

Massive infestation by *Amyloodinium ocellatum* (Dinoflagellida) of fish in a highly saline lake, Salton Sea, California, USA

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ABSTRACT: Persistent fish infestation by the parasitic dinoflagellate *Amyloodinium ocellatum* was found at a highly saline lake, Salton Sea, California, USA. The seasonal dynamics of the infestation of young tilapia was traced in 1997–1998. First appearing in May, it became maximal in June–August, decreased in October and was not detectable in November. Outbreak of the infestation and subsequent mortality of young fish was registered at the Sea at a water temperature and salinity of 40°C and 46 ppt, respectively. Some aspects of the ultrastructure of parasitic trophonts of *A. ocellatum* and their location on the fish from different size groups are considered. The interactions of parasitological and environmental factors and their combined effect upon fish from the Salton Sea are discussed.

KEY WORDS: Parasite · Dinoflagellate · *Amyloodinium ocellatum* · Tilapia · Infestation · Salton Sea

INTRODUCTION

The peridinean dinoflagellate *Amyloodinium ocellatum* (Brown, 1931) Brown & Hovasse, 1946 is global in distribution and infects over 100 species of marine and brackish-water fish. Its biology, morphology and pathogenicity have been subjects for long-term studies since the 1930s (Brown 1934, Nigrelli 1936, Brown & Hovasse 1946, Lom & Lawler 1973, Lawler 1977a,b, Paperna 1980, 1984, Bower et al. 1987, Noga et al. 1991). *A. ocellatum* has a direct life cycle consisting of 3 intermittent stages. The actively feeding parasitic trophont is attached to fish gills and skin; the reproductive encysted tomtom is inserted into sediments; and the free-swimming infective dinospores develop after the tomtom divides. Precise identification of the dinospores in order to determine the taxonomic characteristics of *A. ocellatum* has been established by means of electron microscopic investigations (Steidinger et al. 1989, 1996, Landsberg et al. 1994, 1995).

Amyloodinium ocellatum is known as a very persistent and destructive agent causing massive mortality in aquarium-held fish. Healthy fish are killed within 12 h after being exposed to a high concentration of dinospores (Lawler 1977b). Epizootics of amyloodiniosis in public aquaria in London (Brown 1934), New York (Nigrelli 1940), Singapore (Laird 1956), Denmark (Hojgaard 1962), Taiwan (Chien & Huang 1993) and elsewhere resulted in the loss of 40 to 60% of marine fish. Initially identified as a parasite of aquarium fish, *A. ocellatum* has been recognized as a major pathogen of cultivated marine warmwater fish. In some years, great losses of fish stock from aquaculture facilities that varied from 50 to 80% of the population were registered in Israel, Italy, Spain, France, Yugoslavia, Mexico, Taiwan, and the southern part of the United States (Lawler 1977b, 1980, Paperna & Baudin-Laurencin 1979, Paperna 1980, Noga et al. 1991, Alvarez-Pellitero et al. 1993, 1995, Chien & Huang 1993, Sandifer et al. 1993). Different methods for removing *A. ocellatum* from aquaculture systems and aquaria have been tested. Besides the traditional method of decreasing water salinity, using solutions of copper sulfate and formalin, they include fish immunization with antigens

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of the dinospore stage or anti-*A. ocellatum* serum (Smith et al. 1992, 1993, 1994) and use of nauplii of brine shrimp *Artemia salina* as a bioremediation measure (Oestmann et al. 1995). In recent years, antibiotics have been recommended for the prevention and treatment of amyloodiniosis in mariculture facilities (Oestmann & Lewis 1996).

There have only been a few reports on the occurrence of *Amyloodinium ocellatum* in the wild (Lawler 1980, Overstreet 1982, 1993, Alvarez-Pellitero et al. 1993). Usually, fish infestation by *A. ocellatum* in natural bodies of water has not exceeded 30% and could not be a likely cause of mortality. Only 1 outbreak of fish kill had been attributed to *A. ocellatum* (Overstreet 1993).

In June 1997, we found high infestation by *Amyloodinium ocellatum* among young tilapia in the Salton Sea, California. This body of water is infamous for frequent fish kills and bird dieoffs. High salinity, high and low temperatures, high ammonia levels and low oxygen tension have long been suspected as the causes of these mortality events (California Regional Water Quality Control Board 1994). In recent years the list of dangerous factors has been enlarged by algal toxins and microbial diseases such as avian botulism, avian cholera and Newcastle disease. The possible role of parasites has remained unknown as no previous parasitological investigations had been performed at the Salton Sea. We report here the first systematic examination of the infestation of the tilapia *Oreo-*

chromis mossambicus by *A. ocellatum* from the Salton Sea. We present and discuss information on seasonal variation in fish infestation by *A. ocellatum*, the influence of the Salton Sea environment, some aspects of trophont morphology, and their location on the fish body.

MATERIALS AND METHODS

Characteristics of the lake. The Salton Sea (33° 25' N, 115° 50' W) is the largest lake in California (Fig. 1). It was formed accidentally in 1905–1907 when flood water was diverted from the Colorado River, broke a temporary levee, and filled the large desertic Salton Sink. The modern-day Salton Sea has an area of 980 km² and a 153 km shoreline. Two major tributaries (the Alamo River and New River) which collect wastewaters from agriculture and municipalities provide current freshwater input into the Sea. The Salton Sea lacks outlets and water leaves it only by evaporation. Due to rapid accumulation of salts, water salinity had risen from 4 ppt in 1905 to 46 ppt in 1997. Extremely high nutrient loads have created eutrophic conditions and low oxygen tension that vary in summer months from 0 on the bottom to 20 mg l⁻¹ on the water surface. Contaminants such as selenium, boron, and DDE and its metabolites have been found in the Salton Sea biota (Setmire et al. 1993).

Sampling and preparation. A total of 664 specimens of young tilapia *Oreochromis mossambicus* (Peters), the most abundant species of fish at the Salton Sea, were caught in spring, summer, and autumn in 1997–1998 (Table 1). In 1998 fish were collected from 4 sites along the Salton Sea shoreline: Varner Harbor, Bombay Beach, Red Hill and Salton City (in 1997 from Varner Harbor only) (Fig. 1). Sampling was carried out in shallow harbors inhabited by large schools of young fish. Water temperature was measured in the littoral area of fish collection sites. After being caught with landing net and seine, fish were immediately transported to the field laboratory on the lake shore or were placed into aerated tanks or buckets with Salton Sea water and transported to San Diego State University. Total lengths of fish were measured to the nearest millimeter.

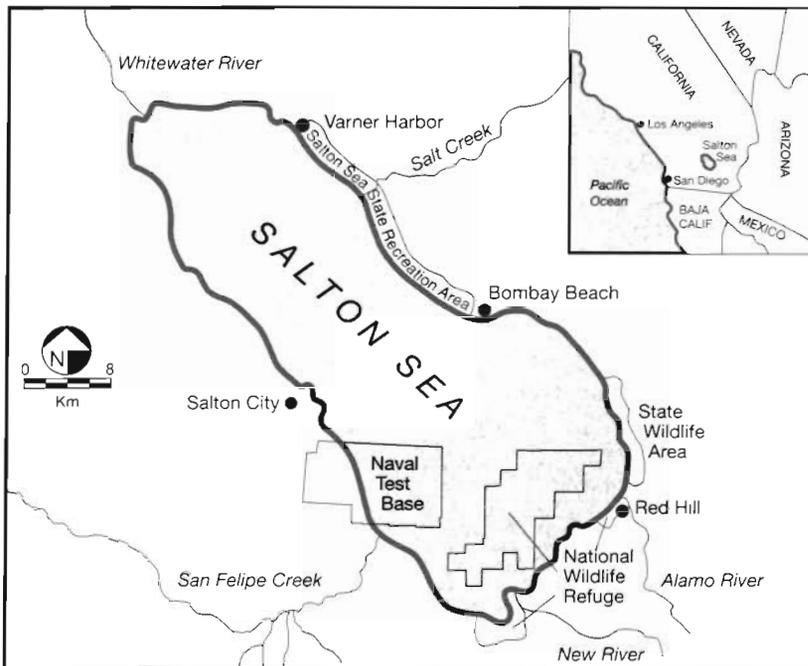


Fig. 1. Map of the Salton Sea. (●) Sites of fish sampling

Three size groups of tilapia were distinguished on the basis of their body length: Group 1: 1.1 to 2.5 cm, so called baby fish (499 fish); Group 2: 2.6 to 6.8 cm (150 fish) and Group 3: 7.0 to 13.0 cm (27 fish). Additionally, specimens of young croaker *Bairdiella icistia* (Jordan and Gilbert) were collected from Varner Harbor and Salton City in July and October 1998, respectively. Two size classes of fish were represented: Group I: 2.9 to 3.7 cm (40 fish) from Varner Harbor; and Group II: 10.5 to 11.0 cm (6 fish) from near Salton City.

Body surfaces, fins, and gills were carefully examined for the presence of ectoparasites under dissecting and compound microscopes. Prevalence and intensity of infestation were defined in fresh unstained samples of fish. Prevalence of infestation was defined as percentage of fish infected. Intensity of infestation was defined as a number of trophonts per fish and was recorded as high (+++, hundreds of parasites per fish), medium (+, dozens of parasites), and low (+, few parasites). Trophonts of *Amyloodinium ocellatum* were measured with an ocular micrometer and photographed using Kodak film and a Zeiss light photomicroscope. Fish infected by trophonts of *A. ocellatum* were selected for examination by scanning electron microscopy (SEM).

Electron microscopy. Whole bodies of the infected tilapia (Group 1) and gill arches of tilapia (Groups 2

and 3) and croaker were fixed in cold Karnovsky fixative for at least 2 h, postfixed in 1% osmium tetroxide for 1 h to increase specimen conductivity, and dehydrated in a graded ethanol series with the final change in absolute ethanol. Then the samples were critical-point-dried with liquid CO₂ and mounted on the stubs. Gills from the smallest tilapia (1.1 to 1.3 cm) were prepared for SEM by removing the left operculum from these fish with fine-tipped forceps to expose the gill baskets. Fish gills and whole fish bodies were sputter-coated with palladium and examined with a scanning electron microscope (Hitachi S 2700) at the accelerating voltage of 10 kV.

RESULTS

Seasonal dynamics of fish infestation

Persistent infestation of young fish by *Amyloodinium ocellatum* was found at the Salton Sea in 1997–1998. The seasonal dynamics of infestation was followed more closely in 1998 at 2 sites, Varner Harbor and Bombay Beach.

In Varner Harbor, initial infestation by parasitic trophonts of *Amyloodinium ocellatum* was observed

among recently hatched tilapia in May 1998, when daytime water temperature in this shallow harbor was 20 to 22°C (Table 1). At that time the prevalence and intensity of infestation were very low (Table 1). Infection of tilapia from Groups 1 and 2 was gradually increased in June and reached a peak in July when water temperature was 40°C (Table 1). In July, 100% prevalence and high intensity of infestation by *A. ocellatum* were found not only in tilapia but also in young croakers. In August, the prevalence of tilapia infestation was the same but intensity was lower (Table 1). In late October and early November, when water temperature decreased to 24 and 21°C, respectively, only tilapia from Group 1 were caught and examined. None of them were found infected by *A. ocellatum* (Table 1).

In general, the pattern of *Amyloodinium ocellatum* infestation of tilapia from the Bombay Beach was very close to that for fish from

Table 1. Seasonal dynamics of infestation by *Amyloodinium ocellatum* of tilapia from the Salton Sea in 1997–1998. G: gills; F: fins; S: skin

Time	Location	Water temp. (°C)	Fish		Infestation		Infected organ
			No.	Size (cm)	Prevalence (%)	Intensity	
1997							
Jun	Varner Harbor	27.9	23	1.4–2.0	100	+++	G,F,S
Aug	Varner Harbor	33.0	21	1.8–2.2	100	+++	G,F,S
Sep	Varner Harbor	29.4	24	2.7–3.9	75	++	G,F
1998							
May	Varner Harbor	21.9	50	1.0–1.2	20	+	G,S
	Bombay Beach	21.6	40	1.0–1.3	25	+	G,S
Jun	Varner Harbor	25.8	20	1.2–1.8	50	+	G,F,S
Jul	Varner Harbor	40.0	25	1.2–1.3	100	+++	G,F,S
			25	3.1–5.3	100	+++	G
			45	1.2–1.5	50	+	G
	Bombay Beach	40.0	25	1.0–1.5	100	+++	G,F,S
			20	4.0–6.0	100	+++	G
			20	1.2–1.5	100	++	G,F
Aug	Varner Harbor	38.2	15	2.6–5.0	100	++	G
			27	1.0–1.5	100	+++	G,F,S
Sep	Bombay Beach	32.2	27	1.0–1.5	100	+++	G,F,S
Oct	Salton City	27.7	40	1.0–1.2	100	+++	G,F,S
			16	7.5–13.0	100	+++	G
			31	1.3–2.2	0		
			40	2.0–2.2	45	+	F,S
			16	2.7–6.8	80	+	G
			11	7.0–10.8	70	+	G
			50	3.0–6.1	85	+	G
Nov	Varner Harbor	21.0	25	1.0–2.5	0		
			25	1.0–2.2	0		
	Bombay Beach	21.0	25	1.0–2.2	0		

Varner Harbor (Table 1). However, in Bombay Beach, the prevalence and intensity of infestation increased more slowly and a peak of infection was registered only in September. By the end of October, when water temperature had declined, infection began to decrease. Intensity of infestation was equally low for tilapia from all size classes while the prevalence of infestation varied from 45% in the smallest fish (Group 1) to 70–80% in fish from Groups 2 and 3 (Table 1). In early November, in Bombay Beach as in Varner Harbor, no fish examined were infected by *A. ocellatum* (Table 1).

In Red Hill, a peak of infestation of tilapia from Groups 1 and 2 by *Amyloodinium ocellatum* was found in July (Table 1). In October, high prevalence was combined with low intensity of infestation (Table 1). At the same time, tilapia collected near Salton City, where water temperature was higher than at other sampling sites, demonstrated 100% prevalence and high intensity of infestation by *A. ocellatum* (Table 1).

In the summer-early autumn period of 1997, tilapia from Groups 1 and 2 were examined only at Varner Harbor (Table 1). The maximal prevalence and intensity of infestation of young fish were maintained during June–August. In September, when daytime water temperature was decreasing, prevalence and intensity of fish infestation began to decrease (Table 1).

Massive mortality of young tilapia and croaker infected by *Amyloodinium ocellatum* was observed in the shallow harbors of the Salton Sea in July 1998. Naturally infected fish demonstrated the same general symptoms of infestation by these parasites described for fish from aquaculture (Lawler 1977b). Fish were rapidly gasping for air at the water surface, swimming spastically and constantly at the water surface before sinking back to the bottom, jumping out of the water, and finally losing their equilibrium and dying. Typically, mortality events developed very rapidly. For example, on July 30, 1998, we observed the death of a massive number of heavily infected young tilapia at Varner Harbor within 2 h after they first exhibited signs of aberrant behavior.

Ultrastructure and localization of trophonts of *Amyloodinium ocellatum*

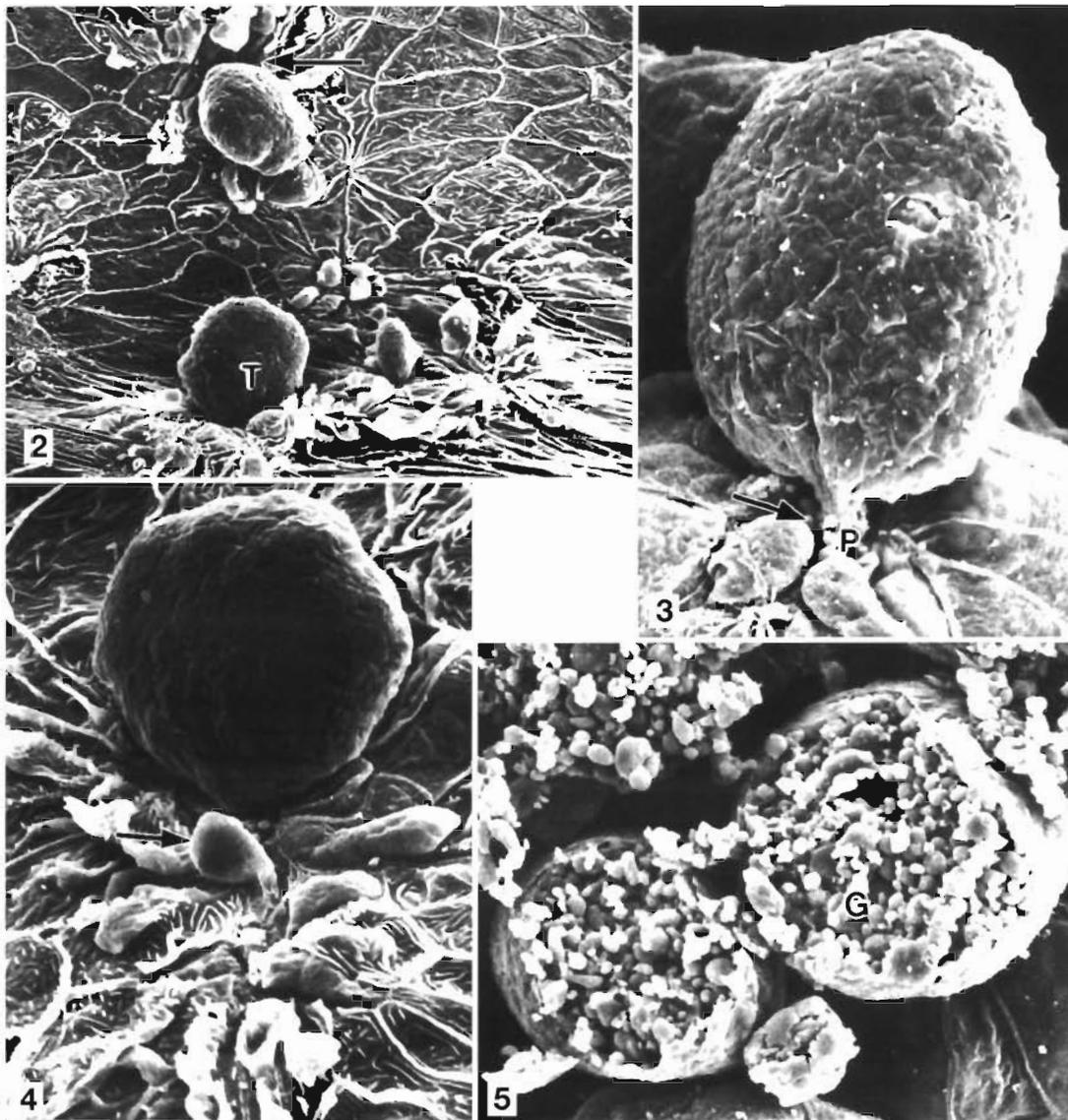
Live parasitic trophonts are brownish or yellowish with small red stigma near the base. The trophonts found on the infected fish varied in size (Fig. 2). The largest ones measured $129 \times 79 \mu\text{m}$ and the smallest $49 \times 26 \mu\text{m}$. The observed ratio between small and large trophonts varied among different individual fish; presumably, it depends on the duration of the fish infestation.

Under the scanning electron microscope, trophonts of *Amyloodinium ocellatum* look like elongated, oval or spherical sacs filled with small granules (Figs. 3 to 5). Their basal portion is narrow and forms a very short stalk or peduncula that ends in a flattened attachment disk (Figs. 3 & 6). Numerous filiform projections, rhizoids, and mobile tentacle-like stopomode protrude from the disk (Figs. 6 to 8). Rhizoids fuse with the surface of epithelial cells providing tight adhesion of trophonts to fish tissues. The long stopomode that is inserted into the cells provides stronger anchoring of the parasites (Figs. 7 & 8). Mature trophonts of *A. ocellatum* easily detach from fish tissues. Numerous impressions at the attachment sites of trophonts can be found on the surface of epithelial tissues of infected fish (Fig. 9).

Trophonts of *Amyloodinium ocellatum* were found only on the external organs of fish studied (Table 1). The location of parasites on the fish depended on host size and level of infestation. In highly infected small tilapia (Group 1), parasites were distributed on the skin, fins, tail, and on the gills in particular (Table 1). Numerous trophonts of *A. ocellatum* were located along gill filaments, between respiratory lamellae, and sometimes on the gill arches (Fig. 10). Groups consisting of 3 to 6 parasites were tightly attached to the tips of gill filaments (Fig. 11). Some *A. ocellatum* were found in the branchial cavity, on the internal surface of gill covers and in the mouth. On the skin, trophonts usually formed clusters of 2 to 5 individuals (Fig. 12). Sometimes *A. ocellatum* on fins, tail, and skin were associated with the ciliate peritrichs *Ambiphrya ameyuri* (Fig. 13). The latter species may also attach to the superficial epithelial tissues of young tilapia and cause significant infestation of fish from the Salton Sea (B. Kuperman & V. Matey unpubl. data). In small tilapia with a medium level of infestation, parasites were concentrated on the gills and fins; fish with low levels of infection had trophonts only on the gills (Table 1).

In medium size tilapia (Group 2) and croaker (Group I) with different intensities of infestation, parasitic trophonts were located mainly on the gills and sometimes on the fins (Table 1). In large tilapia (Group 3) and croaker (Group II), only gills were infected (Table 1). Occasionally, a few *Amyloodinium ocellatum* specimens could be found on the fins and almost none on the body surface, which was tightly covered with scales. It must be noted that the natural picture of trophont distribution on the external organs of fish can be observed only with fresh, unfixed samples because standard fixing procedures used for LM or EM studies cause rapid detachment of trophonts.

We have revealed a definite negative impact of *Amyloodinium ocellatum* on the structure of external



Figs. 2 to 5. *Amyloodinium ocellatum*. Scanning electron microscope microphotographs. Fig. 2. Localization of parasitic trophonts (T) on the skin of tilapia. Epithelial distortion (arrows); $\times 700$. Fig. 3. Frontal view of elongated trophont attached to fish gills showing displacement of peduncula (P). Beginning of a local erosion of epithelial tissue (arrow); $\times 2000$. Fig. 4. Frontal view of spherical trophont. Flattening of the epithelial cells around site of attachment (arrow); $\times 1500$. Fig. 5. Transverse section of trophonts filled with numerous granules (G); $\times 2500$

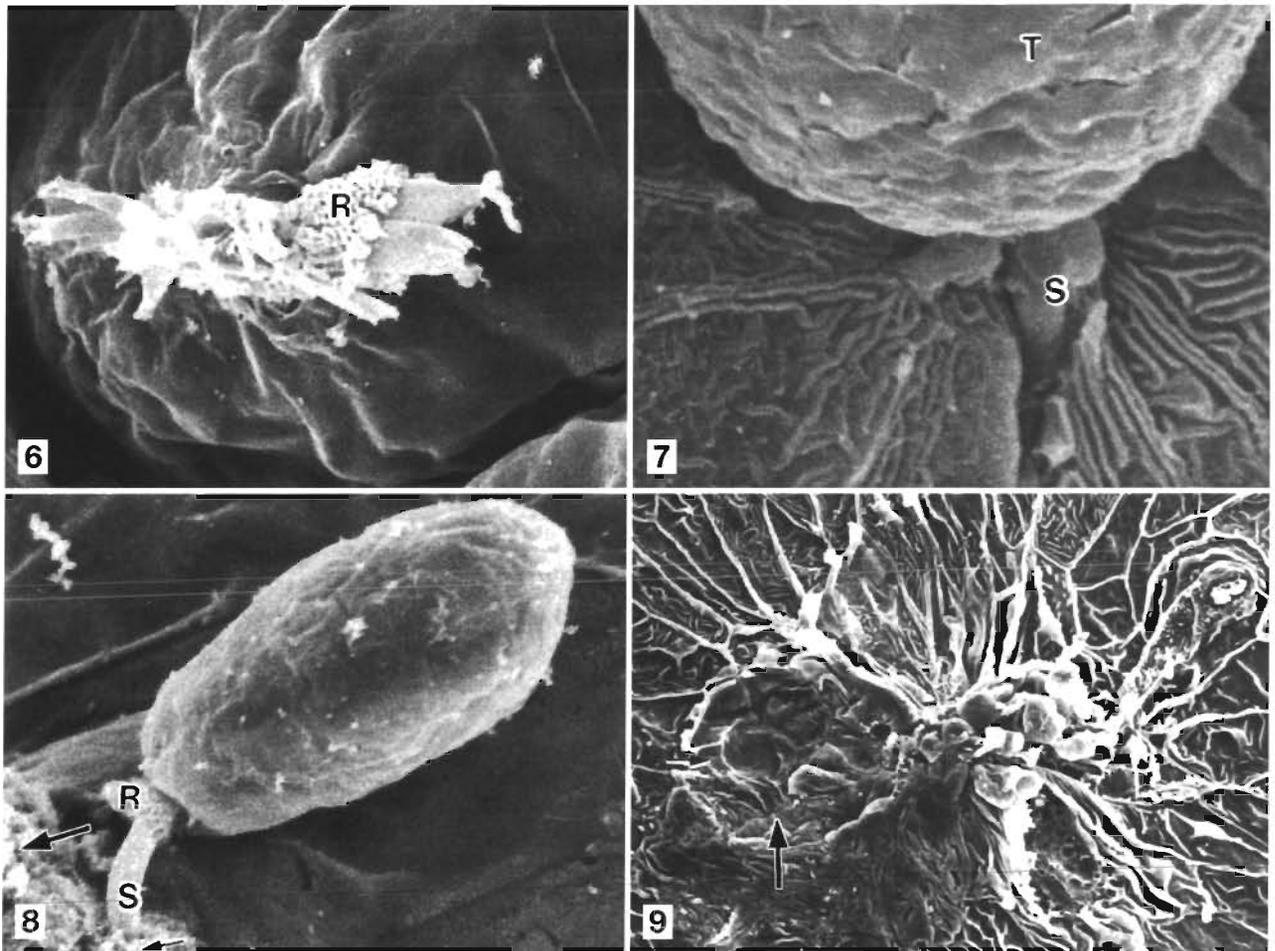
organs of infected fish from the Salton Sea. Fish gills were altered more than other organs. Typically, gill filaments were enlarged and swollen, and partial or full fusion of respiratory lamellae transformed them into asymmetric, club-like structures (Fig. 10). Local epithelial erosion was found in all infected organs at the sites of parasite attachment to the host tissue (Figs. 3, 8 & 11). Flattened and partially or completely destroyed epithelial cells were concentrated around pedunculae of *A. ocellatum* (Figs. 2, 4 & 12). These may represent toxic or digestive effects of substances released from the trophont attachment organ on the

host's epithelial tissues, though this remains speculative (Lom & Lawler 1973).

Ultrastructural alterations in fish infected by *Amyloodinium ocellatum* are of special interest and will be considered in our next paper.

DISCUSSION

A strong and persistent infestation of fish by *Amyloodinium ocellatum* has been found at a highly saline lake, the Salton Sea, in the southwestern part of the



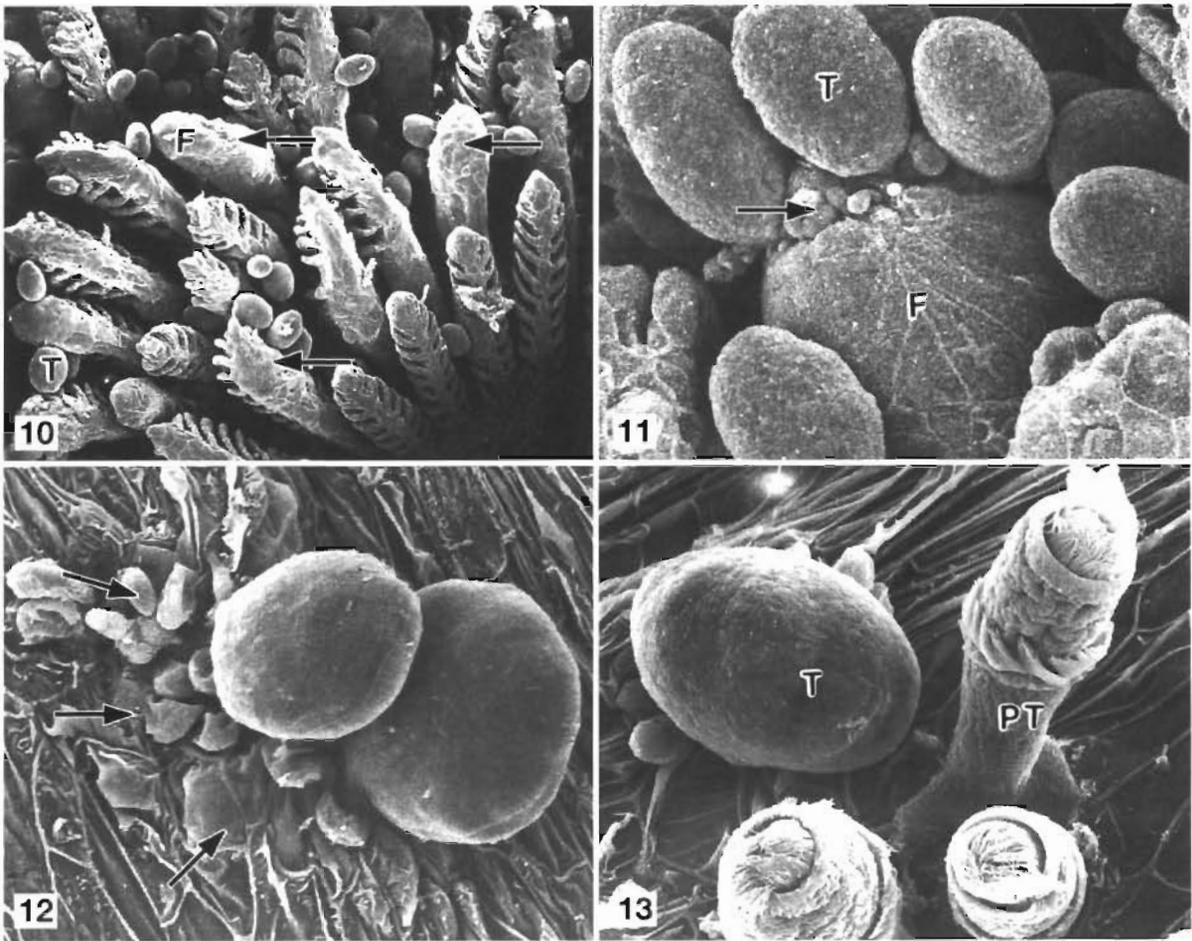
Figs. 6 to 9. *Amyloodinium ocellatum*. Scanning electron microscope microphotographs. Fig. 6. Inner side of the trophont's attachment disk with rhizoids (R); $\times 7000$. Fig. 7. Penetration of a stopomode (S) into epithelial cell of a tilapia gill; $\times 5000$. Fig. 8. Attachment of trophonts to the surface of fish body. Erosion of epithelium (arrow); $\times 5000$. Fig. 9. Tracks of detached trophonts on the surface of fish skin (arrow). Distortion of epithelial tissue; $\times 1300$

USA. Such long-term infection of fish by this dinoflagellate has not been reported before in natural bodies of water. To our knowledge, only Overstreet (1993) has recorded a significant fish mortality caused, at least in part, by infection with *A. ocellatum*. This occurred in the Orange Beach Marina and Shotgun Canal in Alabama, USA, in 1984.

Seasonal variation in fish infestation by *Amyloodinium ocellatum* was traced in 1997–1998. Infection started in May, became maximal in June–July, remained at a high level up to September, decreased in October and disappeared in November. The period of massive infestation of tilapia and croaker by *A. ocellatum* coincided with massive fish kills at the lake. At that time heavy infection by *A. ocellatum* was found not only in young fish as reported here. In late August 1997, Dr Jan Landsberg from the Florida Department of the Environmental Protection Agency recorded the

infestation by *A. ocellatum* of gills of dead and moribund adult fish collected at the Salton Sea by Drs Tonie Rocke and Lynn Creekmore (National Wildlife Health Center, USGS, Madison, Wisconsin [<http://biology.usgs.gov/pr/newsrelease/1997/9-0.html> and pers. comm.]).

The effects of different ecological factors on parasitic trophonts of *Amyloodinium ocellatum* have been studied mainly in the laboratory. High tolerance of this parasite to elevated water salinity and temperature was shown. *A. ocellatum* can live in salinities up to 70 ppt and temperatures up to 35°C, but the optimal ranges for their full development are 30 to 33 ppt and 29 to 34°C, respectively (Brown 1934, Lawler 1977b, Paperna 1980, 1984, Overstreet 1993). Our data obtained in natural conditions demonstrated that a salinity of 46 ppt and temperatures up to 40°C did not limit the completion of the life cycle of this parasite. *A. ocellatum* not only survives but successfully repro-



Figs. 10 to 13. *Amyloodinium ocellatum*. Scanning electron microscopy microphotographs. Fig. 10. Location of trophonts (T) on the gill filaments (F) of tilapia. Fusion of respiratory lamellae and swelling of filaments [arrows]; $\times 200$. Fig. 11. Numerous trophonts on the tip of gill filament. Local erosion of epithelium in the site of trophont attachment (arrow); $\times 1100$. Fig. 12. Trophonts of *A. ocellatum* on the skin. Distortion of epithelial tissue in the site of trophont's attachment (arrows); $\times 1100$. Fig. 13. *A. ocellatum* and peritrichs *Ambiphrya ameiri* (PT) on the surface of a fish tail; $\times 1100$

duces in the Salton Sea. This was confirmed by the appearance of new generations of parasites and their fast maturation on the fish body.

It is well known that environmental factors strongly favor infestation of fish by external parasites (Khan & Thulin 1991). In the Salton Sea, the development of fish infestation by *Amyloodinium ocellatum* is determined by the combined effect of pathogen and such factors as water temperature, salinity, oxygen concentration, and nitrogen level.

Water temperature may be a factor of special importance. The normal thermal range in the natural Mozambique tilapia *Oreochromis mossambicus* habitat varies from 18 to 34°C (Welcomme 1972). Thus, the pattern of fish disease at the peak temperature of 40°C may be explained not only by preferential parasite development at higher temperature, but also by tem-

perature effects on tilapia immunocompetence. It has been suggested previously that thermal stress may reduce the immunity of fish and facilitate infestation (Overstreet 1982, Khan & Thulin 1991). In contrast, high salinity seems less damaging to Mozambique tilapia. In general, this species is considered to be amongst the most salt tolerant among the cichlids (see Watanabe et al. 1997 for review). It grows in ponds at salinities ranging from 32 to 40 ppt, reproduces at salinities as high as 49 ppt (Popper & Lichatowich 1975) and adapts to salinities as high as 120 ppt (Whitefield & Blaber 1979). Adaptation to salinities of 45 to 46 ppt is not crucial for tilapia from the Salton Sea. At the same time, the combination of high water temperature and such salinity levels may have a negative impact on these fish and support their heavy infestation by *Amyloodinium ocellatum*. The question of

whether heavy infestation of fish by *A. ocellatum* correlates to salinity elevation at constant temperatures needs to be further investigated.

Low oxygen tension in the Salton Sea in the summer months may reinforce the negative impact of *Amyloodinium ocellatum*. The shortage of external oxygen, together with destructive alterations of the respiratory organs and distortion of epithelial tissues caused by parasitic trophonts may depress the respiratory functions of fish. The likelihood of death by suffocation is especially great for young fish heavily infected by parasitic trophonts. In this case, not only gas exchange in the gills but also cutaneous respiration as a main source of oxygen for these fish (Rombough & Ure 1990) may have been reduced. Alterations in the water-salt balance processes in the damaged gills were also suspected to occur (Wendelaar Bonga 1997). The developing immune system of such young fish may not be able to fight off infection successfully.

The frequently high levels of ammonia in the Salton Sea (California Regional Water Quality Control Board 1994, J. Watts unpubl. data) may also reduce fish defense mechanisms and the weakened fish may be easily infected by *Amyloodinium ocellatum*.

In the Salton Sea, the parasitic dinoflagellate *Amyloodinium ocellatum* appears to be as an important factor affecting survival of fish populations. We found that parasite infestation of young tilapia increased under unfavorable conditions at the lake. We propose that massive fish mortality events often reported at the Salton Sea may be the result of synergistic effects of parasite load and a complex set of environmental stressors.

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