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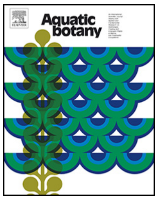

Long-term density dependent effects of the Chinese mitten crab (*Eriocheir sinensis* (H. Milne Edwards, 1854)) on submersed macrophytes

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ABSTRACT

The Chinese mitten crab (*Eriocheir sinensis* (H. Milne Edwards, 1854)), is a highly invasive species and poses a great threat to endemic species and infrastructure in Europe and North America. Although it is partly herbivorous and prefers to live in lakes with abundant submersed macrophytes, little is known about its effect on macrophytes. We used its native range, the mid-lower Yangtze Basin where the species has been cultured intensively for decades, as our study site to test the hypotheses that (1) high crab densities weaken the positive feedback between macrophytes and water transparency, and that (2) the effects of crabs become apparent only on decadal timescales and (3) are density dependent. We used correlative analyses based on 12 years of monitoring and multi-lake comparisons among 20 sub-areas in 4 lakes. High crab densities were found to cause negative effects on submersed macrophytes and transparency, and to weaken the positive relation between macrophytes and transparency. High densities of macrophytes showed resilience to disturbance from crabs. This resilience, however, reduced with continuous presence of high crab densities. Crab densities were strongly positively related with total phosphorus and negatively with transparency and total nitrogen. Phosphorus concentrations and transparency were not related with phytoplankton chlorophyll *a*, suggesting that crab's bioturbation strongly influences water quality. The apparent resilience of the dense macrophyte stands should however, not delay attempts to eradicate the crab where it is invasive as this becomes more difficult once they have become established. When macrophyte abundance is already low at the time of invasion, immediate loss of macrophytes may occur.

1. Introduction

Loss of submersed macrophytes has been widely reported in shallow lakes (Blindow, 1992; Körner, 2002; Schallenberg and Sorrell, 2009; Baastrup-Spohr et al., 2013). Excessive nutrient (nitrogen and phosphorus) loading is a well-known cause (Scheffer, 1998; Carpenter, 2003) but herbivory has received ample attention as a causal factor as well (Lodge, 1991; Wood et al., 2012a,b). Waterfowl, for instance, can exert a strong top-down effect on submerged macrophytes. Its effect increases with waterfowl density and can even lead to an elimination of the above-ground biomass at high waterfowl densities (Wood et al., 2012b). Other herbivores such as grass carp, crayfish, and mammals have been reported to cause negative effects on macrophytes as well (e.g., Lodge and Lorman, 1987; Olsen et al., 1991; Prigioni et al., 2005; Van der Wal et al., 2013; Law et al., 2014), sometimes triggering a complete shift from macrophyte dominance to phytoplankton dominance (Rodríguez-Villafañe et al., 2003).

Although not primarily herbivorous, the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1854), may also contribute to the loss of submersed macrophytes in lakes. The crabs are opportunistic but preferentially feed on macroinvertebrates and detritus (Wen et al., 2000). They also forage on submerged macrophytes, although their importance to the total diet of the crab is limited (3.5–7.5% dry weight) (Jin et al., 2001, 2003). Hence, indirect impacts likely play a more important role than direct consumption. These indirect impacts include damaging the macrophytes while foraging on macroinvertebrates and causing sediment resuspension with their burrowing activity thereby deteriorating

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underwater light conditions. In the absence of crabs, macrophytes typically enhance the water clarity (Kosten et al., 2009) which improves light conditions, thereby enhancing photosynthesis and macrophyte growth. This positive feedback is the primary mechanism maintaining a macrophyte-dominated clear-water state in shallow lakes (Scheffer et al., 1993). We hypothesize that Chinese mitten crabs' bioturbating activities weaken this positive feedback (hypothesis 1).

The crab is a catadromous crustacean native to East Asia. This species has long been a fashionable table delicacy in China. True aquaculture of this species began in the 1980s (Zhao, 2000), characterized by stocking of either hatchery reared or wild propagules to shallow lakes. In its native range, it lives in coastal waters and shallow lakes with abundant vegetation (Zhang et al., 2001; Rudnick et al., 2003). Because of its high reproductive rate and wide range of physiological tolerances (Rudnick et al., 2003), this species successfully invaded Europe over one century ago, mainly through ballast water, and more recently in the 1990s, North America and Western Asia. The crab is still expanding its invasive range (Herborg et al., 2003, 2005, 2007). From reports on the invasive character of the crab, it is known that it negatively impacts biodiversity and fisheries, contributing to the extinction of native invertebrates by preying on them and possibly by competing with native invertebrates and fish (Leppäkoski et al., 2002). Additionally they are capable of destroying dikes and other infrastructure through burrowing (Rudnick et al., 2003; Dittel and Epifanio, 2009). However, little is known about its potential impact on macrophytes in both native and invaded areas.

A short term mesocosm (Jin et al., 2001) and a presence-absence study in the field (Xu et al., 2003) indicated negative effects of high densities of crabs on macrophytes. Instant negative effects of crayfish were also found on macrophytes in short-term enclosure experiments (Lodge and Lorman, 1987; Lodge et al., 1994). In a reservoir where Chinese mitten crab was introduced, however, no effects on submersed macrophyte biomass (*Potamogeton maackianus* and *Ceratophyllum oryzetorum*) were observed until the second year (Yu and Jiang, 2005). The effect of the crabs is likely also density dependent as has been shown in a study in systems where rice and crab culturing is combined: the biomass of plants declined significantly with increasing stocking density (Li et al., 2007). We still lack insight in how crabs affect macrophytes in natural lakes over a decadal scale and at which densities they cause a problem. We hypothesize that the effect of crabs becomes apparent only on a timescale of years and that their effect is likely to be crab and macrophyte density dependent (hypotheses 2 and 3).

In this study, the mid-lower Yangtze Basin was used as an experimental site to study the effects of crabs. In this area, crab culture has been practiced for decades in numerous shallow lakes and many of these lakes have experienced loss of submersed macrophytes, providing an excellent opportunity to look into the effect of crabs on submersed macrophytes. In this study, we relate crab density to macrophytes density and water quality using a dual approach based on (1) monitoring data of a lake with a decadal crab culture (further referred to as the time series analyses) and; (2) a 1-year detailed field-study in 20 sub-areas of 4 large middle-Yangtze shallow lakes supporting crab cultures of different intensity (further referred to as the multi-lake analyses).

2. Methods

2.1. Study sites

Decadal data were available for Lake Biandantang (Hubei Province), a lake in the middle Yangtze Basin. It forms a part of Lake Bao'anhu and has an area of 333 ha, a maximum depth of 2.5 m, and an average depth of 2.1 m. This is the only lake on which decadal (12 years) data of crab and environmental are available. Multi-lake comparisons were conducted on data from isolated sub-areas of the lakes Bao'anhu, Niushanhu, Luhu and Western Liangzihu for which data were gathered in 2001–2002. All four lakes are located in the middle Yangtze Basin (114°08′–48′ E, 30°07′–23′ N) (see Wang et al. (2006) for a detailed description of the locations). Nets and dykes divide the lakes into 20 sub-areas. These divisions are already in place for years or decades. The areas of the sub-areas range from 145 to 6667 ha and they have average depths ranging from 1.8 to 3.3 m (Table 1). Exchange of water among the sub-areas is limited, if occurring at all, which is demonstrated by the considerable variation in environmental conditions such as nutrient levels and transparency among the sub-areas (Table 1). A one-way ANOVA analyses (Wang et al., 2005) underlined the significant difference in environmental conditions. We therefore treated each of the 20 sub-areas as separate water bodies. A warm, humid subtropical climate dominates in this region, with an annual mean air temperature ca. 19 °C and precipitation ca. 1030 mm.

2.2. Stocking and harvest of crabs

Stocking of Chinese mitten crabs started in the late 1980s in Lake Biandantang and in around the year 2000 in the other lakes. Artificially propagated crab juveniles weighing 10 (5–15) (mean (min–max)) g ind^{-1} were transported from Nantong, Shanghai and released into the lakes during winter and early spring (December–May). Their escape is prevented by placing fences made of plastic sheets along the shoreline. Adult crabs are caught with cage traps in autumn of the same year (September–November). Harvest data in Lake Biandantang from 1991 through 1999 were collected from Jin et al. (2001). Data for subsequent years of this and the other sub-areas were obtained from the local farmers directly (Table 1). Stocking rates (SR) were only available for the 20 sub-areas included in the multiple-lake comparison and were calculated as individuals of stocked crabs divided by area. Crab yield (CY) was available for all lakes and calculated as weight of caught adult crabs divided by area. For Lake Biandantang no stocking rate data was available, however, stocking rate tends to be strongly positively correlated to crab yield (between CY and SR in 2001, $r = 0.88$, $n = 16$, $p < 0.001$) when macrophyte biomass is high (with biomass in December 2001 higher than 100 g m^{-2}), we therefore use crab yield here as an indicator for crab density. Harvest effort remained equally high throughout the entire study period (farmers may take up to one month to remove as many crabs as possible).

2.3. Sampling and analyses

Historical data (before 2000) from Lake Biandantang regarding biomass (B_{Mac}, wet weight) of submersed macrophytes were collected from the literature (1991–1993, 1994–1995 and 1996–1999 were collected from Su et al. (1995), Yu and Zeng (1996) and Jin et al. (2001), respectively). Data of Secchi depth (Z_{SD}) and coverage of submersed macrophytes (C_{Mac}) (visual estimation from a boat of the percentage area occupied by submersed macrophytes) were collected from Jin et al. (2001). The data from these references were all reported as averages. The number of sampling sites (n) of Z_{SD} was not specified in Jin et al. (2001); that of B_{Mac} and C_{Mac} was 15 (on 3 transects and 5 for each) in Su et al. (1995), and not specified in Yu and Zeng (1996) and Jin et al. (2001). The methods applied in these references are similar to those we have applied and explained in the following.

Fieldwork was carried out from December, 2001 to December, 2002 (Table 1). Total nitrogen (TN), total phosphorus (TP) and phytoplankton chlorophyll *a* (Chl *a*) were measured seasonally. Winter sampling took place in December 2001 in lakes Bao'anhu,

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Table 1
Stocked populations of Chinese mitten crab in 2001 and 2002 and the environmental variables (annual mean) in 2002 in 20 sub-areas. Area, lake surface area; Z_M, mean water depth; SR, stocking rate of crabs; CY, crab yield; 01 and 02, years of 2001 and 2002; ND, no data; Z_{SD}, Secchi depth; Chl *a*, phytoplankton chlorophyll *a*; TN, total nitrogen; TP, total phosphorus; B_{Mac}, submersed macrophyte biomass.

Lake	Sub-area	Area (ha)	Z_M (m)	SR01 (ind ha^{-1})	CY01 (kg ha^{-1})	SR02 (ind ha^{-1})	CY02 (kg ha^{-1})	Z_{SD} (m)	Chl *a* (mg m^{-3})	TN (mg m^{-3})	TP (mg m^{-3})	B_{Mac} (g m^{-2})
Lake Bao'anhu	Bao'ankou	363	2.10	1321	66	1650	62	1.60	3.91	441	11	3800
	Huangfengkou	188	1.97	1372	96	2390	88	2.03	1.11	257	15	2310
	Changlingzhou	880	2.34	1330	39	1820	82	2.03	1.49	257	9	2240
	Zhuzhou	645	2.57	1441	10	1700	70	1.72	4.29	188	13	1010
	Longwangtou	625	2.45	1289	8	960	38	1.65	4.27	230	17	240
	Lianhuazhou	157	2.69	1915	83	2390	38	1.71	2.47	241	15	80
	Outang	145	2.63	2074	107	2760	111	1.93	3.99	180	13	2740
	Shuimiao	157	2.34	1596	83	2550	105	2.26	2.22	194	11	9480
	Changlingtou	149	1.76	1682	91	2690	111	2.20	1.98	268	16	6800
	Tongshawan	191	2.12	2091	123	2090	78	1.49	2.04	205	16	2430
	Biandantang	333	2.13	1800	38	1290	57	1.37	4.46	270	18	440
	Xiaosihai	150	1.75	1667	60	2000	47	1.29	2.04	190	32	1510
Lake Niushanhu	Eastern	1750	3.29	129	0	1140	34	3.02	2.75	861	8	1070
	Middle	1175	3.33	125	4	1110	38	2.90	1.80	931	5	310
	Western	1333	3.34	338	8	3000	28	2.68	3.30	977	5	1390
Lake Luhu	Wuqianmu	571	2.17	789	16	1260	18	0.80	7.05	783	37	100
	Yiwanwu	1210	2.40	2479	50	2360	16	0.72	4.24	530	33	150
	Hongqicha	451	1.93	3767	22	3100	44	1.00	5.87	254	22	90
	Caimohu	712	1.96	1404	14	ND	ND	1.32	1.85	286	20	50
Lake Liangzihu (Western)		6667	3.21	1266	38	980	26	2.48	2.37	13	438	1120
Mean		893	2.53	1496	48	1961	62	1.81	3.17	16	399	1868
Median		511	2.42	1423	38	2000	60	1.71	2.61	15	262	1095

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Niushanhu and Western Liangzihu and in early January 2002 in Lake Luhu. Spring, summer and autumn samples were taken in April, July and October 2002, respectively. Water depth (Z), Secchi depth (Z_{SD}) and submersed macrophyte biomass (B_{Mac}) were measured monthly. To unify the time scale, when calculating the annual mean of water depth (Z_M), Z_{SD} and B_{Mac}, only the data in the months when TN, TP and Chl *a* were measured were included.

Water samples were collected at 3–16 representative sampling sites within each sub-area, depending on surface area. The samples were taken at 0.5 m below the surface and half way down the water column, and mixed for analyses. Analyses for TN, TP, and Chl *a* were done following the Chinese Water Analysis Methods Standards (Huang et al., 1999). Chl *a* was extracted without grinding from filters (GF/C, Whatman, GE Healthcare UK Limited, Buckinghamshire, UK) using 90% acetone (at 4 °C for 20 h); absorbance was then read at 665 nm and 750 nm, both before and after acidification with 10% HCl using a spectrophotometer (Unico UV-2000, Shanghai, China). TP was measured by an ammonium molybdate-ultraviolet spectrophotometric method after potassium persulphate digestion (at 120 °C for 30 min). TN was determined by alkaline potassium persulphate digestion-UV spectrophotometric method.

The diversity and biomass of submersed macrophytes were measured at 5–31 representative sampling sites within each sub-area, randomly sampled for 2–4 times at each sampling site using a scythe-type sampler (sampling area of 0.2 m^2) with a long handle. Generally, 3 samples were taken. Only two replicates were taken when macrophytes were extremely abundant and variability in density was observed to be low and four replicates were taken when macrophytes were scarce and density varied. After scything, plants were gathered with a 425 μm handnet and put into plastic bags. Samples were then combined, cleaned (removal of extraneous material such as sticks, macroinvertebrates, and substrates), blotted dry, and weighed for wet biomass. Dominant submersed macrophytes in these lakes were *Potamogeton crispus* (A. Benn), *P. maackianus* (A. Benn), *Vallisneria* spp., *Hydrilla verticillata* (Royle), *Ceratophyllum oryzetorum* (Kom), and *Myriophyllum spicatum* (Maxim). When measuring biomass of macrophytes, periphyton was not removed due its extremely low biomass in these lakes. Water depth and Secchi depth were measured once at all the sampling sites of macrophytes. A sounding lead was used to determine water depth.

2.4. Data processing and statistical analyses

To explore the potential resilience of transparency and macrophytes to crab stocking in the time series analysis, we determined the (time-lagged) Pearson's correlation coefficient *r* between crab yield (CY) and the potentially affected variables (Z_{SD}, B_{Mac}, and C_{Mac}). The time-lag consisted of correlating Z_{SD}, B_{Mac}, and C_{Mac} with the CY of the year before.

For the multi-lake comparison, we first tested pairwise correlations among crab culture and environmental variables using Spearman's rank correlation coefficient since they did not follow normal distributions (Shapiro-Wilk test, $p < 0.05$). Using the same dataset, we carried out multiple regression models to quantify the effect of crab stock rate on variables of water quality and macrophytes (while correcting for the effects of other environmental variables). Here we focused on the stock rate of 2001 (SR01) since it had strongest correlations with environmental variables in general (Table 2). All variables except Z_M were log-transformed to avoid highly-skewed distributions. To account for the problem of multi-collinearity of explanatory variables, before the analysis we eliminated the variable with the largest variance inflation factors (VIF) each time until all variables had VIF values <5. Regarding the potential problem of biased parameter estimation caused by any model selection procedure, we fitted the full models that are suggested sufficient for the purpose of parameter estimation (Whittingham et al., 2006). The residuals of each model were tested for normality (Shapiro-Wilk test) after model fitting.

3. Results

3.1. Time-series analyses

Crab yield in Lake Biandantang increased from around 10 kg/ha in 1991 to around 60 kg/ha in 2002, two periods of continuous increases can be identified: 1991–1993 and 1998–2002 (Fig. 1A). Both Secchi depth and coverage of submersed macrophytes showed a clear decreasing trend during the observation periods (Fig. 1B and D). Biomass of submersed macrophytes showed high variation before 1997 and rapid decreases in 1998 and 2003 (Fig. 1C).

Analyses based on data of the same year show a highly significant negative correlation of crab yield with Secchi depth, macrophyte biomass, and macrophyte coverage (Fig. 2A–C). Applying a lag-time of one year (i.e., looking at the relation between crab yield and the environmental variables one year later) decreased the coefficient of correlation of Secchi depth with crab yield by 0.1 (Fig. 2D); crab yield however had closer correlations with macrophytes biomass and coverage in the following year (correlation coefficient increased by 0.13 and 0.02, respectively) (Fig. 2E and F).

3.2. Multi-lake analyses

Although crab stocking rates in 2002 (SR02) were not related to water quality in the same year, stocking rates in 2001 (SR01), were significantly related to water quality variables in 2002 (specifically: Secchi depth, TN and TP; Table 2). This discrepancy suggests a lag time between crab stocking and water quality effects. Further analyses showed a significant ($p = 0.001$) negative effect of SR01 on the positive $\log_{10}(B_{Mac}) - \log_{10}(Z_{SD})$ regression, suggesting the disturbance of crab culture on the link between macrophytes and transparency:

$$\log_{10}(Z_{SD}) = 2.71 + 0.11 \log_{10}(B_{Mac}) - 0.26 \log_{10}(\mathrm{SR01})\ (R^2 = 0.63) \quad (1)$$

The importance of stocking rates in 2001 in explaining the variance of TN and TP was further indicated in multiple regressions (Table 3). Notably the relation between crab stocking rates in 2001 and TP was positive whereas the relation with TN was negative. Crab stocking rates were not significantly related to either phytoplankton concentration (Chl *a*) or macrophytes biomass (Table 2). Both crab yield in 2001 (CY01) and crab yield in 2002 (CY02) correlated significantly only with TN (negatively) and macrophytes biomass (positively) (Table 2).

The water quality variables were generally weakly related to each other, with an exception of the strong relation between Secchi depth and total phosphorus (Tables 2 and 3). Although significant, the relation between Secchi depth and phytoplankton abundance (Chl *a*) was weak. Slightly significant relations were also found between submersed macrophyte biomass and Secchi depth, phytoplankton Chl *a* and TP.

4. Discussion

Macrophyte biomass and water transparency (Secchi depth) were found to decrease with crab yield in the multi-year monitoring (Figs. 1 and 2) and with crab stocking rate in the multi-lake comparison (Fig. A1). Water transparency was positively related to macrophyte biomass and crab stocking rate reduced water clar-

Table 2
Spearman rank correlation (r, upper triangle; p, lower triangle) among crab culture and environmental variables in 20 sub-areas. See Table 1 for explanation of the abbreviations and units; significant correlations ($p < 0.05$) in bold.

$n = 20$	SR01	CY01	SR02	CY02	Area	Z_M	Z_{SD}	Chl *a*	TN	TP	B_{Mac}
SR01		**0.64**	**0.63**	0.36	**−0.58**	**−0.53**	**−0.58**	0.20	**−0.58**	0.53	−0.07
CY01	**0.002**		0.46	**0.65**	**−0.72**	**−0.49**	−0.17	−0.28	**−0.51**	0.18	**0.49**
SR02	**0.004**	0.05		0.37	**−0.50**	**−0.48**	−0.17	0.11	−0.26	0.16	0.13
CY02	0.13	**0.003**	0.12		**−0.78**	**−0.58**	0.09	−0.42	**−0.68**	−0.05	0.70
Area	**0.007**	**<0.001**	**0.03**	**<0.001**		**0.62**	0.23	0.07	**−0.67**	−0.29	−0.33
Z_M	**0.02**	**0.03**	**0.04**	**0.01**	**0.003**		**0.56**	0.10	0.31	**−0.60**	−0.22
Z_{SD}	**0.008**	0.47	0.48	0.73	0.33	**0.01**		**−0.46**	0.20	**−0.88**	**0.47**
Chl *a*	0.39	0.23	0.66	0.07	0.77	0.66	**0.04**		−0.01	0.36	**−0.46**
TN	**0.007**	**0.02**	0.30	**0.002**	**0.001**	0.19	0.39	0.96		−0.20	−0.20
TP	**0.02**	0.46	0.51	0.82	0.22	**0.005**	**<0.001**	0.12	0.39		**−0.45**
B_{Mac}	0.78	**0.03**	0.60	**0.001**	0.15	0.36	**0.04**	**0.04**	0.39	**0.04**	

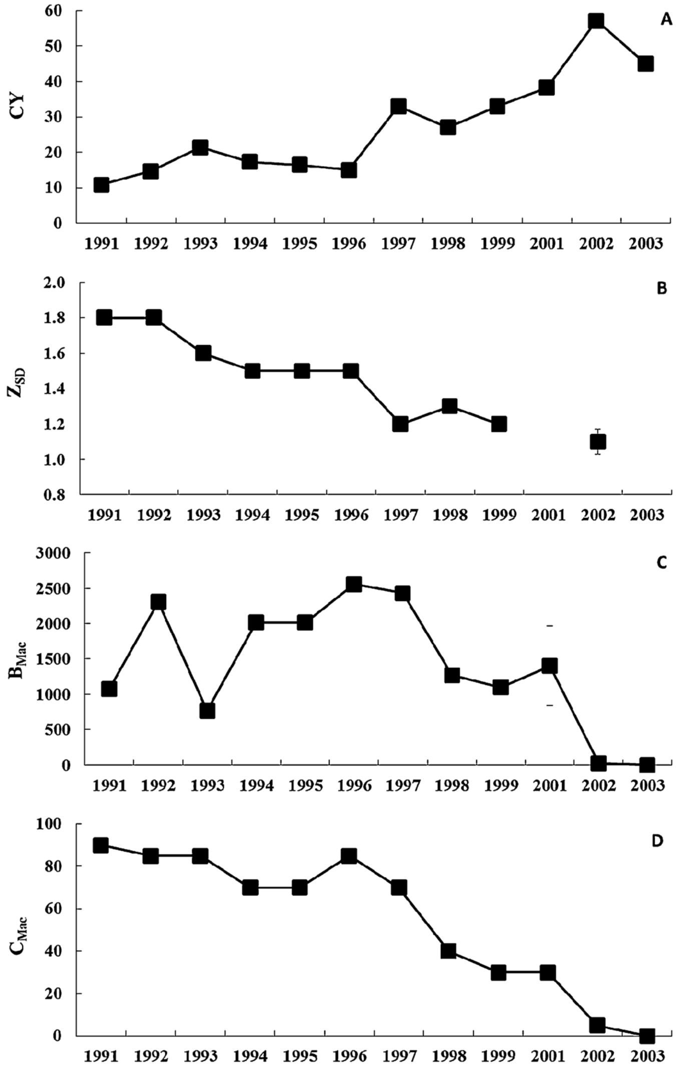

Fig. 1. Variations of crab yield (CY, kg ha^{-1}) ($n = 1$ for each year) and annual mean Secchi depth (Z_{SD}, m), biomass (B_{Mac}, g m^{-2}) and coverage (C_{Mac}, %) of submersed macrophytes in Lake Biandantang during 1991–2003. For the data collected from the references, n of Z_{SD} in 1991–2000 was not specified; that of B_{Mac} and C_{Mac} was 15 in 1991–1993 and not specified in 1991–2000. For the data in 2001–2003 obtained by this study, n was 5 for Z_{SD} and B_{Mac}, and 1 for C_{Mac}. Vertical lines depict standard error bars. All the data from references were reported as averages and no error bar can be made for these data.

ity at a given macrophytes biomass (Eq. (1)). This supports our hypothesis that high densities of Chinese mitten crabs weaken the positive feedback between submersed macrophytes and transparency. The multi-lake comparison suggests that crab density only influences macrophytes biomass at modest initial macrophytes biomass: the regression of macrophyte biomass against crab stocking rate was not significant unless the lakes with high macrophytes biomass (indicated by large circles in Fig. A1E) were excluded. This suggests that the crabs do not affect well-developed macrophyte stands. Although our correlative analyses cannot pinpoint the underlying causal effects of the crab-macrophyte relationship, a mesocosm experiment in which the crabs were found to destroy submersed macrophytes through grazing and uprooting (Jin et al., 2001) confirms the idea that the crabs directly affect the macro-

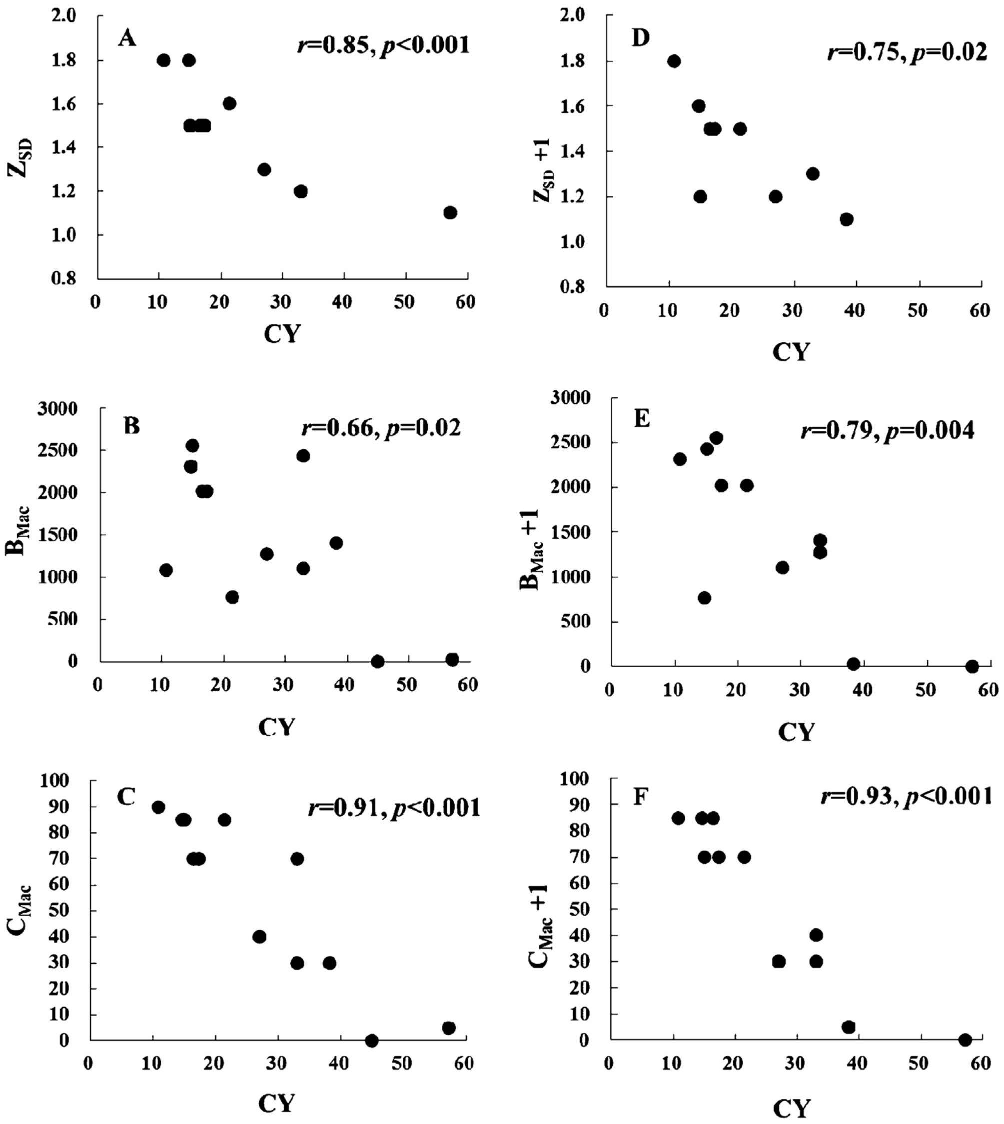

Fig. 2. Relationships of crab yield (CY, kg ha^{-1}) with Secchi depth (Z_{SD}, m), biomass (B_{Mac}, g m^{-2}) and coverage (C_{Mac}, %) of submersed macrophytes in Lake Biandantang during 1991–2003 from analyses with variables determined in the same year (A–C) and *y*-axis variables of one year after (D–F). Vertical lines depict error bars. See Fig. 1 for the explanation on the number of sampling sites and thus no error bars could be included.

Table 3
Multiple linear regressions based on data in Table 1. The complete independent variable set includes SR01, Area, Z_{SD}, Chl *a*, TN, TP, and B_{Mac}; independent variables were removed in case of high VIF (variance inflation factors). $^*p<0.05$, $^{**}p<0.01$. The residuals of all models were normally distributed (Shapiro-Wilk test, $p>0.05$). CI, confident interval. See Table 1 for explanation of the abbreviations and units.

Dependent variable	Independent variables								Adjusted R^2
Z_{SD}		SR01	Area	Chl *a*	TN	TP	B_{Mac}	Intercept	0.84
model	Estimate	−0.42	0.12	−0.13	−0.38	−0.58**	−0.18	1.24×10^{-16}	
	95% CI	(−0.85, 0.01)	(−0.15,0.39)	(−0.38,0.11)	(−0.79,0.02)	(−0.89, −0.27)	(−0.07,0.44)		
Chl *a* model		SR01	Area	TN	TP	B_{Mac}		Intercept	0.09
	Estimate	0.47	−0.04	0.52	0.09	−0.30		1.24×10^{-16}	
	95% CI	(−0.50,1.44)	(−0.67,0.60)	(−0.42,1.42)	(−0.63,0.82)	(−0.88,0.27)			
TN		SR01	Area	Chl *a*	TP	B_{Mac}		Intercept	0.68
model	Estimate	−0.76**	0.29	0.18	0.14	−0.06		-2.54×10^{-16}	
	95% CI	(−1.17,−0.35)	(−0.05,0.63)	(−0.15,0.50)	(−0.29,0.57)	(−0.42,0.30)			
TP		SR01	Area	Chl *a*	Z_{SD}	B_{Mac}		Intercept	0.74
model	Estimate	0.15	−0.05	−0.12	−0.86**	−0.02		5.70×10^{-17}	
	95% CI	(−0.23,0.52)	(−0.35,0.26)	(−0.44,0.19)	(−1.30,−0.41)	(−0.33,0.37)			
B_{Mac}		SR01	Area	Chl *a*	TN	TP		Intercept	0.18
model	Estimate	0.10	−0.22	−0.27	−0.16	−0.46		-3.81×10^{-17}	
	95% CI	(−0.85,1.06)	(−0.81,0.37)	(−0.79,0.25)	(−1.07,0.76)	(−1.10,0.18)			

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phytes at high crab densities. Besides, the first author also observed that the crabs' burrowing activities enhanced resuspension thereby decreasing transparency. The finding of closer correlation of crab yield with transparency but weaker correlation with macrophytes in the-same-year analyses than in time-lag analyses is suggestive of indirect effects of crabs on macrophytes through decreased transparency rather than direct consumption (Fig. 2).

It is unlikely that unknown other factors play a role in the decrease in macrophyte coverage and biomass. Waterfowls are scarce and crayfish are present, but limited to the lakeshore. No or few herbivorous fish are present in the lakes (information provided by the local fish farm). Instead, the fish assemblage is dominated by the planktivores Silver carp (*Hypophthalmichthys molitrix* (Cuvier *et* Valenciennes)) and Bighead carp (*Aristichthys nobilis* (Richardson)). Moreover, the effect of external nutrient loading to the lakes is also limited, as suggested by the abundant macrophytes in winter of 2002 in the sub-areas (B_{Mac} was 3482 g m^{-2} in Bao'ankou and 1408 g m^{-2} in Huangfengkou) receiving the only three water inlets. A macrophyte collapse due to shading by phytoplankton and/or periphyton shading did therefore not occur. First, phytoplankton biomass was only a minor constituent of water turbidity (Fig. A1F) and secondly, periphyton biomass is extremely low in these lakes. In Lake Biandantang, for instance, in 2002—the year of the macrophytes collapse—the annual average phytoplankton chlorophyll *a* was as low as 4.5 mg m^{-3} and periphyton chlorophyll *a* was 9.45 μg g^{-1} macrophyte dry mass (unpublished data, provided by Jian Wang from Institute of Hydrobiology, Chinese Academy of Sciences), which is extremely low when compared to periphyton densities found in other lakes (e.g., 129 μg g^{-1} on average in Quebec and 198 μg g^{-1} in Greenland, (Vadeboncoeur et al., 2006)).

Although well-developed macrophyte stands seem to be unaffected by the disturbance of crabs at first, their resilience reduces with continuous presence of high crab densities. As observed in Lake Biandantang, several years of crab presence above certain threshold densities leads to a gradual decline in macrophytes abundance, especially in macrophyte coverage (Figs. 1 and 2). Our analyses suggest that the short term (1-year) effect of crabs on macrophytes is limited when the macrophyte biomass is high (see Fig. A1E). The inferred resilience of dense macrophyte stands concurs with the period of 7 years during which macrophyte abundance remained high in Lake Biandantang, even though crabs were stocked every year (Fig. 1). The continuous crab stocking as well as the increase in crab densities likely reduced the resilience of the system. This finding corroborates with density dependent effects of other herbivores such as swans Wood et al. (2012a,b) and other waterfowl (Marklund et al., 2002) on aquatic plants and plant bug on strawberry (Rhainds and English-Loeb, 2003). This also concurs with our hypothesis that the effects of Chinese mitten crabs in shallow lakes are plant density dependent. The apparent resilience of well-developed macrophyte stands to the presence of Chinese mitten crabs may be due to different processes. First, at high macrophyte abundance crabs will not damage the entire stand. Remaining unaffected patches may serve as nuclei from which recolonization of the entire lake can take place. Secondly, most macrophytes are evergreen and remain in a vegetative state during winter in mid-lower Yangtze lakes. They start growing in spring, but reach maximum growth rates in August (Wang et al., 2005). The peak of macrophyte growth rate coincides with the crab growth peaks. Mesocosm experiments suggested that the juvenile crabs present in the lake in late winter and early spring have limited impact on the macrophytes, regardless of the stocking densities. Greatest macrophytes damages are caused by adult crabs in summer (Jin et al., 2001). When the initial biomass of the macrophytes is high enough the macrophytes may be resilient to adult crab disturbance. This again, has parallels with the timing effect of herbivorous swans. Swans tend to affect macrophytes less when the macrophyte density is at its peak (Wood et al., 2012b). Thirdly, although the crabs are omnivorous they preferably prey on macroinvertebrates (Zhu et al., 1997). When macroinvertebrates are plentiful, the crab impacts on macrophytes are likely less than when, after successive years of crab presence, macroinvertebrate density is reduced, as illustrated by findings in a reservoir where Chinese mitten crab was introduced (Yu and Jiang, 2005). In this reservoir the biomass and densities of insects and oligochaetes strongly decreased during the first year of crab stocking while no effect on biomass of submersed macrophytes was detected; in the second year with low densities of macroinvertebrates, however, the biomass of submersed macrophytes (*P. maackianus* and *C. oryzetorum*) was reduced (Yu and Jiang, 2005). Fourthly, although the reduced transparency caused by the disturbance of crabs is instantaneous (Fig. 2A and D), its effect on macrophytes is limited. It is only when damaged macrophytes have to re-grow from the sediment surface the next year that light limitation may hamper recovery.

The negative effects of the crab bioturbation on transparency probably work through a complex set of mechanisms. The weak relationships of phytoplankton abundance (Chl *a*) with crab density and transparency (SR01 and Z_{SD}) (Tables 2 and 3) suggest that the declined transparency was largely caused by resuspended non-algal material. This resuspended matter probably contains P, as suggested by the close relationship between crab stocking rate and TP. The phosphorus is likely bound to organic matter and therefore not readily available for phytoplankton, as suggested by the weak TP-Chl *a* relationship. Additionally, bioturbation may improve oxygen conditions at the sediment-water interface reducing phosphate release from the sediment (Lewandowski and Hupfer, 2005; Zhang et al., 2014).

Intriguingly we found a strong negative relationship between crab density and TN (Tables 2 and 3). Although we cannot unravel the underlying causes, increased oxygen concentrations at the sediment surface, due to the bioturbation of the sediment likely enhance denitrification rates (Svensson and Leonardson, 1996; Fanjul et al., 2007; Mchenga and Tsuchiya, 2008). Therefore, although crabs may ultimately enhance phytoplankton densities by eradicating their nutrient competitors, namely macrophytes, they may, at first negatively influence phytoplankton through the reduction of light and through the reduction of N and P availability.

5. Conclusion

In conclusion we argue that, high density of Chinese mitten crabs strongly contribute to loss of submersed macrophytes. The effects of crabs likely work through physical damage of the macrophytes and through a reduction in transparency due to bioturbation. The declined transparency subsequently prevents the re-establishment of macrophytes. In Lake Biandantang, a critical Secchi depth of around 1.3 m in spring is needed for the macrophytes to recover according to the widely accepted critical ratio of Secchi depth to water depth of around 0.6 reported (Wang et al., 2005). Our analyses suggest that when minimum macrophyte coverage or biomass is surpassed, macrophyte stands can endure high crab densities for several years. In order to prevent undesirable loss of macrophytes in the Yangtze shallow lakes, it is therefore important to establish a minimum macrophyte abundance below which lakes should not be stocked with Chinese mitten crabs. Further research into critical thresholds regarding crab stocking densities and minimum macrophyte coverage or biomass is needed to produce guidelines for sustainable crab culture. Additionally our findings imply that in regions where the Chinese mitten crab is invading, sparsely vegetated lakes face a probable immediate total loss of macrophytes, whereas dense macrophyte stands are not at immediate risk. The

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apparent resilience of the dense macrophyte stands should not, however, delay attempts to eradicate this crab where it is invasive, as this becomes more difficult once they have become established.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2016.02.001.

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