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PRIMARY RESEARCH PAPER

Shelter availability reduces the effects of the invasive Red Swamp Crayfish (*Procambarus clarkii*) on eelgrass-dominated clear-water lakes: a mesocosm approach

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Abstract Shelter availability is one of the key features governing crayfish habitat quality. It can directly influence crayfish's individual survival of by lowering the risk of predation, but the ecosystem-wide impacts of sheltering on water quality are largely unknown. To test the effects of shelter availability for *Procambarus clarkii* in clear-water macrophyte-dominated lakes, we performed a 24-day mesocosm experiment in 20 tanks (4 with one crayfish with and without shelters, 4 with two crayfish with and without shelters and 4 controls). The bottom of each tank was almost completely covered by the eelgrass *Vallisneria denseserrulata*. Compared with the treatments with shelters, more broken leaves occurred in the treatments without shelters at both crayfish densities at equivalent crayfish numbers, and total phosphorus was higher in the treatments without shelters. Total suspended solids and total nitrogen concentrations were higher in the treatments with two crayfish without shelters than in those with shelters, whilst these variables did not differ between treatments in the mesocosms

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with one crayfish only. Our results suggest that shelter availability reduces the activity of crayfish (e.g. movement and burrowing) and agonistic behaviour, thereby decreasing the negative effect of the invasive *P. clarkii* on water quality in *V. denseserrulata*-dominated clear-water lakes.

Keywords Biological invasions · *Procambarus clarkii* · Submerged macrophytes · Shelters · *Vallisneria denseserrulata*

Introduction

The Red Swamp Crayfish *Procambarus clarkii* (Girard, 1852) is one of the most widespread invasive species in Chinese subtropical and tropical freshwater ecosystems (Zhan et al., 2016). According to Penn (1954), 100 specimens of Red Swamp Crayfish were carried from New Orleans to Japan in 1927, of which only 20 arrived alive and were introduced to a pond near Tokyo (Penn, 1954; Kawai & Kobayashi, 2005). Two years later, Red Swamp Crayfish were translocated from Japan to Nanjing, China (Dai, 1983) and rapidly spread to most provinces of China where they established dense populations (Wang, 1999). This non-native crayfish has contributed to a decline in submerged macrophyte biomass in aquatic ecosystems in Asia (Jiang et al., 2007; Zeng et al., 2013) and decreased the aquatic insect diversity of invaded wetlands (Watanabe & Ohba, 2022).

As ecosystem engineers, Red Swamp Crayfish impact the aquatic nutrient dynamics, community composition and ecosystem processes through bioturbation. Their feeding, movement and burrowing activities increase sediment resuspension (Angeler et al., 2001; Geiger et al., 2005; Matsuzaki et al., 2009). Red Swamp Crayfish alter the physical habitat of benthic sediments as they forage and/or build shelters (Ottolenghi et al., 2002; Statzner et al., 2000, 2003; Creed & Reed, 2004; Albertson & Daniels, 2016). They reduce the standing stock of macrophytes through direct consumption (Rodríguez et al., 2003; Alcorlo et al., 2004) or by increasing water turbidity through sediment resuspension (Haubrock et al., 2019). They can also diminish the macrophyte biomass through non-consumptive plant shredding (Nyström et al., 2001; Gao et al., 2021). The omnivorous Red Swamp Crayfish also feeds on animals such as amphibians, fish and other invertebrates (Gherardi et al., 2001; Gherardi, 2006). Accordingly, their invasion can lead to a severe increase in phytoplankton abundance, shifting lakes from a macrophyte-dominated clearwater to a turbid state (Rodríguez et al., 2003; Matsuzaki et al., 2009; van der Wal et al., 2013; Oficialdegui et al., 2020).

Agonistic behaviour, i.e. aggressive encounters with conspecifics, of Red Swamp Crayfish can also alter the physical features of benthic habitats (Moore, 2007). Red Swamp Crayfish compete for shelters as a critical resource as they offer protection from predators, conspecifics and environmental changes (Figler et al., 1999; Martin III & Moore, 2010). An increasing number of artificial shelters reduces intraspecific competition and cannibalism amongst Red Swamp Crayfish (Mason, 1979; Matsuzaki et al., 2009). Although the agonistic behaviour of crayfish has been studied extensively in laboratory settings and the field (Issa et al., 1999; Gherardi & Cioni, 2004; Moore, 2007), the impact of shelter presence on water quality is largely unknown.

We conducted a mesocosm experiment to elucidate the effects of the invasive Red Swamp Crayfish on a submerged macrophyte, the eelgrass *Vallisneria denseserrulata* (Makino) Makino, which is widespread in Asia, and the physical and chemical properties of lake water in the presence and absence of shelters. *V. denseserrulata* is distributed mainly in southern China (Chen et al., 2008; Wang et al., 2010). *Vallisneria* species also play important roles in the maintaining and stabilising of freshwater ecosystems, such as providing food for waterfowl, nursery habitats for fishes and substrates for invertebrates. They also contribute to self-purification and water quality (Li et al., 2005) and are therefore used frequently to restore freshwater ecosystems (Korschgen et al., 1997; Liu et al., 2018; Zhang et al., 2021). Nowadays, *V. denseserrulata* is frequently planted to recover Chinese eutrophic shallow lakes because (1) it is green all the year round and (2) it is not growing to the surface, which otherwise might create problems to use the lake for recreation purposes (Liu et al., 2018). We hypothesised that shelter availability would reduce the negative effects of crayfish on *V. denseserrulata* and the water quality in lakes with a clear-water macrophyte-dominated state. Examining the behaviour of Red Swamp Crayfish may increase our understanding of the environmental impacts of this invasive species, which may potentially be important to lake management.

Materials and methods

Experimental mesocosms

The mesocosm experiment was performed in 20 circular plastic tanks containing sediment and water (60 cm upper diameter, 50 cm bottom diameter, 70 cm height, 15 cm sediment depth, 50 cm water depth). The tanks were placed in a transparent organic glass-covered outdoor experimental house without walls. Ground sediment (0.61 mg g^{-1} total nitrogen [TN], 0.65 mg g^{-1} total phosphorus [TP]) was obtained from the Xunsi River, an outlet channel of a shallow lake in Wuhan City. The sediment was air-dried, and coarse debris was removed. Then, it was mixed using a 10.0 mm × 10.0 mm mesh sieve. The characteristics of this sediment were similar to most lakes with Red Swamp Crayfish invasions. An approximately 15-cm-thick layer of homogenised sediment was added to each tank, and the tanks were then filled with tap water (1.08 mg l^{-1} TN, 0.11 mg l^{-1} TP), exposed to natural sunlight and equilibrated for 2 days. Subsequently, 35 V. *denseserrulata* (height≈45 cm) were planted in each tank. After 5 weeks, *V. denseserrulata* covered most of the tank bottom and the experiments began. The experiments lasted from 7 October to 1 November 2019, and the tanks were exposed to natural sunlight for the entire experimental duration. At approximately 10 am every three days, the water temperature was measured using an YSI meter (YSI ProPlus, Yellow Springs, OH, USA). The water temperatures were 19.7 ± 0.2 °C (mean value ± SD), 19.9 ± 0.4 °C, 22.3 ± 0.2 °C, 20.2 ± 0.2 °C,19.4.0 ± 0.3 °C, 19.9 ± 0.3 °C, 19.8 ± 0.2 °C, 19.7 ± 0.2 °C and 19.9 ± 0.2 °C in the nine samplings.

To investigate the effects of shelter availability, four three-way plastic pipes (5 cm diameter, 9 cm length) were added as shelters. The number of plastic pipes was thus greater than the number of crayfish. One adult male Red Swamp Crayfish (≈5 ind. m^{-2}) was randomly added to each of four mesocosms without (1-CF) and with plastic pipes (1-CF + S). Two adult male Red Swamp Crayfish (≈10 ind. m^{-2}) were randomly added to each of another four tanks without (2-CF) and with plastic pipes (2-CF + S). The remaining four tanks held no crayfish and served as a control treatment (CK treatment). The Red Swamp Crayfish were directly collected by cage from a crayfish culture pond and then were maintained in 80 l tanks with *V. denseserrulata* for two weeks before being added to the tanks. We did not feed the crayfish during the experimental period. However, some naturally hatched invertebrates such as snails, zooplankton and dragonfly larvae were observed in the tanks during the experiment period.

Sampling and analysis

At approximately 10 am every three days, water samples were collected from 10 cm below the water surface in the middle of the tank for nutrient and chlorophyll *a* (Chl *a*) analyses. The samples were analysed according to Chinese standard methods (China EPA, 2009), which correspond to US standards (APHA, 1998). TN and TP concentrations were determined spectrophotometrically after digestion with persulphate, TP following the ammonium molybdate method and TN with the hydrochloric acid method. Chl *a* concentrations were determined spectrophotometrically after sample filtration through cellulose acetate filters and extraction of the filtered material with 90% acetone. Suspended material was filtered onto GF/C (pore size 1.2 µm) filters to measure total suspended solids (TSS) after drying at 105 °C for 24 h.

The number of broken leaves floating on the water surface of each mesocosm was recorded every three days. Broken leaves were defined as the opposite of intact leaves, i.e. leaves with an intact leaf tip or a leaf length ≥ 5 cm. At the end of the experiment, all *V. denseserrulata* were harvested by hand after emptying the tank. The biomass (wet weight), mean leaf length and number of *V. denseserrulata* individual plants per tank were recorded.

Statistical analyses

All statistical analyses were performed using SPSS 19.0 (Statistical Product and Service Solutions, USA), and the significance level was $P < 0.05$.

Time series data, including broken leaves, TSS, TN and TP, were analysed by repeated-measures ANOVA (RM-ANOVA) with time as the repeated factor. If a significant difference was observed, a Bonferroni post-hoc test was used to detect which treatments differed. All data sets were examined for homogeneity of variances using Levene's tests. One-way ANOVA was performed to detect differences amongst pairwise comparisons on each sampling occasion. If a significant difference was observed, the Bonferroni test was used to detect differing treatments. For one-way ANOVAs, all data sets were examined for normality. In some cases, the variance was not equally distributed, and Tamhane's T2 test was used to assess the differences amongst groups.

We used Pearson's correlation to test for relationships between the number of broken leaves of *V. denseserrulata* and water quality parameters. Results indicated that the radiance data were significantly correlated with water quality parameters if the significance level was $P < 0.05$.

Results

Effects of crayfish on *V. denseserrulata*

The effects of the various treatments on the number of broken leaves of *V. denseserrulata* differed (RM-ANOVAs, treatment effect, d.f. = 4, $F = 280.0$, $P < 0.01$) (Fig. 1). No broken leaves were observed in the controls during the experimental period. More broken leaves occurred in the 2-CF than in the 2-CF + S, 1-CF, 1-CF + S and treatments (Bonferroni's Multiple Comparison, $P < 0.01$; Fig. 1). The number of broken leaves diminished in the following order: 2-CF > 2-CF + S > 1-CF > 1-CF + S > controls (Bonferroni's Multiple Comparison, $P < 0.01$; Fig. 1).

Effects on TSS and phytoplankton biomass

The effects of the various treatments on TSS concentrations (TSS) differed from each other (RM-ANOVAs, treatment effect, d.f. = 4, $F = 432.1$, $P < 0.01$; Fig. 2). Compared with the treatments, TSS were lowest in the controls during the experimental period (Bonferroni's Multiple Comparison, $P < 0.01$; Fig. 2). Higher TSS occurred in the 2-CF treatment than in the 2-CF + S, 1-CF, 1-CF + S treatments, as well as in the 2-CF + S treatment than in the 1-CF and 1-CF + S treatments (Bonferroni's Multiple Comparison, $P < 0.01$; Fig. 2). TSS did not differ amongst the 1-CF + S and the 1-CF treatments (Fig. 2).

The effects of the various treatments on Chl *a* concentrations (Chl *a*) differed from each other (RM-ANOVAs, treatment effect, d.f. = 4, $F = 61.4$; Fig. 3). Higher Chl *a* concentrations occurred in the 2-CF than in the other treatments, and it was also higher in the 2-CF + S than in the 1-CF + S treatments (Bonferroni's Multiple Comparison, $P < 0.01$; Fig. 3). Chl *a* did not differ between the control and 1-CF + S treatments, or between 1-CF and 1or 2-CF + S treatments.

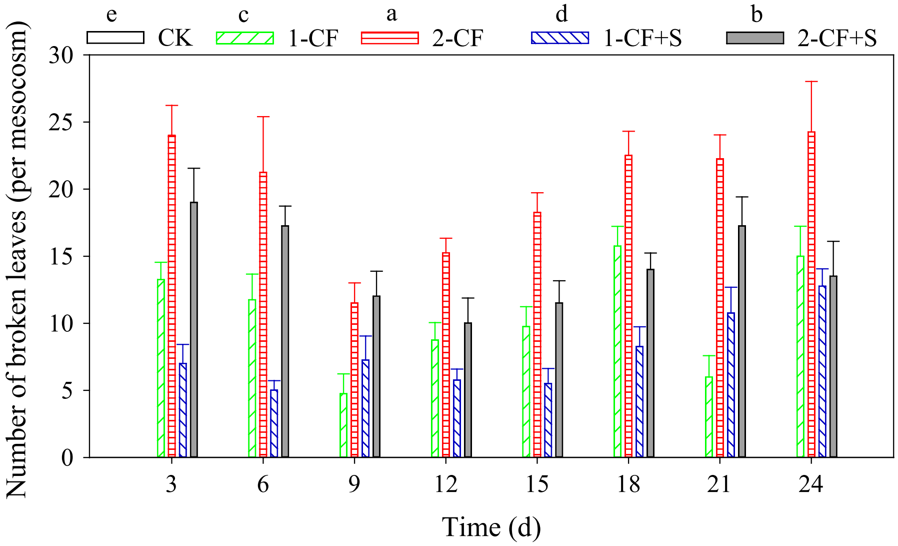


Fig. 1 Mean (± SD, $n = 4$) number of broken leaves of *Vallisneria denseserrulata* in the different treatments. *1-CF* one crayfish, *2-CF* two crayfish, *1-CF + S* one crayfish with shelters, *2-CF + S* two crayfish with shelters and CK control. Treatment labels sharing a lowercase letter indicate no significant differences between treatments at $P > 0.05$

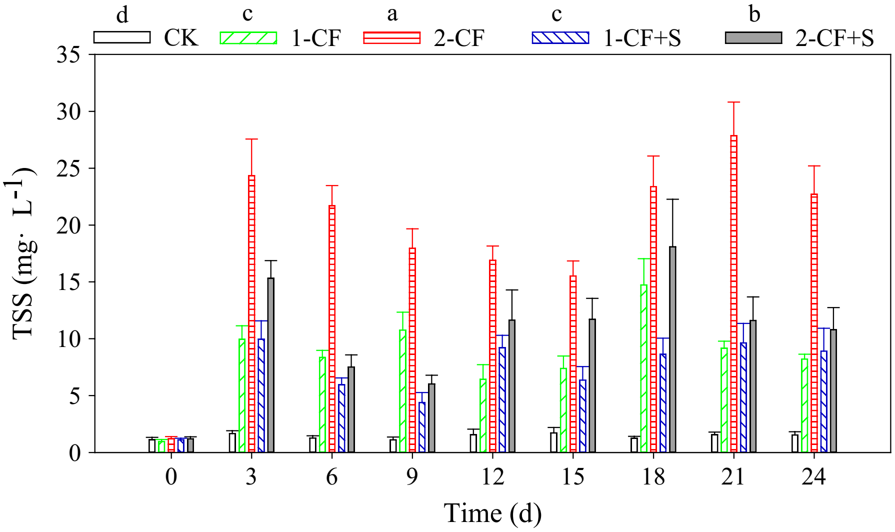


Fig. 2 Mean (±SD, $n=4$) total suspended solid (TSS) concentrations in the different treatments. *1-CF* one crayfish, *2-CF* two crayfish, *1-CF+S* one crayfish with shelters, *2-CF+S* two crayfish with shelters and CK control. Treatment labels sharing a lowercase letter indicate no significant differences between treatments at $P > 0.05$

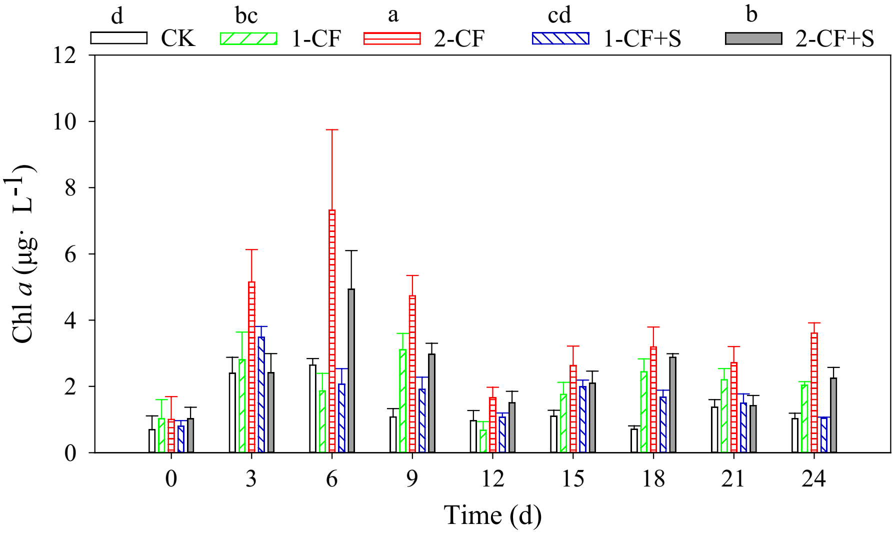


Fig. 3 Mean (±SD, $n=4$) chlorophyll *a* (Chl *a*) in the different treatments. *1-CF* one crayfish, *2-CF* two crayfish, *1-CF+S* one crayfish with shelters, *2-CF+S* two crayfish with shelters and CK control. Treatment labels sharing a lowercase letter indicate no significant differences between treatments at $P > 0.05$

Physical and chemical characteristics

The effects of the various treatments on TN concentrations (TN) differed from each other (RM-ANOVAs, treatment effect, $\text{d.f.}_{\text{TN}} = 4$, $F_{\text{TN}} = 59.6$, $P < 0.01$) (Fig. 4). The effects of the various treatments on TP concentrations (TP) also differed from each other (RM-ANOVAs, treatment effect, $\text{d.f.}_{\text{TP}} = 4$, $F_{\text{TP}} = 208.2$, $P < 0.01$) (Fig. 5). Compared with the crayfish treatments, TN and TP were lower in the controls during the experimental period (Bonferroni's Multiple Comparisons, $P < 0.01$; Figs. 4 and 5). Higher TN and TP occurred in 2-CF than in 1-CF and 2-CF+S (Bonferroni's Multiple Comparisons, $P < 0.01$; Figs. 4 and 5). TN did not differ between 1-CF+S and 2-CF+S or between 1-CF and 1-CF+S (Fig. 4). Higher TP occurred in 1-CF than in 1-CF+S as well as in 2-CF+S than in 1-CF+S (Bonferroni's Multiple Comparisons, $P < 0.01$; Fig. 5).

Relationships between number of broken leaves and environmental factors

Significant positive correlations were observed between environmental factors (TSS or Chl *a* or TN

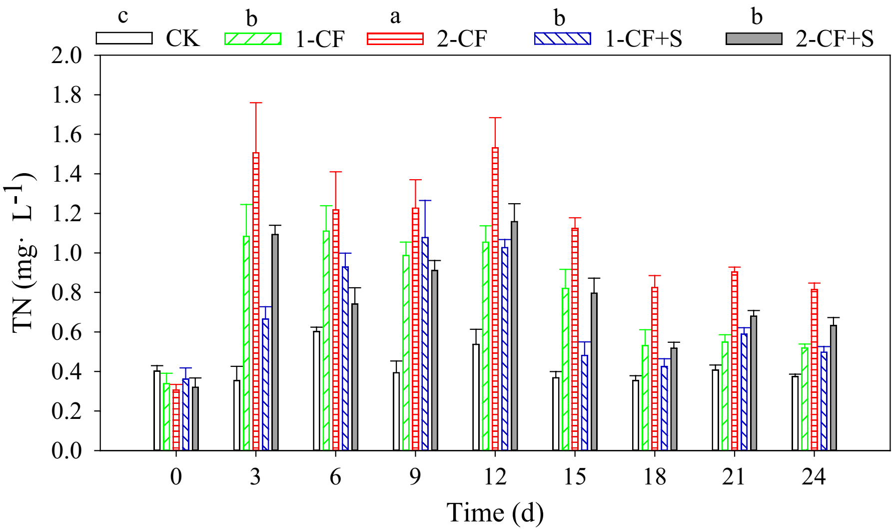


Fig. 4 Mean (± SD, *n* = 4) water column total nitrogen (TN) in the different treatments. *1-CF* one crayfish, *2-CF* two crayfish, *1-CF + S* one crayfish with shelters, *2-CF + S* two crayfish with shelters and CK control. Treatment labels sharing a lowercase letter indicate no significant differences between treatments at $P > 0.05$

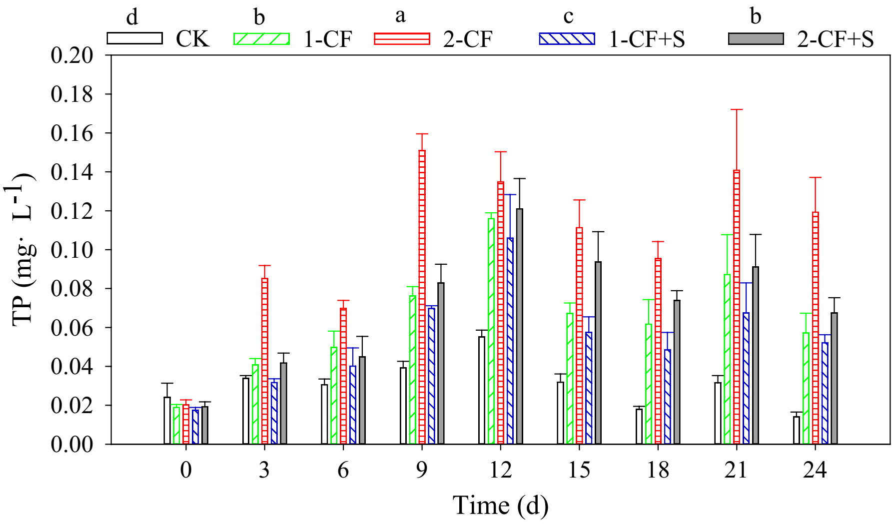


Fig. 5 Mean (± SD, *n* = 4) water column total phosphorus (TP) in the different treatments. *1-CF* one crayfish, *2-CF* two crayfish, *1-CF + S* one crayfish with shelters, *2-CF + S* two crayfish with shelters and CK control. Treatment labels sharing a lowercase letter indicate no significant differences between treatments at $P > 0.05$

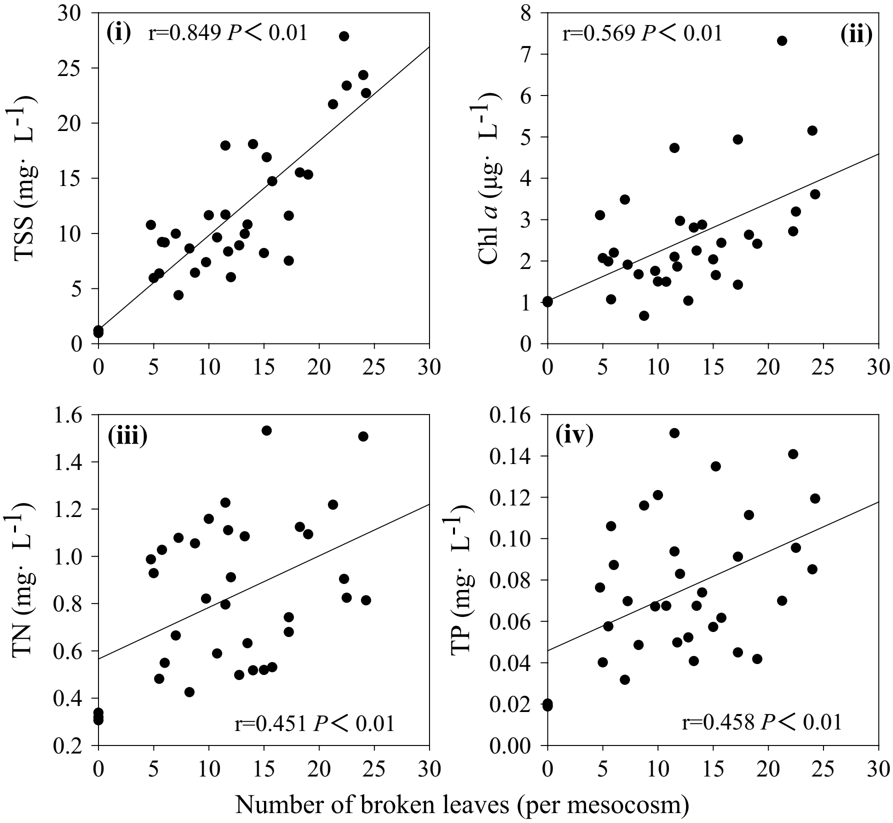
or TP, respective) and the number of broken leaves (Fig. 6).

Discussion

We found that Red Swamp Crayfish degraded water quality by increasing TSS, TN and TP concentrations. The number of broken plant leaves increased at higher crayfish densities, and water quality decreased further. Compared with treatments without shelters, fewer broken leaves occurred in crayfish treatments with shelters at equivalent crayfish numbers, and water quality increased. Our results indicated that shelter availability reduces the negative effects of the invasive Red Swamp Crayfish at equivalent crayfish numbers on clear-water lakes, likely by decreasing their activity (e.g. number of encounters, movement, burrowing, destruction of submerged macrophytes).

The introduction of Red Swamp Crayfish has been shown to decrease submerged macrophyte biomass (van der Wal et al., 2013; Souty-Grosset et al., 2016). Red Swamp Crayfish can induce a significant decline in macrophyte abundance through direct consumption of seedlings (Alcorlo et al., 2004; Cirujano et al., 2004), by diminishing macrophyte

Fig. 6 Scatterplot of the number of broken leaves of *Vallisneria denseserrulata* versus TSS (i), Chl a (ii), TN (iii) and TP (iv) concentrations. The solid line is the linear regression line for all data sets pooled



biomass through non-consumptive plant shredding (van der Wal et al., 2013; Gao et al., 2021) and by increasing water turbidity through sediment resuspension (Angeler et al., 2001; Rodríguez et al., 2003). In our mesocosm experiment, TN, TP, TSS and Chl *a* concentrations, as well as the numbers of broken leaves, were significantly higher after adding Red Swamp Crayfish. Previous research has also shown that water quality could decrease at high crayfish densities (van der Wal et al., 2013; Roessink et al., 2017). Macrophyte destruction under nutrient-rich conditions, particularly in eutrophic shallow lakes, may be followed by a shift from a clearwater to a turbid state dominated by planktonic microalgae, such as *Microcystis* (Rodríguez et al., 2003). In turn, this may further decrease the production of macrophytes and periphyton due to reduced light penetration (Gherardi, 2007). TSS, Chl *a*, TN and TP concentrations increased with the increase of number of broken leaves (Fig. 6), likely reflecting increased release and less uptake of nutrients with increasing number of broken leaves.

We found severe effects occurred at the high density of crayfish (two Red Swamp Crayfish per mesocosm ≈10 ind. m^{-2}), but also major effects at half this density (one Red Swamp Crayfish per mesocosm ≈5 ind. m^{-2}). The natural density range of Red Swamp Crayfish varies depending on geographic range and habitats (Matsuzaki et al., 2009). Lowery and Mendes (1977) showed that the population density of marketable-sized individuals of Red Swamp Crayfish was about 1–3 ind. m^2 in tropical Lake Naivasha, Kenya. Harper et al. (2002) found Red Swamp Crayfish at densities of 6 to 77 ind. m^{-2} in littoral floating vegetation (often comprised mainly of floating water hyacinth) in Lake Naivasha in 1987–1988, and most specimens were juveniles. In the mesotrophic lake Lago della Doccia, Red Swamp Crayfish was first recorded in 2001 and had reached only a relatively low mean density of 0.2 ind. m^{-2} in summer 2003 (Gherardi & Acquistapace, 2007). The population density of crayfish has been suggested as a key factor impacting the magnitude of their ecosystem engineering (Chambers et al., 1990; Statzner & Peltret, 2006;

van der Wal et al., 2011), including negative effects on submerged macrophyte abundance and water quality at higher densities (Chambers et al., 1990; van der Wal et al., 2011). Parkyn et al. (1997) showed that fine sediment removal from stream gravel substrates increased linearly with increasing densities of the New Zealand crayfish. However, most of the observed relationships between response variables and crayfish biomass were non-linear, indicating a saturation of the engineering effects at relatively low animal biomasses (Lodge & Lorman, 1987; Gherardi & Acquistapace, 2007; Matsuzaki et al., 2009).

The effect of crayfish may, however, depend on shelter availability. Rice et al. (2012) found that two similarly sized crayfish spent only slightly more time digging sediment than a single crayfish, and they suggested that crayfish spent time interacting, leading to little additional effect on bed topography or grain entrainment rates than when alone. Our results showed that crayfish behaviour changed markedly when shelters were available, creating weaker negative effects of crayfish on the water quality. Shelters may provide refugia that modify aggressive encounters amongst conspecifics (Hill & Lodge, 1994) and hyperactive behaviour (Statzner & Peltret, 2006). In our study, Red Swamp Crayfish were often found staying alone in the shelters, resulting in less time spent moving, burrowing and foraging (personal observation). Lower movement levels have previously been observed for other crayfish species due to sheltering behaviour when provided with shelters (Statzner et al., 2000). We found support for our hypothesis that shelter availability reduces crayfish-induced water quality changes and macrophyte destruction. Previous studies on the behaviour of other animals have revealed that individuals reared without shelter tended to be more active when placed in a new environment than those reared with shelter (Petrović et al., 2020).

Our results are relevant for invader management and freshwater restoration. Based on natural sheltering behaviour, our study showed that the presence of shelters could reduce crayfish activities (e.g. movement and burrowing) and agonistic behaviour, decreasing the adverse effects of Red Swamp Crayfish on affected aquatic ecosystems. The results indicate that shelter availability should be included as one of the crucial factors in lake management after invasion. For example, large woody debris additions and the restoration of the natural riparian vegetation of lakes could provide additional shelter for crayfish, thereby potentially diminishing their environmental impacts, but could also positively affect the overall crayfish densities due to less interference and loss by predation with a potentially negative effect on the environment on the long term. Therefore, it remains to be clarified how shelter availability affects the stress, behaviour, densities and environmental impacts of crayfish in natural freshwater ecosystems in a longer-term perspective.

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Data availability The data that supports the findings of this study are available in the supplementary material of this article.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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