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Sex-related differences in growth and morphology of blue mussels

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The morphology and growth pattern of male and female blue mussels (*Mytilus edulis*) from the north Norfolk coast, UK, were studied. In allometric terms, the external shell parameters of females grew faster relative to shell length than those of males. In absolute terms, females also grew more quickly than males for all external shell parameters and for most internal body parts. At a given age, females are therefore larger than males. Females had a higher shell to tissue weight ratio and a relatively heavier foot than males. A discriminant function incorporating age, weight and shell length, width, and height correctly sexed 81% of individuals in the sample from which it was derived. Both natural and sexual selection may be involved in the evolution of sexual dimorphism in blue mussels.

INTRODUCTION

Sexual dimorphism, particularly in body size, is common in many animal taxa and is usually interpreted as the result of sexual selection (Hedrick & Temeles, 1989; Andersson, 1994). Differences in morphology between the sexes can also arise through natural selection (Shine, 1989), for example through differential foraging specialization (Hulscher & Ens, 1992; Temeles et al., 2000) or risk of predation (Goetmark et al., 1997).

Sexual dimorphism occurs only sporadically throughout the phylum Mollusca. Sexual differences in shell morphology have been noted in five species of freshwater snails (Kantor & Sysoev, 1991; Brande et al., 1996; Estebenet, 1998; Kurata & Kikuchi, 2000). Some shallow-water octopuses also show secondary sexual dimorphism in body size (Voight, 1995). Among bivalves, reports of sexual dimorphism in shell shape and size appear to be limited to species in freshwater genera, such as *Lampsilis* spp., *Truncilla* spp., *Unio* spp., *Astarte* spp., *Castalia* spp. and *Villosa* spp. (Ortman, 1921; Coe, 1943; Avelar et al., 1991; West & Metcalfe-Smith, 2000). *Transennella* spp. are the only known sexually dimorphic marine bivalves (Ruiz, 1991). It is not clear whether this paucity of records of sexual dimorphism reflects the fact that most bivalves are truly monomorphic, or whether it is simply a consequence of the inherent difficulties in sexing bivalves by visual gonad inspection, particularly during non-breeding periods.

In this paper we investigate sexual dimorphism in the most widely studied marine bivalve, the blue mussel *Mytilus edulis* L.. A vast literature exists on all aspects of the ecology, development and growth of blue mussels (reviews in Bayne, 1976; Gosling, 1992). However, no external signs of sexual dimorphism have yet been reported for this species (Seed, 1969, 1976). We re-examine this conclusion by comparing the shell characteristics, internal tissue weights and growth patterns of male and female blue mussels, collected from a common location and for which gender was determined biochemically.

MATERIALS AND METHODS

Sixty-one mussels were collected haphazardly from the intertidal zone at Stiffkey Marsh on the north Norfolk coast in February 1994. Mussels from that area occur in small clumps embedded in compacted mud, gravel and sand rather than on rock.

Morphological measurements

Shell length (antero-posterior axis), height (dorso-ventral axis), and width (lateral axis) were measured to the nearest 0.1 mm with vernier calipers. Whole live weights with shells closed, and wet weights of shell and total internal tissues were determined to the nearest 0.0001 g. We also weighed mantle tissue, foot and byssus gland (combined and referred to as 'foot'), anterior adductor and byssal retractor muscles, and posterior adductor and pedal retractor muscles (all four combined and referred to as 'muscle') separately. The tissues were then dried to a constant weight at 65°C for 72 hours. For biomass estimation, dry weights of foot, muscles, mantle and total internal tissue were measured at the same standard lengths using an analytical balance.

Sex determination

The sex of mussels was determined visually, when possible, and biochemically. The presence of sperm or ova from gonadal samples was noted during examination under a binocular microscope. To sex mussels in non-breeding condition we used the method described by

Jabbar & Davies (1987). A sample of mantle tissue (20–50 mg wet wt) was placed in a glass tube with 20% (w/v) trichloroacetic acid solution (2 ml) and anti-bumping granules. After the addition of freshly prepared 0.75% (w/v) thiobarbituric acid solution (0.5 ml), the tube was covered with a glass marble and placed in a simmering water bath. The sexes produce two different chromophores (males: yellow; females: pink), allowing a visual determination of gender after 5–10 minutes. Results of the biochemical assay were confirmed by testing a small sample of mussels in breeding condition whose sex was ascertained by visual examination of the gonads.

Age determination

Mussel age was determined using a method modified from Lutz (1976), Richardson (1989) and Richardson et al. (1990). Shell sections were cut anterio-posteriorly and the cut radial sections ground on a glass plate using a silicon carbide grit (600 grit) for 1 min and passed through an ultrasonic cleaner and detergent. Polishing took place on a moving polishing plate with diamond paste (6 micron) on a paper lap for 2 min and cleaned with trichloroethane. The radial sections were etched for 1 min in 2% hydrochloric acid and then rinsed in distilled water and dried. The shell was mounted on an aluminium stub and painted (except for the radial section) with silver paint and coated in gold, 10Å thick. The ultrastructural crystalline patterns within the shell sections were examined with a Hitachi S800 Field emission scanning electron microscope. The number of dark bands, which represent the number of winters experienced since settlement, was recorded. Five control mussels of known age were also analysed and confirmed the accuracy of the method.

Mytilus edulis attains sexual maturity during its first year (Seed, 1969, 1976; Seed & Suchanek, 1992). Therefore, all mussels older than one year were deemed sexually mature.

Allometric and absolute growth models

Using information on shell characteristics and age, we estimated both allometric and absolute growth of male and female mussels. The Gompertz equation ($\text{Length} = K\exp(-\alpha e^{-\lambda t})$) was modelled to the data to estimate absolute growth rates (Theisen, 1973; Bayne & Worrall, 1980).

Statistical analysis

In modelling relative growth, cubic relationships between shell characteristics became linear when data were log-transformed. The slopes and elevations of regression coefficients for males and females were then compared using Student's *t*-tests (Zar, 1999). The Gompertz equation was modelled to the data using the NLIN procedure in SAS (SAS Institute 2001, version 8.02). For ease of graphical representation, predicted values at each 0.5 y interval were plotted against age. To compare the slopes and elevations of regression coefficients between males and females, absolute growth data were plotted on logarithmic scales to obtain linear regressions when necessary. In the analyses of absolute growth, the original sample size of 61 (males = 33, females = 28) was reduced to 28 males and 23 females due to unclear banding pattern in some individuals. The sample was further reduced in some of the analyses owing to difficulties in dissecting some body parts, especially the muscles.

Since male mussels in our sample were slightly older than females (independent samples *t*-test; $t_{60} = 1.67$, $P = 0.099$), the effect of age was removed in comparisons of male and female characteristics by using analyses of covariance (ANCOVA), where age was the covariate.

A discriminant analysis was carried out using shell length, width and height, total weight and age measurements from 26 known males and 21 known females from the original sample. A discriminant function was calculated and the percentage of cases that were correctly sexed by the linear function was established. However, using the same mussels for function estimation and testing can produce overly optimistic estimates of the success of the classification. Therefore, to obtain a better assessment of the discriminant function's reliability, we used the 'leave-one-out' cross-validation or 'jack knifing' method provided by SPSS for Windows (v. 10.1; SPSS Inc. 2000).

RESULTS

Allometric growth

Growth patterns of various body parameters relative to shell length for male and female mussels are shown in Figure 1. In both sexes, shell width and height increased isometrically with shell length. The rates of increase of shell width and height relative to shell length were significantly greater for female mussels than for males (Table 1).

Each sex showed cubic relationships between shell weight and shell length. After log-transformation, a comparison of slopes revealed a significantly greater rate of shell weight increase per unit shell length for females (Table 1). This difference was not driven by the presence of very small females in the sample since the difference remained when these data were omitted (slopes of shell weight relative to shell length: female = 2.70±0.15, male = 2.56±0.1, $t_{52} = 8.26$, $P < 0.001$).

Internal tissue wet weight was related to shell length in a cubic fashion for both male and female mussels (Figure 1). There was no sex difference in either regression slopes (Table 1) or regression intercepts ($t_{52} = 1.13$, $P < 0.05$).

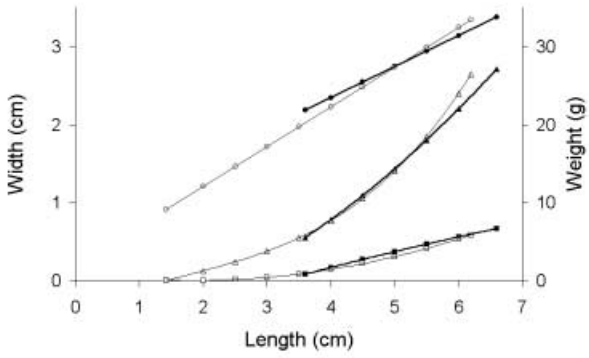

Figure 1. Relationship between various mussel parameters as a function of shell length for blue mussels. ●, shell width; ▲, shell weight; ■, tissue wet weight. Filled shapes, male mussels; open shapes, female mussels. Shell height is not shown as it closely resembles shell width.

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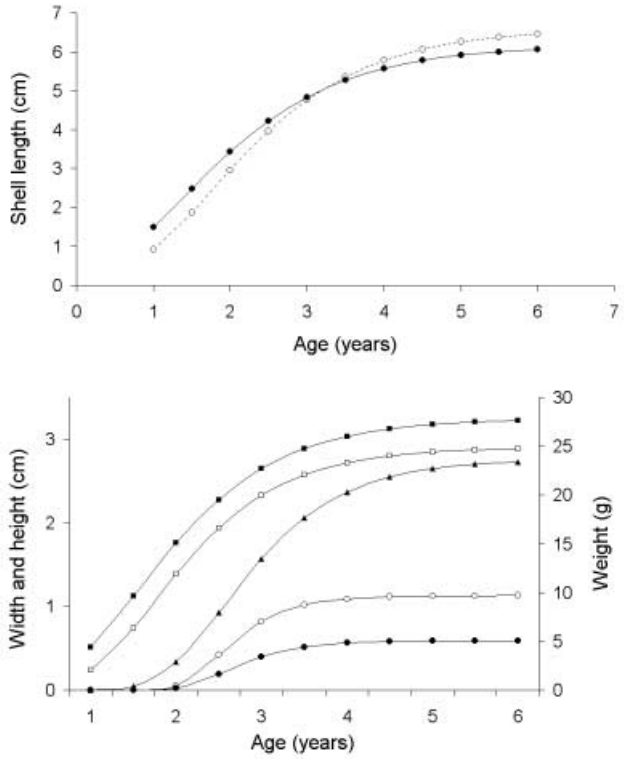

Figure 2. Gompertz growth curves for (A) shell length in male and female mussels, with filled circles, males; and open circles, females; and (B) shell height (■), width (□), weight (▲), tissue wet weight (○) and tissue dry weight (×10, ●) in mussels.

Absolute growth

Shell length

Shell length was variable over all ages, especially for the smaller individuals. Male and female mussels attained lengths of 5–6 cm after 3.5 y (Figure 2A). Female shell length increased significantly faster over time than male length when regression slopes were compared (Table 1). Females are shorter than males until 3 y of age and become longer thereafter.

Shell height, width, shell and tissue weights

All body parameters measured were related to age in a sigmoidal manner (Figure 2B). The relationships became linear when plotted on logarithmic scales and a comparison of slopes showed that there were significant differences between the sexes in all cases. Female shell length, height, width, weight all increased significantly faster over time than those of males (Table 1). Similar differences were observed for total tissue, muscle, mantle and foot wet weights, as well as foot dry weight (Table 1). However, there was no difference between the sexes in rate of increase of dry weights of total tissue, muscle or mantle over time (Table 1).

Relative allocation to body parts

Allocation to specific body parts was calculated as a fraction of the total mussel weight including the shell. Significant differences were revealed between the sexes for shell and total internal tissue allocation independently of age in all mussels. Allocation to the shell was significantly greater in females (ANCOVA, $F_{1,50}=10.77$, $P=0.002$) and allocation to total internal tissue was significantly greater in males (ANCOVA, $F_{1,50}=10.78$, $P=0.002$). Females also showed a greater allocation to both wet and dry foot weight compared to males (foot wet weight: ANCOVA, $F_{1,38}=11.85$, $P=0.001$; foot dry weight: ANCOVA, $F_{1,37}=8.88$, $P=0.005$). No further differences were found between the sexes in their allocations to internal body parameters.

Table 1. *Allometric and absolute growth: comparisons of regression slopes of various body parameters vs shell length or age between male and female mussels.*

Body parameter	Mean female slope	Mean male slope	*t*	N	*P*
Allometric growth					
Shell width	0.51±0.02	0.45±0.04	5.82	61	<0.001
Shell height	0.51±0.02	0.40±0.03	15.53	61	<0.001
Shell weight	2.94±0.07	2.56±0.10	19.99	61	<0.001
Tissue wet weight	1.96±0.10	1.96±0.15	0.02	56	n.s.
Absolute growth					
Shell morphology:					
Length	0.85±0.10	0.45±0.06	8.99	51	<0.001
Height	0.96±0.11	0.44±0.08	10.2	51	<0.001
Width	0.80±0.10	0.37±0.05	9.91	51	<0.001
Wet weights:					
Total shell	2.53±0.28	1.18±0.16	10.69	51	<0.001
Total tissue	2.88±0.34	1.37±0.22	9.67	51	<0.001
Muscle	0.13±0.03	0.07±0.03	−4.67	48	<0.001
Mantle	1.53±0.23	1.23±0.01	−2.66	51	<0.01
Foot	0.06±0.01	0.03±0.01	−8.97	51	<0.001
Dry weights:					
Total tissue	1.33±0.19	1.30±0.22	−0.28	49	n.s.
Muscle	19.8±6.09	16.2±4.46	−1.78	43	n.s.
Mantle	246.8±43.7	228.9±48.1	−0.86	49	n.s.
Foot	13.31±1.75	6.75±1.90	−8.09	50	<0.001

n.s., not significant.

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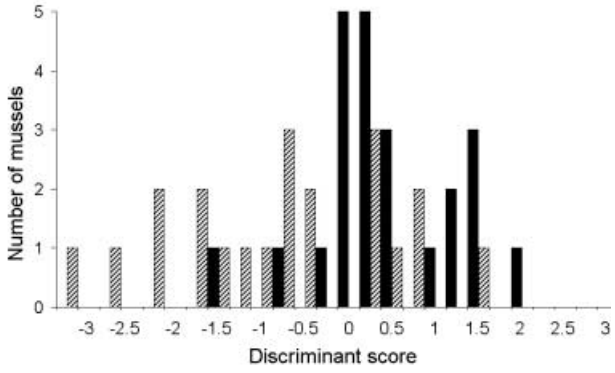

Figure 3. Frequency of male (■) and female (▨) mussels in relation to discriminant scores calculated from a Fisher linear equation. See Results section for details.

Discriminant analysis

Multivariate discriminant analysis of un-standardized scores of five measured characters confirmed the distinctness of the two sexes based on morphometric measurements. An un-standardized discriminant function was obtained for shell length, width and height, age and total weight. The Fisher linear function best discriminating between male and females was as follows:

$$D = 3.5\,L + 0.7\,A + 0.03\,W - 3.0\,H - 0.27\,MW - 6.2 \quad (1)$$

where: L=shell length, A=age, W=shell width, H=shell height, MW=total mussel weight. Positive D scores indicated males and negative D scores indicated females (Figure 3). Using this canonical discriminant function, 81% of the original 47 individuals were assigned to the correct sex. Correct classification was higher for males (92%; 24 males) than for females (67%; 14 females).

DISCUSSION

Male and female blue mussels differ significantly in shell dimensions at comparable ages, in patterns of growth rates and in patterns of weight allocations to shell and internal tissues. In general, mature female mussels were larger and grew faster than males at a given age and they invested relatively more heavily in their shells and foot. These differences were sufficiently marked to allow the correct classification of gender on the basis of external morphology and age for 81% of mussels. Differences between the sexes had not been previously reported for *Mytilus edulis*.

Mussel growth and shape are influenced by factors such as seasonal and annual cycles, temperature, light, population structure, food supply and level on the shore, salinity, genetic predispositions and pollution (Seed, 1976; Seed & Suchanek, 1992). However, none of these factors can explain the differences in mussel growth and morphology between the sexes found in this study since all the mussels were collected at the same time, from the same location.

Sexual dimorphism in blue mussels may reflect differences in either defence or reproductive strategies between the sexes. Mussels have two morphological lines of defence against predators: the active secretion of byssal threads by the foot for anchorage to the substratum and the passive protection afforded by their shells. Byssus production is known to respond quickly to perceived predation risk (Côté, 1995; Reimer & Tendegren, 1997; Reimer & Harms-Ringdhal, 2001). We found no differences in byssus production between males and females under controlled conditions (S.C. Mills, unpublished data), although females exhibited faster absolute foot growth and invested relatively more in their foot compared to males. It is therefore not clear whether foot size is related to capacity for byssal thread production. Blue mussels have also been shown to respond phenotypically to simulated risk of crab predation by producing shorter, thicker, more rounded shells, with relatively more meat per shell volume (Reimer & Tedengren, 1996; Reimer & Harms-Ringdahl, 2001). These characteristics were found in males in our study population. Males had shorter shells and invested relatively more in internal tissues compared to females of similar ages, which may suggest that they are under higher risk of predation.

Sexual dimorphism in *M. edulis* could also be related to sex-related differences in reproduction. Although sexual selection may generally be weaker in broadcast-spawning than in internally fertilizing species, it can act nonetheless. For example, selection on females for high fecundity in the face of high planktonic zygote mortality could result in more voluminous shells for females which can provide more space for eggs. By contrast, gamete production in males is not likely to be constrained by size (Trivers, 1972; Wiklund & Karlsson, 1988). Alternatively, males and females may maximize their fertilization success in different positions or orientations within clumps in the wild. If, for example, the centre of a clump is a better location from which females can release their eggs, the lower risk of predation associated with central locations (Okamura, 1986) may select for longer, wider shells in females rather than the rounder, stouter shells necessary at the edges (Reimer & Tedengren, 1996). Such a scenario would indicate that natural and sexual selection are acting in concert to produce differences between the sexes in mussels. The position of individuals within clumps was not recorded in this study, but sex-specific patterns of distribution within mussel clumps or beds should be considered in the future.

In conclusion, we have shown the presence of sexual dimorphism in both growth rate and allocation to shell and internal tissues in one population of blue mussels. The causes of these sex differences and whether our findings can be generalized to other mussel populations remain to be assessed.

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