Does mating group size negatively affect female investment in the simultaneous hermaphrodite *APLYSIA punctata*?

P. Gianguzza,[1] L. Angeloni[2]

[1]Department of Earth and Sea Sciences, University of Palermo, Italy; [2]Department of Biology, Colorado State University, Fort Collins, CO, USA

Theoretical models and empirical studies have supported the idea that hermaphroditic sex allocation is sensitive to social conditions.[1,2] In general, when the mating group size equals two, both partners are expected to allocate as few resources as possible to sperm production, just enough to fertilize each other's ova, and devote all excess energy to egg production.[1] When the average number of possible partners increases, sperm competition for fertilization leads to greater investment in sperm, and resource allocation to female function is expected to decrease. However to our knowledge the relationship between mating group size and sexual behavior has been investigated in only a few species of outcrossing marine hermaphrodites.

The research presented here explores whether the sea hare *Aplysia punctata* (Cuvier, 1803), a simultaneous hermaphrodite, adjusts its investment in female function in response to social conditions. We experimentally manipulated the group size of mature *A. punctata* to determine whether individuals diminish female investment under different social conditions. We predicted that individual *A. punctata* would diminish egg mass production in larger social groups.

A total of 200 adult *A. punctata* (from 40 to 50 mm long) were collected within the rocky shore of the Ustica Island (Cala Sidoti 038'42.50N; 013°9.00E) from January through March 2006. After collection, sea hares were isolated over a period of 30 days in 35 L aquaria with circulating natural seawater, at ambient light (12 hours light:12 hours dark) and temperature regimes (22°C). *Ulva rigida* (C.Agardh 1823) was supplied daily *ad libitum*, to ensure continuous access to food.

A laboratory experiment was carried out in order to determine if egg mass production of *A. punctata* is related to the size of the social group. Following isolation, animals were randomly assigned to a group of two (P), three (T), four (Q) and eight (O) animals, with 10 replicates of each group size for a total of 170 animals, and constantly monitored for one month. These groups were maintained in tanks (50 cm in diameter, 40 cm in deep) filled with running seawater at a stable temperature and controlled light regime as described previously. All treatments were performed simultaneously and no *A. punctata* was used more than once to ensure the independence of data.

Following the treatments, each individual that had been observed mating as a sperm recipient was isolated and monitored for an additional 30 days in separate cages with continuous access to food. Every day we checked for the presence of a spawned egg mass. When an egg mass was detected, it was gently removed from the cage and its wet weight was recorded to the nearest 0.01 g. The proportions of egg masses showing no development (non viable eggs) and normal development (viable eggs) were determined for each egg mass. Differences in number and width of egg masses (a measure of female investment) were analyzed by two separate one-way Analyses of Variance (ANOVA), with *mating group* (MG), including its four levels (P, T, Q, O) as a fixed factor. Data were tested for normality with a Bartlett test. Homogeneity of variances was also checked with Cochran's C-test. Following the ANOVA, means were compared (at α=0.05) with Student-Newman-Keuls (SNK) tests. The GMAV 5·0 software (University of Sydney, Australia) was used to perform the statistical tests.

All egg masses produced subsequent to the experiment contained fertilized eggs indicating that exogenous sperm transfer had been successful. Egg masses were always laid by individuals that were observed to be the first member of a mating chain (the terminal sperm recipient) and never by an animal that acted as both sperm recipient and sperm donor at the same time. We observed that *A. punctata* laid fertilized egg masses during copulation, after few hours or, at most, 36 hours after copulation.

A total of 23 egg masses were produced: 10 in the P treatment (1 from each pair), 3 in the T treatment (2 from individuals that mated in a pair and 1 from a chain of three), 4 in the Q treatment (all from individuals that mated in a pair) and 4 in the O treatment (2 from individuals that mated in a pair,1 from a chain of three and 1 from a chain of four). All egg masses showed normal cleavage, development of embryos and the hatching of planktonic larvae within 7-10 days. Group size strongly influenced egg production ($F_{3,39}$=6.15; P=0.0017) and SNK results revealed that the number of spawned egg masses was greater for the P treatment than any other treatment (P>T=Q=O) and ranged from a maximum of 1.2±0.1 egg masses per individual (SE) (laid by individuals from P treatments; Figure 1) to a minimum of 0.3±0.1 (SE) (laid by individuals from T and O treatments; Figure 1). Moreover, egg mass size varied significantly as a function of the social condition ($F_{3,39}$=8.26; P=0.0003). In particular, SNK results showed that egg mass width was greater for the P treatment than any other treatment (P>T=Q=O) and ranged from a maximum of 26±3.8 (SE) mm (laid by individuals in the P treatment) to a minimum of 8.0±3.8 (SE) mm (laid by individuals in the T treatment).

Our findings showed that group size strongly influenced female function and that number and width of egg masses were greater for individuals maintained in pairs than for those maintained in larger social groups. These results indicate that *A. punctata* respond as

Correspondence: Paola Gianguzza, Department of Earth and Sea Sciences, University of Palermo, via Archirafi 22, 90123 Palermo, Italy.
Tel: +39.091.23862857.
E-mail: paola.gianguzza@unipa.it

Journal of Biological Research 2015; 88:5161

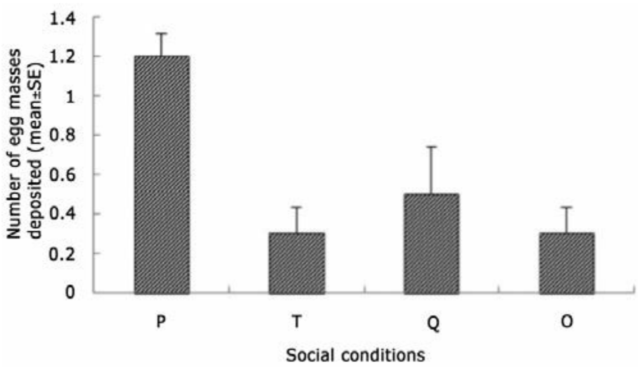

Figure 1. Egg masses laid by individuals from groups of two (P), three (T), four (Q) and eight (O) animals.

expected according to sex allocation theory for simultaneous hermaphrodites.[3] Furthermore, our experiments reveal that female allocation in this species is phenotypically flexible, as fully mature individuals were able to adjust their investment in the number and size of egg masses with the size of the social group.

References

1. Charnov EL. Simultaneous hermaphroditism and sexual selection. P Natl Acad Sci 1979;76:2480-4.
2. Charnov EL. The theory of sex allocation. Princeton, NJ: Princeton University Press; 1982.
3. Michiels NK. Mating conflicts and sperm competition in simultaneous hermaphrodites. In: A.P. Møller (ed.), Sperm competition and sexual selection. London: Academic Press; 1998. pp. 219-54.