Sexual reproduction of the orange dendrophylliid coral *Astroides calycularis* in the Mediterranean Sea

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Fig. 1: *Astroides calycularis: a*dult specimens photographed in Palinuro (Salerno, Campania, Italy, Southern Tyrrhenian Sea, SW Mediterranean)

This study examines the sexual reproduction in a temperate scleractinian coral, living in the southern part of the western Mediterranean Sea. *Astroides calycularis* (Pallas 1766) is an azooxanthellate coral that colonizes vertical walls, overhangs, cave entrances, and sea caverns with strong water movement, from the surface to 30m in depth. The colonies have been collected monthly, from April 2004 to September 2005 at Palinuro (Salerno, Campania, Italy) in the Southern Tyrrhenian Sea. This is the first in-depth investigation of the reproductive biology of this species. As expected for a member of the family Dendrophylliidae, *A. calycularis* was a gonochoric and brooder coral: colonies were sex separated, with all mature polyps in the same colony showing the same sex. Morphological aspects of male gametogenesis were similar to those described in other dendrophylliids coral. Female gametogenesis was characterized by the conspicuous presence of lipid droplets in the oocyte cytoplasm, which were of phagocytic origin. Preliminary quantitative data on the annual reproductive cycle indicate spring fertilization.

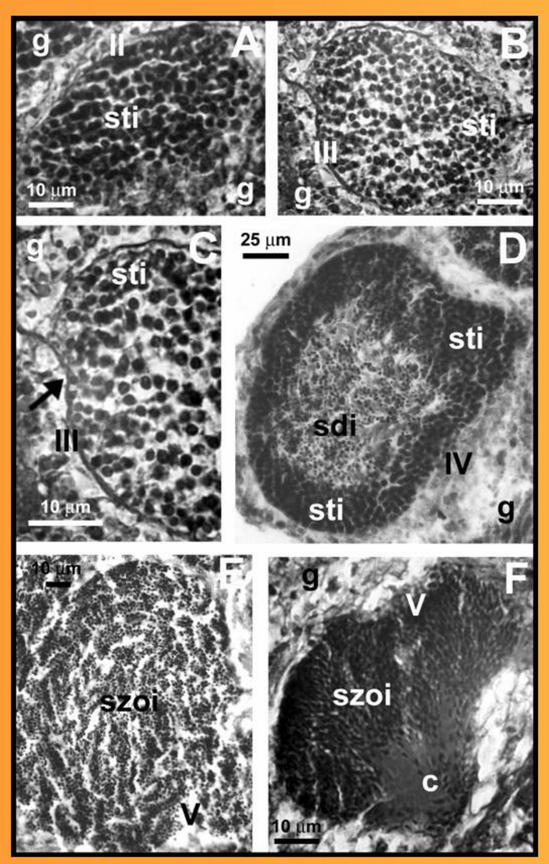

Fig. 2a-e: *Astroides calycularis*. Spermary developmental stages. (a) Stage II: the spermary is made up of a group of spermatocytes undergoing meiosis. (b) Stage III: the spermary, containing spermatocytes undergoing meiosis, is delimited by a wall. (c) Stage III: the wall of the spermary has arisen from the mesoglea (arrows). (d) Stage IV: the spermary presents an external layer of spermatocytes and an internal mass of spermatids. (e) Stage V: the spermary is made up of a mass of spermatozoa. (f) Stage V: shortly before leaving the spermary, mature spermatozoa form "bouquets," with their tails all facing in the same direction. [c: spermatozoa tails; g: gastrodermis; sdi: spermatids; sti: spermatocytes; szoi: spermatozoa; II, III, IV, V: spermary developmental stage]

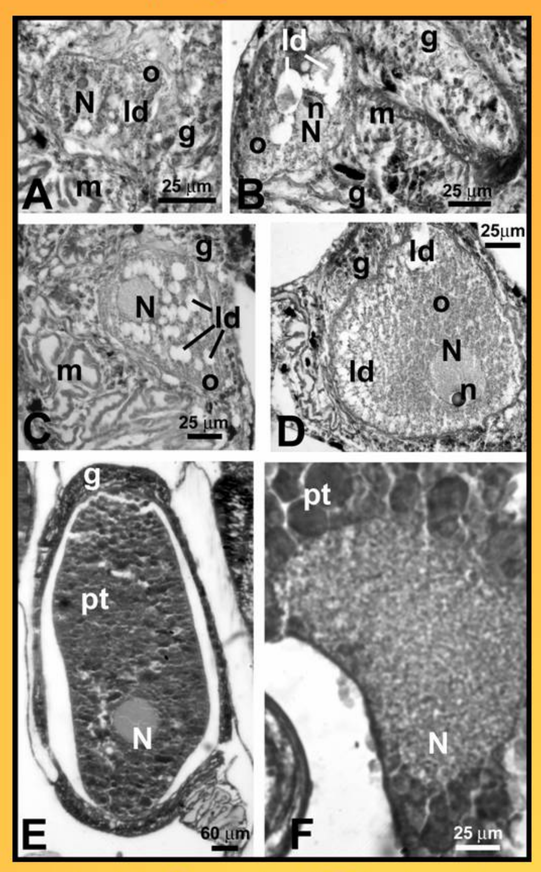

Fig. 3a-f: *Astroides calycularis*. Oogenesis. (a) Early stage: the previtellogenic oocyte located in the mesoglea of a mesentery is characterized by a high nucleus to cytoplasm ratio. Note the granular texture of the gastrodermis surrounding the oocyte. (b) Previtellogenic oocyte located in the central portion of one of the mesenteries. Note the conspicuous presence of lipid droplets in the oocyte cytoplasm near the nucleus. (c) Intermediate stage: vitellogenic oocyte located in the central portion of one of the mesenteries. The ratio nucleus to cytoplasm has diminished. Spherical nucleus is still located in the center of the cell. The cytoplasm has a homogeneous appearance. (d) Late stage: the ooplasm has started to differentiate, yolk plates are now present. The spherical nucleus with a single nucleolus has started to migrate towards the cell's periphery. (e) Late stage: the large mature oocyte, which is still located in a mesentery, is characterized by a differentiated ooplasm full of yolk plates, and by a peripheral nucleus. (f) Late stage: detail of the nucleus in a mature oocyte. It has become concave and is now located on the cell's periphery where it has begun to adhere to an invagination of the plasma membrane. The ooplasm is full of yolk plates. [g: gastrodermis; ld: lipid droplets; m: mesoglea; o: oocyte; N: nucleus; n: nucleolus; pt: yolk plates]

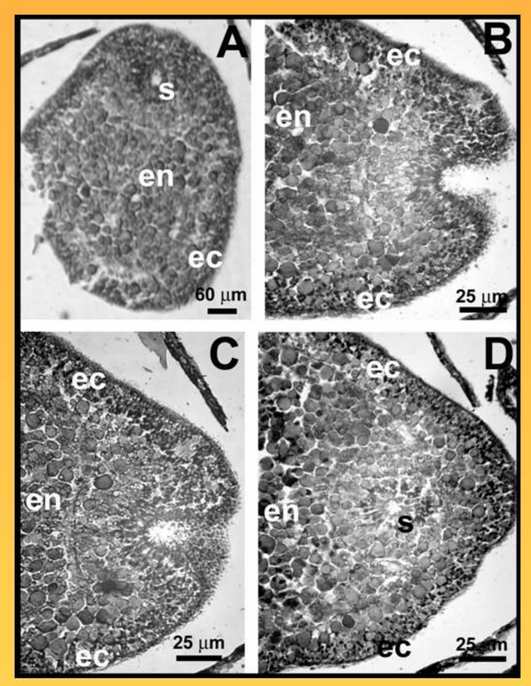

Fig. 4a-d: *Astroides calycularis*. Embryonic development. (a) Stereogastrula (intermediate stage) in the coelenteric cavity. At this stage of development, the ectoderm is clearly distinct from the endoderm. (b - d) Detail of the stomodeal invagination of the stereogastrula. [ec: ectoderm; en: endoderm; s: stomodeal invagination]

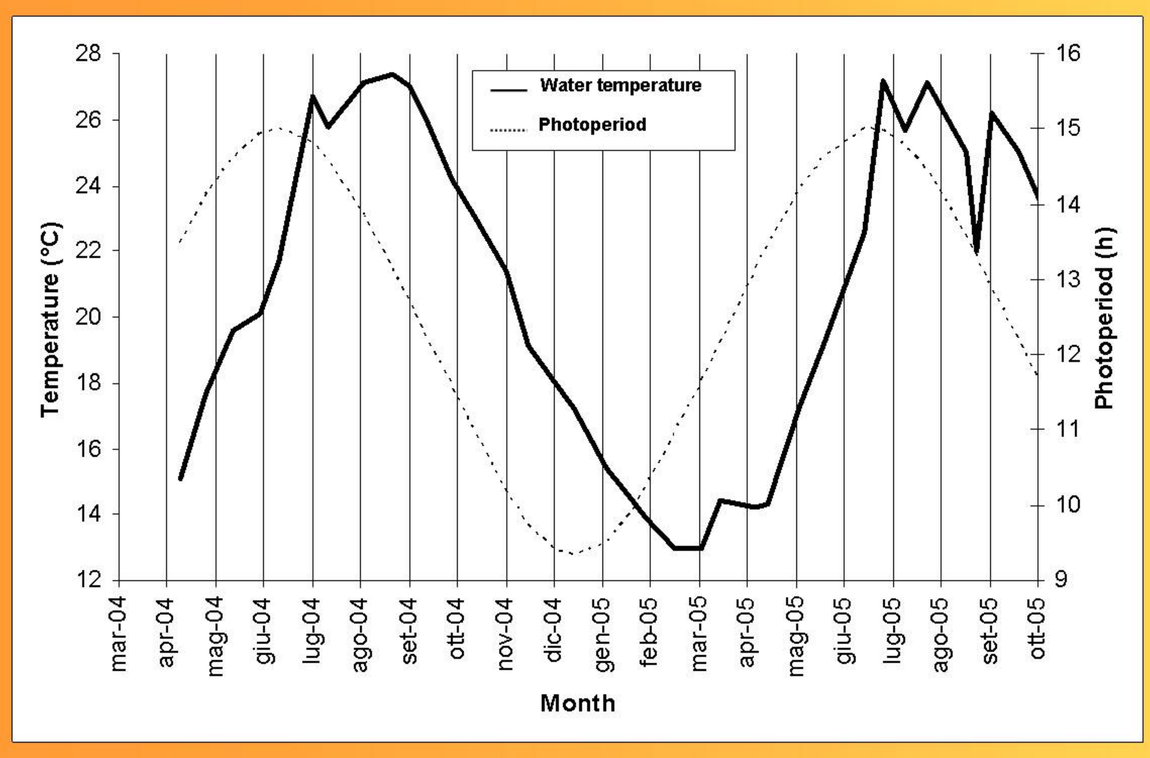

Fig 5: *Astroides calycularis*. Variation of the sea surface temperature and the photoperiod during the 16 months of sample collection.

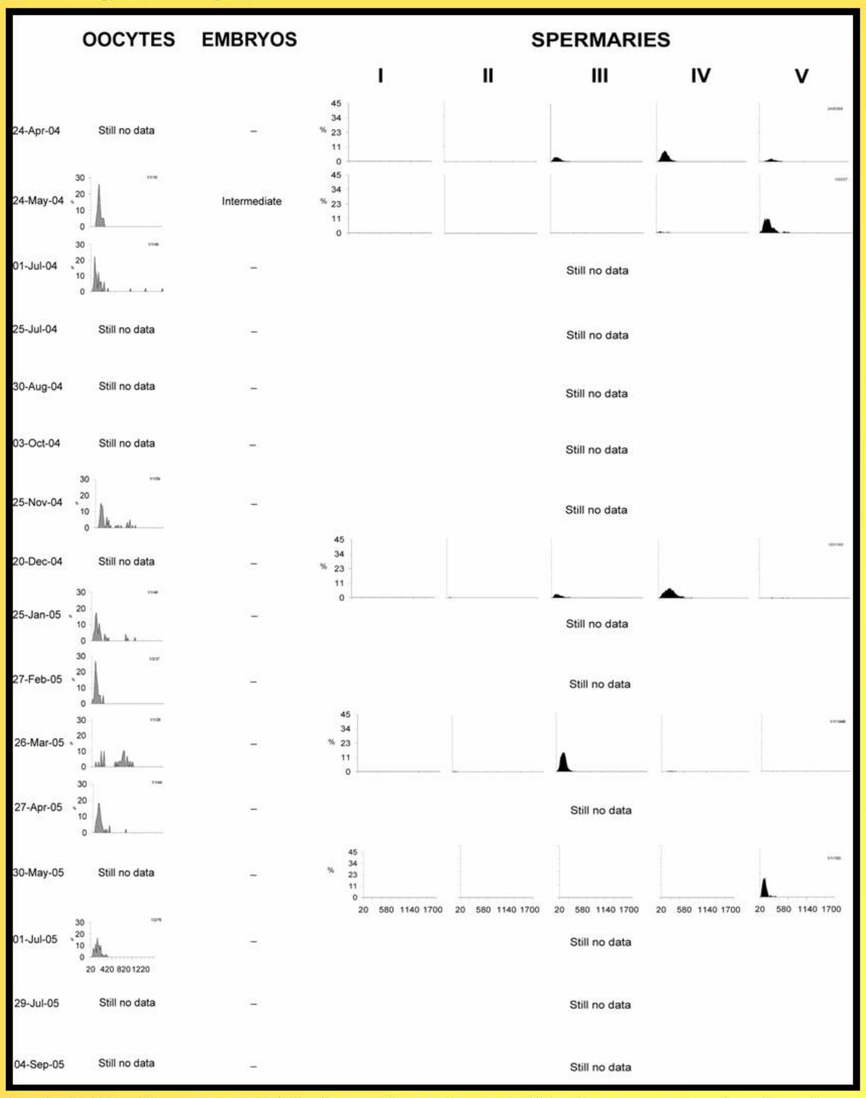

Fig 6: *Astroides calycularis*. Size-frequency distributions of oocyte and of the 5 spermary maturation stages in monthly samples collected off the coast of Palinuro from April 2004 to September 2005.
Values reported in the graphs indicate the number of colonies/number of polyps/total number of oocytes or spermaries measured for monthly sample. The middle column illustrates the presence of embryos in the females coelenteric cavity and their stage of development.

Acknowledgements: We wish to thank R. Navarra, P. Agresta, C. Mattei, M. Meteori, W. Micheli, F. Oliaro, I. Sauirini, V. Bernardelli, M. Galli, E. Manzardo and B. Zoli of the "Pesciolino sub" (www.pesciolinosub.it), diving center of Palinuro, for its logistical and practical support for the dives. Gianni Neto for photographic material. R. Falconi gave us assistance in laboratory guidelines. The Marine Science Group (http://www.marinesciencegroup.org) for organizing and supervising the dives. Our research is supported by the Ministero dell'Istruzione, dell'Universita` e della Ricerca (MIUR), by the Associazione dei Tour Operator Italiani (ASTOI) and by the Canziani foundation of the Department of Evolutionary and Experimental Biology of the Alma Mater Studiorum – University of Bologna.