



## Effect of *Schizothrix calcicola* on white shrimp *Litopenaeus vannamei* (*Penaeus vannamei*) postlarvae

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### Abstract

The noxious effect of *Schizothrix calcicola* strain UTEX B-1936 to white shrimp *Litopenaeus vannamei* (*Penaeus vannamei*) postlarvae is reported. The larvae exposed to this cyanobacterium during a 15-day long experiment grew smaller and weighed less ( $R^2=0.6704$ ) than unexposed organisms ( $R^2=0.9049$ ). Histological analysis of exposed shrimps revealed severe damage to the gastrointestinal lining. We believe that such kind of lesions may interfere with food absorption, which could explain the slow larvae growth and size development observed. Hence, *S. calcicola* blooms in shrimp ponds may jeopardize the culture by slowing shrimp growth rate and biomass yield.

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### 1. Introduction

Cyanophyta (cyanobacteria) blooms (Carmichael, 1992) are now considered part of a general phenomenon known as harmful algae blooms (HABs), that is imposing a serious threat to human health and the environment worldwide (Hallengraeff, 1993). Cyanobacterial toxins or cyanotoxins, in principle, could be aimed to inhibit predators, competing

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algae, and macrophytes, but may also affect other aquatic and terrestrial animals as well as humans (Duy et al., 2000). Based on reports from the past 50 years, it is now recognized that chronic exposure to cyanotoxins occurs in many places, particularly where surface water (such as ponds, rivers, or reservoirs) is directly consumed. Levels as low as one part per billion of certain cyanotoxins (e.g. microcystins and nodularins) have been associated with nonlethal acute or chronic effects, and their monitoring and control, especially in domestic water supplies, is recommended (Scott Yoo et al., 1992).

Most common cyanotoxins exert their effect (biotoxic, neurotoxic, and hepatotoxic) upon ingestion; others have been detected only because of their action on cell cultures (cytotoxins), and still others because they may exert a noxious effect on external or exposed tissues, such as the eye or epidermis after contact. One example of this latter case is a debromoaplysiatoxin produced by *Schizothrix calcicola* which upon contact causes “swimmers itch” and eye irritation and, if ingested, hay fever symptoms, dizziness, fatigue, and acute gastroenteritis (Lippy and Erb, 1976; Mynderse et al., 1977). Association with other cyanophytes considered as assemblages, particularly with *Lyngbya majuscula* and *S. calcicola*, produces both lipid-soluble and water-soluble toxins (Harrigan et al., 1998, 1999; Mynderse et al., 1977). Together they generate a number of metabolites, including lyngbyastatin-1, dolastatin-2, and ypaomide, that have been shown as potent disrupters of cellular microfilament networks and feeding deterrents. All these substances could explain some of the fish and cultured shrimp mortalities associated with cyanobacterial blooms (Banner, 1966; Cortés-Altamirano et al., 1997; Cortés-Altamirano and Licea-Durán, 1999; Harrigan et al., 1998; Karusangar et al., 1997; Nagle and Paul, 1998; Overstreet, 1973; Smith, 1996).

It must be borne in mind that *S. calcicola* is found in many different environmental conditions, including drinking water (Sykora et al., 1980) and, therefore, it is considered as a noxious cosmopolitan species (Skulberg et al., 1993). Some authors have observed mice mortality when the animals are injected with mixed cultures of *S. calcicola* and *Oscillatoria* sp. (Gerwick and Bernart, 1993), and Long and Carmichael (in press) discussed the properties of some oscillatoxins in a combined bloom of *S. calcicola* and *Oscillatoria nigroviridis* showing dermatological and tumor promoter effects. The toxicity and risk assessment of *S. calcicola* blooms in shrimp aquaculture, on the other hand, deserve special attention because Lightner (1982) has already reported that juvenile shrimp develops enteritis after exposure to *S. calcicola*. Here we describe the effect of *S. calcicola* in white shrimp postlarvae feeding and growth.

## 2. Materials and methods

### 2.1. Organisms

*S. calcicola* strain UTEX B-1936 was obtained from the collection of the Botany Laboratory of Texas University. The organism was cultured and maintained in ASN-III medium (Sigma, St. Louis, MO, USA) at 21 °C, with constant stirring (100 rpm) and with light provided by a fluorescent lamp of 3200 lx (62.5 µEi) following the procedure described by Cotteau (1996). Aliquots of this culture were further cultured in larger

volumes (500 or 1000 ml) under identical conditions for 2–3 weeks to obtain the cell biomass necessary for the following experiments. The cells were collected by centrifugation at  $2000 \times g$  for 30 min. The sediments were pooled and suspended in 10 ml of supernatant. The cells were kept frozen at  $-40\text{ }^{\circ}\text{C}$  until use.

*Litopenaeus vannamei* white shrimp PL30, with an average weight of 35 mg/larvae, were acquired from the Laboratory of Aquaculture Genetics of CIBNOR. To attain such developmental stage, 14,400 nauplii 5 were reared in flat-bottomed tanks of 120 l until stage PL1 (organism density: 120/l). At this stage, the organism density was adjusted to support only 25 organisms/l by removing excess or dead animals. UV-treated (180 W, UV-Aquanetic Systems lamp) and filtered (10  $\mu\text{m}$ , pore membrane filter) seawater (35 ppm) was exchanged daily, keeping the temperature at  $28\text{ }^{\circ}\text{C}$  and under constant airming. Feeding was provided three times a day with frozen *Artemia franciscana* and a commercial food preparation (PIASA, La Paz, BCS, Mexico; pellets with 45% protein) in flakes or grains according to shrimp stage development.

## 2.2. Cyanobacteria concentration standard plot

The concentration of *S. calcicola* was found by determining the chlorophyll *a* content in samples extracted with 99.5% acetone. For this, a standard plot using different aliquots of different weights was prepared from the cell suspension of a 250-ml culture. Essentially, the procedure consisted of mixing 0.5 ml of cell suspension with 1 ml of 99.5% acetone and stirring in a Vortex for several seconds. The extract was centrifuged in a microfuge (Beckman microfuge E) for 5 min, and the supernatant absorption at 647, 664, and 630 nm was determined in a Beckman DU 640 spectrophotometer.

## 2.3. Dry weight determination

Concentration was also determined by finding the dry weight of a given suspension as follows. The samples were obtained from 200-ml culture aliquots and the cells collected as indicated above. The wet sediments were weighed and then freeze-dried and weighed again. From this, the percentage of humidity was determined.

## 2.4. Shrimp assays

For an acute toxicity evaluation, 150 ml of a twofold serial dilution (i.e. 100%, 50%, 25%, 12.5%, and 0%) of the cyanobacterium culture was poured in 200-ml bottles in triplicate. Previously fed (2 h) postlarvae (5 organisms per bottle), weighing 35 mg each, were exposed to the different cyanobacteria concentrations for 7 h under constant airming. Afterwards, the organisms were collected manually with a net and fixed in Davidson medium (Lightner, 1996) for 24 h, and afterwards in 70% alcohol for histological observation and storage.

For a chronic exposure evaluation, 30 flat-bottomed plastic recipients of 20 l each, filled up to 50% capacity, were used to grow the shrimp larvae at a density of 6.5 organisms/l for 15 days under dim light and constant airming. Salinity was maintained at  $35.5 \pm 0.7$  ppm and temperature at  $24.5 \pm 1.3\text{ }^{\circ}\text{C}$ , following recommendations of Sánchez

(1996). Water exchange (100%) was carried out daily by siphoning with 1/2-in. plastic tubes holding a 500- $\mu\text{m}$  filter net at the end and the discharge treated with 1–2 ml/l of 6% sodium hypochlorite for 48 h before being drained. Feeding was provided once a day every morning, adding 0.3 g of a finely ground (300–700  $\mu\text{m}$ ) commercial feed to each recipient following recommendations of Cevallos-Orozco and Velázquez-Escobar (1988) and Olivera and Akano-Cohelo (1997). The food was allowed to settle down by interrupting the aeration. When the shrimps approached the feed, 150 mg of cyanobacteria suspended in 0.5 ml of filtered and UV-irradiated seawater were distributed over the food grains to induce their ingestion. After 5–10 min, aeration was carefully reassumed avoiding the resuspension of the feed and/or the cyanobacteria. The shrimps of two recipients (one from the blank group and another from the experimental group) were sacrificed daily and fixed for histological analysis (Lightner, 1996). Surviving animals at the end of the experiment were counted and weighed as a group. Individual weight was estimated by dividing the group weight with the number of surviving organisms.

### 2.5. Histological analysis

Thirty surviving animals in the 1st, 3rd, 8th, and 15th day, from both the control and the experimental recipients, were sampled. For histological analysis, the organisms were injected with Davidson's AFA solution (Humason, 1972) at 1:10 dilution. After 24-h fixation, the samples were stored in ethanol (50%) until analysis. Histopathology was carried out by routine procedure (Lightner, 1996) sectioning at 3–5  $\mu\text{m}$ , mounting on microscope slides, and staining with a variant of Harris' hematoxylin and eosin preparation, as described by Mantoja and Mantoja-Pearson (1967). Histopathological examination of control and infected organisms was performed with a standard light microscope (Luna, 1968).

### 2.6. Statistical analysis

The Student's *t* test was applied (Daniel, 1995; Sokal and Rohlf, 1998) with the aid of the *Statistica* program.

## 3. Results and discussion

The culture medium chosen for *S. calcicola* strain UTEX B-1936 growth proved to be appropriate for our purposes. This medium (ASN-III) allowed an optimum growth of *S. calcicola* in small volumes (200 ml) after 2–3 weeks, and in large volumes (up to 1 l) within 4–5 weeks. Because *S. calcicola* makes enclosures and filaments that easily break and dissociate, it is not possible to estimate directly the number of cells in a Neubauer chamber; hence, the cell amount was found by correlating the chlorophyll *a* content and/or dry weight with the corresponding cell biomass. For example, 200 ml of culture medium averaged 5.5 g of wet weight and 0.21 g of dry weight cell biomass. The optical density at 630 nm of this culture was 0.03. These values were therefore used as references to estimate the amount of cells in each of the experiments.

### 3.1. Shrimp assays

There was no abnormal behavior, neither an unusual mortality, observed in *L. vanammei* postlarvae shrimps exposed to live *S. calcicola* cells in an acute-like dose, or in a repeated subacute dose exposure experiments. Daily mortality was less than 20% in both cases, considered normal for these particular shrimp postlarvae (Lawrence et al., 1998). When exposed to *S. calcicola*, white shrimp postlarvae showed a reduced feeding activity compared to unexposed animals during the test period. This observation was confirmed by the amount of food left and removed from the tanks, and by comparing the group and individual postlarvae weight gain (Fig. 1a and b). The average weight gain in each case showed a significant variation between the test lots and the control group that translated into different development tendencies as expressed by their corresponding linear regression values. For example, as a group, the unexposed postlarvae showed a better growth tendency ( $R^2=0.9345$ ) than exposed animals ( $R^2=0.4631$ ) throughout the study period (Fig. 1a). The sudden increase in weight gain at the end of the experiment (15th day) in the exposed group may be due to differences in the number of organisms provoked by mortality which, nevertheless, is still considered normal at this stage for this particular shrimp species (Lawrence et al., 1998). We confirmed this assumption by determining the average individual weight gain over all the study interval and found that the unexposed postlarvae indeed showed a tendency to gain more weight ( $R^2=0.9049$ ) than the exposed ones ( $R^2=0.6704$ ) at all times. As depicted in Fig. 1b, the exposed organisms showed an important increment in weight in the 15th day, yet such weight was still lower than that of unexposed organisms. It is also worth mentioning that ingestion of *S. calcicola* conferred a blue-green color to the digestive tract of the postlarvae. This was commonly observed 20 min after the cyanobacteria were added to the feed. Altogether, these observations confirm that *S. calcicola* is capable of producing some feed deterrents (Harrigan et al., 1998, 1999; Mynderse et al., 1977).

### 3.2. Histological analysis

A cross-section sample of the dorsal lobule of a normal shrimp postlarvae hepatopancreas is shown in Fig. 2A. As can be observed, the connective tissue surrounding the tubules presents a low number of hemocytes, and the cells forming the tubules are well defined and integrated. In contrast, the dorsal lobule of an abnormal shrimp postlarvae hepatopancreas formed by disrupted tubules with damaged cells and a severe hemocytic infiltration in the surrounding connective tissue after 8 days of exposure is shown in Fig. 2B and exemplifies the effect of *S. calcicola* ingestion.

Fig. 3a shows a longitudinal cross section of a normal shrimp postlarvae hindgut in which the epithelium can be seen composing of a simple layer of cylindrical cells with a central nucleus. The luminal surface appears irregular because of microvilli, but the mucosa maintains its integrity and shape, and the lumen appears empty. Fig. 3b, in contrast, shows the longitudinal cross section of a shrimp postlarvae hindgut that has been exposed to the cyanobacteria during 8 days where the epithelium appears to be losing integrity due to cell desquamation. Some bacteria can be observed in the lumen, indicating tissue disruption. Finally, Fig. 4 also shows a longitudinal cross section of the hindgut of a

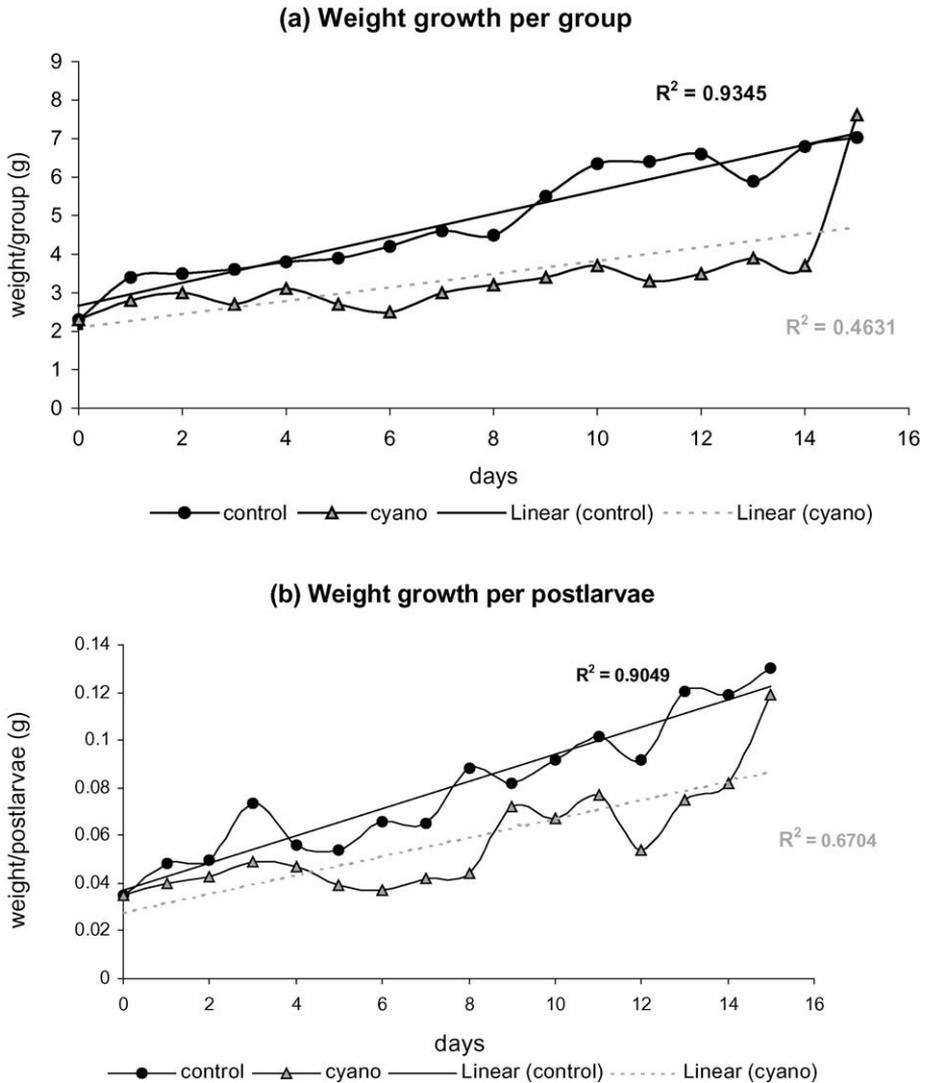


Fig. 1. Growth inhibition of white shrimp (*L. vannamei*) exposed to *S. calcicola*-impregnated feeds: (a) daily weight gain calculated as the weight of pooled surviving larvae; (b) daily individual weight gain calculated from the group weight divided by the number of surviving larvae.

shrimp postlarvae exposed to cyanobacteria after 14 days. In this case, the epithelium appears to be losing integrity, and some hemocytic infiltration in the connective tissue surrounding the gut is observed, revealing a severe damage.

It is worth to mention the human poisoning event registered in Hawaii in September 1994 attributed to consumption of the red alga *Gracilaria coronopifolia* contaminated by aplysiatoxin and debromoaplysiotoxin presumably of cyanobacteria origin (Nagai et al.,

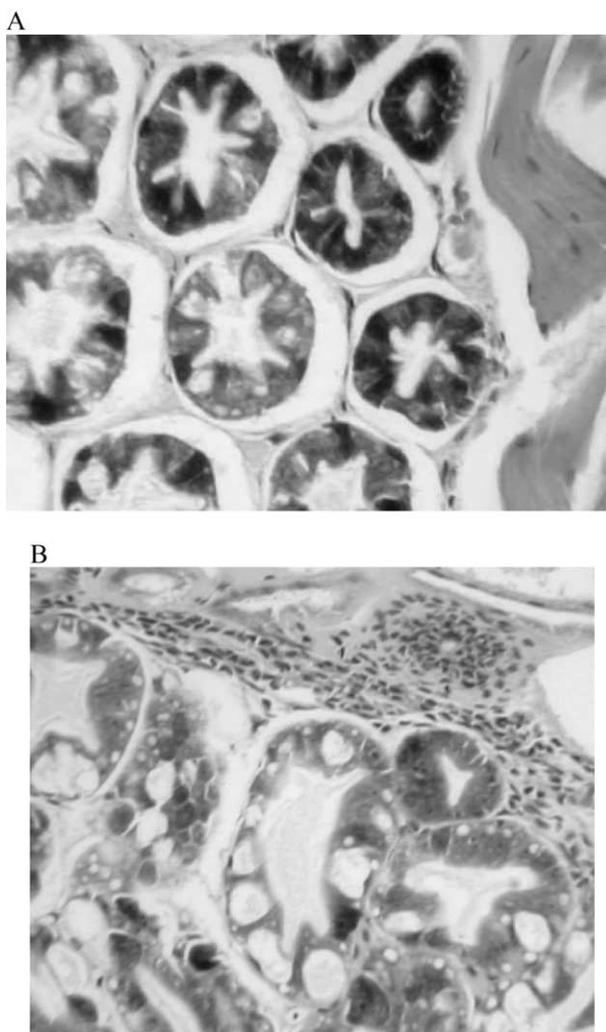


Fig. 2. Cross-section histology of hepatopancreas tissue of (A) unexposed and (B) exposed white shrimp (*L. vanamei*) postlarvae to *S. calcicola* during 15 days (40 $\times$ ).

1996). The symptoms were characterized by vomiting, diarrhea, and a burning sensation of the mouth and throat. Kato and Scheuer (1975) have previously reported that these toxins produce skin irritation, which could explain the throat and mouth burning sensation reported in Hawaii's human poisoning event. In mice, the main signs of toxicity 30 min after intraperitoneal injection of the toxins are diarrhea and lethargy, as well as muscular contractions, and sometimes hind leg paralysis. A dose of 5  $\mu$ g aplysiatoxin was found to be lethal if injected intraperitoneally after 2 h (Ito and Nagai, 1998), and the autopsy revealed bleeding in the small, but not in the large, intestine. The capillaries were congested and the villi eroded and bleeding with an exposed shortened lamina propria

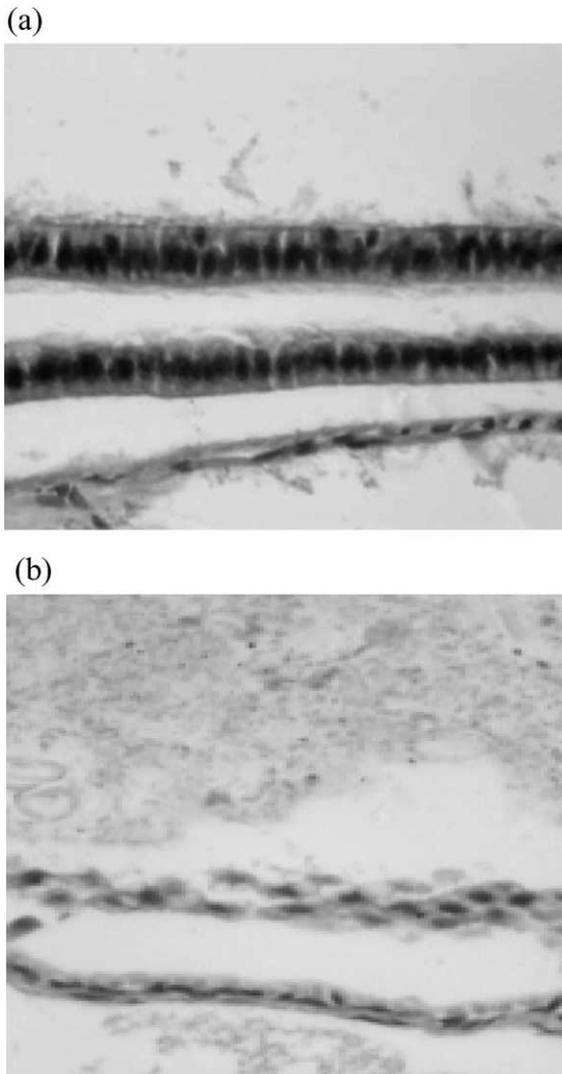


Fig. 3. Histological analysis of white shrimp postlarvae after 8 days of treatment: (a) cross section of normal hindgut tissue; (b) cross section of affected hindgut tissue by *S. calcicola* ingestion (40 $\times$ ).

after exfoliation of surface epithelial cells. The large intestine also showed erosion from where the fluid flowed out. No other organs showed evidence of injury. Animals treated with lower doses recovered after 24 h with some loose muscularis, edema in submucosa membrane tissue, and weight loss. Similar effects have been observed when the toxin is administered via gastric intubation. In this case, after 3 h, the large intestine showed edema and watery substances and, after 8 h, the villi decreased in number and became short. The mechanism by which aplysiatoxin produces all these effects remains unclear and it has

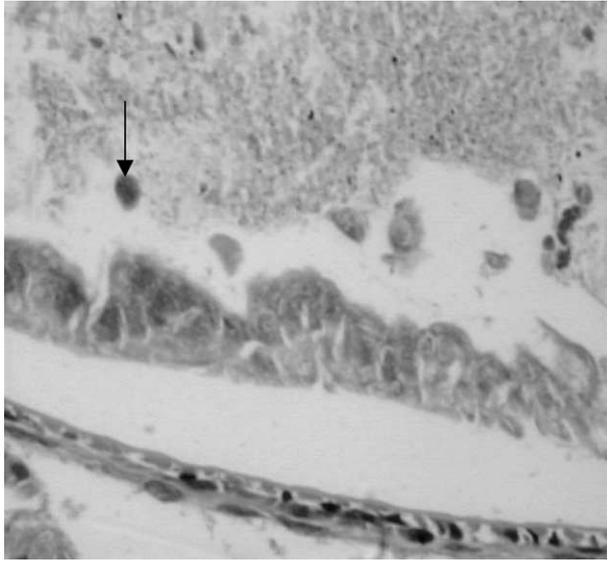


Fig. 4. Histological analysis of white shrimp postlarvae hindgut tissue affected by *S. calcicola* ingestion after 14 days of treatment. The arrow shows a desquamated cell from a disintegrating tissue (40 ×).

been considered reasonable to assume that the inflammatory action of aplysiatoxin is derived from the activation of protein kinase C, which would also explain its tumor promoter capacity (Fujiki et al., 1982). The resemblance of aplysiatoxin-induced lesions in mice and those found with shrimp postlarvae exposed to *S. calcicola* cells suggests that the release of such kind of toxins if the cyanobacteria is ingested is feasible. Moreover, it is also interesting to note that the red alga *G. coronopifolia* becomes toxic because of epiphytic parasitism by noxious cyanobacteria. Thus, these organisms could enter the food chain of many organisms of interest in aquaculture if conditions are favorable for blooming and attachment to their feeds.

#### 4. Conclusions

The above results confirm that *S. calcicola* (UTEX B-1936) is a toxic cyanobacteria to *L. vannamei* shrimp postlarvae. The mechanisms by which *S. calcicola* provokes tissue disruption affecting food absorption and processing and thus, growth and development of the shrimp postlarvae resemble that observed in mice. Several metabolites including oscillatoxins, aplysiatoxin, debromoaplysiatoxin, lyngbyastatin-1, dolastatin-2, and ypaomide are known to be produced in the assemblage of *S. calcicola* and other cyanobacteria. They have been shown as potent cellular disrupters of microfilament networks and feeding deterrents (Harrigan et al., 1998, 1999; Mynderse et al., 1977). Considering the prevailing situation of shrimp aquaculture practice in Northwest Mexico, the possibility of a sudden *S. calcicola* bloom that would affect the animal's growth, thus inducing lower yields or prolonging the necessary culture period to reach a commercial size, is a risk that needs to

be faced. We also believe that shrimp farms may become more susceptible to infectious agents that promote high mortalities as a result of an *S. calcicola* bloom. Therefore, in order to prevent the collapse of the culture, monitoring of noxious and toxic cyanobacteria blooms is highly recommended.

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## References

- Banner, A.H., 1966. Marine toxins from the Pacific: I. Advances in the investigation of fish toxins. *Anim. Toxins* 4, 157–165.
- Carmichael, W.W., 1992. A Status Report on Planktonic Cyanobacteria (Blue-green Algae) and Their Toxins US-EPA, Ohio. 71 pp.
- Cevallos-Orozco, M.L., Velázquez-Escobar, M.A., 1998. Perfiles de la alimentación de peces y crustáceos en los centros y unidades de producción acuícola de México. FAO (México, 139 pp.).
- Cortés-Altamirano, R., Licea-Duran, S., 1999. Florecimientos de microlagas nocivas en estanques de cultivo semi-intensivo de camarón en México. *Rev. Latinoam. Microbiol.* 41, 157–166.
- Cortés-Altamirano, R., Manrique, F.A., Luna Soria, R., 1997. Harmful phytoplankton blooms in shrimp farms from Sinaloa Mexico. 8th International Conference on HABs, Vigo, Spain, June 25–29. Abstract Book, Xunta de Galicia, Spain-IOC-UNESCO.
- Cotteau, P., 1996. Microalgae. In: Lavens, P., Sorgeloos, P. (Eds.), *Manual on the Production and Use of Live Food for Aquaculture*. FAO Fish. Tech. Paper No. 361, Rome, Italy, pp. 7–47.
- Daniel, W.W., 1995. Base para el análisis de las ciencias de la salud. *Bioestadística*. UTEHA, México. 878 pp.
- Duy, T.N., Lam, P.K., Shaw, G.R., Connel, D.W., 2000. Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Rev. Environ. Contam. Toxicol.* 163, 113–185.
- Fujiki, H., Suganuma, M., Nakayasu, M., Hiroshino, H., Moore, R.E., Sugimura, T., 1982. The third class of new tumor promoters, polyacetates (debromoaplysiatoxin and aplysiatoxin) can differentiate biological actions relevant to tumor promoters. *Gann* 73, 495–497.
- Gerwick, H.W., Bernart, M.W., 1993. Eicosanoids and related compounds from marine algae. In: Attaway, D.H., Zaborsky, O.R. (Eds.), *Marine Biotechnology. Pharm. Bioact. Nat. Prod.*, vol. 1. Plenum, USA, pp. 101–152.
- Hallengraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32, 79–99.
- Harrigan, G.G., Yoshida, W.Y., Moore, R.E., Nagle, D.G., Park, P.U., Biggs, J., Paul, V.J., Mooberry, S.L., Corbett, T.H., Valeriotte, F.A., 1998. Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula*/*Schizothrix calcicola* cyanobacterial assemblages. *J. Nat. Prod.* 61, 1221–1225.
- Harrigan, G.G., Luesch, H., Yoshida, W.Y., Moore, R.E., Nagle, D.G., Biggs, J., Park, P.U., Paul, V.J., 1999. Tumonoic acids, novel metabolites from a cyanobacterial assemblage of *Lyngbya majuscula* and *Schizothrix calcicola*. *J. Nat. Prod.* 62, 464–467.
- Humason, G.L., 1972. *Animal Tissue Techniques*, 3rd ed. Freeman, San Francisco.
- Ito, A., Nagai, H., 1998. Morphological observations of diarrhea in mice caused by aplysiatoxin, the causative agent of the red alga *Gracilaria coronopifolia* poisoning in Hawaii. *Toxicon* 36, 1913–1920.
- Karusangar, I., Otta, S.K., Karusangar, I., 1997. Harmful algal blooms in shrimp culture ponds in India. 8th International Conference on HABs, Vigo, Spain, 111. Abstract book, Xunta de Galicia, Spain-IOC-UNESCO.

- Kato, Y., Scheuer, P.J., 1975. The Aplysiotoxins. *Pure Appl. Chem.* 41, 1–14.
- Lawrence, A.L., Castille, F.L., Speed, M., McKee, D.A., Page, K.O., 1998. A simple stress test for *Penaeus vannamei* postlarvae. *Aquaculture* 165, 233–242.
- Lightner, D.V., 1982. Toxic effect of certain marine blue-green algae to penaeid shrimp. Report NOAA-NA-79AA-D-00024. 79 pp.
- Lightner, D.V., 1996. A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultures Shrimps. World Aquaculture Society, USA.
- Lippy, E.C., Erb, J., 1976. Gastrointestinal illness at Srwickley PA. *J. Am. Water Works Assoc.* 68, 606–610.
- Long, B., Carmichael, W.W., 2002. Marine cyanobacteria toxins Chapter 11. Manual on Harmful Marine Microalgae. Monographs on Oceanographic Methodology, vol. 11. UNESCO, Paris. In press. See also: <http://www.cyanosite.bio.purdue.edu/cyanotox/toxins/aplysia.html>.
- Luna, L.G., 1968. Manual of Histological Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. McGraw-Hill, New York.
- Mantoja, R., Mantoja-Pearson, M., 1967. Initiation aux Techniques d'Histologie Animale Masson, Paris.
- Mynderse, J.S., Moore, R.E., Kashiwaga, M., Norton, T.R., 1977. Antileukemia activity in the Oscillatoriaceae: isolation of debromoaplysia toxin from *Lyngbya*. *Science* 196, 538–540.
- Nagai, H., Yasumoto, T., Hokama, Y., 1996. Aplysiatoxin and debromoaplysiatoxin as the causative agents of a red alga *Gracilaria coronopifolia* poisoning in Hawaii. *Toxicon* 37, 753–761.
- Nagle, D.G., Paul, V.J., 1998. Chemical defense of a marine cyanobacterial bloom. *J. Exp. Mar. Biol. Ecol.* 225, 29–38.
- Olivera, A., Akano-Cohelo, M., 1997. Nutricao de larvas e pos-larvas. II Curso Internacional Producao de Pos-larvas de Camarao Marinho. Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo. Sub-programa II: Acuicultura. LCM Univ. Federal de Santa Catarina, Brasil, pp. 95–110.
- Overstreet, R.M., 1973. Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture* 2, 105–140.
- Sánchez, M.R., 1996. Efecto nutricional relativo de cuatro especies de algas como alimento de la larva de *Peneaus vanammei*. *Aquaculture* 58, 139–144.
- Scott Yoo, R., Carmichael, W.W., Hoehn, R.C., Hrudey, S.E., 1992. Cyanobacterial (Blue-green Algal) Toxins: A Resource Guide. American Water Works Association, Denver, CO, USA.
- Skulberg, O.M., Carmichael, W.W., Codd, G.A., Skulberg, R., 1993. Taxonomy of toxic cyanophyceae (cyanobacteria). Algal, Toxins in Seafood and Drinking Water. Academic Press, pp. 145–164. Chapter 9.
- Smith, O., 1996. Toxic effect of blooms of marine species of oscillatoriales on farmed prawns (*Peneaus monodon*, *Peneaus japonicum*) and brine shrimp (*Artemia salina*). *Toxicon* 34, 857–869.
- Sokal, R.R., Rohlf, F.J., 1998. Biometry. The Principles and Practice of Statistics in Biological Research. Freeman, USA. 887 pp.
- Sykora, J.L., Keleti, G., Roche, R., Volk, D.R., Kay, G.P., 1980. Endotoxins, algae and limulus amoebocyte lysate test in drinking water. *Water Res.* 14, 829–839.