

The importance of allochthonous litter input on the biomass of an alien crayfish in farm ponds

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Abstract To evaluate the effects of allochthonous litter input on the population density of invasive red swamp crayfish (*Procambarus clarkii*) in Japanese farm ponds, we analyzed gut contents, stable isotope ratios, and the correlation between crayfish biomass and environmental factors in the ponds. For our correlation analysis, we used Akaike's information criterion (AIC) corrected for small sample size (AIC_C) to select appropriate models within the generalized linear model. Allochthonous litter input was the most influential variable affecting crayfish biomass, followed by pond area. Gut content analysis demonstrated a positive correlation between the percentage of litter in the crayfish gut and the amount of litter input into the pond from which animals were collected. Crayfish $\delta^{13}\text{C}$ became increasingly similar to litter $\delta^{13}\text{C}$ as litter input into ponds increased. Nitrogen isotope signature analysis suggested that microorganisms attached to litter may contribute to crayfish diet. The above results obtained by three complementary approaches demonstrated an important influence of allochthonous litter input on crayfish biomass in farm ponds. We propose that the appropriate management of surrounding forests may be effective in controlling the

abundance of exotic crayfish with minimized impacts on native communities.

Keywords Bottom-up · Detritus · Energy source · Exotic crayfish · *Procambarus clarkii* · Stable isotope

Introduction

Freshwater ecosystems worldwide harbor a wide variety of invasive alien species, which pose serious threats to native biodiversity through biotic and abiotic interactions (Lodge et al. 1998; Strayer 2010). Alien crayfish are one of the notorious invasive organisms in freshwater ecosystems, feeding on benthic invertebrates, aquatic plants, algae, and detritus (e.g., Gutierrez-Yurrita et al. 1998; Stenroth and Nystrom 2003). Furthermore, some crayfish are known to be ecosystem engineers that modify physical structures of substrates by consuming aquatic plants (Maezono and Miyashita 2004; Rodriguez et al. 2005), and by processing leaf litter (Larned et al. 2003). Thus, alien crayfish may be invasive engineers causing much larger impacts in their introduced ecosystems than invasive non-engineers. To predict the risk of spreading and to find appropriate methods for controlling invasive crayfish, it is essential to identify factors that sustain their high abundance.

In contrast to top-down drivers of crayfish abundance (e.g., Englund 1999; Seiler and Turner 2004; Nystrom et al. 2006), bottom-up mechanisms are not well understood. Gut content and stable isotope analyses are useful for identifying major food sources of crayfish. Benthic invertebrates (Hicks 1997; Whitley and Rabeni 1997; Parkyn et al. 2001; Hollows et al. 2002; Olsson et al. 2008) and allochthonous litter (and/or microorganisms attached to the litter; England and Rosemond 2004; Bondar et al. 2005;

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Reid et al. 2008) are thought to be important crayfish energy sources. Algae may also be important dietary components (France 1996; Kennedy et al. 2005). However, these studies have not definitively demonstrated bottom-up effects on crayfish abundance. Another type of studies that examined correlation between crayfish abundance and environmental factors suggested that invertebrates (Usio and Townsend 2000) and phytoplankton productivity (Abrahamsson and Goldman 1970) may limit the local densities of crayfish, but the causality of this relationship is ambiguous because there is no direct evidence for bottom-up effects. Recently, Kennedy et al. (2005) found that removal of the riparian plants led to decreased density of the red swamp crayfish (*Procambarus clarkii*) in a creek through decreasing litter input. However, because the control and treatment sites were within the same creek (several tens of meters apart), and the crayfish population response was measured 1 year after the experimental removal of litter-providing trees, it remained unclear whether the difference in local population densities resulted from a crayfish emigration or change in population size per se.

Here, we focused on the red swamp crayfish which is a notorious invasive species introduced worldwide, reducing the abundance of aquatic macrophytes and insects (Holdich et al. 1988; Maezono and Miyashita 2004; Geiger et al. 2005; Gherardi and Acquistapace 2007). In Japan, the red swamp crayfish was introduced approximately 80 years ago and is now established widely in freshwater ecosystems (Ban 1980). Our objective was to identify factors that limited red swamp crayfish population density in farm ponds. Farm ponds were chosen for two reasons. First, this system provides numerous experimental units at the ecosystem level, which differ in surrounding forest cover, allowing us to test effects of allochthonous resources on crayfish population density. Second, although farm ponds are artificial systems, they have histories spanning centuries and are richly biodiverse, comparable to natural Japanese systems (Kadono 1998); many rare species of odonates, aquatic insects, and aquatic plants rely heavily on farm ponds (Kadono and Yuma 1995; Kadono 1998; Hamasaki et al. 2009). Thus, the exploration of bottom-up factors influencing alien crayfish abundance is important for biodiversity conservation. We used three complementary approaches: gut content analysis, stable isotope analysis, and correlation analysis (between crayfish density and environmental factors) to test our hypothesis that allochthonous leaf litter enhances the population density of crayfish. Specifically, we examined: (1) whether the proportion of allochthonous leaf litter in the crayfish diet increases with increasing litter input; (2) whether crayfish $\delta^{13}\text{C}$ values become closer to litter $\delta^{13}\text{C}$ values in ponds with increasing allochthonous litter input; and (3) whether

there is a positive correlation between crayfish biomass and allochthonous litter input.

Materials and methods

Study area

Studies were performed in farm ponds in Namegawa town, Saitama Prefecture, eastern Japan (36°03'N, 139°22'E). The landscape is composed of hilly hardwood forests, paddy fields, and small residential areas, being typical of the rural landscape in Japan. More than 200 farm ponds were distributed in this town, and the average area and the average maximum depth in these ponds were approximately 2,800 m² and 2 m, respectively (Arai 1993). These ponds were constructed several hundred years ago to irrigate paddy fields by damming streams in the upper parts of small valleys. Thus, the three-dimensional structures of ponds were similar. Due to rainfall and irrigation for rice fields, water levels change temporarily, but the degree is small, mostly less than 1 m (Arai 1993).

We chose study ponds without alien fish predators (largemouth bass and bluegill) because they are known to limit crayfish abundance (Maezono and Miyashita 2003). We confirmed the absence of these alien fish via several methods, including electrofishing, gill netting, interviews, and visual surveys. A total of 20 ponds were selected, each of which was situated >100 m from another, and separated by a ridge covered with forest. Therefore, movement of crayfish between ponds seemed to be infrequent, and each study pond can be regarded as an independent unit. The bottom substrate of all ponds consisted generally of mud, with few large stones that could provide refuges for crayfish. In ponds surrounded by forests, a large amount of litter that had entered from the riparian forests accumulated on the bottom.

The farm pond fauna was relatively simple and consisted of macroinvertebrates (e.g., chironomids, oligochaetes, larval dragonflies, red swamp crayfish, and shrimp) and vertebrates (e.g., goby, topmouth gudgeon, common carp, and bullfrog). The density of the common carp was so low that its effect on crayfish was likely to be very small.

Riparian vegetation was variable among the study ponds; the riparian vegetation of forested ponds was dominated by deciduous trees, such as konara oak (*Quercus serrata*). These trees reached more than 20 m height, and were densely vegetated, hence producing a large amount of litter every autumn. In contrast, the riparian vegetation of open ponds was sparse, primarily graminoids such as silver grass (*Miscanthus sinensis*). Inside the ponds, emergent plants such as reed (*Phragmites communis*) and

cattail (*Typha latifolia*) were occasionally found, but the area occupied by these plants was generally very small compared to the total pond area (Table 1). Many ponds lacked floating and submerged macrophytes, such as lotus (*Nelumbo nucifera*) and water chestnut (*Trapa japonica*), probably due to crayfish consumption (Maezono and Miyashita 2003).

Crayfish abundance and biomass

Crayfish were collected from 20 ponds with various degrees of canopy cover using funnel traps (overall 25 × 40 × 25 cm, funnel mouth diameter 6 cm, mesh size 3 mm; Table 1). Ten traps were randomly set along entire pond shorelines on three consecutive nights from June to September in 1999 and 2000. Commercial carp bait was used in all traps. The trapping period was approximately 3 h to avoid crayfish escape or cannibalism. Trapped individuals were classified according to size based on postorbital carapace length [CL; long (L), CL > 30 mm; medium (M), 30 mm > CL > 20 mm; short (S), 20 mm > CL]; each animal was then released after clipping part of the telson for identification of captured individuals. Population size was estimated using the Petersen method as modified by Chapman's formula (Seber

1982). Because the sex ratio of the captured crayfish was almost unity (male 2,820, female 2,847) and the recapture rates were also very similar between sexes (male 0.148, female 0.151), we pooled individuals of both sexes for population estimation. Crayfish biomass in each pond was estimated by

$$\sum \hat{N} \times \frac{n_i}{n} \times W_i$$

where \hat{N} is the estimated population size, n_i and n are the number of individuals captured in i -th size class and in all size classes, respectively, and W_i is the mean weight of 20 individuals in i -th size class that were randomly (L 34.50 g, M 7.35 g, S 3.00 g). The abundance of small crayfish may have been underestimated because trapping is known to have a bias towards catching of larger crayfish (Price and Welch 2009). However, this does not seem to greatly affect total biomass of crayfish in a pond, as the biomass contribution of small crayfish would be much smaller than that of larger ones.

Environmental factors

We measured four environmental variables that may affect crayfish biomass in farm ponds: pond area, water quality, emergent plant cover, and allochthonous litter input. Pond

Table 1 The estimated number of red swamp crayfish (*Procambarus clarkii*), their biomass, and the physicochemical characteristics in 20 farm ponds

Pond	Area (m ²)	Crayfish numbers/m ² (CI)	Biomass (g/m ²)	Conductivity (μS/cm)	Forest cover (%)	Shoreline with forest (m)	Annual litter input (g)	Emergent plant cover (m ²)
A	2,106.3	1,514 (1,226–1,802)	19.3	131.6	21.5	124.0	130.8	0.0
B	2,777.8	337 (46–628)	3.1	141.7	5.3	52.0	33.5	0.0
C	1,087.5	455 (137–773)	11.2	124.3	2.3	9.0	14.6	35.0
D	1,922.8	336 (192–480)	2.7	136.9	14.1	50.5	81.8	0.0
E	1,235.8	1,449 (716–2,182)	21.1	101.4	65.3	150.0	379.1	0.0
F	1,438.8	2,219 (1,617–2,821)	19.1	72.7	55.7	157.0	325.5	0.0
G	2,980.5	824 (605–1,043)	9.5	162.7	33.6	154.6	191.8	0.0
H	3,153.3	3,935 (2,644–5,226)	43.1	72.5	53.5	219.9	301.3	0.0
I	2,471.8	806 (544–1,068)	11.3	51.6	47.4	218.0	275.2	0.0
J	1,956.1	2,226 (1,660–2,792)	16.5	69.5	82.0	225.3	464.3	0.0
K	1,543.0	982 (699–1,265)	8.9	71.5	33.1	88.0	190.9	67.3
L	3,279.4	1,175 (710–1,640)	7.3	55.6	46.6	237.0	266.1	0.0
M	5,373.0	1,406 (660–2,152)	6.1	243.0	28.8	271.6	166.1	0.0
N	1,383.2	502 (372–632)	11.9	78.5	32.0	130.5	196.9	0.0
O	3,472.9	723 (400–1,046)	4.6	208.0	22.0	188.0	131.8	93.0
P	1,095.8	439 (197–681)	7.1	142.7	63.3	136.8	369.9	23.3
Q	3,541.6	190 (61–319)	1.8	79.1	17.1	126.5	100.4	0.0
R	1,106.5	828 (719–937)	20.3	77.8	48.4	119.2	286.9	0.0
S	1,702.3	1,648 (846–2,450)	20.8	236.0	56.0	189.8	327.8	131.3
T	2,589.3	987 (597–1,377)	12.7	137.5	33.5	180.3	196.8	0.0

Conductivity refers to specific conductivity, CI 95% confidence interval

area and emergent plant cover were estimated between August and October 2000 using a laser rangefinder (Bushnell) and visual methods. Pond depth was not measured because its variability is known to be much smaller than the variability of pond area (Morita and Arai 2003). Water conductivity was measured using a specific conductivity meter (Yokogawa Electric) and used as a representative indicator of water quality, because other physicochemical characters, such as pH and substratum type, were similar among study ponds (Maezono and Miyashita 2003).

Allochthonous litter input into ponds was estimated as follows. First, direct litter fall from canopy trees to the ponds was estimated using litter traps. Each litter trap consisted of a basket (diameter 480 mm, mesh size 1×10 mm) with floats. Five litter traps were set in each of six ponds with different proportions of forest cover. The litter traps were placed on the surface of the water under canopy cover, 2 m from the shoreline. Second, to estimate indirect litter input from, eight traps were arrayed around shorelines of five ponds (40 traps in total). The ponds selected were those having representative riparian structure in our study ponds. Each trap consisted of plastic net and metal frames in a design that resembled a soccer goal (size $50 \text{ cm} \times 10 \text{ cm} \times 20 \text{ cm}$, mesh size 10 mm), which was oriented with the trap entrance facing toward the forest. Litter samples were retrieved once or twice a month from October 1999 to September 2000 and weighed to the nearest 0.1 g after drying at 60°C for 48 h in an oven. The mean (\pm standard error, SE) direct litter inputs under canopy cover and indirect litter inputs from riparian forests calculated for all ponds were $522 \pm 114 \text{ g/m}^2$ and $317 \pm 126 \text{ g/m}$, respectively. Total litter input at the whole pond level (L) was estimated with the following formula:

$$L = (522 \times V_C + 317 \times S_L)$$

where V_C is the area of vegetation cover above each pond (m^2) and S_L is the length of shore line adjacent to the forest for each pond (m). V_C and S_L were measured using the methods by which pond area was estimated.

Gut content analysis

Crayfish were collected with funnel traps containing mashed potato bait. Mashed potatoes were packed into tea bags to prevent crayfish from consuming the bait. Trapping was conducted from May to July in 2000 in seven ponds that varied in terms of riparian forest cover. Crayfish were immediately fixed in 70% ethanol after catching and preserved in 70% ethanol until analysis. Fifteen adult crayfish from each pond were used for gut content analysis. Foregut contents were placed in Petri dishes containing water and observed under a stereomicroscope (magnification

$\times 13.4\text{--}80$) after removing the amorphous matters (Hollows et al. 2002; Bondar et al. 2005). Under the microscope, food items larger than 0.5 mm located at the intersections of 1-mm^2 grids were classified into 13 food categories: 7 animal materials (fish, crayfish, shrimps, chironomids, zooplankton, other insects, or unknown animals), 5 plant materials (dicotyledons, monocotyledons, filamentous algae, diatom, or unknown plants), and others including unknown materials. A total of approximately 500 grid intersections containing food items were analyzed for each individual. We considered dicotyledonous material to be allochthonous litter because the greater part of this material consisted of canopy litter such as oak; we did not treat monocotyledonous matter as allochthonous litter because it was included in the emergent macrophyte litter fraction.

Stable isotope analysis

We used stable isotope analyses to identify energy sources for crayfish in farm ponds. Analyses were performed on organisms and materials that (1) we found in our gut-content analysis, or (2) had been found in previous studies (e.g., Momot 1995; Gutierrez-Yurrita et al. 1998). Samples were collected from four ponds that differed in terms of percent forest cover (with subsequent differences in allochthonous litter input): 2.3% (pond C), 17.1% (pond Q), 55.7% (pond F), and 65.3% (pond E). Sampling was performed in June and September 2000 [see Table S1 in Electronic supplementary material (ESM) for sample sizes], taking into account the seasonal change in phytoplankton stable isotope signatures (Yoshioka and Wada 1994). Plant material (litter from konara oak, lotus, reed, filamentous algae *Spirogyra* sp., and kudzu *Pueraria lobata*), benthos (chironomids and oligochaetes), dragonfly nymphs (Odonata: Libellulidae), and mud were collected by dip net from the bottom or surface of ponds. Decapod crustaceans (red swamp crayfish and shrimp *Palaemon paucidens*) and fish (topmouth gudgeon *Pseudorasbora parva* and goby *Rhinogobius* sp.) were collected with funnel traps. *Cyclops* spp. were collected with a plankton net. Particulate organic matter (POM) was collected from pond water, which was filtered onto glassfiber filters (Whatman GF/C), after which large zooplankton such as *Cyclops* spp. were removed; the filters were incinerated at 500°C for 5 h to calculate organic matter content. The mean CL (\pm standard deviation, SD) of crayfish was 35.1 ± 38.1 mm. Muscle tissues were separated from fish and crustacean bodies, and the lipid content was extracted using a chloroform:methanol (2:1) solution. Mud and *Cyclops* spp. samples were washed with 1 N HCL and deionized water to remove carbonates. Several individuals of small benthos, such as chironomids and *Cyclops* spp., were combined to form a single sample. The samples were

kept at -40°C in a freezer until stable isotope analysis. All samples were ground after drying at 60°C for 24 h. The stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were measured with an isotope ratio mass spectrometer (EA-IRMS system, NC2500-ConFloII-MAT252) at Nagoya University.

Isotope ratios were expressed as the deviation (‰) from a standard Pee Dee Belemnite for carbon, and from atmospheric nitrogen gas for nitrogen, as follows:

$$\delta X = \left\{ (R_{\text{sample}}/R_{\text{standard}}) - 1 \right\} \times 1000$$

where X is either ^{13}C or ^{15}N , and the corresponding ratio is either $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. A laboratory standard for DL-alanine was used as the running standard for isotope measurements. Carbon and nitrogen isotopes are known to increase by 0.4 and 3.4‰, respectively, per trophic level through food webs (Post 2002).

Statistical analysis

To examine the relationship between crayfish biomass and environmental factors in farm ponds, we performed model selection in regression analyses using Akaike's information criterion (AIC) corrected for small sample size (AIC_C ; Burnham and Anderson 2002). The independent variables were pond area, litter input, specific conductivity, emergent plant cover, and the interaction between pond area and litter input. This interaction term was included because the effect of litter on crayfish density was expected to saturate in small ponds with large perimeter/area ratios. Crayfish biomass and litter input were standardized by unit area for each pond. We constructed 20 models (including all possible combinations of variables) to identify the one which best explained crayfish biomass in farm ponds. Interaction terms were included when pond area and litter input were among the independent variables. Each variable of any interaction term was centered to reduce the problem of multicollinearity (Burnham and Anderson 2002). Crayfish biomass was log-transformed to improve homoscedasticity. We

calculated the Akaike weight (w_i) for each model; w_i is the probability that model i is the best model among those considered.

Gut content data were arcsine-transformed and analyzed by two-way analysis of variance (ANOVA) to test for the effects of pond and month. Likewise, carbon and nitrogen isotope ratios of crayfish were analyzed using two-way ANOVA using the pond and month as factors. All analyses were performed using the software package R for Windows, v.2.4.1.

Results

Relationships between crayfish biomass and environmental factors

The mean values ($\pm\text{SD}$) for crayfish biomass, allochthonous litter input, pond area, and specific conductivity in farm ponds were $12.9 \pm 9.5 \text{ g/m}^2$, $221.6 \pm 121.0 \text{ g/m}^2$, $2,310.9 \pm 1,103.5 \text{ m}^2$, and $119.7 \pm 57.9 \text{ }\mu\text{S/cm}$, respectively (Table 1). Multiple regression analyses showed that 11 models had AIC_C values smaller than that of the null model. Ten of these models included litter input as an independent variable, and eight of these included pond area, suggesting the importance of these variables. The best model included litter input, pond area, and the interaction term litter \times pond area as independent variables. The absolute Z values of regression coefficients (estimate/SE) for litter input and the interaction term were all larger than 2 (Table 2; only top five models are shown), indicating significant effects of these variables. The Akaike weight (w_i) of the top model was nearly 0.47, while those of the other models were less than 0.16, indicating the superiority of the top model.

Gut contents analysis

Dicotyledonous plant material was found in the guts of all crayfish. The percent occurrence of this material (range

Table 2 Results of model selection for crayfish biomass in farm ponds

Model	AIC_C	ΔAIC_C	w_i	Z values					
				Intercept	L	A	C	$L \times A$	Ep
1	42.80	0.00	0.47	18.11	4.18	−0.25		2.68	
2	44.99	2.19	0.16	15.70	3.23				
3	45.37	2.57	0.13	18.16	4.35	−0.47	1.12	2.92	
4	46.59	3.79	0.07	15.85	2.80	−1.18			
5	46.95	4.15	0.06	17.54	4.05	−0.23		2.60	0.13
Null	51.33	8.53	0.01	12.88					

Only the top five models are shown. Regression coefficients were expressed as Z values (estimate/SE)

w_i Akaike weight, L annual litter input in pond (g/m^2), A pond area, C specific conductivity, Ep emergent plant cover

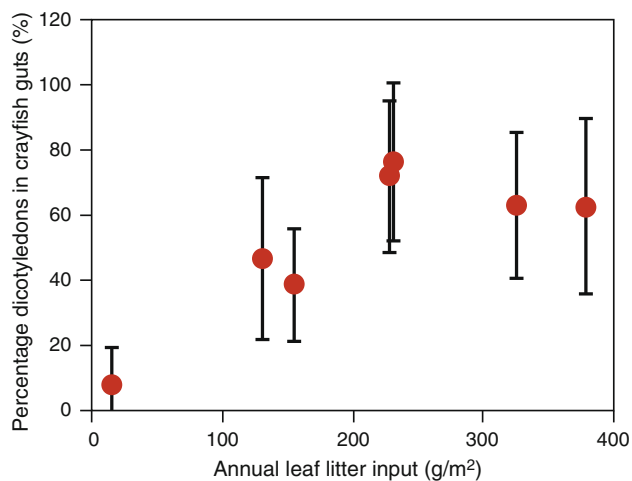


Fig. 1 Relationship between the mean percentage occurrence ($\pm 1SD$) of dicotyledons in red swamp crayfish (*Procambarus clarkii*) guts and annual litter input in Japanese farm ponds

2.1–86.5%) differed significantly among ponds, but did not differ among months (pond: $F_{6,84} = 14.47$, $P < 0.0001$; month: $F_{2,84} = 0.1482$, $P = 0.8625$; pond \times month: $F_{12,84} = 1.123$, $P = 0.3531$; Table S2 in ESM). When the samples collected over 3 months were pooled, a significant positive relationship was detected between the percent occurrence of dicotyledonous material in crayfish gut and the amount of litter input ($r = 0.7691$, $df = 5$, $P = 0.0433$; Fig. 1). Fish made up the highest percent occurrence of animal material in crayfish guts (range 0.0–68.8%). The interaction term pond \times month had a significant effect on the proportion of fish in the gut, and so did the main effect of pond (pond: $F_{6,84} = 12.04$, $P < 0.0001$; month: $F_{2,84} = 0.1782$, $P = 0.8371$; pond \times month: $F_{12,84} = 3.049$, $P = 0.0013$; Table S2). However, no significant relationship was observed between the percent occurrence of fish in crayfish gut and the amount of litter input ($r = -0.4345$, $df = 5$, $P = 0.33$). The percent occurrence of chironomids (range 0.0–2.5%) did not differ among farm ponds (pond: $F_{6,84} = 0.8268$, $P = 0.5524$; month: $F_{2,84} = 0.6135$, $P = 0.5439$; pond \times month: $F_{12,84} = 0.9772$, $P = 0.4771$).

Stable isotope analysis

Crayfish stable isotope ratios differed significantly among farm ponds, but not between sampling periods, for both carbon (pond: $F_{3,28} = 91.78$, $P < 0.001$; month: $F_{1,28} = 0.4877$, $P = 0.48$; pond \times month: $F_{3,28} = 0.677$, $P = 0.58$) and nitrogen (pond: $F_{3,28} = 64.90$, $P < 0.001$; month: $F_{1,28} = 0.0221$, $P = 0.88$; pond \times month: $F_{3,28} = 1.687$, $P = 0.19$). Stable isotope signatures indicated that the major crayfish food and energy sources differed among ponds (Fig. 2; Table S1). Primary producers (filamentous

algae and lotus) were likely major sources in pond C. Mud was a potential energy source in pond Q in June. Allochthonous litter appeared to be the major energy source in ponds F and E. The chironomid $\delta^{13}C$ ranged from -28.5 to -40.0‰ , and values were lower in ponds F and E, in which litter input was large (Fig. 2). In addition, $\delta^{15}N$ signatures showed that fish and shrimp belonged to higher trophic levels than crayfish.

A significant negative relationship was detected between crayfish $\delta^{13}C$ and the amount of allochthonous litter input in both seasons (June: $F = 90.86$, $df = 2$, $P = 0.0108$, $r = 0.892$; September: $F = 133.7$, $df = 2$, $P = 0.0074$, $r = 0.993$; Fig. 3). No relationship was found between crayfish $\delta^{13}C$ and pond area (June: $F = 1.91$, $df = 2$, $P = 0.3010$, $r = 0.6989$; September: $F = 3.84$, $df = 2$, $P = 0.1891$, $r = 0.811$). Furthermore, $\delta^{13}C$ values for crayfish and allochthonous leaf litter were similar in ponds with elevated leaf litter input. These results indicate that crayfish depend more heavily on terrestrial litter as an energy source in ponds with a large amount of litter input.

Discussion

We used three complementary approaches to show that allochthonous litter is an important energy source for pond-dwelling crayfish, which in turn positively affects crayfish population density through bottom-up processes. We obtained three major lines of evidence in support of this finding: (1) the dietary proportion of litter estimated from gut content analysis increased with increasing litter input; (2) crayfish $\delta^{13}C$ values were more closely associated with litter $\delta^{13}C$ values in ponds with greater litter input; and (3) crayfish density was positively correlated with litter input even after taking into account potentially confounding environmental factors. To our knowledge, this is the first study providing sound evidence for the effects of bottom-up processes on crayfish populations.

Positive relationships between the amount of allochthonous litter input and the dependence of crayfish on litter have been reported in studies of gut contents and/or stable isotope signatures (England and Rosemond 2004; Kennedy et al. 2005). In an American stream, Kennedy et al. (2005) demonstrated a positive relationship between the amount of allochthonous litter input and crayfish dependence on litter in autumn or winter when litter was abundant. However, no positive association was observed in spring (Kennedy et al. 2005), suggesting that terrestrial–aquatic coupling might not be as important during the spring and summer in the stream studied. In contrast, our study was conducted in the early summer and early autumn when litter was less abundant, and we have provided stronger evidence than previous studies for bottom-up effects on crayfish.

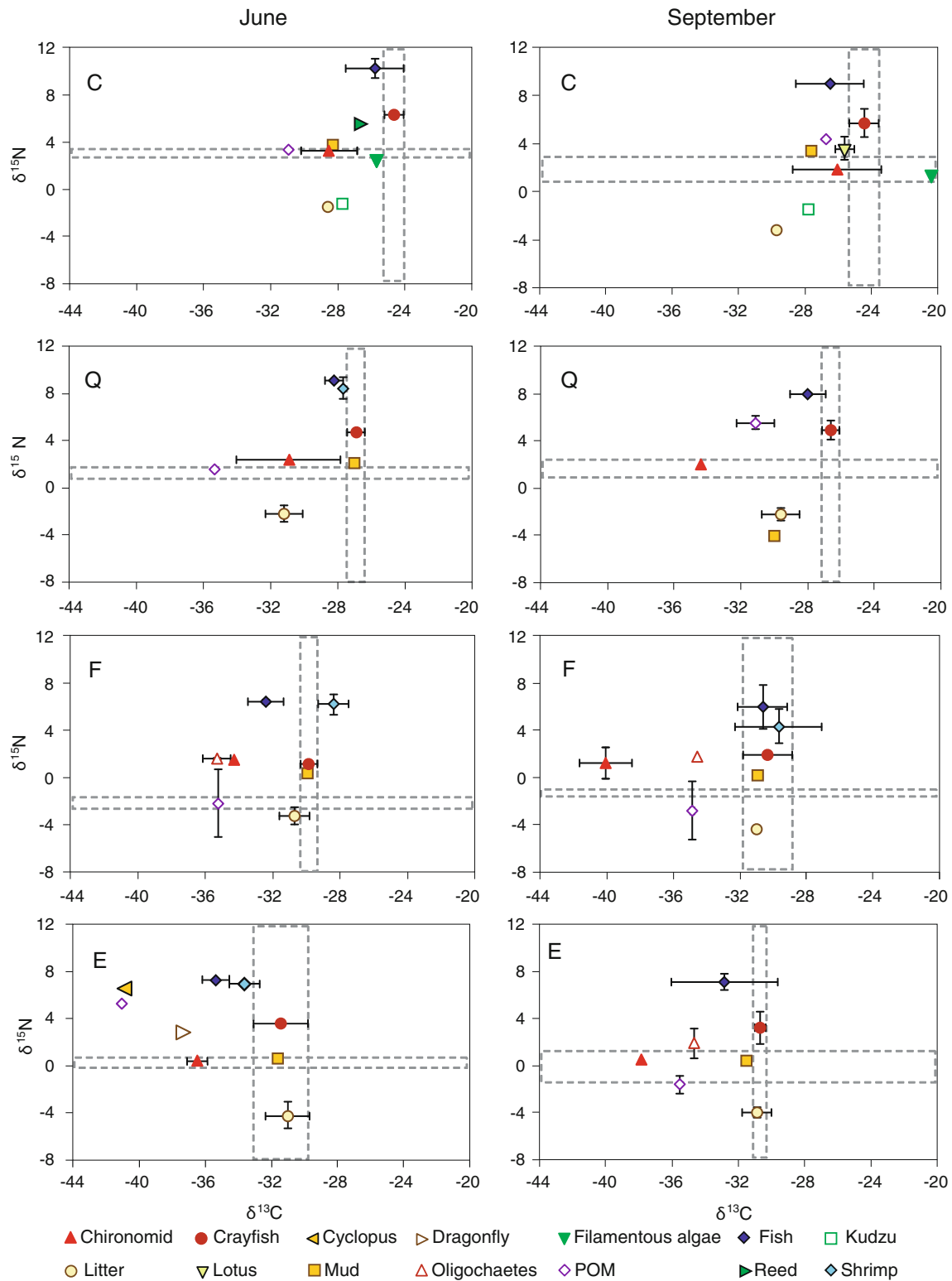
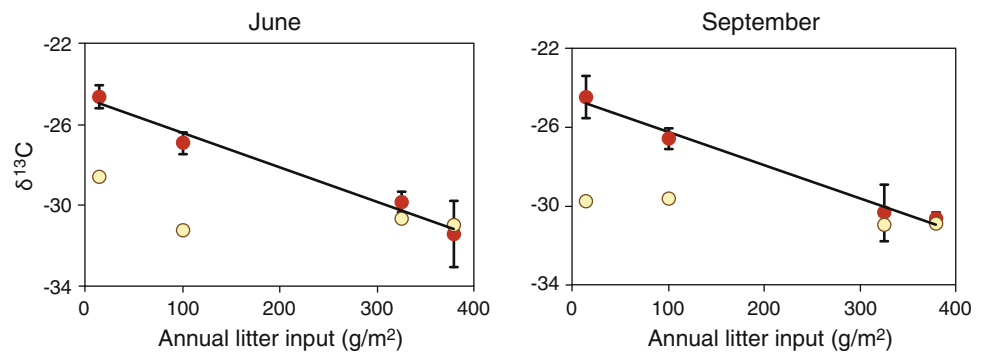


Fig. 2 Stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of red swamp crayfish and their possible dietary components in farm ponds (C, Q, F, E) in June and September 2000 (mean $\pm 1\text{SD}$). Vertical and

horizontal boxes represent the range of mean ($\pm 1\text{SD}$) nitrogen and carbon isotope ratios of crayfish diets expected from the empirical rule of the stable isotope change between prey and predator

Fig. 3 Relationship between carbon isotope signature of crayfish and litter input in June and September 2000 (mean \pm 1SD). Closed and open circles represent stable isotope ratios of crayfish and litter, respectively, in each pond



Detritus is considered to be a low quality resource (Momot 1995). If this is the case, it is unclear whether litter makes a direct energetic contribution to crayfish. Stable $\delta^{15}N$ differences between litter and crayfish in ponds with large amounts of litter input ranged from 4.38 to 8.94. Because these values exceed 3.4‰ (i.e., the standard distance between adjacent trophic levels), there may be intermediate energy sources that we did not examine in our analysis. Previous stable isotope analyses suggested that bacteria and biofilms may be candidate energy resources for crayfish (Hollows et al. 2002; Bondar et al. 2005; Reid et al. 2008). Furthermore, laboratory experiments show that crayfish growth rates are enhanced when detritus enriched with bacteria is provided as food (Wiernicki 1984; McClain et al. 1992a, b). Attached microbes are known to change $\delta^{15}N$ values of plant detritus without changing $\delta^{13}C$ signatures (Fellerhoff et al. 2003). Thus, allochthonous litter may indirectly provide an energy source for crayfish through biofilm formation. This postulate is corroborated by our finding that the $\delta^{15}N$ value of mud, a mixture of detritus and bacteria, was at an intermediate trophic level between crayfish and litter in some ponds. Benthic invertebrates such as chironomids, considered to be a major energy source for crayfish in past studies (Momot 1995), were probably not important as an energy source because their $\delta^{13}C$ values differed by $>4\text{‰}$ from those of crayfish in most ponds. Similarly, small fish that occupied a significant portion of gut content in some ponds appeared to have little contribution to energy source for crayfish, since fish belonged to higher trophic levels than crayfish as evidenced by $\delta^{15}N$ values.

We also showed that crayfish biomass was positively correlated with allochthonous litter input, and the strength of the association increased with pond size. As mentioned above, this increase with pond size can be explained by the negative correlation between pond area and perimeter/area ratio. As small ponds have larger perimeter/area ratios, the amount of substrate litter per unit area should generally be larger, hence the positive effect of litter on crayfish density may be weaker, or even become saturated in small ponds. In other words, the limitation according to litter input appears to be stronger in larger ponds. It should be noted,

however, that a positive association between crayfish biomass and allochthonous litter input does not necessarily imply bottom-up effects, because litter accumulated in ponds may have increased the carrying capacity for crayfish through increased substrate complexity and/or habitable space. Although the relative importance of bottom-up versus habitat effects are unknown, the increased energetic contribution of detrital-based food webs with increasing litter input, as well as a superabundant food supply from forests, suggests the existence of bottom-up effects. Interspecific interactions such as competition and predation appeared to be less important than allochthonous litter subsidy. First, potential competitors such as native crayfish were absent, and shrimps were minor in terms of biomass. Second, predatory fish that are likely to influence crayfish abundance were absent (bass and bluegill) or in low densities (carp) in our study ponds.

Our finding that allochthonous resources control crayfish abundance has important implications for community dynamics in farm ponds. Because crayfish appeared to be subsidized by allochthonous resources in a donor-controlled manner, crayfish likely exert continued impacts on pond communities even after the abundances of constituent members of pond food webs have fallen steeply. This indirect negative effect between litter provided by riparian forests and organisms in ponds, which is mediated by generalist crayfish, is analogous to apparent competition (Holt 1977), whereby the density of a particular prey species reduces another prey by increasing the abundance of a shared predator species. This type of indirect interaction is also similar to “hyperpredation” when the shared predator is an exotic species (Smith and Quin 1996). Hyperpredation is a notorious mechanism causing severe declines and even extinctions of native species (Norbury 2001; Roemer et al. 2002; Courchamp et al. 2003; White et al. 2006), and this mechanism may explain why exotic red swamp crayfish have large impacts on native ecosystems (Maezono and Miyashita 2004; Geiger et al. 2005; Gherardi and Acquistapace 2007).

The mechanisms we identified have also important implications for ongoing attempts to mitigate the impact of exotic crayfish in pond ecosystems. The eradication of

exotic crayfish solely by trapping is usually impractical in terms of cost and labor (Hein et al. 2006). Control of crayfish by predatory fish would be effective (Hein et al. 2006), but the introduction of non-native predatory fish is not recommended due to strong secondary impacts on native communities, as demonstrated for largemouth bass in farm ponds (Maezono and Miyashita 2003). As Kennedy et al. (2005) showed, crayfish abundance would be expected to decline in our pond systems were we to reduce allochthonous litter input from surrounding forests. Until the 1960s, lowland hardwood forests in Japan were traditionally maintained by periodic coppicing for fuel, but the subsequent lack of human management has allowed overgrowth of canopy trees (Kobori and Primack 2003). This has probably enhanced leaf litter input into farm ponds over the past several decades. Thus, selective or periodic tree cutting is a restoration practice not only for ponds but also for secondary forests. We propose that the appropriate management of surrounding forests, coupled with crayfish trapping, would likely mitigate crayfish impacts while having a minimal secondary impact on farm pond communities.

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