Variable success of biological control of *Lythrum salicaria* in British Columbia

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Abstract

Purple loosestrife, *Lythrum salicaria*, has invaded North American wetlands over the last 200 years. A biological control project was started in British Columbia, Canada, in 1993 with the introduction of *Galerucella calmariensis*, a leaf-feeding beetle of European origin. To evaluate the success of the biological control project in the Lower Mainland of British Columbia, we monitored the defoliation levels of *Lythrum* at 10 release sites of the control agent, identified factors associated with among-site variability in defoliation levels, and determined the effect of defoliation on the performance and abundance of *Lythrum*. In tidal areas, the beetles could not establish due to tidal disturbances. The control agent established at all non-tidal sites, but defoliation levels varied considerably. Defoliation levels were negatively associated with predation on *Galerucella* eggs, suggesting that general predators may be able to prevent or delay successful biological control of *Lythrum*. Eggs on *Lythrum* stems surrounded by water experienced lower predation rates than eggs on stems on land, indicating that standing water acts as a partial refuge against predators for *Galerucella* eggs. Flower-bud density, length of inflorescence, and plant biomass per m^2 were all negatively related with the intensity of beetle feeding, and stem height was marginally reduced with increased beetle grazing. Dry biomass per m^2 of *Lythrum* appeared to decline drastically only at sites where beetle grazing was intense.
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1. Introduction

It is not uncommon for the success of a biological control project to vary with geographic region and habitat, associated with differences in the biotic and abiotic environments. When biocontrol projects are not successful, it is generally due to the failure of a biological control agent to establish or to exert enough predation pressure on the weed to lower its abundance or biomass (Sheppard, 1992). Among other factors, habitat stability is well known to influence control-agent establishment, with stable habitats such as forests showing higher rates of establishment of control agents than disturbed habitats such as agricultural fields (Cameron et al., 1993; Hall and Ehler, 1979). Once established, a prerequisite for the control agent's ability to suppress the weed at a given release site is a sufficiently high density of the agent. Local parasitoids and predators are frequently blamed for preventing the populations of the control agents from reaching high densities (Goeden and Louda, 1976). Alternatively, host-plant quality can play a decisive role in determining the abundance of the control agent: an agent can be successful in one environment, but not in another due to the poor quality of the host plant at one site that does not allow population growth of the control agent (e.g., Room, 1990).

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Even when high population densities are reached, however, some control agents do not control their hosts (Cullen and Snowball, 1979) because they do not cause debilitating damage to the host plant or kill it. Among the most successful control agents are leaf-chewing insects (Crawley, 1989) because repeated, severe defoliations deplete a plant's reserves. The attacked plants eventually die and a decline in plant density can result. In many plant species, a threshold level of defoliation exists below which individual performance is not significantly affected due to compensation following herbivory (Hendrix, 1988), a response facilitated by the existence of stored food reserves and axillary buds that allow regrowth. In such plant species, weak herbivory, even when consistent, is unlikely to affect plant survival and density.

The biological control of purple loosestrife, *Lythrum salicaria* L. (Lythraceae; referred to herein simply as *Lythrum*), in North American wetlands by European leaf-feeding chrysomelid beetles is a recent example of repeated biological control success (reviewed in Blossey et al., 2001). Yet, the beetles have not successfully established at all North American sites, nor do they control the abundance of their host plants in all locations. In the Lower Mainland of British Columbia, *Lythrum* has infested wetlands including sites along the Fraser River that are strongly influenced by tides. Here only one control agent, *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae), has been widely introduced during the past 11 years. Casual observations have indicated a high variation in beetle densities among release sites. Recent studies have documented that even moderate levels of feeding by *Galerucella* spp. impact the reproductive and vegetative performance of *Lythrum* (e.g., Katovich et al., 1999, 2001) in the same year. *Lythrum* stem density, however, may only be affected after several years of herbivory and when a threshold beetle density has been reached (Lindgren, 2003) due to inaccessible nutrient reserves that allow the plant to compensate for the feeding damage.

Our aims in this study were: (1) to document the spatial and temporal variability in *G. calmariensis*' feeding damage among the release sites; (2) to identify environmental factor(s) that can explain the variability, and (3) to determine the effects of beetle feeding on *Lythrum*. As recommended by McEvoy et al. (1991), we used a combination of long-term monitoring and short-term experiments to investigate the success of this biological control project.

2. Materials and methods

2.1. Study system

Lythrum salicaria is a perennial plant of Eurasian origin that was introduced to North America in the early

1800s and was first recorded in British Columbia as a herbarium specimen, collected in 1897. However, it did not persist and was not reestablished until the 1920s (F. Ganders, University of British Columbia, Vancouver, personal communication). The biology, ecology, history of invasion into North America, and the environmental impacts of *Lythrum* have been described extensively (Anderson, 1991; Blossey et al., 2001; Gaudet and Keddy, 1988; Keddy et al., 2000; Mal et al., 1992; Shamsi and Whitehead, 1974; Thompson et al., 1987). *Lythrum* grows in a wide range of environmental conditions (Mal et al., 1992 and references therein). The species grows rapidly and up to 2.7 m in height (Mal et al., 1992) with a high biomass, conferring a strong competitive ability (Gaudet and Keddy, 1988). With increasing age, the number of annual stems produced by individual plants increases to a maximum of 30–50 (Mal et al., 1992). Individual plants are long-lived (>20 years) and have a high survival once they are more than 2 years old (Anderson, 1991). *Lythrum* seed production is high (estimates range from 100,000 to 2,700 000 per plant per year) and the seeds form an abundant seed bank; the small and light seeds are easily dispersed by water, wind, and animals (Mal et al., 1992 and references therein). *Lythrum* successfully colonizes open soils and responds positively to many kinds of human and natural disturbances (Rachich and Reader, 1999). Given the tendency of *Lythrum* to occupy sensitive, wet habitats such as stream banks, lakes, and ditches, Hight and Drea (1991) argued that biological control is the most suitable and environmentally safe technique for managing this exotic plant.

To date, mainly one species of insect, *G. calmariensis*, has been introduced in the Lower Mainland of British Columbia, beginning in 1993 (Table 1). There is an extensive literature on the biology, ecology, and taxonomy of this species (Blossey, 1991, 1995; Blossey and Schat, 1997; Hight and Drea, 1991; Malecki et al., 1993; Manguin et al., 1993; McAvoy et al., 1997; Medina, 1995; Nechols et al., 1996; Sebolt and Landis, 2002; Velarde et al., 2002). The following is a brief summary of the species' life history as it relates to this study.

The adult beetles emerge from the soil in late April to mid-May and feed on leaf tissue of the host plant. Following a period of 7–10 days of feeding and mating, the main oviposition period occurs during May and June with reduced egg production until the end of July. Each female can lay up to 500 eggs in small batches of 2–10 eggs on the stems, leaves, and leaf axils of *Lythrum*. Small neonate larvae appear first in early June and crawl to the top leaves to feed. The larvae molt three times with more than 80% of the larval growth and feeding damage occurring in the last larval instar (Loos and Ragsdale, 1998). Developing larvae move to the underside of leaves and feed extensively on leaf and stem tissue. After 2–3 weeks of feeding, the larvae move into

Table 1
Introductions of *Galerucella* spp. to the experimental sites in the Lower Mainland of British Columbia

Site	Coordinates	Year of release	Number released	Species	Site conditions
Boundary Bay Park	49°01.40′N, 123°03.17′W	1995	475	90% *G. calmariensis*, 10% *G. pusilla*	Non-tidal
Boundary Bay Park	49°01.40′N, 123°03.17′W	1996	216	*G. calmariensis*	Non-tidal
Chilliwack	49°07.77′N, 122°05.00′W	1997	200	*G. calmariensis*	Non-tidal
Colony Farm	49°14.27′N, 122°47.46′W	1997	300	*G. calmariensis*	Non-tidal
Deas Island	49°06.59′N, 123°03.45′W	1997[*]	310	*G. calmariensis*	Tidal
Iona marshes	49°13.2′N, 123°12.7′W	1993[*]	?	*G. calmariensis*	Non-tidal
Iona North marsh	49°13.2′N, 123°12.7′W	1997	300	*G. calmariensis*	Non-tidal
Iona South marsh	49°13.2′N, 123°12.7′W	1997	300	*G. calmariensis*	Non-tidal
Jericho Beach Park	49°16.30′N, 123°11.99′W	1993	38	*G. calmariensis*	Non-tidal
Jericho Beach Park	49°16.30′N, 123°11.99′W	1997	300	*G. calmariensis*	Non-tidal
Ladner Marsh	49°07.54′N, 123°05.05′W	1999[*]	500	*G. calmariensis*	Tidal
MacDonald slough	49°12.62′N, 123°12.18′W	1997[*]	600	*G. calmariensis*	Tidal
Westham Island	49°06.53′N, 123°10.99′W	1993[*]	270	*G. calmariensis*	Non-tidal
Westham Island	49°06.53′N, 123°10.99′W	1997	100–150	*G. calmariensis*	Non-tidal

? unknown. [*] Failed control agent introduction.

the soil underneath the host plant to pupate. The adults of the next generation emerge in July, 2–3 weeks after the larvae have entered the soil. Adult beetles hibernate in the soil or in hollow plant stems when flooding occurs (Schooler, 1998). A low density of new egg masses found in August 1999 at two of the monitoring sites suggests that this species is partially bivoltine in southwest British Columbia.

2.2. Study sites

Monitoring and experiments were conducted at 10 sites in the Lower Mainland of British Columbia (Fig. 1), at which the *Galerucella* spp. beetles had been released by Roy Cranston, the B.C. Ministry of Agriculture, Food and Fisheries, in collaboration with Dr. Judith Myers from the University of British Columbia (UBC) from 1993 to 1999. The sites are described in

more detail in Denoth (2004). Table 1 summarizes the information about the sites and the control agent's releases.

2.3. Monitoring of Lythrum and Galerucella

To determine the performance of *Lythrum*, all sites except MacDonald slough and Iona North marsh were monitored at least once a year, in July or August, from 1999 to 2002. Here, we only report part of this data set, as it pertains to this study.

Following a slightly modified procedure described in Blossey and Skinner (2000), we estimated *Lythrum* performance at each site by averaging the results recorded in each of 10 to 15 1-m² quadrats. At three sites with little variability the number of quadrats sampled was as low as five in 2002. Quadrats were 5 m apart on straight line transects that were laid in the same general area
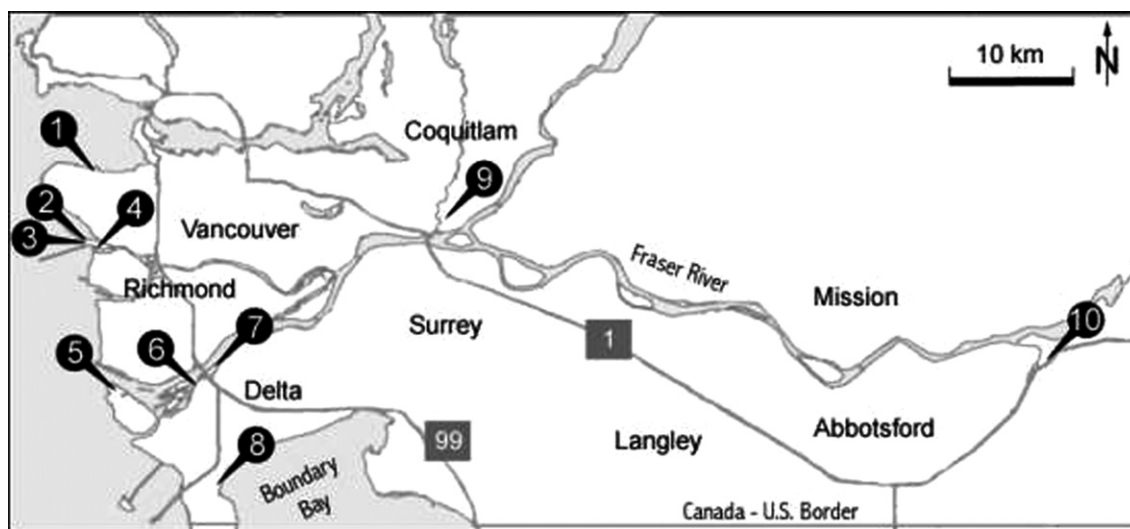

Fig. 1. Map showing experimental sites. ❶ Jericho Beach park; ❷ Iona North marsh; ❸ Iona South marsh; ❹ MacDonald slough; ❺ Westham Island; ❻ Ladner marsh; ❼ Deas Island; ❽ Boundary Bay park; ❾ Colony Farm; and ❿ Chilliwack roadside (Yale road exit); ▮ and ▨ are Highways.

each time a site was visited. Transect starting points were selected subjectively to facilitate the accessibility to sampling quadrats. *Lythrum* abundance was estimated by counting the number of stems in each quadrat. To estimate biomass, 30 shoots consisting of the three tallest and the three shortest *Lythrum* stems in the first five sampling quadrats were cut at their base and dried at 80 °C for 48 h. Average *Lythrum* plant biomass per m^2 for the jth site, A_j, was computed as:

$$A_j = \frac{d_j}{30} \sum_{i=1}^{n} \frac{a_{ij}}{n_j},$$

where d_j is the combined dry biomass of the three tallest and the three shortest stems sampled in each of five quadrats at the jth site, a_{ij} is the number of *Lythrum* stems in the ith quadrat, and n_j is the number of quadrats sampled at the jth site.

The reproductive performance of *Lythrum* was determined by taking the following measures of the five tallest stems in each quadrats: the length of the inflorescences, the density of flower buds on the inflorescences (number per 5 cm), and the number of inflorescences per stem. Vegetative performance of *Lythrum* was estimated by recording the number of stems per quadrat and by measuring the height of the five tallest stems. Only quadrats with a minimum of five stems were selected to allow an equal number of measurements in each quadrat.

Galerucella feeding damage was determined at each site by subjectively estimating the amount of *Lythrum* foliage removed. One of six damage categories based on the estimated percentage removal of foliage, A: 0%; B: ⩽5%; C: 6–25%; D: 26–50%; E: 51–75%; and F: 76–100%, was subjectively assigned to the *Lythrum* stems in each quadrat. The overall feeding damage at a site was computed by averaging the midpoints of all damage categories over all sample quadrats. Therefore, the theoretical maximum feeding damage was 88%.

Galerucella densities were estimated by counting the number of eggs, larvae, and adult beetles separately during 1 min each in each quadrat. Density estimates of all quadrats per site were averaged.

2.4. Beetle survival in tidal areas

To test the hypothesis that disturbance associated with the water movement causes failure of beetle establishment in tidal areas, we carried out a field experiment to determine the effect of tidal flooding on the beetle larvae.

We infested 12 individual *Lythrum* stems with five beetle larvae each in late afternoon of June 14, 2001, at MacDonald slough and determined the number of remaining larvae the next morning, after a high tide. Two treatments were applied: second- and third-instar larvae were either set on the tips of tall plants, which

were not submerged during the high tide, or on small plants that would be submerged. Maximum tide level relative to the *Lythrum* stems was recorded during the high tide to ensure discriminating treatments to the larvae on tall and small stems, respectively. The data were analyzed using a Mann–Whitney U test.

2.5. Variation in predation and food-plant quality among sites

To contrast predation on the beetles and plant quality among sites with high and low defoliation levels, we conducted experiments at four non-tidal release areas in 2001. Two of these, Iona North marsh and Jericho Beach Park, had experienced substantial to complete defoliation of *Lythrum* in 2000, whereas the sites Iona South marsh and Westham showed average defoliation levels of less than 10% in 1999 and 2000. The densities of *Galerucella* adult, egg, and larval stages were estimated in 0.25-m^2 quadrats according to the monitoring procedure described above. *Lythrum* defoliation levels, predation on the beetles, and plant quality, measured as the weight and mortality, respectively, of larvae protected from predators, were determined at each site.

2.5.1. Egg predation

Egg predation was assessed in mid-May. First, we determined the height distribution of the egg masses at each site from 10 arbitrarily selected *Lythrum* stems. We then selected one experimental egg mass on each of 50 arbitrarily chosen *Lythrum* stems such that the height distribution of the experimental egg masses reflected the natural distribution. The position of each egg mass (in 5-cm intervals from the ground) and the number of eggs in the egg masses were recorded. Each egg mass was marked by placing a small dot of Liquid Paper correction fluid just above it (Nechols et al., 1996). After a 7-day period, we recorded the condition of the eggs. Shrunken eggs, missing eggs, and eggs that had been opened were considered to be preyed upon. The average level of predation for each site was computed for the 50 egg masses.

2.5.2. Predation on larvae

To estimate predation on larvae, larvae were exposed to predators at each of the four experimental sites. To assure equal plant quality, the *Lythrum* plants to receive the experimental larvae were grown in the greenhouse from stem cuttings. Four weeks before the experiment started, *Lythrum* shoots were harvested at Iona South marsh and immediately transferred to the UBC greenhouse. Here, all except three leaves at the shoot tips were removed, and the shoots were planted singly in plant pots filled with mineral soil and put in a mist bed to develop roots. Water sprinklers and heating cables for bottom heat maintained a humid (80% relative humidity)

and warm (25 °C) climate that encouraged plant growth in the mist bed.

We used a total of 20 plants per site. The plants were paired to produce 10 sets: one of the plants was selected randomly and received 10 second-instar larvae; the other plant served as a control for immigrating larvae because larvae disperse from plants when severe defoliation leads to food shortage (personal observations). The control plant was placed at a distance of 20 cm from the experimental plant. Each pair of plants was considered to be a replicate and was at least 1 m from other replicates. The potted plants were placed in the soil such that the containers were completely buried. Water for the plants was provided when necessary, usually daily. After a 6-day period, the plants were recovered, and the larvae were counted. Missing larvae were considered to have been killed by predators. We assumed that the larval immigration rate did not vary between control and experimental plants. The number of surviving larvae was determined by computing the difference in the number of larvae between each pair of plants to correct for larval immigration. To compute the mean number of surviving larvae at each site, we averaged the number of surviving larvae of all plant pairs.

2.5.3. Predation on pupae

To measure predation on pupae, we buried 20 sets of four pupae 1–1.5 cm under the soil surface, in their natural pupation environment, at each of the four experimental sites. To facilitate recovery of the pupae, we placed them on a 10 cm × 10 cm piece of mesh screen and marked their location with a small wooden stick. The number of recovered pupae was recorded after a week. Missing pupae were considered to have been preyed upon.

2.5.4. Plant quality

Plant quality was assessed indirectly by measuring the weight and survival of caged larvae after feeding on individual plants. We caged 10 arbitrarily selected plants in early May to prevent oviposition by adult beetles. When larvae were available, we infested each plant with 10 first-instar larvae from nearby plants. Since most of the larval growth takes place in the third instar (Loos and Ragsdale, 1998), differences in initial larval weight were considered insignificant and were not recorded. Crawling predators were prevented from accessing the plants by applying Stickem (Phero Tech, Richmond, B.C.) to the base of the cages and the base of the plant stems. After 13 days, the larvae were recovered, counted, and weighed on a Mettler laboratory scale.

2.5.5. Data analysis

Each of the four variables examined was correlated individually with the percentage plant damage at each site using Pearson linear correlation. All variables were transformed to meet the assumptions of linear correla-

tion analysis. Angular transformation was used to transform proportions (percentage plant damage, predation rate of eggs), square root transformation was applied to the count variables (surviving larvae, surviving pupae), and larval weights were log transformed. The data could not be analyzed with the use of analysis of variance techniques, since the response variable (defoliation level, measured after the experiments were conducted) was continuous in 2001, with defoliation levels ranging from low (Iona South) to intermediate (Iona North, Jericho) to high (Westham).

2.6. Influence of water level on egg predation

To determine if standing water had a protective influence on the eggs of *Galerucella*, we carried out a predation experiment at Iona South marsh. The site was chosen because individual plants in the water had shown high defoliation levels in 2001, but not plants on land. In 2002, we measured the predation rates of egg masses on *Lythrum* stems surrounded by water or not surrounded by water. A total of 15 individual *Lythrum* plants were selected in mid-May, seven in standing water, eight on land. Five egg masses were chosen at regular intervals on each stem and marked with a small dot of Liquid Paper just above the egg mass. The number of eggs of each egg mass was recorded. After 1 week, we recorded if predation (yes or no) had occurred to each of the egg masses. The mean predation levels per plant stem were compared using a Mann–Whitney *U* test.

2.7. Effects of Galerucella feeding on Lythrum

We examined the effect of beetle feeding on the reproductive and vegetative performance of *Lythrum* at six non-tidal sites in 2000 by relating the observed feeding damage to *Lythrum* performance indicators using Spearman rank correlation. To examine the cumulative effect of 4 years of *Galerucella* feeding (1999–2002) on *Lythrum* density, we examined the relationship between *Lythrum* feeding damage and the percent change in stem densities from 1999 to 2002 at the six sites using Spearman rank correlation. We used *Lythrum* flower-bud density as a surrogate, after establishing that it was a reliable measure of feeding (see Section 3). Changes in *Lythrum* dry biomass over the monitoring period were only evaluated qualitatively, since dry biomass was estimated from only one sample per site.

3. Results

3.1. Variation in beetle's feeding damage

Feeding damage to *Lythrum* varied in space and time (Fig. 2). The beetles failed to establish at any of three
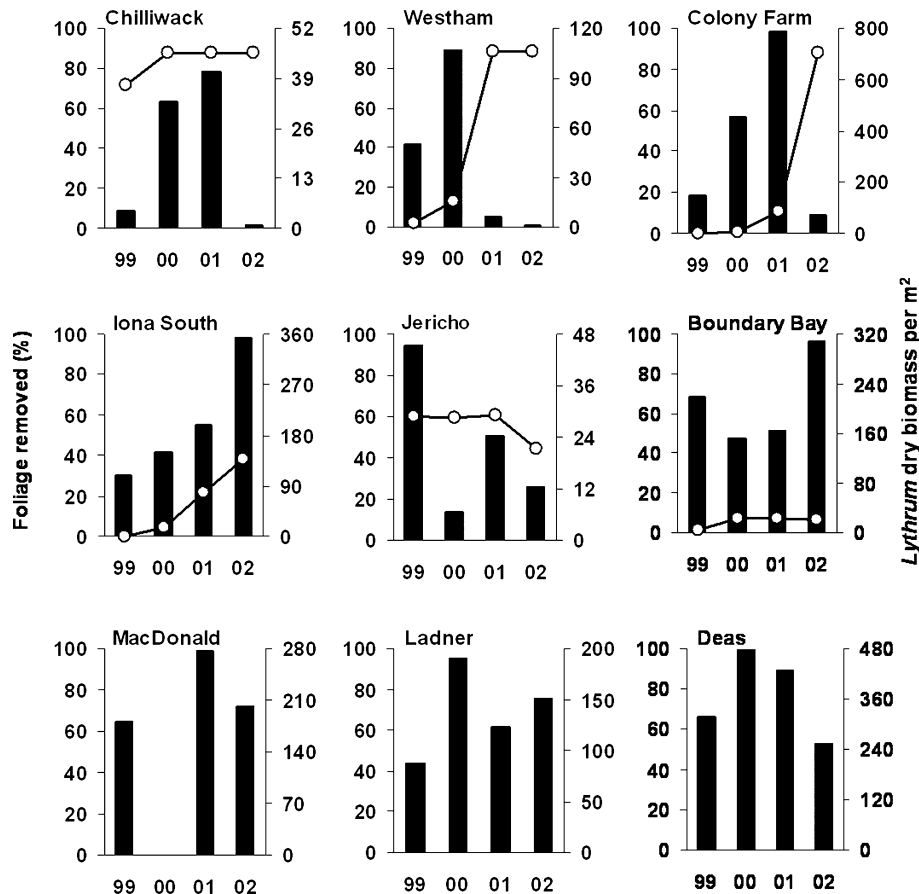
Fig. 2. Average percentage foliage removed (line graph, left y axis) and plant dry biomass per m^2 (bar graph, right axis) in populations of *Lythrum* from 1999 to 2002. Plant dry biomass was not recorded at MacDonald in 2000. Dry biomass was estimated by the average dry biomass per stem per year and per site multiplied by the average number of stems at each site in August. Note different scales of plant biomass.

tidal sites. At the MacDonald site only, individual adult beetles and negligible feeding damage (below 5% of foliage removed in all years), but no eggs, larvae, or pupae, could be observed at the edges up to three years after the release. The number of sites where beetles occurred at sufficient density to cause complete plant defoliation (henceforth referred to as beetle outbreaks) increased to three by 2002.

A rapid increase in feeding damage, from low feeding damage to complete defoliation, can occur within a year, as shown at the Westham and Colony Farm sites. Once outbreak densities were reached, high *Lythrum* defoliation levels were usually sustained in the following years. Consistently low herbivory levels were found only at one site, Boundary Bay Park. Defoliation levels increased at all other non-tidal sites over the monitoring period.

3.2. Beetle survival in tidal areas

Survival of larvae in tidal areas was related to plant height. The tide in the night of June 14 was so high as to completely submerge the short *Lythrum* plants, but left the upper parts of the tall plants uncovered. Significantly more larvae disappeared from the short than from the tall stems ($Z = 2.49$, $P = 0.013$) during the high tide. Starting with five larvae, 4.5 ± 0.34 larvae were recovered from the tall plants, on average, compared with only 2.2 ± 0.54 from the short plants.

3.3. Variation in predation and plant quality among sites

Average levels of beetle feeding damage on *Lythrum* at the four experimental sites ranged from a low of 22% at Iona South to a high of 88% at Westham (Table 2). Beetle density was highly variable between sites. On a per-stem basis, adult beetle density was highest at Iona South marsh, the site with the lowest larval feeding damage, with 1.3 beetles observed per minute in May of 2001 (Fig. 3). This was likely due to dispersal of adult beetles from the nearby Iona North site where severe defoliation resulted in a food shortage. Relative egg mass and larval densities were highest at Westham with 5.9 egg masses and 4.8 larvae observed per stem and per minute, respectively, but considerably lower at the other sites,

Table 2
Feeding damage on *Lythrum salicaria* (proportion foliage removed), *Galerucella calmariensis* egg, larval, and pupal predation levels (measured as the proportion of eggs, larvae, and pupae preyed upon, respectively) and plant quality (measured as larval weight in 10^{-6} g and the number of larvae recovered out of 10 larvae, respectively) at each of four experimental sites in 2001[a]

Variable	Iona South	Jericho Beach	Iona North	Westham
Feeding damage	0.22 ± 0.06	0.61 ± 0.10	0.63 ± 0.05	0.88 ± 0.00
Egg predation	0.60 ± 0.06	0.26 ± 0.05	0.34 ± 0.06	0.07 ± 0.03
Larval predation	0.46 ± 0.07	0.22 ± 0.05	0.34 ± 0.12	0.30 ± 0.07
Pupal predation	0.25 ± 0.06	0.23 ± 0.06	0.11 ± 0.05	0.45 ± 0.10
Plant quality (larval weight)	3494 ± 501	3369 ± 385	4269 ± 348	2365 ± 270
Plant quality (larval survival)	9.30 ± 0.33	6.80 ± 0.80	9.00 ± 0.33	9.20 ± 0.29

[a] Experimental methods and results of the statistical tests are given in the text. Values are means ± SEM.
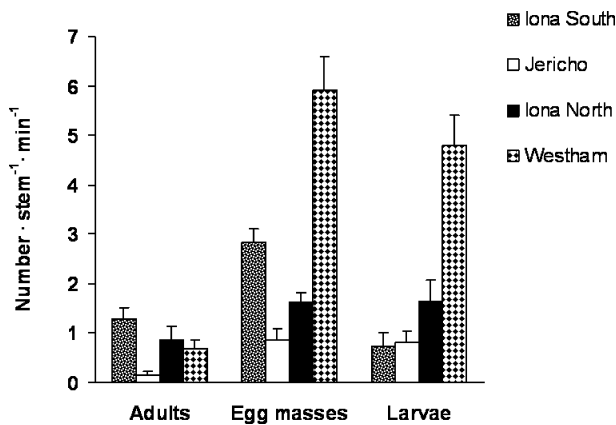

Fig. 3. Number (+SEM) of adults, egg masses, and larvae of *Galerucella* observed per *Lythrum* stem and per minute at four experimental sites in 2001. Adult and egg-mass densities were recorded in mid-May, larval density in mid-June.

approximately reflecting the subsequent defoliation levels.

Levels of predation on eggs were highly variable, ranging from a low of 7% at Westham to a high of 60% at Iona South (Table 2). Plant damage varied with the level of predation on eggs ($r = -0.98$, $n = 4$, $P = 0.02$). Predation on eggs on stems surrounded by water was lower than for stems on land with 20% versus 67%, respectively ($Z = 2.57$, $P = 0.01$).

Predation on larvae was highest at Iona South marsh, where, on average, 46% of all larvae disappeared (Table 2), and lowest at Jericho with a disappearance of 22%. Predation was similar and intermediate at Iona North marsh and Westham. Levels of predation on the larvae among sites were not related to levels of plant feeding damage ($r = 0.71$, $n = 4$, $P = 0.29$).

Predation on pupae was lowest at Iona North marsh where, on average, 11% of all pupae disappeared. The predation rates of pupae were intermediate at Iona South marsh and Jericho, respectively. The pupal predation rate was highest at Westham, where, on average, only 55% of the experimentally deployed pupae were retrieved. Levels of pupal recovery were not related to levels of plant defoliation ($r = -0.5$, $n = 4$, $P = 0.5$).

Plant quality, measured as the weight and survival, respectively, of larvae on caged plants was not associated with the mean levels of feeding damage to plants among sites (larval weight: $r = -0.56$, $n = 4$, $P = 0.44$; larval survival: $r = -0.07$, $n = 4$, $P = 0.93$).

3.4. Effect of Galerucella feeding on Lythrum

Larval densities (Table 3) were strongly correlated with *Lythrum* defoliation levels ($r_s = 0.94$, $n = 6$, $P = 0.005$). Larval feeding apparently had a negative impact on the performance of *Lythrum* plants (Table 3): the flower-bud densities of the inflorescences

Table 3
Average ± SEM *Galerucella* larval density, defoliation, and measures of *Lythrum* reproductive and vegetative performance at six non-tidal sites[a]

Variables	Boundary Bay	Chilliwack	Colony Farm	Iona South	Jericho	Westham
Larval density	9.5 ± 1.2	44.5 ± 6.9	4.6 ± 1.1	14.3 ± 3.0	21.4 ± 3.5	18.1 ± 6.4
Defoliation (%)	7.3 ± 1.6	88.0 ± 0.0	0.6 ± 0.4	4.8 ± 1.5	59.4 ± 6.5	13.1 ± 2.4
Flower-bud density	28.4 ± 8.7	0.0	68.9 ± 2.3	26.4 ± 6.1	0.0 ± 0	18.6 ± 4.5
Length inflor. (cm)	5.8 ± 1.8	0.0	39.7 ± 3.6	14.2 ± 3.2	0.0 ± 0	7.6 ± 2.3
Number inflor. per stem	6.7 ± 1.4	0.0	4.4 ± 0.5	5.4 ± 0.9	0.0	6.3 ± 0.9
Dry biomass (g)	151.4	32.5	399.0	133.4	5.5	97.8
Stem density	22.3 ± 3.3	32.5 ± 5.1	76.7 ± 7.0	29.5 ± 2.4	21.1 ± 2.9	15.6 ± 1.6
Stem density 1999	20.7 ± 4.3	14.1 ± 2.0	36.9 ± 4.4	20.8 ± 2.4	18.5 ± 2.9	15.9 ± 2.0
Stem density 2002	36.6 ± 5.2	6.7 ± 2.0	87.0 ± 8.8	29.6 ± 3.3	12.3 ± 1.6	20.0 ± 0.6
Stem density ch.	+77	−52	+136	+42	−33	+26

[a] All estimates were recorded in 2000, unless indicated otherwise. Stem density ch. refers to the change in stem densities from 1999 to 2002 in percent of 1999. See Section 2 for details.
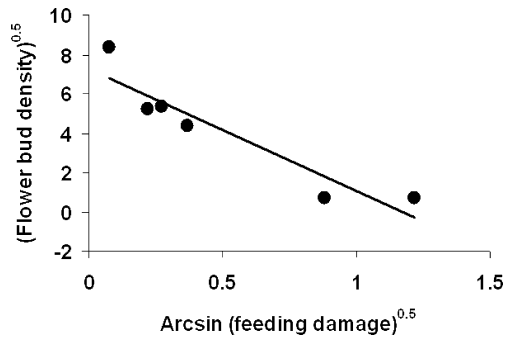
Fig. 4. Relationship between beetle feeding damage (arcsine transformed) and the mean flower-bud density per 5 cm of inflorescence of *Lythrum* (square-root transformed) ($r_s = -0.93$, $n = 6$, $P = 0.008$).
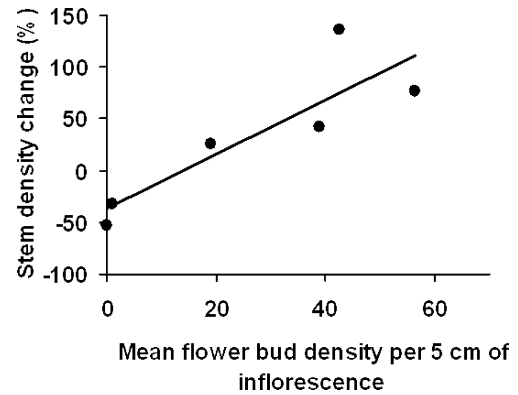

Fig. 5. Relationship between the average beetle feeding damage, measured by the mean flower-bud density per 5 cm of inflorescence of *Lythrum* from 1999 to 2002, and changes in stem densities (in percent of stem density in 1999) between 1999 and 2002 at non-tidal sites ($r_s = 0.94$, $n = 6$, $P < 0.005$).

($r_s = -0.93$, $n = 6$, $P = 0.008$; Fig. 4), the length of the inflorescences ($r_s = -0.93$, $n = 6$, $P = 0.008$), and dry biomass of *Lythrum* per m^2 ($r_s = -0.89$, $n = 6$, $P = 0.02$) were all negatively correlated with the observed feeding damage levels in the same year. Flower-bud densities (square-root transformed) did not vary among the three tidal sites without *Galerucella* (MacDonald: 50.8 ± 3.7; Deas: 49.5 ± 1.8; Ladner Marsh: 53.3 ± 4.2; Welch ANOVA, $F_{2, 23.3} = 0.21$, $P = 0.81$), and thus, variation in flower-bud density at sites with beetles is likely due to beetle feeding damage. Stem height was marginally related to the observed feeding damage ($r_s = -0.77$, $n = 6$, $P = 0.07$). Both the number of inflorescences per stem ($r_s = -0.1$, $n = 6$, $P = 0.87$) and the stem densities ($r_s = -0.37$, $n = 6$, $P = 0.47$) were not related to the feeding damage among sites in 2000.

Considering all non-tidal sites, there was a significant and positive association between the average feeding damage between 1999 and 2002, measured by the mean flower-bud density (flower-bud density is negatively associated with feeding density, Fig. 4), and the relative changes in stem density ($r_s = 0.94$, $n = 6$, $P > 0.005$, Fig. 5). Stem densities only decreased when the feeding activity was so intense as to inhibit flowering for four years, otherwise they increased; stem densities increased less when the average feeding damage was more intense. The stem density only decreased significantly at one site, Chilliwack (Mann–Whitney test, $Z = -2.30$, $P = 0.021$; Table 3), where *Lythrum* plants had experienced complete defoliation for at least four consecutive years. Plant biomass apparently increased initially when the feeding damage started to increase at the Chilliwack, Westham, Iona South, and Colony Farm sites, but declined drastically when the grazing reached outbreak levels (Fig. 2). There were no apparent trends at sites where the beetle did not establish (MacDonald, Ladner, and Deas), or where beetle attack was negligible (Boundary Bay). At the Jericho site, *Lythrum* plants experienced consistent and complete defoliation in an unflooded part, while plants in a flooded area were not consistently attacked. The overall average defoliation level masks a high variability at this site.

4. Discussion

4.1. General discussion

The biological control of *L. salicaria* by the beetle *G. calmariensis* is generally considered to be successful (Blossey et al., 2001; Katovich et al., 2001; Landis et al., 2003; Lindgren, 2003), but may only be partially so in coastal British Columbia. In tidal areas, the beetle failed to establish. Since the tidal water movement displaced *Galerucella* larvae over the short term, repeated disturbance events could severely impact larval survival, especially in early June, when *Lythrum* plants are small and completely submerged during high tide. Neonate larvae may be especially vulnerable due to their presumably reduced ability to hold on to the plant surface. Given the high rate of disappearance of larvae, we suggest that tidal disturbances may explain the failure of beetles to establish at any of the tidal sites. It is likely that the frequent disturbances also affect other life-history stages, including the oviposition behavior of *Galerucella*, since no eggs were found on plants in tidal areas. The observation that *Galerucella* was present at the MacDonald site for several years after the release was likely due to repeated dispersals to this site from the nearby Iona sites. We suggest that the beetle's inability to reproduce and develop in tidal areas prevents *Galerucella* from successfully establishing in this environment.

Four years of monitoring revealed high spatial and temporal variability in beetle feeding damage among non-tidal sites. The rapid increase in the number of outbreak sites shows that the beetles are not generally

prevented from reaching high densities and that they can exhibit rapid population growth. However, the presence of generalist, crawling predators may delay or hinder beetle outbreaks. Among the factors studied, only egg predation was significantly associated with the levels of herbivore feeding damage in a given year. Thus, this study suggests that predation influences beetle densities among sites. A range of generalist predators, among them ladybird beetles (Coleoptera: Coccinellidae), earwigs (Dermaptera: Forficulidae), and wolf spiders (Arachnida: Lycosidae), are known to effectively attack immature stages of *G. calmariensis* and *Galerucella pusilla*, and may significantly reduce the populations of *Galerucella* (Sebolt and Landis, 2004; Wiebe and Obrycki, 2004). The level of egg predation appears to be influenced by an abiotic factor, the water levels surrounding *Lythrum* stems in early spring. The strongly reduced egg predation on plants in standing water indicates that water can act as a barrier that prevents crawling predators from climbing the stems and preying on the beetle eggs. Such refuges from predators may allow small-scale (individual plant) beetle outbreaks, and could be potential starting points for larger outbreaks. If this finding applies generally, we predict that beetle outbreaks should start more commonly in years when winter precipitation is plentiful and in patches of plants in standing water, where high water levels in the spring protect the eggs from crawling predators. A significant decrease of the water level by the end of June observed at most sites would allow the beetles to pupate successfully in the soil. Adult overwintering success may not be affected by high water levels given the beetle's ability to use hollow plant stems as refuges in case of flooding (Schooler, 1998). While larval predation has been suggested as a force that could impact beetle populations (Sebolt and Landis, 2002), we did not find evidence for a consistent role of larval or pupal predation, or plant quality, in influencing beetle populations. However, the statistical power of these tests was low.

The results of this study suggest that the beetle is effective at reducing seed output and biomass of *Lythrum*. Our findings are in agreement with the results of other studies that have documented negative effects of herbivore feeding on the reproductive and vegetative performance of *Lythrum* (Blossey and Schat, 1997; Katovich et al., 1999, 2001; Lindgren, 2003). At least in the short term the reduction in seeds is not likely to translate into changes in the plant's population dynamics, given the plants' longevity and large seed bank in the soil (Welling and Becker, 1990). Nevertheless, the potential of *Lythrum* to spread is reduced because it occurs mainly by means of seeds (Mal et al., 1992). In addition, the reduced biomass of *Lythrum* under beetle attack will likely reduce its competitiveness (Gaudet and Keddy, 1988). The observation that plant stem height seemed only marginally related to the beetle feeding damage

may be due to a lack of power in our analysis because the average stem height of *Lythrum* varied due to site conditions before the release of the beetle, but was not measured and thus, could not be included in the analysis. Katovich et al. (1999) and Lindgren (2003) found in their studies that beetle feeding reduced the height of *Lythrum* stems significantly.

Previous research has shown that *Lythrum* is able to tolerate intense feeding of *Galerucella* spp. for more than 2 years before mortality occurs (Blossey and Skinner, 2000; Katovich et al., 1999). Consistent with this result, we found that *Lythrum* stem density decreased significantly at the Chilliwack site, which experienced the most consistent and intense feeding of all sites over the 4-year monitoring period. However, since quadrats with fewer than five stems were not considered for the monitoring, the estimates of decreases of plant density are conservative, and this may have prevented finding a significant reduction in stem densities at other sites.

To our knowledge, this is the first study to suggest that cumulative feeding may affect density and abundance of *Lythrum*. While 4 years of complete plant defoliation resulted in a reduction of stems, herbivory that was less intense was associated with an increase in stem density over the same time period. A possible explanation is that herbivory that does not result in complete defoliation causes seedling mortality only, thus limiting the growth of the population, while repeated, complete defoliation results also in the death of well-established plants and reduces the plant's population size. Alternatively, beetle feeding that does not result in complete defoliation may cause initial increases in the stem density of *Lythrum*, potentially by stimulating a morphological response in *Lythrum*, such as increased branching close to the soil surface. Clearly, experimental manipulations are needed to investigate the general applicability of the observed pattern and to determine the mechanism by which it is produced, since we cannot rule out the possibility that stem densities would also have increased in the absence of beetle feeding.

A reduction in plant biomass may be more important than a reduction in stem density alone in the context of competitive interactions. The reduction of dry biomass at the Chilliwack, Jericho, Westham, and Colony Farm site could benefit native species by reducing resource competition before *Lythrum* stem density is significantly reduced. The changes in plant biomass at the three tidal sites are likely due to yearly variation in abiotic conditions, since the beetle did not establish at these sites. This suggests that the consistent reductions of plant biomass at sites with considerable defoliation levels are caused by beetle attack. The observation that *Lythrum* biomass increased initially with increasing beetle grazing at many sites could indicate that *Galerucella* populations respond rapidly numerically to an increased plant abun-

dance, and can successfully control the host plant at many non-tidal sites.

4.2. Implications for biological control

Galerucella calmariensis appears to be only partly effective as a control agent of *L. salicaria* in southwest British Columbia. The beetle does not appear to be a suitable control agent in tidal areas where tidal disturbances prevent its establishment. While the beetle established at all non-tidal release sites and reduced the weed's seed output, it did not reduce *Lythrum* biomass and density at all sites by 2002. However, given the observed increase in sites with beetle outbreaks, we expect a decline in *Lythrum* abundance at more non-tidal sites over time.

Our results highlight the importance of the abiotic environment at the introduction sites for the weed's and the beetle's population dynamics. While interference with native, generalist predators appeared to influence the abundance of *Galerucella* at a local scale, the abiotic environment may ultimately determine the balance of the trophic interactions and thus, the success of this biological control project at a regional scale. Biological control was apparently facilitated by the creation of temporary refuges from predation for the control agent in non-tidal areas when water levels were high in the spring. In contrast, tidal areas act as a refuge for the weed, since disturbances associated with the water movement appear to prevent control-agent establishment.

The failure of biological control in tidal areas raises concerns about the potential of these areas to serve as a consistent seed source from which new areas can be infested. The question of how *Lythrum* is regulated in tidal areas in its native range provides a valuable opportunity for ecological study and could also guide future management decisions such as the introduction of a suitable control agent into tidal areas for effective management of *Lythrum* on a regional scale.

Acknowledgment

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