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Ostracod Reactions to Non-Toxic and Toxic Algae

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Summary. When fed algae continuously, laboratory ostracods had a life span of 21–28 days; without food, they were able to survive about 7 days. When exposed to thick suspensions of gas-vacuolate blue-green algae, survival rates were generally low. Twenty five % of our euplanktonic strains killed entire ostracod populations within 24–48 hrs; about 30% of other waterbloom strains were dangerous to ostracods causing either death or coordination loss. Some amphibious non-gas-vacuolated strains were also toxic to ostracods. Toxins from broken cells were not persistent in effect; ostracods surviving the initial shock often appeared recovered within 2–4 days. In di-algal systems, ostracods generally fed on a single species and did not always choose green algae over blue-green. Unicellular green algal strains (*Ankistrodesmus, Scenedesmus, Kirchneriella*) were generally preferred. Some chlorophycean forms (*Zygnema, Hormidium, Trentepholia*) were shunned in favor of bluegreens such as *Tolypothrix* and *Westiella*. Erect and branched strains (*Fischerella, Westiella, Tolypothrix*) were eaten before *Anabaena* and *Nostoc* with the latter genus being least preferred. Intact *Nostoc* colonies were usually not ingested, but some loosely growing, non-toxic strains of both *Nostoc* and *Anabaena* provided adequate food for ostracod populations indefinitely.

Introduction

The common ostracod, *Cyprinotus incongruens*, lives a typical benthic existence along shallow shorelines of inland waters. Since development of blue-green algal waterblooms is a frequent occurrence in many impoundments, ostracod populations are periodically exposed to windrowed algal masses. The effects of shoreline pile-ups of blue-green algae on higher animals are well documented (*e.g.*, Francis, 1878; Deem and Thorp, 1939; Stewart *et al.*, 1950; Rose, 1953; Senior, 1960), but responses of benthic microcrustaceans to waterblooms have seldom been reported.

Because *C. incongruens* is a voracious feeder (maximal rate ≈ 1 fecal pellet X ostracod^{-1} X hr^{-1}) and is easily cultured in the laboratory, we decided to test its response to euplanktonic, gas-vacuolate blue-green algal strains—all of which are regarded as being potentially hazardous because of waterbloom origins. In addition, since several amphibious cyanophycean strains were also available for testing, it was possible to examine in some depth the adage (*e.g.*, Sorokin, 1971) that blue-green algae are usually unfit as food sources for invertebrates (Gibor, 1956; Hargrave, 1970; Arnold, 1971; Porter, 1973).

Materials and Methods

Experimental ostracod (*Cyprinotus incongruens*) populations were reared in 4 l glass containers of aged tap water fortified with ASM-1 (Gorham *et al.*, 1964) nutrients. The dominant forage algal species varied but were generally *Ankistrodesmus*, *Kirchneriella*, *Selenastrum* and *Scenedesmus*. Before testing, all ostracods were washed free of algal cells and debris in Coors ceramic spot plates.

Unialgal strains of euplanktonic blue-green algae were cultured in 70 ml of ASM-1 medium in 125 ml Erlenmeyer flasks on reciprocal or rotary shakers at about 100 rpm. Cultures were maintained at a temperature of $24 \pm 1°$C and illuminated by continuous "cool white" fluorescent lighting of about 2500 lux. Cells were harvested by centrifugation after 4–7 days of growth, and 2–4 mg (dry wt) preparations of whole- or sonificated-cells suspended in 7 ml culture medium were added to 10 ml Erlenmeyer test flasks. Duplicates of whole- and disrupted-cell suspensions of each algal strain were tested simultaneously; each test was repeated 3–5 times. In all cases, 5 active ostracods (20 per test) were placed in each flask with the algal strain to be tested. The ostracods were observed for distress symptoms for a maximum of 120 hours or until dead. A high intensity light-beam rigged as a flask probe (B&L Micro Lamp, 6.5 v bulb) was used as a stimulus.

Lawns of amphibious, nitrogen-fixing, blue-green algae were established on 1.5% agar plates that were prepared with nitrate-free ASM-1 medium. When heavy lawns had developed, the plates were flooded with nitrate-free ASM-1 medium and inoculated with 15–20 washed ostracods. Observations were made daily for successful colonization; if none developed, inoculations were repeated a minimum of 3 times. Except for non-agitation and axenic algal inoculations, algal-plate growth conditions were the same as for liquid cultures.

Tests for ostracod food-preferences were carried out similarly except that nitrate was furnished and most algae were selected primarily for their ability to establish luxuriant lawns on agar plates; a few poor lawn-forming green algae were included because they grow in inland waters and are the usual forage for laboratory ostracods. After algal lawn development, the plates provided 2-strain systems—each plate-half being composed of a different alga. For a period of 8–10 days, intermittent microscopical examinations of fecal pellets of grazing ostracods were made.

Results and Discussion

a) Toxicity

Since methods for determining specific responses of ostracods to algal toxins were unestablished, we first determined the laboratory life-span of *Cyprinotus incongruens* by placing adults and newly hatched individuals into 10 ml Erlenmeyer flasks containing green algal forage species. While some individuals lived longer than 2 months, the average life span was 21–28 days. Since young ostracods were found to be more active and faster than older adults, sluggish or inactive individuals were discarded during the pre-test washing. This precaution should have eliminated virtually all natural deaths during the 120 hrs test period. Additionally, we realized that while not necessarily toxic, certain blue-green algae might be inadequate as foodstuffs (*e. g.*, Arnold, 1971) causing death from starvation rather than poisoning. However, since ostracods usually survived at least 7 days without food on sterile, flooded, agar plates (a period which exceeded the maximum testing time by 48 hrs), we assumed the death rate due to starvation to be zero.

About the only special requirement for *C. incongruens* during maximal test runs (120 hrs) was a tractable surface on the bottom of the flask; *C. incongruens* does not seem to be able to remain upright on slick surfaces. Algal coverings or thin layers of sterile agar provided excellent traction.

Most algal strains tested (Table 1) can be grouped into 3 fairly distinct categories: *toxic*, *slowly toxic*, and *non-toxic*. Both disrupted and whole-cell preparations of *toxic* strains affected *C. incongruens* by producing coordination loss and partial paralysis during the first 24 hrs and, within 48 hrs, almost complete paralysis

Table 1. The effect of gas-vacuolate euplanktonic blue-green algal strains on *Cyprinotus incongruens*

a) *Toxic* strains; severe, rapidly occurring symptoms usually ending in death of test animals			
NT69-13	*Microcystis aeruginosa*	ET71-2	*Microcystis aeruginosa*
ET72-17	*Anabaena baltica*	IUCC 1303	*Gloeotrichia echinulata*
NT69-5	*Anabaena affinis*		
b) *Slowly-toxic* strains; symptoms usually occurring after 48 hrs delay			
ET73-1	*Microcystis aeruginosa*	ET72-28	*Cylindrospermum doryphorum*
ET72-18	*Anabaena levanderi*	NT69-10	*Oscillatoria agardhii*
NT69-2	*Anabaena circinalis*	ET71-7	*Oscillatoria lacustris*
NT71-20	*Cylindrospermum doryphorum*		
c) *Non-toxic* strains; no toxicity symptoms produced in test animals			
ET73-3	*Microcystis aeruginosa*	ET72-19	*Anabaena macrospora*
NRC-44	*Anabaena flos-aquae*	NT68-23	*Anabaena flos-aquae*
NT69-25	*Anabaena orientalis*	NT69-51	*Anabaena sphaerica*
NT69-6	*Anabaena limnetica*	NT69-9	*Aphanizomenon flos-aquae*

or death; all ostracods were either dead or paralyzed after 72 hrs (Fig. 1). The *slowly toxic* strains caused about the same effects, but onset did not usually occur until after 48 hrs (Fig. 2).

The obvious symptoms of acute algal poisoning involved rapid disruption of normal ostracod activities; subsequently, feeble appendage movements and irregular circular swimming patterns developed and were followed by apparent loss of coordination, equilibrium, balance, and ultimately, paralysis and death. On the other hand, ostracods exposed to *non-toxic* strains seemed to feed and behave normally without distress over the entire 120 hrs test period.

Ostracod responses to the 2 groups of poisonous algae (Figs. 1 and 2) appeared to vary mostly in the time lapse before symptoms appeared which suggested that toxins from the two groups were similar. Such an effect seems to indicate that the more rapidly poisonous forms produced and excreted toxin at higher levels. Further substantiating this explanation was the lack of distinction in ostracod responses to disrupted-cell preparations of both *toxic* and *slowly toxic* strains. In these disrupted-cell mixes, symptoms were acute with rapid onset; however, ostracods surviving the initial shock frequently appeared completely recovered by the end of the test period. Close parallels to these results have been reports from studies of natural waterblooms (Fritch *et al.*, 1934; Deem and Throp, 1939; Grant and Hughes, 1953) where toxins rapidly lost their effect after algal decomposition began.

In contrast to a case of non-excretion of poisons by a toxic strain of *Aphanizomenon flos-aquae* (Gentile and Maloney, 1969), in these experiments toxin excretion into the growth medium seemed to be a habitual or regular process; filtered media from rapidly growing cultures usually produced equal but ephemeral toxicity symptoms in *C. incongruens*. Perhaps this explains why toxicity symptoms appeared in ostracods during brief exposures to toxic whole-cell suspensions even though fecal pellet and gut analysis failed to indicate actual consumption of the algae. Also, whether or not *C. incongruens* has the ability to deto-

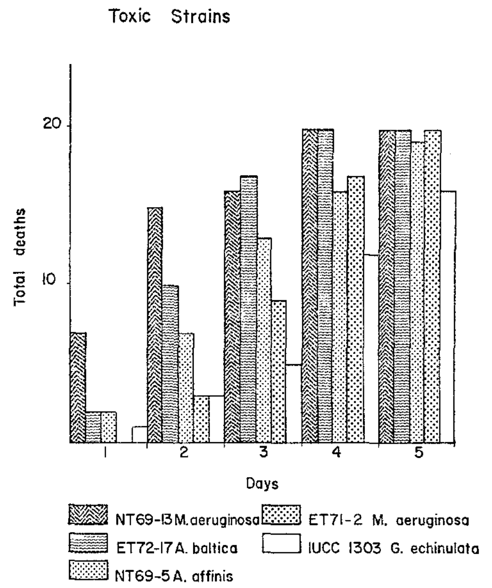

Fig. 1

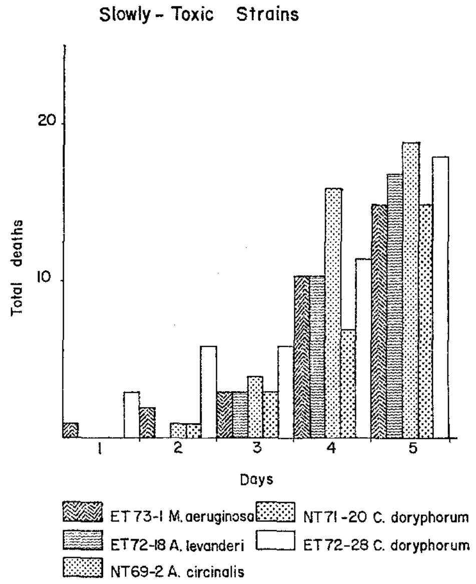

Fig. 2

Figs. 1. and 2. Toxicity of whole-cell preparations of euplanktonic blue-green algae to *Cyprinotus incongruens*. Results show total kills in two parallel test-pairs

xify algal poisons in the gut was not determined during these experiments. However, since exposures to whole-cells of less toxic strains often lacked the impact of their sonificated mixes, some resistance to consumed toxins could be indicated.

Benthic organisms such as *C. incongruens* probably do have resistance to blue-green algal toxins due to exposure in proximal habitats. When compared to reported responses of vertebrates (*e. g.*, Wheeler *et al.*, 1958; Sawyer *et al.*, 1967), ostracods are highly resistant; note that strain NRC 44 *Anabaena flos-aquae*, though highly toxic to mice (Gorham *et al.*, 1964), did not evoke apparent symptoms of distress in *C. incongruens*. But, toxic waterblooms and algal pile-ups along shorelines may decimate ostracod populations without being directly lethal—coordination losses would render individuals helpless and defenseless against predation and wave action.

b) Algal Food Sources

Twenty-four strains of nitrogen-fixing algae, randomly selected, were grown into luxuriant lawns on nitrate-free agar plates; after flooding, each plate was inoculated with 15-20 ostracods. The results summarized in Table 2 show that while a single strain, *Fischerella epihytica* (Martin and Wyatt, 1974a), was extremely toxic, four others were lethal to entire test groups of ostracods within 5 days. While parallels to this 20% toxicity in our experimental algae may not normally exist in nature, our results do indicate that a substantial number of blue-green algae may be toxic to some invertebrates.

Table 2. Ostracod survival on nitrogen-fixing algal lawns: categories of experimental strains

I. Proven food sources; Ostracod populations maintained for 3 or more months			
ET70-2	*Anabaena catenula*	ET71-5	*Nostoc macrosporum*
NT69-20	*Anabaena torulosa*	ET71-14	*Nostoc spongiaforme*
NT69-22	*Anabaena ambigua*	ET71-22	*Nostoc* sp.
NT71-23	*Nostoc pruniforme*	NT69-52	*Westiella intricata*
II. Weakly toxic or non-toxic, poor food sources; Ostracod populations not supported much longer than 7 days			
IUCC 1444	*Anabaena flos-aquae*	ET71-1	*Fremyella* sp.
ET71-19	*Anabaena variabilis*	ET71-18	*Nostoc rivulare*
NT69-14	*Anabaena torulosa*	ET72-7	*Nostoc coeruleum*
IUCC 1301	*Fischerella muscicola*	ET72-16	*Nostoc culticulare*
IUCC 1829	*Fischerella muscicola*	ET71-11	*Nostoc rivulare*
IUCC 424	*Tolypothrix distorta*		
III. Toxic; Ostracods die within 5 days			
NT69-35	*Anabaena inaequalis*	ET72-3	*Anabaena doliolum*
IUCC 377	*Anabaena variabilis*	ET70-5	*Nostoc zetterstedtii*
VI. Very toxic; entire Ostracod population usually killed in about 12 hrs			
NT69-32	*Fischerella epiphytica*		

Equally important, perhaps, is the fact that about 50% of the strains tested may not have been toxic *per se* but, as the only available foodstuffs, failed to adequately support ostracod colonies despite the high protein content (40–50%) of blue-green algae (Rohlich and Sarles, 1949; Boyd and Lawrence, 1967; Boyd, 1973). Most of these algae are similar to strains which have served as adequate food sources, and our experiments did not suggest reasons for their non-suitability. A few strains may have been non-ingestable because they grew in colonies with firm slime encasements (Martin and Wyatt, 1974b); they were available as food only when colonies were disrupted.

Table 3 presents results from studies of ostracod food selections from lawns of two contrasting algal types. In blue-green algal systems, fecal pellet analyses generally indicated a preference for Stigonemataceae, Scytonemataceae, Nostocales and Chroococcales in the order listed. Surprisingly, the heavy ensheathments of the first two groups did not hinder grazing; the encasing slime of some Nostocales appeared to have discouraged feeding. Erect, freely-growing filaments usually were eaten before closely packed algal forms.

Predictably, green algae were the usually selected food. However, blue-green algae were consumed in preferenced to green algae about 30% of the time. When cyanophyceae were preferentially consumed, the rejected green alga either had a growth form which inhibited grazing or was not an aquatic form.

Since it is virtually impossible for both strains of di-algal systems to have simultaneously reached equivalent growth stages and since sporadic fecal pellet analyses showed only the immediate patterns of feeding, these results should probably be interpreted only in broadest terms. Ostracods can and do utilize blue-green algae as food under some conditions. Noteworthy was the fact that in di-algal systems one alga was usually grazed while the other was untouched, even

Table 3. Ostracod food preferences; strain selection in 2-phase algal systems

I. Blue-green algal combinations				
NT69-52	*Westiella intricata*	over	IUCC 424	*Tolypothrix distorta*
NT69-52	*Westiella intricata*	over	ET72-7	*Nostoc coeruleum*
IUCC 1301	*Fischerella muscicola*	over	NT69-20	*Anabaena torulosa*
NT68-14	*Scytonema archangelii*	over	ET70-2	*Anabaena catenula*
IUCC 424	*Tolypothrix distorta*	over	ET71-18	*Nostoc rivulare*
ET70-2	*Anabaena catenula*	over	ET71-14	*Nostoc spongiaforme*
ET71-14	*Nostoc spongiaforme*	over	NT68-4	*Chroococcus* sp.
ET71-5	*Nostoc macrosporum*	over	NT69-27	*Gloeocapsa* sp.
II. Green and blue-green algal combinations				
a) Blue-greens over greens				
NT69-22	*Anabaena ambigua*	vs.		*Zygnema* sp.
NT69-52	*Westiella intricata*	vs.		*Trentepohlia* sp.
IUCC 1829	*Fischerella muscicola*	vs.		*Zygnema* sp.
ET70-2	*Anabaena catenula*	vs.		*Protoderma* sp.
NT69-52	*Westiella intricata*	vs.		*Trentepohlia* sp.
IUCC 424	*Tolypothrix distorta*	vs.		*Mougeotia* sp.
ET70-2	*Anabaena catenula*	vs.		*Zygnema* sp.
b) Greens over blue-greens				
	Scenedesmus sp.	vs.	NT69-27	*Gleocapsa* sp.
	Scenedesmus sp.	vs.	ET72-7	*Nostoc coeruleum*
	Tribonema sp.	vs.	NT69-3	*Anabaena laxa*
	Mougeotia sp.	vs.	NT69-35	*Anabaena inaequalis*
	Protoderma sp.	vs.	IUCC 1444	*Anabaena flos-aquae*
	Ankistrodesmus sp.	vs.	ET70-2	*Anabaena catenula*
	Crucigena sp.	vs.	NT68-4	*Chroococcus* sp.
	Scenedesmus sp.	vs.	ET72-16	*Nostoc culticulare*
	Hormidium sp.	vs.	NT69-22	*Anabaena ambigua*
	Crucigena sp.	vs.	ET70-2	*Anabaena catenula*
	Crucigena sp.	vs.	ET73-3	*Microcystis aeruginosa*
	Ankistrodesmus sp.	vs.	ET71-18	*Nostoc rivulare*
	Zygnema sp.	vs.	NT69-14	*Anabaena torulosa*
	Mougeotia sp.	vs.	IUCC 1823	*Anabaena randhawae*
	Mougeotia sp.	vs.	IUCC 941	*Gloeotrichia* sp.
c) Preference about equal				
IUCC 1829	*Fischerella muscicola*	vs.		*Trentepohlia* sp.
NT71-23	*Nostoc pruniforme*	vs.		*Protoderma* sp.

if both species were possible food sources. This is contrary to feeding patterns in multi-algal combinations during laboratory cultivation where many different organisms are eaten by *Cyprinotus incongruens*.

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