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Chattonella marina (Raphidophyceae), a potentially toxic alga in the Salton Sea, California

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Abstract

Chattonella marina was found in high abundance in the phytoplankton of the Salton Sea from April to November, 1997. Maximum mean density was over 600 cells ml^{-1} mid-lake. It was not detected from January 1997 to March 1997 or in January and February 1998. Transmission electron microscope studies show the typical *Chattonella* features: a teardrop-shaped nucleus, numerous radially arranged chloroplasts and lack of a cell wall. Gills of fish collected at the height of the *Chattonella* bloom showed damage to the primary and secondary lamellae and increased numbers of mucus cells. To our knowledge this is the first report of a species of *Chattonella* in a salt lake.

Introduction

Marine raphidophycean algae such as *Chattonella, Fibrocapsa* and *Heterosigma* have been implicated in major fish kills in various parts of the world (e.g. Subrahmanyan, 1954; Imai & Itoh, 1987; Shumalin et al., 1994; Hallegraeff et al., 1998; Tomas, 1998). Species in the genus *Chattonella* have been of special concern because of monetary losses due to mass mortality of finfish raised in aquaculture, especially yellowtail (*Seriola quinqueradiata* Temmick & Schlegel) in Japan (Imai & Itoh, 1987; Onoue et al., 1989), southern bluefin tuna (*Thunnus maccoyi* Castelnau) in Australia (Hallegraeff et al., 1998) and salmon (*Salmo salar* Linneaus) in Norway (Elbraechter, 1999). The toxic effects of *Chattonella marina* (Subrahmanyan) Y. Hara et Chihara have been ascribed to the production of brevetoxins similar to those of dinoflagellates (Onoue et al., 1987; Ahmed et al., 1995; Khan et al., 1995) and to superoxide radicals (Ishimatsu et al., 1996; Oda et al., 1997). Exposure of fish to *C. marina* causes gill epithelium to become swollen with massive mucous production (Endo et al., 1985, 1992), and fish appear to smother even in well-oxygenated water.

In laboratory experiments on the strain found in Australian waters (Marshall & Hallegraeff, 1999) *C. marina* was shown to grow optimally at 25 °C and at a salinity of 30 p.s.u. (p.s.u=practical salinity units which are essentially equivalent to g kg^{-1}, several percent lower than g l^{-1}). Cells were capable of good growth from 10 °C to 30 °C and from 15 to 45 p.s.u., however at 50 p.s.u. growth only occurred from 15 to 25 °C. High irradiance (450 μmol m^{-2} s^{-1}) also increased the growth.

The life cycles of *C. marina* and *C. antiqua* (Hada) Ono have been studied in the Seto Inland Sea of Japan (Imai & Itoh, 1987; Imai et al., 1991). This waterbody is a eutrophic marine embayment with only a narrow connection to the ocean and limited circulation. There these species over-winter by forming cysts in late summer and early fall. They excyst into the flagellated form in early summer when the water at the sediment interface warms to ca. 20 °C and then reproduce by asexual division, often forming dense blooms. In laboratory experiments, the cysts required a dormancy period of several months at 11 °C before excystment occurred.

Whereas excystment of some dinoflagellates appears to be inhibited by conditions of low dissolved oxygen at the sediment interface, *Chattonella* spp. were found to excyst even at O_2 levels of 2 mg l^{-1} (Montani et al., 1995). This ability to excyst at low oxygen levels was used to explain the occurrence of

red tides of *Chattonella* spp. in the Seto Inland Sea in the summer, a time of strong stratification.

Another factor strongly controlling the extent of *Chattonella* spp. red tides is the degree of eutrophication and anthropogenic inputs of nutrients especially to shallow marine ecosystems (Watanabe et al., 1995).

Description of site

The Salton Sea is below sea level and located in a closed basin in Riverside and Imperial counties in the southeastern corner of California. It formed in 1905–1907 by flooding from the Colorado River. It is the largest lake in California with an area of 980 km^2 and a maximum depth of about 15 m (Ferrari & Weghorst, 1997).

The salinity is approximately 43 g l^{-1} (as of 1999) and varies somewhat depending on proximity to freshwater inflows and season. It is primarily maintained by agricultural and municipal wastewaters from the Whitewater River in the north and the Alamo River and New River to the south. There is very little precipitation and most of the inflow originates from Colorado River water used for crop irrigation. The resultant high input of nutrients has historically caused dense blooms of algae (Carpelan, 1961; Bain et al., 1970). Dinoflagellates and diatoms, most of which are of marine origin, are abundant year round. *Chattonella* has not been previously reported from this or any other lake but two unidentified 'motile green algae' were detected in abundance in 1968–1969 (Bain et al., 1970) and one or both of these may have been raphidophytes.

Marine organisms from the Gulf of California and Pacific Ocean have been deliberately and accidentally introduced as part of an effort to establish a sport fishery in the lake. These introductions were mostly in the 1940s and 1950s at which time the salinity was similar to that of the ocean (Walker, 1961). This may have been the main source of the marine phytoplankton species presently found in the lake. In addition to deliberate fish introductions, tilapia (*Oreochromis mossambicus* Peters) has established itself in the lake and has become the dominant fish in the system (Costa-Pierce & Doyle, 1997). The juveniles of this fish are abundant in nearshore areas of the lake in mid-summer.

The presence of toxic algae has been considered as a possible cause of wildlife mortality at the Salton Sea. Therefore, a project to monitor the phytoplankton of the lake was started in January 1997. The purpose of this project was to determine if any known toxic species were present, and if so, whether present in densities known to be harmful to fish or birds.

Materials and methods

Phytoplankton sampling

Samples for enumeration of phytoplankton species were taken approximately monthly beginning in January of 1997 at the following three mid-lake locations located along the main axis of the lake (see map in Watts et al., 2001):

S-1	33° 25′ 00″ N	115° 55′ 00″ W
S-2	33° 21′ 00″ N	115° 51′ 00″ W
S-3	33° 18′ 00″ N	115° 48′ 00″ W

The present findings are part of a larger study of the plankton dynamics during 1997–1999 (Reifel et al., 2001, 2002; Tiffany et al., 2002).

Integrated samples of whole water from the lake surface to 9 m were taken with a 3 m long tube sampler and fixed in the field with 1% Lugol's solution. *C. marina* was enumerated with the standard technique (Utermöhl, 1958) using a Leitz Diavert inverted microscope and two crossed diameters at 400×. On some dates (May 20, and from October 17 forward), the phytoplankton samples from discrete depth intervals of 0–3 m, 3–6 m and 6–9 m were analyzed separately to determine vertical distributions differences in the three strata. On these dates the arithmetic mean densities for the three strata were obtained, so as to have values comparable to those calculated for integrated 0–9 m samples. A constant of 1.5 individuals ml^{-1}, the lowest non-zero density that could be observed given the volume of water examined, was added to each value. Geometric mean density of*C.marina* and its among-station standard error were calculated for those dates where two or three stations were sampled. Not all three stations were sampled on every date due to logistical difficulties, primarily weather conditions that made boat operations unsafe. On dates when only one station was sampled, that value is given without an indication of error.

Temperature and oxygen were measured at each station and date at 1 m depth intervals starting at the surface using a YSI model 57 oxygen meter. Temperature could be accurately read to 0.1 °C. The oxygen probe was calibrated in air at 100% humidity, corrected for a salinity of 40 g l^{-1} and an altitude of 69 m below sea level. Oxygen readings were accurate to

0.01 mg l^{-1} in the range 0–10 mg l^{-1} and 0.1 above 10 mg l^{-1}. Variation in barometric pressure on different dates was not taken into account, a source of 2–4% error.

Species identification

Live *Chattonella* cells were collected in summer 1997 and observed with light microscopy using brightfield and Nomarski illumination for confirmation of identification to the genus level. This is necessary because raphidophytes do not generally preserve well due to their fragile nature and tendency to shed flagella upon preservation (Throndsen, 1997). Scanning and transmission electron microscope studies of *Chattonella* cells were carried out to confirm the identification to species level. One ml water samples from the Salton Sea were fixed for electron microscopy by the sequential addition of 1 ml of 0.1 M cacodylate buffer (pH 7.2) containing 4% glutaraldehyde and 800 mOsm sucrose followed by 0.5 ml of 4% aqueous osmium tetroxide. After 20 min fixation at room temperature, cells were rinsed with fresh buffer. For scanning electron microscopy, cells were collected onto Nucleopore filters, dehydrated in ethanol, critical point dried, then sputter-coated with Au/Pd and viewed in a Hitachi S-2700 SEM. For transmission electron microscopy, cells were collected by centrifugation, then dehydrated in ethanol, and embedded in Spurr's resin. Silver sections were stained with uranyl acetate and lead citrate, then viewed with a Philips 410 TEM.

Examination of fish gills

Seven specimens of tilapia (*Oreochromis mossambicus*) (length 3.7–5.2 cm) were collected from Varner Harbor at northeast corner of the Salton Sea in August 1997, when *C.marina* was abundant lake-wide, and examined using SEM. Gill arches were fixed in cold Karnovsky fixative for 2 h, postfixed in osmium tetraoxide for 1 h and dehydrated in a graded ethanol series with the final change in absolute ethanol. Then the samples were critical point dried with liquid CO_2, mounted on stubs, sputter-coated with Au/Pd and examined with a scanning electron microscope (Hitachi S-2700) at an accelerating voltage of 10 kV.

Results

Species identification

These cells most closely resemble *C.marina* as described by Hara & Chihara (1982). Cells are yellow-brown, with two subequal, heterodynamic flagella which emerge from a sub-apical depression (Fig. 1A, D). The posterior end of the cell exhibits some variation in shape from pointed to blunt-ended, but a groove in the posterior tip of the cell is commonly observed (Fig. 1A). Swimming cells divide by binary fission (Fig. 1B). A large, tear-shaped nucleus is situated in the center of the cell (Fig. 1A, D). There are numerous elliptical chloroplasts arranged radially around the cell periphery (Fig. 1D). Pyrenoids are located in the internal end of the chloroplasts, and thylakoids are observed penetrating the pyrenoid matrix (Fig. 1E). Electron-dense osmophilic bodies (Fig. 1D, E) are widely distributed at the cell surface and appear as small bumps on the cell surface (Fig. 1B,C), but no 'oboe-shaped' mucocysts or ejectosomes have been observed in cells examined to date. The cell periphery is highly vacuolated, and cells are fragile, since they are bounded only by a cell membrane.

Fish gill damage

Gills from tilapia collected during the *C.marina* bloom were distorted due to severe swelling, shortening and fusion of secondary (respiratory) lamellae (Fig. 2A). Similar distortions have been observed in other species of fish exposed to *C. marina*, *C. antiqua* and other *Chattonella* spp. (Tyoshima et al., 1985; Endo et al., 1992; Ishimatsu et al., 1995). These alterations were associated with hypertrophy, hyperplasia and edema of the epithelium of the secondary lamellae.

In contrast with Shimada et al. (1991), we found that the epithelium of gill filaments had a much larger than normal number of mucus cells. Numerous pores of these cells were seen mainly along the trailing edge of filaments and between lamellae (Fig. 2B). Hishida et al. (1997) obtained similar results in yellowtail (*Seriola quinqueradiata*). They suggested that mucus discharged from the filamental epithelium can block lamellar water channels and may be one of the causes of fish asphyxiation when fish are exposed to *C. marina*.

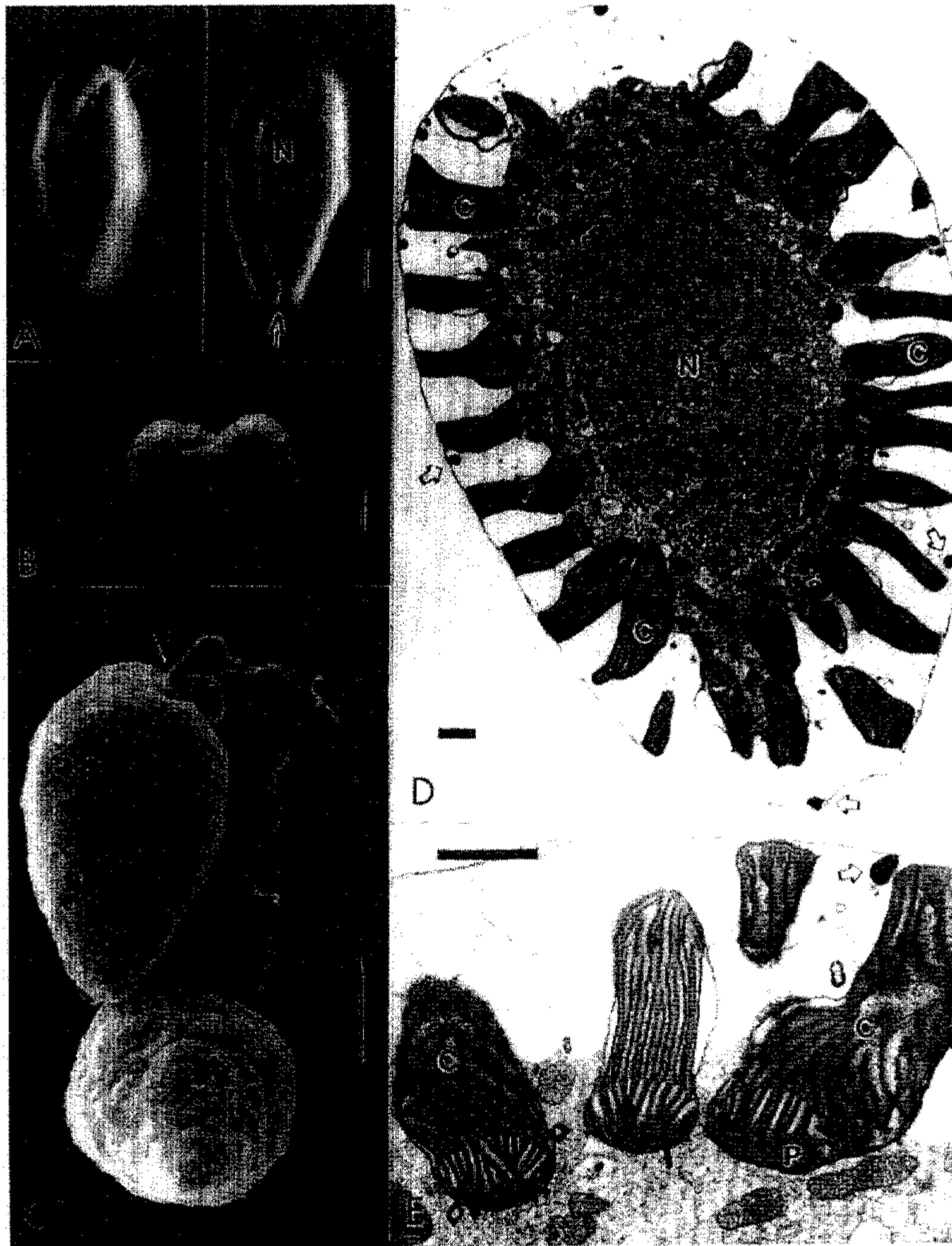

Figure 1. Light and electron microscope images of *Chattonella marina.* (A) Images of a swimming cell focussed at two levels, showing the sub-apical flagellar depression (arrowhead) and the posterior groove (arrow); bar = 10 μm. (B) A cell undergoing binary fission in the swimming stage, with the replicating flagella indicated (arrows); bar=10 μm. (C) SEM image of cells, with two flagella emerging from a sub-apical pit (arrowhead). Numerous bumps on the cell surface are the electron-dense bodies observed in TEM images; bar=10 μm. (D) TEM image of a longitudinal section, depicting the radially arranged chloroplasts (C), and the large, centrally located nucleus (N); electron dense bodies subtend the cell surface (open arrows); bar = 1 μm. (E) The pyrenoid (P) is located in the inner end of the chloroplast (C), and thylakoid membranes can be seen penetrating the pyrenoid matrix (arrows); bar = 1 μm.

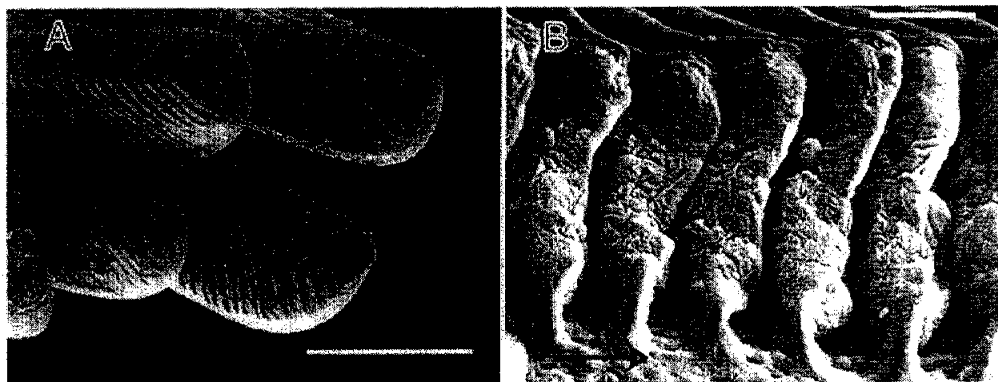

Figure 2. SEM of gills of young tilapia collected during a bloom of *Chattonella marina*. (A) Distal parts of gill filaments with fused secondary lamellae (fi – filament, sl – secondary lamellae; arrow indicates fusion of secondary lamellae); bar = 500 μm. (B) Swelling of secondary lamellae (sl) and excessive development of mucus cells (pore of mucus cell indicated by arrow); bar = 50 μm.

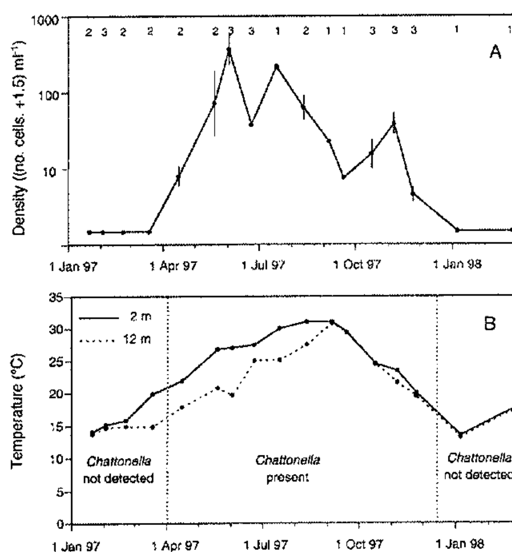

Figure 3. (A) Geometric mean density of *Chattonella marina* (± 1 SE) in the Salton Sea in the 0–9 m portion of the water column, January 1997–February 1998. (B) Mean Salton Sea mid-lake water temperatures at 2 and 12 m. Numbers across the top of A indicate the number of stations monitored on each sampling date for *C.marina* and temperature.

Seasonal variation in abundance

Abundance of *C. marina* was correlated with the annual temperature regime (Fig. 3A, B). Water temperatures in 1997–98 varied seasonally, with lowest temperatures occurring in January 1997 and 1998 and warmest in early September 1997. Thermal stratification of the water column existed most of the time during spring and summer, interrupted by brief episodes of wind-induced mixing (Watts et al., 2001). This is reflected in the difference in temperature between 2 m and 12 m (Fig. 3B). Dissolved oxygen was low at depth most of the summer although sporadic mixing allowedsome dissolved oxygen to be mixed into the lower strata at times of high winds (Watts et al., 2001). A breakdown of thermal stratification and an increase in mixing due to convectional circulation occurred starting in September 1997 as the lake began to cool and became nearly isothermal.

No cells of *C. marina* were seen in samples from January to March 1997 when the temperatures at 12 m were less than 15 °C (Fig. 3A, B). Cells were first observed in low numbers on April 16. Peak density of 624 cells ml^{-1} for the integrated 0–9 m depth interval was observed at station S-2 on June 3. On that date the other two stations also reached their maximum measured densities (367 and 138 cells ml^{-1} at S-1 and S-3, respectively). By November, very few cells of *C. marina* were found and in January 1998 (temperature at all depths < 15 °C) no cells were found in the counts or seen in qualitative samples. The environmental conditions of the Salton Sea during the bloom (15°C to 35 °C and 46 g l^{-1}) fell into the range of good growth for *C. marina* given by Marshall & Hallegraeff (1999).

C. marina has been measured at even higher densities in the Salton Sea. In July 1998, a 0–3 m integrated sample at station S-1 had a density of over 2200 cells ml^{-1} and a nearshore sample in August 1999 had 11 500 cells ml^{-1} (K. Reifel, unpublished data).

Cell densities generally decreased with depth (Fig. 4A) on three dates (May 20, Oct 17 and Nov

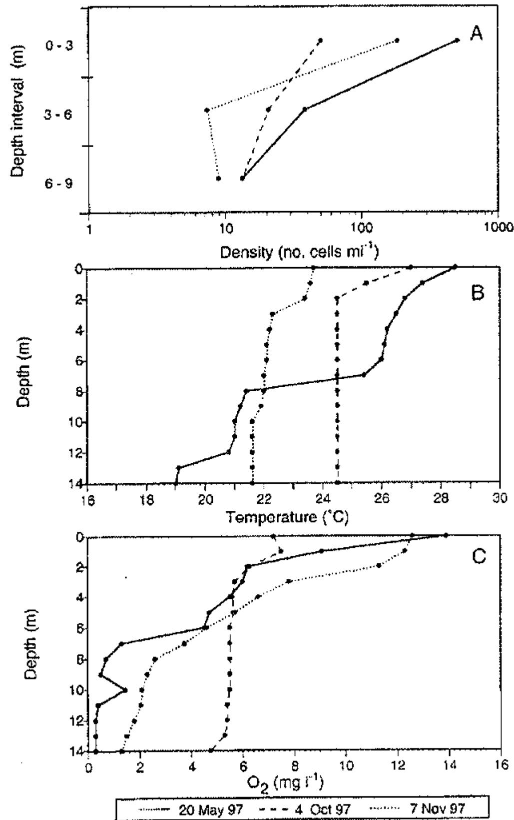

Figure 4. Vertical gradients in (A) density of *Chattonella marina*, (B) temperature and (C) dissolved oxygen at the sampling station S-1 in the center of the northern basin, on three dates.

7, 1997) where phytoplankton samples from 0 to 3 m, 3 to 6 m, and 6 to 9 m at station S-1 were analyzed separately. The highest densities were found in the 0–3 m depth interval. At least for these three dates, the degree to which C. *marina* was concentrated in the surface waters (0–3 m) was positively correlated with the stability of the water column as indicated by the steepness of the temperature gradient (Fig. 4B). Oxygen profiles at the same station on these three dates (Fig. 4C) showed the greatest degree of anoxia in bottom waters occurred in May, coincident with very high density of *C. marina* in the 0–3 m stratum.

Discussion

We have identified our species of *Chattonella* as *C. marina* as described by Hara & Chihara (1982). *C. marina* is separated from a related species, *C. subsalsa* Biecheler, primarily on the basis of two ultrastructural characteristics: presence of mucocysts and the relationship between thylakoid membranes and the chloroplast pyrenoid matrix. *C. subsalsa* contains 'oboe-shaped' mucocysts or ejectosomes, and the thylakoids of these cells do not penetrate the pyrenoid. By comparison, *C. marina* has thylakoids in the pyrenoid matrix and does not contain the distinctive mucocysts.

The strong seasonal variation in abundance of *C. marina* in the Salton Sea plankton, with maximum densities in summer and apparent absence in winter, is similar to findings in the Seto Inland Sea of Japan (Imai et al., 1991). In both locales, it appears in the plankton as the flagellated, vegetative form in spring at about the time bottom waters reached 15–20 °C. The flagellated form disappears from the water column in the colder months (January through March). The temperature regimes are similar in the two locations, varying from about 12 °C in winter to over 25 °C in summer. This suggests a similar life cycle occurring in both water bodies with cells over-wintering in cyst form in the sediments. Cysts of *C.marina* have not been confirmed in the sediments of the Salton Sea, but it seems probable they are there in the colder months when vegetative cells of *C.marina* were not observed in the water column of the Salton Sea.

C. marina may contribute to fish kills that occur in the spring and summer at the Salton Sea. Densities of *C. marina* over 500 cells ml^{-1} are believed to cause high mortality in yellowtail in the Seto Inland Sea of Japan (Okaichi, 1989). Some of our integrated (0–9 m) mid-lake samples exceeded this value. Preliminary data on the depth distribution of this species in the lake indicate that at least daytime densities of *C. marina*, like many dinoflagellates, are highest in surface waters (Fig. 4A). *Chattonella* spp. are known to undergo vertical migration to the surface in the daytime (Watanabe et al., 1995). This raises the strong possibility of synergistic effects on fish in the Salton Sea by this raphidophyte and hypoxia. In summer, high water temperatures and water column stratification result in low oxygen concentrations at depth (Fig. 4 C). This forces the fish into surface waters where *C. marina* densities are maximal.

The occurrence of swollen primary and fused secondary lamellae in small tilapia (*Oreochromis mossambicus*) and frequent fish kills during the summer when *C. marina* is present in the Salton Sea plankton suggests the use of controlled experiments using the

Salton Sea strain of *C. marina* to determine if it can cause these effects.

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