

SHELL SIZE VARIATION IN *LITTORINA LITTOREA* IN THE WESTERN SCHELDT ESTUARYHANS DE WOLF,<sup>1,\*</sup> RONNY BLUST,<sup>1</sup> AND THIERRY BACKELJAU<sup>1,2</sup><sup>1</sup>University of Antwerp (RUCA), Groenenborgerlaan 171 B-2020, Antwerp, Belgium; <sup>2</sup>Royal Belgian Institute of Natural Sciences (KBIN), Vautierstraat 29, B-1000, Brussels, Belgium

**ABSTRACT** *Littorina littorea* was collected along a salinity gradient in the Scheldt estuary, located in the South of the Netherlands. Its morphological population structure was investigated to see whether salinity was correlated with shell size and shell weight. Shell size did not increase along the salinity gradient, as was expected, but rather showed a clear size transition between two salinity ranges (i.e., 10–20‰ and 21–30‰). Animals attain their largest size within a salinity range of 21–30‰. Relative shell weight did not vary consistently with salinity.

**KEY WORDS:** environmental stress, estuary, *Littorina littorea*, salinity, Scheldt river, shell size

## INTRODUCTION

Although littorinids show high levels of intraspecific shell polymorphisms (see Reid 1996 and references therein), *Littorina littorea* (Linnaeus, 1758), the largest species in the genus, shows relatively little morphological variation (Janson 1987). However, morphological differences were noted between populations of *L. littorea* on the West Somerset coast (Crothers 1992). Along this coastline, a weak correlation was found between shell shape, as expressed by the shell length/aperture length ratio, and wave exposure (Crothers 1992). The differences were explained by differential growth and/or survival rates in response to the effects of wave exposure (Crothers 1992). In contrast, Janson (1987) found almost identical shapes between exposed rocky and boulder shore specimens. Apparently, the only consistent shell variation is found between marine and sheltered brackish forms, with the latter being smaller and thinner-walled (see Reid 1996). This variation is supposed to be ecophenotypic (Reid 1996) because *L. littorea* is a planktonic developing animal that is presumed to have a high dispersal and gene flow potential (Janson 1987, Reid 1996), minimizing the likelihood of selection as a possible impetus for the observed shell variation (Chapman 1995). Nevertheless, predation experiments with the oystercatcher *Haematopus ostralegus* have shown that the aperture size of *L. littorea* may be susceptible to selection, although field observations have never confirmed these experimental results (Robertson 1992).

*L. littorea* is widely distributed, occurring in the eastern (White Sea to southern Portugal) and western (Labrador to Virginia) Atlantic (Reid 1996). Unlike other littorinids, it does not solely occur on hard substrates but is also able to crawl over sand and soft mud (references in Reid 1996). This ability, along with its planktonic development and its tolerance to low salinity conditions (9.5‰), enables it to penetrate far into estuaries (Reid 1996).

In the Scheldt estuary, situated in the south of The Netherlands, *L. littorea* is found from Vlissingen (i.e., rivers' mouth) to Bath, 50 km inward from the mouth, where it occurs along a gradually decreasing salinity gradient, ranging from marine to brackish (Fig. 1). The Scheldt estuary thus forms an ideal setting to test whether salinity is indeed correlated with the shell morphology of *L. littorea*. If, under brackish conditions, *L. littorea* has a smaller, thin-

ner-walled shell, then we expect a shell-size, weight gradient in the estuary following the salinity gradient.

## MATERIALS AND METHODS

On 8 August 1998, *L. littorea* was collected at seven sites along the western Scheldt estuary, covering its entire range in the western Scheldt (Fig. 1). These sites included, in order of increasing salinity: Bath, Waarde, Hansweert, Hoedekenskerke, Ellewoutsdijk, Borssele, and Vlissingen (Fig. 1). One population was collected at each site. Each sample consisted of 40 animals. Each of the 280 specimens was morphometrically characterized. Five shell traits were measured to the nearest 0.05 mm using a caliper: shell height (HS), shell width, aperture height, aperture width, and shell-top height (De Wolf et al. 1997). In addition, total wet weight (i.e., shell + soft body parts) and body wet weight (soft body parts) were determined to the nearest mg, and all individuals were sexed on the basis of the presence or absence of a penis.

A seven-by-two contingency table was constructed to test whether the sex distribution differed from site to site, employing the Metropolis algorithm to obtain unbiased estimates of the exact *P* value (Miller 1997). Morphometric patterns were investigated by means of a two-way multivariate analysis of variance (MANOVA), contrasting the fixed factor "sex" with the random factor "sampling site." Morphological patterns were further investigated by means of a standard canonical discriminant analysis (CDA). Finally, an analysis of covariance (ANCOVA) of shell weight was performed using shell height as a covariate. Of special interest in this analysis is the interaction with the covariate because it tests whether the slopes of the shell weight on shell length are homogenous for the seven sites. Except for the contingency table analysis, all statistical analyses were performed using the software package Statistica v. 5.0 (Statsoft 1995).

## RESULTS

At each site, except for Hoedekenskerke, males outnumbered females (Fig. 1). The number of males differed significantly at the different sites ( $P = 0.0003$ ). The results of the two-way MANOVA are summarized in Table 1. A significant part of the total variation can be explained by the random factor "sampling site" (Table 1), whereas the fixed factor "sex" did not contribute significantly, nor did the interaction (Table 1).

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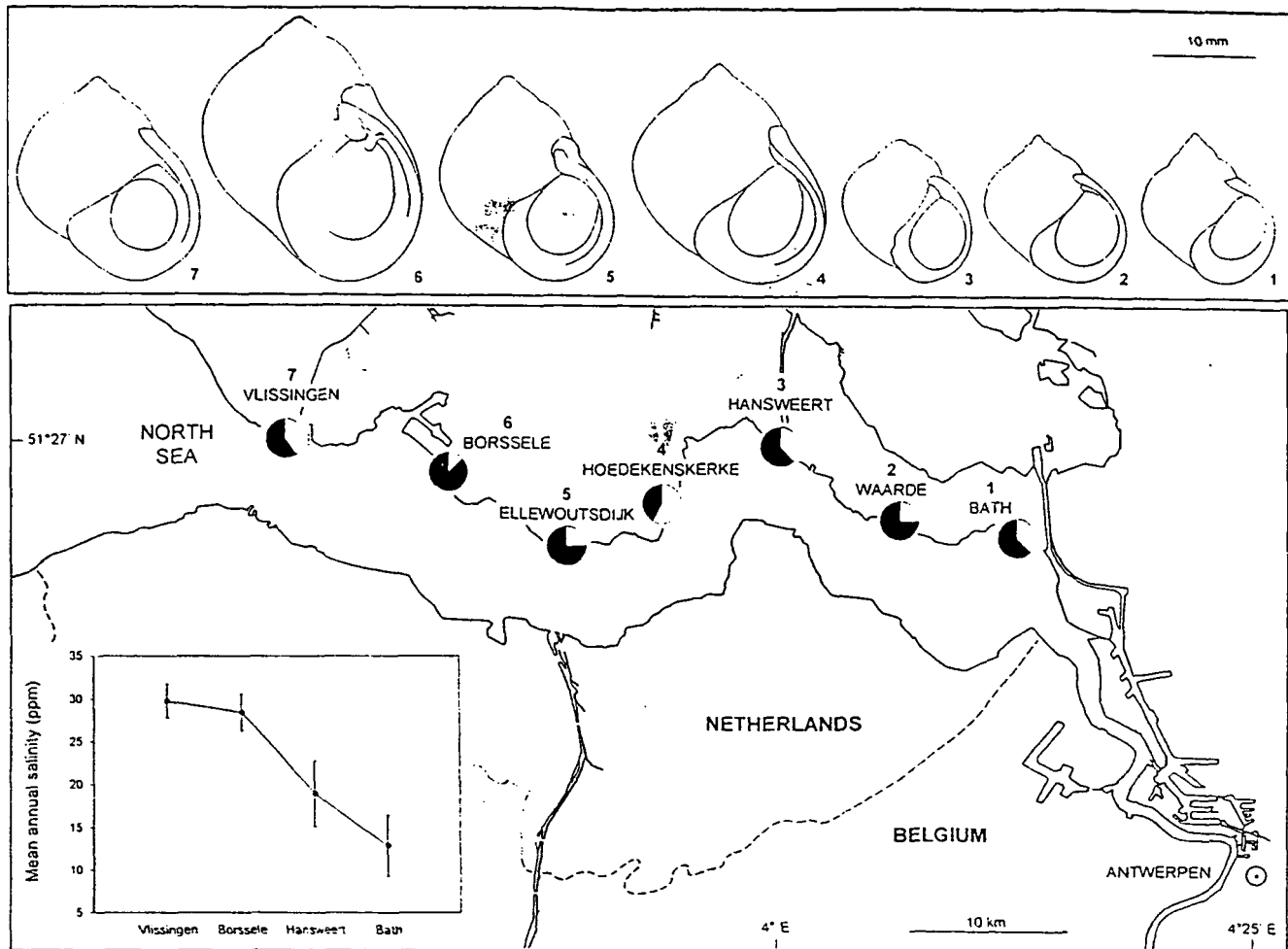


Figure 1. Sampling area, sites, and shells typical of each of the sampling sites (sites 1-7). Pie diagrams represent number of males (black zone) and females (white zone) collected at each site ( $n = 40$ ). The line graph represents mean salinity values and standard deviations along the Scheldt estuary on the basis of seasonal measurements from 1990-1997.

These results are illustrated in Figure 2, where the mean shell height and standard deviations for males and females are plotted for each sampling site. Mean shell height values for both sexes overlap but simultaneously reveal a structuring at the sampling site level. Mean shell height of specimens collected at the less marine-like sites (i.e., salinity range 10-20‰; Fig. 1) are on average smaller compared with the shell height of specimens collected more downstream at a salinity range of 21-30‰ (Fig. 1). However, due to individual variation this observation is merely a trend because post-hoc Sheffé tests failed to significantly discriminate both groups.

Given that, with respect to the measured shell characteristics,

TABLE I.

Results of the two-way MANOVA, contrasting the random factor "sampling site" and the fixed factor "sex."

Effect	Wilks' $\lambda$	df1	df2	P value
Site	0.183218	42	1,227	<0.0001
Sex	0.957741	7	261	0.1230
Site $\times$ Sex	0.844134	42	1,227	0.3448

males do not differ significantly from females, a single CDA was performed without considering the factor "sex." The mean values of the first two canonical variables (CV) are used to plot all sampling sites, as shown in Figure 3. The first CV describes 64.83% of the total variation and is mainly an expression of shell height (Table 2; HS = -1.11614). Shell height decreases with decreasing CV1 values, discriminating the different sampling sites, so that specimens collected at the least marine-like sampling sites are in general smaller than specimens collected at more marine-like sites (Fig. 3). The second CV describes an additional 19.83% of the total variation and is mainly an expression of the shell weight (Table 2; SW = 1.91514). Shell weight increases along the positive CV2 axis. Four groups can be distinguished along both CV axes: Bath and Waarde, consisting of specimens with small and light shells; Hansweert, consisting of specimens with small and relatively heavy shells; Ellewoutsdijk and Vlissingen, consisting of specimens with intermediate-sized shells and intermediate shell weights, and Borssele and Hoedekenskerke, consisting of specimens with larger and heavier shells. Hence, relative shell weight does not follow the salinity gradient. This is also illustrated in the ANCOVA, where the regression slopes of the shell weight on shell height are not homogeneous at the different sampling sites (Table

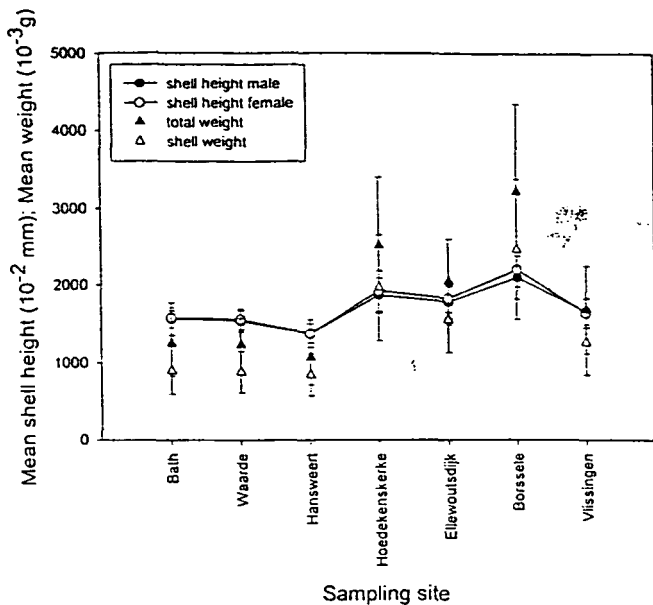


Figure 2. Mean shell height (HS), total weight (TW) (i.e., soft body weight + shell weight), shell weight (SW), and standard deviations for males and females of *Littorina littorea* collected at the seven sites.

3) and in Figure 2, where mean shell weight, mean total weight, and mean shell height are plotted at the different sampling sites.

#### DISCUSSION

As was predicted, *L. littorea* had smaller and lighter shells in more brackish conditions. However, shell size did not decrease clinally away from the sea, following the salinity gradient. Instead, specimens were either small (approximately <15 mm) or large (approximately >19 mm). No intermediate-sized animals were found at sites having intermediate salinity levels (i.e.,  $\pm 15\%$ ). If salinity is indeed an environmental factor that affects shell size in *L. littorea*, it seems that its effect is either present or absent with a threshold salinity value of approximately 15–20‰. Above this salinity threshold, animals are able to attain a large shell; below this value, animals never have comparable shell sizes. In either case it must be clear that, at brackish sites, *L. littorea* is likely to

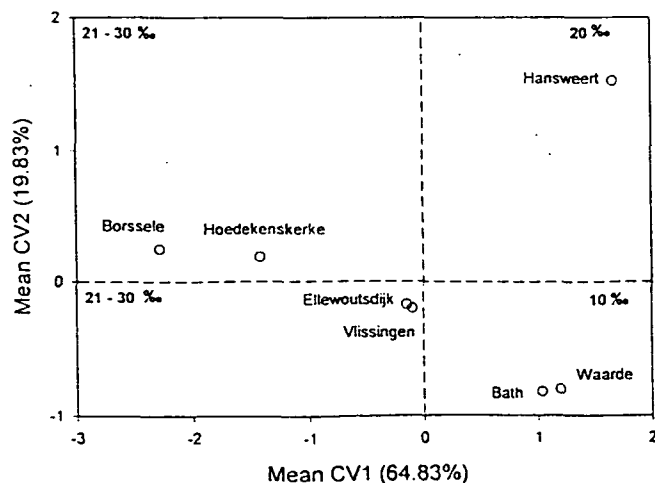


Figure 3. Mean values for the first two canonical variables.

TABLE 2.

Standardized coefficients for the first two canonical variables (CV) in a canonical discriminant analysis contrasting all eight sampling sites.

Dependent Variable	CV1	CV2
Shell height	-1.11614	-1.92037
Shell width	0.28414	0.81833
Aperture height	-0.71504	0.03916
Aperture width	0.68485	-1.47668
Shelltop height	0.57448	0.61353
Shell weight	-0.58913	1.91514
Eigenvalue	1.83935	0.56258
Explained variation, %	64.83	19.83

encounter less favorable living conditions, which might result in a decrease of growth and/or survival rate (Crothers 1992), shifting the less marine-like populations toward smaller-sized individuals. It must also be noted, however, that the largest shells were not recorded at Vlissingen (i.e., highest salinity) but at Borssele (i.e., second highest salinity). At Borssele, specimens were collected in the direct vicinity of a nuclear power plant. Possible water temperature differences, due to the outflowing cooling water, might affect the species shell growth. Indeed, larger shells can be produced at higher temperatures due to the lower energy cost of calcification because calcium carbonate dissolves less well at higher temperatures (Graus 1974, Clarke 1983). However, on a macrogeographical scale, *L. littorea* attains its largest size in the northern parts of its distribution range (i.e., cooler waters) (Reid 1996). Clearly, further experimental work is needed to clarify and explain the possible effects of water temperature and salinity on shell deposition in *L. littorea*.

In the case of shell weight, the presumed relationship with salinity was even less clear. Moreover, it seemed unlikely that relative shell weight differences can be explained by salinity differences. The top right positioning of the Hansweert population in the CDA graph (Fig. 3) indicates that specimens with the highest relative shell weight occur under the third lowest salinity conditions.

TABLE 3.

Results for the interaction of the dependent variable shell weight (SW) and the covariate shell height (HS) in the ANCOVA (i.e., test for parallelism) and corresponding regression equations at each of the seven sampling sites.

	SS	df	MS	F Value	P Value
Effect	3.811.414	6	635.235.6	20.06198	<0.0001
Error	8.454.196	264	31663.7		

Site	Regression equation	r <sup>2</sup>
Bath	HS = 1.059.5 + 0.5614 SW	0.8928
Waarde	HS = 1.133.3 + 0.4672 SW	0.8832
Hansweert	HS = 913.2 + 0.5549 SW	0.8485
Hoedekenskerke	HS = 1.258.3 + 0.3239 SW	0.8452
Ellewoutsdijk	HS = 1.082.9 + 0.4553 SW	0.9103
Borssele	HS = 1.398 + 0.2897 SW	0.9078
Vlissingen	HS = 1.169.3 + 0.3844 SW	0.8737

Abbreviations: SS = Sum of squares; MS = Mean square.

Sexual dimorphism is common in the genus *Littorina*, with females being larger than males (Reid 1996), which is presumed to be related to growth and/or longevity differences (Reid 1996). However, sex-related shell height differences have not been found in *L. littorea* (Reid 1996), even though sexual selection for female shell size has been documented, with males preferring to mate with larger, and thus more fecund, females (Erlandson & Johannesson 1994). The fact that we did not find sex-related shell size differences is thus in agreement with what was previously found. However, our sex assignment, made on the basis of the presence or absence of a penis, leads to a sex ratio of almost 2:1 (male/female), which differs markedly from a previously published sex ratio of 1:1 (Daguzan 1977). The fact that we found twice as many males might be related to the presence of imposex—the development of male sex characteristics on females (e.g., a penis and/or vas deferens) (Bauer et al. 1997)—and/or intersex—the disturbance of the phenotypic sex determination between the gonad and genital tract—which is known to occur in *L. littorea* (Bauer et al. 1997). Imposex has never been recorded in *L. littorea*. In contrast, intersex gradually transforms the female pallial tract such that the female pallial organs are supplanted by a male prostate gland, and a seminal groove and a small penis occur (Bauer et al. 1997). The fact that penis shedding and/or regression also occur in *L. littorea* (Deutsch & Fioroni 1992) makes it even more difficult to distinguish between an intersex female and a male with a shed or regressed penis. Therefore, it could be that some specimens that were classified as males were in fact intersex females. However, female intersex expression occurs only in juvenile stages or during sexual immaturity (Bauer et al. 1997). As a consequence, intersex females are expected to have a smaller shell. Hence, if intersex females were included in the male population, they would not have increased the mean male shell height, masking possible shell size

differences with the presumed larger females, but rather would have decreased the mean shell height of the male population. In any event, the occurrence of penis shedding, penis regression, and intersex make basing sex assignment in *L. littorea* on the presence of a penis unreliable. Similarly, the presence or absence of a prostate gland is also an unreliable sex-determining character (Bauer et al. 1997).

Finally, salinity is not the only environmental factor that may be correlated with the morphology of *L. littorea*. In an estuary, which is structured by a complex of gradients, a wide variety of natural and human-induced stresses are present that may affect the shell morphology of estuarine gastropods. In this respect, in the period 1981 to 1983, the Scheldt estuary was ranked among the most heavily polluted estuaries around the world for both the dissolved as well as the particulate metal phase (Bayens 1998). Dissolved metal concentrations measured at Hansweert and Vlissingen differ significantly, with Vlissingen being less polluted (Rijksinstituut voor Kust en Zee, RIKZ, pers. comm.). Concentrations of volatile organic compounds are high and decrease along the estuary as well (De Wulf et al. 1998). Clearly, pollution is an important potential stressor in the Scheldt estuary, and its effect on the morphological population structure of the estuarine organisms must be investigated.

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