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NOTE

The *Leptasterias* (Echinodermata: Asteroidea) species complex: variation in reproductive investment

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ABSTRACT: Egg diameter, the amount of protein per egg, and the number of eggs per individual produced did not differ between 2 morphologically and genetically distinct species of seastars, *Leptasterias epichlora* (Brandt) and *L. hexactis* (Stimpson). In these 2 closely related sympatric species, variability in egg quality might be mostly attributed to environmental factors rather than genetic constraints.

KEY WORDS: Egg size · Egg number · Protein content · Seastar

The systematics of small six-rayed seastars of the genus *Leptasterias* in the Puget Sound region (Washington, USA) has been controversial (Bush 1918, Fisher 1930, Chia 1966a, Kwast et al. 1990). Chia (1966a), Kwast et al. (1990), and Stickle et al. (1992) identified 3 morphologically distinct species, *L. epichlora* (Brandt), *L. aequalis* (Stimpson) and *L. hexactis* (Stimpson). Chia (1966a) found no differences in their reproductive cycle, embryology and brooding behaviour. He concluded that all six-rayed seastars in the Puget Sound region belong to one biological species, *L. hexactis*. Kwast et al. (1990) and Stickle et al. (1992) identified 2 genetically distinct forms of *Leptasterias* as species, *L. hexactis* and *L. epichlora*. The third form, resembling *L. aequalis*, was considered a hybrid between *L. hexactis* and *L. epichlora*. These authors stressed that these species are distinct. Most studies on the development, brooding behaviour, reproductive cycle and ecology did not distinguish among these 3 co-occurring forms of *Leptasterias* (Chia 1966a, b, 1968a, b, Menge 1972, 1974, 1975). This leads to the question as to whether the reproductive output varies among these 3 species and whether data on one of these species can be compared to the pooled data on all 3 species found in the literature.

Egg size, egg numbers, and the organic content of the eggs may vary within a single spawn of a single individual, among individuals from the same population, and among individuals from different populations or species (Emlet et al. 1987, McEdward & Carson 1987, George et al. 1990, McEdward & Chia 1991). Closely related species can have similar egg sizes or a broad range of egg sizes (Emlet et al. 1987). The present paper investigates the use of these reproductive parameters to clarify the *Leptasterias* species complex. *L. epichlora* and *L. hexactis* were used because they were the most abundant species in the Puget Sound region.

Materials and methods. *Leptasterias epichlora* and *L. hexactis* were collected on 27 September 1991 from Deadman Bay (48° 30' N, 123° 09' W). Individuals were examined under a dissecting microscope and identified to species by ray shape, coloration, body size, and arrangement and abundance of minor and major pedicellaria (Kwast et al. 1990).

Wet body weight, egg size, numbers of eggs, and protein content of the eggs for both species were compared. Eggs were removed from the gonads by vigorously shaking and tearing the gonadal tissue in 0.45 µm filtered sea water. All eggs released incidentally during dissection were collected. The total number of eggs produced by a female was counted using a dissecting microscope. The diameters of 40 to 80 eggs per female were measured using a compound microscope equipped with an ocular micrometer. For protein analysis, samples of 10 eggs per female were held at − 80 °C. Five such samples were prepared for each female. The concentration of protein was measured by the method of Lowry et al. (1951).

Statistical tests were carried out with SAS® software (SAS Institute, Inc. 1988) and SYSTAT (Wilkinson 1989), using methods described by Sokal & Rohlf (1981)

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and Neter et al. (1990). Nested ANOVAs (balanced designs) with species as a fixed factor and females as a random factor were carried out to show variation in egg diameter and protein content among females and between species on untransformed data. In order to have the same number of levels for the nested factor (females), some females were randomly eliminated from the nested ANOVA analyses. Analysis of covariance (ANCOVA) tested the relationship between body wet weight (the independent variable) and the number of eggs produced. The number of eggs produced per female was square-root-transformed to correct heteroscedasticity.

Results. Nested analysis of variance on balanced data showed no significant differences in egg diameter and protein content per egg between the 2 species (Table 1). The mean egg diameter was 976.9 ± 110.9 µm (n, the total number of eggs measured = 576) and the mean protein content per egg was 40.5 ± 4.9 µg egg^{-1} (n, the total number of samples = 42) for *Leptasterias epichlora* and 971.9 ± 125.0 (n = 448) and 40.1 ± 8.6 (n = 41) for *L. hexactis*. The major part of the variation in egg size (42.6% of the total variation) and protein content per egg (45.0% of the total variation) was due to significant variation among females. The rest of the variation was among eggs produced by a single female and thus protein content per egg (Table 1).

For both species, the separate regressions of the number of eggs per female on wet body weight were linear ($F = 38.8$, $p < 0.0001$) and did not differ in slope ($F = 2.0$; Table 2, Fig. 1). No significant differences were observed in the number of eggs per female between the 2 species when wet body weight was kept constant ($F = 2.4$, $p = 0.1$; Table 2). The mean number of eggs produced was 541 ± 170 (n = 9) for *Leptasterias epichlora* and was 409 ± 275 (n = 11) for *L. hexactis*.

Discussion. The present study suggests that comparing data on one of these species with pooled data on all 3 species of *Leptasterias* as in Chia (1966a, 1968a) and Menge (1972, 1974, 1975) is valid. Despite morphological and genetic differences between these co-occurring species (Kwast et al. 1990, Stickle et al. 1992, Foltz & Stickle in press, Stickle & Foltz in press) egg sizes, egg numbers, and protein content per egg are similar. This might be because of the extensive hybridisation between these 2 co-occurring species (Kwast et al. 1990).

The present results are consistent with those of Emlet et al. (1987) in that significant variation in egg size, egg number, and the amount of protein in the egg was observed among females. In agreement with Chia (1966a) a strong correlation between the number of eggs produced and female body weight was observed. Large females of the seastar *Leptasterias epichlora* produce more large, higher quality eggs than small females (George 1994). The large seastar *L. polaris* (70 to 200 g wet wt) produces over 3000 eggs (Himmelman et al. 1982). Thus the major part of the variation

Table 1. *Leptasterias epichlora*, *L. hexactis*. Nested analyses of variance of egg diameter (µm) and protein content (µg egg^{-1})

Source of variation	SS	df	MS	*F*	p	Percentage of variance component
Egg diameter						
Species	82174.1	1	82174.1	0.5	0.5	
Females	3224310.3	18	179128.4	24.8	<0.0001	42.6
Error	4479752.1	620	7225.4			57.4
Total	7786236.5	639	12185.0			
Protein content per egg						
Species	13.8	1	13.8	0.1	0.7	
Females	1890.0	16	118.1	4.3	<0.0001	44.9
Error	1495.4	54	27.7			55.1
Total	3399.2	71	47.9			

Table 2. Analyses of covariance of the number of eggs per female (square-root-transformed) for *Leptasterias epichlora* and *L. hexactis*. Wet body weight is the covariate

Test	Source of variation	SS	MS	*F*
Test for linearity within species	Common slope within species	404.453	404.453	38.8***
	Deviations from a common slope	177.203	10.424	
Test for homogeneity of slopes of regression coefficients	Differences between separate slopes	19.887	19.887	2.0 ns
	Deviations in each species from its separate slope	157.238	9.827	
	Total	655.233		
Final ANCOVA	Adjusted means between species	24.636	24.636	2.4 ns
	Deviations from a common slope	177.203	10.424	

***p < 0.0001, ns: not significant

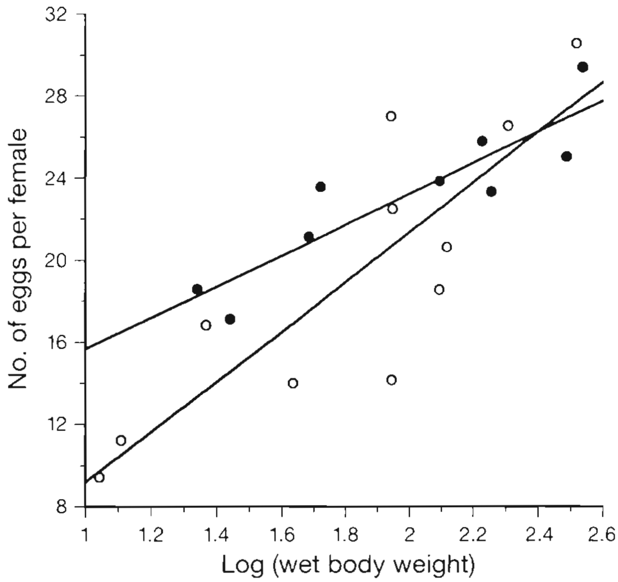

Fig. 1. *Leptasterias epichlora, L. hexactis.* Number of eggs per female (square-root-transformed) versus wet body weight (g) for the seastars *Leptasterias epichlora* (●) and *Leptasterias hexactis* (○)

observed in the reproductive parameters measured among females might be due to differences in female body size. Variation in egg number and quality might result from both genetic and environmental factors. As Stickle (1985) pointed out, production of somatic tissue and gametes by the seastar *L. hexactis* is strongly influenced by environmental factors. The degree of influence from environmental sources depends largely on conditions experienced by a female during the time energy is being acquired and converted towards oogenesis. Female *L. epichlora* from a favourable site produce large, higher quality eggs than females from a less favorable site (George 1994).

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