

APPLIED ISSUE

A laboratory study of the effects of shelter availability and invasive crayfish on the growth of native stream fish

SHIN-ICHIRO S. MATSUZAKI*, MASAKI SAKAMOTO[†], KIYOSHI KAWABE[‡] AND NORIKO TAKAMURA*

*Center for Environmental Biology and Ecosystem, National Institute for Environmental Studies, Tsukuba-shi, Ibaraki, Japan

[†]Department of Environmental Engineering, Toyama Prefectural University, Imizu-shi, Toyama, Japan

[‡]Hodogaya Contract Laboratory Co Ltd, Tsukuba-shi, Ibaraki, Japan

SUMMARY

1. The alteration of stream habitats by urbanisation reduces the availability of shelter. Reduced shelter availability may increase both predation risks and metabolic costs, negatively affecting the growth performance of stream fish. Although urbanised streams often allow the establishment of invasive species, the additive or interactive effects of shelter availability and invasive species are rarely explored. The invasive red swamp crayfish (*Procambarus clarkii*) is a strong shelter competitor and predator in streams.
2. We conducted a stream-channel experiment to investigate how shelter availability and this invasive crayfish species affect the growth performance (measured as the loss in body mass under starved conditions) of two native benthic species, Japanese crucian carp (*Carassius auratus* complex) and an endangered cobitid fish (*Lefua echigonia*).
3. We found that both the absence of shelter and the presence of crayfish increased body mass loss in the crucian carp. However, the growth performance of the crucian carp was improved by the presence of shelter except in the presence of crayfish, in which case the shelter available did not ameliorate the negative effect of the crayfish on growth performance (i.e. an interactive effect). This result suggests that crayfish may affect the growth performance of the crucian carp through chemical cues as well as through shelter occupancy.
4. In contrast, shelter availability is the primary factor affecting the body mass loss of the cobitid fish. However, the growth performance of these fish was unaffected by the presence of crayfish. The change in the growth performance of the cobitid fish in response to the absence of shelter was greater than that of the crucian carp. This finding suggests that the cobitid fish may be more vulnerable than the crucian carp to a reduction in shelter availability.
5. Our study demonstrates that reduced shelter availability and/or crayfish invasion can have significant, indirect negative effects on the growth performance of native fish, but whether those effects are interactive might vary depending on the species. Our findings have clear implications for stream restoration and habitat assessment.

Keywords: habitat complexity, non-lethal effects, specific growth rates, ventilation rate, vigilance

Introduction

Numerous streams worldwide have been radically modified by human activities over the centuries (Paul &

Meyer, 2001; Allan, 2004), and the severe physical disturbance produced by urbanisation (i.e. habitat destruction and degradation of habitat quality) is among the most serious threats to stream organisms (Taniguchi, Inoue &

Correspondence: Shin-ichiro S. Matsuzaki, Center for Environmental Biology and Ecosystem, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba-shi, Ibaraki 305-8506, Japan. E-mail: matsuzakiss@nies.go.jp

Kawaguchi, 2001; Riley *et al.*, 2005; McKinney, 2006; Bernhardt & Palmer, 2007). Fundamental alterations of the streambed, bank and surrounding habitats through the clearing of vegetation, concrete-lined channels, dredging, an increased sediment load and the compaction of the substrata can reduce shelter availability and habitat complexity, negatively affecting the survival, physiology, growth and, ultimately, the fitness of many stream fauna such as fish and amphibians (Lowe, Nislow & Bolger, 2004; Suttle *et al.*, 2004; Millidine, Armstrong & Metcalfe, 2006; Finstad *et al.*, 2007). Although ecological restoration to recreate the in-stream habitats or riparian zones that are needed to support a persistent population of stream fauna has received increasing interest (Bernhardt & Palmer, 2007), the importance of shelter availability has received much less attention in habitat assessment and restoration planning (Finstad *et al.*, 2007).

Many freshwater fish use shelters to hide from potential predators (Valdimarsson & Metcalfe, 1998; Steele, 1999). However, a growing body of evidence suggests that the benefit of shelter is not restricted to the obvious reduction in predation risk and that the presence of shelters may also reduce the metabolic costs associated with other mechanisms for avoiding predators, such as camouflage, vigilance and preparedness to escape attacks by predators or competitors (Fischer, 2000; Millidine *et al.*, 2006; Finstad *et al.*, 2007). For example, Millidine *et al.* (2006) showed that the absence of shelter increases the metabolic cost (oxygen consumption rate) of juvenile Atlantic salmon (*Salmo salar* L.) by as much as 30%. High metabolic costs can have substantial effects on the growth rates, and some recent studies have reported a positive relationship between shelter availability and growth rates (Finstad *et al.*, 2007; Olsson & Nystrom, 2009). Thus, a reduction in the availability of shelter can have indirect negative impacts on the metabolism, growth performance and population demographic rates of stream fishes.

Recent reports indicate that the massive disturbances induced by urbanisation also promote the establishment of invasive species, in addition to degrading and destroying the physical habitats of streams (Riley *et al.*, 2005; McKinney, 2006; Johnson, Olden & vander Zanden, 2008; Matsuzaki *et al.*, 2011). Furthermore, habitat modification and species invasion can interact synergistically to produce detrimental effects on native species (Didham *et al.*, 2007). Urban streams allow an increased presence and abundance of invasive crayfish, which often exhibit a high biomass in highly urbanised streams (Riley *et al.*, 2005; Matsuzaki *et al.*, 2011). It is well known that invasive crayfish profoundly affect native freshwater communities through multiple mechanisms including predation, engi-

neering, shelter occupancy and chemical cues (Guan & Wiles, 1997; Larson & Magoulick, 2009; Matsuzaki *et al.*, 2009; Cai *et al.*, 2011). Shelter is a highly valued resource for crayfish, and the competition for shelters with native fish is intense (Guan & Wiles, 1997; Griffiths, Collen & Armstrong, 2004). Guan & Wiles (1997) showed that crayfish affect the abundances of native benthic fish through both shelter competition and predation. In degraded or urbanised stream habitats where shelter availability is limited, the competition for shelters with crayfish can be expected to have detrimental effects on the survival and growth of native fish. Furthermore, crayfish destroy submerged macrophytes, which provide shelter for stream fish (Matsuzaki *et al.*, 2009). Therefore, the effects of both shelter availability and crayfish invasion are important issues that need to be addressed in urban stream restoration efforts.

In this study, we conducted a stream-channel experiment to investigate the relative importance of shelter availability and the presence of invasive crayfish (*Procambarus clarkii* Girard, hereafter termed crayfish) on the growth performance of two native fish, Japanese crucian carp (*Carassius auratus* complex) and cobitid fish (*Lefua echigonia* Jordan & Richardson), which are known to use shelters. The red swamp crayfish is native to the southern central United States and north-eastern Mexico and is one of the world's most widespread and harmful invasive crayfish species. This crayfish has invaded numerous streams, ponds, lakes and paddy fields throughout the Japanese archipelago (Matsuzaki *et al.*, 2011; Miyake & Miyashita, 2011). In the experiment reported here, the presence or absence of shelter was cross-factored with the presence or absence of crayfish. We evaluated the growth performance of the native species individually by measuring loss in body mass under starved conditions during the experimental period. We predicted that an increase in the metabolic costs associated with the absence of shelter and the presence of crayfish would be accompanied by an increase in the overall loss in body mass of the native fish species.

Methods

Study species

Many Japanese stream and river habitats are degraded as a consequence of hydropower development, road building, deforestation and agriculture (Taniguchi *et al.*, 2001). The streams are profoundly impacted by the changes associated with the urbanisation and suburbanisation of catchments. In urban catchments, the stream beds and

vegetation areas are replaced with concrete, the sediment loads increase, and the hydrology and geomorphology of the streams are fundamentally altered (Fig. 1a), leading to decreased shelter availability and increased substratum embeddedness. Such stream degradation is accompanied by increase in invasive red swamp crayfish. The detrimental impacts of this crayfish on the native species and ecosystems through trophic and non-trophic mechanisms are causing increasing concern.

We selected two native benthic fish, Japanese crucian carp (hereafter termed crucian carp) and cobitid fish, which differ in morphology, diet, swimming behaviour and habitat use. The cobitid fish is invertivorous, has a cylindrical body shape and prefers upstream or mid-stream reaches and small irrigation ditches with cool water temperatures, dense submerged macrophytes and sand substratum. The species is currently considered

endangered (Category IB: endangered) by the Ministry of the Environment of Japan, because of habitat degradation, water quality deterioration and impacts from invasive species. The crucian carp is omnivorous, has a laterally compressed body shape and prefers midstream or downstream reaches, ponds and lakes with warm water temperatures and mud substratum. This species is also a commercially important fish species in Japanese inland fisheries.

The crucian carp and cobitid fish were collected in the southern Ibraki Prefecture, Japan, from Lake Kasumigaura and the Sakura River, respectively. These fish were transferred to the National Institute for Environmental Studies (NIES) and were housed in 250-L flow-through fibreglass reinforced plastic tanks in a glasshouse under a natural photoperiod at 15–20 °C with supplemental aeration. A large number of circular polyvinyl chloride (PVC) pipes of various sizes were added into the tanks to serve as shelters. The fish were fed to satiation with dry, pelleted food once a day. The crayfish were bought from a local aquarium retailer and were maintained in the same conditions used for the fish.

We used cobitid fish (adult) and crucian carp (juvenile) of approximately equal size (a standard length of 5 cm) in the experiment. The mean wet mass (\pm SD) of the crucian carp and cobitid fish were 1.50 ± 0.24 g ($n = 20$) and 2.35 ± 0.21 g ($n = 20$), respectively. We selected small and medium-sized crayfish (a mean orbital carapace length of 12.5 ± 1.0 mm, $n = 21$) because large crayfish can act as predators (Guan & Wiles, 1997).

Channel experiment

The experiment was performed in February 2009, using 14 parallel experimental stream channels with a flow-through system built in the glasshouse at the NIES under natural light conditions (Fig. 1b). Owing to logistical and space constraints, two types of channels with different lengths were used: ten short channels (0.21 m wide \times 0.9 m long, water depth 10 cm) and four long channels (0.21 m wide \times 2.7 m long, water depth 10 cm). The stream channels were constructed with PVC troughs. Using a 2.0-mm plastic mesh, parts of the short and long channels were divided into two and six test arenas (0.21 m wide \times 0.45 m long), respectively. The experiment was conducted with no other substrata (e.g. sand, stones or aeration tube) to accurately evaluate the effect of shelter. To maintain a constant water quality, ambient oxygen levels and water temperature was kept constant, ground water (0.29 mg L⁻¹ total phosphorus, 0.33 mg L⁻¹ total nitrogen) was continuously trickled from the top of each

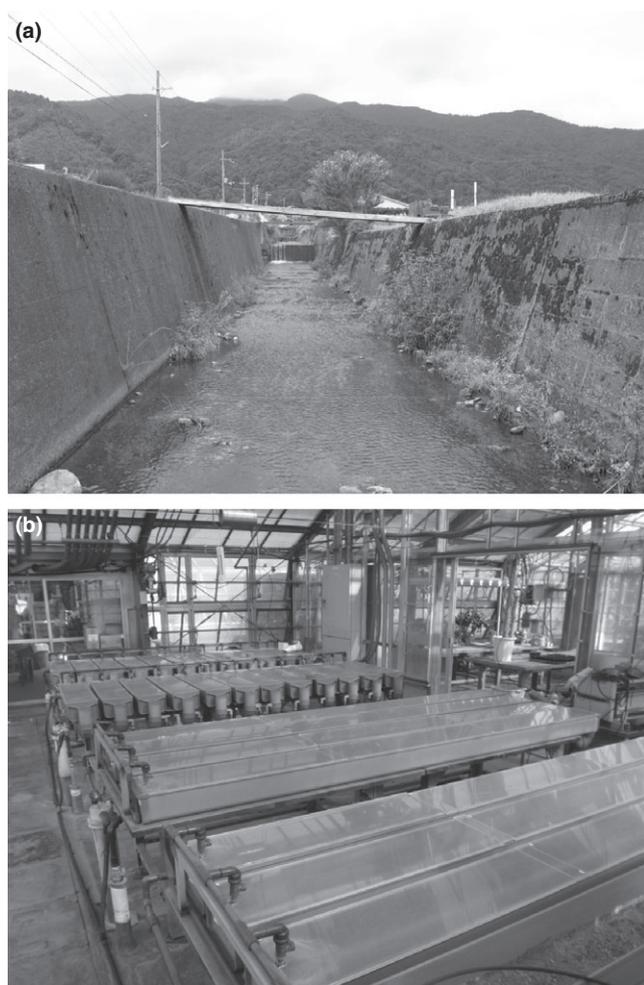


Fig. 1 (a) A typical urbanised stream covered with concrete materials (i.e. a habitat with a low amount of shelter availability) in Japan (the Kannon River, Fukui Prefecture), (b) The short (background) and long (foreground) experimental channels used in this study. Photograph by S. Matsuzaki.

arena using 6-mm flexible PVC tubes with valves. The mean flow in the experimental channels was 0.3 L min^{-1} , and the oxygen concentration was $7.6 \pm 0.3 \text{ mg L}^{-1}$ (mean \pm SD). The water temperature was measured continuously using an automated data logger (mean \pm SD: $16.2 \pm 0.5 \text{ }^\circ\text{C}$). Each channel was covered by a 3-mm-thick clear acrylic plate to prevent the animals from escaping. Food was not provided during the experiment, and no natural food items entered through the inlet water or from the top of the arena.

Experimental design and procedure

For each native fish species, the experiment involved a 2×2 factorial design comprising the presence or absence of shelter crossed with the presence or absence of crayfish. Each treatment was replicated five times. The treatments with and without crayfish were randomly assigned to the 20 arenas in the long and short channels, respectively, to avoid the effects of any chemical cues from either the crayfish or other fish. In the shelter treatments, one PVC grey cylinder pipe (internal diameter of 2 cm, length of 7 cm) was placed in the centre of the arena (not fixed). In the shelter-competition treatments, one small or medium-sized crayfish was introduced into each arena. To prevent starvation of the crayfish, new crayfish were introduced to replace those previously used throughout the experiment.

On 9 February (day 0), we released one cobitid fish or crucian carp, which had been starved for 48 h before the experiment, into the arena. After 1 h, one crayfish was added in each crayfish treatment to begin the experiment. No mortality occurred during the experiment or at the end of the experiment. To quantify the growth performance, the change in mass over time was used as the growth rate for this experiment. We measured the wet mass ($\pm 0.01 \text{ g}$) of the fish individually on the initial day (day 0) and at the end of the experiment (day 8). The fish were dried with Kimwipes® (Kimberly-Clark, Roswell, Georgia, U.S.A.), placed in a 50-mL beaker containing water and weighted using a Mettler & Toledo microbalance. We calculated the daily specific growth rates (SGRs) according to the following equation (Krause *et al.*, 1998; Finstad *et al.*, 2007; Slos & Stoks, 2008; Olsson & Nystrom, 2009):

$$\text{SGR} = (\ln M_{\text{end}} - \ln M_{\text{initial}}) / (t_{\text{end}} - t_{\text{initial}})$$

where M_{end} and M_{initial} denote the fish wet mass at the final (t_{end}) and initial (t_{initial}) days of the experimental periods. This measure of the growth rate was independent of the initial fish mass; fish that suffered higher metabolic costs were expected to show higher absolute losses in mass and more negative SGR.

On day 4 (09:30 AM), day 6 (03:00 PM) and day 7 (09:00 AM), we observed the presence or absence of shelter use by native fish and by the crayfish by viewing from the top of the arenas without opening the cover.

Statistical analysis

We examined the effects of the availability of shelter, the presence of crayfish and their interactions (shelter \times crayfish) on the SGRs of the two native fish species, using generalised linear models (GLMs) with a gamma distribution and log link. To control for the effect of the position of the arena in the channel, we numbered the arenas from upstream (one) to downstream (six) (one to two in the case of the short channels) and then incorporated this variable into the models. Following Burnham & Anderson (2002), we used an information-theoretic model selection approach to rank and evaluate the models. The models for all the possible parameter subsets were compared in terms of parsimony and prediction based on the Akaike information criterion (AIC) (Burnham & Anderson, 2002). The Akaike weights W_i were calculated for each model. A given W_i can be interpreted as the expected probability of that model being selected as the most appropriate if the data were collected again under identical circumstances. If one of the models was clearly the best fit (e.g. if $W_i > 0.9$), then model inference can be made on the selected best model (Burnham & Anderson, 2002). Often, however, no single model is clearly superior to the others in the set. Therefore, a multimodel inference approach was adopted for estimating the relative importance of variables (RVI) (Burnham & Anderson, 2002). The relative importance of the predictor variable j was estimated by adding W_i across all the models in the set where variable j occurs. The relative importance of variable j is reflected in the sum of Akaike weights ($\sum W_i$). The larger the $\sum W_i$, the more important variable j is relative to the other variables. The predictor variables with a $\sum W_i < 0.5$ are considered relatively unimportant (Burnham & Anderson, 2002). All the analyses were conducted in R version 2.11.0 (R Development Core Team 2010) using the package MuMIn (Bartoń, 2009).

Results

Competition for limited shelter

In the shelter treatments without the crayfish, all the crucian carp and cobitid fish consistently entered into the shelter. However, in the shelter treatments with the crayfish, the crayfish consistently occupied the shelter.

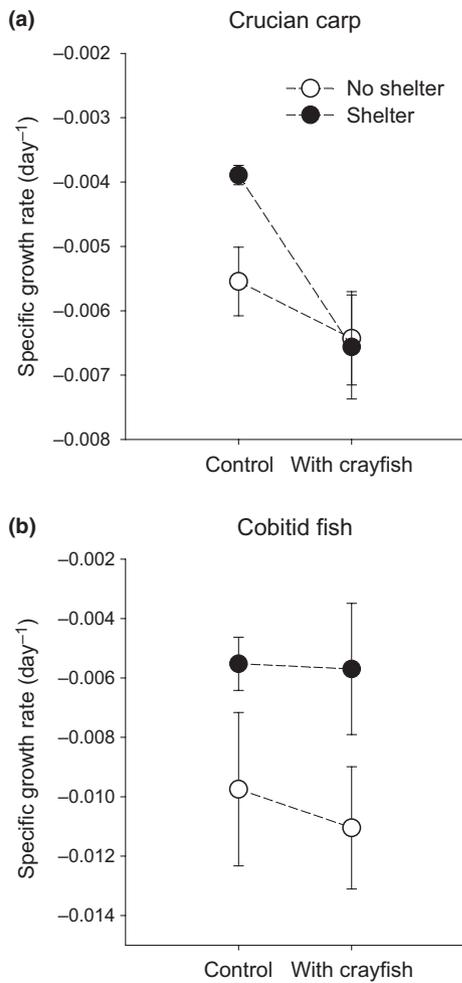


Fig. 2 The mean (\pm SE) specific growth rate (SGR, day⁻¹) of the (a) crucian carp and (b) cobitid fish. Treatments ($n = 5$ fish each) consisted of the presence or absence of shelter cross-factored with the presence or absence of non-native red swamp crayfish. A lower SGR indicates a greater loss in body mass during the experimental period.

Although the native fish species rested into the shelter before the introduction of crayfish, the crayfish were observed forcing native fish to leave the shelter as soon as they entered. The expelled fish usually rested outside and adjacent to the cylindrical shelter occupied by the crayfish. However, neither aggressive attacks on the fish nor consumption of the fish by the crayfish was observed.

Growth performance

All of the crucian carp and cobitid fish showed negative SGRs (Fig. 2). In the model selection for growth performance, more than one model had a Δ AIC value of <2.0 (Table 1). These model selection results indicated that a multimodel inference approach was needed for this data set.

For the crucian carp, the multimodel inference approach showed that both the shelter and the crayfish variables were found in the highest-ranked models for explaining the variation in the SGRs, and showed large $\sum W_i$ values (Table 1). The $\sum W_i$ value was greater for the crayfish variable than for the shelter variable. The reduction in body mass increased with the absence of shelter and with the presence of crayfish (Fig. 2a). However, the interaction term between the shelter and crayfish variables also appeared in the set of models selected ($\sum W_i = 0.56$, Table 1), and this value indicated that this interaction was moderately important as an explanatory variable ($\sum W_i > 0.5$). This result indicates that the effects of shelter availability and crayfish presence on growth performance of crucian carp were interactive. The growth performance of the crucian carp was improved by the presence of shelter except in the presence of crayfish; the

Table 1 The three most highly ranked generalised linear models and relative importance (0 = no evidence, 1 = strong evidence) of individual variables affecting the specific growth rates (day⁻¹) of each native fish species. The relative variable importance (RVI) was based on the sum of the Akaike weights (W_i) across all models in candidate set in which the variable occurs

Species	Rank	Explanatory variables (estimate \pm SE)				K	AIC	Δ AIC	W_i
		Shelter	Crayfish	Shelter : Crayfish	Arena position				
Crucian carp	1	76.7 \pm 34.8	-24.8 \pm 25.2	-79.8 \pm 40.9		5	-184.8	0.00	0.35
	2	78.0 \pm 35.0	-15.7 \pm 28.0	-81.5 \pm 41.2	-5.7 \pm 6.9	6	-183.7	1.10	0.20
	3		-58.0 \pm 22.0			3	-183.2	1.59	0.16
	RVI*	0.73	0.95	0.56	0.36				
Cobitid fish	1	82.4 \pm 37.4				3	-145.0	0.00	0.41
	2	82.2 \pm 38.0	-10.6 \pm 32.4			4	-143.2	1.87	0.16
	3	82.9 \pm 38.4			-2.7 \pm 10.0	4	-143.1	1.91	0.16
	RVI*	0.86	0.34	0.08	0.27				

AIC, Akaike's information criterion; Δ AIC, difference in the AIC value of each candidate model from that in the best model; K, number of parameters; W_i , Akaike weight.

*RVIs shown in bold type (> 0.5) are considered relatively important (Burnham & Anderson 2002).

presence of shelter did not ameliorate the negative effect of crayfish on the growth of the crucian carp. Averaged for conditions of shelter present and absent, crayfish presence was associated with a 38% greater loss in body mass of the crucian carp.

For the cobitid fish, the multimodel inference approach revealed that only the shelter variable had a large $\sum W_i$ and a positive effect on the SGR (Table 1, Fig. 2b). The absence of shelter increased the loss of body mass of the cobitid fish by approximately 86% (Fig. 2b), regardless of the presence or absence of the crayfish. The change in SGR for the cobitid fish was greater than that for the crucian carp. In addition, the crayfish variable and the interaction term between the shelter and crayfish did not appear in the selected models and exhibited small $\sum W_i$ values.

The arena position term showed a small $\sum W_i$ value in both of the native fish species (Table 1), indicating that the position of the arena in the channel had no effect on the SGRs.

Discussion

Our experiment showed that the availability of shelter and the presence of invasive crayfish affected the growth performance of native benthic fish species, but the relative importance of those effects differed between the crucian carp and cobitid fish. We found that shelter availability is the primary factor affecting mass loss in the cobitid fish, whereas both shelter availability and crayfish presence influence mass loss in the crucian carp (Fig. 2, Table 1). Our results suggest that decreased shelter availability and/or crayfish invasion may have significant negative effects on the growth performance of fish but that the magnitude of those effects and whether those effects are additive might vary depending on the fish species. Reductions in growth performance have potentially serious repercussions for the future fitness of stream fish species (Yamahira *et al.*, 2007).

Our results are consistent with previous laboratory and field studies, in which a lack of sheltering opportunities was shown to have negative effects on the metabolic or growth rate (Millidine *et al.*, 2006; Finstad *et al.*, 2007; Olsson & Nystrom, 2009). It is possible that the enhanced loss of body mass in the absence of shelter resulted from increased mental alertness: a state of heightened antipredator vigilance that increases resting metabolism and energetic demands (Fischer, 2000; Roulin, 2001; Millidine *et al.*, 2006). The change in the SGRs of the cobitid fish in response to shelter absence was greater than that of crucian carp, suggesting that the cobitid fish may be more vulnerable than the crucian carp to a reduction in shelter

availability. Furthermore, the costs associated with the absence of shelter might be higher in food-limited environments, such as those in our experiment. Metcalfe, Huntingford & Thorpe (1987) reported that increased antipredator vigilance might decrease foraging opportunities.

More importantly, the effect of shelter availability on growth performance in the presence of crayfish differed substantially between the native fish species. Both the crucian carp and the cobitid fish expelled from the shelter appeared to use the habitat outside of and adjacent to the shelter when crayfish occupied the shelter, but the body mass loss of only the crucian carp increased. For the cobitid fish, therefore, it seems that even in the presence of the crayfish, a small amount of space around the shelter might have the potential to reduce the metabolic costs, such as the cost of vigilance. However, the occurrence of invasive crayfish can be equally deleterious to the growth performance of the crucian carp whether shelter is available or not. In aquatic environments, chemical cues tend to be more important than visual cues as indicators of changes in predation risk (Brönmark & Hansson, 2000). A number of previous studies on a diverse set of aquatic species have shown that the non-lethal effect of predation alters the morphology, physiology and behaviour of the prey (Sakamoto, Chang & Hanazato, 2006; Beckerman, Wieski & Baird, 2007; Hawkins, Magurran & Armstrong, 2007; Slos & Stoks, 2008). Crucian carp (*Carassius carassius*, L.) change their behaviour and body shape in response to chemical cues from piscivorous predators (e.g. Brönmark & Pettersson, 1994). Johansson & Andersson (2009) have reported that cues from pike (*Esox lucius*, L.) reduce activity in crucian carp, although the observed loss in body mass was small in magnitude. However, the crucian carp in the current study usually rested immobile on the floor of the arenas, and differences in activity were not observed. However, the crucian carp lost more weight when crayfish was present. Van Uitregt, Hurst & Wilson (2012) have demonstrated that chemical predation cues limit activity but also cause a reduction in growth and development. Accordingly, we suggest that cues from the crayfish may have negative effects on the growth performance of native fish.

One possible mechanism to explain these results is an increase in the gill ventilation rate and mental alertness. Atlantic salmon fry increase their gill ventilation rate in response to predator odour cues, and their ventilation rate increases with increasing concentrations of predator odours (Hawkins, Armstrong & Magurran, 2004; Hawkins *et al.*, 2007). It is possible that this response prepares the fish for possible sustained activity, such as

fleeing. Yet, increasing the ventilation rate is energetically expensive for fish, and this metabolic cost can reach 25% of the total metabolic rate of a fish (Hughes & Shelton, 1962; Yamamoto, 1991; Millidine *et al.*, 2006). Our results suggest that through chemical cues and shelter competition, crayfish could alter the allocation of energy to growth in the fish, resulting in a reduction in growth performance.

In contrast, the presence of the crayfish did not affect the SGRs of the cobitid fish. Recent reviews report that some naïve prey respond appropriately to a novel predator, whereas other prey fail to recognise predation threats and display inappropriate or ineffective antipredator responses (Banks & Dickman, 2007; Sih *et al.*, 2010). We hypothesise that antipredator responses might not be appropriate in cobitid fish, and this species may therefore experience heavy predation under natural conditions if this predator is present.

Limitations, future directions and conservation implications

At least three caveats apply to this study and should be the focus of future research. First, our study examined only one invasive crayfish and two native fish species, but our results highlight the importance of considering species-specific vulnerability to the absence of shelter and to the presence of an invasive predator or competitor. The first priority for future research is to assess the effects of shelter availability and crayfish on other native fish species and to investigate the impacts of other invasive shelter competitors such as channel catfish (*Ictalurus punctatus*, Rafinesque). The use of shelter has been reported in many stream fish species including Atlantic salmon (e.g. Millidine *et al.*, 2006), burbot (*Lota lota*, L.) (Fischer, 2000), stone loach (*Noemacheilus barbatulus*, L.) (Guan & Wiles, 1997; Fischer, 2000), bullhead (*Cottus gobio*, L.) (Guan & Wiles, 1997) and spinous loach (*Cobitis shikokuensis*) (Kawanishi, Kubo & Inoue, 2010). Furthermore, shelter competition commonly occurs between crayfish and native fish in streams (Guan & Wiles, 1997; Griffiths *et al.*, 2004). Thus, we believe that other native fish species (especially the benthic fish species that use shelters continually) may respond negatively to reduced shelter availability and the presence of crayfish, but the magnitude of the associated costs may differ among native fish species. Future work should also emphasise the interactive effects of other stresses, such as global warming and hypoxia, which can also potentially affect metabolism (Nilsson *et al.*, 2009). An emphasis on the interactive effects is important because many native aquatic species suffer from multiple pressures, rather than a single pressure (Didham *et al.*, 2007).

Second, our experiment used only small-sized native fish and crayfish and did not consider the effect of predation. Large crayfish reduce the survival of benthic fish through predation as well as shelter competition (Guan & Wiles, 1997). Small-sized native fish and juvenile fish may be negatively impacted by crayfish predation in limited shelter conditions, and the availability of shelters influences the mortality directly. In addition, in natural streams, fish expelled from the shelter by crayfish would be more vulnerable to piscivorous fish (Griffiths *et al.*, 2004). In contrast, larger fish that are not influenced by crayfish predation are likely to be severely influenced by a reduction in shelter availability (Finstad *et al.*, 2007). Further study is needed to examine how the relative importance of predation, chemical cues and shelter availability on growth performance changes with fish size.

Third, our experiment was conducted in small arenas in a stream-channel system and was conducted over a short period. This study is a first step towards understanding the additive effects of shelter availability and invasive species on growth performance, but extrapolation of our results to a field situation requires caution, especially if multiple trophic interactions are being considered. A large-scale experiment, in combination with other methods, could help relate the experimental results to natural communities.

Our findings have important implications for habitat restoration. River development and land-use changes (e.g. urbanisation) have the potential to alter physical habitat characteristics rapidly and can facilitate the establishment of invasive species (Taniguchi *et al.*, 2001; Riley *et al.*, 2005; McKinney, 2006). Unfortunately, the metabolic and growth cost associated with reduced shelter availability have received little attention in habitat assessments or stream restoration programmes aimed at improving stream environments to maintain native fish diversity. In view of the importance of shelter availability, there is a need to identify and prioritise the suitable habitats (i.e. those with a high habitat complexity) for endangered fish species such as cobitid fish. A method developed by Finstad *et al.* (2007) that measures shelter availability using flexible PVC tubes can be effective for this purpose. In addition, the provision of artificial physical structures can enhance shelter availability in highly degraded or urbanised stream habitats (Miller *et al.*, 2009).

Simultaneously, the control and removal of crayfish should be considered in stream restoration, because crayfish have detrimental impacts on several native taxa through both lethal and non-lethal effects (i.e. shelter competition and chemical cues). In real ecosystems, crayfish may alter physical habitats and reduce shelter

availability via ecosystem engineering, because they directly cut submerged macrophytes that provide shelter for various aquatic animals (Matsuzaki *et al.*, 2009). In Japan, alien species that cause damage to native biodiversity, human safety and agriculture are designated as invasive species (IAS) in the IAS Act by the Ministry of the Environment of Japan, but the red swamp crayfish is not currently designated as an IAS. Few regulations exist to prevent the further introduction of red swamp crayfish in Japan. We recommend the establishment of effective methods to prevent invasions, remove this invasive species and develop public education programmes about the potential hazards that these crayfish pose to Japanese streams.

Acknowledgments

We are most grateful to the staff at the NIES Ecosystem Field Station, including Yoshio Suzuki and Chizuko Yoshida, Yutaka Ogamino, Takamaru Nagata and Takahiro Morosawa, for their valuable assistance during the experiments. We would like to thank Akira Yoshioka and Munemitsu Akasaka for their statistical advice, as well as Richard Johnson and two anonymous referees for their helpful comments on an earlier draft of this manuscript. This study was partially supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan to S. Matsuzaki (nos. 1811493 and 23710288). The present study was also supported by the Environment Research and Technology Development Fund (S9) of the Ministry of the Environment, Japan.

References

Allan D.J. (2004) Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 257–284.

Banks P.B. & Dickman C.R. (2007) Alien predation and the effects of multiple levels of prey naivete. *Trends in Ecology & Evolution*, **22**, 229–230.

Bartoń K. (2009) MuMIn: Multi-Model Inference. R Package, Version 0.12.2. Available from <http://r-forge.rproject.org/projects/mumin/>.

Beckerman A.P., Wieski K. & Baird D.J. (2007) Behavioural versus physiological mediation of life history under predation risk. *Oecologia*, **152**, 335–343.

Bernhardt E.S. & Palmer M.A. (2007) Restoring streams in an urbanizing world. *Freshwater Biology*, **52**, 738–751.

Brönmark C. & Hansson L.A. (2000) Chemical communication in aquatic systems: an introduction. *Oikos*, **88**, 103–109.

Brönmark C. & Pettersson L.B. (1994) Chemical cues from piscivorous induce a change in morphology in crucian carp. *Oikos*, **70**, 396–402.

Burnham K.P. & Anderson D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York.

Cai F., Wu Z., He N., Wang Z. & Huang C. (2011) Can native species crucian carp *Carassius auratus* recognizes the introduced red swamp crayfish *Procambarus clarkii*? *Current Zoology*, **57**, 330–339.

Didham R.K., Tylianakis J.M., Gemmill N.J., Rand T.A. & Ewers R.M. (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology & Evolution*, **22**, 489–496.

Finstad A.G., Einum S., Forseth T. & Ugedal O. (2007) Shelter availability affects behaviour, size-dependent and mean growth of juvenile Atlantic salmon. *Freshwater Biology*, **52**, 1710–1718.

Fischer P. (2000) An experimental test of metabolic and behavioural responses of benthic fish species to different types of substrate. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 2336–2344.

Griffiths S.W., Collen P. & Armstrong J.D. (2004) Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology*, **65**, 436–447.

Guan R.Z. & Wiles P.R. (1997) Ecological impact of introduced crayfish on benthic fishes in a British lowland river. *Conservation Biology*, **11**, 641–647.

Hawkins L.A., Armstrong J.D. & Magurran A.E. (2004) Predator-induced hyperventilation in wild and hatchery Atlantic salmon fry. *Journal of Fish Biology*, **65**, 88–100.

Hawkins L.A., Magurran A.E. & Armstrong J.D. (2007) Innate abilities to distinguish between predator species and cue concentration in Atlantic salmon. *Animal Behaviour*, **73**, 1051–1057.

Hughes G.M. & Shelton G. (1962) Respiratory mechanisms and their nervous control in fish. In: *Advances in Comparative Physiology and Biochemistry* (ed. O.E. Lowenstein), pp. 275–364. Academic Press, New York.

Johansson F. & Andersson J. (2009) Scared fish get lazy, and lazy fish get fat. *Journal of Animal Ecology*, **78**, 772–777.

Johnson P.T.J., Olden J.D. & vander Zanden M.J. (2008) Dam invaders: impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and the Environment*, **6**, 359–365.

Kawanishi R., Kubo R. & Inoue M. (2010) Habitat use by spinous loach (*Cobitis shikokuensis*) in southwestern Japan: importance of subsurface interstices. *Ecological Research*, **25**, 837–845.

Krause J., Loader S.P., McDermott J. & Ruxton G.D. (1998) Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **265**, 2373–2379.

- Larson E.R. & Magoulick D.D. (2009) Does juvenile competition explain displacement of a native crayfish by an introduced crayfish? *Biological Invasions*, **11**, 725–735.
- Lowe W.H., Nislow K.H. & Bolger D.T. (2004) Stage-specific and interactive effects of sedimentation and trout on a headwater stream salamander. *Ecological Applications*, **14**, 164–172.
- Matsuzaki S.S., Usio N., Takamura N. & Washitani I. (2009) Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia*, **158**, 673–686.
- Matsuzaki S.S., Terui A., Kodama K., Tada M., Yoshida T. & Washitani I. (2011) Influence of connectivity, habitat quality and invasive species on egg and larval distributions and local abundance of crucian carp in Japanese agricultural landscapes. *Biological Conservation*, **144**, 2081–2087.
- McKinney M.L. (2006) Urbanization as a major cause of biotic homogenization. *Biological Conservation*, **127**, 247–260.
- Metcalfe N.B., Huntingford F.A. & Thorpe J.E. (1987) The influence of predation risk on the feeding motivation and foraging strategy of juvenile Atlantic salmon. *Animal Behaviour*, **35**, 901–911.
- Miller M.W., Valdivia A., Kramer K.L., Mason B., Williams D.E. & Johnston L. (2009) Alternate benthic assemblages on reef restoration structures and cascading effects on coral settlement. *Marine Ecology-Progress Series*, **387**, 147–156.
- Millidine K.J., Armstrong J.D. & Metcalfe N.B. (2006) Presence of shelter reduces maintenance metabolism of juvenile salmon. *Functional Ecology*, **20**, 839–845.
- Miyake M. & Miyashita T. (2011) Identification of alien predators that should not be removed for controlling invasive crayfish threatening endangered odonates. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **21**, 292–298.
- Nilsson G.E., Crawley N., Lunde I.G. & Munday P.L. (2009) Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology*, **15**, 1405–1412.
- Olsson K. & Nystrom P. (2009) Non-interactive effects of habitat complexity and adult crayfish on survival and growth of juvenile crayfish (*Pacifastacus leniusculus*). *Freshwater Biology*, **54**, 35–46.
- Paul M.J. & Meyer J.L. (2001) Streams in the urban landscape. *Annual Review of Ecology, Evolution, and Systematics*, **32**, 333–365.
- R Development Core Team (2010) *R2.11.0: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. <<http://www.R-project.org>>.
- Riley S.P.D., Busteed G.T., Kats L.B., Vandergon T.L., Lee L.F.S., Dagit R.G. *et al.* (2005) Effects of urbanization on the distribution and abundance of amphibians and invasive species in southern California streams. *Conservation Biology*, **19**, 1894–1907.
- Roulin A. (2001) On the cost of begging vocalization: implications of vigilance. *Behavioral Ecology*, **12**, 506–511.
- Sakamoto M., Chang K.H. & Hanazato T. (2006) Inhibition of development of anti-predator morphology in the small cladoceran *Bosmina* by an insecticide: impact of an anthropogenic chemical on prey-predator interactions. *Freshwater Biology*, **51**, 1974–1983.
- Sih A., Bolnick D.I., Luttbeg B., Orrock J.L., Peacor S.D., Pintor L.M. *et al.* (2010) Predator-prey naivete, antipredator behavior, and the ecology of predator invasions. *Oikos*, **119**, 610–621.
- Slos S. & Stoks R. (2008) Predation risk induces stress proteins and reduces antioxidant defense. *Functional Ecology*, **22**, 637–642.
- Steele M.A. (1999) Effects of shelter and predators on reef fishes. *Journal of Experimental Marine Biology and Ecology*, **233**, 65–79.
- Suttle K.B., Power M.E., Levine J.M. & McNeely C. (2004) How fine sediment in riverbeds impairs growth and survival of juvenile salmonids. *Ecological Applications*, **14**, 969–974.
- Taniguchi Y., Inoue M.M. & Kawaguchi Y. (2001) Stream fish habitat science and management in Japan: a review. *Aquatic Ecosystem Health & Management*, **4**, 357–365.
- Valdimarsson S.K. & Metcalfe N.B. (1998) Shelter selection in juvenile Atlantic salmon or why do salmon seek shelter in winter? *Journal of Fish Biology*, **52**, 42–49.
- Van Uitregt V.O., Hurst T.P. & Wilson R.S. (2012) Reduced size and starvation resistance in adult mosquitoes, *Aedes notoscriptus*, exposed to predation cues as larvae. *Journal of Animal Ecology*, **81**, 108–115.
- Yamahira K., Kawajiri M., Takeshi K. & Irie T. (2007) Inter- and intrapopulation variation in thermal reaction norms for growth rate: evolution of latitudinal compensation in ectotherms with a genetic constraint. *Evolution*, **61**, 1577–1589.
- Yamamoto K. (1991) Relationship of respiration to body-weight in the carp *Cyprinus carpio* under resting and normoxic condition. *Comparative Biochemistry and Physiology. Part A, Physiology*, **100**, 113–116.

(Manuscript accepted 9 January 2012)