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MEASURING GEOGRAPHICAL VARIATION IN *ACANTHOSCELIDES MACROPHTHALMUS* (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE) SEED PREDATION ON *LEUCAENA LEUCOCEPHALA* (JIMBAY) AND PARASITOID WASPS ON SAN SALVADOR, BAHAMAS

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ABSTRACT

Leucaena leucocephala (Jimbay) is a highly invasive plant species native to Mexico and of concern in tropical climates worldwide. There is particular interest in seed predators that may be effective in controlling this species in sensitive habitats. Jimbay has been used for several centuries as cattle fodder, and was likely brought to the island of San Salvador, Bahamas for this purpose. This species is now present in disturbed areas on the periphery of the island as well as the interior of the island on remnants of 18th century plantations and other agricultural sites. We investigated predation on Jimbay by collecting Jimbay pods from 26 locations on San Salvador Island. Seed predation by *Acanthoscelides macrophthalmus* is easy to identify and we compared the seed predation to proximity of populated areas. We analyzed these data by dividing the island into four sectors of north, south, east, and west, and analyzed data within and among sectors. These data show that seed predation was not associated with proximity to populated areas on San Salvador Island. Parasitoid wasp numbers were strongly correlated with the number of *A. macrophthalmus* (the host) and parasitism rates were far higher in our study than published from other locations. In the future, we will further explore ecological and phenological factors that may lead to these results. This research may lead to a better understanding of the seed predator and inform potential management of Jimbay in sensitive areas.

INTRODUCTION

Leucaena leucocephala (Jimbay) is an invasive plant species that was likely introduced to San Salvador Island, Bahamas in the early 19th century and was used as cattle fodder. This species has spread throughout the island and is now abundant in many disturbed areas. This leguminous plant bears pods that grow in groups of three to five on a stalk. The mature pod is brown with brown seeds that average 18 to 25 per pod (ISSG 2006). Jimbay is known to invade disturbed native vegetation, due to its tolerance of a range of rainfall of 500-3500 mm and being able to stand strong seasonal dry climates (ISSG 2006). This invasive plant has the capability to produce between 40,000 to 80,000 seeds per year (Egli et al. 2012). Jimbay can flower throughout the year and may have a 2-4 poddings cycle per year (Tuda et al. 2009). *Leucaena leucocephala* also has allelopathic properties due to the production of mimosine, which is concentrated in young leaves and mature seeds, and although the plant was used as fodder, it is toxic to cattle (Tuda et al. 2009).

There are at least two insect species that use the Jimbay pods and seeds as food and protection and at least 6 parasitoid wasps that use those insects as hosts on San Salvador Island (Sanders et al. 2013, pers. obs.). Since this plant species is invasive throughout the tropics, it is important to understand if seed predators, native as well as introduced, may be able to control the spread of this species (Vassiliou and Papadoulis 2008, Tuda et al. 2009, Shoba and Olckers 2010, Sharratt and Olckers 2012). After emerging from a pod, the female bruchid beetle, *Acanthoscelides macroph-*

thalmus, mates and oviposits on the pod surface, or on the seed itself (Vassiliou 2008). The larvae hatches and as a 1st instar burrows through the pod and into a seed, consuming the inside of a single seed passing through multiple instars. The larvae pupates within the seed and the adult beetle emerges burrowing out of the seed and the pod (Vassiliou and Papadoulis 2008). The life cycle within the pod ranges from 27-59 days (Shoba and Olckers 2010).

There is also predation by an unidentified moth species (Lepidoptera) (Sanders et al. 2013), that causes extensive damage to the pod and seeds. This is easily identified by the extensive silk combined with frass within the pod and the multiple seeds that the moth has partially consumed. The frass and damage appears to cause fungal growth and further seed damage within the pod.

The multiple wasp species emerging from the pods (Sanders et al. 2013) are not seed predators; they are parasitoids on the beetle and moth. There are roughly five species of wasp attacking the beetle and at least one species using the Lepidoptera as a host (Figure 1). Parasitoid wasps lay their eggs within the larvae of the host species. The larval wasp then slowly consumes the living host until it is nearing pupation. The parasitoid then kills and consumes the remainder of the host and, in the wasps found in this study, pupates within the host chrysalis (e.g. Figure 2c). Parasitoid wasps are frequently used to control insect populations (Orr 1988).

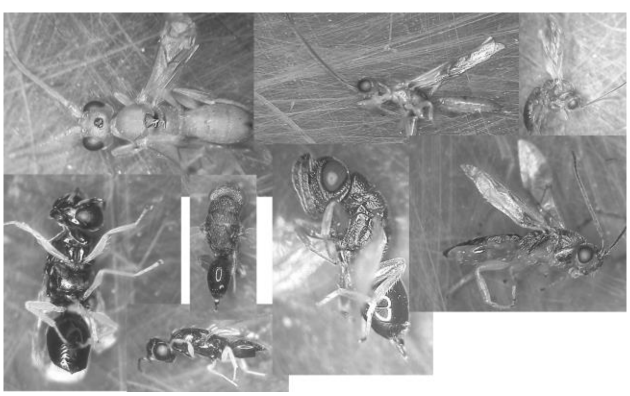

Figure 1. Parasitoid wasp species found emerging from Jimbay seeds and pods.

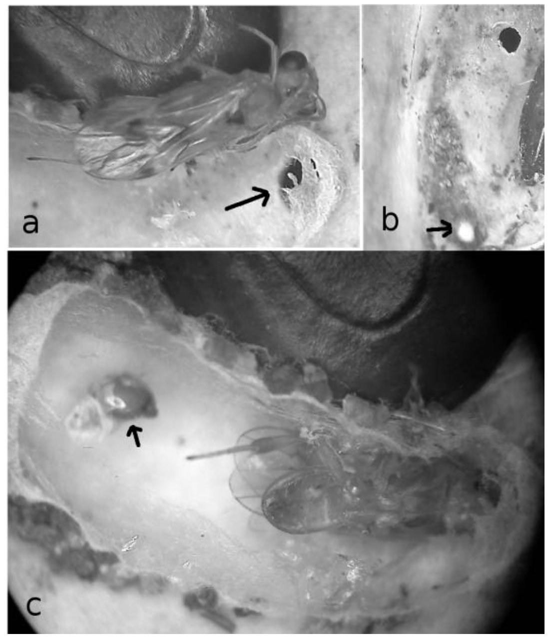

Figure 2. Wasp and moth parasitism evidence on seed. a: wasp emergence hole, b: moth pre-cut emergence hole covered in silk, c: remains of caterpillar, wasp cutting exit hole seen in (a).

Here we examine the presence of seed predation on Jimbay by *A. macrophthalmus* at different locations throughout Sal Salvador. Authors of previous studies have speculated on the potential biological implications that the bruchine could have on the spread of Jimbay (Egli and Olckers 2012). Although the abundance of seeds produced has led others to suggest that the beetle is not sufficient to reduce the spread of the plant (Sharratt and Olckers 2012). For *A. macrophthalmus* to be a useful biocontrol agent for Jimbay it is essential we determine what causes the variation in seed predation we find in Jimbay pods. We hope that by understanding what influences seed predation on San Salvador we can contribute to the global effort to control the spread of Jimbay. Based on the results of fieldwork in 2011 with seven sites, we found that beetles appeared to be more prevalent on the Jimbay closer to towns and decreased in number further away from towns or populated areas. Here we test the hypothesis that Jimbay seed predation by *A. macrophthalmus* increases with proximity to populated areas due to the fact that Jimbay was introduced in agricultural areas bordering populated areas on San Salvador and remains dense in these disturbed habitats.

During 2012, we compared Jimbay seed predation in four sectors on the island of north, south, east, and west. In each sector, seed predation was compared to the distance from a populated or previously populated area. We also determined if predation was different among sectors. We did not find a statistical difference in seed predation rate by the beetle among sectors. However, we did find that parasitoids were consuming a large proportion of the beetles in all sectors

MATERIALS AND METHODS

During late April and early May of 2011, Jimbay pods were collected at various locations around San Salvador Island, Bahamas: Fortune Hill, Long Bay, Owl's Hole, South Jetty, Light House, South Victoria, and Snow Bay (Figure 3, Appendix 1). Pods were removed from multiple plants at each site (5-10), and placed in Ziploc® storage bags, labeled with the date and location. The insects were allowed to emerge for 25 days in the bags and the bags were frozen in a -80°C freezer. After freezing the pods were assessed for seed damage, pod damage, and insect emergence.

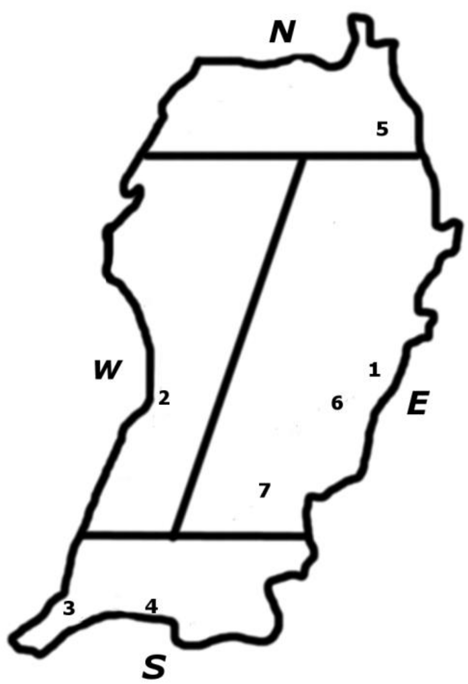

Figure 3. San Salvador sample locations from the 2011 study.

We removed and counted the seeds from each pod. We then examined the pods under a dissecting microscope to determine predation, seed damage, pod damage, and maturity. Previous research established that *Acanthoscelides macrophthalmus*, a bruchid beetle, create circular exit holes with smooth edges averaging 1.7 mm in diameter in both the seed and the pod (Sanders et al. 2013). Wasp holes were similar to the bruchid exit hole, but smaller, averaging about 0.6 mm in diameter with course edges. The Lepidoptera produce irregularly shaped exit holes, averaging 1 mm in diameter, in the pod and left the seeds chewed, and surrounded them with frass and silk (Sanders et al. 2013). Parasitoid wasps hatching from lepidopteron larvae were identified by a hole chewed through the chrysalis and pod (Figure 2a). Prior to pupating it appears that the lepidopteron larvae chews a hole in the pod and covers it with silk (Figure 2a, b). We suspect that this is used by the moth after hatching if the pod has not dehisced (Lepidoptera do not have chewing mouth parts). If the larvae is parasitized the wasp ignores the silk covered hole and chews its own exit hole (Figure 2a, c). It is also possible that the Lepidoptera larvae enters the pod at a later instar by chewing a hole through the pod and then covers the hole after entry. Field observations will need to be done to better understand the lepidopteran's life history.

During late April and early May of 2012, we used similar methods in order to test the hypothesis that seed predation on Jimbay is greater near populated areas on San Salvador Island. The methods we used in 2012 were altered due to time constraints and the increased number of sites. Only the pods were examined due to limited time available and we did not remove plant material from the Island for laboratory study. Therefore, more pods and locations were studied and were collected compared to the 2011 study but we did not collect seed or emergence data. Instead, each slot in a pod (the location of the seed within the pod) was counted, which represented a seed. Slots were examined for pod damage, immaturity, and frass, all of which are easily recognized with only the pod. The same insect species were identified using the types of damage associated with each species (Sanders et al. 2013). We found it easy to identify immature seeds or aborted seeds using this method because a slot, which contained an immature or aborted seed, was very shallow or had no indentation at all. An immature seed slot would also, in some cases, have the seed still in the area shriveled and connected to the pod. We collected pods by driving to different locations on the island and removing pods from multiple Jimbay plants (15-45 pods) and placing them in Ziploc® bags.

The locations that the pods were collected from in 2012 are: The Gulf, Monument, 0.8 km north of Monument, Watling's Castle, French Bay, 1.6 km north of Farquharson's, 1.6 km west of Gerace Research Centre, Priest Monument, 0.8 km west of Gerace Research Centre, Lindsay Reef, Cockburn Town, past Priest Monument before Rocky Point, East Beach, the Thumb, Six Pack Pond Trail, the Old Church by Pigeon Creek

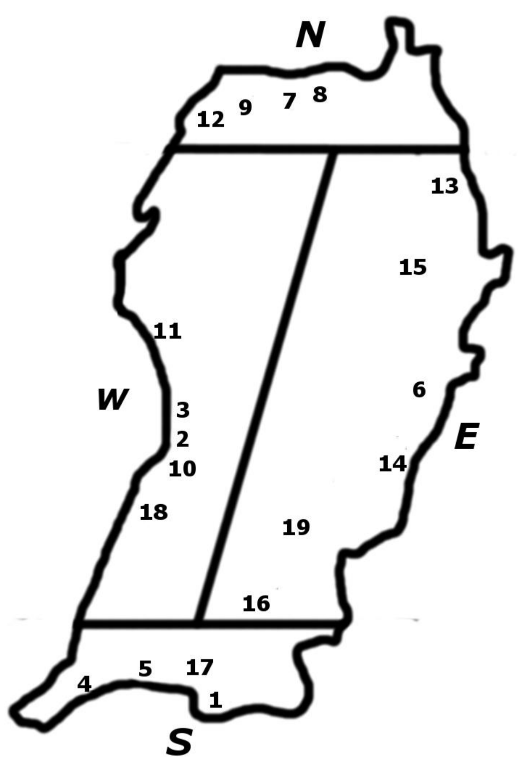

Figure 4. San Salvador sample locations from the 2012 study.

turnoff, 2.4 km south of Old Church, Sugar Loaf, and Pigeon Creek (Figure 4, Appendix 2).

We divided our study sites into four sectors: north, east, south, and west. Each of these sectors had a populated area, or previously populated area in or near the sector that was used as a focal point to measure a distance to each of the sample sites. This was done by putting the sample sites onto a Google Earth map of San Salvador, Bahamas, and then using the ruler tool to measure from the specified main area in each sector to the different sample sites and recording the distance in kilometers.

In the northern sector, there were four sites: 1.6 km west of GRC, Priest Monument, 0.8 km mile west of GRC, and past Priest Monument (Rocky Point) which were all measured from the populated area of the Gerace Research Centre/United Estates (Table 1). For the eastern sector, there were six locations: 1.6 km north of Farquharson's, East Beach, the Thumb, Six Pack Pond Trail, the Old Church, and Pigeon creek, which the distances were measured from the Fortune Hill settlement (an old loyalists plantation) (Table 2). In the southern sector there were four locations: the Gulf, Watling's Castle, about 2.4 km south of the Old Church, and French Bay which were all measured from the Sugar Loaf Settlement (Table 3). The western sector had five locations: the Monument, 0.8 km north of the Monument, Lindsay Reef, Cockburn Town, and Sugar Loaf, which were measured from the populated area of Cockburn Town (Table 4).

Table 1. Distance from GRC- Northern

Location	Distance (km)
7-1.6 km W. GRC	1.6
9-Priest Monument	3.9
8-0.8 km W. GRC	0.8
12-Past Priest Monument	5.3
Average	2.9

Table 2. Distance from Fortune Hill-Eastern

Location	Distance (km)
6-1.6 km N.Farquharson's	1.3
13-East Beach	7.6
14-The Thumb	1.0
15-Six Pack Pond Trail	4.7
16-Old church	7.2
19-Pigeon creek	5.0
Average	4.5

Table 3. Distance from Sugar Loaf-Southern

Location	Distance (km)
1-The Gulf	6.0
4-Watlings Castle	5.3
17-2.4 km S. old church	5.6
5-French Bay	3.5
Average	5.1

Table 4. Distance from Cockburn-Western

Location	Distance (km)
2-Monument	4.3
3-0.8 km N. Monument	3.7
10-Lindsay Reef	4.8
11-Cockburn Town	0.0
18-Sugar Loaf	5.6
Average	3.7

RESULTS AND DISCUSSION

During the 2011 study, we found seed and pod damage associated with *A. macrophthalmus*, an unidentified moth, and six parasitoid wasp species (Figure 2). The locations and number of seeds collected can be found in figure 3, table 5, and appendix 1. In a South African study, 5% of the beetle mortality was due to parasitoids (Egli and Olckers 2012). In the 2011 study, we found 44% beetle mortality from parasitoids (Table 5).

Therefore, parasitoids likely had an impact on *A. macrophthalmus* populations in our sites and the beetle's potential as a biological control for Jimbay on San Salvador. There was also a 35% predation on the moth by parasitoid wasps. We found an average of 34% seed predation by beetles (Table 5). The highest percentage of beetle seed predation was found at Long Bay (69%) and South Victoria (64%) (Table 5). These are populated areas and these data led us to speculate that there will be a higher percentage of beetle predation on Jimbay closer to such areas.

The same predation types from the 2011 study were examined in the 2012 study; however, the beetle species and its parasitoids were the focus of the study. The locations that were studied as well as the number of seed slots can be found in figure 4, table 6, and appendix 2.

The distance from a populated area in each sector was not associated with beetle predation (R^2 = 0.05, Figure 5). Beetle seed predation was much lower than the 2011 study, on average only 1% (Table 6). These results do not support our hypothesis that there will be more beetle predation on seeds near populated areas. This result could have been due to differences in the length of time a pod was available for oviposition by *A. macrophthalmus*. This would result in variation in seed predation in our samples unrelated to actual differences among sites. In the future, we will determine a way to ensure that the pods are of equal age and level of maturity and therefore exposed to ovipositing beetles for an equal amount of time.

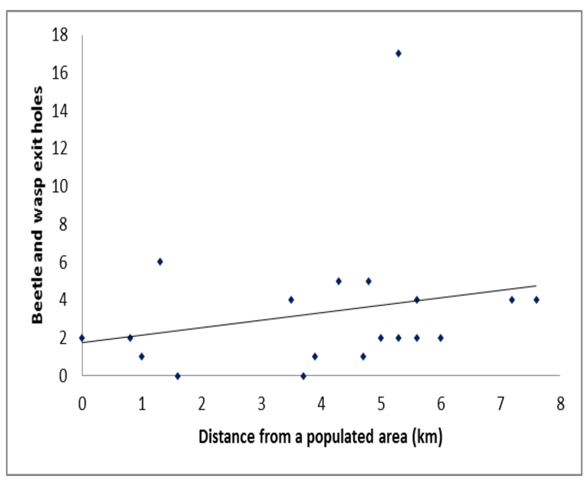

Figure 5. Distance from populated areas explained little variance in beetle and wasp exit hole numbers in Jimbay pods (R^2= 0.05).

We found a high rate of wasp parasitism (44%) in the 2011 study for nearly all of the seven

Table 5. 2011 Beetle, wasp, and moth exit holes and beetle and moth parasitism rates

Location	Beetle seed predation rate	Total beetle and wasp exit holes	Beetle parasitism rate	Moth and wasp exit holes	Moth parasitism rate	Seeds
Fortune Hill	0.27	23	0.39	9	0.44	147
Long Bay	0.69	25	0.48	12	0.42	55
Owls Hole	0.38	18	0.17	22	0.32	154
South Jetty	0.4	3	0.67	21	0.33	71
Light House	0.29	3	1.00	25	0.16	62
South Victoria	0.64	31	0.39	7	0.43	88
Snow Bay	0.9	0	0.00	16	0.38	33
Average	0.34	15	0.44	16	0.35	87.1

Table 6. 2012 Sector distances, beetle and wasp exit holes, and beetle parasitism rates

Sector	Average Distance from populated area (km)	Beetle seed predation (%)	Total beetle and wasp exit holes	Beetle parasitism rate	Seed slots
Northern	2.9	1.8	20.0	0.5	1376
Eastern	4.5	0.5	18.0	0.4	4333
Southern	5.1	1.0	12.0	0.8	1952
Western	3.5	0.9	14.0	0.4	2945
Average	4.0	1.1	16.0	0.5	2652

locations studied (Table 5). In the 2012 study, we found that the wasp predation rate on the beetle was also high and ranged between 39% - 75% among the sectors even though seed predation was much lower (Table 6). The highest parasitism rate was found to be in the southern sector (75%) which also had the greatest average distance (5.1 km) from a populated area (Table 6). However, the lowest average distance in the northern sector did not match with the lowest average parasitism rate (Table 6). This high parasitism rate across two studies, one using emergence data and the other using pod data, increases our confidence in our measure of beetle parasitism regardless of methods. In a South African study, it was found that hymenoptera parasitoids only affected 7-9% of the beetles (Sharratt and Olckers 2012). Although the South African study is far from the beetle and plant's native range, the study still recorded 10 species of parasitoid wasp attacking *A. macrophthalmus*.

The average beetle parasitism rate for the 2012 study was 50% (Table 6). In this study, the parasitism rate of the beetle is so high that it appears unlikely that the beetle alone could be capable of controlling Jimbay on San Salvador. In order for *A. macrophthalmus* to be considered as an agent for invasive plant control, the beetle would need to destroy 95-99% of the seeds produced annually by the Jimbay (Sharratt and Olckers 2012). However, the combination of the lepidopteron and *A. macrophthalmus* may be worth investigating further as potential biocontrol agents of Jimbay on San Salvador even though both suffer high mortality from parasitoids (Table 5). If the lepidopteron is a specialist on Jimbay, the combination of the two seed predators in areas without the parasitoid wasps might result in Jimbay control.

In future studies on the ecology of these seed predators and parasitoids we will control for pod exposure time, and the samples will be stored to allow for insect emergence. Pod data will also be used for tracking the lepidopteron activity and parasitism. We hope that our future research on Jimbay seed predators and their parasitoids can determine if biocontrol of Jimbay may be possible.

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REFERENCES

Egli, D., and T. Olckers. 2012. Oviposition patterns and egg mortality in *Acanthoscelides macrophthalmus* (Chrysomelidae: Bruchinae), a biological control agent of *Leucaena leucocephala* (Fabaceae) in South Africa. African Entomology, 20(1):111-118.

ISSG. 2006. Ecology of *Leucaena leucocephala*. In: Invasive Species Specialist Group (ISSG) (Eds) *Global Invasive Species Database*. *http://www.issg.org/database/species/ecology.asp?fr=1&si=23* (accessed 27 April 2013).

Orr, D. B. 1988. Scelionid Wasps as Biological Control Agents: A Review. *The Florida Entomologist*, *71*(4):506-528.

Sanders, B. S., D. S. Kjar, and T.P. Egan. 2013. Beetle (Coleoptera: Bruchidae: *Acanthoscelides macrophthalamus*) seed predation on *Leucaena leucocephala* (Leguminosae: Mimosoideae) seeds and pods at two sites on San Salvador Island, Bahamas. Pp. 53-58 *in* C. Tepper and R. Shaklee, eds., Proceedings of the 14th Symposium on the Natural History of the Bahamas. Gerace Research Centre, San Salvador, Bahamas.

Sharratt, M. E., and T. Olckers. 2012. The biological control agent *Acanthoscelides macrophthalmus* (Chrysomelidae: Bruchinae) inflicts moderate levels of seed damage on its target, the invasive tree *Leucaena leucocephala* (Fabaceae), in the KwaZulu-Natal coastal region of South Africa. African Entomology, 20(1):44-51.

Shoba, Z., and T. Olckers. 2010. Reassessment of the biology and horst range of *Acanthoscelides macrophthalmus* (Chrysomelidae: Bruchinae), a seed-feeding beetle released for the biological control of *Leucaena leucocephala* in South Africa. Aftrican Entomology, 18(2):1-9.

Tuda, M., L. Wu, Y. Tateishi, C. Niyomdham, S. Buranapanichpan, K. Morimoto,W. Wu, C. Wang, Z. Chen, H. Zhu,Y. Zhang, K. Murugan, L. Chou, and C.D. Johnson. 2009. A novel host shift and invaded range of a seed predator, *Acanthoscelides macrophthalmus* (Coleoptera: Chrysomelidae: Bruchinae), of an invasive weed, *Leucaena leucocephala*. Entomological Science, 12:1-8.

Vassiliou, V. A., and G. Papadoulis. 2008. First record of *Acanthoscelides macrophthalmus* (Schaeffer) (Coleoptera: Bruchidae) in Cyprus. Entomologia Hellenica. 17:52-55.

APPENDICES

Appendix 1. Sample locations from the 2011 study.

Location	Latitude	Longitude
1-Fortune Hill	24° 1'17.61"N	74°27'19.65"W
2-Long Bay	24° 0'55.25"N	74°31'21.38"W
3-Owl's Hole	23°57'14.55"N	74°33'18.58"W
4-South Jetty	23°57'16.90"N	74°31'32.28"W
5-Light House	24° 5'56.48"N	74°27'5.78"W
6-South Victoria	24° 0'49.89"N	74°28'1.12"W
7-Snow Bay	23°57'13.74"N	74°29'18.86"W

Appendix 2. Sample locations from the 2012 study and sector category.

Location	Latitude	Longitude	Sector	Distance from pop. area (km)
1-The Gulf	23°56'51.08"N	74°31'0.75"W	Southern	6.0
2-Monument	24° 0'42.20"N	74°31'35.88"W	Western	4.3
3-0.8 km N. Monument	24° 1'4.70"N	74°31'24.78"W	Western	3.7
4-Watlings Castle	23°57'14.71"N	74°32'48.79"W	Southern	5.3
5-French Bay	23°57'15.08"N	74°31'59.75"W	Southern	3.5
6-1.6 km N.Farquharson's	24° 1'56.66"N	74°27'1.74"W	Eastern	1.3
7-1.6 km W. GRC	24° 6'53.26"N	74°28'52.59"W	Northern	1.6
8-0.8 km W. GRC	24° 7'3.43"N	74°28'30.41"W	Northern	0.8
9-Priest Monument	24° 6'46.15"N	74°30'5.77"W	Northern	3.9
10-Lindsay Reef	24° 0'26.53"N	74°31'52.99"W	Western	4.8
11-Cockburn Town	24° 3'4.69"N	74°31'50.48"W	Western	0.0
12-Past Priest Monument	24° 6'22.58"N	74°30'58.91"W	Northern	5.3
13-East Beach	24° 5'20.97"N	74°26'38.71"W	Eastern	7.6
14-The Thumb	24° 0'47.20"N	74°27'22.13"W	Eastern	1.0
15-Six Pack Pond Trail	24° 3'47.54"N	74°27'14.16"W	Eastern	4.7
16-Old church	23°58'14.52"N	74°30'3.46"W	Eastern	7.2
17-2.4 km S. old church	23°57'25.69"N	74°31'12.97"W	Southern	5.6
18-Sugar Loaf	23°59'54.84"N	74°32'10.98"W	Western	5.6
19-Pigeon creek	23°59'8.60"N	74°29'6.03"W	Eastern	5.0