction towards vegetative growth. Reductions in flowering would likely impact seed set. Recruitment and ongoing persistence of *Ruppia* can depend on recurrent seed set (Strazisar et al. 2016). We conclude that it is unlikely that grazing pressure would result in significant loss of *Ruppia* from the VWWS, but the potential complexity of outcomes warrants further investigation, with emphasis on sexual reproductive stages of the life cycle and ideally, extending over multiple annual cycles where seasonal variation in environmental factors, consumption and herbivory can be taken into account (Ren et al. 2012; Heck et al. 2021).

Salinity and temperature are important drivers of the lifecycle of Ruppia

Note that less than 5% of the total number of germinated seeds found were *R. megacarpa*, making it difficult to draw conclusions about the germination requirements of this species (although freshwater has been reported as a requirement (Brock 1982)), and so the remainder of this discussion pertains to *R. polycarpa*. Low salinities generally promote germination of estuarine seagrass seeds (Orth et al. 2000), seedling growth and survival (Strazisar et al. 2013). Germinated seeds of *R. polycarpa* were first observed in June when conditions were hypersaline and remained present at all sites over the entire experiment, with surface water salinities ranging from 1 to 60 ppt. The number of germinated seeds also rose with both increasing average salinity and when there was more variation in salinity over a month. Similarly, Vollebergh and Congdon (1986) observed in situ germination of *R. polycarpa* when salinity was 40‰ TDS in autumn which was the start of the growing season. The authors concluded that the ability to germinate under

hypersaline conditions had important implications for population persistence since seeds that germinated early in the season would be exposed to favourable conditions for longer, be more likely to mature and reproduce. Early stages of the lifecycle, including seed germination and seedling establishment, are vital for species persistence (Donohue et al. 2010). We predict that early timing of germination and the ability to germinate over a broad range of salinities could increase the resilience of *R. polycarpa* to higher and more variable salinities associated with a drying climate.

Temperature is recognised as an important regulatory factor for seagrass growth (Lee et al. 2007) and we assumed that it would control adult plant abundance (Moore et al. 2014). Seedling density increased with rising average temperatures from ~ 14 to 25 °C. This wide optimal range for seedling emergence is beneficial, given temperatures continuously rise over the growing season. Variation in seedling emergence is another form of bet hedging as it spreads the risk of seedling failure and increases the likelihood of a proportion surviving and reproducing in habitats characterised by temporal unpredictability (Fan et al. 2018). Gu et al. (2018) reported 30 °C as being optimal for seedling establishment of *R. sinensis* so it is possible that seedlings of *R. polycarpa* may tolerate higher temperatures than were observed here. The abundance of adult plants rose with increasing average temperature and increasing variation in temperature and then declined at temperatures ≥ 21 °C suggesting the temperature for optimum growth may be closer to 20 °C. This is similar to the values reported for *R. maritima* (Koch and Dawes 1991) and *R. drepanensis* (Santamaría and Hootsmans 1998). We do not consider the apparent sensitivity of the adults to temperature concerning for resilience as the plants grew rapidly, reached sexual maturity within 3 months and were able to set seed before the negative effects of warming became evident. However, sexual reproduction can be inhibited by temperature and the ability of *R. polycarpa* to reproduce under warmer temperatures associated with climate change will likely be a key determinant for resilience. For *R. drepanensis,* the optimal temperature for flowering and seed production was 20 °C with no flowers produced at 14 °C and only a few flowering at 30 °C but no seeds produced after approximately 4 months (Santamaría and Hootsmans 1998). We recommend that future studies investigate the likelihood of seeds and/ or seedlings transitioning to become reproductive adults under different temperature scenarios.

Complete lifecycle assessment reveals bet-hedging strategies of Ruppia

In habitats characterised by fluctuating environmental conditions, survival and reproduction are likely to be greatest for individuals able to complete their lifecycles quickly and over

a broad range of environmental conditions (Brock 1982). Early life history stages, including dormant seeds, germinated seeds and seedlings, were present over the entire study period (Fig. 5) and under different combinations of salinity, temperature and water depth: high salinity/low temperature/ high water depth, low salinity/high temperature/high water depth and high salinity/high temperature/low water depth. Prolonged germination and seedling emergence under multiple combinations of conditions reduce the risk of losing an entire cohort when conditions suddenly change (Cohen 1966) and is proposed to maintain shoots, and overall fitness, during fluctuations with seasonal wetting and drying (Mannino and Graziano 2016). These strategies are indicative of bet hedging and may confer resilience to more persistent hypersaline conditions associated with drying, but for this to be realised, the resultant juveniles must survive and reproduce. Seed set occurred between September and November (Fig. 5) under conditions of low salinity–warm temperatures and, combined with the rapid decline of adults from November onwards, suggests that reaching sexual maturity during early spring (September) may be critical for the persistence of *R. polycarpa* populations. This could be due to the sensitivity of flowering and seed production to temperature, as has been reported for *R. drepanensis* (Santamaría and Hootsmans 1998). Sim et al. (2006) observed higher adult mortality under increased salinity conditions. The same individuals were not tracked in our study; however, we did observe adult senescence when temperature and salinity had both increased in December and January (Fig. 5). The conditions in this study likely do not represent those predicted to emerge under a more extreme climate so we stress the need for experiments to be designed with these scenarios in mind and with more focus on determining the salinity and temperature thresholds that enable sexual reproduction. Experiments should use the same individuals from the start, through each transition, to completion of the lifecycle to determine the overall response to climate change (Radchuk et al. 2013).

Conclusion

By examining the complete lifecycle, our results confirmed that swan grazing does not negatively impact seagrass resilience. We revealed a benefit of swan grazing through enhanced seedling recruitment that was not previously known as assessments tend to focus swan grazing effects on later life stages. The continuous seed germination and seedling emergence of *R. polycarpa* over the study period, linked to a broad range of salinity and temperature conditions, indicates bet-hedging strategies that increase resilience to variable environmental conditions. Since early life stages are often key determinants of population persistence,

these results, in combination with rapid growth and seed set of adult plants, imply that *R. polycarpa* may be well placed to cope with drier and warmer conditions. These findings lay the foundation for examining how emerging salinity and temperature regimes may impact seagrasses. Gathering information pertaining to abiotic and biotic thresholds for each lifecycle stage is recommended as part of the toolbox for predicting seagrass response to climate change.

Declarations

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