A review of fish-killing microalgae: causes, impacts and management with emphasis on *Prymnesium*

Jan Landsberg

Florida Marine Research Institute (FMRI)

Fish and Wildlife Conservation Commission (FWC)

St. Petersburg, Florida

Etiology of aquatic animal mortalities

- toxic microalgae
- contaminants
- water quality
- pathogens
- fishery by-catch
- mechanical damage
- natural

Toxic/harmful microalgae

- dinoflagellates**
- diatoms
- cyanobacteria
- raphidophytes**
- prymnesiophytes**
- dictyophytes
- chrysophytes

** ichthyotoxic species

HABs and fish kills

- global
- all habitats
- > 60 ichthyotoxic species known
- > 30 species harmful to fish


Fish kills

Harmful mechanisms

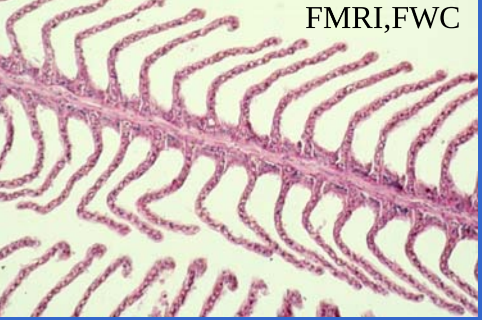
- toxins
- enzymes
- reactive oxygen species
- mechanical
- physical
- anoxia/hypoxia
- NH_4 toxicity
- allelopathy
- starvation
- predation

Ichthyotoxic species

- *Karenia brevis*
- *K. mikimotoi*
- *Karlodinium micrum*
- *Gymnodinium pulchellum*
- *G. aureolum*
- *Amphidinium* spp.
- *Cochlodinium* spp.
- *Pfiesteria piscicida*
- *P. shumwayae*
- *Alexandrium monilatum*
- *A. tamarense*
- *Chrysochromulina* spp.
- *Heterosigma* sp.
- *Fibrocapsa* spp.
- *Prymnesium* spp.
- *Chattonella* spp.

Impacts of ichthyotoxic species

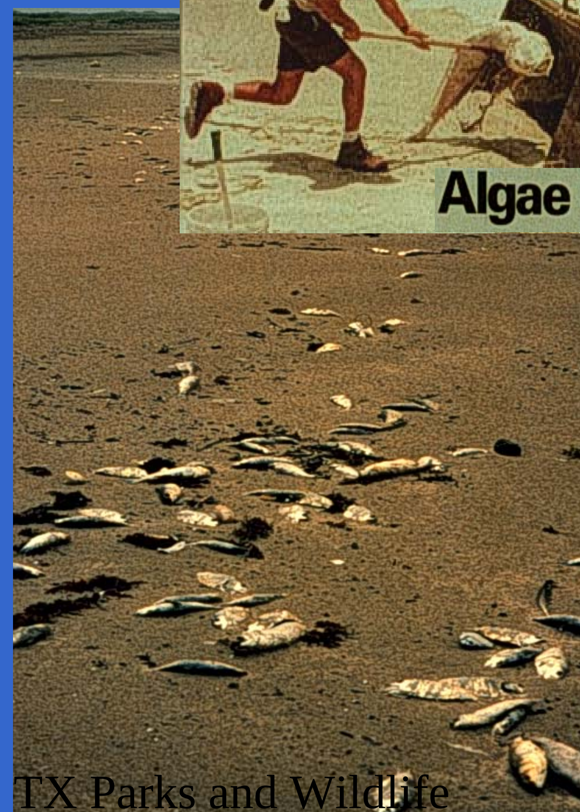
- public health
- direct mortalities
- indirect losses – disease, growth, fecundity, loss of recruitment
- economic
- ecological

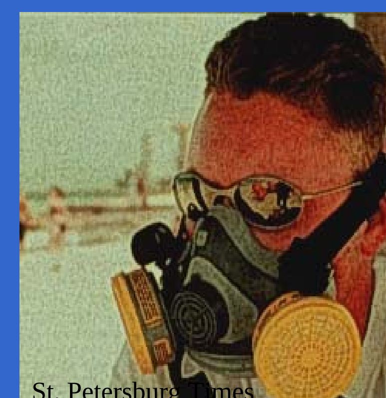


Exposure routes

- gills – absorption of soluble toxins from water
- skin – absorption of soluble toxins from water
- ingestion – direction consumption of cells/ bioaccumulation of toxins

Karenia brevis

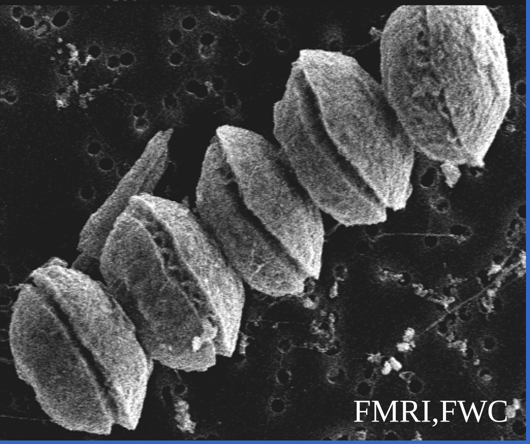




Brevetoxins - neurotoxins and hemolysins

Gymnodinium pulchellum (brevetoxins)

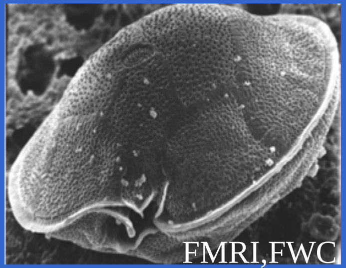
- natural mortalities of fish and mortalities in aquaculture
- respiratory irritation in humans



Alexandrium monilatum (hemolysins)

- reduced filtration in oysters and clams
- decreased byssus production in molluscs
- moribund shellfish
- mortality in oysters
- fish mortalities

Reef fish disease - Caribbean, Florida

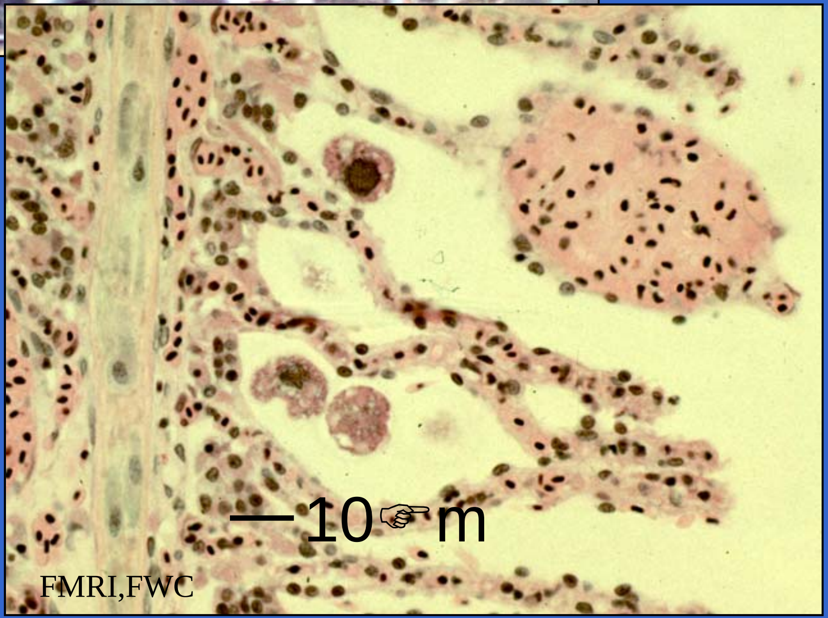


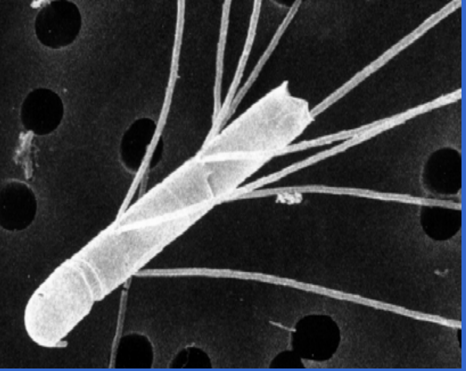
Microalgae

From Landsberg 1995

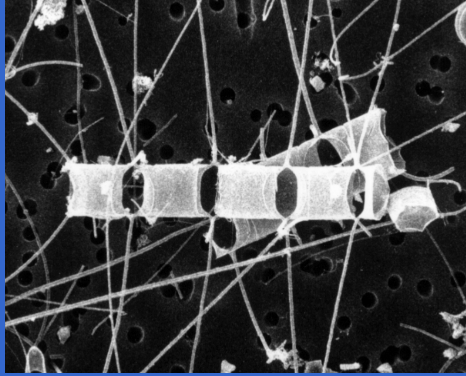
Scrippsiella sp.

- fish mortalities
- ?toxic
- low dissolved oxygen
- acute gill pathology





Diatoms

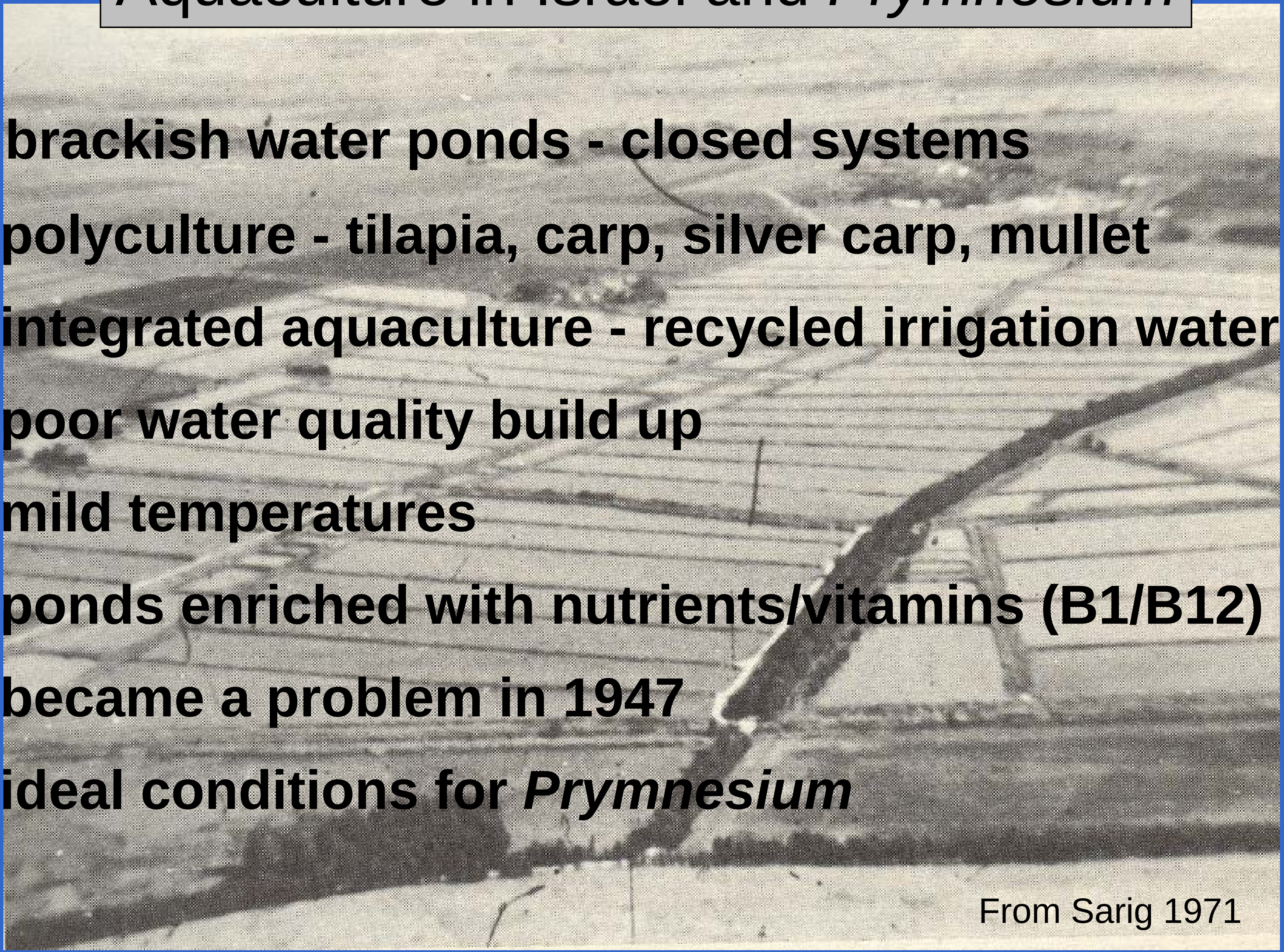


- physical damage to gills by spines, barbs
- gill lesions, excessive mucus, asphyxiation
- marked edema
- change in blood parameters
- immunosuppression - susceptibility to vibriosis

Prymnesium

- at least 3 ichthyotoxic species - globally
- primarily *P. parvum* associated with kills
- brackish water – aquaculture systems
- fish exposed to prymnesins in the water
- no transfer of toxins up the food chain or in drinking water

Aquaculture in Israel and *Prymnesium*



- **brackish water ponds - closed systems**
- **polyculture - tilapia, carp, silver carp, mullet**
- **integrated aquaculture - recycled irrigation water**
- **poor water quality build up**
- **mild temperatures**
- **ponds enriched with nutrients/vitamins (B1/B12)**
- **became a problem in 1947**
- **ideal conditions for *Prymnesium***

From Sarig 1971

From Sarig 1971

Heavy carp mortality in 5 hectare pond due to *Prymnesium parvum* in Israel

Prymnesium parvum from Israel

Fig. 2. The phytoflagellate *Prymnesium parvum* Carter × 4000.

From Sarig 1971

Prymnesium impacts

- gill breathing organisms sensitive –
  larval amphibians, finfish, bivalves
- non selective
- restricted by habitat type
- seasonality
- acute effects only – direct through the gills

Prymnesium parvum blooms in Israel

- lack of correlation between blooms, toxin, fish kills
- sporadic fish kills
- requires vitamins B_{12} and thiamine
- can tolerate freshwater with chloride 250-625 ppm
- no growth below 0.1% salinity
- typically rare in natural habitats

Prymnesium parvum toxin

- hemolytic and ichthyotoxic components
- toxin synthesized during late stage of logarithmic growth and in early stationary phase
- intracellular > extracellular
- biosynthesis and extracellular stability affected by environmental conditions
- light essential for toxin formation
- phosphate limitation > toxin production
- toxin inactivated by change in pH, absorption on various colloids, exposure to UV and short wave light

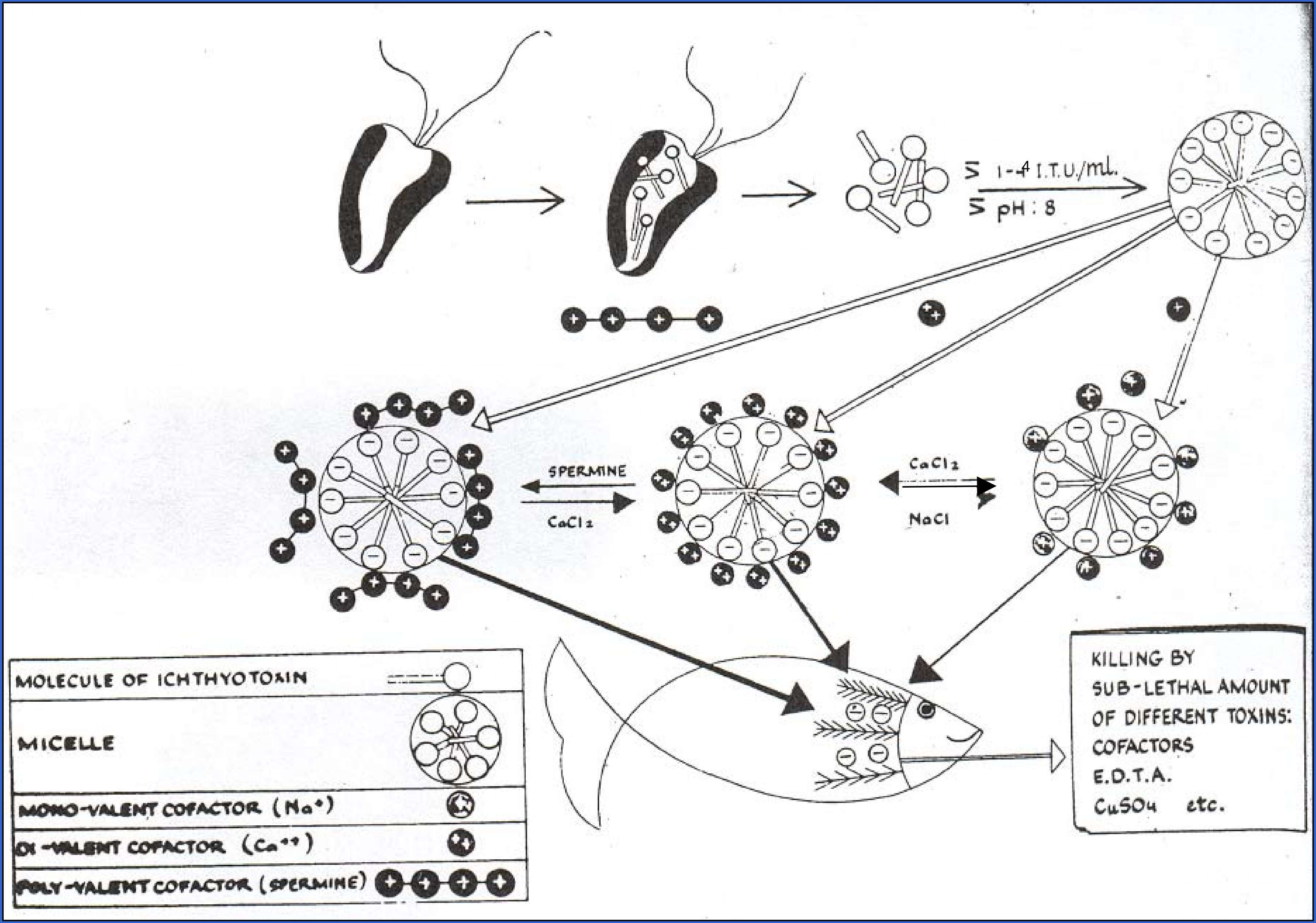
From Sarig 1971

Prymnesium parvum toxicity

- activity of prymnesin requires cationic cofactors
- Na, Mg, Ca and salinity determine toxicity
- non toxic by dialysis or cationic exchange column
- ichthyotoxicity restored on addition of the dialyzate
- dialyzed cation salts e.g. Ca/Mg restore fish toxicity
- streptomyin, spermine, detergents (DADPA)
  enhance toxicity of *Prymnesium* preparations
- relative activities of various cations different
- inverse relationship between toxicity and salinity

From Sarig 1971

Mode of action of *Prymnesium* toxin on fish



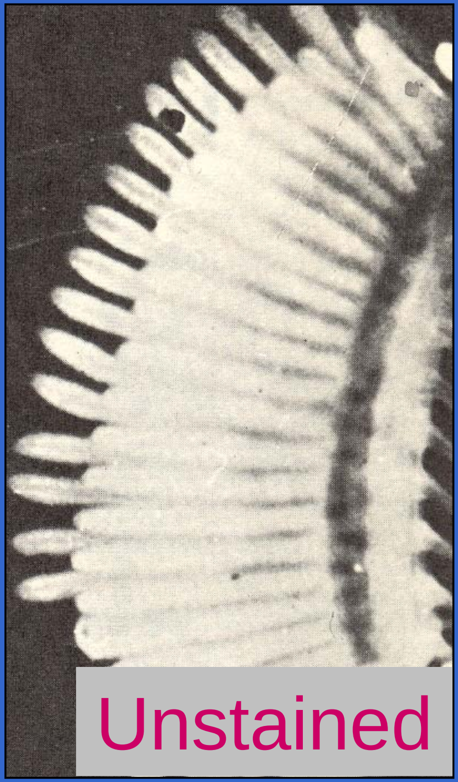
From Sarig 1971

Mode of toxin action

- fish affected within minutes of exposure

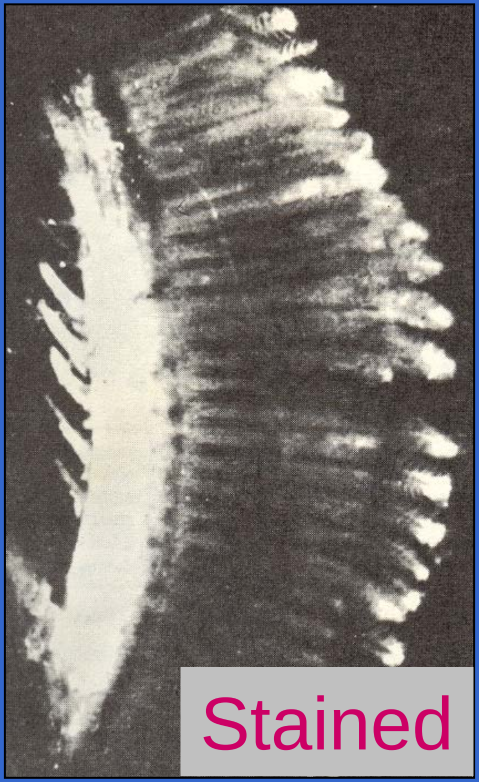
unexposed fish

fish exposed to toxin-cation mixture



trypan blue

increase in gill permeability



From Sarig 1971

Mode of toxin action

- increased gill permeability only in conditions in which ichthyotoxin activity is cation activated
- pH dependent -- requires higher pH
- toxic activity inhibited by NaCl
- damage to gill permeability and consequent sensitization to toxic agents is reversible
- intoxication in two stages

1) reversible specific damage resulting in the loss of selective gill permeability

2) response of sensitized fish to an array of toxins

Fish bioassay

- dependence of toxin activity on various cations lead to sensitive bioassay
- assay based on minimal toxin concentration killing *Gambusia*
- in the presence of 3’3 diaminodipropylamine (DADPA) as a cationic activator

From Sarig 1971

Add 1 ml DADPA (0.003M) + tris buffer (0.02M), pH = 9

$28^{o}C$

2 hours exposure

50 ml pond H_2O

40 ml distilled H_2O
+ 10 ml pond H_2O

50 ml distilled H_2O
(control)

Death = 1 ITU

Death = 5 ITU

1/25 lethal
dose in ponds

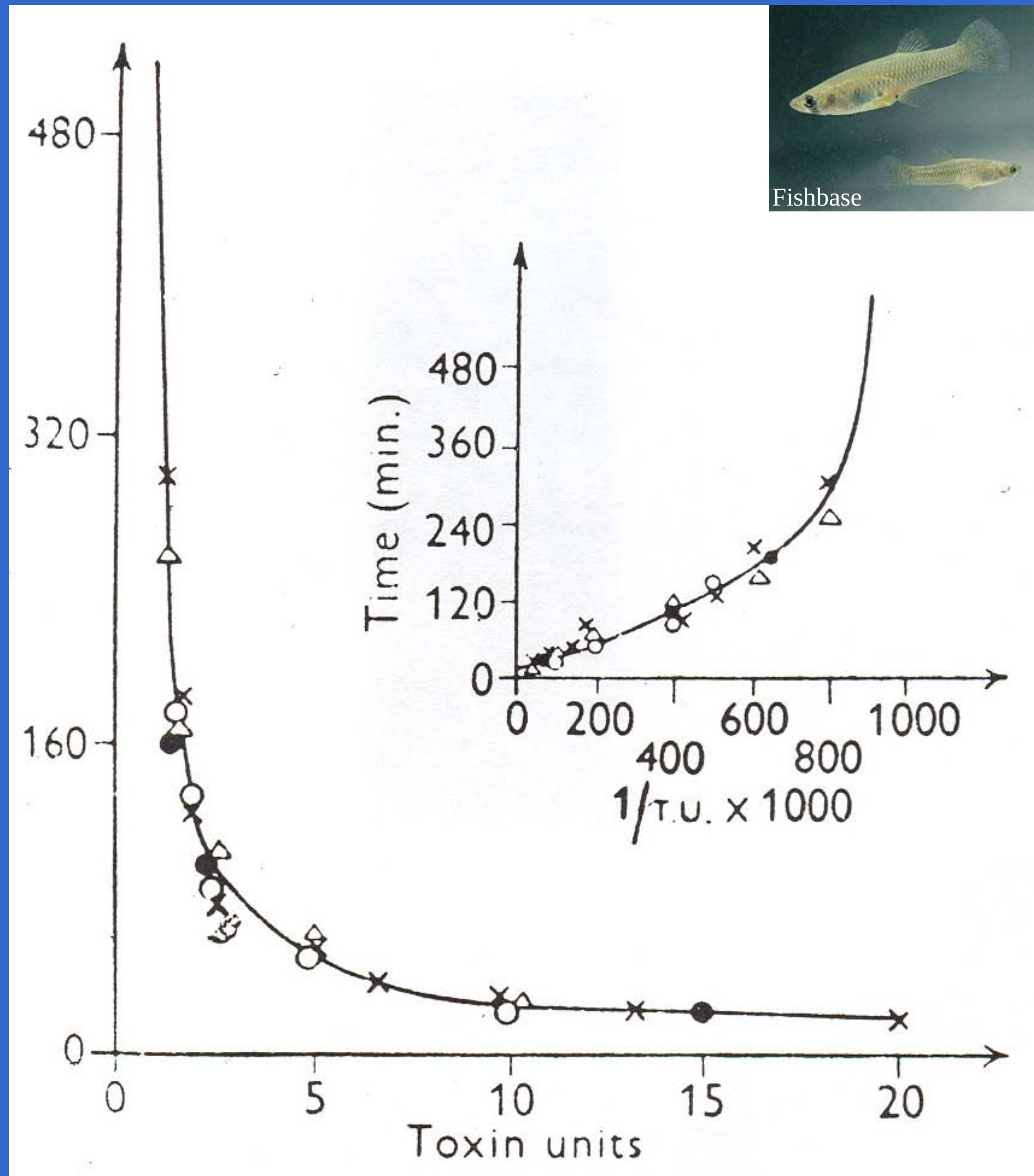
1/5 lethal
dose in ponds

Recommend treatment

1 ITU = minimal amount of ichthyotoxin/ml that kills fish

Relationship # toxin concentration and time for loss of equilibrium

Time (mins)



From Sarig 1971

Testing chemical applications on *Prymnesium*

- 10 ppm $(NH_4)_2SO_4$ lytic effect
- low cost, high solubility, ease of dispersion
- *Prymnesium* lysis > with temperature and pH
- decreased activity of $(NH_4)_2SO_4$ in winter
- ammonia responsible for cell lysis
- diurnal changes in pH max. at noon
- control added few hours before pH peak
- Cu_2SO_4 not dependent on pH or temp
- $(NH_4)_2SO_4$ (acid fertilizer) lowers pH
- treatment strategy varies with conditions

From Sarig 1971

Management of *Prymnesium* blooms in Israel

- proactive monitoring
- fish bioassays
- test for sublethal *Prymnesium* concentrations
- treat ponds with liquid ammonium
- ammonia concentrates in *Prymnesium*
- shift in pH > water entry > swelling > cell lysis
- best results at temps < 20ºC and pH < 8.5
- aqua ammonia is alkaline and raises the pH

From Sarig 1971

From Sarig 1971

Algicides and *Prymnesium*

pH	Temp (°C)	Liquid ammonia	Ammonium sulfate	Copper sulfate
>9.0	> 20	-	10-12	-
	17-20	10-12	15	2
	10-17	14	25	2-3
8.6-9.0	> 20	10-12	13-15	-
	17-20	12-14	20	2
	10-17	14	-	2-3
<8.6	> 20	12-13	15-17	-
	17-20	13-14	25	2-3
	10-17	-	-	2-3

per Kg/1000m^3 pond water = 1million liters or 265,000 US gallons

Management strategies

- prevent blooms
- inactivate or remove toxin
- separate fish from blooms

Needs

- dynamics of toxin production in different systems
- determine triggers for bloom formation
- spatial and temporal variations in toxicity
- are *Prymnesium* effects only acute?
- impacts on recruitment?
- economic assessment for management strategies