A POPULATION OF THE ROUGH WINKLE *LITTORINA SAXATILIS* (OLIVI, 1792) (CAENOGASTROPODA, LITTORINIDAE) ISOLATED FROM THE OPEN SEA

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Abstract *A population of the Rough Winkle,* Littorina saxatilis, *is described that is isolated from the sea. The site is an abandoned limestone quarry next to the sea in East Lothian, Scotland, which became flooded by the sea and later cut off from the sea again. Some seawater seeps into the site at high tide, and subsequently drains away. The winkles at this site are very small, and have different patterns of shell colour polymorphism from winkles on the nearby open shore. These differences from the nearby population on the open shore must have developed since the site was cut off from the sea between about 60 and 110 years ago. The population size is of the order of 100,000 animals.*

Key words Littorina saxatilis, *polymorphism, habitat, population size, speciation*

INTRODUCTION

The Rough Winkle, *Littorina saxatilis* (Olivi, 1792), is a common species on the upper shore in the North Atlantic, and has spread to other parts of the world. It occurs on rocky shores, where it may extend above the high water mark, being moistened only by spray. It can also be found in a variety of more sheltered habitats, including sheltered inlets, lagoons, salt marshes, and estuaries (Reid, 1996). It is ovoviviparous, and thus has poor powers of dispersal, and shows extreme morphological and genetic variation. Different ecotypes have been described, varying in size, shell shape, and colour, and these have been correlated with ecological factors such as degree of exposure, and predation. There is a large ecotype, found on shores less exposed to wave action; a smaller wave-exposed ecotype; a very small barnacle ecotype, adapted for living among barnacles; and a sheltered/brackish ecotype of intermediate size, that lives on more sheltered shores (Reid, 1996).

Because of this great variability, *L. saxatilis* has been studied intensively to see if the variation is associated with habitat. Factors such as exposure to waves, availability of crevices in rocks for the winkles to shelter in, and predation by crabs are among factors that have been found to affect size, shell thickness and shell shape in this species (Janson, 1982; Raffaelli, 1982). In various places contiguous populations have been studied that differ in shell size and shape, as well as physiological and behavioural characteristics, and that appear to have a partial reproductive barrier between them (Johannesson *et al.*, 1993, 1995; Hull *et al.*, 1996; Grahame *et al.*, 2006; Rolán-Alvarez, 2007). These observations have led to consideration of the possibility of sympatric or "ecological" speciation (Rolán-Alvarez, 2007; Johannesson *et al.*, 2010; Galindo & Grahame, 2014; Johannesson, 2016).

As well as variations in size and morphology, the shells of *Littorina saxatilis* also show extensive polymorphism for colour and colour pattern. Common colour forms have been explained as being cryptic, the result of selection by predators using visual recognition, though there could well be other factors involved (Atkinson & Warwick, 1983; Heller 1975b; Byers, 1990; Raffaelli, 1979; Johannesson & Ekendahl, 2002). In addition, there are uncommon, conspicuous morphs that are maintained in populations, apparently by balancing selection (Johannesson & Butlin, 2017).

Several years ago, some specimens of *Littorina saxatilis* were found at what appeared to be a terrestrial site near the sea. It was originally supposed that their occurrence was purely adventitious, but more recently it was realised that there was in fact a thriving population of this species, cut off from the open sea, but moistened by seawater from time to time. In this paper the site where this population occurs is described, estimates of the population given, and some characteristics of the winkles themselves are described.

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Material and Methods

The identity of the winkles as *Littorina saxatilis* was determined by Simon Taylor, the Honorary Marine Recorder of the Conchological Society of Great Britain and Ireland. The site was mapped using Garmin Etrex handheld GPS units, taking 10-figure British National Grid readings at intervals of a few metres. The individual readings thus had a precision of one metre. The readings were then plotted using Microsoft Excel software to produce a basic map. Salinity of water was measured using a hand-held refractometer (obtained from All Pond Solutions, Uxbridge, UK) calibrated in refractive index and salinity.

Population estimates were made by marking out an area, approximating to a regular geometrical figure, that was believed to include the vast majority of winkles on the site; because the winkles were very sparse at the edges of the site, it was not possible to be sure that every winkle was included, but this should not affect the results significantly. Two axes were laid out across the marked area, at right angles to each other. The area where the winkles were found was covered with stones (see Results), and selected stones were lifted at measured distances along each of the axes, the approximate area of each stone measured, and the number of winkles on and under each stone counted. To calculate the total number of winkles, the whole area was divided into three concentric areas of equal width. This was done because the density of winkles was greatest in the centre of the area, and least at the outermost region. The total number of winkles in each of the concentric areas was calculated by multiplying the counts of the winkles on the selected stones by the ratio of the area of each concentric region to the area of the stones on and under which the winkles were counted. Finally, the totals for each of the concentric areas were added together.

Shell heights only were measured, from the apex of the shell to the lowest point on the aperture, parallel to the columella, as described by previous authors (e.g. Raffaelli & Hughes, 1978; Janson, 1982; Sundberg, 1988). For larger shells, vernier callipers were used. As this was not practicable for the smallest shells, these were measured using an eyepiece micrometer in a binocular dissecting microscope. To ensure the correct orientation of the shells, they were mounted on Blu-Tack (Bostik Findley Ltd., Stafford, England) to hold them in place.

Results

The site

The site where the population of *Littorina saxatilis* occurs is in an abandoned limestone quarry close to the sea at Catcraig, East Lothian, Scotland (British National Grid reference NT717772), about 4 kilometres east of Dunbar (Fig. 1). It appears that the quarry was at one stage connected to the sea; whether this was deliberate or the result of storm damage is not known. Latterly the connection to the sea was blocked off again by the build up of a substantial shingle bank (Fig. 2). The dates of these events are not known. However, the Ordnance Survey map of the site surveyed in 1853 and published in 1854 (Haddingtonshire Sheet 7, 6-inch, first edition) shows no inlet of the sea at this site. Later maps (Haddingtonshire Sheet VII. SE. 6-inch, 2nd edition, surveyed in 1893 and 1906, and published in 1895 and 1908 respectively) do show an inlet of the sea at this point that corresponds with the site of the quarry. These maps can be consulted on-line at the National Library of Scotland (maps.nls.uk). The 25-inch map (Plan NT7077 & Plan NT7177), revised in 1963 and published in 1964 shows a continuous coastline at this point, without any inlet of the sea. Thus the quarry must have become flooded some time after 1853, and must have been cut off from the sea again before 1963. (Note that revision of smaller scale Ordnance Survey maps is not necessarily complete, so that they are not reliable for showing details such as that with which this paper is concerned.)

The shape of the site is elongated, with grassy slopes running down from the surrounding land to the bottom of the quarry (Figs 1 and 3). The floor of the quarry slopes down towards the eastern end, where it is clearly below the sea level at high tide. The winkles are restricted to a small area (approximately 5.9m×4.6m) covered with bare stones, under which the ground is usually damp (marked with an asterisk on the map) (Figs 3 & 4). This area is approximately 40m from the sea at high tide. Surrounding the stony area is a region covered with a green film of algae. Beyond this is an area of typical saltmarsh plants (Figs 5 & 6; Table 1).

Figure 1 A general view of the abandoned limestone quarry at Catcraig, East Lothian, UK, looking west. The site where the population of *Littorina saxatilis* is found is the stony area on the left.

The site had been visited several times before any water was seen in the stony area. However, around the time of high tide, water seeps up from underneath the stones and reaches up to the tops of the stones (Fig. 5). This was observed on 18th June 2016 and 2nd August 2016. On 24th June 2017, at a high spring tide, the water welled up from underneath the stony area, and also ran down the slope between this area and the shingle bank, covering a much larger area (Fig. 6); however, no water was seen coming through the shingle bank. On 18th June 2016, the salinity of the water was measured as 28 ppt (parts per thousand). On 24th June 2017, when there was a particularly high spring tide, and a considerable quantity of water was coming into the site, salinity levels of 32.5 to 34 ppt were measured. Samples of seawater from the open seashore nearby gave a value of 35 ppt on both occasions, as expected.

As well as *Littorina saxatilis*, live specimens of the land snails *Cepaea nemoralis* and *Cornu aspersum* were sometimes found on and around the stony area where *Littorina saxatilis* was found, as well as some amphipod crustaceans. Elsewhere in the dry grassy areas of the quarry there were also snails typical of such a habitat: *Candidula intersecta, Punctum pygmaeum, Pupilla muscorum, Truncatellina cylindrica, Vallonia costata, V. excentrica* and *Vertigo pygmaea*.

The animals

The winkles are abundant at this site. On 2nd August 2016, the population was estimated as

Table 1 Saltmarsh plants indentified around the site where *Littorina saxatilis* lives.

Sea Milkwort, *Glaux maritima*
Greater Sea-spurry, *Spergularia media*
Sea Wormwood, *Artemisia maritima*
Annual Seablite, *Suaeda maritima*
Common Saltmarsh-grass, *Puccinella maritima*
Buckshorn Plantain, *Plantago coronopus*
Red Fescue, *Festuca rubra*

Figure 2 The shingle bank separating the abandoned quarry from the open sea.

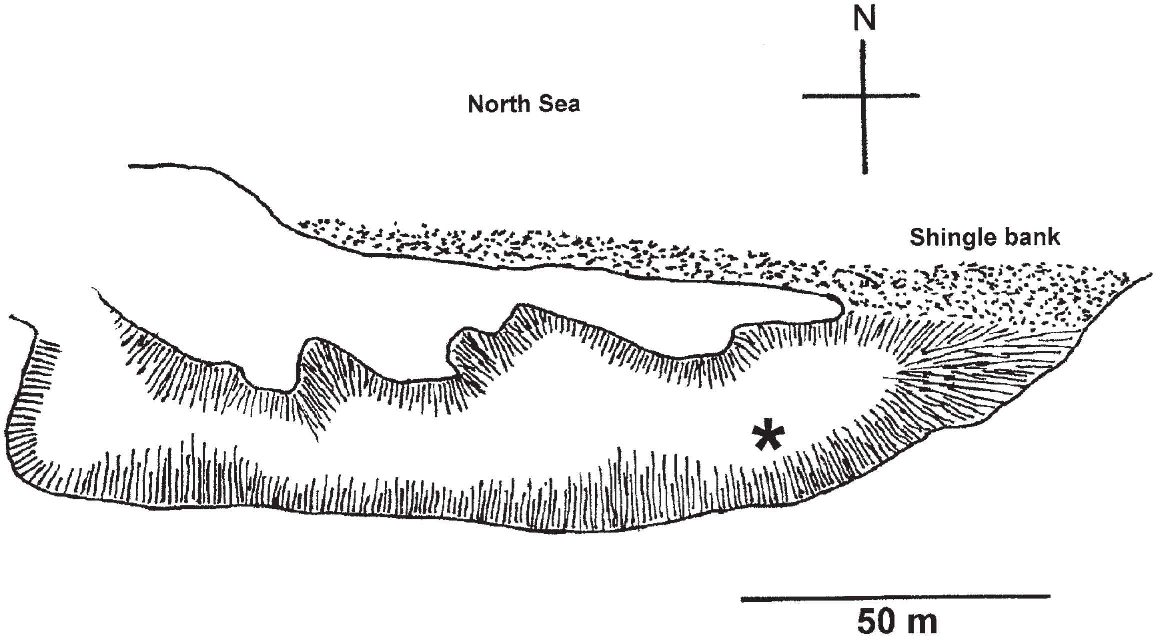

Figure 3 A map of the abandoned quarry at Catcraig. The site where *Littorina saxatilis* was found is indicated by an asterisk.

Table 2 Shell heights of populations of *Littorina saxatilis* from various points along the East Lothian coast.

Site	Grid reference	Habitat	Shell height ± standard deviation (n)
Catcraig (adults)	NT717772	quarry	3.78±0.62 (59)
Catcraig (juveniles)	NT717722	quarry	1.56±0.30 (38)
Catcraig	NT718772	rocky shore	10.59±2.63 (63)
North Berwick	NT553855	harbour wall	10.19±1.35 (68)
Aberlady Bay	NT465801	mudflats & saltmarsh	8.44±2.12 (47)

Figure 4 A close-up view of the stony area where *Littorina saxatilis* lives.

Figure 5 A view of the *L. saxatilis* site at around the time of high tide on 2nd August 2016, with water coming up to top of the stones. The pale plants behind the stony area are Sea Wormwood, *Artemisia maritima.* Between and around the stones is Annual Seablite, *Suaeda maritima.*

Figure 6 A view of the *L. saxatilis* site on 24th June 2017, at the time of a high spring tide, with the stony area and surrounds flooded with sea water. The shingle bank separating the site from the open sea is in the distance. In the foreground is a patch of the saltmarsh plant Sea Wormwood, *Artemisia maritima.*

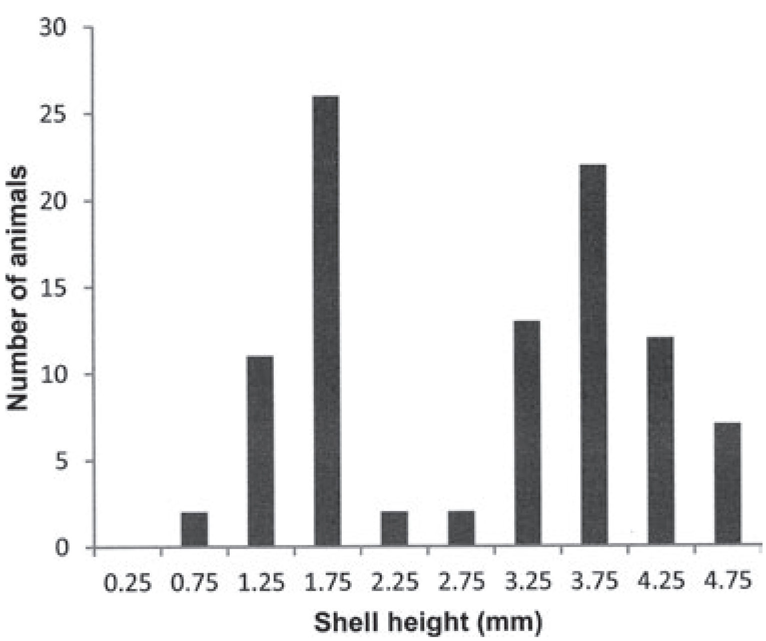

Figure 7 Histogram of the heights of shells from the quarry site. Note the peak in the 1.75mm class for juveniles, and in the 3.75mm class for adults.

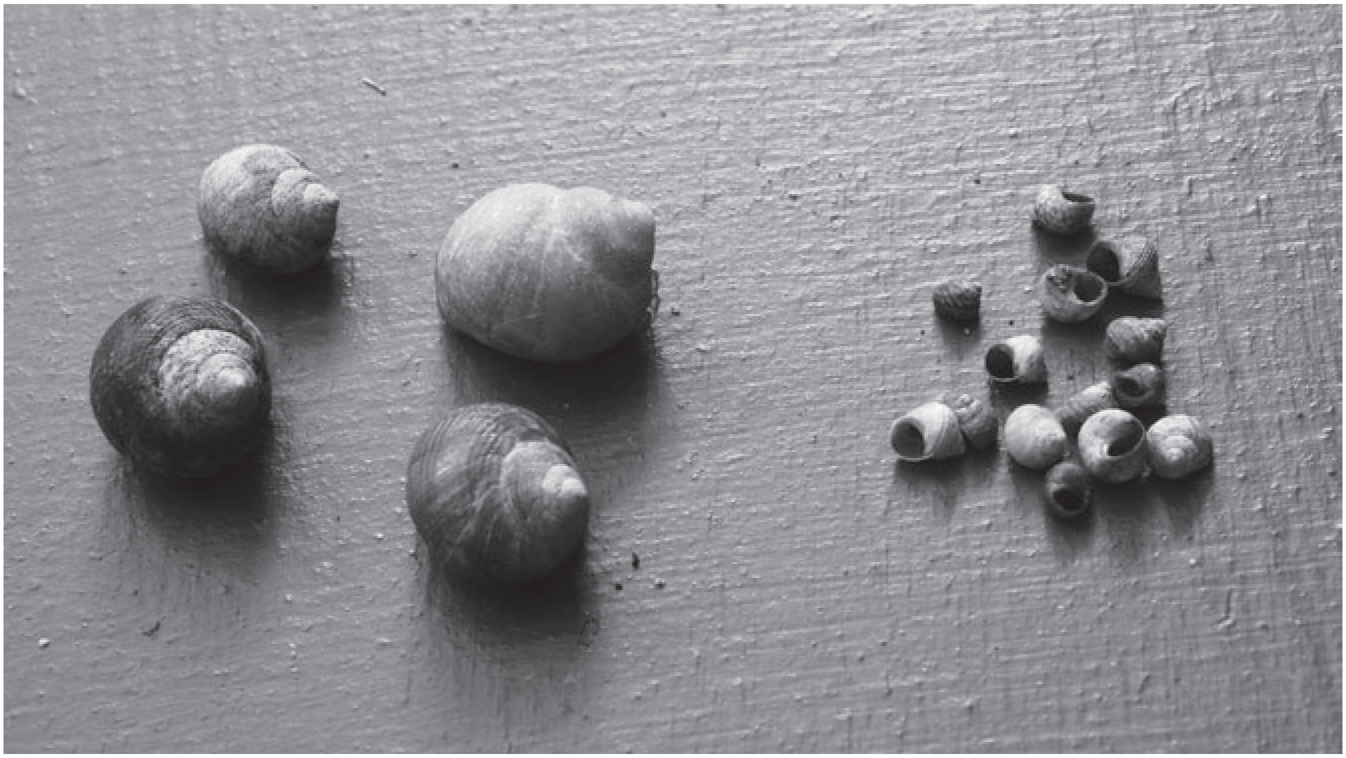

Figure 8 Specimens of *L. saxatilis* from the open shore (grid reference NT718772) at Catcraig (left) and from the quarry (NT717772) (right), showing the great difference in their sizes.

Figure 9 *Littorina saxatilis* on the stones at the site in the quarry at Catcraig.

Figure 10 A specimen of *L. saxatilis* with a tessellated shell, obtained from the abandoned limestone quarry at Catcraig.

335,622 animals; on 3rd September 2016, a figure of 95,514 was obtained. Maximum densities of winkles in the centre of the area were calculated as 6,500 and 3,500 m^{-2} respectively. Although there are various sorts of potential errors in these measurements (see Discussion), these results indicate that the population size is of the order of 100,000. Both adult animals and juveniles are present; a sample of the former gave a mean height of 3.78mm, while the latter had a mean height of 1.56mm (Table 2 & Fig. 7). The shells of this population are much smaller than those of other populations on the East Lothian coast that are open to the sea (Table 2 & Fig. 8). The shells of winkles from the rocky shore at Catcraig, and from the harbour wall at North Berwick average over 10mm in height, while specimens from mudflats and saltmarsh at Aberlady Bay are rather smaller. The winkles from the quarry at Catcraig are about half the height of those at Aberlady Bay.

The winkles at the quarry are found all over the stones which occupy most of the site. They occur on the top (Fig. 9), sides and underneath the stones; some are found lying on the damp soil underneath the stones, and a few are found further from the centre of the area under small items of debris among the surrounding algal film.

Shells of *Littorina saxatilis* are very variable in colour, and different authors have attempted to classify the colours, not always consistently (Pettit, 1973; Ekendahl & Johannesson 1997). In this study, it was found impracticable to do an objective analysis of shell colour, as there were many different colours and patterns that often seemed to grade into each other. However, in all the populations examined, including that in the quarry, "dark" shells were one of the commonest forms. Yellow shells were fairly frequent at North Berwick, and on the open shore at Catcraig, but were never found in the quarry. However, one form was common in the quarry; this was the tessellated form (Fig. 10), which apart from a single shell on the harbour wall at North Berwick, was only found elsewhere on the mudflats at Aberlady Bay. Of a sample of 91 adult winkles from the quarry, 39 were tessellated, and 38 were dark.

Discussion

In this paper a population of *Littorina saxatilis* is described that is isolated from the sea, only receiving sea water by percolation through the substrate at high tide. The population is large, and reproducing, as shown by the presence of distinct size classes in the population, and the animals are unusually small, and show characteristic shell colour polymorphisms. The site where the winkles occur seems to have become flooded not more than about 160 years ago, and then cut off from the sea some time between about 60 and 110 years ago. The winkles presumably entered the site when it was open to the sea, and have managed to survive there since it was cut off from the sea, although the possibility that the population could have been founded by a single fertilised female, dropped by a bird for example, should not, perhaps, be entirely discounted. Although brackish sea water percolates into the site from time to time at high tide, for much of the time there is no open water. Of the various *Littorina* species, only *L. saxatilis*, being ovoviviparous, could survive in such an environment; species with planktonic larvae would of course be unable to survive, and species that produce spawn would suffer from the spawn drying out. The site bears some resemblance to saltmarsh, although the winkles live almost entirely in a stony area.

Two estimates were made of the population size, giving rather different numbers, although it seems safe to say that the population is of the order of 100,000. Figures for population density are quite moderate compared with some published values (Reid, 1996), although much lower densities can also be found, depending of the habitat (Berry, 1961; Johannesson *et al.,* 2010). There is inevitably some uncertainty in these estimates: the relatively small size of the areas sampled compared with the total must inevitably give rise to stochastic errors; the total area occupied by the winkles is difficult to define precisely; and neither the complete area, nor the individual stones, are in reality regular geometrical forms, and are therefore difficult to measure accurately. Nevertheless, there does seem to have been a reduction in the number of individuals on the second occasion when the population was estimated, as the concentrations per unit area, especially in the centre of the site, were lower. *Littorina saxatilis* is believed to breed throughout the year (Heller, 1975a; Hannaford Ellis, 1983), so it would seem unlikely that large changes in the size of the population would be the result of an older generation dying off, leaving only younger individuals. However, the evidence of clearly defined size classes in this population suggest that breeding might be more seasonal in this population. More detailed analyses of the population size and structure would be required to understand this.

Two characteristics of this population are the small size of the individuals, and the distinct shell colour morphs, particularly tessellated shells. There are many factors affecting shell size in *Littorina saxatilis.* Several ecotypes have been recognised, with differences in shell size and shape, size of aperture, and shell thickness (Reid, 1996; Johannesson *et al.,* 2010). The largest specimens of *Littorina saxatilis* are found on shores with moderate exposure. On exposed rocky shores, shell size is correlated with the size of the crevices into which the animals can retreat to shelter from wave action (Raffaelli & Hughes, 1978). The barnacle ecotype, which inhabits barnacle shells on rocky shores, has very small shells (Johannesson *et al.* 1993; Reid, 1996). Lagoonal populations also tend to be composed of small individuals (Barnes, 1993; Reid, 1996; Wilson *et al.,* 1999), but the characteristic lagoonal form of this species lives permanently submerged. Winkles from sheltered and brackish water habitats are medium sized (Reid, 1996). The habitat of the winkles described here is perhaps most similar to saltmarsh, but the winkles are much smaller than those obtained from a typical saltmarsh site nearby (Table 2). Although the stony habitat occupied by these winkles provides many crevices for the winkles to shelter in, they are not subjected to violent wave action, and so do not need to shelter from it. Presumably they have become adapted to local conditions, and their small size is a result of factors that have still to be established.

Shell colour in *Littorina saxatilis* is generally held to be cryptic, at least to some extent, though other factors may be involved (Heller, 1975b; Raffaelli, 1982; Atkinson & Warwick, 1983; Byers, 1990; Ekendahl & Johannesson, 1997). There were clear differences in the proportions of the different shell colour morphs between the winkles found in the quarry, and those occurring in other sites; most notable was the high frequency of tessellated shells, not found on the adjacent rocky shore. In fact, a high proportion of tessellated shells is typically found in brackish and marshy habitats (Reid, 1996). It might be supposed that the tessellated form is particularly cryptic in the quarry site, though there is no evidence for this. Various animals prey upon winkles: carnivorous molluscs, crabs, fish, a variety of birds, and mammals (Pettitt, 1975). Carnivorous molluscs, crabs and fish are, of course, absent from the site, and most of the birds listed by Pettitt (1975) that prey on Littorinids are marine species, mainly ducks (Anatidae) and a variety of waders. However, predation by small passerines, especially pipits and wagtails (Motacillidae) seems a possibility. It is not clear whether the tessellated form of *L. saxatilis* would appear more cryptic than other colours to such predators. The winkles are often to be found on the surface of the stones in the quarry, where their shells dry out, and appear greyish, regardless of their actual colour. Although the colour of dry shells often resembles that of the stones to which they are attached, the shells are easily visible because of the shadows they cast (Fig. 9). It may therefore be that the high proportion of tessellated shells in the quarry population results from a pleiotropic effect of some other factor not immediately apparent.

This isolated population of *Littorina saxatilis* is of particular interest because of substantial evidence that this species can show remarkable differentiation even between contiguous populations, and that in some cases a partial reproductive barrier has developed between them (Johannesson *et al.*, 1993, 1995; Hull *et al.*, 1996; Grahame *et al.*, 2006; Rolán-Alvarez, 2007). In the situation described in this paper, the separation of the quarry population from that on the nearby open rocky shore appears to be complete. Although it is possible that during severe storms a few winkles from the shore might be washed into the quarry, or that individuals might be transported into the quarry attached to birds, this is clearly not significant, as the two populations are clearly differentiated. The winkles in the quarry population are much smaller than those on the open shore, and have a substantial proportion of tessellated shells, which are absent on the shore. This differentiation seems to have occurred in a period of between about 60 and 110 years, since the population was cut off from the sea. In view of the idea that incipient speciation might be occurring in various rocky shore populations (Rolán-Alvarez, 2007; Johannesson *et al.*, 2010; Galindo & Grahame, 2014; Johannesson, 2016), it would seem worthwhile investigating in more detail the degree of differentiation between the shore and quarry populations at Catcraig to determine whether a similar process of incipient speciation might be occurring here.

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