

Can native species crucian carp *Carassius auratus* recognizes the introduced red swamp crayfish *Procambarus clarkii*?

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Abstract *Procambarus clarkii* is native to the south-central United States (Louisiana) and northeastern Mexico, and is a highly efficient predator that poses a damager to native species after its introduction or invasion. In its natural habitat, *P. clarkii* consumes *Carassius auratus*, however, whether *C. auratus* recognizes *P. clarkii* as a predator is not yet clear. In laboratory experiments, we investigated whether experienced and inexperienced *C. auratus* recognize *P. clarkii* as a predatory threat and the specific sensory modality used by *C. auratus* to respond to chemical and visual stimuli from *P. clarkii*. In the chemical stimuli experiment, two kinds of chemical stimuli were used, water from a tub containing *P. clarkii* previously fed with *C. auratus* (*C. auratus* diet cues) and water from a tub containing unfed *P. clarkii* (*P. clarkii* cues). In the visual experiment, experienced *C. auratus* decreased activity, but inexperienced *C. auratus* avoided the predator compartment. When *C. auratus* diet cues were presented, both experienced and inexperienced *C. auratus* increased the use of shelter, decreased activity in the initial response phase. Compared with the blank treatment, experienced *C. auratus* responded to *P. clarkii* cues by decreasing activity; however, inexperienced *C. auratus* showed no reduction in activity. *C. auratus* appears to recognize *P. clarkii* as a predator both through visual and chemical cues. Further analysis revealed that *C. auratus* may recognize *P. clarkii* visually through the disturbances caused by *P. clarkii* movement and chemically by detecting conspecific alarm cues in the diet of *P. clarkii*. The results also indicate that experienced *C. auratus* can recognize *P. clarkii* by innate chemical cues from *P. clarkii*, whereas inexperienced *C. auratus* cannot [Current Zoology 57 (3): 330–339, 2011].

Keywords *Procambarus clarkii*, *Carassius auratus*, Chemical cues, Visual cues, Non-native predator

The introduction of non-native species into new regions and habitats is one of the major challenges to biodiversity (Soulé, 1990; Vitousek et al., 1996). Non-native species are one of the major causes of the decline in native species, and non-native predators in particular present a greater threat on the native prey population than on native predators (Mack et al., 2000; Kats and Ferrer, 2003; Salo et al., 2007). In communities where predators and prey have coexisted for long periods, the prey respond to predatory pressure by developing behaviors or morphologies that reduce the chance of encounters with predators or that enhance the chance of escape once detected (Lima and Dill 1990). In contrast, prey in communities with novel alien predators is more likely to be predator-naïve and to lack specific avoidance behaviors. Such naïveté facilitates greater hunting efficiency by novel predators and so threatens naïve prey (Salo et al., 2007).

Animals in freshwater systems are more sensitive to introduced predators than those in terrestrial or marine

systems (Cox and Lima, 2006). Continental terrestrial animals are seldom naïve about novel predators owing to the homogenizing effects of historical biotic interchanges. Comparable biotic interchanges could also preclude prey naïveté in most marine systems. In freshwater systems, however, persistent isolation from predator archetypes occurs at local, regional, and continental scales. The resulting multiscale pattern of prey naïveté renders freshwater systems more sensitive to introduced predators than terrestrial systems (Cox and Lima, 2006). Therefore, studying the interaction between native prey and introduced predators is important for the protection of native species in freshwater systems. Early detection by native prey is essential for their survival in such systems.

To avoid predation, prey species utilize such mechanisms as vocalization, visual warning displays, subtle postural changes, vibratory mechanostimuli, and a variety of chemical stimuli (Wisenden et al., 2009). In aquatic environments, the presence of predators and

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predation risk is conveyed by various chemical cues released during successive stages of predation (Wisenden et al., 2010), including predator kairomones, chemical alarm cues and disturbance cues from conspecifics (chemicals released by startled or disturbed prey), and diet cues (Ferrari et al., 2010). In aquatic environments, visual cues are considered important for predator detection by prey (Smith and Belk, 2001; Brown and Magnavacca, 2003). If effective predator detection systems are effective, the prey exploits such resource-rich aquatic environments with minimum risk (Petranka et al., 1987). To respond adaptively to predation threats and increase the chances of survival, the prey must detect and respond to cues emitted from each predator species that constitutes significant risk. When the prey detected the presence of a predator, prey species are usually considered to exhibit anti-predator defenses, such as decreased activity or mobility (Lawler, 1989) and withdrawal into spatial refuges (Rahel and Kolar, 1990).

The red swamp crayfish *Procambarus clarkii* is native to the south-central United States (Louisiana) and northeastern Mexico. The species has been introduced, sometimes deliberately, outside its natural range to countries in Asia, Africa, Europe, and elsewhere in the Americas (Huner, 1988; Barbaresi and Gherardi, 2000). The invasion of *P. clarkii* can have both structural and biological effects on the new ecosystem (Barbaresi and Gherardi, 2000). Burrowing may result in damage to agricultural and recreational areas (Barbaresi et al., 2004). More significantly, *P. clarkii* is a polytrophic species that adjusts its diet to resource availability, including the consumption of fish like *Carassius auratus* and *Gambusia holbrooki* (Correia, 2003). Feeding on mollusks, insects, amphibians, fish, and macrophytes (Smart et al., 2002; Alcorlo et al., 2004; Pérez-bote, 2005) may alter local food chains, and even result in the disappearance of some native species. In some Californian streams, *P. clarkii* predation on eggs, larvae, and adult newts *Taricha torosa* has been suggested as one factor leading to the disappearance of this species (Gamradt and Kats, 1996; Gamradt et al., 1997; Kats and Ferrer, 2003). The arrival of *P. clarkii* coincided with the local extinction of the indigenous species *Austropotamobius pallipes* in Granada (Gil-Sánchez and Alba-Tercedor, 2002). *Procambarus clarkii* was introduced to the Chinese Jiangsu Province in 1929; in recent decades, it has become widely distributed in watercourses and marshes in more than 10 provinces in the south of China (Li and Xie, 2002). Salo et al. (2007)

proposed that predator origin (native vs. alien) had a highly significant effect on predator avoidance by prey, resulting in alien predators having a hunting efficiency double that of native predators. As an alien predator, *P. clarkii* usually exerts strong predation pressure on native species (Cruz and Rebelo, 2007). However, there are few studies examining whether native species can recognize *P. clarkii* as a predator.

Carassius auratus belongs to Cypriniformes, Cyprinidae, *Carassius* Jarocki, a freshwater fish common to lakes and ponds in China (Yue, 2000). As *P. clarkii* could prey on *C. auratus* (Correia, 2003; and our own unpublished data), it is important for *C. auratus* to recognize *P. clarkii* as a predator. However, there are no reports on whether *C. auratus* can recognize *P. clarkii* as a predator. Can *C. auratus* recognize the introduced *P. clarkii* through visual cues or chemical cues? In our experiments, we investigated the specific sensory modalities used by *C. auratus* to detect the presence of *P. clarkii* by examining a number of possible chemical and visual stimuli under controlled conditions.

1 Materials and Methods

1.1 Animals

Experienced *C. auratus* (712 individuals, body length 3.24 ± 0.56 cm) were collected from the ditches in Oujian village, Qixing District, Guilin City, Guangxi Province, China, where *C. auratus* has coexisted with *P. clarkii*. *P. clarkii* (74 individuals, body length 8.56 ± 1.04 cm) were collected from the same area. Inexperienced *C. auratus* (830 individuals, body length 3.19 ± 0.40 cm) were obtained from a fish hatchery in Nanning, Guangxi Province, China, where no *P. clarkii* are found. Given that we collected our stimulus predator animals from an area containing *C. auratus*, the stimulus animals had the opportunity to feed on *C. auratus*. To minimize the effect of wild dietary cues in the study, both *C. auratus* and *P. clarkii* were kept starved in the laboratory under a nature photoperiod at 20–30 °C for at least 3 days before the experiment. Afterwards, *C. auratus* were fed *ad libitum* with fish meal. *P. clarkii* were divided into two groups, one fed with *C. auratus*, and the other fed nothing.

1.2 Visual experiment

The experimental methods were modified from Kiesecker et al. (1996). In this series of experiments, we tested whether experienced and inexperienced *C. auratus* responded to the presence of *P. clarkii* when exposed to visual cues only. We tested *C. auratus* in a rectangular aquarium (100×60 cm²) with opaque walls.

A line divided the tank into width-wise halves, and a 5 mm thick glass was placed across both ends of the tank, creating a 25×60 cm² cage on each end of the tank. We filled the tank with dechlorinated tap water to a depth of 10 cm. Five test *C. auratus* were simultaneously placed in a cylinder (10 cm diameter) and allowed to acclimate for 10 min. During blank treatment, both end cages were empty. The five *C. auratus* which just used in blank treatment were used in visual treatment. During the visual cues treatment, five *P. clarkii* were placed in one of the end cages randomly 40 min prior to the start of each test.

The *C. auratus* were released after the 10 min acclimation period and the test began. Each test included two 5 min trials (an initial and a final response) separated by a 5 min interval. *Carassius auratus* activity and distribution were measured by a camera controlled monitor. As a measure of activity level, the number of times any one of the five *C. auratus* crossed the center line during each trial was counted. To assess the avoidance of the predator compartment, we counted the number of *C. auratus* on each end of the tank at 30 s intervals. These 30 s counts were averaged for each test. The position of *P. clarkii* at one end or the other was random from trial to trial. A total of 40 replicates were taken for experienced *C. auratus* and 30 replicates for inexperienced *C. auratus*. The five test *C. auratus* in each replicate were never used in more than one test.

1.3 Chemical Experiment

Bouwma and Hazlett (2001) suggested that most prey recognize predators through chemical cues released either by the predator itself or by the predator's recent diet. In this experiment, two kinds of chemical cues were used: one derived from *P. clarkii* fed with *C. auratus* (diet cues) and the other derived from *P. clarkii* fed nothing (innate *P. clarkii* cues)

The methods used in this experiment were also modified from Kiesecker et al. (1996). We tested *C. auratus* in a gravitational flow-through system composed of three 25 L plastic tubs measuring 50 × 37 × 20 cm³. The tubs were placed at different heights so that water flowed from one to another at 0.6 L/min. The two lower-most tubs had both input and output openings and did not contain more than 12 L of water. The upper container was filled with 23 L of water. Before the chemical experiment, *P. clarkii* were starved at least 72 h to minimize other food effects. Since the mean net hindgut evacuation rate for *P. clarkii* is 81% after 48 h (McClain, 2000), the effect of the former food of *P. clarkii* can be presumed to be negligible after 72 h. In the diet cue treatment, we made sure that *P. clarkii* had

fed on *C. auratus* not longer than 8 h previously. In the *P. clarkii* cues experiment, *P. clarkii* were starved at least 72 h. In the blank treatment, no *P. clarkii* were added to the middle tub. From the middle tub, water flowed to the lowermost tub that contained the test *C. auratus* and was marked with a line that divided the tank into width-wise halves. A shelter made of opaque board (25 × 37 cm²) was installed on the output side of the lower tank. The water in the lower tank was maintained at a depth of 10 cm. The shelter was placed at a depth of 5 cm, giving the *C. auratus* has the choice to swim above or below the shelter. The five *C. auratus* which just used in blank treatment were used in chemical treatment. In chemical treatment, we added *P. clarkii* to the middle tub 40 min prior to the start of each test, and the experimental procedures were the same as blank treatment.

A test began 10 min after the flow was initiated. Each test included two 5 min trials separated by a 5 min interval. *Carassius auratus* activity and distribution were measured by a camera controlled monitor. As a measure of activity level, we counted the number of times a test animal crossed the center line during each trial as well as the number of test individuals under the shelter at 30 s intervals. These 30 s counts were then averaged for each test. A total of 30 replicates of each cue treatment were obtained. Test individuals were never used in more than one test. In all experiments, the *P. clarkii* used in the tests were arbitrarily drawn from the stock tanks and then returned after testing.

1.4 Statistical Analyses

Repeated measures ANOVA was used to analyze the number of individuals distant from the predator *P. clarkii* (arbitrarily determined for blank treatments) in the visual experiment and under the shelter in the chemical experiment. Simple effects tests were follow-up tests when the interaction was significant. In the visual and chemical experiments, we separately summed up the number of times *C. auratus* crossed the center line in the initial and final responses of each trial. The *t*-test was used to determine whether there were significant differences in the initial and final responses of each trial, followed by paired *t*-tests to assess differences in the activity level.

2 Results

2.1 Visual experiment

Repeated measures ANOVA revealed no significant difference in the average number of experienced *C. auratus* in the area distal to the *P. clarkii* compared to blank treatment with no predator (Table 1). However,

after the predator was presented, inexperienced *C. auratus* spent more time in the distal half of the tank (Fig.1). For either experienced or inexperienced *C. au-*

*ratu*s, the interaction between response phase and treatment has no significant effect on the average number of using area distal to the *P. clarkii* (Table 1).

Table 1 Repeated measurements analysis of variance testing the effects of visual cues on area use (away from the predator compartment) of experienced and inexperienced *C. auratus*, effects of *C. auratus* diet cues, *P. clarkii* cues on area use (under the shelter)

Source	Experienced				Inexperienced			
	df	Mean Square	F	P	df	Mean Square	F	P
<i>Visual cues</i>								
Response phase (initial × final)	1	22.563	3.023	0.084	1	10.453	0.813	0.369
Treatment(blank × cues)	1	4.203	0.563	0.454	1	884.083	68.733	<0.001
Response phase × Treatment	1	7.023	0.941	0.334	1	3.000	0.233	0.630
Error	156	7.463			116	12.863		
<i>C. auratus</i> diet cues								
Response phase (initial × final)	1	38.521	3.921	0.050	1	65.801	6.054	0.015
Treatment(blank × cues)	1	68.641	6.986	0.009	1	58.521	5.384	0.022
Response phase × Treatment	1	51.668	5.259	0.024	1	3.308	0.304	0.582
Error	116	9.825			116	10.869		
<i>P. clarkii</i> cues								
Response phase (initial × final)	1	58.963	7.541	0.007	1	39.968	3.862	0.052
Treatment(blank × cues)	1	14.083	1.801	0.182	1	18.501	1.788	0.184
Response phase × Treatment	1	0.333	0.043	0.837	1	0.521	0.050	0.823
Error	116	7.819			116	10.349		

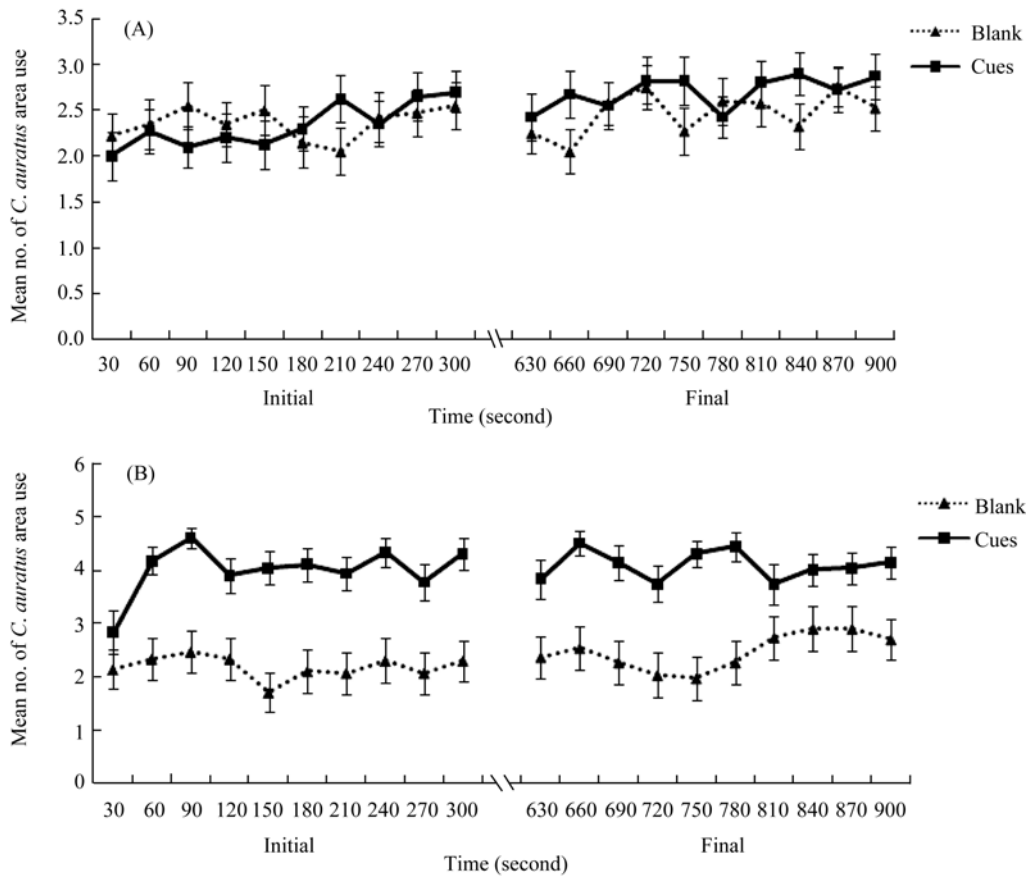


Fig. 1 Changes in area use (away from the predator compartment) by *C. auratus* that cooccur with *P. clarkii* (experienced: Panel A) and those that do not (inexperienced: Panel B), following blank treatment and visual cues treatment of *P. clarkii*. Data is expressed as mean ±SE.

The paired-samples *t*-test showed that the presence of *P. clarkii* in the end compartment affected the behavior of experienced *C. auratus*. The movement of experienced *C. auratus* decreased significantly when *P. clarkii* were presented ($t_{79} = 3.187, P = 0.002$). In contrast, *P. clarkii* did not affect the movement of inexperienced *C. auratus* ($t_{59} = 0.014, P = 0.989$) (Fig. 2).

2.2 Chemical Experiment

Repeated measures ANOVA revealed that the use of shelter was significantly different between the initial and final responses for both experienced and inexperienced *C. auratus*, when *C. auratus* diet cues were presented (Table 1). Since the interaction between responses phase and treatment was not significant for inexperienced *C. auratus* (Table 1), inexperienced *C. auratus* used the shelter more frequently in response to diet cues than during blank treatment (Fig.3B). However, the interaction between responses phase and treatment was significant for experienced *C. auratus* (Table 1). Followed simple effects test showed that more

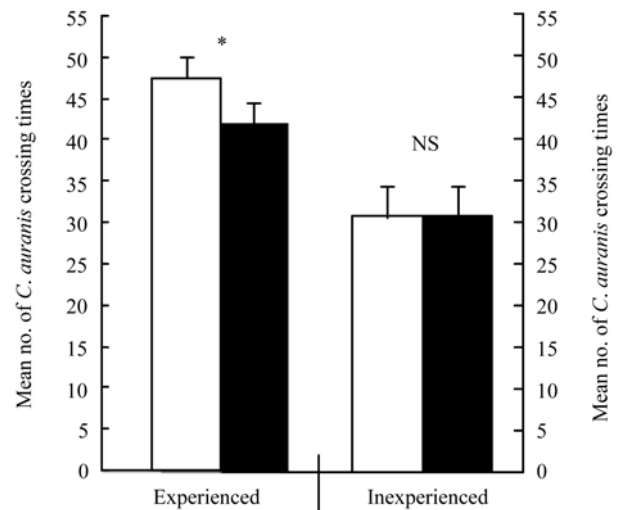


Fig. 2 Changes in activity level (crossing the centre line) of *C. auratus* that cooccur with *P. clarkii* (experienced) and those that do not (inexperienced), following exposure to nothing (open bars) and visual cues of *P. clarkii* (solid bars) Data is expressed as mean \pm SE. NS: $P > 0.05$, *: $P < 0.05$.

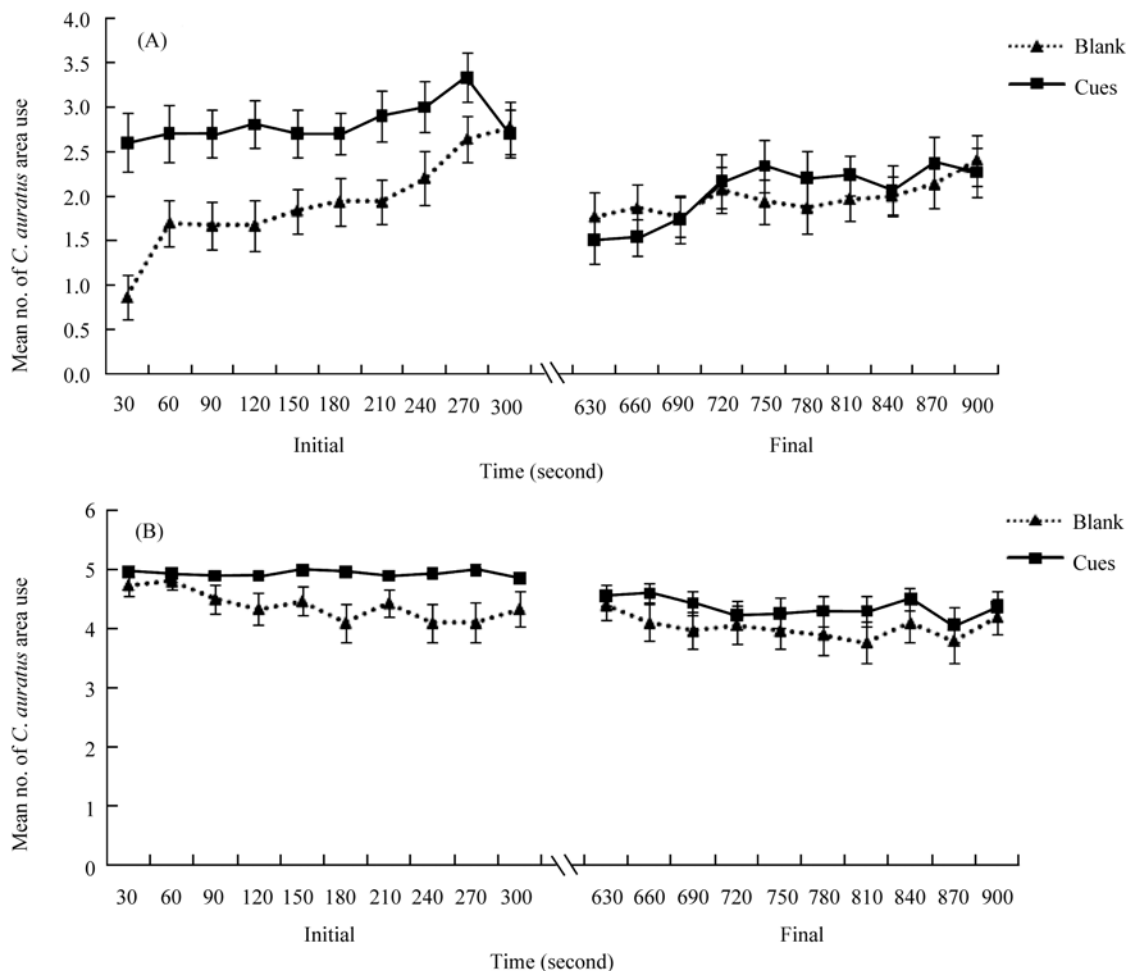


Fig. 3 Changes in area use (under the shelter) by *C. auratus* that cooccur with *P. clarkii* (experienced: Panel A) and those that do not (inexperienced: Panel B), following blank treatment and cues treatment of *P. clarkii* fed *C. auratus* Data is expressed as mean \pm SE.

experienced *C. auratus* used the shelter in diet cues treatment than that in blank treatment during the initial phase ($F_{1,117}=11.89, P=0.001$), not during the final phase ($F_{1,117}=0.060, P=0.807$) (Fig. 3A).

There were also significant differences in activity between the initial and final responses for both experienced and inexperienced *C. auratus*, when *C. auratus* diet cues were presented (experienced: $t_{58} = 4.211, P = 0.021$; inexperienced: $t_{58} = -2.951, P < 0.001$), so the data were separated for further analysis. Compared with the blank treatment, both experienced and inexperienced *C. auratus* reduced their activity in response to the *C. auratus* diet cues during the initial response (experienced: $t_{29} = 6.239, P < 0.001$; inexperienced: $t_{29} = 2.826, P = 0.008$). However, there was no significant difference in the final response (experienced: $t_{29} = 1.454, P = 0.157$; inexperienced: $t_{29} = 1.814, P = 0.080$) (Fig. 4).

Although there was a significant difference in the average number of experienced *C. auratus* in the shelter between the initial and final responses, *P. clarkii* cues did not alter shelter use by experienced *C. auratus* (Table 1). Similarly the average number of inexperienced *C. au-*

ratus in the shelter was not altered by *P. clarkii* cues (Fig. 5). The interactions between responses phase and treatment were not significant for both experienced and inexperienced *C. auratus* (Table 1).

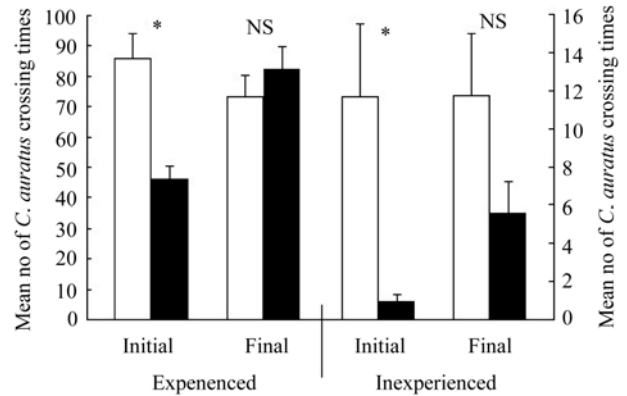


Fig. 4 Changes in activity level (crossing the centre line) of *C. auratus* that cooccur with *P. clarkii* (experienced) and those that do not (inexperienced), following exposure to dechlorinated tap water (open bars) and cues of *P. clarkii* fed *C. auratus* (solid bars) in initial and final responses phase

Data is expressed as mean \pm SE. NS: $P > 0.05$, *: $P < 0.05$.

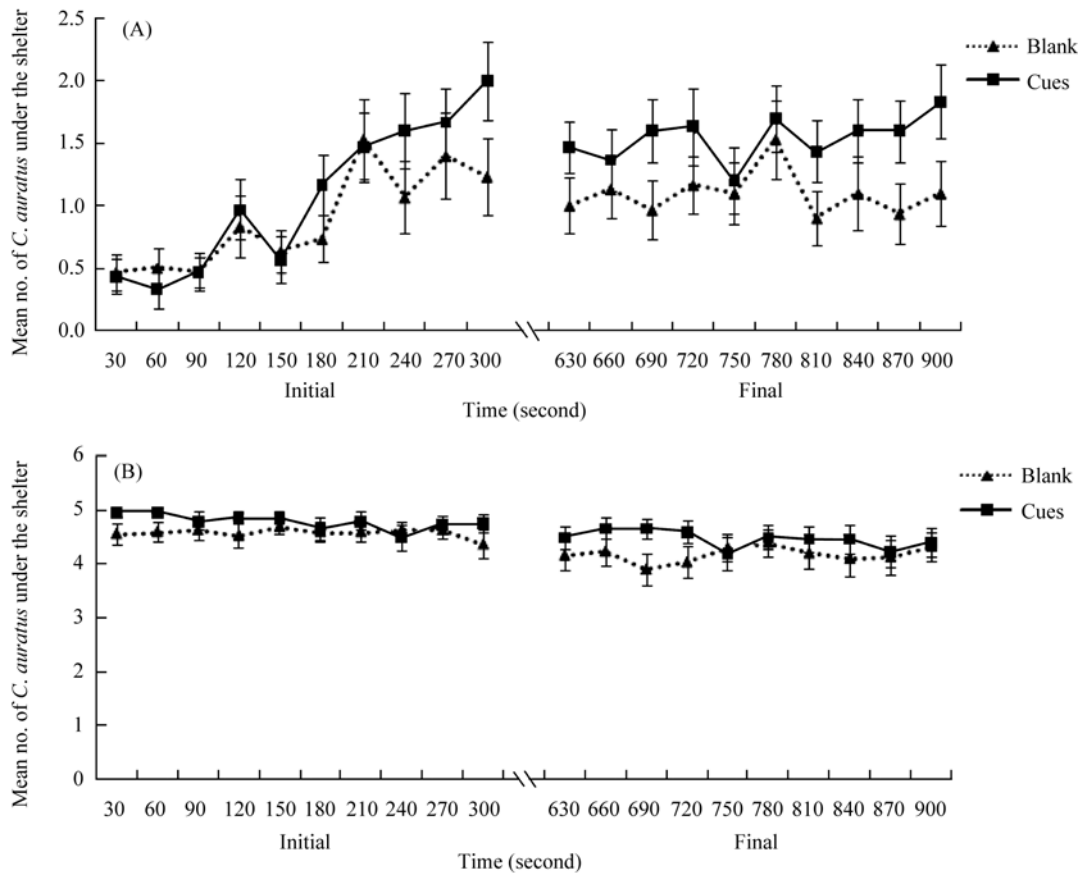


Fig. 5 Changes in area use (under the shelter) by *C. auratus* that cooccur with *P. clarkii* (experienced: Panel A) and those that do not (inexperienced: Panel B), following blank treatment and cues treatment of *P. clarkii* itself that fed nothing

Data is expressed as mean \pm SE.

Paired-samples t-tests revealed that the movement of experienced *C. auratus* decreased when *P. clarkii* cues were presented ($t_{59} = 2.990$, $P = 0.004$). Although there was a significant difference in the activity of inexperienced *C. auratus* between the initial and final responses ($t_{58} = 1.788$, $P = 0.001$), *P. clarkii* cues did not affect the activity of inexperienced *C. auratus* (initial responses: $t_{29} = 1.354$, $P = 0.186$; final responses: $t_{29} = 1.195$, $P = 0.242$) (Fig. 6).

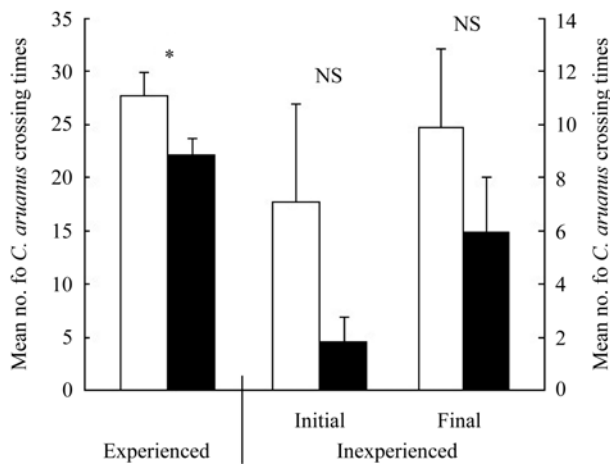


Fig. 6 Changes in activity level (crossing the centre line) of *C. auratus* that cooccur with *P. clarkii* (experienced) and those that do not (inexperienced), following exposure to dechlorinated tap water (open bars) and cues from *P. clarkii* itself that fed nothing (solid bars) in initial and final responses phase

Data is expressed as mean \pm SE. NS: $P > 0.05$, *: $P < 0.05$.

3 Discussion

Anti-predator responses include a reduction in movement, avoidance of the predator compartment, and increased in shelter use (Sih, 1987; Lima and Dill, 1990). When exposed to the visual cues of *P. clarkii*, experienced *C. auratus* decreased their activity, and inexperienced *C. auratus* avoided the predator compartment. In the diet cues experiment, both experienced and inexperienced *C. auratus* increased the use of shelters, decreased their activity in the initial responses phase. In the *P. clarkii* cues experiment, experienced *C. auratus* decreased their activity. Our study confirms and extends previous reports (Smith and Belk, 2001) by demonstrating that both experienced and inexperienced *C. auratus* demonstrate anti-predator responses to visual and chemical cues. Indeed, Smith and Belk (2001) found that both chemical cues (predator diet) and visual cues (predator behavior) evoked antipredator responses in shoals of mosquitofish, and that the effects of these

cues were additive.

In the visual experiment, experienced *C. auratus* did not avoid the predator compartment, but instead decreased their activity when exposed to the visual cues of *P. clarkii*. Similar results were also reported by Kiesecker et al. (1996). Although the western toad *Bufo boreas* tadpoles examined in their study reduced their activity in response to the sight of garter snakes *Thamnophis sirtalis*, the authors suggested that this response was due to the high levels of snake activity rather than a response to visual cues. In the present study, *P. clarkii* were very active in the tanks during the experiment; thus, the avoidance behavior of *C. auratus* could have been evoked by the disturbance caused by the movement of *P. clarkii* rather than by the specific recognition of *P. clarkii* as a predator. However, it is strange that inexperienced *C. auratus* avoided the predator compartment but did not decrease their activity. This could be a stress reaction rather than an anti-predator response as Dai et al. (2004) showed that inexperienced *Rana daunchina* demonstrated rambling and aimless responses when exposed to the visual cues of *P. clarkii*.

Our experimental data provide evidence indicating that both experienced and inexperienced *C. auratus* can recognize *P. clarkii* as a predator based on chemosensory cues associated with the predator's diet, specifically the feeding on conspecifics. Similarly, previous studies have demonstrated that the chemical cue for predator avoidance was diet based, and that a prey species exhibited fright responses more efficiently and quickly if the predator's diet contained individuals from the same prey species (Laurila et al., 1997). Stabell and Lwin (1997) found that crucian carp *Carassius carassius* exhibited an adaptive change in body morphology in response to carp-fed predators. Some studies also documented that native prey animals can learn the identity of unknown predators by detecting conspecific alarm cues in the diet of the predator. For example, Mathis and Smith (1993) found that predator-native fathead minnows *Pimephales promelas* exhibited anti-predator behavior in response to the cues from of fathead minnow-fed pike. Similarly, Chivers et al. (1996) showed that larval damselflies (*Enallagma* spp.) exhibited anti-predator behavior in response to the cues from damselfly fed pike. In our study, both experienced and inexperienced *C. auratus* recognize *P. clarkii* based on the chemosensory cues released by *P. clarkii*, however, this finding does not coincide with that of Wisende et al. (1997), who reported that damselflies in the same habitat as northern pike adopted anti-predator behaviors in

response to chemical stimuli from pike, but that the chemical stimuli from the pike had no behavioral influence on damselflies from an area where no pike were found.

In the diet cues experiment, both experienced and inexperienced *C. auratus* reduced their movement in the initial response (first 5 minutes of exposure) but not in the final response. The risk-sensitive predator-avoidance hypothesis predicts that as the concentration of chemical cue decreases, the intensity of the anti-predator response also decreases (Mirza and Chivers 2003). Thus, the reduction in avoidance behavior may be due to a decrease in the concentration of the conspecific chemical alarm cues. Indeed, Brown et al. (2006) found that juvenile convict cichlids *Archocentrus nigrofasciatus* exhibited more intense responses to a high background level of alarm cue and less intense responses to a lower alarm cue concentration.

In our study, *C. auratus* were collected from two populations, one native to a region where *P. clarkii* had been established, and the other from a habitat without *P. clarkii*. Experienced *C. auratus* responded to both diet cues and *P. clarkii* cues, whereas inexperienced *C. auratus* responded only to diet cues. Individuals from populations that frequently encounter predators typically exhibit stronger anti-predator responses than individuals from populations that rarely experience predators (Mathis and Smith, 1993). Apparently, previous experience is required for *C. auratus* to recognize and respond to *P. clarkii* cues while the response to chemosensory cues is innate. It is possible that experienced *C. auratus* learn to recognize the odor of *P. clarkii* or alarm signals diet. Wisenden et al. (1997) found that the differences in northern pike *Esox lucius* recognition of the damselfly (*Enallagma* spp.) population resulted from experience. In our study, the diet cues came from the conspecific alarm cues of *C. auratus*. These conspecific alarm cues may be common in the environment of *C. auratus* (with or without *P. clarkii*) as other predators prey on *C. auratus*. Thus, inexperienced *C. auratus* can recognize and respond to *P. clarkii* based on conspecific alarm cues. Indeed, many other species have been shown to possess and respond to conspecific alarm cues (Ferrari et al., 2010).

When exposed to the *C. auratus* diet cues, *C. auratus* increased shelter use and reduced activity (Fig. 3, Fig. 4), which demonstrated that *C. auratus* display anti-predator behaviors in response to chemical cues released from conspecifics. Many aquatic animals, including some invertebrates, fish, and amphibians, use

chemical cues to assess predation risk (Dodson et al., 1994; Kiesecker et al., 1996; Chivers and Smith, 1998), as prey often hide in structurally complex refuges, in the bottom sediment, or in other habitats that restrict the visual detection of approaching predators (Peckarsky and Dodson, 1980; Stauffer and Semlitsch, 1993). Aquatic media are well suited for chemical signals because many compounds can dissolve in water, allowing a large number of potential chemical signals to be detected (Kleerekoper, 1969; Hara, 1994). Therefore, individuals that can use chemical cues from nearby conspecifics should receive earlier and more reliable information on potential dangers than those that rely solely on visual cues (Mathis, 2003). It was good for the survival of *C. auratus* using chemical cues to assess predation risk of *P. clarkii*.

The interaction between responses phase and treatment in *C. auratus* diet cues experiment was significant for experienced *C. auratus*, but not for inexperienced *C. auratus* (Table 1). The response process of experienced and inexperienced *C. auratus* to the diet cues was different between the initial and final phase (Fig. 3). In the initial phase, the number of experienced *C. auratus* under the shelter in diet cues treatment was significant higher than that in blank treatment. However, in the final phase, it was not significant different between the two treatments. For inexperienced *C. auratus*, both the numbers under the shelter for the two phases in diet cues treatment were significant higher than those in blank treatment. Since experienced and inexperienced *C. auratus* came from different population, the anti-predator behaviour of them might have adaptive variation (Huntingford et al. 1994).

In the results, the activities of experienced *C. auratus* in the blank treatment differed between the two chemical cue experiments (Fig. 4 and Fig. 6). This phenomenon may be caused by the different temperature during the process of the two experiments. The *C. auratus* diet cue experiment (Fig. 4) was conducted in the first third of June and the indoor temperature was around 24°C. The *P. clarkii* cues experiment (Fig. 6) was conducted in the last third of June and the indoor temperature was around 27°C. The activities of five *C. auratus* on 24°C was two times as that on 28°C, and decreased rapidly from 24°C to 28°C (Reynolds and Casterlin, 1979). So, *C. auratus* showed a much lower response to the treatment in the *P. clarkii* cues experiment compared to *C. auratus* diet cue experiment. However, the effects of temperature on activities of *C. auratus* were removed in this study, since we made blank treatment for each experiment.

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