J. moll. Stud. (1977), 43, 241-254

INTER- AND INTRASPECIFIC VARIATION IN *LITTORINA OBTUSATA* AND *L. MARIAE* (GASTROPODA : PROSOBRANCHIA)

B. J. GOODWIN* and J. D. FISH
Department of Zoology, University College of Wales, Aberystwyth
(Received 24 February 1977)

INTRODUCTION

The taxonomy of British species of *Littorina* has frequently been in dispute especially as regards the highly polymorphic 'species' commonly known as *Littorina saxatilis* (L.) (= *rudis*) and *Littorina obtusata* (L.) (= *littoralis*). The complex nomenclatural situation which has prevailed in the rough periwinkle *L. saxatilis* (James, 1968) has been clarified by the recent study of Heller (1975) who has put forward evidence that '*L. saxatilis*' from the Welsh coast is a complex of four sympatric species: *L. rudis* Maton, *L. patula* Jeffreys, *L. nigrolineata* Gray, and *L. neglecta* Bean. The confused nomenclatural history of the flat periwinkle, *L. obtusata,* partly stems from the fact that Linnaeus (1758) placed two apparently similar shells in different genera, namely *Turbo obtusatus* and *Nerita littoralis.* These designations were followed by a proliferation of forms described variously as species, sub-species or varieties and have been catalogued by Dautzenberg & Fischer (1914) who reduced the number of species to one *(L. obtusata),* although they retained *littoralis* as a sub-species.

More recently, Colman (1932), in a study of shell characters, demonstrated that the British form, *L. littoralis,* and the American form, *L. palliata* (Say), were synonymous with the Scandinavian *L. obtusata,* which name has priority by pagination from the 10th edition of the *Systema Naturae* (Linnaeus, 1758). In an investigation of polymorphism and its relation to the ecology of *L. obtusata* Sacchi (1961a,b, 1963, 1964, 1966a,b) recognized a size dimorphism in which he termed the morphs "dwarf" and "normal". As a result of this division a number of other morphological, physiological and ecological differences were revealed and Sacchi & Rastelli (1966) separated *L. obtusata* into two sympatric units and described the dwarf form as a new species, *Littorina mariae.* Interspecific differences have been elaborated by Sacchi (1967, 1969a,b, 1972) but most subsequent authors have either noted the division and then ignored it or were apparently unaware of it. The present paper is the result of investigations designed to study inter- and intraspecific variation of *L. obtusata* and *L. mariae* from a wide range of localities.

MATERIALS AND METHODS

The localities visited by the authors are shown in Table 1 together with their exposure values (Ballantine, 1961). These values were assigned as closely as possible to the specific area from which the main collection of each species was taken and in some cases they were judged to be different for the two species on the same beach, for example Trevone in Cornwall and College Rocks, Dyfed. In addition, samples were sent from the following locations for which exposure values are not known: Wishing Well and Farland Bight on the Isle of Cumbrae, Scotland; Tromsø, Bergen and Drøbak, Norway; Reykjavik, Iceland; and Deer Island, New Brunswick, Canada.

Shell Variation

Individual snails were assigned to one of the colour varieties described for *L. obtusata* subsp. *littoralis* by Dautzenberg & Fischer (1914). Variations in size and shape of the shells were studied using a similar method to that of Colman (1932). Dimensions *a* and *b* were used as described by Colman but the value *c* was found to be difficult to measure with consistent accuracy (see Sacchi, 1961a) and thus shell height was substituted (see Fig. 1). Colman's ratios *a/b, a/c* and *c/b* were also

*Present address: Portway School, Penpole Lane, Shirehampton, Bristol.

Table 1. Collection sites listed in order of decreasing exposure

Littorina obtusata		*Littorina mariae*	
Locality and National Grid Reference	Exposure Value	Locality and National Grid Reference	Exposure Value
Hartland Quay, Devon (SS 224 249)	4-5	Hartland Quay, Devon (SS 224 249)	3-4
Constitution Hill Rocks, Dyfed. (SN 583 828)	5	Blackrock, Brighton (TQ 345 030)	3-4
Lee Bay, Devon (SS 479 469)	5	Trevone, Cornwall (SW 888 759)	4-5
Church Reef, Wembury, Devon. (SX 518 484)	5	Lee Bay, Devon (SS 479 469)	5
Sandy Beach, Anglesey (SH 283 851)	5-6	Church Reef, Wembury, Devon. (SX 518 484)	5
Ynys Traws, Anglesey (SH 272 749)	6	Constitution Hill Rocks, Dyfed. (SN 583 828)	5
College Rocks, Dyfed (SN 578 816)	6	New Quay (South), Dyfed (SN 385 605)	5
Trevone, Cornwall (SW 888 759)	6	College Rocks, Dyfed (SN 578 816)	5-6
Watchet, Somerset (ST 077 437)	6-7	Sandy Beach, Anglesey (SH 283 851)	5-6
St. Mary's Island Northumberland (NZ 352 750)	6-7	Ynys Traws, Anglesey (SH 272 749)	6
		New Quay North (Dyfed) (SN 395 595)	6

used as indicators of differences in shell shape. Differences in the values of parameters *a, b* and *c* and the ratios *a/b, a/c* and *c/b* both between sexes and between species, were subjected to statistical analysis. For locations where only one species was present a one-way analysis of variance (ANOVA) was used to test the significance of differences between males and females. For locations where both *L. obtusata* and *L. mariae* were collected an initial ANOVA was carried out using all four groups (males and females of both species) and if a significant difference at the 5% level was obtained the groups were further investigated by means of Scheffé's multiple comparison test. For each sample the appropriate comparisons were made from the following:- 1) male *L. mariae v.* female *L. mariae;* 2) male *L. obtusata v.* female *L. obtusata;* 3) male *L. mariae v.* male *L. obtusata;* 4) female *L. mariae v.* female *L. obtusata.*

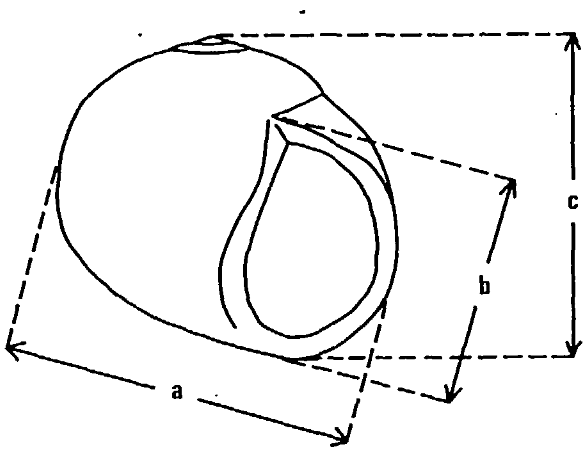

Fig. 1. Adult specimen of *Littorina obtusata* showing the shell parameters *a, b* and *c* used throughout this study.

Radular morphology

Radulae were cleaned in 30% KOH, rinsed in distilled water followed by absolute alcohol and dried overnight in a desiccator. They were attached to scanning electron microscope stubs with 'Bostik' and coated with a layer of carbon, and a layer of gold/palladium mixture for examination at 7.5kV using a Cambridge Stereoscan 600. A total of 29 radulae of *L. obtusata* (from College Rocks, Constitution Hill Rocks, East Haven and Tromsø) and 27 radulae of *L. mariae* (from College Rocks, Constitution Hill Rocks and Drøbak) were examined, care being taken to study only the unworn teeth. In addition, radular length/shell parameter ratios were determined for both species from Constitution Hill Rocks and Sandy Beach. The shell measurements *a*, *b* and *c* were recorded as above and the radulae were measured to the nearest 0.5mm.

Breeding Cycle

Attempts to elucidate the breeding cycle of the two species by studying seasonal changes in the external features of the reproductive organs and the histology of the gonad proved unsuccessful. The most reliable indication of spawning activity was the presence or absence of egg masses on intertidal fucoid algae. Monthly collections were made at College Rocks *(L. obtusata)* and New Quay *(L. mariae)* and the number of egg masses collected per hour was used as an estimation of their abundance. Observations were also made on the shape of the egg masses, the species of alga on which they were laid, and their distribution on the algae.

RESULTS

Sacchi & Rastelli (1966) used penis morphology as a means of distinguishing males of the two species. This observation has been confirmed in the present study, *L. mariae* invariably showing an extended tip to the penis and a single row of penial glands, whereas *L. obtusata* has a short tip and two or three poorly defined rows of glands. The mean and range of the number of penial glands for all populations examined are shown in Table 2.

In the present study a valuable distinction between females of the species has been made. In *L. mariae* the ovipositor lacks pigmentation whereas in *L. obtusata* it is to varying degrees black pigmented. This character, which does not show seasonal variation, is an important means of separating the females of both species and has been used with an accuracy greater than 99% on living specimens from several populations in Wales. Observations on fixed specimens proved to be less satisfactory since the body surface was often poorly fixed and liable to slough off. This can be

Table 2. The number of penial glands in populations of *L. obtusata* and *L. mariae*

	Littorina obtusata			*Littorina mariae*		
Locality	*n*	Mean ± SE	Range	*n*	Mean±SE	Range
College Rocks	36	27.3 ± 0.9	17-34	17	11.7 ± 0.5	8-16
Constitution Hill Rocks	25	27.3 ± 0.9	16-35	37	12.1 ± 0.2	9-16
Ynys Traws	13	29.3 ± 2.0	18-43	24	10.8 ± 0.3	8-13
Sandy Beach	27	34.2 ± 1.2	19-42	23	12.2 ± 0.4	9-16
Wishing Well	19	29.1 ± 1.5	18-45	52	9.7 ± 0.3	6-13
Farland Bight	20	24.9 ± 1.1	17-34			
St. Mary's Island	30	37.0 ± 1.2	24-51			
Blackrock				10	11.7 ± 0.5	9-14
Church Reef	59	29.5 ± 0.7	20-42	20	12.1 ± 0.5	8-17
Hartland Quay	10	29.9 ± 1.6	20-38	72	11.3 ± 0.3	6-17
Lee Bay	43	30.7 ± 1.4	18-46	48	11.5 ± 0.3	7-16
Trevone	42	27.9 ± 0.8	20-38	55	12.5 ± 0.3	7-16
Watchet	58	33.3 ± 1.0	22-53			
Drøbak				41	7.7 ± 0.2	5-10
Bergen (Sample 1)	58	30.1 ± 0.8	18-49	9	11.6 ± 0.9	8-16
Bergen (Sample 2)	55	31.2 ± 1.0	16-45			
Tromsø	33	29.0 ± 1.8	19-46			
Deer Island	23	24.7 ± 0.8	19-36			

prevented if the animals are killed by immersion in boiling water prior to fixation as this causes slight relaxation of the foot retractor muscles allowing the fixative to penetrate rapidly behind the operculum. When adequate fixation was achieved the above observations were confirmed.

Shell variation between L. obtusata *and* L. mariae

Data on shell colour are in agreement with the observations made by Sacchi (1967, 1969b, 1974) and show that the dominant colour morphs of *L. obtusata* are olivacea and reticulata, whereas *L. mariae* has reticulata and citrina as the dominant morphs. The morph olivacea is rare in *L. mariae* and none were encountered in the present study. The observation that the dominant colour morph on sheltered shores was olivacea for *L. obtusata* and citrina for *L. mariae,* whereas on more exposed beaches reticulata was the commonest morph in both species (Sacchi, 1974) was confirmed.

The mean values of shell parameters *a, b* and *c* for adult males and female specimens of both species are given in Tables 3 and 4. Adult snails are here defined as mature individuals in which thickening of the shell lip has commenced or is complete. With the exception of the small sample from Bergen (sample 1) the mean values for male and female *L. obtusata* treated separately, were greater than those for male and female *L. mariae.* Using Scheffé's mutiple comparison test, these differences were significant at the 5% level or better. Although *L. obtusata* is larger than *L. mariae* at a given locality, size is not an absolute criterion as overlap does occur if the whole geographical range is studied. Populations from the British Isles, however, showed no overlap of mean adult size although individual shells of adult *L. mariae* may be larger than those of *L. obtusata.*

Sexual dimorphism is present in both species and is more pronounced in *L. mariae* where, with the exception of the small sample from Bergen, comparison of male with female values for parameters *a, b* and *c* are all significantly different at the 5% level or better (Scheffé's test). In *L. obtusata,* although the majority of values for parameters *a, b* and *c* for females are greater than those for males, few of these differences are significant.

Table 3. Mean values of shell parameters *a, b* and *c* for adult male and female *Littorina obtusata.*
* indicates parameters where the male value is greater than that of the female.

Locality	Sex	*n*	*a* mean	*a* variance	*b* mean	*b* variance	*c* mean	*c* variance
Constitution Hill	m	26	14.81	0.66	10.47	0.38	12.72	0.55
Rocks	f	24	14.91	0.36	10.63	0.21	12.73	0.31
Sandy Beach	m	29	14.96	0.43	10.58	0.25	13.38	0.58
	f	34	15.07	0.49	10.78	0.38	13.48	0.74
Lee Bay	m	44	15.74	0.65	11.09	0.25	13.71	0.44
	f	62	15.86	0.50	11.29	0.25	13.90	0.43
Church Reef, Wembury	m	60	14.66	0.58	10.36	0.30	13.13	0.62
	f	51	14.93	0.64	10.61	0.39	13.39	0.73
Bergen (sample 1)	m	61	12.57	0.81	9.10	0.47	11.04	0.81
	f	70	12.82	0.79	9.31	0.40	11.20	0.83
College Rocks	m	37	15.07	0.41	10.51	0.18	13.14	0.55
	f	36	15.31	0.51	10.74	0.23	13.36	0.55
Wishing Well	m	20	13.91	0.76	9.91	0.35	12.38	0.88
	f	30	13.91	0.86	10.05	0.46	12.31*	0.76
Trevone	m	44	16.12	0.38	11.23	0.22	13.79	0.47
	f	64	16.30	0.60	11.40	0.30	13.85	0.58
Hartland Quay	m	10	15.41	0.66	10.95	0.18	13.44	0.62
	f	9	15.75	0.97	11.44	0.52	13.57	0.63
Bergen (sample 2)	m	58	13.41	0.48	9.76	0.30	12.01	0.65
	f	59	13.51	0.58	9.87	0.23	11.93*	0.55
Tromsø	m	33	11.56	3.12	8.02	1.44	11.22	2.57
	f	34	12.31	1.43	8.46	0.80	11.85	1.70
Deer Island	m	23	9.47	0.84	6.36	0.37	9.35	0.66
	f	31	9.93	1.15	6.81	0.51	9.76	1.20
Watchet	m	60	17.06	0.46	12.09	0.30	14.97	0.58
	f	48	17.29	0.39	12.44	0.21	15.08	0.55
St.Mary's Island	m	53	17.02	0.68	12.62	0.29	15.03	0.74
	f	56	17.44	0.47	12.74	0.23	15.25	0.86
Farland Bight	m	20	12.77	3.71	9.27	1.59	11.19	2.71
	f	19	13.57	1.46	9.85	0.78	11.91	1.23
Ynys Traws	m	13	15.89	0.37	11.36	0.14	14.03	0.63
	f	14	16.54	0.50	11.79	0.45	14.25	0.73

Table 4. Mean values of shell parameters *a*, *b* and *c* for adult male and female *Littorina mariae*.

Locality	Sex	*n*	*a* mean	*a* variance	*b* mean	*b* variance	*c* mean	*c* variance
Constitution Hill Rocks	m	37	12.08	0.87	8.67	0.45	10.12	0.71
	f	46	12.73	0.47	9.27	0.23	10.67	0.48
Sandy Beach	m	23	10.06	1.36	7.29	0.65	8.47	1.05
	f	28	11.76	1.39	8.52	0.75	9.81	1.08
Lee Bay	m	48	11.19	0.53	8.11	0.27	9.62	0.41
	f	45	12.06	0.53	8.89	0.32	10.35	0.42
Church Reef, Wembury	m	20	11.44	0.74	8.27	0.34	9.94	0.60
	f	14	12.89	0.38	9.39	0.16	11.10	0.46
Bergen (sample 1)	m	9	12.10	1.12	8.66	0.44	10.18	0.77
	f	14	12.96	1.33	9.45	0.69	10.99	1.48
College Rocks	m	17	11.64	1.14	8.44	0.56	9.86	0.86
	f	14	12.89	0.68	9.45	0.25	10.92	0.28
Wishing Well	m	52	8.92	1.19	6.44	0.65	7.65	0.89
	f	62	10.16	0.89	7.52	0.49	8.69	0.64
Trevone	m	56	11.88	0.65	8.51	0.36	9.97	0.59
	f	52	12.70	0.89	9.15	0.48	10.61	0.83
Hartland Quay	m	72	11.68	0.54	8.35	0.28	9.85	0.46
	f	28	12.53	0.53	9.09	0.28	10.62	0.42
Blackrock, Brighton	m	10	12.22	0.48	9.07	0.21	10.40	0.60
	f	11	12.99	0.12	9.65	0.11	11.07	0.15
Drøbak	m	41	6.14	0.73	4.58	0.41	5.21	0.52
	f	76	7.04	1.77	5.23	1.04	6.11	1.40
Ynys Traws	m	24	8.33	0.79	6.15	0.43	7.02	0.54
	f	21	10.13	0.98	7.45	0.42	8.35	0.68
New Quay (N)	m	43	9.42	0.63	6.77	0.31	7.89	0.51
	f	43	10.74	0.60	7.86	0.30	8.88	0.41

The use of shell parameter ratios to investigate shell shape has revealed several differences between the two species. The mean value of *a/b* is, with one exception, higher in *L. obtusata* than *L. mariae* and this may be attributed to the smaller relative size of the last body whorl *(b)* of *L. obtusata.* Although in both species the male ratio *a/b* is in the majority of populations studied higher than that of the female, the differences are statistically significant in only a few cases. Comparison of the ratios *a/c* and *c/b* shows a reversal between the two species so that in *L. obtusata* the ratio *c/b* is greater than that of *a/c* for all populations studied, while in *L. mariae* (with the exception of males from Wishing Well and Lee Bay and both sexes from Church Reef) the ratio of *a/c* is greater than *c/b.* Even allowing for the slightly greater relative value of parameter *b* in *L. mariae,* the value of *c,* denominator in one ratio and numerator in the other, would appear to be the critical parameter. Since parameter *c* is here defined as shell height, where this is relatively large (as in *L. obtusata)* a low value for the ratio *a/c* and a high value for the ratio *c/b* will be obtained. The converse is true for *L. mariae,* and the relative flatness of the shell of *L. mariae* may thus be deduced from these ratios.

Shell variation within L. obtusata *and* L. mariae

In addition to the size dimorphism between *L. obtusata* and *L. mariae* for a particular locality, considerable variation of shell dimensions is also apparent within species. If the parameters *a, b* and *c* are considered in relation to the exposure value of the locality, an interesting relationship is revealed (Fig. 2). There is a size gradient in both species such that mean adult size increases with decreasing exposure in *L. obtusata,* but decreases in *L. mariae.*

The degree of sexual dimorphism in *L. mariae* also appears to be related to the exposure of the habitat such that there is an increase in sexual dimorphism as exposure decreases. This is illustrated in Fig. 3 in which the percentage difference in size between male and female is considered in relation to the exposure value for each of the parameters *a, b* and *c.* If the mean values of *a, b* and *c* (pooled for each exposure value) for both sexes are graphed against the degree of exposure (Fig. 4) it is clear that both males and females of *L. mariae* decrease in size with decrease in exposure, but that the males become smaller relatively more quickly. Similar trends are not apparent in *L. obtusata* where the level of sexual dimorphism is, in any case, very low (Fig. 3).

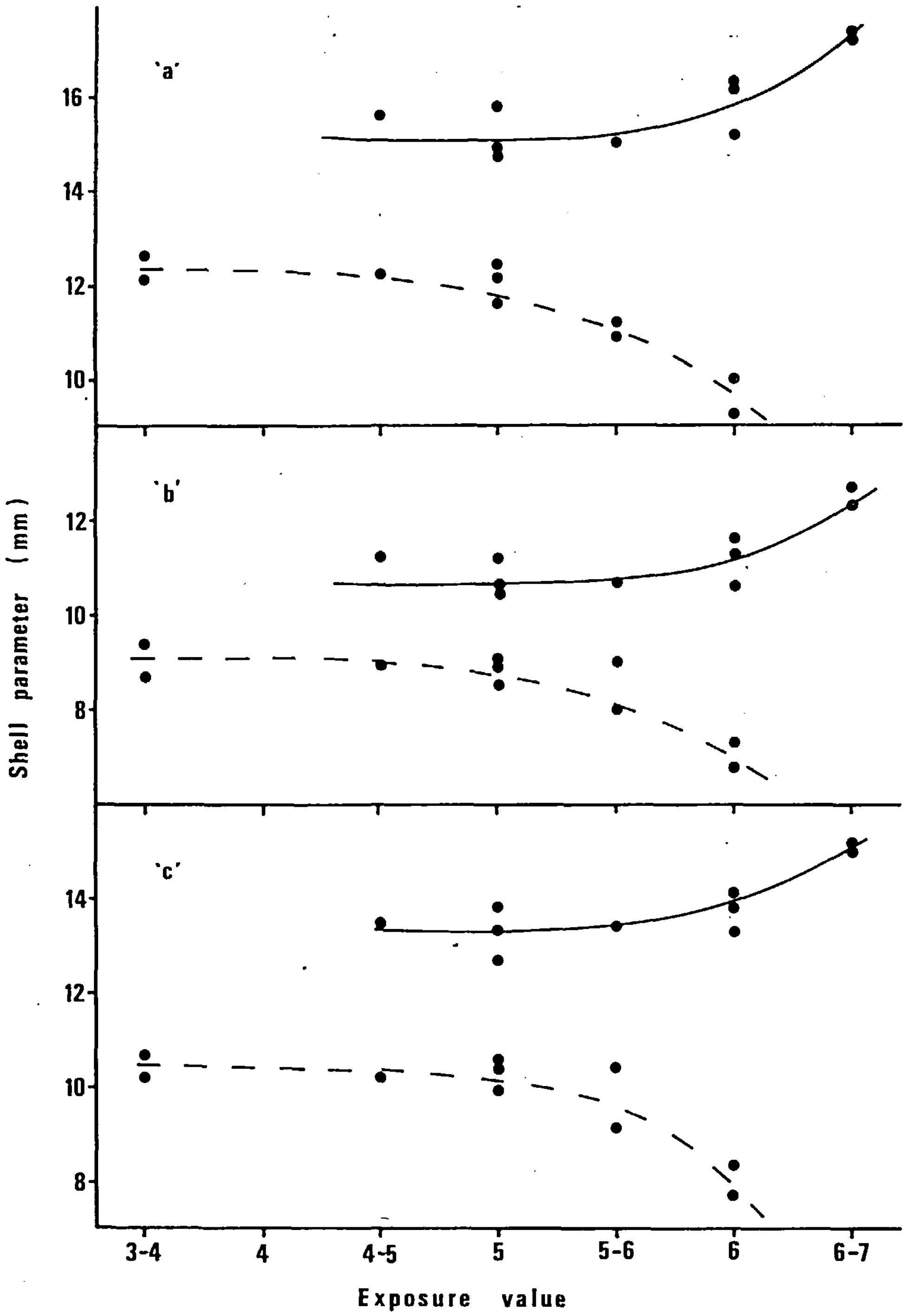

Fig. 2. Variation in the mean value of shell parameters *a*, *b* and *c* in relation to exposure for *Littorina obtusata* (●—●) and *L. mariae* (● – – ●). Exposure decreases from left to right.

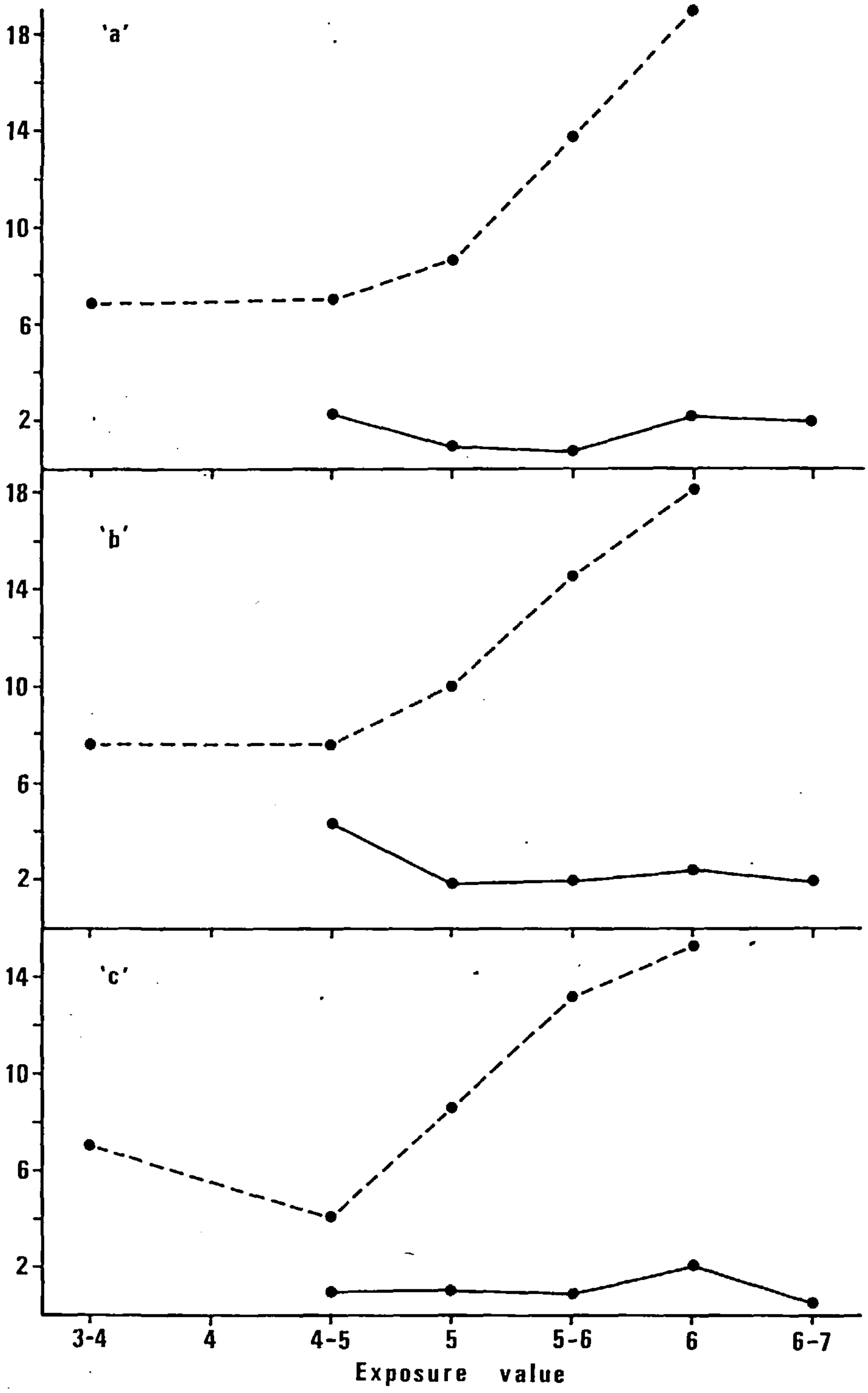

Fig. 3. Difference in size (mean values of parameters *a*, *b* and *c*) by which females are larger than males, expressed as a percentage of the male value. *Littorina obtusata* ●—●; *L. mariae* ●- - -●.

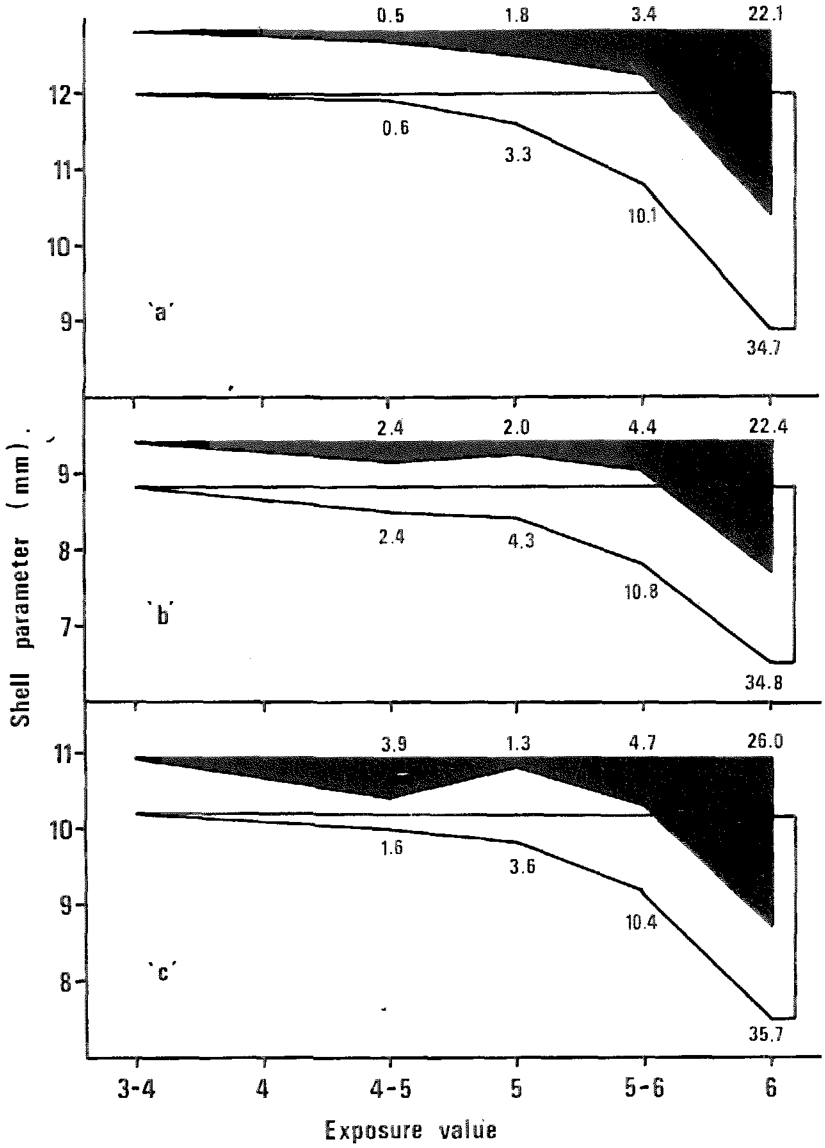

Fig. 4. Decrease in size of shell parameters *a, b* and *c* for males (lower, open) and females (upper, shaded) of *Littorina mariae* in relation to decreasing exposure. Figures refer to the percentage decrease in mean size in relation to the mean size at exposure value 3-4.

Radular morphology

There is considerable variation in the nature of the radular teeth such that intra- as well as interspecific differences have been encountered. Nevertheless, a tentative division on the basis of radular characters is proposed and this is based on observations made on specimens from as far apart as Wales and Norway. Interspecific differences useful in the separation of the species are summarized in Fig. 5 and Table 5.

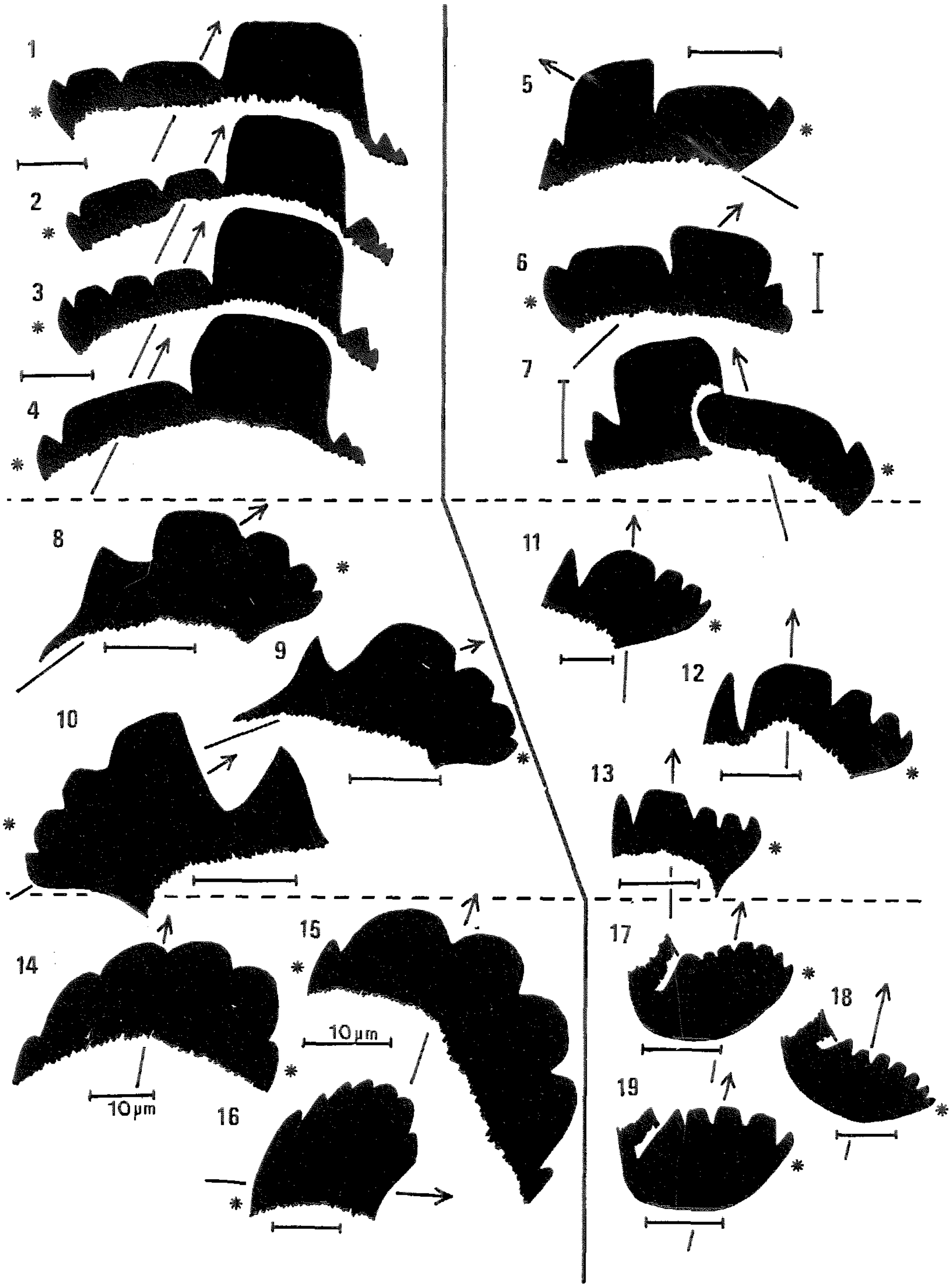

Fig. 5. Lateral (1-7), inner marginal (8-13) and outer marginal teeth (14-19) from radulae of *Littorina obtusata* (left side of page) and *L. mariae* (right side of page). * denotes side of tooth nearest to the rachidian tooth. → longitudinal axis of radula; arrow points anteriorly. Bar equals 20µm except where stated.

Table 5. Radular characters of *L. obtusata* and *L. mariae.* Those of greatest significance in separating the species are in italics. See Fig. 5.

	Littorina obtusata	*Littorina mariae*
Rachidian Tooth	Similar	Similar
Lateral Tooth	Highly variable. *Often bearing three cusps* on the inner portion but ranging from two to four.	Less variable. There are *normally only two cusps* on the inner portion. The outer part may lack the smallest cusp.
Inner Marginal	The outermost cusp (5th) is triangular and forms (more or less) a *right angle* with the outer margin of the fourth cusp.	The outermost cusp is *more pointed* more slender, and forms an *acute angle* with the fourth cusp.
Outer Marginal	Normally six cusps, sometimes seven, which are *usually truncated with shallow notches* between.	More variable, with from five to seven cusps which are *often rounded with deep notches* between, less often truncated.
Radular length/shell parameter ratios	Comparison of the radular length/shell parameter ratios of the two species from each locality showed significant differences: (Scheffé's test) at the 5% level or better. The ratios of *L. obtusata* are greater than those of *L. mariae.*	

Breeding cycle

The shell height (parameter *c*) at which *L. obtusata* and *L. mariae* reached sexual maturity was determined throughout the year for populations from College Rocks, and it is clear that maturation of the two species takes place over a different size range. No overt sexual differentiation takes place before 7.0mm shell height in *L. obtusata* and 4.0mm in *L. mariae,* although the size at which development begins (and at which full sexual maturity is attained) is presumably dependent on the mean adult size which itself varies on different beaches (Tables 3 and 4). Although some development of the reproductive organs takes place between a shell height of 7-10mm in *L. obtusata* and 4-8mm in *L. mariae,* the most rapid development is apparent as the snails approach the adult size of 10-12mm in *L. obtusata* and 8-10mm in *L. mariae.* Development of the male is slightly in advance of that of the female for a particular shell height.

The spawn of both species is characterized by wide variation in size and shape. Although differences in spawn size cannot be used to separate the species, minor interspecific differences do occur in spawn shape and the substratum on which the spawn is laid. Egg diameter also differs between the species and these differences are summarized in Table 6. It has so far proved impossible to separate the egg masses using histological and histochemical techniques.

The results of the monthly collection of egg masses are shown in Fig. 6. There is no significant seasonal trend in the number of embryos per egg mass in either species.

DISCUSSION

Sacchi found that, as in many closely related species, it was difficult to pinpoint a single character by which individuals of *L. obtusata* and *L. mariae* could be positively identified and much of his work was concerned with differences at the population level. The criteria listed by Sacchi have been confirmed in the present study and, in addition, the distinction between females of the species on the basis of pigmentation of the ovipositor has proved to be a most useful character.

A diagnostic feature valuable for use in the field is the relatively flatter shell of *L. mariae.* This is often the most noticeable visual difference between shells of the two species and is indicated by the analysis of shell parameters *a, b* and *c* and their ratios described on p. 244. Correlated with the relative flatness of *L. mariae* is the fact that the insertion of the outer lip on the final body whorl is often higher in *L. mariae* than *L. obtusata.* The higher insertion of the lip is also apparent in juvenile *L. obtusata* since it is primarily during the final phase of growth that the more elevated spire of L. obtusata becomes apparent. Other differences, however, rule out the possibility of confusing adult

Table 6. Spawn characteristics of *Littorina obtusata* and *Littorina mariae*

Character	*Littorina obtusata*	*Littorina mariae*
Spawn shape	Occasionally almost round (up to 10%). Rarely kidney-shaped (less than 2%).	Round spawn not observed. Often kidney-shaped (more than 20%).
Mean egg diameter	210-255μm	195-200μm
Substrate to which spawn attached	*Fucus spiralis; F. vesiculosus; Ascophyllum nodosum.* Rock. May be laid on thallus, stipe or holdfast.	*Fucus serratus* Laid on thallus.

L. mariae with juvenile *L. obtusata* of the same size:- (1) there is a thin growing lip in juvenile *L. obtusata;* (2) the junction of the outer and columellar lips is extended downwards in *L. obtusata;* (3) in *L. mariae* with a fully thickened growing edge the outer lip joins the body whorl in a straight line, whereas the incompletely thickened lip of *L. obtusata* is notched at the junction (Fig. 7).

In addition to the above observations two subjective differences are apparent and these are useful for identification in the field. The relative thickening of the shell of *L. mariae* gives a reduced aperture size, and the angle of insertion of the outer lip on to the body whorl is often close to 90° for *L. mariae* but usually more acute in *L. obtusata* (Fig. 7).

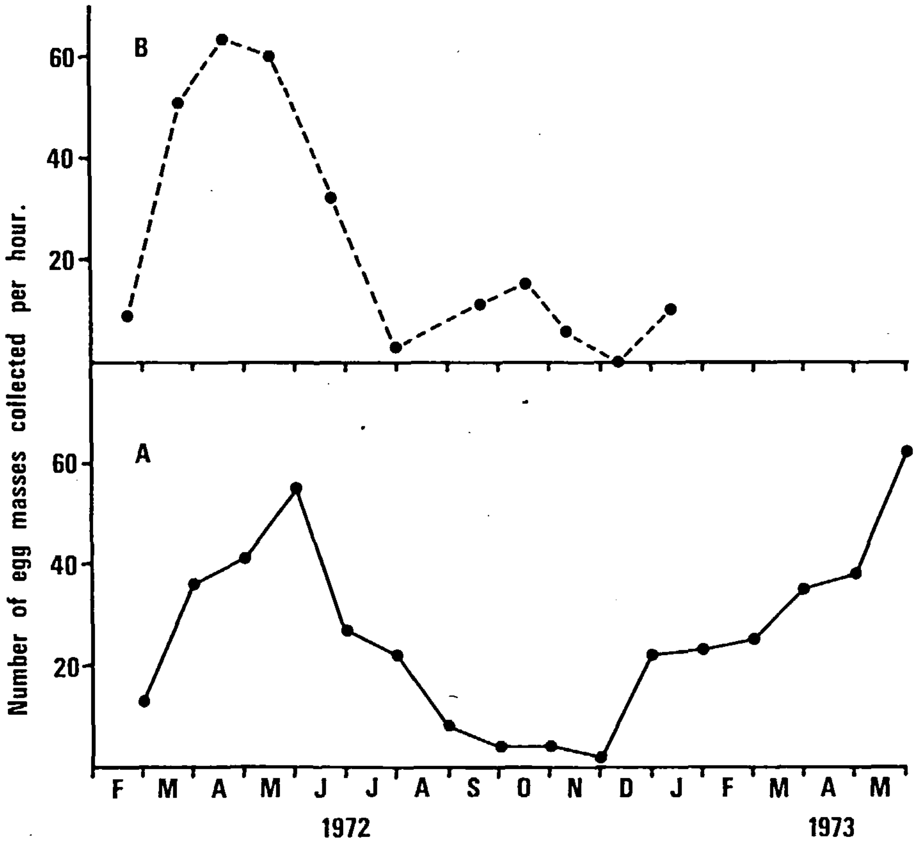

Fig. 6. The seasonal abundance of the spawn of *Littorina obtusata* (A) and *L. mariae* (B).

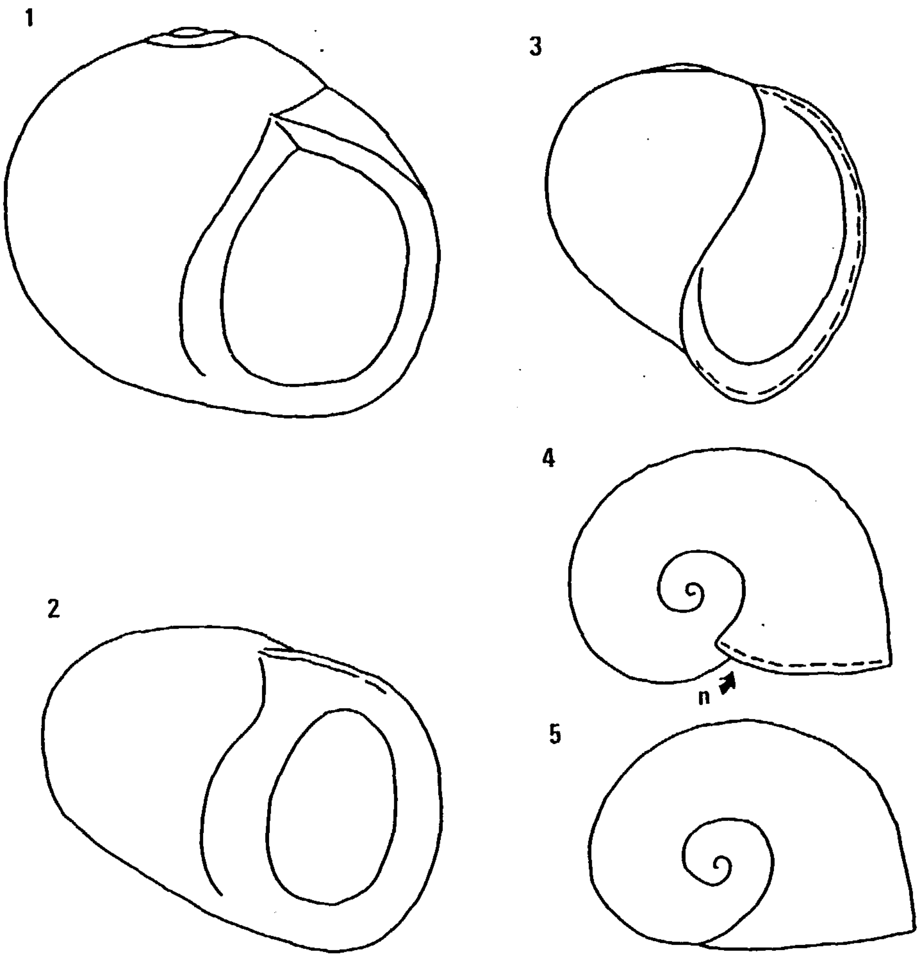

Fig. 7. (1) Adult *Littorina obtusata.* (2) Adult *L. mariae.* Note the relatively lower insertion of the outer lip of *L. obtusata* and the relatively larger aperture. (3 & 4) Juvenile *L. obtusata.* Note the downward extension of the unthickened growing lip in (3) and the notch (n) at the junction of the growing lip and the body whorl in (4). (5) Adult *L. mariae.* Compare with juvenile *L. obtusata.* Not to scale.

Variation in shell size has been studied by a number of authors and correlation between reduction in size and increased wave exposure has been recorded for several species although there is little evidence that wave action *per se* is the causative factor. In *Nucella lapillus* (L.) the effects of the environment in producing a large degree of variation have long been recognized (Cooke, 1895; Colton, 1916; Agersborg, 1929), although Moore (1936) did not detect correlation between shell shape and wave exposure and believed that diet was responsible for the observed variability. Despite the influence of a genetic component in the morphological variation of *Nucella* (Staiger, 1957), Kitching, Muntz & Ebling (1966) suggested that the variation between two forms of *Nucella* at Lough Ine could be accounted for by the selective forces of wave action and crab predation. Crothers (1973) has confirmed that there is a correlation between variation in shell shape and change in exposure to wave action but it is clear that in addition to wave action and crab predation, other components may also be important. In *Dicathais orbita* (Gmelin) Phillips, Campbell & Wilson (1973) found that water temperature, diet, substratum and degree of exposure to wave action were all associated with variations in shell characteristics.

Small size associated with increased exposure has been reported several times for *Littorina* spp. including *L. obtusata* (Sacchi, 1969b). The environmental selective agents responsible for larger size on more sheltered shores are not obvious although Sacchi (1969b) suggested that in *L. obtusata* it was the result of more favourable growth conditions. In a recent study, Reimchen (1974) investigated the causes of shell variation in *L. obtusata* and *L. mariae* and has suggested different strategies by which the observed size variation in both species might be explained in terms of the selective effects of wave action and crab predation.

The clinal variation in size and shape of *L. obtusata* along the United States Atlantic Coast (Colman, 1932) is presumably dependent, at least partially, on factors other than wave action and crab predation. Colman demonstrated that shells from Bergen, Plymouth and Rhode Island were biometrically similar and that shells from Rhode Island were connected to the thinner and more pointed-spired *L. obtusata palliata* or *L. palliata* of Maine by a continuous series of intermediate forms. In the present study the similarity of *L. obtusata* from Bergen to typical *L. obtusata* from Britain has been confirmed as regards shell shape although the specimens from Bergen were slightly smaller (Table 3), while to the north, specimens from Tromsø were not only smaller but had a much more elongated spire and thinner shells. These few observations suggest the possibility of a gradient, similar to that demonstrated by Colman for the United States Atlantic coast, extending up the Norwegian coast and perhaps to the U.S.S.R. where *L. palliata* is common (see Zenkevitch, 1963).

With the exception of penis structure and ovipositor pigmentation, the reproductive systems of *L. mariae* and *L. obtusata* are similar in external morphology and histological appearance and interspecific differences in the level of reproductive development throughout the year are slight. It is clear from this and the time of reproduction, as indicated by the appearance of egg masses, that the possibility of interspecific copulation between *L. obtusata* and *L. mariae* is not ruled out by differences in the breeding season. However, interspecific copulation between these species was not observed during the present study although it has been reported for other littorinids (Thompson, 1852; Sauvage, 1873; Linke, 1933). Probably one of the most important isolating mechanisms is the occurrence of *L. obtusata* and *L. mariae* on different zones on the beach, although penis morphology may be important in this context and is worthy of further study. It is possible that morphological, distributional and perhaps behavioural barriers similar to those described for *L. picta* Phillipi and *L. pintado* (Wood) (Struhsaker, 1966) act as effective isolating mechanisms.

SUMMARY

Studies on *Littorina obtusata* and *L. mariae* from a wide range of localities have confirmed the criteria used by Sacchi & Rastelli in separating the species. In addition, the females of the species can be identified by the pigmentation of the ovipositor which in *L. mariae* lacks pigmentation whereas in *L. obtusata* it is to varying degrees black pigmented. Shell characters useful for identification in the field, such as the relatively flatter shell of *L. mariae*, are outlined. Mean adult shell size of both species varies with the exposure of the habitat, in *L. obtusata* increasing with decreasing exposure, decreasing in *L. mariae*. These and other aspects of intra- and interspecific shell variation are discussed. Intra- and interspecific differences have been recorded in the nature of the radular teeth and differences useful in the separation of the species are described. There are few interspecific differences with respect to the reproductive biology of the snails.

ACKNOWLEDGMENTS

We wish to thank Miss S. Lutkins of the Department of Statistics for guidance on statistical procedures and Dr I. Inglis, Dr P. G. Moore, Dr Finn Walvig, Dr Wim Vader, and the staff of the Bergen Marine Institute for kindly providing specimens.

REFERENCES

AGERSBORG, H. P. K. 1929. Factors in the evolution of the prosobranchiate mollusc *Thais lapillus*. *Nautilus*, **43**, 45-59.

BALLANTINE, W. J. 1961. A biologically defined exposure scale for the comparative description of rocky shores. *Field Studies*, 1(3), 1-19.

COLMAN, J. 1932. A statistical test of the species concept in *Littorina*. *Biological Bulletin*, **62**, 223-43.

COLTON, H. S. 1916. On some varieties of *Thais lapillus* in the Mount Desert Region, a study in individual ecology. *Proceedings of the Academy of Natural Science, Philadelphia*, **68**, 440-54.

COOKE, A. H. 1895. *Cambridge Natural History.* Vol. III. Mollusca. London, Macmillan.
CROTHERS, J. H. 1973. On variation in *Nucella lapillus* (L.): shell shape in populations from Pembrokeshire, South Wales. *Proceedings of the Malacological Society, London,* **40**, 319-27.
DAUTZENBERG, P. H. & FISCHER, H. 1914. Étude sur le *Littorina obtusata* et ses variations. *Journal de Conchyliologie,* **62**, 87-128.
HELLER, J. 1975. The taxonomy of some British *Littorina* species, with notes on their reproduction (Mollusca: Prosobranchia). *Zoological Journal of the Linnaean Society,* **56**, 131-51.
JAMES, B. L. 1968. The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. *Field Studies,* **2**, 615-50.
KITCHING, J. A. MUNTZ, L. & EBLING, F. J. 1966. The ecology of Lough Ine XV. The ecological significance of shell and body forms in *Nucella. Journal of Animal Ecology,* **35**, 113-26.
LINKE, O. 1933. Morphologie und physiologie des Genitalapparates des Nordseelittorinen. *Wissenschaftliche Meeresuntersuchungen,* **19**, 1-60.
LINNAEUS, C. 1758. *Systema Naturae.* 10th Edition, Vol. 1. Stockholm.
MOORE, H. B. 1936. The biology of *Purpura lapillus.* I. Shell variation in relation to environment. *Journal of the Marine Biological Association of the U.K.,* **21**, 61-89.
PHILLIPS, B. F., CAMPBELL, N. A. & WILSON, B. R. 1973. A multivariate study of geographic variation in the whelk *Dicathais. Journal of Experimental Marine Biology and Ecology,* **11**, 27-69.
REIMCHEN, T. E. 1974. *Studies on the biology and colour polymorphism of two sibling species of marine gastropod* (Littorina). Unpublished Ph.D. thesis, University of Liverpool.
SACCHI, C. F. 1961a. Contribution à l'étude des rapports écologie/polychromatisme chez un prosobranche intercotidal, *Littorina obtusata* (L.) à Roscoff. *Cahiers de Biologie Marine,* **2**, 271-90.
SACCHI, C. F. 1961b. Relazioni ecologia-policromatismo nel Prosobrancho intercotidale *Littorina obtusata* (L.) a Roscoff. II. — Richerche biometriche. *Bollettino di Zoologia (Publicata dall Unione Zoologica Italiane, Napoli),* **28**, 517-28.
SACCHI, C. F. 1963. Contribution à l'étude des rapports écologie/polychromatisme chez un prosobranche intercotidal, *Littorina obtusata* (L.) III. — Données expérimentales et diverses. *Cahiers de Biologie Marine,* **4**, 299-313.
SACCHI, C. F. 1964. Relazioni tra ecologia e policromatismo nel Prosobrancho intercotidale *Littorina obtusata* (L.) IV. — Studio sulla Ria de Vigo. *Archivo Zoologico Italiano,* **49**, 93-156.
SACCHI, C. F. 1966a. *Littorina obtusata* (L.) (Gastropoda, Prosobranchia): a problem of variability and its relation to ecology. *Simposia Genetica et Biologica Italica,* **13**, 521-72.
SACCHI, C. F. 1966b. Sur le dimorphisme du pênis chez *Littorina obtusata* (L.) (Gastr. Pros.). *Comptes rendus hebdomaires des Séances de l'Académie des Sciences,* D, **262**, 2370-72.
SACCHI, C. F. 1967. Variabilità ed ambiente nella coppia di specie intertidali *Littorina obtusata* (L.) e *Littorina mariae* Sacchi e Rastelli (Gastropoda Prosobranchia) a Concarneau (Bretagne Meridionale). *Studia Ghisleriana,* **2**, 339-55.
SACCHI, C. F. 1969a. Recherches sur l'écologie comparée de *Littorina obtusata* (L.) et de *Littorina mariae* Sacchi et Rast. (Gastropoda, Prosobranchia) en Galice et en Bretagne. *Investigaçion Pesquera,* **33**, 381-414.
SACCHI, C. F. 1969b. Ricerche biometriche sul dimorfismo sessuale della coppia di specie intertidali *Littorina obtusata* (L.) e *L. mariae* Sacchi e Rast. (Gastropoda Prosobranchia) in Bretagne ed in Galizia. *Bollettino di Pesca Piscicoltura e Idrobiologia,* **24**, 33-47.
SACCHI, C. F. 1972. Recherches sur l'écologie comparée de *Littorina obtusata* (L.) et de *L. mariae* Sacchi et Rast. (Gastropoda, Prosobranchia). II. — Recherches sur la valence thermique. *Bollettino di Pesca Piscicoltura e Idrobiologia,* **27**, 105-37.
SACCHI, C. F. 1974. Le polychromatisme des littorines (Gastropodes, Prosobranches) d'Europe: points de vue d'un écologiste. *Mémoires de la Société Zoologique de France,* **37**, 61-101.
SACCHI, C. F. & RASTELLI, M. 1966. *Littorina mariae,* nov. sp.: les différences morphologiques et écologiques entre "nains" et "normaux" chez l'espèce *L. obtusata* (L.) (Gastr., Prosobr.) et leur signification adaptive et évolutive. *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano,* **105**, 351-69.
SAUVAGE, H. E. 1873. Note sur l'accouplement des *Littorina rudis* et *Littorina littorea. Journal de Conchyliologie,* **21**, 122.
STAIGER, H. 1957. Genetical and morphological variation in *Purpura lapillus* with respect to local and regional differentiation of population groups. *Année Biologique,* **33**, 251-58.
STRUHSAKER, J. W. 1966. Breeding spawning, spawning periodicity and early development in Hawaiian littorines: *L. pintado* (Wood) *L. picta* Phillipi and *L. scabra* (Linné). *Proceedings of the Malacological Society, London,* **37**, 137-66.
THOMPSON, W. 1852. On *Littorina palliata. Annals and Magazine of Natural History,* **10**, 75-76.
ZENKEVITCH, L. 1963. *Biology of the seas of the U.S.S.R.* London, George Allen & Unwin.