
ZOOPLANKTON, ZOOBENTHOS,
ZOOOPERIPHYTON

Factors Inducing the Crayfish *Procambarus clarkii* Invasion and Loss of Diversity in Caohai Wetland

Qing Li^{a,b}, Bin He^{a,*}, Yongxiang Chen^a, Haitao Zhao^a, Xiaohui Xue^a, and Tu Feng^a

^a College of Ecological Engineering, Guizhou University of Engineering Science, Guizhou Province, Bijie City, 551700 China

^b Department of Biology, Carleton University, Ottawa, Canada

*e-mail: hebin123kewen@163.com

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Abstract—Through comparing three survey reports of Caohai since 1986 once a decade in the past thirty years, we confirm that some physical and chemical factors directly (e.g. a decrease of water salinity, pH variability) have provided opportunities for the invasion of *Procambarus clarkii* (Girard, 1852) in Caohai (a wetland situated in Guizhou, South-West China) in 2010. In addition, some physical and chemical factors (e.g. an increase of organic oxygen consumption and total nitrogen) reflect the richness of herbivorous food (vascular plants residues) for this exotic crayfish. Furthermore, we suggest that the successful invasion of exotic crayfish is related to human activity and the presence of Anatidae waterflows. Direct and indirect effects of crayfish invasion on Caohai have been evaluated by comparing data between before 2010 and after 2010. Although it is an omnivorous species, eating animal food is more conducive to its growth and reproduction. Invasion of red swamp crayfish had a negative effect on submerged plants, Annelida, aquatic insects and amphibians. By contrast, increases in this exotic crayfish provides a richer food source for Ciconiiformis birds. Further research is needed to solve crayfish invasion thoroughly.

Keywords: crayfish invasion, omnivores, fauna, macrophyte

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INTRODUCTION

Biotic invasions refer to the not exclusively human-driven phenomena of non-native species (non-indigenous in taxonomic categories and geographic origins) being transferred to new, often distant, ranges where their descendants proliferate, spread, and establish a self-sustained population that gradually develops into a dominant population. The consequence of biotic invasions could be deemed more detrimental generally, with altering dominant species in a community, a physical feature of ecosystem, nutrient cycling, plant productivity, and decaying of biodiversity (Mack et al., 2000). The adverse effects of biotic invasion have been ranked among the most important drivers of biodiversity losses (Sala et al., 2000) and species extinctions in freshwater ecosystems (Foden et al., 2008). Invasion of aquatic alien species is the second largest threat to the reduction or extinction of freshwater fish populations (Foden et al., 2008).

Procambarus clarkii (the red swamp crayfish) is native to Eastern North America, but has been introduced worldwide because of its economic importance and value as a food source (Barbaresi et al., 2000). *P. clarkii* was introduced from Japan to Nanjing, China in 1929, from where it has spread to almost every province and city. This species has established

wild populations in some rivers, ponds, ditches, paddy fields and other water bodies. Multiple features determine the high invasiveness of *P. clarkii*: firstly, it has a habit of burrowing, as main prerequisite for the invasiveness of non-indigenous species, for feeding, reproductive, and defensive purposes. The burrows allow crayfish to withstand environmental extremes, such as high temperatures and dehydration, and protects them from predators. In addition, they usually live in the cracks of masonry and caves, and use grasses as natural shelters. *P. clarkii* can adapt to a wide range of environment, with the suitable pH range is 7.5–8.5, the suitable dissolved oxygen amount is not less than 2 mg/L, and the salinity is less than 10 g/L, and temperature from 5°C to 38°. This invasive species can thus withstand a wide range of physical, chemical, and biological conditions, demonstrating great ecological plasticity (Matteo et al., 2011). Further, it is a generalist omnivores and opportunistic crayfish, feeding on vegetal and animal detritus, submerged vegetation, molluscs, amphibians, fish and insect larvae (Matteo et al., 2011; Gherardi et al., 2007). Some adult individuals even show cannibalism. Thirdly, it has high fertility, with *P. clarkii* can produce more than 700 eggs based on female size, and the complex mother-offspring relationships enhance descendant survival increasing the populations growth (Gherardi, 2006;

Aquiloni and Gherardi, 2008). Finally, it has strong resistance to plague of *Aphanomyces astaci* (Souty-Grosset et al., 2006; Gherardi and Panov, 2009) and parasite of *Paragonimus* genus (Gherardi, 2006; Gherardi and Panov, 2009), and able to accumulate and transfer heavy metals, pesticides and toxins of cyanobacteria to its consumers, therefore it can withstand extreme environmental conditions and outcompete native species.

Given the *P. clarkii* is a generalist omnivore (Parkyn et al., 2001), the effect of introducing it into aquatic systems is not restricted to displacement of the autochthonous crayfish but also extends to the rest of the trophic chain levels (Donato et al., 2018). Various types of impact have been documented in response to the introduction of *P. clarkii*. Invasion of this species is a known cause for the decline of several native freshwater species, such as competitive removal of autochthonous fish species, the disappearance of amphibians due to predation, declines in macroinvertebrates (Correia, 2002), and destruction of the aquatic plant cover (Rodríguez et al., 2003). By contrast, *P. clarkii* has also become a new and rich food source for a number of vertebrate predators, such as fish and birds, and may thus provide positive change for some native species (Ilhéu et al., 2007).

It is not known exactly when and how the *P. clarkii* entered the Caohai Lake, but we learned, from local residents and experts, that crayfish first appeared in Caohai in approximately 2010. Since that time, it has widespread throughout whole Caohai conservation reserve, becoming the dominant species in some sewers, rivers and river channels. This study reviews the existing three times comprehensive survey reports on Caohai wetland, comparing the survey data from its early recovery stage in 1985 (Xiang, 1986), before the introduction of crayfish in 2005 (Zhang et al., 2007) with later one after crayfish successful invasion in 2017, we outline hypotheses on the causes and consequences of this crayfish invasion, which could help to estimate its potential future distribution area and identify effective management practices to contain its spread. At the same time, this information may provide evidence of the effects of predation by alien crayfish on the trophic web of Caohai wetland.

Study area. Caohai wetland is known for its high conservation value and is important for several populations of migratory birds, especially *Grus nigricollis*, plateau cranes endemic to China. Caohai National Nature Reserve is located in the southwest of Weining Yi and Hui Autonomous County, Guizhou Province, China. It is a typical Karst shallow lake (1980 hm²; maximum depth 5.0 m), and its geographic coordinates are from 26°47'35" N to 26°52'10" N, and from 104°9'23" E to 104°20'10" E. The Caohai was traditionally used as an irrigation pool for agriculture, the inhabitants of the area have traditionally used it for uncontrolled hunting of birds, amphibians and fish. In

the early 1970s, in order to get a little more land, a devastating human intervention "released water to make land" caused the plateau freshwater lake ecosystem that has been running for centuries to collapse and disintegrate. In years, this intervention resulted in ecological imbalances, abnormal climate, bird migration, soil desertification, as well as crop diseases and insect's plague. These consequences of this change demonstrate the important function of this lake ecosystem in maintaining and regulating the local ecological environment. Not only rich in biological resources, but also a large number of paleontological fossils and human cultural relics, it has long attracted the attention of domestic and international scholars. The Guizhou government decided to restore the Caohai area and has been regularly monitored since 1980 onwards.

MATERIALS AND METHODS

Physical and chemical water data from Caohai, and the aquatic plant and fauna inventories in periods before and after exotic crayfish introduction, were extracted from the two comprehensive survey reports on Caohai wetland in 1985 and 2005 (Xiang, 1986; Zhang et al., 2007), as well as the later survey in 2017. Due to the fact that these studies partially overlap, the inventory with the largest record of species was used to establish the number of species affected and the decrease. Due to the lack of data on Zoobenthos after 2007, the related data of Zoobenthos after crayfish invasion was extracted from the published article (Chen et al., 2015). However, due to their short survey time and small survey scope, the statistically obtained species of Zoobenthos were not complete.

Due to the lack of data referring to the *P. clarkii* for Caohai wetland, we estimated the Caohai crayfish densities in October of 2018 and 2019. Due to the high conservation value of Caohai, the local government has banned vessels from entering Caohai core waters. Therefore, six sites of marginal water (Jiangjia Wan, Yangguan Mountain, Baijia Zui, Dengjia Yard, Louluo Mountain and Gujia Dixia) (Fig. 1) were selected within the Caohai. Three sampling stations were selected within each site, making a total of eighteen sampling stations. At each station, three non-baited fyke traps were set up for a period of 24 h. The abundance and proportion of capture were estimated on the basis of the presence and absence of crayfish in each fyke-trap (Meineri et al., 2014).

Statistical analysis. The captured abundance and proportion of *P. clarkii* and other fauna were calculated as means \pm standard deviation (SD), and evaluated by one-way analysis of variance (ANOVA) using GraphPad Prism 5 version software. Significance in the mean values was set at $p < 0.05$.

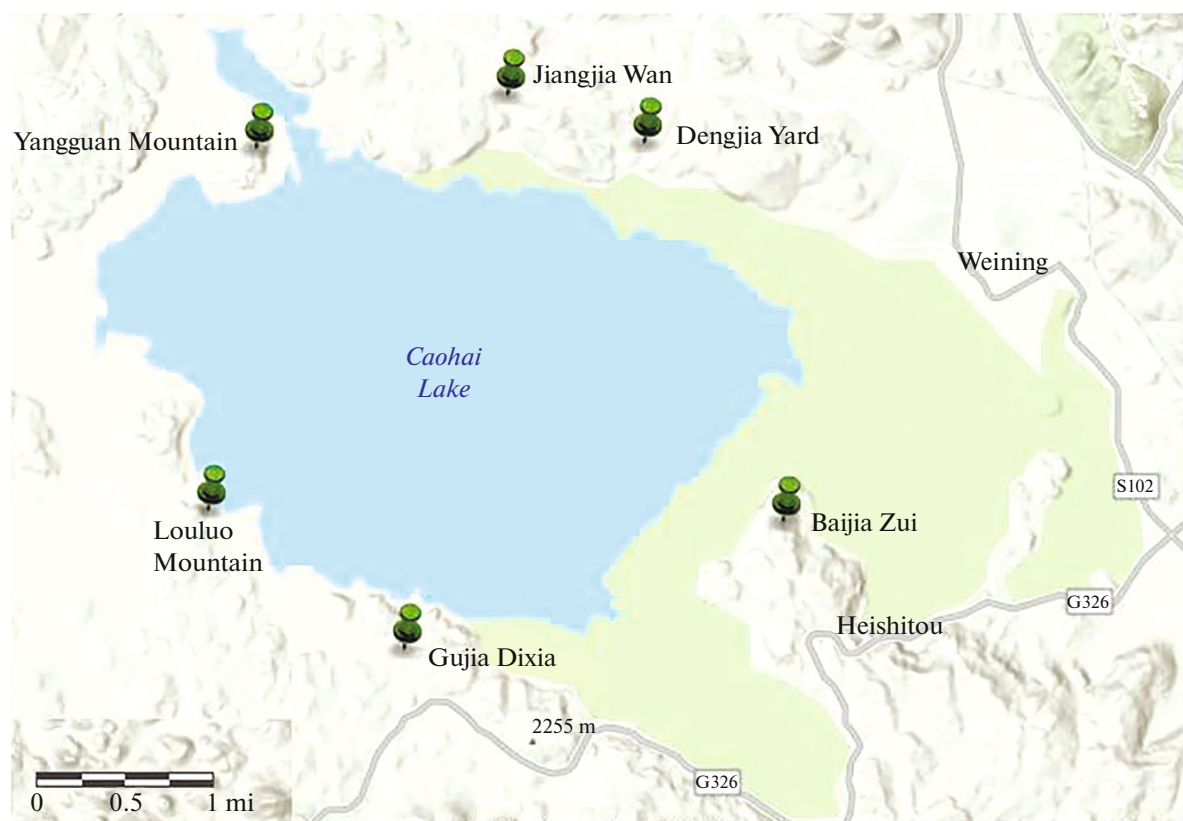


Fig. 1. Map of the sampling sites in the Caohai Lake. The pushpin shows the location of six sampling sites (Yangguan Mountain, Louluo Mountain, Gujia Dixia, Baijia Zui, Dengjia Yard and Jiangjia Wan).

RESULTS

Abundance and proportion of *P. clarkii* and other fauna in Caohai. The abundance of fauna ranged from five to eight species in one trap. The abundance of fauna at Jiangjia Wan reached the peak (Fig. 2). From *Palaemon sinensis*, *Pseudoperilampus lighti*, *Procambarus clarkii*, *Pelteobagrus fulvidraco*, *Bellamya aeruginosa*, *Rhodeus ocellatus*, *Ctenogobius giurinus*, *Pseudorasbora parva*, *Carassius auratus*, *Parafossarulus striatulus*, to *Misgurnus anguillicaudatus*, the abundance was decreased. The proportion of *P. sinensis* at all the sites was significantly higher than other species (Fig. 3). *P. clarkii* relative proportion varied, but on the whole the proportion of crayfish at sampling sites near Weining Country (Figs. 3a, 3c, 3d) was significantly higher than other sampling sites (Figs. 3a, 3e, 3f), human activities are presumed to play a major role in influencing the crayfish abundance and proportion, and enrich the local species.

Changes in physical and chemical variables. Water and air temperature both were significantly increased from 1986 to 2007, may resulting in the decrease of water dissolved oxygen and oxygen saturation from 2007 to 2017. After the arrival of this crayfish, air temperature decreased by 41% and water temperature by 23%, respectively. The same trend was also reflected

in pH, silicon and total ferrum. There was not significant change of pH before and after crayfish arrived, but the water body is always in a weak alkaline state, which is conducive to the survival of crayfish. To the opposite, the water depth and total nitrogen first decreased and then increased. These physical and chemical characterizations showed a wavy change. From 1986 to 2017, the organic oxygen consumption presents a continuous upward trend, whereas the total phosphorus and water salinity decline constantly, especially from 1986 to 2007, the salinity dropped by 67%. As necessary nutrient elements, Cl^- , Mg^{2+} and Na^+ also increased constantly, while Ca^{2+} and SO_4^{2-} showed the opposite trend (Suppl. Table S1).

Phytoplankton. Total biomass of phytoplankton decreased after crayfish invasion, although almost every species amount of phytoplankton increased. Before crayfish invasion, the ratio of Chlorophyta coverage and biomass were the highest of all phytoplankton. After the crayfish invasion, the ratio of Chlorophyta coverage decreased but still the highest (Suppl. Table S2). The ratio of biomass declined by 52% significantly, with the Pyrrophyta biomass reached the highest of all phytoplankton. Followed by, the ratio of coverage of Cyanophyta was decreased by 54%, but with biomass increasing by 56%. The ratio of

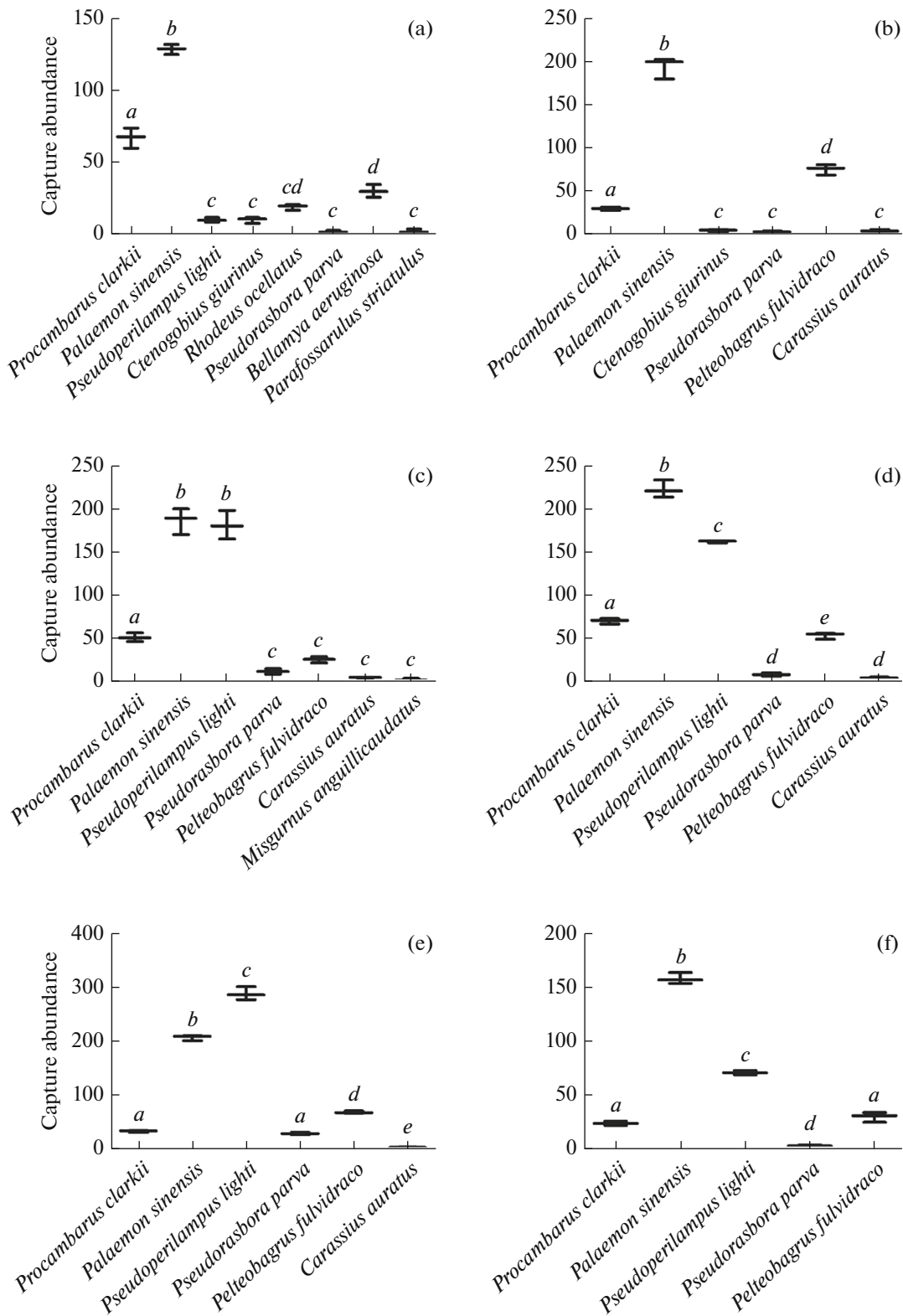


Fig. 2. Captured species abundance in one trap at different sampling sites. For each species, the upper and lower transverse lines represent the maximum and lowest abundance of the captured species in one site, respectively. The mean values of every species are plotted as a middle longer transverse line. (a) Jiangjia Wan; (b) Yangguan Mountain; (c) Baijia Zui; (d) Dengjia Yard; (e) Louluo Mountain; (f) Gujia Dixia. Here and Fig. 3 letters (a–e) in common indicate no significant difference ($p < 0.05$).

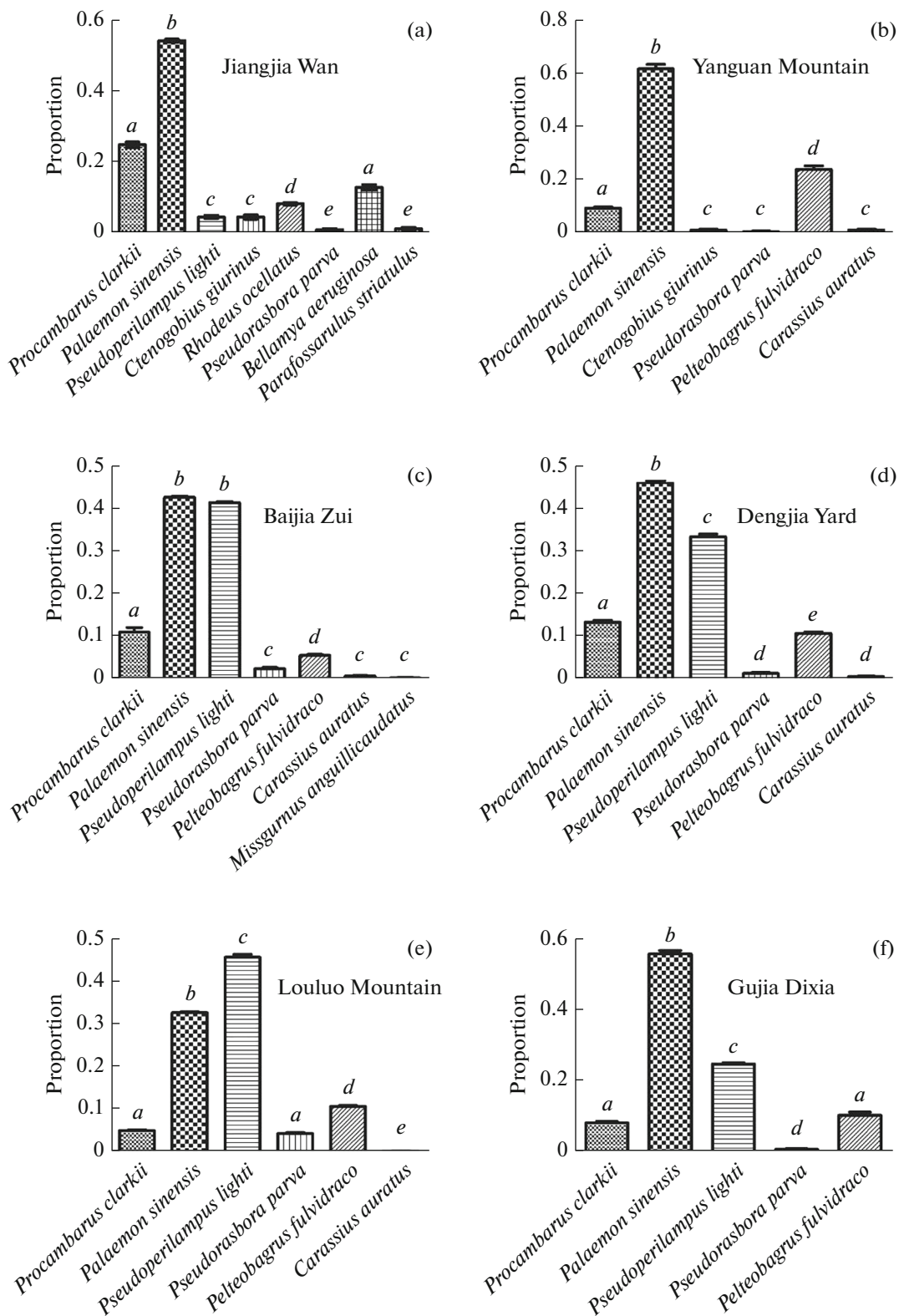


Fig. 3. Captured species proportion in the one trap at different sampling sites. (a) Jiangjia Wan; (b) Yanguan Mountain; (c) Baijia Zui; (d) Dengjia Yard; (e) Louluo Mountain; (f) Gujia Dixia. Vertical segment on the top of each bar represents the standard error.

Table 1. The ratios of coverage and biomass of aquatic macrophytes in Caohai Lake in periods before and after exotic crayfish introduction

Type of aquatic macrophytes	Before 2010		After 2010	
	Total biomass, g/m ² : fresh weight: 4819.5 dry weight: 658.3		Total biomass, g/m ² : fresh weight: 12284 dry weight: 1370.8	
	cover, %	species amount	cover, %	species amount
Emergent plants	59.19	29	61.77	42
Floating-leaved plants	6.12	3	4.41	3
Submerged plants	28.57	14	27.94	19
Floating plants	6.12	3	5.88	4

coverage of Bacillariophyta increased by 96%, but with the ratio of biomass decreased by 69%. Density defined as the biomass divided by coverage, the density of Cyanophyta and Pyrrophyta increased by 80 and 68%, respectively. The others phytoplankton density decreased by 90–98%, except for a 46% reduction in Chlorophyta.

Zooplankton. After crayfish invasion, there were five genera and four species in total missing in Zooplankton, which were Trichoderma, Solanum, Phytophthora, Pyrus and Cucurbita, and the added genera were Amoeba, Epistylis, Vorticella and Euplotes. Rotifera missing genera are Argonotholca, Collotheca, Hexarthra, Squatinella and Ploesoma, and those added were Pompholyx, Proales, and Testudinella. As for Cladocera, the missing genera were Dadaia, Eurycerus and Polyphemus, those added were Bosminopsis, Chydorus, Rhynchotalona and Simocephalus. Copepoda missing genera were Eodiaptomus and Neodiaptomus, the added were Canthocamptus, Acanthocyclops and Limnoithona. Compared the survey between before and after crayfish invasion, the dominant species in Zooplankton are *Arcella vulgaris* and *Lecane luna*, there was no significant change for the composition of Zooplankton species from 2007 to 2017 (Suppl. Table S3).

Aquatic macrophytes. According to vegetation morphological characteristics and ecological habits, the aquatic macrophytes of Caohai are divided into emergent plants, floating-leaved plants, submerged plants and floating plants. After the crayfish invasion, the total biomass increased, with a 61% in fresh weight and a 52% in dry weight. And the amount of species had increased significantly, especially the emergent plants has increased by 31%, but the coverage ratio of different aquatic plants types almost decreased except emergent plants, with gone down from emergent, submerged, floating and floating-leaved plants (Table 1). Before and after the crayfish invasion, the changes in dominant species were mainly reflected in emergent and submerged plants, the added species were *Phragmites australis* and *Typha angustifolia* of emergent

plants, and *Potamogeton perfoliatus*, *Myriophyllum spicatum* and *Potamogeton pectinatus* of submerged plants (Suppl. Table S4). The *Sparganium stoloniferum* of emergent plants and *Trapa maximowiczii* of floating-leaved plants, listed in 1986 survey report, were not appeared in the dominant species of 2007 and 2017 survey reports (Suppl. Table S4).

Zoobenthos. It shows that species amount had decreased, especially Annelida and Aquatic insect, but increased in Mollusc after this exotic crayfish invasion (Suppl. Table S5). In addition, the dominant species also changed, mainly in Gastropods and Crustacea. Before crayfish invasion, the dominant species including *Cipangopaluding cathayensis*, *Radix lagotis*, *Neocaridina dentiolata*, *Pantala flavescens* and *Tendipes* sp. There was the appearance of *Sinodina gregoriana*, but not in the dominant species list. After crayfish invasion, there were many Gastropods added in dominant species, including *Parafossarulus striatulus*, *Bellamya aeruginosa*, *Lymnaea stagnalis*, *Bellamya purificata*, *Radix ovata* and *Tricula*. As for Crustacea, the Caohai native species *N. dentiolata* had not been found, and *S. gregoriana* had become the dominant species. In addition, two exotic crustacean species *P. sinensis* and *P. clarkii* had been listed in the dominant species (Table 2).

Amphibian. The findings have shown that crayfish will prey on the eggs and larvae of amphibians, leading to a decline in quantity and species of amphibians (Kats and Ferrer, 2003; Cruz and Rebelo, 2005; Rodríguez et al., 2005). Before crayfish invasion, fourteen species of amphibians were found in Caohai Lake, but after appearance of crayfish, an alien species (*Lithobates catesbeiana*) of amphibian was found. Native species *Tylotriton kwichowensis* and *Pseudorana weiningensis* have disappeared (Suppl. Table S6).

Birds. Among the composition of bird resources in Caohai, the water bird group is the most distinctive. The water birds, especially Anatidae, were the most abundant and speciose birds in Caohai. The Caohai's bird resources can be classified into migrant, summer migrant, passing migrant, winter migrant, migrant

Table 2. Dominant species change of Zoobenthos in periods before and after exotic crayfish introduction

Group	Species	Before 2010	After 2010
Gastropods	<i>Cipangopaludina cathayensis</i>	+	–
	<i>Radix lagotis</i>	+	–
	<i>Parafossarulus striatulus</i>	–	+
	<i>Bellamya aeruginosa</i>	–	+
	<i>Lymnaea stagnalis</i>	–	+
	<i>Rithynia fuchsiana</i>	–	+
	<i>Bellamya purificata</i>	–	+
	<i>Radix ovata</i>	–	+
	<i>Tricula</i>	–	+
Crustacea	<i>Neocaridina dentioulata</i>	+	–
	<i>Sinodina gregoriana</i>	–	+
	<i>Palaemon sinensis</i>	–	+
	<i>Procambarus clarkii</i>	–	+
Oligochaeta	<i>Branchiura sowerbyi</i>	–	+
Aquatic Insect	<i>Pantala flavescens</i>	+	–
	<i>Tendipus</i> sp.	+	+

Table 3. Bird species amount before and after crayfish introduction in Caohai Lake

Type	Before 2010		After 2010	
	species amount	cover, %	species amount	cover, %
Migrant	88	43.3	94	38.21
Summer migrant	21	10.3	33	13.41
Passing migrant	13	6.4	13	5.28
Winter migrant	69	34	86	34.96
Migrant status Unclear	12	5.9	22	8.94
Waterfowl	79	39	140	56.91

status unclear and waterfowl species. Before the crayfish invasion, most species were migrants, while waterfowl is a little less. Following the *P. clarkii* invasion, however, waterfowl species increased significantly more than other species, and became the largest group birds in Caohai (Table 3). After 2010, although the dominant species changed significantly, only including the *Anas penelope*, *Anas strepera*, *Tadorna ferruginea*, *Fulica atra*, *Gallinula chloropus*, *Anser indicus*, *Grus nigricolis*, *Grus grus* and *Egretta garzetta*, there increased 25 newly bird species (Table 4).

DISCUSSION

Here, we reviewed and compared the past three 10-year surveys of Caohai wetland to summarize and analyze the potential losses in biodiversity following the invasion of *P. clarkii* in this region. In Fig. 4, we attempt to summarize these findings and thereby lay a foundation for future monitoring this exotic species

invasion. While we can summarize these correlative patterns in the environment and species composition, quantitative determination of the causal relationships among these factors are only possible through future research.

Considering crayfish habitat, our analysis showed that changes of physical conditions, such as salinity, pH and water temperature, may explain the invasion of crayfish successfully a decade ago. The pH (7.7–10.2) and water temperature (20–29°C) around 2010 were near optimal crayfish growth and reproduction. Huner and Barr study has demonstrated that *P. clarkii* adult individuals are found in water up to 10 g/L salinity and that reproduction is limited to water less than 5 g/L salinity (Huner and Barr, 1991). The recent study that traps were placed in water bodies that had a salinity level above 5 g/L captured with no crayfish, further confirms that low salinity can increase the occurrence, the abundance and the reproduction success of *P. clarkii* (Meineri et al., 2014). Caohai water salinity is not only

Table 4. Bird dominant species change and newly increased species in periods before and after exotic crayfish introduction

Species	Period	
	before 2010	after 2010
Dominant species		
<i>Aythya ferina</i>	+	–
<i>Anas penelope</i>	+	+
<i>A. strepera</i>	+	+
<i>A. platyrhynchos</i>	+	–
<i>A. poecilorhyncha</i>	+	–
<i>A. acuta</i>	+	–
<i>A. crecca</i>	+	–
<i>A. falcata</i>	+	–
<i>Tadorna ferruginea</i>	+	+
<i>Fulica atra</i>	+	+
<i>Gallinula chloropus</i>	+	+
<i>Anser indicus</i>	+	+
<i>Grus nigricollis</i>	+	+
<i>Grus grus</i>	+	+
<i>Ardea cinerea</i>	+	–
<i>Egretta garzetta</i>	+	+
Newly increased species		
<i>Bubulcus ibis</i>	–	+
<i>Ixobrychus eurhythmus</i>	–	+
<i>Cygnus columbianus</i>	–	+
<i>Aix galericulata</i>	–	+
<i>Rallus aquaticus</i>	–	+
<i>Amaurornis phoenicurus</i>	–	+
<i>Chlidonias leucopterus</i>	–	+
<i>Clamator coromandus</i>	–	+
<i>Aerodramus brevirostris</i>	–	+
<i>Cecropis daurica</i>	–	+
<i>Coracina melaschistos</i>	–	+
<i>Pycnonotus sinensis</i>	–	+
<i>Oriolus chinensis</i>	–	+
<i>Sturnia sinensis</i>	–	+
<i>Sturnus sericeus</i>	–	+
<i>S. cineraceus</i>	–	+
<i>Ficedula albicilla</i>	–	+
<i>Cisticola juncidis</i>	–	+
<i>Prinia crinigera</i>	–	+
<i>P. inornata</i>	–	+
<i>Cettia fortipes</i>	–	+
<i>Phylloscopus fuscatus</i>	–	+
<i>P. reguloides</i>	–	+
<i>Parus venustulus</i>	–	+
<i>Emberiza tristrami</i>	–	+

less than this threshold limit (5 g/L) since record in 1986, but also constantly decreasing from 1986 to 2017. The crayfish abundance from sampling sites near the residence area is higher than others (Fig. 2). This may result from more domestic water pouring into the lake leading to lower salinity. It speculated that the increase in freshwater inputs and crayfish introduction from other places for unknown reasons lead to its invasion.

P. clarkii is a generalist and opportunistic omnivore (Parkyn et al., 2001), the effect of its introducing into aquatic system is not restricted to displacement of the autochthonous macroinvertebrates, such as the disappearance of *N. dentioulata* after its invasion (Table 2). But can also extends to the rest of the trophic chain levels through predating, grazing and burrowing. *P. clarkii* diet includes submerged macrophytes, invertebrates, algae and detritus. However, in preference experiments, it shown that crayfish prefer animal food over detritus or plants (Gherardi and Barbaresi, 2007). Animal protein is a chief substance promoting crayfish rapid growth, experimental studies show that crayfish survival and growth increase when fed animal food as compared to a diet based on plants or detritus (Gherardi and Barbaresi, 2007). In our study, we saw many remains of predated *P. sinensis* in containers during we captured and shipment of crayfish back to the lab, and our results (Figs. 2 and 3) showed that *P. sinensis* could be captured at every sampling site and its biomass and proportion were the highest. *P. sinensis* is another exotic crustacean and its body length is from 20–40 mm, preferring to live in freshwater lakes or ponds with aquatic plants, has become the dominant species in Caohai wetland. The data of relationship between *P. sinensis* and *P. clarkii* are consistent with the results given in the previously studies, with *P. clarkii* change their diet from plant food and detritus to an animal diet in relation to the availability of macroinvertebrates (Correia, 2002). It is uncertain which invaded the Caohai Lake earlier as for *P. clarkii* and *P. sinensis*, but given the abundance and wide distribution of *P. sinensis* providing high occurrence of crayfish large-scale expansion. Our analyzed data further demonstrated that this species is selecting its animal prey as the animal food is abundance, it prefers on the most profitable prey items available with more energy and less consumption, resulting in the decrease of aquatic insect and Crustacean, increase of Gastropods and Oligochaeta in dominant species of Zoobenthos (Table 2). The increase of Oligochaeta may be due to its living in sediment, *P. clarkii* must be exhausted from burrowing the sediment to obtain food. Gastropods are protected by their hard shells.

The impacts of exotic crayfish invasion also on the decrease of Amphibian through direct predation on its eggs and larvae, e.g. on *Taricha torosa* (Kats and Ferrer, 2003), decreasing its larvae activities (Cruz and Rebelo, 2005), preventing its successful mating and spawning, and destroying its spawning grounds by destroying the aquatic plants. Three native amphibian

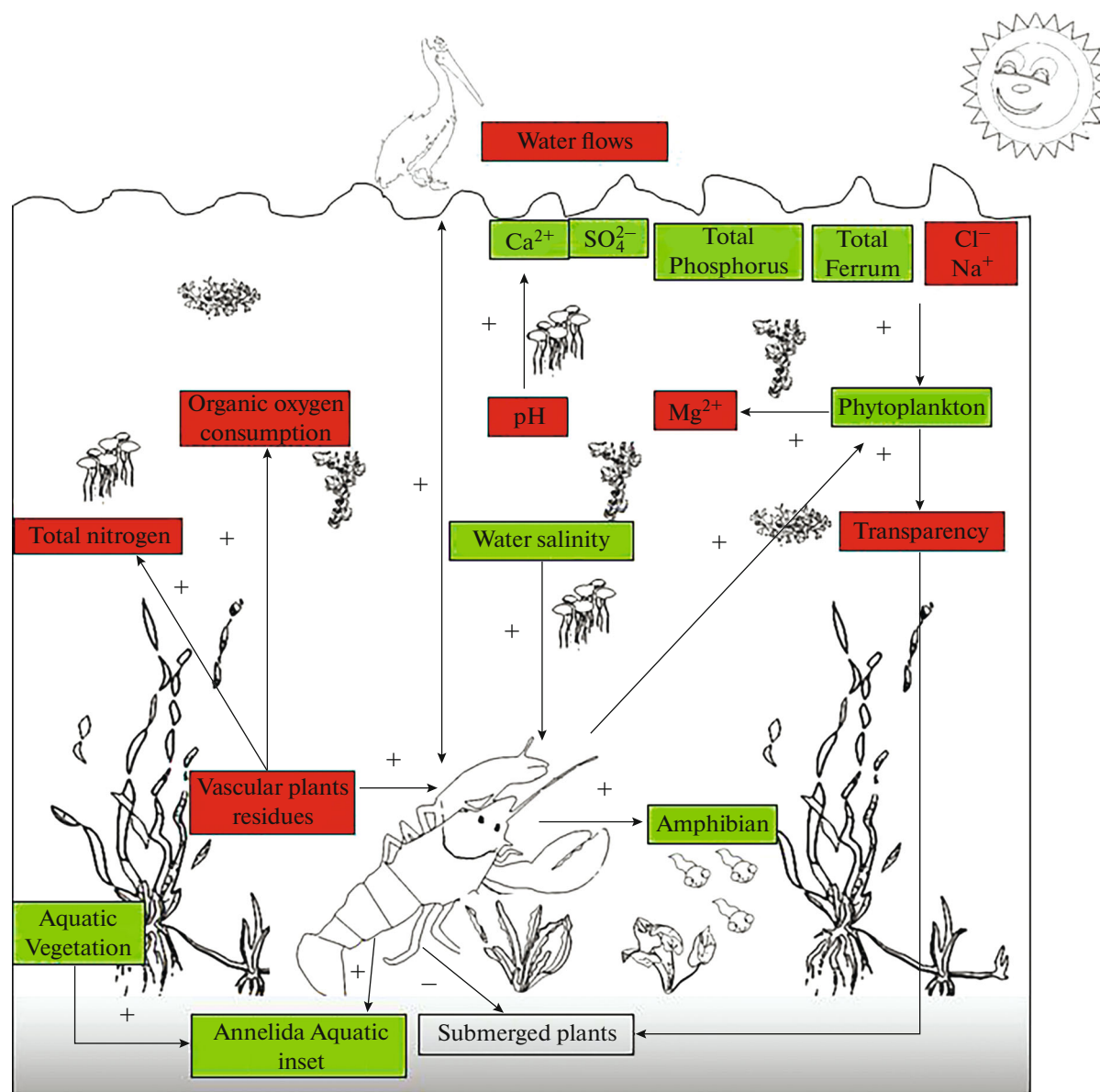


Fig. 4. Outline of putative direct and indirect causal factors and consequences of exotic crayfish invasion in Caohai ecosystem. Red box indicates the increased biomass, species amount, or value of physical and chemical characterisation of Caohai water body after crayfish invasion, the green box stands for the decreased ones. “+” and “-” shows the positive and negative effect, respectively.

species disappeared, and one exotic amphibian species appeared after *P. clarkii* been introducing in Caohai wetland. Disappearance of these species may be due to the alternative predation of *P. clarkii* on eggs and larvae of amphibians, or disturb on its spawning and mating. Furthermore, it is the result of another exotic amphibian species invasion, e.g. *L. catesbeianus*. *L. catesbeianus*, native to the eastern United States, is considered one of the 100 most invasive species in the world (Lowe et al., 2000), it can threaten native amphibians through direct predation (Jankowski and Orchard, 2013), competition (Snow and Witmer, 2011), sexual interference (Pearl et al., 2005) and

transmission of pathogens (Martel et al., 2013). Among *L. catesbeianus* food, *P. clarkii* is not only preyed upon by bullfrog, but also probably as bullfrog first source of food (Vannini et al., 2015). The phenomenon that the areas where *P. clarkii* and *L. catesbeianus* live in sympatry observed in Caohai also confirm this point. So another possible explanation for the decrease in amphibian species observed is related to the invasion of *P. clarkii* inducing an expansion of invasive *L. catesbeianus* and indirectly leading to the disappearance of native amphibian species in Caohai.

In addition to human activities, birds may be another vector for dispersal of *P. clarkii* in Caohai wet-

land except human activities. The characteristics of Caohai bird distribution before crayfish invasion (Tables 3 and 4) is consistent with the evidence given that Anatidae are of particular importance for the dispersal of exotic crayfish due to their abundance, widespread distribution, and the capacity for long-distance movements (Green et al., 2002). Furthermore, the evidence given in the study of *P. clarkii* was readily preyed upon by species of Ciconiiform birds (Correia, 2001) providing some explanations on the distribution traits of dominant bird species in Caohai after crayfish invasion (Table 4).

The impacts of *P. clarkii* invasion impacts on flora are less pronounced than those on fauna. Phytoplankton, as the primary producer, is the basis of the material cycle and energy flow in the entire aquatic ecosystem. Due to its respond quickly to changes in the nutrition status of the water body, phytoplankton is usually a common indicator for evaluating eutrophication. Silicon, the main component of diatom cells, is very important for the total production of organisms in water. It has shown that diatoms cannot reproduce below 0.13 mg/L. From 1986 to 2017, Caohai silicon content is always higher than 0.13 mg/L, so it will not likely affect diatom reproduction. Ferrum, an important trace element of phytoplankton, can promote the normal production of chlorophyll, and involved in respiration as the main component of animal heme, cytochrome oxidase, catalase and peroxidase. The optimal concentration of ferrum for phytoplankton growth is 0.14–1.47 mg/L. From 2007 to 2017, the ferrum content decreased from 0.5 mg/L to 0.06 mg/L, which is not conducive to the growth of phytoplankton. Phosphorus is an essential nutrient element for all phytoplankton. Carbohydrates and phosphoric acids (directly related to photosynthesis) form phosphate esters, which are the main cellular components and involved in energy transmission of respiration and fermentation process. That total phosphorus presented continuous decline from 1986 to 2017 maybe a factor to inducing the decrease of phytoplankton (Goldyn et al., 2007). Na^+ mainly comes from human activities and is discharged into the water body as domestic sewage. Cl^- , Ca^{2+} , Mg^{2+} and SO_4^{2-} are necessary for phytoplankton metabolism. As the components of chlorophyll, Ca^{2+} has important effects on the synthesis and metabolism of proteins, the conversion of carbohydrates, and the absorption and transformation of nitrogen and phosphorus, and Mg^{2+} is mainly involved in the metabolism of carbohydrates. When there are more Ca^{2+} and Mg^{2+} in the water body, the organisms absorb them less. Therefore, the increase of Mg^{2+} in water body indicated that the amount of Mg^{2+} used by phytoplankton in the water is reduced, and it is speculated that the increase of Mg^{2+} is caused by the reduction of phytoplankton. However, as for Ca^{2+} decrease, it may be the result of the rise of pH, inducing the increase of

CO_3^{2-} , increasing likelihood of Ca^{2+} present as precipitates of CaCO_3 . Sulfur is an essential nutrient element for algae, which absorbs and utilizes SO_4^{2-} to meet the needs of sulfur. SO_4^{2-} is one of the main anions in natural waters, which is high in content and generally not lacking. In total, increase of Cl^- and Na^+ , decrease of Ca^{2+} , SO_4^{2-} , total ferrum and total phosphorus, and the appearance of exotic crayfish may together have reduced phytoplankton abundance, which would contribute to the higher water transparency and $[\text{Mg}^{2+}]$.

Higher water transparency is beneficial to the growth of submerged plants. However, the compared data show that the submerged plants biomass seems stable before and after crayfish invasion. Thus, our analysis is consistent with many other studies have shown that even relatively low crayfish densities ($<1 \text{ ind/m}^2$) can completely remove submerged plants from shallow lakes (Rodríguez et al., 2003). However, success invasion of crayfish plays different consequences on macrophytes (Fernández-Aláez et al., 2002). Another significant change of aquatic macrophytes is the increase of emergent plants, one of the vascular plants (Table 4). Examination of stomach contents emphasizes indigestible residues, such as plant fragments and detritus, has confirmed that vascular plants residues is one of the herbaceous food sources for this species. The increase of emergent plants providing enough food for exotic crayfish, while the burrow behavior of crayfish boosts the inorganic nutrient resolve in favor of emergent plants growth. The amount of oxygen consumption by organisms (called organic oxygen consumption) in Caohai is mainly determined by the amount of aquatic vascular plant residues, which consumes a lot of oxygen when being decomposed by microorganisms. The more organic matter deposited on the lake bottom, the greater the oxygen consumption potential. The continuous upward trend of organic oxygen consumption strongly manifests the richness of aquatic vascular plant residues on the bottom, improving the fertility of the water body and facilitating the growth of aquatic macrophytes. The increase in total nitrogen after the occurrence of crayfish may be also the results of increasing in aquatic vascular plant residues.

In conclusion, some observed factors (e.g. decrease of water salinity, rich vascular plants residues, vast Anatidae waterflows and human activity) together likely facilitated the successful invasion of the red swamp crayfish in Caohai. Because of its omnivorous diet and burrowing behavior, *P. clarkii* invasion appears to have direct and indirect effects on the Caohai wetland ecosystem through a trophic cascade. Ultimately, the invasion of this species may be driving declines in Amphibians, Zoobenthos and aquatic macrophyte, and facilitating invasion of some Ciconiiformis bird species, and stabilization of sub-

merged plants (Fig. 4). In order to control the *P. clarkii* invasion more reasonably and effectively, however, the quantitative relationships among these factors and species is in urgent need of further study.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material (Table S1–S6) available at <https://doi.org/10.1134/S199508292204040X>.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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