

REPRODUCTIVE BIODIVERSITY IN MEDITERRANEAN AND TROPICAL POPULATIONS OF SCLERACTINIAN AND GORGONIAN ANTHOZOANS (CNIDARIA)

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RESEARCH PROJECT

INTRODUCTION

Cnidaria (Coelenterates) appeared for the first time during the Precambrian. Today the phylum is comprised of more than 10,000 living species. They are prevalently marine species and are subdivided into three major classes: Hydrozoa, Scyphozoa and Anthozoa (Brusca & Brusca 1990). Many organisms may be said to represent this phylum such as hydroids (*Hydra*), well-known to students and biologists, large jelly fish, popular and familiar to bathers, precious corals (for ex., the Mediterranean red coral, *Corallium rubrum*) and hermatipic (builders of the great tropical coral reef). All the organisms of this phylum possess stinging cells which they discharge on contact with prey (Hessinger & Lenhoff 1988).

Cnidaria have complex life cycles. Most species are able to reproduce both sexually and asexually (Fautin et al. 1989). Sexual and asexual reproductive phases alternate in a life cycle known as metagenesis. In reality, the metagenetic paradigm – jelly fish (sexual phase) – larvae (dispersal phase) - polyp (asexual phase) – jelly fish – has, in many cases, been modified through the reduction or loss of certain stages and the adoption of sexual reproductive behavior by polyps and in some cases the adoption of the asexual stage by jelly fish. Particularly, while the jelly fish stage is generally present, albeit in varying complexity, in hydrozoans and scyphozoans, it is totally absent in anthozoans.

Compared to the objective complexity of the biological and reproductive cycles of Cnidaria, their anatomy is not complex, with a relatively simple tissue arrangement (Scrutton 1979). Individuals, whether they be polyps or jelly fish, are composed of two layers of cells, ectoderm and endoderm, separated by a non-cellular mesoglea. In particular, the “gonads” are basically a mass of germ cells grouped together. The germ cells originate from the ectoderm in Hydrozoans and from the endoderm in Scyphozoans and in Anthozoans.

Generally, in the field of zoology today, Cnidaria are among the least studied animals, so “little” so that in a recent review, they were defined as “neglected animals” (Fautin 2002; Müller & Leitz 2002). In particular, information on the biology of scleractinian anthozoans has been collected basically over the past 20 years and deals mainly with tropical or subtropical species, while there is precious little, if any, information on species

living in temperate climates, especially on Mediterranean species (Veron 2000). Thus far, their population structure and dynamics have been compared and correlated to certain environmental parameters and with the phenomena of symbiosis with unicellular algae (zooxanthellae) that are necessary to the forming of compact calcareous skeletal tissue and as trophic support in polyps. (Bak & Meesters 1999; Kinzie 1999; Fagoonee et al. 1999). We have but fragmentary information on their reproductive strategies which include asexual phenomena such as budding, fragmentation, and fission, and phenomena of gonochoric (separate sexes), hermaphroditic, and parthenogenetic sexuality (Fautin 2002). Development also appears to change when we look at the study of larvae with planktonic or bentonic dispersal and different nutritional habits: lecithotrophic, planktotrophic, and simbiotrophic (Hughes et al. 1999; Müller & Leitz 2002).

COLLECTED DATA

Our lab has been investigating reproductive biology and population dynamics in various animal groups for many years (Cnidaria, Platyhelminthes, Anellida, Vertebrates [Fish and Amphibians]). Regarding the phylum Cnidaria, in recent years attention has been focused mainly on scleractinian anthozoans, and particularly on solitary corals (= not colonial). Flanked by a research project being carried out in collaboration with the laboratory of Dr. Nanette E. Chadwick-Furman (Interuniversity Institute for Marine Science of Eilat – Israel) on the study of tropical species of the family Fungiidae living in the northern Red Sea (Chadwick-Furman et al. 2000; Goffredo & Chadwick-Furman 2000, 2003; Gektidis et al. 2004), a main line of investigation focused on the population structure and reproductive biology of the Mediterranean scleractinian *Balanophyllia europaea* (Goffredo & Telò 1998; Goffredo & Zaccanti 2004; Goffredo et al. 2000, 2002, 2004a, b). Surprisingly, we found the species to be hermaphroditic although it is part of a genus that has thus far been described as comprising sexually separate species. Even in the case of the larger family of Dendrophylliidae, which is comprised of about 29 genera with 349 species, the hermaphroditic sexual condition is an exception in that 96% of the species investigated is gonochoric (Harrison & Wallace 1990).

In short, we collected data on the biology of *Balanophyllia europaea* regarding:

- a) sexual condition, reproductive modality and embryonic development (Goffredo & Telò 1998): we found that *Balanophyllia europaea* is a simultaneous hermaphroditic brooding coral (the only one of its genus);
- b) ultrastructures of spermatogenesis (Goffredo et al. 2000): we found sperm morphology in *Balanophyllia europaea* to be the same as that in species of separate sexes (Harrison 1985; Steiner & Cortes 1996); this fact supports the hypothesis that hermaphroditism in *Balanophyllia europaea* is a secondary, adaptive condition;
- c) the annual cycle of sexual reproduction (Goffredo et al. 2002): this study is the first investigation to be carried out of the annual cycle of sexual reproduction in a Mediterranean solitary coral; the differential distribution of the germ cells of the opposite sex that we found along the oro-aboral axis in hermaphroditic polyps of *Balanophyllia europaea* (we found that by and large the distribution of mature testicles was towards the polyp's oral pole while that of mature oocytes was towards the aboral pole) was interpreted as a possible barrier to self-fertilization as it would diminish encounters between gametes of the opposite sex, produced by the same individual;
- d) behavior and larval dispersal capacity (Goffredo & Zaccanti 2004): zooxanthellate larval dispersal capacity (those that live in symbiosis with unicellular algae) of *Balanophyllia europaea* turned out to be significantly superior to the capacity estimated for azooxanthellate larvae (that do not live in symbiosis with algae) of *Balanophyllia elegans* (Gerrodette 1981), a species living in the western coastal waters of North America; we suggested that the symbiosis with the zooxanthellae that trophically support the larvae supplying them with nutrients of photosynthetic origin favors larval dispersal and briefly delays adhesion and metamorphosis;

- e) skeletal densitometry, growth rates of individuals living in the wild, population structure and dynamics (Goffredo et al. 2004a): the population structure of *Balanophyllia europaea*, compared to that of *Balanophyllia elegans* (Fadlallah 1983), had a lower numerical density of individuals; we suggested that the difference between the two species with respect to population density be linked to the differences in sexual condition (hermaphroditism in *Balanophyllia europaea* vs. gonochorism in *Balanophyllia elegans*, Fadlallah & Pearse 1982) and in larval dispersal capacity (high in *Balanophyllia europaea*, low in *Balanophyllia elegans*, Goffredo & Zaccanti 2004);
- f) population genetics (Goffredo et al. 2004b): the genetic structure of *Balanophyllia europaea* was found to deviate notably from the Hardy-Weinberg equilibrium, with a significant lack of heterozygotes, supporting the hypothesis that self-fertilization characterizes the reproductive biology of *Balanophyllia europaea*.

Comparing the data we collected on *Balanophyllia europaea* with that collected by American scientists on *Balanophyllia elegans*, we have surmised that there is a correlation between reproductive and colonization modalities in these organisms and that it is in agreement with evolutionary models of sexuality which consider hermaphroditism as an adaptive strategy to cope with conditions of low population density and gonochorism as an adaptive strategy to cope with conditions of high population density (Ghiselin 1969).

WORK PLAN

As a research group, it is our intention to deepen investigation on the relationship between reproductive and environmental colonization modalities in anthozoans. We plan to continue study of solitary as well as colonial forms. Our investigation of solitary corals has continued in the Mediterranean with the study of another two species of scleractinian anthozoans, *Leptopsammia pruvoti* and *Balanophyllia regia* [reported to be sexually separate (Goffredo et al. 2004c and personal observations)], and in the Red Sea with the continuation of our collaboration with the above-mentioned Israeli laboratory for the study of the reproductive biology and population dynamics in tropical fungiids. The extension of our investigations to colonial forms has recently begun with our study of the Mediterranean scleractinian anthozoan *Astroides calycularis* in the area of Cape Palinuro (Salerno) [polyps found to be sex separate (personal observations)]. We also plan to work jointly with the laboratory of Prof. Howard R. Lasker (The State University of New York – University at Buffalo), specialized in the reproductive biology and population dynamics of tropical colonial gorgonian anthozoans (Coffroth & Lasker 1998; Lasker & Coffroth 1999; Castanaro & Lasker 2003; Lasker 2003; Lasker et al. 2003; Sanchez & Lasker 2003; Sanchez et al. 2003a, b; Gutierrez-Rodriguez & Lasker 2004a, 2004b; Sanchez & Lasker 2004; Sanchez et al. 2004). In order to begin our work with Prof. Lasker, we have forecast a 4-month study program in his laboratory in the U.S. to be supported by funds from the *Programma Marco Polo*. The stay in the U.S. will allow us to deepen our knowledge and study work protocols regarding important fundamental research methodology in the investigation of reproductive biology and population dynamics. In fact, the program we have discussed with Prof. Lasker will allow us to train in the following:

Underwater research using oxygen tanks in shallower depths (0-40 m) and tanks using a mix of gases (trimix: oxygen + nitrogen + helium) in greater depths (40 – 125 m) – for observation and sample collection (to further train in these methods of scientific underwater sampling, we have scheduled a three-week study period aboard the oceanographic ship *F. G. Walton Smith* of the University of Miami);

Biometry of colonial forms in the field and in the laboratory– in order to define population structure and dynamics parameters;

Histochemical techniques – to identify growth sites of colonial forms;

Study of algal density (symbiont zooxanthellae) in animal tissue – to characterize the dynamics of the symbiosis, with particular attention to the trophic role played by photosynthetic processes in the gametogenesis of anthozoan polyps;

Engineering and fluid dynamics techniques – to make a model of gamete dispersal and fertilization processes in underwater environments, within the scope of investigations on reproductive success;

Population dynamics mathematical models – to assess reproductive success in the wild, in terms of density and dispersal in colonial forms;

Highly variable genetic markers – to assess self-fertilization phenomena in hermaphroditic forms and for the study of population genetics;

Sequencing of protein coding mitochondrial gene – to develop studies of molecular phylogenesis.

This project, through the intra-and inter-specific comparison of sexual condition (gonochoric or hermaphrodite), of fertilization strategies (cross- or self-fertilization) and environmental colonization modalities (larval dispersal capacity and population density) in solitary and colonial forms, should produce an array of data that will allow us to verify:

- germ cell differentiation processes, with special emphasis on the quantity of energy deployed to spermatogenesis in relation to fertilization systems;
- the existence and extent of self-fertilization in hermaphroditic populations;
- the relationship between sexual condition and environmental colonization modalities, in terms of population density;
- the relationship between reproductive modality and intraspecific biodiversities, in terms of the genetic structure of the populations.

The body of data collected on the “ Anthozoan system” will then be compared to the models of “Breeding System, Sex Allocation and Evolution” that have been suggested for animal and vegetable organisms (Cruden 1977; Charnov 1982; Shoen & Brown 1991; Jürgens et al. 2002).

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