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SNAIL TRAILS: USE OF RFID TECHNOLOGY TO DISCOVER HOW FAR INTERTIDAL SNAILS TRAVEL AND WHERE THEY LIVE

2016 | AINSLIE JILANE MCLEOD

B.Sc. Honours thesis

THOMPSON RIVERS UNIVERSITY

SNAIL TRAILS: USE OF RFID TECHNOLOGY TO DISCOVER HOW FAR INTERTIDAL SNAILS TRAVEL AND WHERE THEY LIVE

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF BACHELOR OF SCIENCE (HONS.)

in the

DEPARTMENT OF BIOLOGICAL SCIENCES

(Environmental Biology & Ecology)

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Dated this 31st of May, 2016, in Kamloops, British Columbia, Canada

ABSTRACT

Mark and recapture (MR) is a common technique used to study animal behaviour, however, conventional tagging methods involving direct observation have difficulties in locating small, cryptic animals in complex environments. Radio frequency identification (RFID) technology can solve this problem, to offer a promising advantage in the intertidal zone. This project represents the first use of RFID technology to discover which habitats snails use throughout the summer, how far throughout the intertidal zone they move per day, and how widely they disperse towards other populations. When mounted to snails, RFID technology, which uses small passive integrated transponder (PIT) tags, allows a researcher to detect individual snails even when they are hidden from view. The specific goals of this study were to determine: 1) the effectiveness of using RFID to study snail behaviour, 2) which microhabitats were used most often by the snails, 3) how far the snails travelled each day, and 4) how far the snails dispersed over the summer. This research was conducted on the intertidal snail, *Nucella ostrina*, near the Bamfield Marine Sciences Center, in British Columbia, Canada. In summer 2015, I attached one 12 mm PIT tag to each of 64 snails and located their position in the intertidal zone daily using an RFID reader. PIT tags had no detectable effect on snail movement or survival; thus, using RFID technology is an effective technique for tagging snails to study their behavior. Intertidal snails occupied a variety of hidden microhabitats throughout the study, which made 30% of them invisible to the human eye without the use of RFID technology. In addition, intertidal snails moved very little each day (13.82 ± 7.01 cm) and moved non-directionally, which led to limited displacement over the study period where majority of snails (78%) only dispersed up to 1 m from their starting position. Consequently, snail populations may have limited gene flow between neighboring populations.

Thesis Supervisor: Dr. Louis Gosselin

ACKNOWLEDGEMENTS

Special thanks goes to my supervisor, Dr. Louis Gosselin, for his support and guidance during this study. Also to Dr. Matthew Reudink and Dr. Karl Larsen for agreeing to be on my committee to provide their shared knowledge and support. Finally, a huge thanks goes to the staff at BMSC and to my field assistants including, Luke Andersson, Hilary Hamilton, Brianna Iwabuchi, and Elena Buscher. Funding was provided by and NSERC Discovery Grant to Dr. Louis Gosselin and a UREAP award and grant to myself.

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INTRODUCTION

Animal movement is a key factor in habitat selection, access to food, and finding mating partners (Leidvogel et al. 2013), and also an influence of population density and gene flow between populations (Rochette & Dill 2000; Pardo & Johnson 2004; Liedvogel et al. 2013). Gene flow is particularly important because it can constrain local adaptation by reducing inbreeding depression and increasing genetic divergence, or promote local adaptation by spreading new alleles throughout a species' range (Slatkin 1987; Peterson & Denno 1997). The level of gene flow between populations is heavily correlated with the dispersal ability of individuals (Peterson & Denno 1997). Dispersal abilities, however, vary considerably among animal species. For instance, some species of marine intertidal animals have a life cycle that includes planktonic larvae that spend days or weeks in the water column and therefore have the potential to disperse great distances with the water currents (Grantham et al. 2003; Hellberg 2009). Many other species, however, have no widely dispersing phase in their life cycle. For example, the intertidal snail *Nucella ostrina* has direct development: adults encapsulate embryos within egg capsules and attach these to rocks, where embryos develop until they become juveniles that emerge and crawl away (Gosselin & Chia 1994; Allen 2014). However, the dispersal abilities of juveniles and adults of direct developing intertidal species, such as *N. ostrina*, remain poorly understood.

The most frequently used approach to studying animal movement is the mark and recapture (MR) method (Gibbons & Andrews 2004; Henrya & Jarne 2007). MR is useful for quantifying growth rate, movement distance, activity patterns, habitat use, and survival of tagged individuals (Gibbons & Andrews 2004; Henrya & Jarne 2007). The most common MR technique used for snails involves adhering numbered tags to their shell (Pardo et al. 2004; Curtis 2005; Pardo &

Johnson 2006; Henrya & Jarne 2007; Seuront et al. 2007; Wright & Nybakken 2007; Kovach & Tallmon 2010; O'Dwyer et al. 2014), although a few studies marked the shell with paint or ink (Gosselin 1993; Henrya & Jarne 2007), attached metal tags to the shell (Crowe et al. 2001) or, more recently, inserted a small radio tag into the body (Conery 2012; Kreis 2012). Previous studies using these MR techniques suggest that snails can potentially travel long distances, but dispersal away from their place of birth remains limited by non-directional, random patterns of movement (Pardo et al. 2004; Curtis 2005; Michel et al. 2007; Seuront et al. 2007).

For a tagging method to be reliable it must be long lasting, make it easy to detect the animal, have no effect on the animal, and allow for MR with minimal disturbance to the animal. External marking techniques used for snails, such as the ones described above, have important limitations to these guidelines. First, printed tags may become lost or unreadable due to damage or environmental exposure (Gibbons & Andews 2004; Henrya & Jarne 2007; Riley et al. 2010). Second, and possibly most important, an individual must be found and visually inspected in order to ascertain its identity and location. This can be difficult in the structurally complex environments of the marine benthos because tags may become covered in debris or algae, and snails are often hidden under rocks or in crevices and out of sight of the observer at recapture sessions (Gibbons & Andews 2004; Pardo & Johnson 2006; Henrya & Jarne 2007; Riley et al. 2010; Yamane & Gilman 2009; Marshall et al. 2013; Dahlhoff et al. 2001). The loss of tags and inconsistent recovery of animals has lead to unreliable recapture data and incorrect estimates of population distribution, dispersal, abundance and survival (Gibbons & Andews 2004; Henrya & Jarne 2007).

Radio frequency identification (RFID) technology, using passive integrated transponder (PIT) tags, is a promising approach that may be able to resolve both of these problems and improve our knowledge of movement in intertidal animals. RFID technology is a powerful and robust technique (see Appendix A), that provides many advantages over other tagging techniques (Gibbons & Andrews 2004; Kurth et al. 2007; Cookingham & Reutz 2008; Hale et al. 2012; RFID Canada 2012; Turner et al 2012). For example, PIT tags can be detected and their unique numbers can be read from short distances without actually seeing the individual (Gibbons & Andrews 2004; Kurth et al. 2007; Cookingham & Reutz 2008; Hale et al. 2012; Kurth et al. 2012; RFID Canada 2012; Cooke et al. 2013; Lapointe et al. 2013; Turner et al. 2013), so the use of PIT tags can potentially increase the efficiency and accuracy of MR techniques when searching for small animals in complex intertidal habitats. In addition, PIT tags are minimally disruptive and can work for the entire lifetime of the individual (Cookingham & Reutz 2007; Gibbons & Andrews 2004; Hale et al. 2012; RFID Canada 2012; Gianasi et al. 2015). Due to recent advances in tag size, RFID technology is suitable for studying the movement of smaller individuals than previously possible (Cooke et al. 2013; Lapointe et al. 2013). PIT tags have been tested on invertebrates in the laboratory including abalone (Hale et al. 2012), freshwater mussels (Kurth et al. 2007), coconut crabs (Sato et al. 2013), and crayfish (Bubb et al. 2002) with high tag retention and no significant effects on growth, movement, or survival of the individuals. PIT tags have also been used to study the effectiveness of using RFID technology for MR with coconut crabs (Drew et al. 2012) and freshwater mussels (Kurth et al. 2007) in the field with high tag retention and no adverse effects on mortality or behaviour. RFID technology has not been used to study intertidal animal movement in the field, which leaves a gap in knowledge of the effectiveness of using RFID technology because animals can react differently in their natural environment compared to when they are held in an artificial laboratory environment (Lauzon-

Guay & Scheibling 2008). In the laboratory, animals are normally kept in a controlled environment with no external stress, whereas field conditions expose animals to environmental conditions. These stressful conditions may have an additive effect on an animal, resulting in a greater impact from the PIT tags.

Although RFID technology can provide advantages over conventional tagging methods used with intertidal snails, the effectiveness of using RFID to find and follow animals in the intertidal zone is unknown. Furthermore, it is unknown what impacts RFID technology has on the behaviour or survival of intertidal snails. This was the first study to use RFID technology to track the movements of intertidal invertebrates in the field. The purpose of this study was to determine the effectiveness of RFID technology for studying intertidal invertebrates, and to use RFID technology to quantify the movements, dispersal, and microhabitat use of adult *N. ostrina* in the intertidal zone. The specific goals of the project were to: 1) determine whether PIT tags affect snail movements or survival, 2) document microhabitat use by snails, 3) determine how far snails travel each day, 4) establish how far snails disperse over the summer, and 5) determine the influence of environmental conditions on snail movements in the intertidal zone.

METHODS

Study location and species

This study was carried out at the Bamfield Marine Sciences Center (BMSC) on the west coast of Vancouver Island, British Columbia, Canada and at a field site in Barkley Sound near BMSC from June to August 2015. The intertidal snail *Nucella ostrina* was chosen for this study because it is common in the mid intertidal zone amongst mussel beds, barnacles, seaweeds and rocky substratum along the pacific coastline from Alaska to California. This is a predatory snail that feeds primarily on mussels (*Mytilus trossulus and M. californianus*) and barnacles (*Balanus glandula*) within its habitat (Gosselin & Chia 1994). Adults snails (19-26 mm shell length) were collected from Prasiola Point (48°49'03"N, 125°10'07"W), and brought back to the laboratory to be sexed, weighed, and tagged. To quantify adult movement, this study included two components. First, a laboratory experiment, examining the effectiveness of the tagging method, involved 40 snails, 20 of which were tagged with a 12 mm PIT tag. Then, a field experiment examining snail movements using 64 snails, each tagged with a 12 mm PIT tag. Tagged snails were returned to Prasiola Point, and placed within the study grid to monitor their movement in the field. A large proportion of the study grid was covered by a mussel bed (*M. californianus*), which gave snails a large area to find a suitable microhabitat amongst the mussels.

RFID and tagging

To monitor snail movement, a PIT tag (Oregon RFID 12.0 mm x 2.1 mm HDX PIT tag ISO 11784/11785) was attached to each snail and these snails were then relocated on a daily basis using a hand-held RFID reader (Oregon RFID DataTracer) with attachable antenna. The smallest PIT tags currently available are 7 mm long; such small tags, however, have a limited range of detection (RFID Canada 2012). Their limited read range was confirmed by a preliminary trial that I carried out with 8 mm PIT tags, which were found to have a detection range of ~5 cm. Consequently, 12 mm PIT tags, which have a detection range of ~10 cm, were used in this study. One PIT tag was cemented to the anterior dorsal part of the shell of each snail using Gorilla® Super Glue Gel (Figure 1). The position of the PIT tag on the snail was chosen so as to cause the least interference on movement (e.g. getting caught in algae). Prior to tag attachment, the shell was cleaned and dried with paper towel to remove algae, dirt and water. The snail was then weighed to the nearest 0.0001 g to determine its mass without the PIT tag. Next, a thin line of glue was placed on the shell and the PIT tag was held in the glue for roughly 20 seconds. When the glue was dry, the PIT tag was fully encapsulated in a second layer of super glue. While the glue dried, the unique PIT tag number, along with the animal's shell length, gender, mass, color, and shell condition (e.g. damage) were recorded for each individual. After the glue was fully dried, the snail was reweighed to assess the added mass of the tag and glue for each individual and thus determine the added body weight of the PIT tag to each snail. The snails were then kept overnight in flowing seawater to ensure tag retention.

Figure 1. Adult *N. ostrina* bearing a 12mm PIT tag attached on the anterior dorsal part of the shell using Gorilla® Super Glue Gel

Laboratory study of PIT tag effects on snail mortality and movement

Mortality

The survivorship of 40 snails held in the laboratory was monitored for 60 days starting on June 28 and ending on August 27. Snails were kept in a submerged container with rocks, barnacles, mussels, algae, and flowing seawater to simulate their natural environment. To determine the effects of PIT tags on snail mortality, 20 of these snails were randomly selected and tagged with one 12 mm PIT tag each on June 28, and 20 other snails were left untagged. Mortality was compared between tagged and non-tagged snails using a contingency table analysis.

Movement

To determine the effect of PIT tags on snail movement, 10 tagged and 10 non-tagged snails were placed on a submerged sheet of acrylic, marked with a 5 cm X 5 cm grid, and then monitored for the next ten minutes by recording the x and y coordinates of each snail once every minute. This allowed for the calculation of total travel distance, net distance displaced (NDD; Pardo & Johnson 2004), and directionality for each snail. Travel distance was calculated using the formula [$\sqrt{\ }((x_2-x_1)^2+(y_2-y_1)^2)$] for each one minute interval and then adding up the distances of all ten intervals. Snail NDD was calculated using this formula to compare the snail's final position in the grid with its initial position when first placed in the grid. The snail's directionality was calculated using the formula: directionality = (NDD/total travel distance). A directionality value of 1 indicates movement in a straight line away from the starting position; as the snail travels in a more convoluted path, the directionality value gets smaller. The experiment consisted of sequentially monitoring four groups of five snails, each group consisting of tagged and non-tagged snails, and then repeating the trial a second time using the same groups of animals. For data analysis, a Friedman randomized block test was first used to determine whether snail movement differed among the five groups of snails, using the two trials as blocks. Then given that there was no significant difference among groups of snails, all data was pooled to compare the movements of tagged and non-tagged snails using a T-test.

Field experiment

Study Grid

A permanent grid was established in the intertidal zone at Prasiola Point, within which the position of individual snails was monitored on a daily basis. The grid was 12 m wide horizontally along the shore, and 5-6 m high vertically up the slope of the shore (Figure 2). The lowest edge of the grid was 2.24 m above mean lower low water (MLLW), and the highest edge of the grid was 3.48 m above MLLW. The grid was permanently marked in the intertidal zone by drilling holes in the rocky substratum and inserting stainless steel screws at 1 m intervals along the horizontal axis and at 2 m intervals along the vertical axis. Where needed, mussels and algae were cleared from the rock surface within a ~5 cm radius around each screw. In a few places, where the mussel bed (*M. californianus*) was too deep for viewing surface-mounted screws, long galvanized nails were cemented in place using marine epoxy. In other places, the rock beneath the intertidal community was rotten or cracked and would not hold a screw or nail; in those locations, marine epoxy was used to cement the screw in place. PIT tagged snails were returned to the field site and placed ~1 m from each other within the study grid. The field site was revisited daily between July 7 and August 21 (43 days) to search the grid for PIT tagged snails using the RFID detector. The surrounding intertidal zone was also searched, up to roughly 5 m away from the study grid to ensure no snails had left the grid. During the course of this field experiment, some snails were lost from the study grid due to dislodgement, mortality, or predation; therefore, more snails were collected and tagged to replace them as needed. Initially, 30 tagged snails were placed in the grid on July 5th. Additional tagged snails were added to the study grid on July 12 (n=6), July 26 (n=10), and July 28 (n=13), for a total of 59 snails that were tagged and placed in the study grid

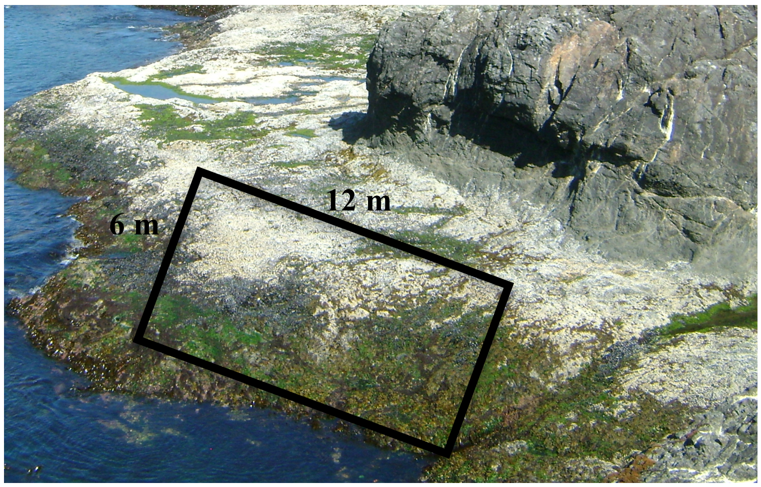

Figure 2. An aerial view of the field site, Prasiola Point, indicates the approximate location of the study grid, which covered an area 12 m horizontally along the shore by 5-6 m vertically up the slope of the shore, where snail movement was tracked in the intertidal zone.

Microhabitat use

Once detected with the RFID reader, each PIT tagged snail was recorded as visible or hidden to the human eye after physically searching for the snail. This gave a conservative estimate as to how many snails would be visible to the human eye if using other external tagging techniques because the location of the detected snail was first known by using RFID technology. The microhabitat occupied by the detected snail was also recorded as being exposed (e.g. on a rock, on algae, on mussels, or on barnacles) or covered (e.g. under rocks, under algae, under mussels, or under barnacles).

Snail movement

To determine the position of each snail in the grid each day, the x and y coordinates of each detected individual were recorded to an accuracy of 2.5 cm by using a 1 m x 1 m quadrat and lining up its corners to the screws of the intertidal grid (Figure 3). This allowed daily travel distances to be calculated using the distance formula: [$\sqrt{((x_2-x_1)^2+(y_2-y_1)^2)}$]. Snail horizontal distance displaced (NHDD) along the shore over the study period was also calculated using the simplified distance formula: $|x_{final}-x_{initial}|$ to compare the snail's final position in the grid at the end of the study with its initial position when first placed in the grid earlier in the summer. The snail's directionality was calculated using the formula: directionality = (NDD/total travel distance).

Figure 3. To locate the position of each snail in the grid, RFID technology was used to determine the x and y coordinates of each detected individual to an accuracy of 2.5 cm by using a 1 m x 1 m quadrat lined up on the screws of the intertidal grid. This allowed for their movements distances to be calculated.

Factors affecting snail movement in the field

Tidal amplitude

Tidal height and tidal amplitude data were obtained from the online tide tables provided by the Department of Fisheries and Oceans (DFO) Canada. Tidal amplitude was calculated based on the highest high tide of the day and subtracting the lowest tide of the day, either before or after the highest tide.

Temperature data

Temperature was monitored at 15 minute intervals between July 16 and August 23 using three temperature probes (iButton®, Thermochron model D5 1921G) placed at 1.5 m above MLLW, near the bottom of the study grid. The temperature probes were secured to the rock surface by placing them in gray mesh bags, which resembled the color of the rock surface, and bolting these to the rock surface ~6 m apart (Jenewein & Gosselin 2013). These data were used to determine substratum temperature at low tide and seawater temperature at high tide. To calculate low tide substratum temperature for a given day, I used the average temperature measured over a 2 h period centered on the low tide time over two daily low tides; the correlation between substratum temperature and average daily snail movement during the 24 h period following exposure to these conditions was then examined. Seawater temperature at high tide for any given day was calculated as the average temperature measured over a 2 h period centered on the high tide time over two daily high tides; the correlation between water temperature and average daily snail movement during the 24 h period following exposure to these conditions was then examined.

Gender

The gender of each snail was determined prior to attaching a 12 mm PIT tag by gently lifting the shell of snails while their foot remained attached to a hard surface. The absence (female) or presence (male) of a penis located behind the right tentacle was recorded. To effectively compare movements between genders using T-tests, the movements of 27 tagged males and 37 tagged females were monitored in the grid at Prasiola Point.

RESULTS

Laboratory study of PIT tag effects on snail mortality and movement

During the 60 days of the laboratory experiment, none of the 20 snails lost their tag (100% tag retention). PIT tags had no significant effect on the mortality of *N. ostrina* in the laboratory (Contingency table analysis: χ^2=0.11, df=1, n=40, p=0.74). During the two month period when these snails were held in the laboratory, 30% (6/20) of tagged snails died and 35% (7/20) of non-tagged snails died. In the movement experiment, there was no significant different in average distance travelled (Friedman block test: S=1.00, df=1, n=8, p=0.32), NDD (S=1.00, df=1, n=8, p=0.32), or directionality (S=1.00, df=1, n=8, p=0.32) among the five groups when blocked by trial. The data from these five groups were then combined to compare the movement of tagged (n=10) and untagged (n=10) snails. There was no significant difference between tagged and untagged snails in average distance travelled (t=-1.29, df=17, n=20, p=0.22), NDD (t=-1.65, df=16, n=20, p=0.12), or directionality (t=-0.39, df=17, n=20, p=0.70) (Figure 4).

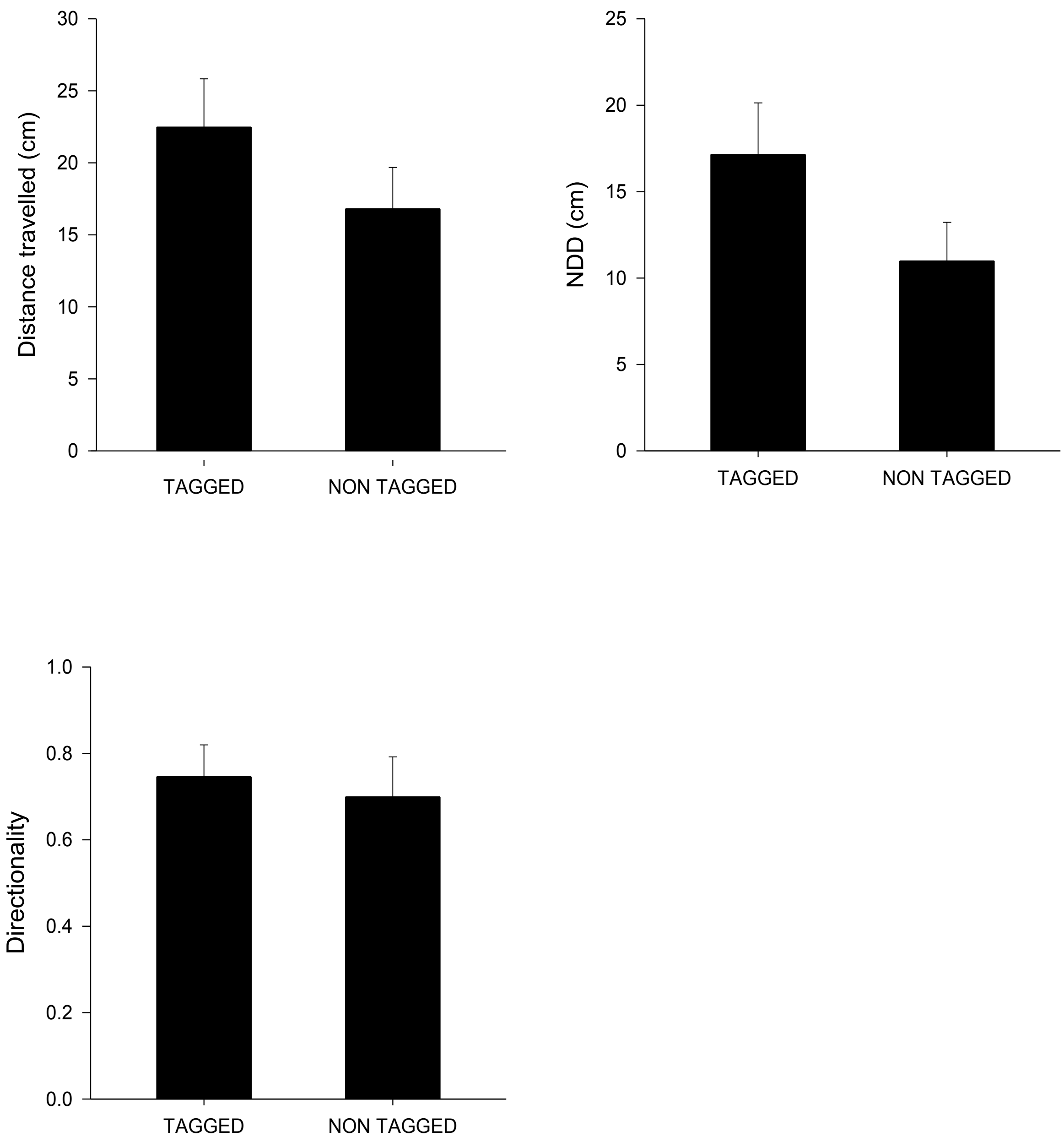

Figure 4. Results from the laboratory study of the effect of PIT tags on snail movement: (A) total distance travelled, (B) net distance displaced (NDD; i.e. the straight-line distance between first and last position), and (C) directionality of tagged (n=10) and non-tagged (n=10) snails. A directionality value of 1 indicates movement in a straight line away from the starting position. There was no significant difference between tagged and non-tagged snails in distance travelled, dispersal distance, or directionality.

Field study of *N. ostrina* behaviour

Of the 59 snails that were tagged and placed in the study grid, 40 snails died or could not be relocated for at least a few days during the study. Five of the 40 snails (12.5%) disappeared during the first 24 h after being placed in the grid and had likely been swept away by waves before being able to firmly attach to the substratum. Seventeen snails were found again after they had been lost from the grid; of those 17 snails, eight were found dead (20%), three (7.5%) were found dead and caught in mussel (*M. californianus*) byssus, three (7.5%) were dead and their shell inhabited by a hermit crab, two (5%) were ingested by sea anemones and one (2.5%) was ingested by a sea star. Finally, 18 snails (45%) disappeared at some point after the first 24 h and were never relocated despite regular searches outside the grid; their fate is unknown.

Effects of PIT tags on snails in the field

PIT tag size and mass had no significant effect on snail movement in the field. Snails tagged with 8 mm and 12 mm PIT tags for a preliminary experiment showed no difference in movement (see Appendix B). Furthermore, there was no relationship between the proportional increase in body mass resulting from the added PIT tag and the snail's travel distance (Pearson correlation: $R=-0.146$, $n=55$, $p=0.287$), dispersal from their starting position ($R=-0.482$, $n=11$, $p=0.113$), or directionality of movement ($R=-0.202$, $n=11$, $p=0.551$)

Microhabitat use

Nucella ostrina occupied a wide range of microhabitats in the intertidal zone during the field study. The highest proportion of snails (36% ± 10%, average ± SD) were found sheltering beneath mussels each day, but many snails were also found on rock surfaces (21% ± 10%) or on exposed surfaces of mussels (21% ± 10%). On average, 54% ± 11% of detected snails inhabited microhabitats that were sheltered from the sun and from sight (e.g. under rocks, under mussels, under barnacles, and under algae). I also physically searched for detected snails to visually confirm their microhabitat, but a large proportion of snails (30% ± 9%, average ± SD) could not be found without destructively dismantling the microhabitat.

Snail movement

Snail movement was highly variable during the field study. During days 1 and 2 after placement in the field, distance travelled was high, at 60.9 ± 98.1 cm (average ± SD) and 26.2 ± 58.6 cm respectively, whereas the daily travel distance for all subsequent days was 13.82 ± 7.01 cm (Figure 5). Based on these results, movement data from days 1 and 2 after placement in the field were excluded from further data analyses for all snails to give a better estimate of the crawling ability of *N. ostrina* and to better understand their biology. Total distance travelled by snails during the study period, the warmest period of the year, was then calculated for those snails that had been tagged and placed in the field grid at the very start of the experiment (July 9), survived the entire study period (43 d), and remained in the grid throughout that time. Those snails (n=11) travelled an average total distance of 268.2 ± 125.8 cm, with individual travel distances ranging from 70 cm to 476 cm.

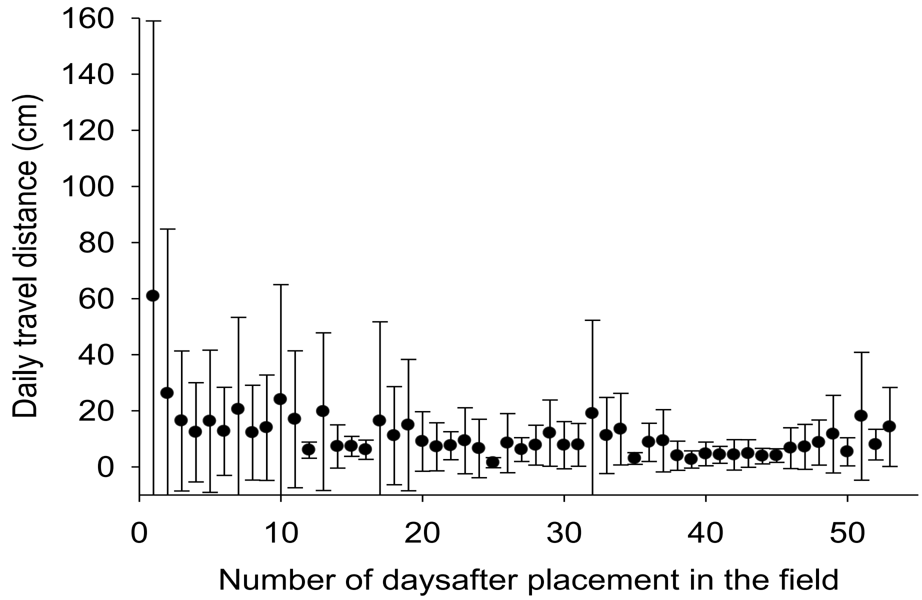

Figure 5. Average daily movement distances as a function of the number of days since the tagged snail was placed in the study grid. Error bars represent standard deviation.

NHDD of each snail (n=58) was quantified by comparing its final position in the grid at the end of the study with its initial position when first placed in the grid earlier in the summer. Snails were present in the study grid for differing amounts of time because some became lost from the grid due to dislodgement, mortality, or predation and others were collected and tagged to replace them; therefore, the analysis was broken down based on how long each snail was present in the grid (Figure 6). Fourteen snails were present in the grid for less than 10 days and most of these snails (n=13) only moved ≤1.5 m from their starting position. Only one of these snails displaced more than 4 m and this snail was observed being dislodged by a wave and was relocated 4.4 m away from its starting position. Twelve snails were present in the study grid for 10-20 days and all of these snails only moved ≤1.5 m from their starting position, but half of these snails (n=6) travelled ≤0.5 m from their starting position during the study. Seventeen snails were present in the grid for 20-30 days and over half of these snails (n=11) travelled ≤1 m from their starting position whereas the rest travelled ≤2 m. Fifteen snails were in the grid for >30 days; most of these snails (n=14) only moved ≤1.5 m. Only one of these snails travelled over ≤2.5 m.

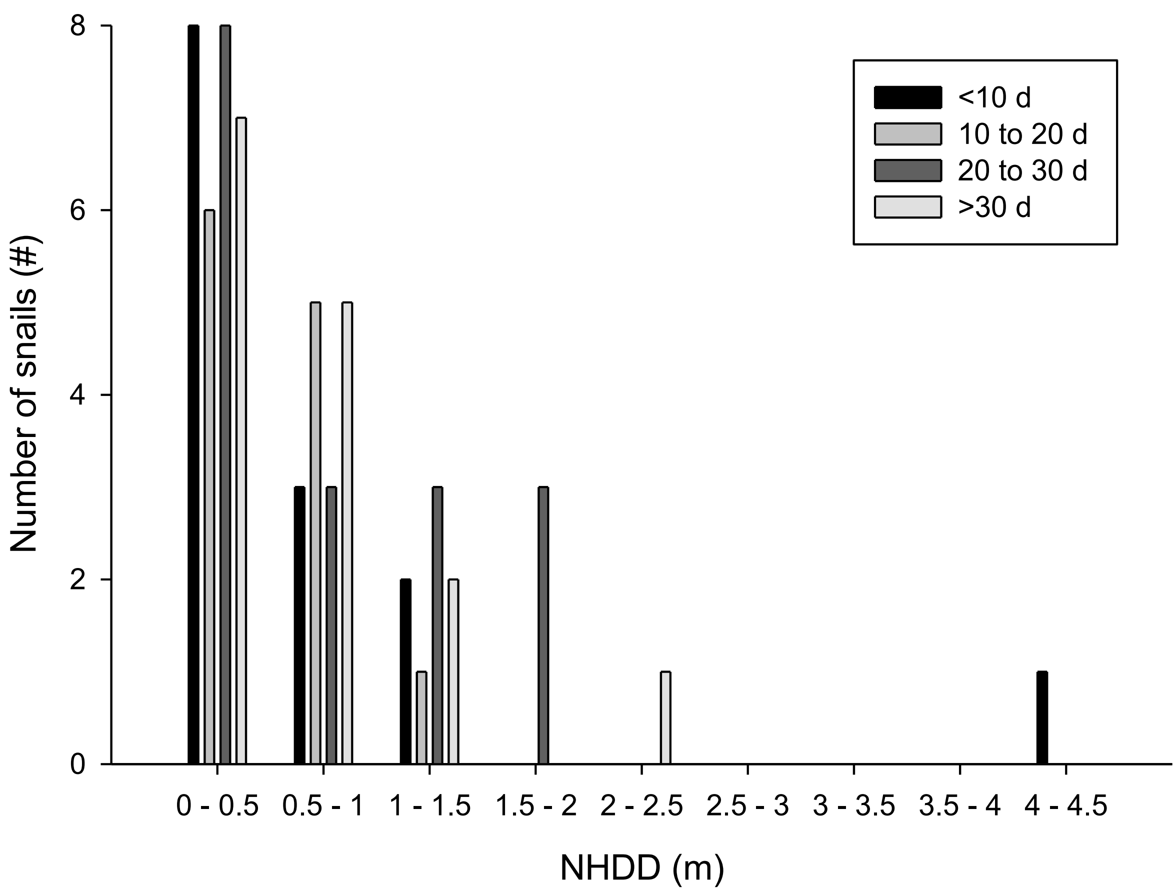

Figure 6. The net horizontal displacement distance (NHDD) for snails present in the study grid for less than 10 days (n=14), between 10-20 days (n=12), between 20-30 days (n=17), and over 30 days (n=15) during the study period (49 days). NHDD was calculated as the snail's straight-line horizontal distance travelled from their starting position over the study.

The directionality of snail movement was calculated by using the data on total travel and dispersal distance for individual snails. This analysis was carried out for those snails that had been tagged and placed in the field grid at the very start of the experiment (July 9) and remained in the grid throughout the entire study period (43 d). For those snails (n=11), directionality was low with an average directionality value of 0.299 ± 0.233 (average ± SD).

Finally, I examined the relationship between movement distance and factors such as gender, temperature, and tidal amplitude. There were no significant differences in movements between genders (see Appendix C). Similarly, average daily travel distance was not correlated with tidal amplitude or seawater temperature (see Appendix D). There was a significant correlation between substratum temperature and daily travel distance when data when all snails were pooled, but this relationship was not significant when analyzing male and female snails separately (see Appendix D).

DISCUSSION

Effectiveness of using RFID technology for studying intertidal snails

In order for a tagging technique to be effective, it must have minimal or no effect on the tagged individual. The laboratory experiment comparing tagged and untagged snails revealed that PIT tags had no effect on snail mortality or movement. In addition, the 12 mm PIT tags (~0.1 g) used in this study were somewhat heavy relative to the weight of the snails, adding 4-13% to their body mass, but the proportional increase in mass had no detectable effect on distance travelled by snails in the field. These results are consistent with those of Crowe et al. (2001), who attached relatively heavy metal tags (0.3 g) to juveniles (16-25 mm) of the marine snail *Trochus niloticus* and yet achieved high recapture rates and detected no short-term effect on snail mortality over a period of five days in the field. The metal tags used by Crowe et al. were three times heavier than the 12 mm PIT tags used in the present study, and both studies used similar-sized snails (*N. ostrina* in this study; 19-26 mm shell length). Although PIT tags increased the body weight of *N. ostrina* by more than the recommended 4% used for terrestrial (Wilson et al. 2011) and aquatic vertebrates, such as sea turtles (Renaud & Carpenter 1994; Revelles et al. 2007), snails may not be as affected by the added weight because their movements take place mainly when submerged. Most intertidal snail species, including *N. ostrina*, are most active when they are underwater at high tide (Pardo et al. 2004; Pardo & Johnson 2006). Objects weigh less under water because water exerts a buoyant force on submerged objects, counteracting the effects of gravity and making the object lighter; therefore, snails may not feel the full weight of the attached PIT tag when they are moving underwater. In addition, shell-bearing intertidal animals often have debris or epiphytic organisms encrusted on their shell; thus, snails are used to carrying extra weight and an external tag attached to their shell may have less of an affect these animals. PIT tags have been successfully used on other invertebrates in the laboratory, including coconut crabs (Drew et al. 2012; Sato et al. 2013), crayfish (Bubb et al 2002), freshwater mussels (Kurth et al. 2007), and sea cucumbers (Gianasi et al. 2015) with no significant effects on growth, movement, or survival of the individuals. Similarly, PIT tags had no significant effect on snail survival or movement in this laboratory study. This technique was therefore deemed effective for studying *N. ostrina* in the field.

Microhabitat use

To confirm each snail's location, the study grid was physically searched to visually observe each snail. Snails often inhabited sheltered microhabitats (e.g. under mussels, under rocks, under algae, or under barnacles), making them difficult to view in the intertidal zone. On average, RFID technology increased recapture rates by 30% over visual searching because the observer was unable to view 30% of the detected snails at recapture sessions. This is a conservative estimate as to how many snails are missed by observers when using conventional tagging methods involving direct observation because the location of these detected snails was known by using RFID technology and the observer was still unable to see the snails. RFID technology also increased detection rates for snails that were ingested by a predator; two snails were eaten by sea anemones and one was eaten by a sea star, making them invisible to the observer. These results are consistent with those of Kurth et al. (2007) in a study of freshwater mussels where PIT tag detection exceeded the recapture rate of visual searches by 25% for tagged mussels buried beneath the substrate. Overall, PIT tags substantially increased the detection rate of this cryptic species relative to earlier tags or labels that require direct observation of the animal. The use of RFID technology expands the ability to study intertidal animals because PIT tags allow for snails to be detected in environments where traditional surveys would be difficult or impossible and observers using other tagging techniques would miss all hidden as well as ingested snails.

In this study, a large proportion of snails were found beneath mussels, indicating that this was a preferred microhabitat. Snails likely chose this microhabitat because it provided adequate shelter from unfavorable conditions such as high light intensity, high wave action, and predation. Sheltered microhabitats help intertidal animals reduce water loss, and energy consumption, which increases their chance of survival (Johnson & Black 2008; Jones & Boulding 1999; Kovach & Tallmon 2010; Martin et al. 2013; Marshall et al. 2013; Smoothey 2013).

Snail movement

In the field study, RFID technology was found to be an important technology to track the movement of *N. ostrina* because their daily location could be determined to an accuracy of 2.5 cm, which allowed for their travel distances to be calculated. Snails moved noticeably more on days one and two after placement in the field than on subsequent days. This increase in movement could be due to the stress from handling process or because snails were placed in an undesirable location in the study grid and they needed to move to a more suitable microhabitat. The effects of handling have not been investigated in many animals (Henrya & Jarne 2007), but handling is known to affect animal movement and freshly released individuals are more susceptible to mortality (Henrya & Jarne 2007; Ryser et al. 2011). In this study, snails can be impacted from the tagging process due to adhesive drying time and risk of desiccation. To account for the possibility that movements during the first two days after placement in the field were not normal behavior, movement data from these days were excluded from further data analyses for all snails. The daily movement of *N. ostrina* was limited during the study, as snails often only moved on average 14 cm each day, and snails often remained in the same for position for two or more consecutive days. Movement may be limited because snails did not need to travel great distances to acquire food or shelter among the mussel bed (*M. californianus*) that covered a large proportion of the study grid. Prasiola Point offered a high prey density area for *N. ostrina*, a predatory snail who feeds on mussels and barnacles (Gosselin & Chia 1994) and snails were often seen on, or in close proximity to, these prey species.

Most snails never travelled father than 1.5 m from their starting position during the study and the farthest a snail travelled was less than 5 m; therefore, *N. ostrina* has limited dispersal ability by crawling. The study species also does not have a widely dispersing larval phase in their life cycle. Consequently, it appears that a very limited amount of gene flow would result from adult crawling movement during the summer. When gene flow is limited, the rate of local adaptation may increase, causing variation between populations over a small geographic scale (Slatkin 1987; Peterson & Denno 1997). For example, populations of *N. ostrina* at eleven field sites spanning the southern half of the species range can show differences in their response to wave action (Sagarin & Somero 2006). Geographic variation has also been studied in *N. canaliculata,* a closely related species, and seven populations that were over 100 km apart were found to have geographic variation in heat stress tolerance among populations present in hotter locations compared to populations located in cooler locations (Kuo & Sanford 2009). Although crawling may limit the dispersal ability of *N. ostrina*, crawling is not the only mechanism by which this species can disperse and contribute to gene flow. For example, juveniles are known to travel by "drifting" in the water column (Martel & Chia 1991) and both juveniles and adult snails are able to relocate by "rafting" on a piece of floating debris in the water (Coe & Rogers 2012). In addition, wave action dislodged one snail in the present study, moving it 4.4 m away from its starting position, so relocation of individuals by waves can also contribute to dispersal. Snails had limited dispersal ability by crawling in this study, however, little is known about the rate by which snails disperse via these other methods and this should be further investigated to determine its effect on gene flow. In addition, future studies should quantify the amount of gene flow occurring between populations by performing a nested clade analysis of the special distribution of genetic variation among populations (Templeton 1998).

Snails had a low directionality value of 0.299 ± 0.233, which means they travelled in a highly convoluted path over the summer. These results are consistent with previous studies conducted on snails that also conclude that snails can potentially disperse large distances, but are limited by their non-directional movement patterns (Pardo et al. 2004; Curtis 2005; Michel et al. 2007; Seuront et al. 2007). Their non-directional movement may further limit the dispersal ability for *N. ostrina* because snails can move large distances over time, but do not move in a straight line and travel short distances from their starting position.

Conclusions

This was the first study to use RFID technology to study the movement of intertidal animals. By using RFID technology, this study was able to determine the effectiveness of using RFID technology for tracking snail movement in the intertidal zone. A successful tagging technique for mark recapture studies needs to be reliable, long lasting, and have no impact on the individual being studied (Gibbons & Andrews 2004; Henrya & Jarne 2007). RFID technology fits these criteria for intertidal snails because PIT tags had no tag loss, no effect on the individual in the laboratory, and effectively increased both the efficiency and accuracy of data collection in the intertidal zone. These criteria are important because they indicate that the results from the study were not influenced by the tagging technique, resulting in biased data.

In the field experiment, RFID technology was a powerful technique that increased the detection rates of snails by 30% compared to visual searches. Snails were hard to find in the intertidal zone because they often inhabit hidden microhabitats that provide shelter from the sun, waves, and predation. By using RFID technology to monitor daily activity of *N. ostrina*, snails were found in a short amount of time (~1-2 h) and more accurate and reliable movement and dispersal data was obtained. Snails crawled limited distances each day and they travelled in a convoluted path over time; therefore, they did not travel far from their starting position over the study. When dispersal is limited to a short distance across the shore, gene flow may also be limited such that populations of snails are better able to adapt to their local conditions.

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APPENDICIES

Appendix A: Information on RFID technology

Appendix B: Comparing 8 mm and 12 mm PIT tags

Appendix C: Male and female movement

Appendix D: Other factors potentially affecting snail movement

APPENDIX A: INFORMATION ON RFID TECHNOLOGY

RFID technology is a simple technology that was initially used in fisheries research, but is now used for biological field research with birds, reptiles, mammals, amphibians, and invertebrates (Gibbons & Andrews 2004; RFID Canada 2012). Transponders (tags) can be active or passive and work at different frequencies depending on their use (RFID Canada 2012). High frequency (13.56 MHz), passive RFID is ideal for animal studies because it travels through most materials including water, substrate, and body tissue (RFID Canada). Passive transponders, such as PIT tags, have no internal power source and remain dormant until they are powered by a short-range electromagnetic signal generated by the reader (Gibbons & Andrews 2004; RFID Canada 2012). PIT tags are small, electronic microchips encased in glass or plastic that can be injected under the skin or into a body cavity or can be attached externally (Gibbons & Andrews 2004). Internal placement of the PIT tags offers a more permanent strategy; however, high tag rejection and mortality has been observed in invertebrates such as sea cucumbers, mussels, abalone and, crayfish with internal placement (Hale et al. 2012; Kurth et al. 2007; Turner et al. 2013).

RFID technology does have some limitations for MR studies. First, the size range of animals that can be fitted with currently available PIT tags is limited by the weight and size of the tags (Gibbons & Andrews 2004; Hale et al. 2012). A study of sea urchins revealed adverse effects of PIT tags on growth rate, gonadal index, food consumption, movement, and survival rate (Lauzon-Guay & Scheibling 2008). A more recent study of freshwater pearl mussels also revealed adverse effects of PIT tags on feeding periods, burial depth, and movement (Wilson et al. 2011). The PIT tags used were less than 1% of the animal's body mass, which is within the recommended threshold of 4% (Wilson et al. 2011). Consequently, the size of the animal must be considered before implementing this tagging technique. PIT tags have also been tested in abalone (Hale et al. 2012), coconut crabs (Drew et al. 2012; Sato et al. 2013), crayfish (Bubb et al. 2002), mussels

(Kurth et al. 2007), and juvenile zebrafish (Cousin et al. 2012), in the laboratory with high tag retention and had no major effects on growth, movement, or survival of the individuals. Small PIT tags are available for use with smaller individuals, but their read range to communicate with the reader limits use of these tags. Communication with the RFID reader is another limitation because read range depends on tag size and orientation relative to the reader (Burnette et al. 2013; Drew et al 2012; RFID Canada 2012; Cooke et al. 2013).

APPENDIX B: EXPERIMENT COMPARING 8 mm PIT TAGS to 12 mm PIT TAGS

Snails were tagged with 8 mm and 12 mm for a preliminary experiment. The purpose of the experiment was to determine the smallest tag size that could be easily to detect in the intertidal zone, making it suitable for use in the field study. The preliminary experiment revealed no significant difference in NDD (T-test: $t=0.4$, $df=6$, $n=8$, $p=0.706$) or daily travel distance (T-test: $t=0.72$, $df=6$, $n=8$, $p=0.489$) between snails tagged with 8 mm or 12 mm tags (Figure 7). The 12 mm PIT tags were chosen for this study because they had a greater read range, making snails easier to find in the intertidal zone. Furthermore, these tags were consistently found during recapture sessions whereas the 8 mm PIT tags were harder to detect and were often missed in a preliminary field experiment.

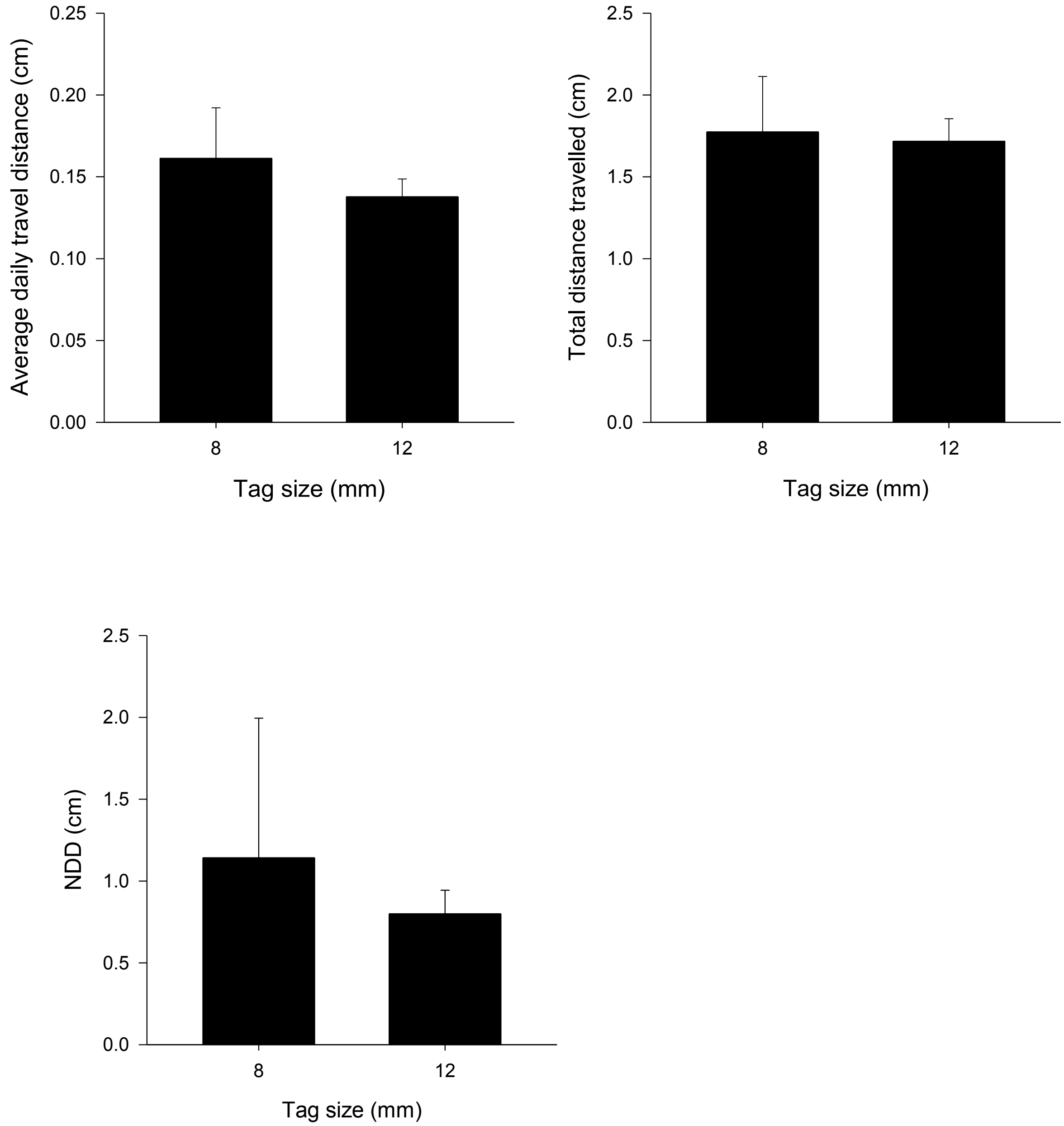

Figure 7. Results from the preliminary field experiment on the effect of PIT tag size on snail movement: (A) total distance travelled, (B) net distance displaced (NDD; i.e. the straight-line distance between first and last position), and (C) directionality of snails tagged with 8 mm (n=4) and 12 mm (n=4) PIT tags. A directionality value of 1 indicates movement in a straight line away from the starting position.

APPENDIX C: MALE AND FEMALE MOVEMENT

Snail gender was determined by looking for an appendage (penis) located behind their right tentacle. Gender had no significant effect on daily movement distance (T-test: $t=-0.36$, $df=53$, $n=55$, $p=0.720$), total travel distance (T-test: $t=-0.39$, $df=9$, $n=11$, $p=0.707$), NDD (T-test: $t=1.12$, $df=9$, $n=11$, $p=0.291$), or directionality of movement (T-test: $t=0.77$, $df=9$, $n=11$, $p=0.463$). Daily movement distance was averaged for each individual in the study grid (n= 31 females and 24 males), but total distance travelled, NDD, and directionality were only calculated for snail present in the grid during the entire study (n= 6 females and 5 males) period from July 9-August 21 (43 days).

APPENDIX D: OTHER FACTORS POTENTIALLY AFFECTING MOVEMENT

Previous studies suggest that the tidal cycle plays a major role in the ecology of intertidal animals because they are exposed to harsh low tide conditions for differing amounts of time (Pardo & Johnson 2006; Seuront et al. 2007; Kreis 2012). Snails are expected to move more as tidal amplitude increases because they are submersed in water for longer periods of time (Seuront et al. 2007; Kreis et al. 2012). Tidal height data were obtained from the online tide tables provided by the Department of Fisheries and Oceans (DFO) Canada. This data was used to calculate tidal amplitude, which was later correlated with the daily distance travelled by snails to determine its effect. Tidal amplitude showed no significant relationship with snail travel distance (all snails pooled; Pearson correlation: R=0.34, n=30, p=0.066). This relationship was even less significant when examining only the movements of males (R=0.199, n=27, P=0.555), but there was a significant relationship with female snail daily travel distance (R=0.442, n=30, P=0.02). Snails in this study may not be affected by the tidal cycle because there was high wave action at the study site. High wave action and water flow are two important factors known to limit snail activity because they are at a greater risk of dislodgement from the substratum while moving or foraging (Curtis 2005; Pardo & Johnson 2006; Wright & Nybakken 2007; Dahlhoff et al. 2001). Female snails have to find places to spawn or to find food due to an increased energy demand from laying egg capsules throughout the study (pers. obs), and therefore they are likely to move more in search of food or to find places to spawn.

Temperature is another important factor known to affect snail behavior (Pardo & Johnson 2006; Yamane & Gilman 2009; Chapperon & Seuront 2011). The effects of temperature in this study were examined using temperature probes (iButton®, Thermochron model D5 1921G) to determine the substratum temperature at low tide and seawater temperature at high tide. There was a significant relationship between substratum temperature and the daily travel distance of all snails (R=0.541, n=19, p= 0.017). This relationship was marginal when examining the relationship with daily travel distance of only males (R=0.426, n=19, p= 0.069) and it was not significant when examining the relationship with female daily travel distance (R=0.262, n=19, p= 0.279).

Temperature of the seawater was analyzed in a similar way, but high tide water temperature had no significant correlation with daily snail travel distance (R=0.137, n=19, p= 0.575). It is slightly unexpected that seawater temperature had no effect on movement because snails move mostly during high tide. One explanation could be that snails were highly affected by thermal stress during low tide causing them to feed and recover their energy during high tide. Substratum temperature was expected to affect snail movement because the snail's foot is in direct contact with the substrate and is highly correlated with animal body temperature (Chapperon & Seuront 2011). Movement was correlated with increasing substratum temperature because snails were exposed to physiologically stressful conditions, which increases their body temperature (Chapperon & Seuront 2011; Marshall et al. 2013). Movement increased because snails need to forage or to find shelter from these conditions (Curtis 2005; Pardo & Johnson 2006; Michel et al. 2007; Yamane & Gilman 2009; Chapperon & Seuront 2011;).