

***Littorina littorea* (Linnaeus, 1758) (Gastropoda, Littorinidae) considered as an indicator of recovery from sewage pollution**

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The effect on *Littorina littorea* of removal of a short sea sewage outfall (SSSO) at Forness Point (FP) in South East England was studied between 1994 and 2001. Controlled harvesting suggested that intense grazing of littorinids affected the return of macroalgae to the mid- and upper-shore area. Nearby shores with different densities of *L. littorea* differed markedly in the composition of marine, littoral, macroalgae. The population structure at two of these sites suggested there was some nutritional and competition stress. Body condition (the regression coefficient when dry soft-body mass is regressed on maximum shell length) declined during winter at three shores. The littorinid population at FP did not appear to have suffered, or benefited, from the change in the outfall. However, by contrast, adjacent shores which carry more pollution burden showed statistically significant reductions in body condition. These results suggest that body condition of *L. littorea* might be an effective biomarker for pollution assessment on rocky shores.

Key words: Gastropoda, Littorinidae, *Littorina*, macroalgae, pollution.

INTRODUCTION

After fifty years of discharging untreated sewage, a short-sea outfall (SSO) at Foreness Point in the South East of England was replaced in 1989 by a 1900m long-sea outfall (LSO). In 1994, the chalk platform (about 0.25 ha) supporting the SSO had few algal macrophytes but had large numbers of *Littorina littorea* (Linnaeus, 1758). By contrast, the adjacent Walpole Bay was covered by dense macroalgae but few *L. littorea*. Littorinids feed on diatoms and macrophytic algae such as *Enteromorpha* (Wilhelmsen & Reise, 1994), although they avoid eating the tougher fucoids (Reid, 1996). On the upper shore, plant detritus becomes an important food (Watson & Norton, 1985).

Since sewage pollution reduces algal diversity and encourages growth of green algae such as *Enteromorpha* (Borowitzka, 1972), the outfall change might have affected the algal community. However, four years after the SSO stopped, macrophyte cover on the shore had not changed significantly. On the mid/upper shore, *Littorina littorea* population density remained high at approximately 161/m² and algal cover was sparse. By contrast, on the lower shore, algae continued to be abundant, despite a *L. littorea* population of 100-200/m². In low intertidal zones, settlement by algal sporelings can swamp consumption by littorinid grazing (Dayton, 1975).

It was possible that before the change in outfall, the upper shore littorinids had been partially sustained by the sewage, and loss of this resource might have ecologically stressed the population. Bilton (1974) observed that starved salmon could maintain body length yet lose body mass. The ratio of body mass to shell size ('body condition') of individuals in the littorinid population might have changed in a similar way.

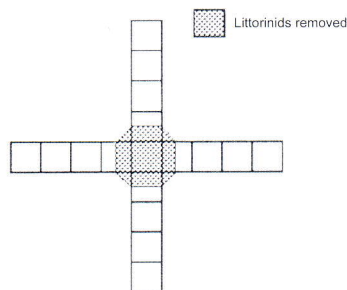


Fig. 1. Design to test migration of *Littorina littorea* from adjacent positions after clearing of *L. littorea* from a 1 m² quadrat.

Unfortunately, no detailed information was available on the *L. littorea* populations prior to the change in the outfall, though recovery time for mature rocky intertidal communities can exceed two years (Dayton, 1971). The aim of the current investigation was therefore, in a post hoc study, to investigate whether the *L. littorea* population at Forness Point had been stressed by the change in discharge. This would be achieved by, firstly, investigating the relationship between *L. littorea* and the algal community and secondly, comparing population structure and body condition of *L. littorea* populations at Forness Point with populations at nearby sites.

METHOD

The shores investigated comprised Forness Point (FP), Beresford Gap (BG), Walpole Bay (WP) and Ramsgate Undercliff (RU). Each shore comprises a gently sloping, Cretaceous, chalk platform which is exposed at low tide. For all parametric statistical analyses, data were confirmed to be normal.

In order to investigate the relationships between *L. littorea* and macrophyte cover at FP, sixteen sites were randomly selected at FP. Eight sites of 1m² were situated in the upper shore intertidal band and eight in the mid shore. Of these eight, four were randomly assigned as experimental sites and four as controls. Numbers of *L. littorea* and macrophyte percentage cover were recorded at each site and the experimental sites were then each cleared completely of algae and littorinids on 29/6/1994. Daily to 19/8/1994, numbers of *L. littorea* and macrophyte percentage cover were recorded at each site.

It was possible that littorinids were migrating into and out of the cleared areas on each tide so that unidentified *L. littorea* would be responsible for any grazing effects in the previous experiment. Therefore, four extra experimental sites were randomly selected at FP on 24/8/1994. In a depletion experiment, numbers of *L. littorea* /m² were recorded over several tides in four orthogonal directions for a distance of four metres using a metre quadrat (fig 1). Data were investigated by analysis of variance.

To confirm results from the previous experiments, eight 1m² quadrats were randomly chosen on the mid-shore at each of three shores (FP, WB and BG). Macrophyte percentage cover and density of *L. littorea* were recorded on 5/8/94 and compared between the shores.

In order to investigate the population structure of *L. littorea* in relation to macroalgal density, data for FP were compared with data from BG. Specimens were randomly selected from the mid-shore on 9/8/94. The maximum shell length of all the *L. littorea* in a ran-

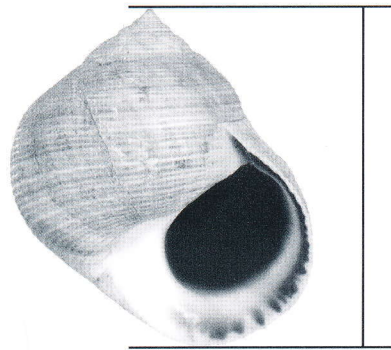


Fig. 2. Dimension of shell length measurement.

domly selected quadrat on the lower and upper shore at FP was measured. The slope of the chalk platform at BG was so low that upper and lower shore could not be distinguished. Population structures were investigated by using an adaptation of the Cassie curve (Cassie, 1954).

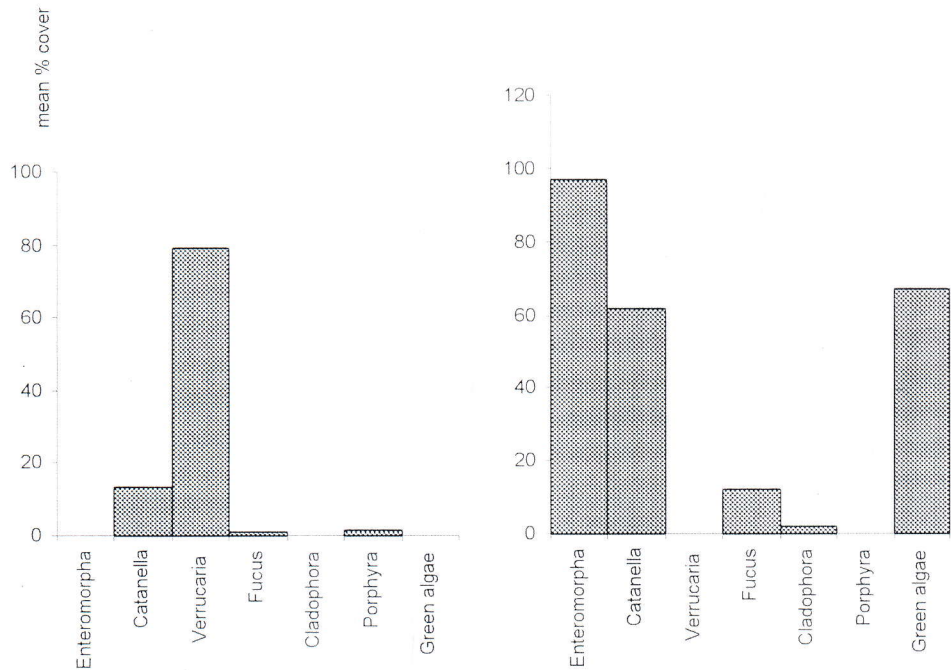
On the basis of data collected in the previous experiment, body condition of *L. littorea* at FP, BG and RU was compared. To do this, fifty specimens of *L. littorea*, ranging in size, were randomly selected from each shore on 29/9/94. Maximum shell length was measured (fig. 2) and bodies were removed after immersion in boiling water for 1 minute. The wet-weight of each body was recorded. Dry body weight was then obtained after drying at 60°C for 24 hours. For reasons of scale, body mass was initially plotted against shell length cubed. However, the regression fit was no improvement on a simple plot of shell length on dry mass and therefore, the latter was used as an index of body condition.

RESULTS

In order to investigate relationships between *L. littorea* and macrophyte cover, before the experimental areas were cleared, the relationships between abundance of *L. littorea* and algae was investigated by multiple regression. This is not statistically legal, since each algal taxon is not independent of the others, nor are any relationships known to be linear. Nevertheless, the approach served as a pointer to relationships which were then investigated in other ways. Thus Table 1 indicates significant, positive relationships between *L. littorea* density and abundance of both *Enteromorpha* and other green algae such as *Ulva*. (nb since this work was done, it has been suggested by Hayden et al. (2003) that

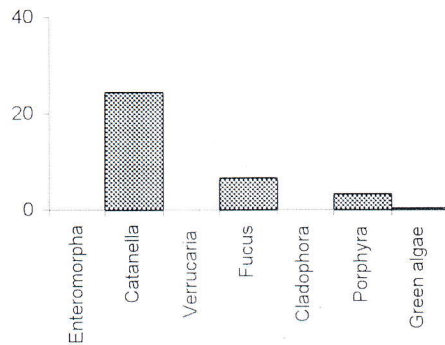
Table 1. Results of multiple regression analysis where number of *Littorina littorea* is the y variable and algal abundances are the x variables.

	Regression Coefficient	t	P
Miscellaneous green algae	-37.50	-1.98	0.050
<i>Verrucaria maura</i>	-0.33	-1.85	0.066
<i>Catenella caespitosa</i>	0.24	0.42	0.674
<i>Fucus vesiculosus</i>	16.38	3.62	0.000
<i>Enteromorpha</i> spec.	-1.29	-9.13	0.000
<i>Laurencia pinnatifida</i>	-5.63	-0.66	0.513
<i>Porphyra umbilicalis</i>	-0.99	-0.25	0.802



Mean no. of *Littorina littorea* / m² = 167.1

Mean no. of *Littorina littorea* / m² = 4.6



Mean no. of *Littorina littorea* / m² = 270

Fig. 3. Mean percentage plant cover for eight replicate quadrats on each of three shores which differ in density of *Littorina littorea*.

Enteromorpha and *Ulva* are, in fact, ecotypes of the same species). After removal of both *L. littorea* and algae, percentage coverage of *Enteromorpha* increased rapidly in most experimental quadrats, reaching 100% cover by 6/7/1994. The control sites showed fluctuating numbers of *L. littorea* and moderate algal presence, except for *Catenella caespitosa* (Withering) Irvine and the lichen, *Verrucaria maura* Wahlenb.

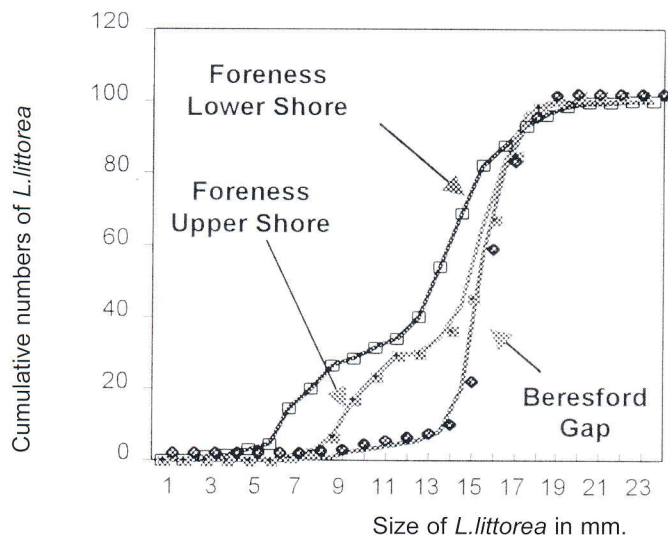


Fig. 4. Comparison of the population structures of *Littorina littorea* populations at Foreness Point and Beresford Gap.

A two-way analysis of variance was used to test whether harvesting from 4 separate quadrats at FP caused significant depletion of littorinids from adjacent areas. The results indicate that aspect (North, South, East or West) had no effect on the distribution ($F_{1,56} \text{ df}=3 \text{ } P=0.212$). Harvesting caused significant depletion but only from quadrats in the adjacent position ($F_{4,56} \text{ d.f.}=3 \text{ } P=0.007$). There was no significant interaction between aspect and position ($P=0.96$).

When *Littorina littorea* density in relation to vegetation cover was compared at FP, BG and WB, the algal community composition was quite different between the three shores (fig. 3). With only 4.6 *L. littorea* /m², WP had the highest plant cover, mainly comprising Chlorophyta. BG with 270 *L. littorea* /m² supported fucoids and *Porphyra umbilicalis* (L.), whereas at FP, *Catenella caespitosa* was most abundant.

FP and BG both had high densities of *Littorina littorea*, but differed in their algal communities. It seemed possible that the intensity of grazing was higher at BG than at FP, the former having a higher density of littorinids. In order to investigate whether the population structures of *L. littorea* differed between these two shores, a "Cassie" curve investigation was carried out at FP and BG (fig. 4). The curve for BG suggests there is a single cohort of large *L. littorea*. By contrast, the Foreness Lower Shore curve suggests two cohorts averaging approximately 9mm and 20mm. The curve for the Foreness Upper Shore population lies between the two.

The previous experiment suggested that due to competition, the slope coefficients for dry mass on shell length of *L. littorea* at BG might be lower than at FP. In addition, the slope coefficients might also be low at RU, where there was relatively more pollution from the nearby port of Ramsgate and sewage treatment works on the river Stour. These three

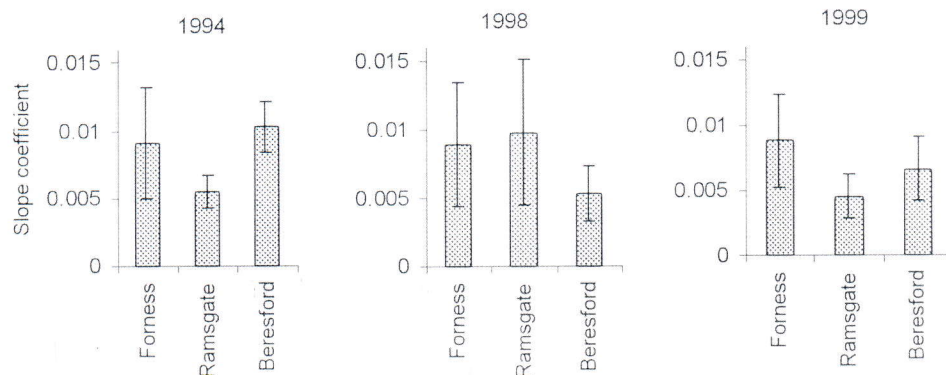


Fig. 5. Comparison of regression slope coefficient (a measure of body condition) for dry weight of soft body, regressed on the maximum shell length.

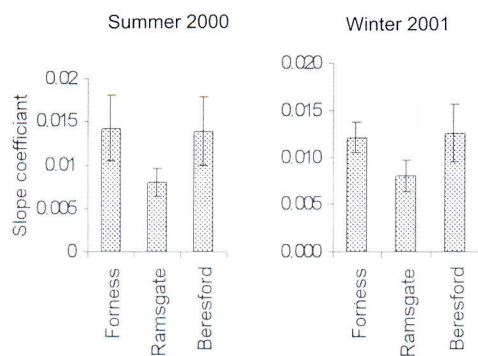


Fig. 6. Comparison of regression slope coefficient (a measure of body condition) for dry weight of soft body, regressed on the maximum shell length. Summer is compared with winter.

sites were visited in 1996, 1999 and 2000 in order to investigate possible changes in body condition which might reflect change in nutritional stress. Fig. 5 compares the regression slope coefficients. At FP, the slope coefficients stayed remarkably constant across the study period, whereas BG and RU showed significant fluctuations. Two-way anova indicated significant differences between the sites ($F_{2,841} \sim 65$ $P < 0.0001$), between the times ($F_{3,841} \sim 103$ $P < 0.0001$) and with significant interactions ($F_{6,841} \sim 33$ $P < 0.0001$).

A comparison of summer and winter data for 2000 (fig.6) shows patterns similar to 1994 and 1999, but the mean slope coefficient at each site is lower in winter. A two-way anova showed significant differences between seasons ($F_{1,300} \sim 14.7$ $P < 0.0001$) and sites ($F_{2,300} \sim 122$ $P < 0.001$).

DISCUSSION

In the investigation of the relationships between *L. littorea* and macrophyte cover, harvesting caused an immediate increase in the abundance of *Enteromorpha intestinalis*, leading to the development of dense patches of this green alga. Although littorinids eat

E. intestinalis, invasion by *Littorina littorea* into these dense patches was then limited by the excessive plant production. On other cleared areas, the results support the suggestions of Lodge (1948), Southward (1962) and Underwood (1980) that mollusc grazing is an important control on macro-algal establishment and production. The depletion experiment showed that there was only a local effect of harvesting on the population density of *L. littorea* in adjacent areas. The results of the previous experiment were, therefore, unlikely to be due to littorinids migrating in and out of the harvested area on a single, or few tides.

Once *Enteromorpha intestinalis* established itself densely in cleared areas, there was only a limited migration of *Littorina littorea* into such areas. Therefore, the investigation was extended to compare three sites with different littorinid densities (FP, WB and BG). The macrophyte community composition was quite different between these shores and it seemed that particularly at BG, there was such intense grazing that the littorinid population might have been under nutritional stress. To some extent, this was borne out by the investigation of population structure. Thus the population structure of *L. littorea* on upper- and mid-shore at FP, and at BG, all differed from each other. The lower shore at FP (150/ m²) had a more pronounced juvenile cohort than the upper shore (167.1/ m²), where there might have been more nutritional stress (Fischer-Piette & Seone-Camba, 1962). At BG (270/m²), the high densities might have led to intra-specific competition for food which could have reduced the viability of a juvenile cohort. However, these densities were not extreme compared with values of 350-480 recorded by Wilhelmsen & Reise (1994). The possibility of a single cohort at BG raises a question about the source of this population if it did not develop in situ. Curry & Hughes (1992) note that because *L. littorea* produce pelagic eggs, populations connected by tidal currents might be in genetic continuity. Thus animals from FP could stock BG.

Initially, we hypothesised that the relatively large numbers of *L. littorea*, yet low amounts of vegetation, at FP were evidence that other forms of food must have been available to support the population after the change in the SSO. The original food supply may have come from the organic matter contained in the sewage. Since the removal of the sewage discharge, the amount of food available for the littorinid population may have reduced. Thus there might have been competition for any macroalgae sporlings that might settle. This hypothesis of nutritional stress at FP was not supported by the investigation of body condition.

The regression coefficient of dry body mass on shell length was used here as a measure of body condition. The summer-winter comparison showed firstly, a consistent pattern of relationships between the three sites, and secondly, a small but significant reduction in body condition in Winter compared with summer. This might be due to lower seasonal temperatures causing some nutritional stress whereas the metabolic costs of respiration have to be maintained through the winter. Body condition in *L. littorea* thus seems to be a sensitive indicator of environmental stress. Littler and Murray (1978) found that *Littorina* sp. from areas polluted by domestic waste had reduced gut volumes and suggested that *Littorina* sp. may need to consume less food volume to meet their energetic requirements in an enriched environment. This might explain the variation in body condition recorded for RU between 1994 and 1998. Both Evans (1948) and Smith & Newell (1955) suggest that chalk substrata do not favour the development of littorinid populations, which conflicts with the observation of dense littorinid populations at three of these chalk platform sites. However, periwinkles feeding on the thin film of algal sporelings and diatoms could consume sufficient amounts of soft chalk that there might be a significant metabolic cost to its disposal.

In conclusion, it appears that *L. littorea* can be used as an indicator of environmental stress. In this context, the change in the sea outfall does not appear to have adversely

affected the population at Foreness Point. However, the fact that adjacent populations seem to be affected by local conditions needs further investigation. It would be useful to extend this work to other shores, and for longer periods, to further test the value of body condition as an effective biomarker.

REFERENCES

- BILTON H.T., 1974. Effects of starvation and feeding on circulus formation on scales of young Sockeye salmon of four racial origins, and of one race of young Kokanee, Coho and Chinook salmon. In: T.B. BAGNAL, ed., The ageing of fish: 40-70. London.
- BOROWITZKA, M.A., 1972. Intertidal algal species diversity and the effect of pollution. – Australian Journal of Marine and Freshwater Research 23: 72-84.
- CASSIE, R.M., 1954. Some uses of probability paper in the analysis of size frequency distributions. – Australian Journal of Marine and Freshwater Research 5: 513-522.
- CURRY, J.D. & R.N. HUGHES, 1982. Strength of the dogwhelk *Nucella lapillus* and the winkle *Littorina littorea* from different habitats. – Journal of Animal Ecology 51: 47-56.
- DAYTON, P.K., 1971. Competition, disturbance and community organisation: The provision and subsequent utilisation of space in a rocky intertidal algal community. – Ecological Monographs 41: 351-389.
- DAYTON, P.K., 1975. Experimental evaluation of ecological dominance in a rocky shore intertidal algal community. – Ecological Monographs 45: 137-159.
- EVANS, R.G., 1948. The intertidal ecology of selected localities in the Plymouth neighbourhood. – Journal of the Marine Biological Association of the United Kingdom 27: 173-218.
- FISCHER-PIETTE, E & J. SEONE-CAMBA, 1962. Sur un facteur de prospérité de *Littorina littorea* et son effet sur la frontière supérieure de l'espèce. – Bulletin du Centre d'Études et de Recherches Scientifiques, Biarritz 4: 171-185.
- HAYDEN, H.S., J. BLOMSTER, C. MAGGS, P. SILVA, M. STANHOPE & J. WAALAND, 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct evolutionary entities. – European Journal of Phycology 38: 277-294.
- LITTLER M.M. & S.N. MURRAY, 1978. Influence of domestic waste on energetic pathways in rocky intertidal communities. – Journal of Applied Ecology 15: 583-595.
- LODGE S.M., 1948. Algal growth in absence of *Patella* on an experimental strip of foreshore, Port St. Mary, Isle of Man. – Proceedings and Transactions of the Liverpool Biological Society 56: 78-83.
- REID, D.G., 1996. Systematics and evolution of *Littorina*. Ray Society Monograph: i-ix, 1-457. London.
- SMITH, J.E. & G.E. NEWELL, 1955. The dynamics of the zonation of the common periwinkle, *Littorina littorea* (L.) on a stony beach. – Journal of Animal Ecology 24: 35-56.
- SOUTHWARD, A.J., 1962. Limpet grazing and the control of vegetation on rocky shores. In: D.J. CRISP, ed., Grazing terrestrial and marine environments: 265-274. Oxford.
- UNDERWOOD, J.A., 1980. The effects of grazing by gastropods and physical factors on the upper limits of distribution of intertidal macroalgae. – Oecologia 46: 201-213.
- WATSON D.C. & T. NORTON, 1985. Dietary preferences of the common periwinkle *Littorina littorea*. – Journal of Experimental Marine Biology and Ecology 88: 193-211.
- WILHELMSSEN, U. & K. REISE, 1994. Grazing on green algae by the periwinkle *Littorina littorea* in the Wadden Sea. – Helgoländer Meeresuntersuchungen 48: 233-242.