

藻類要聞 \* 繁楷體 ~ Algues \* Avancées récentes  
~ Algas \* Alto interés ~ Algae \* Highlights ~  
ВОДОРΟΣЛИ \* ВАЖНЕЙШИЕ МОМЕНТЫ ~ أخبار الطحالب \*

DINOFLAGELLATE LIFE-CYCLE COMPLEXITIES

The dinoflagellates are a group of predominately marine, alveolate protists whose structural organization, morphological diversity, and novel behaviors have captured the imaginations of phycologists and protozoologists for 250 years. The 2000 or so extant species that comprise the dinoflagellates sort almost equally as phototrophs and heterotrophs; however, a growing awareness that many species are really mixotrophic, engaging in some combination of phototrophy and phagotrophy, is eroding the conventional view of dinoflagellate nutrition (Stoecker 1999). The intricate jigsaw puzzles formed by the thecal plates of armored species (Fensome et al. 1993, Steidinger and Tangen 1996), the complex light-sensing organelles of the Warnowioids (Gaines and Elbrächter 1987), and unique feeding structures, including zipper-like “mouths” for swallowing food, peduncles or feeding tubes for “sucking-up” whole prey or their cytoplasmic contents, and feeding veils for encasing and externally digesting large prey (Jacobson and Anderson 1986, Buck et al. 1990, Hansen and Calado 1999), are but a few of the remarkable features that make dinoflagellates truly exceptional. Equally fascinating are the colors exhibited by dinoflagellates, ranging from the opalescent blues, greens, and pinks of heterotrophic forms to yellows, reds, and browns of photosynthetic species (Kofoid and Swezy 1921, Lebour 1925). This amazing array of pigmentations led Kofoid and Swezy (1921) to comment that dinoflagellates “. . . are most brilliantly colored, vying with orchids and butterflies in variety of color and delicacy of shading.”

The study of dinoflagellates, however, has not been without its drawbacks. Difficulties encountered when examining delicate, sometimes rare specimens are not only frustrating, but can lead to misinterpretation and erroneous conclusions. Changes in temperature and osmotic stress associated with desiccation during microscopic examination can cause cellular distortion, ecdysis, or loss of integrity. Many dinoflagellates, particularly athecate species, are notoriously difficult to preserve, with chemical fixatives producing misshapen cells, swollen membranes, and clumping of specimens. Such phenomena may account for some of the unusual reports that have failed to be corroborated by subsequent investigators. For example, the

pseudopodial network illustrated by Hofender (1930) as an anastomosing feeding structure of *Ceratium hirundinella* is likely an artifact of cell trauma, while the juxtaposition of a small *Protoperidinium* within the sulcus of *Ceratium lunula*, interpreted by Norris (1969) as evidence of feeding, may represent spurious, post-mortem placement of specimens (Jacobson 1999).

Successful cultivation of dinoflagellates was not achieved until early in the twentieth century when *Cryptocodinium cohu* was first grown on rotting pieces of the brown algae *Fucus* (Taylor 1987). Since then, only a small percentage of dinoflagellate species have been successfully brought into culture. Most of these are neritic, photosynthetic species whose growth requirements are tolerant of high cell densities typically achieved in culture. Oceanic forms seem more difficult to grow, perhaps due to their intolerance of contaminants associated with culture glassware (Loeblich 1984), while heterotrophic dinoflagellates often require the availability of appropriate prey or host species. Not surprisingly, therefore, early studies of dinoflagellate autecology were based on observations of living or preserved field samples and were thus limited by availability of suitable material and the occurrence of ephemeral life-history stages. Linking presumed life-history stages in the absence of cultures often requires assumptions that can lead to inaccurate life cycles and taxonomic confusion. The case of *Pyrocystis lunula* and *Dissodinium pseudolunula* is a good example. The taxonomic history of these genera is long and varied, dating back to the early observations of Murray during the voyage of the Challenger Expedition (see Elbrächter and Drebes 1978 for details). For almost a century, life-history stages of *P. lunula* and *D. pseudolunula* were mistakenly aggregated into a single incomplete life cycle. Only after cultures were established (Swift and Durbin 1971; Drebes 1978) was it possible to demonstrate the existence of two species, one free-living and one parasitic, and to complete their life cycles and reveal that the two organisms belonged to separate orders (Elbrächter and Drebes 1978; Drebes 1978).

The availability of cultures has also proved important in demonstrating the relevance of sexuality and encystment in the life cycles and ecology of dino-

flagellates (Walker 1984 and references therein). Except for *Noctiluca*, all dinoflagellates examined thus far have haplontic life cycles dominated by asexual reproduction of haploid vegetative cells (Pfiester and Anderson 1987). Temporary or pellicle cysts formed in response to adverse environmental conditions and division cysts produced following feeding or growth are known to occur within the asexual cycle of several species (von Stosch 1973, Dale 1983, Drebes and Schnepf 1988, Skovgaard 1996). A sexual phase has also been documented for many dinoflagellates and involves the fusion of asexually generated gametes to produce a motile zygote, the planozygote. Planozygotes may undergo meiosis to reestablish the haploid phase, or produce a resting cyst (the hypnozygote), with cells reentering the asexual cycle upon germination. Polymorphism is pronounced in some dinoflagellates, particularly the parasitic species (Cachon and Cachon 1987), and can result in rather elaborate life histories. Among the more remarkable dinoflagellate life cycles are those reported for members of the Phytodiniales. For example, *Cystodinedria inermis* appears to have some 35 life-history stages encompassing biflagellate cells, a variety of lobose and filose amoeboid forms, and encysted stages (Pfiester and Popovský 1979, Popovský and Pfiester 1982). Unfortunately, the life cycle of *C. inermis* is known only from field collections and should be viewed with caution. This is of particular concern as some stages in the life cycle of *C. inermis* are believed to be identical to *Actinophrys sol* and other protists that are well documented in the literature as distinct and valid species (Popovský and Pfiester 1982).

The toxic dinoflagellate *Pfiesteria piscicida* Steidinger et Burkholder (Steidinger et al. 1996) also has a highly complex life cycle, with the 24 or more life-history stages including a number of flagellated cell types, smooth walled and spiny, chrysophyte-like cysts, and amoebae with filose and lobose pseudopods (Burkholder and Glasgow 1995, 1997). Stages differ greatly in size ranging from <10  $\mu\text{m}$  flagellates to 400  $\mu\text{m}$  amoebae. Some of the amoeboid forms are apparently identical to species of planktonic and benthic amoebae described by earlier researchers (Burkholder and Glasgow 1997). While *P. piscicida* can be maintained in culture with algal prey, induction of toxicity and transitions between certain stages appear to require the presence of live fish, a condition that inherently carries concerns about contamination. To help alleviate those concerns, confirmation of transitions between stages has relied on the use of single cells or aggregates of individually isolated cells (Burkholder and Glasgow 1997). Nonetheless, controversy over the validity of life-history stages within the life cycle of *P. piscicida* remains.

In an attempt to validate the complex life cycle of *Pfiesteria piscicida*, Litaker et al. (2002) studied clonal cultures isolated from sites in North Carolina and Maryland where fish kills have been reported. To insure correct identification of their isolates, the au-

thors sequenced the small subunit ribosomal RNA gene and confirmed that it matched sequence data previously published for *P. piscicida* (Litaker et al. 1999, Oldach et al. 2000). Life-cycle processes were examined for cultures fed algal prey (*Rhodomonas* sp.) and for cultures grown in the presence of fish (40–80 mm goldfish or tilapia). Conventional light microscopy, video microscopy, fluorescent nuclear stains, and electron microscopy were eloquently combined to determine the morphology and nuclear complement of life-history stages and to document morphogenetic events associated with stage transformations. The work required development of a new protocol to permeabilize cysts for application of nuclear fluorochromes. Results demonstrate that the life cycle of *P. piscicida* is haplontic, including asexual and sexual phases that resemble patterns previously reported for other dinoflagellates. Furthermore, life-cycle events were the same whether cultures were grown on algal prey or in the presence of fish. The life cycle of *P. piscicida* as described by Litaker et al. (2002) differs greatly from prior reports (Burkholder and Glasgow 1995, 1997). It has relatively few life-history stages, lacks amoeboid forms, and does not include spiny, chrysophyte-like cysts.

Litaker et al. (2002) never observed amoeboid forms or flagellate-amoeba transitions in *Pfiesteria piscicida* cultures grown on algal prey. They did, however, recover amoebae from cultures grown in the presence of fish and from fish tanks that contained *P. piscicida*. Amoebae from both sources resembled amoeboid stages previously reported as part of the life cycle of *P. piscicida* (Burkholder and Glasgow 1995, 1997). To test the hypothesis that these amoebae were life-history stages of *P. piscicida*, Litaker et al. (2002) developed a suite of fluorescently labeled peptide nucleic acid probes for use in *in situ* hybridization studies. A probe specific for the SSU rRNA of *P. piscicida* failed to react with amoebae associated with *P. piscicida* and fish, while a group-specific probe for the SSU rRNA of the amoebae failed to react with *P. piscicida*. The authors thus concluded that amoebae present in their cultures were not part of the life cycle of *P. piscicida*.

Results of studies like that of Litaker et al. (2002) are always constrained by the number of isolates that can be examined and by the apparent conflict of trying to establish that something does not exist. Thus, debate will likely continue over what the true life cycle of *Pfiesteria piscicida* really is. Would other clones of *P. piscicida* isolated under different circumstances form amoeboid stages? Might gametes from different clones be required to produce planozygotes capable of amoeboid transformation as illustrated by Burkholder and Glasgow (1995, 1997)? Resolution of such issues will require time, patience, and hard work. In the meantime, Litaker et al. (2002) have given very sound reasoning for approaching *P. piscicida* as a dinoflagellate with a relatively simple life cycle. That reasoning should facilitate efforts to understand the potential environmen-

tal and health consequences of this harmful “algal” species.

D. W. COATS

P. O. Box 28

Smithsonian Environmental Research Center

Edgewater, MD 21037, USA

e-mail: coats@serc.si.edu

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