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A Brief History of Dinoflagellate Feeding Research[1]

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ABSTRACT. Our understanding of the great diversity and novelty of dinoflagellate feeding ecology (summarized in Fig. 1) is progressing rapidly after a slow, erratic beginning a century ago. This advance has not been based primarily on technical innovations. Rather, basic microscopy of wild material, like that employed a century ago, has continued to yield many important insights. Indeed, several of the most exciting discoveries (including the pallium of thecate heterotrophs and the ingestion of ciliates and dinoflagellates by both naked and thecate dinoflagellates) are actually rediscoveries that have expanded upon reports published sixty to ninety years earlier. The elucidation of feeding strategies among thecate species has advanced particularly rapidly, with the recent addition of over a dozen widespread and important thecate genera to the rank of phagotroph, leading to a significant paradigm shift: the theca can no longer be considered an insurmountable or even a significant barrier to phagotrophy. This research is now developing from a descriptive and anecdotal stage to an experimental and quantitative stage, involving analysis of rates, ecological roles, and survival strategies; however, important new descriptions are still emerging. It has been shown that dinoflagellates, despite having relatively low densities and growth rates compared to ciliates, are ecologically significant. They can even compete with and prey upon microcrustacean grazers. Among many future advances in this field, perhaps the most significant will be an understanding of nano-sized dinoflagellates, particularly "*Gymnodinium*" and "*Gyrodinium*" spp. that have already been shown to play major roles in marine food webs. This development awaits a massive taxonomic overhaul of this diverse, polyphyletic assemblage. In addition, the feeding habits of well characterized thecate taxa still await characterization.

Key Words. Engulfment, heterotrophy, mixotropy, pallium phagotropy, tube feeding.

OUR understanding of the diversity and novelty of dinoflagellate feeding ecology (summarized in Fig. 1) is progressing rapidly after a slow, erratic beginning a century ago. In fact, the current pace of discoveries, each seemingly more remarkable than the last, is sufficiently rapid to leave one feeling a bit disoriented and profoundly amazed. For example, out of a list of 60 of the most important reports over a span of 90 years, 40 have appeared in the last 10 years. The number of investigators that focused on dinoflagellate phagotrophy in this century prior to the mid-80's could be counted on one hand, including the Cachons (1974), Lee (1977), Kimor (1981), Spero (1982), and Drebes and Schnepf (1982). In an early review by Dodge (1983), over 200 years after the earliest studies of dinoflagellates by Müller (1773), all known phagotrophs where athecate, and thecate groups such as *Protoperidinium* were thought to be "chemo-saprophytic," absorbing dissolved organics. The recent review by Gaines and Elbrächter (1987) was ignorant of widespread predation upon ciliates by both thecate and athecate forms, of the phagopod, and of the dinophysoid peduncle. Undoubtedly, the reviews that follow this short history will likewise soon become outdated. There are now several dozen investigators currently or recently active in this field, and the diversity of phagotrophic phenomena that has been revealed is truly astonishing. Like any good story, the history of this multifaceted field is rich with delightful ironies and reversals of assumptions, serendipitous discoveries, and seemingly perverse and repeated reversals of conventional food web linkages.

Interest in dinoflagellates is obviously a part of the growing interest in the microbial food web. The emerging importance of the microbial food web, the protists and prokaryotes of which produce the majority of the ocean's respiration, came as a result of an iterative process wherein the latest "most important" component of the plankton was shown to be a group long overlooked due to past methodological deficiencies. Thus, the novelty of nanophytoplankton was followed by that of picoplankton such as *Synechococcus* and finally *Prochlorococcus,* subsequent systematic breakthroughs following a falling size gradient.

In contrast, dinoflagellate taxonomy was well, although imperfectly, described many decades ago (Schiller 1933), yet knowledge of feeding capabilities would only slowly emerge due to the delicate nature of these notoriously finicky organisms. A good example is provided by the plastidic *Dinophysis* spp.: even with new insights concerning their phagotrophic requirements (Jacobson and Andersen 1994), none has yet been cultivated. Not surprisingly, the nutritional requirements of two omnivorous "weedy" species, the quasi-dinoflagellate *Oxyrrhis marina* and the true dinoflagellate *Crypthecodinium cohnii,* were thoroughly examined long before other dinoflagellates (e.g. Provasoli and Gold 1957; Droop 1959). Many advances have occurred haphazardly through risky "fishing expeditions," the success of which appear to depend on serendipitously favorable conditions of high predator and prey abundance that facilitated the difficult and unpredictable task of witnessing fleeting and unpredictable feeding behavior in a wild, natural assemblage—in other words, being at the right place at the right time. The unpredictable, challenging labor of working with living material remains of key importance.

THE EARLY YEARS

The earliest observations of feeding were understandably made with large naked forms (Dangeard 1892 and earlier workers) such as *Gyrodinium spirale* that often contain large, conspicuous food inclusions (including diatoms over 50 μm in diameter). These early workers, including Pouchet (1883), referred to dinoflagellates as "cilioflagellates" since the unusual transverse flagellum was once thought to be a ring of separate cilia. Non-thecate dinoflagellates do not preserve well, however, and are often overlooked in formalin-fixed samples. Given the difficulty of working with fragile, living material, one cannot help admiring the watercolor depictions of phagotrophic cells presented by Kofoid and Swezy (1921), truly inspiring work. Other reports of the ingestion of diatoms and other prey by naked species were made by Elbrächter (1972), Irish (1979), Frey and Stoermer (1980), Kimor (1981), and Popovski (1982). The more robust thecate cells, including conspicuous heterotrophs such as *Protoperidinium,* eluded conclusive documentation of feeding until recently. Ironically, this and other recent 'discoveries' were actually confirmations of wonderfully detailed and accurate observations of early workers. For example, Schütt (1895) provided drawings, unsurpassed in detail and intricacy, of apparent *Podolampus* feeding (very similar to *Protoperidinium* pallium feeding), and Biecheler (1936a, b; 1952,

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published posthumously two decades after the observations) illustrated feeding by a photosynthetic/mixotrophic *Gyrodinium pavaillardii* on a *Strombidium* ciliate, and *Peridinium gargatua,* complete with theca, ingesting a large *Alexandrium* cell. So many decades passed before these strange behaviors were finally confirmed and photo-documented (Skovgaard 1996; Uchida, Kamiyama and Matsuyama 1997) that Biecheler's singular work seemed to represent oddities and consequently seemed to have had little influence on the field. Thus, the brilliant, detailed observations of Kofoid and Swezy, Schütt, and Biecheler remain a humbling reminder to those of us working in a highly capitalized, high-tech environment that important work can arise from a simple light microscope, coupled with patience, luck and the appropriate search image.

Some early observations of feeding, being unconfirmed, needed to be treated with appropriate caution and skepticism. For example, Hofeneder's (1930) drawings of *Ceratium* pseudopodia could have been artifacts of membrane swelling following cell trauma, and Norris' (1969) figure of *Ceratium* engulfing a small *Protoperidinium* within the sulcus could have been the result of spurious placement post-mortem. One of Hofeneder's (1930) illustrations depicts a ciliate held within a pseudopod-like compartment. Now that numerous *Ceratium* species are known to prey on ciliates (Bochstahler and Coats 1993a, Jacobson and Anderson 1996), it appears that Norris may have been the first and only witness of this elusive event. Likewise, Bursa (1961) shows a *Protoperidinium globulus* ingesting an unidentified, spiny flagellate, a finding that has not yet been confirmed. It is now known that most species of *Protoperidinium* surround diatom prey with a pseudopod called the pallium, although some species feed on dinoflagellates, and the smallest species, *P. bipes,* can also feed on prasinophytes. Bursa's drawing closely resembles the partially retracted pallium, with the likely diatom prey already detached, so his interpretation is likely, and understandably, in error.

THE MODERN ERA

With the exception of the few works noted above, the postwar years saw an eclipse of interest in dinoflagellate phagotrophy, perhaps due to the compelling need to understand harmful algal blooms caused by phototrophic 'red-tide' dinoflagellates, and the revolution in culture techniques that resulted in the work of Droop (1959), Provasoli, McLaughlin and Droop (1957), Guillard (1975), and others. Indeed, several species that have been maintained in long-term culture (spanning several decades) have been shown (with wild material, not with cultured cells) to have mixotrophic capabilities. Studies on heterotrophy involved not phagotrophy but uptake of dissolved organics in culture (e.g. Morrill and Loeblich 1979). Because available texts often overlooked holozoic forms and treated dinoflagellates simply as algae, those students (including the author) who were fortunate enough to be exposed to dinoflagellates at all might well be ignorant of the very idea of dinoflagellate phagotrophy unless pointed to the relatively inaccessible early work.

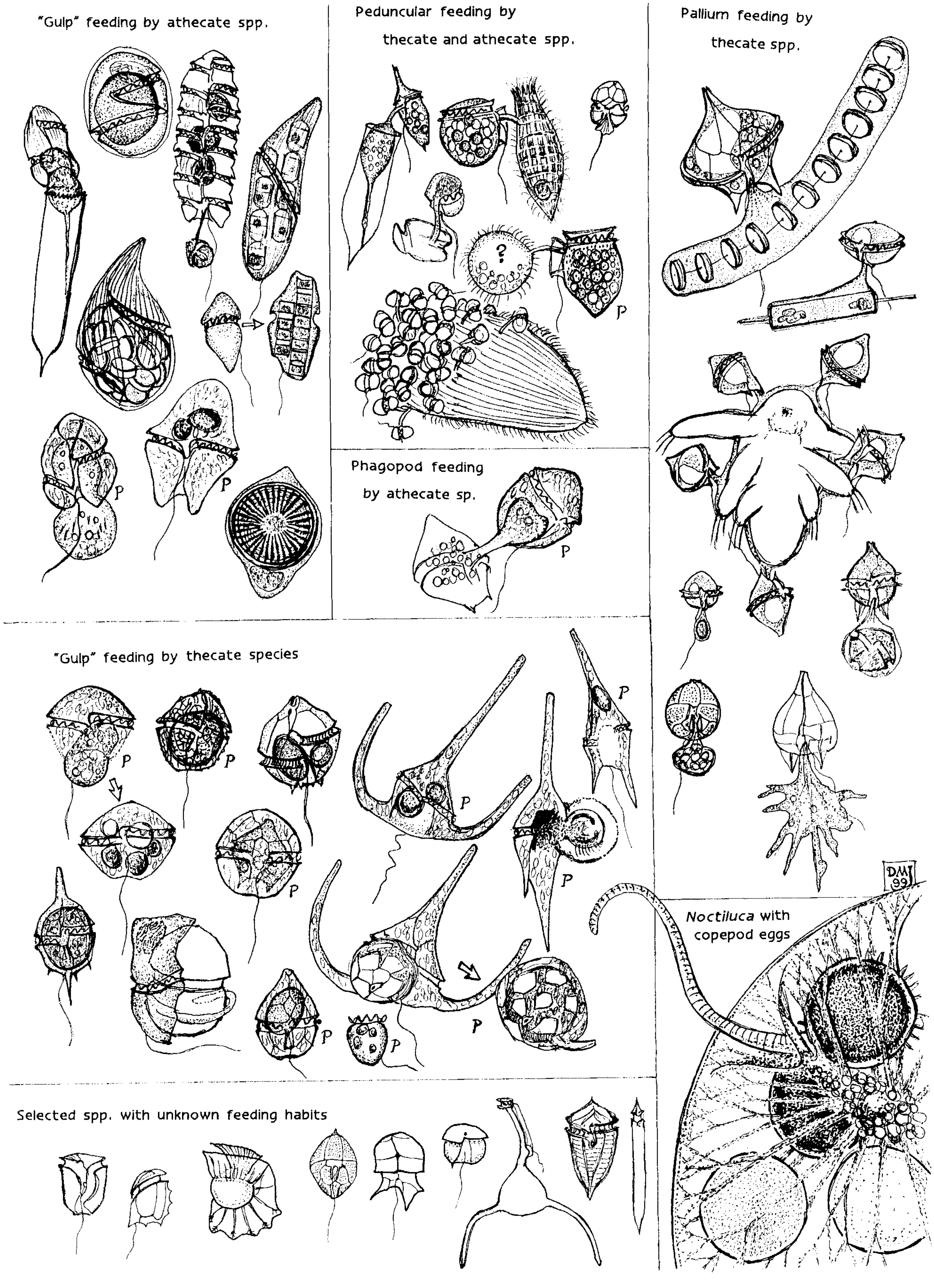
The often overlooked, but powerfully evocative works of Kofoid and Swezy (1921), Schütt (1895), Biecheler (1936a,b; 1952), and others, together with the intellectual challenge offered by such enigmatic holozoic groups as *Protoperidinium* (large, ubiquitous and abundant, yet with no known prey), provided compelling motivation for new generations of workers to take interest in dinoflagellate phagotrophy during the eighties and nineties. In this apparent "golden age" of dinoflagellate feeding research, an area of substantial progress is that of phagotrophy of thecate or armored species, previously assumed to be deprived of phagotrophic capabilities. In an influential study, Smetack (1981) highlighted the abundance of heterotrophic dinoflagellates in a coastal community.

The many feeding strategies that have been and are now being revealed among the enigmatic thecate species, both aplastidic and plastidic, represent a substantial paradigm shift. Feeding among the large, ubiquitous thecate heterotrophic groups (including *Protoperidinium* and the diplopsaloids) was long thought to involve saprotrophy of dissolved organic compounds, despite evidence of spectacular phagotrophy, including Schütt's (1895) convincing observations of external pseudopodia in *Podolampus* and other tropical dinoflagellates, the confirmation of extracellular feeding by *Blepharocysta* (Steidinger, Davis and Williams 1967), and Beicheler's (1936a) observations of feeding by the thecate *Peridinium gargantua* on *Alexandrium* sp. Therefore, the discovery of grazing upon large spiny diatoms via the pallium (Gaines and Taylor 1984; Jacobson and Anderson 1986) came as a marvelous surprise, the spirit of which was perhaps best captured by Greg Gaines' memorable slide of a *Protoperidinium* pallium enveloping a whale skeleton. These reports failed, however, to anticipate the remarkable predation upon copepod eggs and nauplii by certain species of *Protoperidinium* (Jeong 1994). The mystery of phagotrophy among thecate species was deepened by a rare observation in the 1980's of a *Fragilidium* sp. containing several intact *Dinophysis acuminata* cells (Fukuyo, Y., pers. commun.).

The peduncle, known in naked species since the 1970's, was also shown to be employed by thecate dinoflagellates, including *Peridiniopsis berolinense* (Wedemayer and Wilcox 1984) and the dinophysoid genera *Dinophysis* and *Oxyphysis,* which ingest ciliates piecemeal (Inouye, Fukuyo and Nimura 1993; Hansen 1991). The presence of a functional peduncle in plastidic *Dinophysis* cells was surmised, prior to the discovery of food vacuoles, from the presence of the ultrastructural signature of the inactive peduncle, namely the microtubular ribbon (Jacobson and Andersen 1994), even though this same structure was judged to be vestigial and nonfunctional by Lucas and Vesk (1990). However, this structure may not be limited to cells having a protrusive peduncle, since such a microtubular ribbon has been observed in *Alexandrium tamarense* (Doucette, G., pers. commun.) and in *Ceratium* (Dodge and Crawford 1970), long before mixotrophy was demonstrated or reconfirmed in these genera.

The presence of large food vacuoles within thecate cells, as mentioned above for *Fragilidium,* was recently shown to be widespread, involving *Gonyaulax, Scrippsiella, Amylax* and *Alexandrium,* the latter of which could also ingest a *Dinophysis* sp. (Jacobson and Anderson 1996). Finally, feeding by *Fragilidium* spp. upon dinoflagellates was confirmed twice, with prey including *Protoperidinium* (Jeong et al. 1997) and *Ceratium* spp. (Skovgaard 1996). This last observation, involving the nearly unbelievable and complete ingestion of an elongate, horned thecate cell by a relatively small, thecate dinoflagellate with intact but locally disarticulated plates, represents a spectacular coup de gras to the paradigm that the theca represents a insurmountable or even substantial barrier to phagotrophy.

A truly ground breaking report demonstrated that Biecheler's early discovery of mixotrophic feeding on ciliates was not an isolated incident. Bochstahler and Coats (1993a, b) showed by using the protargol silver-staining technique (a specialty of ciliologists) that the dominant phototrophs of Chesapeake Bay (*Gyrodinium uncatenum, Gymnodinium sanguineum,* and *Ceratium furca*) were ingesting large numbers of small choreotrich ciliates. Thus, two intertwined issues, feeding by thecate and athecate mixotrophs and the predation by a wide variety of dinoflagellates upon ciliates, were highlighted. The ingestion of larger ciliates, many containing plastids, was found by Jacobson


"Gulp" feeding by athecate spp.
Peduncular feeding by thecate and athecate spp.
Pallium feeding by thecate spp.
Phagopod feeding by athecate sp.
"Gulp" feeding by thecate species
Selected spp. with unknown feeding habits
Noctiluca with copepod eggs
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and Anderson (1996) in species of *Alexandrium, Amylax, Gonyaulax, Scrippsiella,* and *Ceratium,* but the observation of an inclusion within *Prorocentrum micans* (found only in two cells) likely involved an endo-parasitic infection of the dinoflagellate. However, Li et al. (1996) have documented the feeding of *Prorocentrum minimum* on cryptophytes (the latter having a convenient yellow autofluorescence). While only recently 50% of dinoflagellates where thought to be phagotrophic, the percentage has clearly risen substantially from a veritable flood of mixotrophic discoveries. It may ultimately be difficult to identify very many dinoflagellates that are entirely free of phagotrophic capabilities.

While food vacuoles containing ingested ciliates are now routinely found, the process of actual ingestion is rarely witnessed. After Biecheler's striking observations (1936a, b; 1952), no ingestion behavior was observed until Uchida, Kamiyama and Matsuyama (1997) skillfully documented the direct and entire engulfment of large tintinnid ciliates by *Gymnodinium instriatum.* In the same year, the author (unpubl. data) found an *Alexandrium pseudogonyaulax* with a half-ingested *Mesodinium rubrum* cell (the hypotheca appearing naked). Both instances involve the sulcus with no apparent protrusive structures.

Until very recently, all heterotrophic dinoflagellates were thought to be non-toxic. Now, a toxic heterotrophic dinoflagellate responsible for harmful "algal blooms" has emerged: *Pfiesteria piscicida* (Burkholder and Glasgow 1997). Its ability to intoxicate and immobilize large schools of fish (and, incidentally, human bystanders) is unlike any feeding strategy yet encountered. While the implications for estuarine environmental health are sobering, this discovery raises the question of the possible role of toxins in phagotrophy among such toxic mixotrophs as *Dinophysis* and *Alexandrium.*

THE FUTURE

In addition to basic light microscopy coupled to a sharp eye, which still permits the bulk of relevant discoveries, the following techniques and technologies have become indispensable: epifluorescent microcopy, video microscopy, transmission and scanning electron microscopy (coupled to single cell manipulation techniques), gentle mixing of cultures using roller bottles or Ferris wheels, and radioisotope incorporation. The possible future role of molecular biology is difficult to predict, although fluorescent DNA or RNA probes used to characterize amorphous food vacuoles may soon be employed. On a futuristic note, perhaps in situ video microscopy at depth, facilitated by baiting or trapping, will reveal new feeding phenomena. However, one cannot be confident that the ultimate elucidation of the genetic foundations of feeding, including cytoskeletal proteins and assembly processes involving feeding structures, will occur in our lifetimes.

Since evolutionarily related organisms often share a common feeding mechanism, an accurate systematic foundation is vital to continued progress in this field. Therefore, at this late date, it is rather frustrating to be shackled by an unworkable taxonomy involving the so-called 'naked' forms, some which have cryptic tabulation resembling those of thecate genera. When a suitable taxonomy is realized, based on a wealth of new surface structures (distinctive acrobase or apical pore complexes, singular pores at a variety positions, etc.) and gene sequences, the study of the *Gymnodinium/Gyrodinium/Amphidinium/*etc. group (especially the small, most abundant species) will become highly productive. Further, it is hoped that data on adequately characterized species is eventually made available on the internet.

In conclusion, the future of dinoflagellate feeding research is, in part, easy to predict. Further discoveries of unexpected and remarkable feeding mechanisms and prey interactions are a certainty. To imagine what sort of new prey may be revealed, one needs only to look at the known prey of dinoflagellates, which includes protists (dinoflagellates, ciliates, diatoms, and nanoflagellates), microcrustacea (copepod eggs and larvae), and wounded metazoa (nematodes, fish, etc.). Perhaps the last phagotrophic process to be revealed might involve difficult-to-sample benthic species preying on macroalgae or large foraminifera.

The following topics, among others, remain to be investigated:

1. The nature of the possible role of the apical pore complex in prey detection;
2. The degree and mechanism of prey selectivity in both mixotrophs and heterotrophs;
3. The differentiation of residual food vacuoles and autophagosome accumulation bodies;

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Fig. **1**. Selected examples of dinoflagellate feeding techniques, drawn roughly to scale. Species are listed for each panel starting with top left cell and proceeding clockwise. P = photosynthetic; all others are non-photosynthetic. **"Gulp" feeding by athecate spp.**: *Gyrodinium* sp. with unknown food (Taylor, F. J. R., pers. commun.); *Polykrikos kofoidii* with *Scrippsiella* sp., note slender capture filament (DMJ., pers. observ.); *Gyrodinium* sp. with *Melosira* (DMJ., pers. observ.); *Gyrodinium helveticum* (Irish 1979); *Gymnodinium* sp. with diatom (Frey and Stoermer 1980); *Gymnodinium sangunium* with ciliates (Bockstahler and Coats 1993); *Gyrodinium* with *Strombidium* sp. (Biecheler 1952); *Gyrodinium instriatum* with *Helicostomella* sp. (Uchida, Kamiyama, and Matsuyama 1997); *Gyrodinium spirale* (center) with *Thalassiosira* sp. (Buck & Newton 1995). **Peduncular feeding by thecate and athecate spp.**: *Oxyphysis oxytoxoides* on tintinnid (Inouye, Fukuyo, and Yoshida 1987); *Dinophysis (Phalacroma) rotundata* with *Tiarina* sp. (Hansen 1991); *Pfiesteria piscicida* with large amoeboid stage, fish gill, and epithelial tissue not shown (Burkholder and Glasgow 1997); *Dinophysis norvegica* on undetermined ciliate (Jacobson and Andersen 1994); *Gymnodinium fungiforme* swarm on large ciliates (Spero 1982); *G. fungiforme* on *Amphidinium* sp. (Taylor, F.J.R., pers. commun.). **Pallium feeding by thecate spp.**: *Protoperidinium depressum* with *Thalassiosira* sp. (DMJ., pers. observ.); *Diplopsalis lenticula* with *Ditylum brightweillii* (Naustvoll 1998); *Peridinium divergens* with copepod nauplius (Jeong 1994); *Protoperidinium steinii* with *Alexandrium* sp. (Jacobson and Anderson 1986); *Podolampus,* no known prey (Schütt 1895); *Blepharocysta* sp. with mass of cyanobacteria (Schütt 1895; Steidinger, Davis, and Williams 1967); *Oblea rotunda* with *Pyramimonas* sp. (Jacobson and Anderson 1986). ***Noctiluca* with copepod eggs**: *Noctiluca miliaris* with ingested *Acartia tonsa* eggs (Kimor 1981). **Selected spp. with unknown feeding habits**: *Amphidiniopsis* and an unnamed psamophilic heterotroph, *Ornithocercas* sp., *Lissodinium* spp., *Heterodinium* sp., *Triposolenia* sp., *Oxytoxum* spp. **"Gulp" feeding by thecate species**: *Alexandrium psuedogonyaulax* ingesting *Mesodinium rubrum* (DMJ., pers. observ.); *Gonyaulax grindleyi* with unknown prey (Jacobson and Anderson 1996); *Gonyaulax alaskensis,* a coprophage (DMJ., pers. observ.); *Ceratium longipes* with ciliates (Jacobson and Anderson 1996); *Ceratium* sp. ingesting ciliate (Hofeneder 1930); *Ceratium furca* with ciliate (Bockstahler and Coats 1993); *Fragilidium subglobosum* with *Ceratium* sp. (Skovgaard 1996); *Prorocentrum minimum* with cryptophytes (Li et al. 1996); *Scrippsiella* sp. with ciliate (Jacobson and Anderson 1996); *Peridinium gargantua* with *Alexandrium* sp. (Biecheler 1952); *Amylax* sp. with ciliate (Jacobson and Anderson 1996); *Fragilidium mexicanum* (center) with *Protoperidinium divergens* (Jeong et al. 1997). **Phagopod feeding by athecate sp.**: *Amphidinium cryophilum* with *Peridinium* sp. (Wilcox and Wedemayer 1991).

4. The possible role of feeding prior to the induction of sexuality and encystment;
5. The nature of the force-generating mechanisms in various phagotrophs;
6. The possible presence and nature of paralytic toxins involved in prey capture;
7. The nature of phagotrophy in a wide range of enigmatic dinoflagellates: *Ornithocerocus, Amphisolenia* and other tropical dinophysoids, *Oxytoxum, Heterodinium,* etc.

There will be more than enough to keep students of phagotrophic dinoflagellates busy for generations to come, funding permitting. The fun is just beginning.

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UPCOMING MEETINGS

11th International Congress of Protozoology

2001 Jerusalem, Israel

5th International Chrysophyte Symposium

July 26–31, 1999 Edwardsville, Illinois
Southern Illinois University

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3rd European Congress of Protistology, and

9th European Conference on Ciliate Biology

July 26–30, 1999 Helsingor, Denmark

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