Cysts as Factors in *Pyrodinium bahamense* Ecology

DONALD M. ANDERSON
Biology Department
Woods Hole Oceanographic Institution
Woods Hole, MA 02543 USA

ANDERSON, D.M. 1989. Cysts as factors in *Pyrodinium bahamense* ecology, p. 81-88. *In* G.M. Hallegraeff and J.L. Maclean (eds.) Biology, epidemiology and management of *Pyrodinium* red tides. ICLARM Conference Proceedings 21, 286 p. Fisheries Department, Ministry of Development, Brunei Darussalam, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Pyrodinium bahamense, like many red tide-forming dinoflagellates, includes a dormant cyst stage in its life history. Given the important role of such resting stages in bloom initiation, species dispersal, genetic recombination, survival through environmental or nutritional stresses, and as vectors of toxicity, there is a clear need to study the distribution, abundance, and general physiological ecology of *P. bahamense* cysts. This paper summarizes what little is known about the cysts of this important species and suggests a variety of research programs that would add much to our understanding of the spatial and temporal dynamics of *P. bahamense* red tides.

Introduction

The resting cyst is an important factor in the ecology of many neritic dinoflagellates. These highly resistant cells can survive in the sediment for extended periods of time and then germinate to release vegetative cells that serve as an inoculum to initiate blooms. Cysts are considered important with respect to species dispersal, bloom timing, bloom location, survival through adverse conditions, and, for toxic species, as sources of toxin (Wall 1971; Anderson 1984). Twenty years ago, Wall and Dale (1969) demonstrated that a microfossil called *Hemicystodinium zoharyi* (Rossignol 1961) was in fact the resting cyst of *Pyrodinium bahamense.* Aside from the preliminary germination experiments conducted by Wall and Dale (1969), all other published work on the living form of this cyst has been taxonomic in nature (e.g., Matsuoka et al. 1989). The objective of this paper is to describe the information that has been obtained for other cyst-forming dinoflagellates and to discuss how such data can be useful in studies of *P. bahamense* bloom dynamics and distribution.

Life Histories

The dominant reproductive mode in dinoflagellates is asexual fission (reviewed in Pfiester and Anderson (1987)). Those species that form true resting cysts are also capable of sexual reproduction, forming gametes that fuse into a swimming zygote (planozygote) which, in most cases, transforms into a nonmotile, thick-walled resting cyst (Beam and Himes 1980). The morphological characteristics of these different life history stages and the duration of the transitions between stages and of the stages themselves differ between species. Examples of complete dinoflagellate life history descriptions are those of *Gymnodinium pseudopalustre* (von Stosch 1973), *Peridinium cinctum* (Pfiester 1975), and *Gyrodinium uncatenum* (Tyler et al. 1982).

Induction of sexuality in laboratory cultures is most often accomplished by nutrient limitation (e.g., Pfiester 1975; Turpin et al. 1978; Anderson et al. 1984; Anderson et al. 1985), but cyst formation has also been observed in nutrient-replete media (Yoshimatsu 1981; Watanabe et al. 1982). One of the problems in studying dinoflagellate sexuality in culture is that not all cultures will produce cysts, even when many potential mating types are crossed (Anderson et al. 1984). This may relate to unknown culture stresses or to genetic changes in species maintained in culture for extended intervals.

None of the information described above is known for *P. bahamense.* Research is needed to describe the details of the *P. bahamense* life cycle, with emphasis on: mating types; morphology of gametes and planozygotes; and mechanisms of sexual induction. The ability to recognize these life cycle stages in natural samples will provide important information on the stage of the bloom (i.e., initiation, development or decline). Likewise, studies that work out the steps needed to produce cysts in laboratory cultures may be necessary for subsequent efforts to understand the physiological or biochemical characteristics of the cysts of this species.

Dormancy and Quiescence

A newly formed cyst generally has a "maturation" interval during which it cannot germinate, even when growth conditions are optimal (Pfiester and Anderson 1987). Termed "dormancy", this stage should be distinguished from "quiescence", which refers to the subsequent interval when maturation is complete, but the resting state continues due to the absence of suitable growth conditions. Domancy is under internal (endogenous) control, whereas quiescence is maintained by external (exogenous) factors.

The duration of dormancy varies significantly between species. For some, it can last several months (e.g., *Alexandrium tamarense (Protogonyaulax tamarensis)* Anderson 1980), a few weeks (*Scrippsiella trochoidea;* Binder and Anderson 1987), or a few days (*Gymnodinium catenatum;* Blackburn et al. 1989). This maturation time interval has major implications on the bloom dynamics of a species. For example, the relatively long dormancy requirements of *A. tamarense* mean that cysts deposited after a bloom cannot germinate for several months, sometimes leading to two distinct bloom events in the spring and fall, respectively, in temperate waters (Anderson and Morel 1979). In contrast, the much shorter dormancy of *S. trochoidea* allows that species to cycle rapidly between the sediments and the water column, resulting in nearly continuous presence of motile cells when temperatures are favorable for growth (Binder and Anderson 1987).

Wall and Dale (1969) described a series of germination experiments using *P. bahamense* cysts that suggest the existence of a dormancy interval. Sediment collected in February contained cysts that did not germinate in mid-March under conditions that did result in germination several weeks later. Much more detailed experiments are required to obtain further information.

When dormancy is complete and the cyst is capable of germination, ɋuiescence can be maintained by a variety of external factors. Three of the most important are sub- or supra-optimal temperatures, anoxia and inadequate light. In temperate waters, where cysts can experience dramatic seasonal changes in bottom temperatures, data now being collected show that many species have a temperature range outside which germination is inhibited (Pfiester and Anderson 1987). For example, cysts of *A. tamarense* from Cape Cod (USA) germinate between 6 and 20°C, whereas cyst of *Gyrodinium uncatenum* from the same waters only germinate between 15 and 21ÁC (D.M. Anderson, unpub. data). It is thus not surprising that the former species first appears in the spring and the latter in the summer/early fall in that region.

Similar data, if obtained for *P. bahamense,* would provide insights into the role of temperature in bloom initiation. Such information would be especially interesting given the relatively narrow range of temperature fluctuations in tropical waters. A hint of low temperature inhibition of *P. bahamense* cyst germination is found in the study by Wall and Dale (1969) which indicated that there was no cyst germination during 6 months of storage at 16°C. A more complete study is clearly warranted.

A study by Anderson et al. (1987) demonstrated that the cysts of several different dinoflagellates could not germinate in anoxic sediments. Another study (Anderson et al. 1982) showed that most cysts in core samples are not located at the sediment surface but are buried by bioturbation and sedimentation and are thus below the oxidized layer at the surface where germination is possible. *P. bahamense* may be no exception and special experiments on this species need not be a high priority. On the other hand, vertical cyst distribution needs to be studied to provide the background to evaluate the potential impact of anoxia on *P. bahamense* bloom initiation.

Another factor that affects cyst quiescence is light, which would be absent or low due to cyst deposition in deeper waters or burial within the sediments. A wide range of responses to light have been observed between species. Some will not germinate in the dark, others require only a brief pulse of light to initiate germination, while most can germinate in the dark but at rates that are slower than those observed with illumination (Binder and Anderson 1986; Anderson et al. 1987). Here again, nothing is known of the light requirements of *P. bahamense* cysts.

Cysts that have matured through their dormancy phase can reenter the resting state and be unresponsive to favorable growth conditions (Anderson and Keafer 1987). In higher plants, this process is termed secondary dormancy, with the external environment (e.g., seasonal temperature changes) causing the transition. The process discovered in cysts of *A. tamarense* is similar, except that the evidence suggests that the re-induction of true dormancy is under internal control - that there is an endogenous annual clock that determines germination potential (Anderson and Keafer 1987). An endogenous rhythm if present in *P. bahamense* cysts, could explain the timing of blooms given the relatively small temperature ranges of tropical waters.

Cyst Distributions

Two types of cyst distribution studies have been conducted - qualitative and quantitative. The former typically determine the presence or absence of the cyst of a species in a series of samples along a coastline (e.g., Anderson et al. 1982). These studies are valuable in describing the general geographic distribution of a species and can even indicate areas where toxicity might be an unrecognized problem. For example, the detection of cysts of *A. tamarense* in areas of Connecticut (USA) with no history of shellfish toxicity suggested a re-evaluation of monitoring station locations and eventually led to the detection of toxicity in shellfish in areas that were previously not monitored (Anderson et al. 1982). Although inherently quite valuable and easy to obtain, presence versus absence cyst data should be interpreted with caution. The presence of cysts of a species of interest does suggest the potential for bloom initiation, but the converse is not true. The absence of detectable cysts of a species does not mean an area is free from the threat of toxic blooms. An excellent example of this point emerged during this *Pyrodinium* workshop, where multiple sediment samples collected in Brunei Bay were found to contain very few living dinoflagellate cysts. Since the cores were taken in areas where large *P. bahamense* blooms had occurred in recent years, the paucity of cysts clearly reflects factors such as cyst resuspension and transport and not the continual absence of the species from the plankton.

Quantitative cyst mapping is a much more time-consuming, tedious process that should only be attempted if specific goals justify the effort. Such data provide countour plots of cyst distributions that can reveal important patterns. One example that may be especially relevant to the *P. bahamense* problem in the Indo-West pacific region is a study of *A. tamarense* cysts in an estuarine system on Cape Cod (Anderson et al. 1982). That mapping effort showed how cysts were numerous within one lagoon or embayment but undetectable in adjacent waters. This suggested a "point source" for the species - a location where blooms can originate before being transported elsewhere. Other cyst surveys (Tyler et al. 1982; White and Lewis 1982; Anderson and Keafer 1985) provide examples of how widespread cyst distributions can be depicted with quantitative mapping techniques. Consistent with our general lack of knowledge on all aspects of *P. bahamense* biology and ecology, there have been no cyst distributional studies of any type. In addition to the motivations described above, the tantalizing relationship that is often suggested between *P. bahamense* blooms and mangrove systems could be strengthened by cyst surveys that reveal an abundance of cysts in nearshore waters, possibly even in localized "point sources" near mangrove-dominated areas.

The distributions depicted by mapping surveys should be interpreted with caution. High cyst concentrations in certain locations may well indicate important seedbeds for bloom initiation. However, the cysts may be accumulating in areas where light levels or temperatures are unfavorable for germination, or where germination might produce motile cells that are consistently carried away from shellfish or fish resources. One means to assess the magnitude of the inoculum provided by cysts at a location is to conduct a quantitative survey of the site, and then to monitor the extent of germination. This can be accomplished by monitoring the chlorophyll fluorescence that is a sign of impending germination for some species (Yentsch et al. 1980; Anderson and Keafer 1985). The highly resistant cyst wall of *P. bahamense* offers an even easier alternative - that of monitoring the ratio between living and empty cysts through time. Since the *P. bahamense* cyst wall will not degrade after germination (it is fossilizable) and is easily recognizable even when empty, the deposition of new cysts and the germination of living cysts would be seen as increases and decreases in the ratio through time. This approach has been used to study the cyst dynamics of *Gonyaulax polyedra* in Scottish sea lochs (Lewis et al. 1985; Lewis 1988), and is already being used in a study of *P. bahamense* in the Samar Sea of the Philippines (K. Matsuoka, pers. comm.).

The need for *P. bahamense* cyst mapping surveys is apparent. No distributional data of any type have been collected, yet the pattern of species dispersal throughout the Indo-West Pacific region may reflect cyst transport as much as the movement of established blooms between countries by surface currents. Similarly, the origin of blooms remains unknown in all affected countries. Do the blooms originate in localized areas nearshore, and then move with currents to adjacent locations? Or alternatively, are there offshore seedbeds (as is the case with *A. tamarense* in some regions (Anderson and Keafer 1985) that can initiate blooms that are then transported towards shore? These important questions can be addressed in part through cyst mapping surveys.

Discussion

Although the cyst of *P. bahamense* has been known for twenty years, suprisingly little is known of its germination requirements, physiological tolerances and geographic distribution. The research issues described above should be investigated in any program attempting to understand *P. bahamense* bloom dynamics, but the results must be interpreted with caution. The fact that *P. bahamense* has a cyst indicates that the cells necessarilly spend some of their existence in a dormant state in the sediment. What is not clear is how important those cysts are in general bloom dynamics. In temperate waters where cysts provide a mechanism for over-wintering, the dormancy strategy is of great importance if a species is to persist from year to year. In tropical waters where temperature fluctuations are less severe, encystment may provide other more subtle benefits such as those arising from genetic recombination. It is conceivable that careful scrutiny of the water would find at least a few *P. bahamense* motile cells present at all times, with the cyst inoculum being relatively insignificant compared to the growth of the low level vegetative population. This speculation is probably incorrect, but is offered to emphasize that cysts can be important for different reasons in different regions.

Acknowledgements

This work was supported in part by the National Science Foundation (OCE-86-149210) and by the Donaldson Charitable Trust. Contribution No. 7128 from the Woods Hole Oceanographic Institution.

References

Anderson, D.M. and F.M.M. Morel. 1979. The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. Est. Coast. Mar. Sci. 8: 279-293.

Anderson, D.M., D.M. Kulis, J.A. Orphanos and A.R. Ceurvels. 1982. Distribution of the toxic red tide dinoflagellate *Gonyaulax tamarensis* in the southern New England region. Est. Coast. Shelf Sci. 14: 447-458.

Anderson, D.M., D.G. Aubrey, M.A. Tyler and D.W. Coats. 1982. Vertical and horizontal distributions of dinoflagellate cyst in sediments. Limnol. Oceanogr. 27: 757-765.

Anderson, D.M. 1984. The roles of dormant cysts in toxic dinoflagellate blooms and shellfish toxicity, p. 125-138. *In* E. Ragelis (ed.) Seafood Toxins. Am. Chem. Soc. Symposium Series. Washington, D.C.

Anderson, D.M., D.M. Kulis and B.J. Binder. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis:* Cyst yield in batch cultures. J. Phycol. 20: 418-425.

Anderson, D.M. and B.A. Keafer. 1985. Dinoflagellate cyst dynamics in coastal and estuarine waters, p. 219-224. *In* D.M. Anderson, A.W. White and D.G. Baden (eds.) Toxic Dinoflagellates. Elsevier, New York.

Anderson, D.M., D.W. Coats and M.A. Tyler. 1985. Encystment of the dinoflagellate *Gyrodinium uncatenum:* temperature and nutrient effects. J. Phycol. 21: 200-206.

Anderson, D.M. and B.A. Keafer. 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis.* Nature 325: 616-617.

Anderson, D.M., C.D. Taylor and E.V. Armbrust. 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. Limnol. Oceanogr. 32: 340-351.

Beam, C.A. and M. Himes. 1980. Sexuality and meiosis in dinoflagellates, p. 171-206. *In* M. Levandowsky and S.H. Hutner (eds.) Biochemistry and Physiology of Protozoa. 2nd Ed. Vol. 3, Academic Press, London.

Binder, B.J. and D.M. Anderson. 1987. Physiological and environmental control of germination in *Scrippsiella trochoidea* (Dinophyceae) resting cysts. J. Phycol. 23: 99-107.

Blackburn, S.I., G.M. Hallegraeff and C.J. Bolch. 1989. Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum* from Tasmania, Australia. J. Phycol. 25: 577-590.

Lewis, J., P. Tett and J.D. Dodge. 1985. The cyst-theca cycle of *Gonyaulax polyedra (Lingulodinium machaerophorum)* in Creran, A Scottish West Coast sea-loch, p. 85-90. *In* D.M. Anderson, A.W. White and D.G. Baden (eds.) Toxic Dinoflagellates. Elsevier, New York.

Lewis, J. 1988. Cysts and sediments: *Gonyaulax polyedra (Lingulodinium machaerophorum)* in Loch Creran. J. Mar. Biol. Ass. U.K. 68: 701-714.

Matsuoka, K., Y. Fukuyo and C.L. Gonzales. 1989. A new discovery of cysts of *Pyrodinium bahamense* var. *compressum* from the Samar Sea, Philippines, p. 301-304. *In* T. Okaichi, D.M. Anderson and T. Nemoto (eds.) Red Tides: Biology, Environmental Science and Toxicology. Elsevier, New York.

Pfiester, L.A. 1975. Sexual reproduction of *Peridinium cinctum* f. *ovoplanum* (Dinophyceae). J. Phycol. 11: 259-265.

Pfiester, L.A. and D.M. Anderson. 1987. Dinoflagellate life cycles and their environmental control, p. 611-648. *In* F.J.R. Taylor (ed.) The Biology of Dinoflagellates. Blackwell Scientific Publications, Ltd., Oxford.

Rossignol, M. 1961. Analyse pollinique de sediments marins Quaternaires en Israel II. Sediments Pleistocenes. Pollen Spores 4: 121-148.

Tyler, M.A., D.W. Coats and D.M. Anderson. 1982. Encystment in a dynamic environment: deposition of dinoflagellate cysts by a frontal convergence. Mar. Ecol. Prog. Ser. 7: 163-178.

Turpin, D.H., P.E.R. Dobel and F.J.R. Taylor. 1978. Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. J. Phycol. 14: 235-238.

von Stosch, H.A. 1973. Observation on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* and *Woloszynskia apiculata* sp. nov. Br. Phycol. J. 8: 105-134.

Wall, D. and B. Dale. 1969. The "hystrichosphaerid" resting spore of the dinoflagellate *Pyrodinium bahamense* Plate, 1906. J. Phycol. 5: 140-149.

Wall, D. 1971. Biological problems concerning fossilizable dinoflagellates. Geoscience and Man. III: 1-15.

White, A.W. and C.M. Lewis. 1982. Resting cysts of the toxic, red tide dinoflagellate *Gonyaulax* excavata in Bay of Fundy sediments. Can. J. Fish. Aquat. Sci. 39: 1185-1194.

Watanabe, M.M., M. Watanabe and Y. Fukuyo. 1982. Encystment and excystment of red tide flagellates. I. Induction of encystment of *Scrippsiella trochoidea,* p. 27-42. *In* Nat. (Japan) Inst. Environ. Stud., Res. Rep. No. 30. Eutrophical and Red Tides in the Coastal Marine Environment.

Yentsch, C.M., C.M. Lewis and C.S. Yentsch. 1980. Biological resting in the dinoflagellate *Gonyaulax excavata*. Bioscience 30: 251-254.

Yoshimatsu, S. 1981. Sexual reproduction of *Protogonyaulax catenella* in culture: I. Heterothallism. Bull. Plank. Soc. Japan 28 131-139.