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Intraspecific egg size variation and sperm limitation in the broadcast spawning bivalve *Macoma balthica*

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ABSTRACT

Broadcast spawners are exceptionally suited and simple models for studying parental investment in offspring, because direct post-spawning investment is nonexistent. However, a comprehensive understanding of the large variation that exists in their egg sizes is still lacking. One of the main hypotheses states that variation in fertilization conditions underlies some of the egg size variation, as larger eggs are larger targets for sperm. Here, we test the hypothesis that egg size may be locally tuned to expected ambient sperm concentrations during fertilization. In accordance with the hypothesis, we find that in the bivalve *Macoma balthica* (L.) adult density as a proxy for sperm concentration correlates strongly (correlation coefficient −0.87) with egg size in the field. Optimisation modeling confirms the negative relationship between optimal egg size and sperm concentration for *M. balthica* and this is independent of the fertilization model used. Discrepancies between models and observations remaining include larger egg sizes overall and a concave predicted relationship that is not obvious in the data. The results suggest that in *M. balthica* sperm limitation may play a role in fertilization success and in shaping egg size variation, and that locations with high population densities may make disproportionately large contributions to the next generation.

1. Introduction

One of the central themes in the study of life history evolution is parental investment in offspring (Trivers, 1972). A principal trait in this respect is egg size. Marine broadcast spawning invertebrates (i.e., with external fertilization) have formed excellent empirical study systems, in which post-spawning maternal investment is necessarily entirely contained within the eggs themselves. This implies an important simplification of a complex problem that includes post-spawning or post-natal investment. In spite of this simplification, understanding natural egg size variation in broadcast spawners remains a challenge (Bernardo, 1996; Marshall et al., 2000; Levitan, 2006; Bode and Marshall, 2007; Marshall and Keough, 2008a,b).

Egg sizes in broadcast spawning taxa tend to vary considerably among (e.g. Lessios, 1987; Levitan, 2000; McEdward and Morgan, 2001) as well as within species (e.g. Lessios, 1987; Bertram and Strathmann, 1998; George, 1999; Miles et al., 2007; Phillips, 2007; Marshall and Keough, 2008a,b). Concepts based on optimality theory first and foremost explain egg size as the product of a trade-off with egg number (Vance, 1973a,b; Smith and Fretwell, 1974): females should always produce the maximum number of eggs that are, as a consequence, of minimal size for development, unless larger egg size conveys benefits that increase disproportionally with size. The two benefits of larger size to the offspring are higher fertilization probability and an increased chance of zygote survival. Sperm is necessary to start egg development in the majority of species and can be a limiting resource for broadcast spawning females (Levitan, 1993, 1995, 1998; Yund, 2000); however, sperm can also be too abundant and lead to polyspermy (Styan, 1998). A change in sperm availability could lead to a different optimal egg size (Levitan, 1993). In the case of sperm limitation there are strong indications that intensified sperm limitation should theoretically translate into a larger optimal target size (Jantzen et al., 2001; Podolsky, 2002, 2004; Luttikhuizen et al., 2004; Levitan, 2006), and this relationship should reverse in case of adverse effects of polyspermy (Styan, 1998). Larger target size may be achieved by increasing egg size, or, alternatively, by increasing the size of accessory structures such as jelly coats (Podolsky, 2004) or, chemically rather than physically, by increased secretion of chemo-attractants (Jantzen et al., 2001). Remarkably, the hypothesis that egg size varies intraspecifically with sperm limitation has not yet been directly tested (see also Levitan, 2006). However, Crean and Marshall (2008), having set out to test egg size plasticity in the ascidian *Styela plicata* in relation to sperm competition, reached the conclusion that sperm limitation might better explain their data.

Pleas for examining intraspecific in addition to interspecific patterns to test life history theories were made by Bernardo (1996) and several others (see references in Bernardo, 1996), because the transfer of interspecific patterns to the intraspecific level has often proven elusive. At the interspecific level, many factors (including phylogenetic, environmental, morphological, physiological) covary to

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produce patterns among groups that are well separated by space and time. Ideally, tests should take place at a level where selection may reasonably be assumed to take place. This is the case for the populations studied here, as gene flow as well as differential selection have been demonstrated to occur at this scale (Luttikhuizen et al., 2003a,b; Drent et al., 2004).

Here we present the results of an intraspecific test of the hypothesis that increased sperm limitation selects for larger egg sizes in the sedentary, broadcast spawning bivalve *Macoma balthica* (L.). This taxon, the Baltic tellin, is a circumpolar species of the northern hemisphere that lives in the shallow subtidal to the high intertidal, burrowed in mud or sand. It has a single spawning season per year in most places (Harvey and Vincent, 1989) and fertilization of its eggs, that bear no accessory structures, is external. The fertilized eggs develop for about three days without feeding and the larvae are planktotrophic (see Drent 2002). It occurs in a range of natural population densities of approximately 1–500 adult individuals per m^2 (see Bocher et al., 2007) and equal sex ratio (Caddy, 1967). Adult density may be regarded as an inverse measure of sperm concentration during external fertilization because with decreasing adult density, male density decreases similarly, and, all else being equal, the ambient sperm concentration during fertilization can be expected to be lower. We measured egg sizes in three different years at several locations and correlated this with population densities, predicting a negative correlation. Verification of the interpretation of the data is done by optimisation modeling, determining optimal egg size by maximizing expected reproductive success of female *M. balthica* as a function of sperm limitation.

2. Materials and methods

In early spring, at which time the eggs are ripe and the animals are ready for spawning (Caddy 1967; Honkoop et al., 1999), of three different years (1995, 1996 and 1999) we collected *M. balthica* using a grab and sieve from a boat (for subtidal sites) or a corer and sieve (for intertidal sites). In both 1995 and 1996 samples were taken at three intertidal sites, and in 1999 at two intertidal and three subtidal sites (see Table 1). Sampling sites were distributed over the western Dutch Wadden Sea and the adjacent North Sea (Fig. 1). The bivalves were individually induced to spawn by administering a temperature shock of about 10 °C (Honkoop and van der Meer, 1997; Honkoop et al., 1999). A female's batch of eggs was photographed twice from a flat microscopic slide, under a Zeiss stereo microscope at 63× magnification. Eggs were measured from the projected slides as described in Honkoop and van der Meer (1997). Two perpendicular measurements of the diameter of each egg were taken and averaged per egg, for 30 (1995 and 1996 data) or 15 (1999 data) eggs per female. For each site, eggs of on average 28 females were measured (for exact numbers see Table 1).

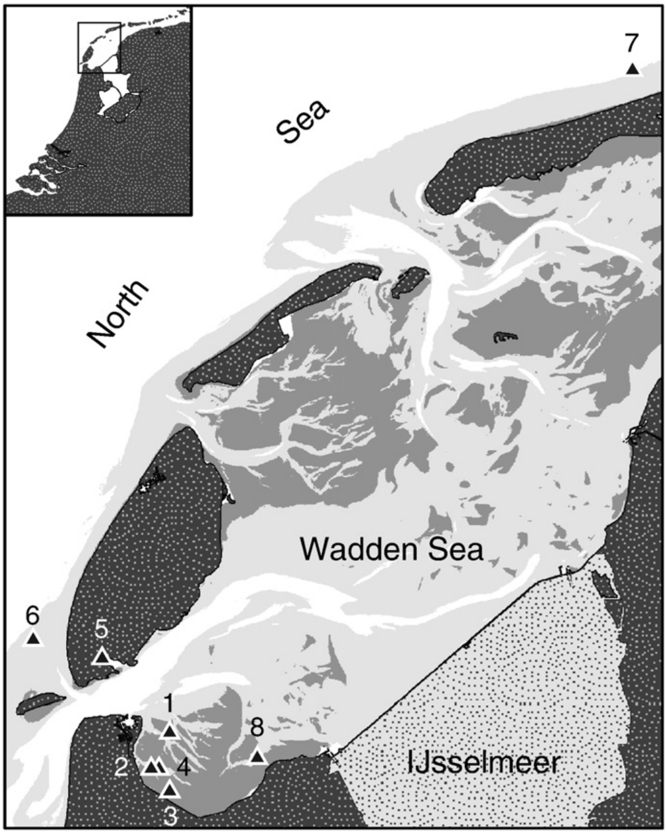

Fig. 1. Map showing sampling locations (triangles) of *Macoma balthica* in Dutch Wadden Sea and North Sea. Location numbers: see Table 1.

For 1995 and 1996, local adult density (number of individuals per m^2 with shell length exceeding 10 mm) at the time of spawning was obtained from the benthos monitoring data base of J. Beukema and R. Dekker (Royal Netherlands Institute for Sea Research, see e.g. Beukema et al., 2002). In 1999, local adult density was estimated at the time of collection for our spawning experiments.

Fitness (f) of individual females producing eggs of variable size was defined as the expected number of recruits per female and modeled as the product of number of eggs produced (n), fertilization probability per egg (p) and survival probability per zygote (s):

$$f(x,y) = n(x)\cdot p(x,y)\cdot s(x)$$

where x = egg volume (mm^3) and y = sperm concentration (μL^{-1}). Optimal egg size was found by maximizing f. This was done for a range

Table 1
Egg size of *Macoma balthica*: summarized data per year per site. 'se' = standard error among female averages; 'CV' = coefficient of variation. Egg size data for 1995 and 1996 were reanalyzed from Honkoop and van der Meer (1997).

Year	Site	Position	$n_{females}$	Adult density (ind/m^2)	Av. egg diameter (μm) (se)	CV overall	CV among female
1995	1 Balgzand A	52°57.00′N 4°50.39′E	23	66	107.8 (1.19)	6.7	5.3
	2 Balgzand B	52°55.30′N 4°48.84′E	20	189	104.1 (0.81)	5.0	3.5
	3 Balgzand C	52°54.28′N 4°50.54′E	24	224	100.5 (0.49)	3.7	2.4
1996	1 Balgzand A	52°57.00′N 4°50.39′E	46	73	108.5 (0.90)	7.3	5.6
	2 Balgzand B	52°55.30′N 4°48.84′E	34	150	106.0 (0.82)	6.6	4.5
	3 Balgzand C	52°54.28′N 4°50.54′E	40	165	104.3 (0.76)	6.2	5.0
1999	4 Balgzand D	52°55.36′N 4°49.15′E	11	22	107.0 (0.64)	2.7	1.9
	5 Mok	53°05.00′N 4°45.80′E	43	56	106.6 (0.47)	3.8	2.9
	6 Molengat	53°01.44′N 4°40.08′E	30	5	108.4 (0.88)	6.4	4.4
	7 Terschelling	53°27.33′N 5°25.00′E	7	150	105.5 (1.99)	4.9	5.0
	8 Wierbalg	52°56.00′N 4°57.05′E	29	115	106.7 (0.59)	3.9	2.9
overall			307		106.1	6.5	4.7