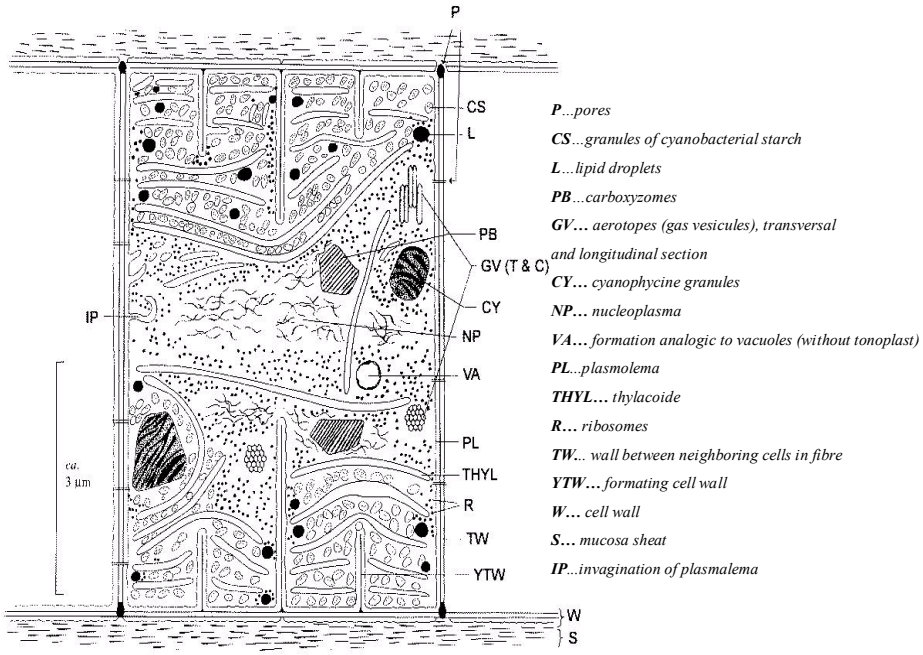
Toxins of cyanobacteria and algae (cyanotoxins)

- Cyanobacteria and algae
  - In prevalence autothrophic organisms
  - Uni-cellular and multi-cellular body
  - Cyanobacteria prokaryonta
  - Algae eukaryonta

- Cyanobacteria (Cyanophyta, Cyanobacteria)

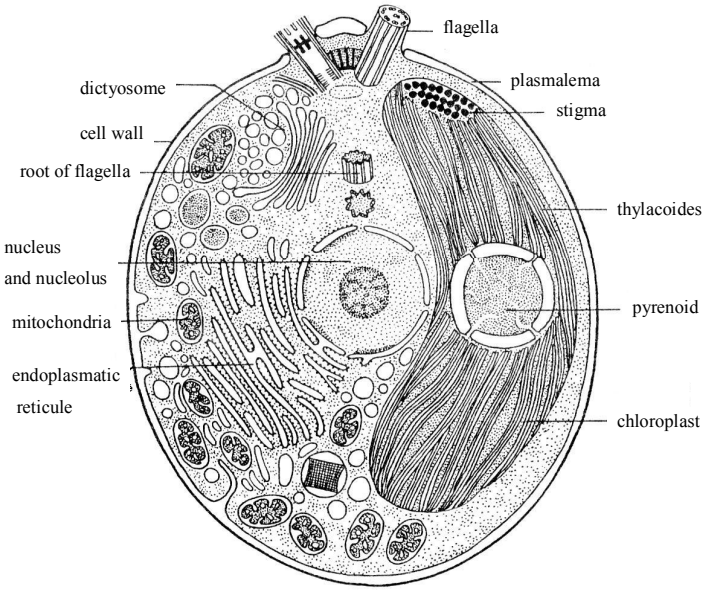
- Colonies of photosyntethising cells
  - Fibers or fluffs
- Prokaryotic organisms
  - No nucleus, no chloroplasts nor mitochondria
  - DNA in nucleoplasmatic area
  - Photosynthesis similar to plants
- Classification:
  - Bentic – sessile to surfaces and bed
  - Planktonic – freely floating colonies
- Algal bloom
  - Concentration over 10000 cells/mL
  - Visible color of water
  - Summer period
    - Czech republic: *Microcystis aeruginosa, Aphanisomenon phlos-aquae, Anabaena* spp.
    - Northern Europe *Oscillatoria rubescens*
- Some species
  - Gas vacuoles
    - Flotation
    - Formation of foam over 10^6 cells/mL
- High toxicity
  - Monitoring of levels in water
  - Different levels of monitoring



•Obr. 1: Transversal section of cell Pseudanabaena species (Pankratz & Bowen 1963).

• Algae

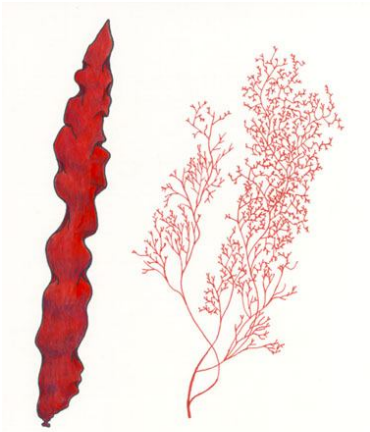
- Eukaryotic organisms
- Numerous subdivisions:
  - Submicroscopic variability in morphology
  - Composition of photosynthetic pigments
  - Composition of reserve compounds
  - Cross-penetration of types
- Developmental branch
  - Autotrophic organisms
  - Connected with water
- Bentic or planktonic
- Frequent toxicity
  - *Rhodophyta* red algae
  - *Dinophyta* dinophlagelates
  - *Cryptophyta* cryptomonads
  - *Chromophyta* brown algae



http://vydavatelstvi.vscht.cz/knihy/uid_es-006/hesla/img__d10e3894.html

Rhodophyta (Red Algae)

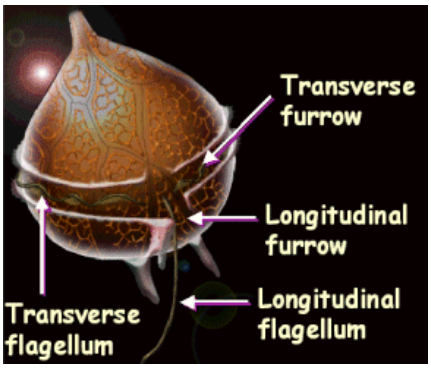
- In majority marine organisms
  - Only few of them in sweet water
- Lots of different structural patterns of thallus
  - Unicellular microscopic
  - Complex tissue-like thalluses of microscopic parameters
  - Never possess flagellate stadium
- Pigments:
  - chlorophyll **a** and **d**, posses also phycobillines (similarly to Cyanobacteria).
- Very complicated life cycles
  - reproduction both non-sexual and sexual (oogamy).
- Cell surface is covered by thick polysaccharide wall
  - Polysaccharides industrial materials (for example agar).
  - Food
    - Marine species *Porphyra* - nori in Japan
- Representatives:
  Corallina – very common marine species, easy incrustation with calcite (very huge geological sediments from dead bodies)
  Batrachospermum – verticile branched sweet water species
  Lemanea – similar to *Batrachospermum*, looks tubular, but possess verticile branched thallus. Similar also ecology - grows epilitic (on stones) in fast flowing pure waters
  Gelidium – one of the most used industrial sources of agar



Rhodophyta

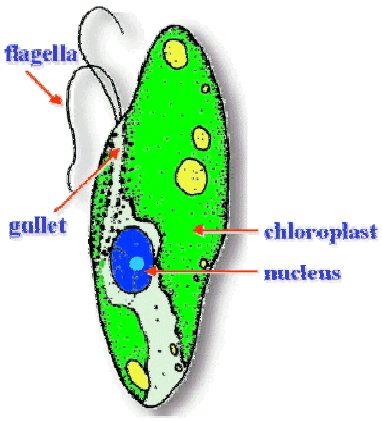
Dinophyta

- Freely living flagellates
- Very complicated life cycles
  - both sessile and amoeboid stadium
- Part of Dinophyta does not possess photosynthetic apparatus and nutrition is therefore heterotrophic
  - Possible active „hunt“ and phagothropia
- Pigments
  - Chlorophylls **a** and **c**
- Cell surface is usually covered by huge cellulose casing
- Very strange nucleus, called dinokaryon.
  - Very big, cca 10x bigger amount of DNA, then usual
  - Chromosomes of dinokaryon are permanently condensed and possess no histoproteins
- Dinophyta are able to produce large amounts of toxic compounds
  - Very toxic flos acquae, so called „red tide“
- Most common:
  - *Ceratium, Peridinium* – both genera include many species, both sweet water and marine
  - *Noctiluca miliaris* – component of marine luminescent plankton



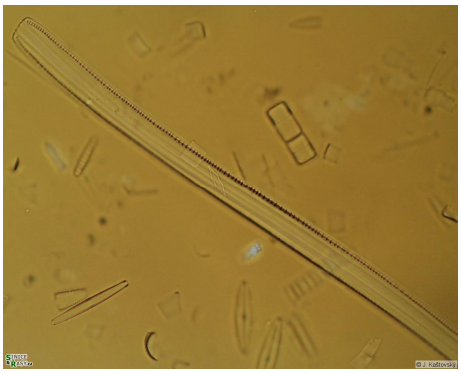
Cryptophyta

- Group of small flagellates
- Pigments:
  - chlorophyll **a** and **c** and fycobillines as cyanophyta
- In addition to own nucleus:
  - Organel called nucleomorph
    - degenerated nucleus of endosybiont
    - chloroplast
- Their surface is soft
  - well digestible for different plankton heterotrophs.
  - psychrophilic.
- Representative is genus *Cryptomonas*



Chromophyta

- Very large division
  - Includes seven classes
  - Very different organisms
    - microscopic diatoms
    - multimeter marine seaweeds
- Identical microscopic, ultrastructural and biochemic parameters
- Pigments
  - Chlorophyll **a** and **c** , mostly also xantophyll fucoxanthine
  - Chloroplasts 4 membranes
    - Two of them are membranes of endoplasmatic reticule, mostly connected with nucleus
    - Under surface of chloroplast wreath-like lamela – showing position of chloroplast DNA
    - thylacoids are connected in triplicates
- Storage compound is chrysolaminaran, deposited outside of chloroplast, never starch (further storage compounds: oils, polyphosphate grains – volutine and others)
- Flagellate stadia possess two heteroconte (non-comparable)) flagella, which are different in longitude, function snd strucutre of mastigonemates



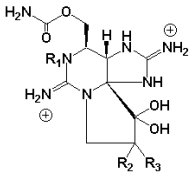
Classification of Cyanobacterial and Algal Toxins

- Neurotoxins and paralytic poisons
- Hepatotoxins
  - alkaloid
  - peptidic
- Tumor promoting factors, genotoxines and mutagens
- Cytotoxins, prymnetoxins
- Embryotoxins
- Dermatotoxic alkaloids
- Lipopolysaccharides
- Immunotoxins and alergens

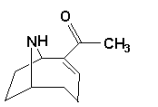
- Mixed bioactivity common
- Population of one species produces more different toxins

Neurotoxins and paralytic poisons
(Paralytic shellfish poisons)

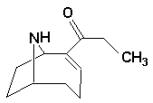
- Representative compounds:
  - anatoxin a, anatoxin a(s), anatoxin b, homoanatoxin
  - saxitoxin, neosaxitoxin
  - aphantoxins 1-5
  - gonyautoxins
  - Chemical structure:
    - Purine derivatives
      - Saxitoxins, aphantoxins, gonyautoxins
      - Tricyclic perhydropurine
      - Different substitution
    - Derivatives of cyclic *N*-hydroxyguanine
      - Anatoxin a(s)
    - Simple bicycles
      - Anatoxin a, homoanatoxin a



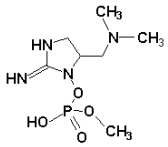
STX	R_1	R_2	R_3
STX	H	H	H
GTX-II	H	H	OSO_3^-
GTX-III	H	OSO_3^-	H
NeoSTX	OH	H	H
GTX-I	OH	H	OSO_3^-
GTX-IV	OH	OSO_3^-	H



ANATOXIN-a



HOMOANATOXIN-a

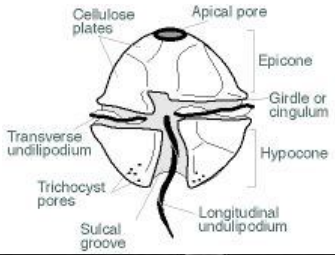


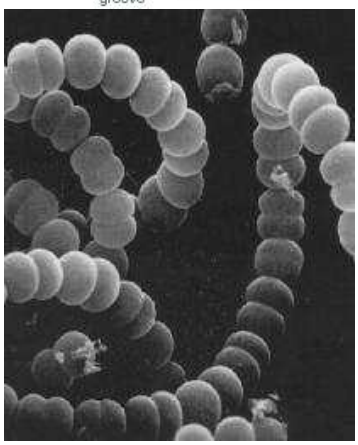
ANATOXIN-a(s)

BRIEF REVIEW OF NATURAL NONPROTEIN NEUROTOXINS Jiri Patocka and Ladislav Stredab

Sources:

- ***Gonyaulax*** Dinophyta
  - Marine algae
- ***Anabaena, Aphanizomenon***
  - Cyanobacteria
- **Principles of effect:**
  - **Aphantoxins, saxitotin, neosaxitoxin** – blockade of transfer of neural excitements via blocking of Na+ channels. No influence on K+ channels
  - **Anatoxin A and homoanatoxin** causes change of function in preganglial neural terminations, acetylcholine receptors, increases the flow of Ca2+ ions into cholinergic neural terminations
  - **Anatoxin a(s)** acts as blocker of cholinesterase, causes depolarisation of postsynaptic terminations, affects nicotinic, muscarinic and acetylcholine receptors
  - **Saxitoxin** is je blocker of Na+ channels (first toxin with essential influence for explanation of Na+ and K+ channels function and neurobiology), tetrodotoxin disrupts action potential of neural and muscular fibers



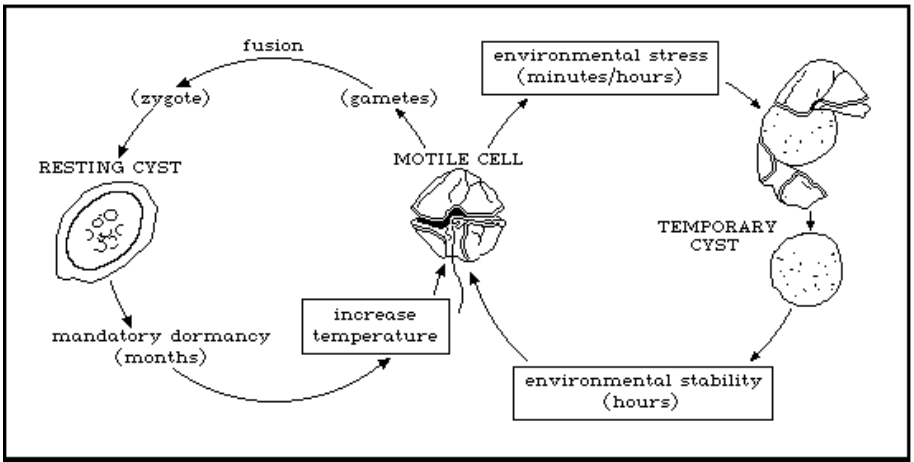


- **Symptoms of intoxication by anatoxins**
  - **Anatoxin-a, homoanatoxin-a, anatoxin-a(s)**
    - *Anabaena flos-aquae*
    - postsynaptic depolarizing neuromuscular blockers
    - inhibitors of acetylcholinesterase
    - Strong interaction with nicotine receptor
    - Hypersalivation
    - Diarrhea
    - Paralysis
    - Death caused by respiratory failure

- **Potential war poisons (chemical warfare)**
  - Absorption
    - Inhalation
    - Intact skin
    - Per oral

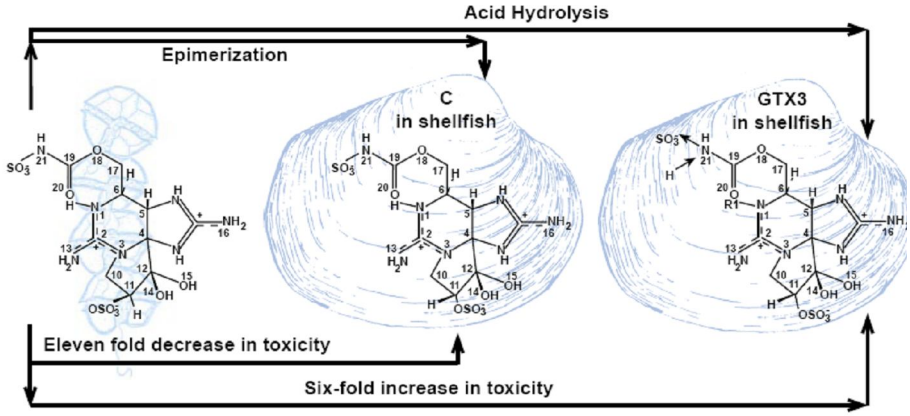
- Introduction into food chain
  - Accumulation in Crustaceans and fishes
  - Both dependent and independent on climate
- Intoxication PSP
  - Relaxation of smooth muscles
  - Depression of action potential in heart
  - Block of sodium channel
    - Guanidine ring condition of effect
    - Block from outer side of channel
    - Blocked both open and closed channel

http://www.pac.dfo-mpo.gc.ca/ops/fm/shellfish/Biotoxins/closures/default_e.htm



Cyst development in Gonyaulax excavata: (A) resting cyst; (B) motile cell; (C) temporary cyst. Changes in environmental factors which stimulate the formation and conversion of cysts (encystment and excystment) are indicated (Adapted from: Yentsch & Incze, 1980).

•Cysts contain possible 1000 times higher amounts of toxines



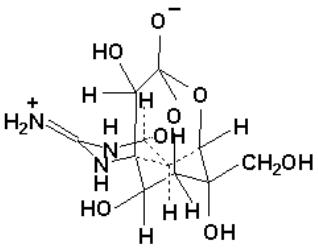
Paralytic Shellfish Poisoning: The Alaska Problem

Raymond RaLonde, Marine Advisory Program, Aquaculture Specialist

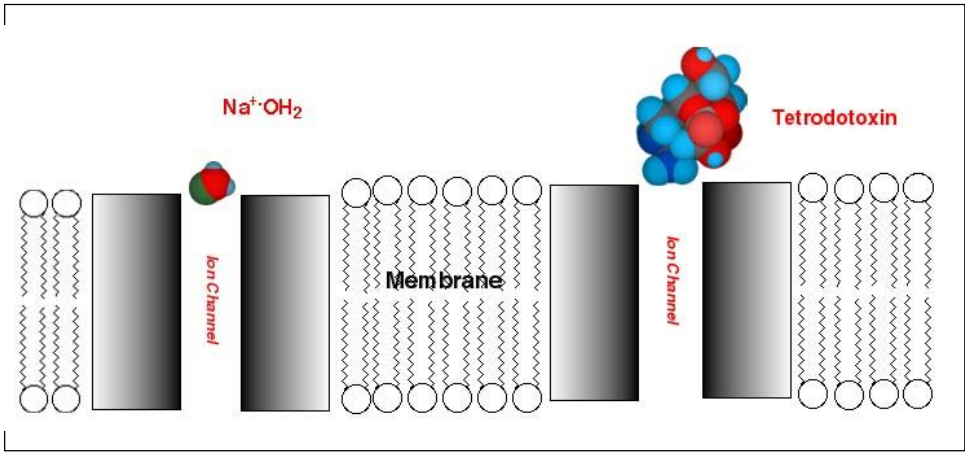
- Symptoms of saxitoxin intoxication
  - Consumption of contaminated food
    - Oysters, Crustaceans
    - Very rapid onset
  - LD *p.o.* 0.5 mg, *i.v.* 0.05 mg
  - Anesthesia and immobility of tongue and fingers
  - Sense of thirst
  - Pain in tips of fingers
  - Massive intoxication
    - GIT disorders
    - Headache
    - Disorder of movement coordination
    - Ascendant type of paralysis
    - Disorders of cognitive functions
    - Respiratory paralysis
- For differential diagnostics absence of hypotension
- PSP compounds
  - saxitoxin, neosaxitoxin, gonyautoxin I, gonyautoxin III, and decarbamoyl saxitoxin
    - Toxicity similar
  - gonyautoxins II, IV, V, VI, VIII, VIII-epimer, sulphocarbamoyl gonyautoxin I, IV
    - Substantially less toxic
  - Toxicity strongly dose-dependent
- Usage
  - Chemical warfare
  - Experimental compounds

Tetrodoxin TTX

- Potent and rapid action
- *Tetraodontiformes*
  - tetraodon, pufferfish
  - ovaria, liver, guts highest content
  - skin traces only
  - In Japan 646 of cases between 1974 nda 1983 (179 mortal), in present time 30-100 per year
- Some frogs, octopuses, snails and slugs
- Unusual tricyclic structure
  - guanidinium toxins
  - aminoperhydroquinazoline
- Specific blocker of Na+ channels of neurons
  - Tetrodotoxin – Na+ binding site extremely narrow
  - TTX acts as hydrated Na^+
  - Inters the channel orifice, binding to a glutamate residue in channel peptide
  - Conformation changes
  - Electrostatic binding to an open channels



TETRODOTOXIN

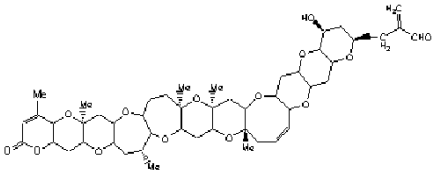


http://www.life.umd.edu/grad/mlfsc/zctsim/ionchannel.html

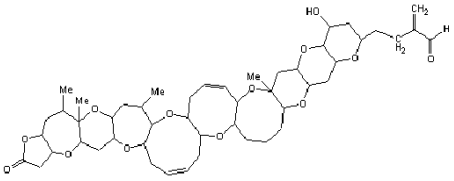
- Extreme toxicity TTX
  - Minimal *p.o.* is 30 µg/kg
    - Decomposition in acidic environment in stomach
  - Termostable, decomposition in acids and bases
- Symptoms of intoxication
  - In minutes or hours
  - Trembling, tingling and paresthesia of tongue, lips and tips of finger
  - Headache, nausea, vomiting, diarrhea
  - Second degree
    - Continuous paresthesia
    - Paralysis
    - Impossibility of movements
    - Convulsions, arrhythmia, mental confusion
  - Death caused by respiratory arrest aprox. In 8 hours
    - Possible full consiousness close before death

Brevetoxins

- *Gymnodinium breve* (*Ptychodiscus brevis*)
  - –So called red tide
    - Massive death of fishes
    - Mexican gulf, Australia, coast of N. America
  - –Polycyclic ethers
    - Lipophilic
    - 10 and 11 rings
    - All-*trans* arrangement
    - Relatively stable compounds (only very high and very low pH can cause decomposition)
  - –Mechanism
    - Depolarisation, opening ofotevření napěťově řízených Na+ kanálů
    - Uncontrolled influx of Na+ into cell
    - Change of voltage necessary for channels opening, hyperexcitability
  - –Symptoms:
    - Often confused with ciguatoxins intoxication
    - Tingling of face, throath, fingers
    - Trembling, nausea, vomiting, diarrhea, headache
    - Mydriasis
    - Slowering of heart rate
    - No mortal cases described



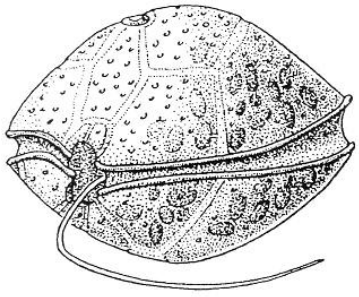
BREVETOXIN-A, a type I brevetoxin

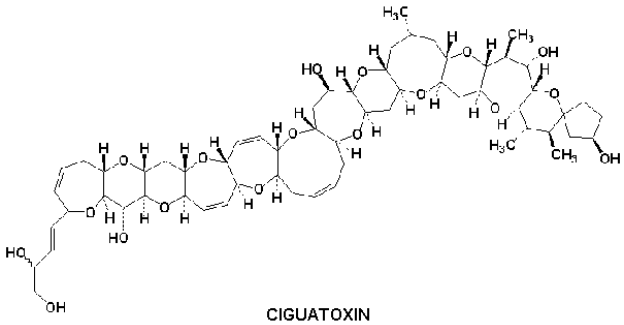


BREVETOXIN-B, a type II brevetoxin

•Ciguatera toxins

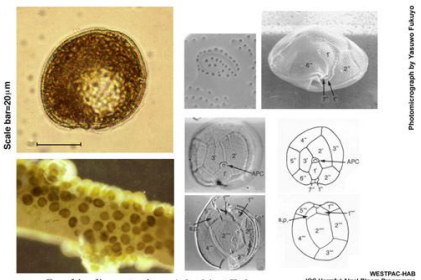
- –Mixture of compounds
  - In present time 24 relative compounds (ciguatoxin, maitotoxin, scaritoxin, okadaic acid)
- –Dinoflagellate *Gambierdiscus toxicus*
  - Corall reefs
- –Found in tropical fish
  - Tropics and subtropics
- –Low molecular lipid polyethers
- –Resistant to temperature
- –Stimulation of Na+ transitic through
- membrane
- –Neurotoxins
- –4 categories of symptoms
  - Neurologic - 7 days
  - Cardiovascular
  - Gastrointestinal 1-2 days
  - General 1-7 days





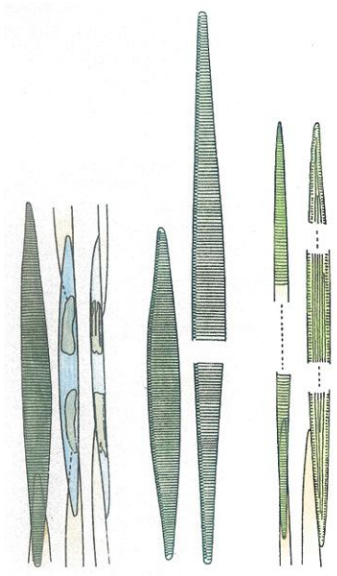
CIGUATOXIN

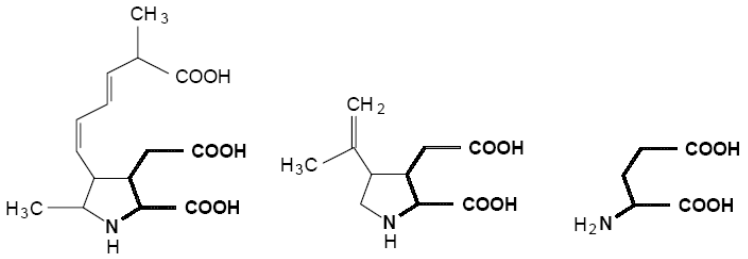
- Onset of intoxication:
  - 10 minutes to 12 hours after contact, after intake of contaminated fishes possible 36 hours
- Beginning of poisoning
  - Vomiting, general weakness, diarrhe
  - Decreased sensitivity to painful stimules
  - Tingling and burning of fingers
  - Sense of changing cold and heat
- Further stadia
  - Hypotension, mydriasis, arrhythmia
  - Convulsions, circulatory collapse, respiratory failure, death
- Possibility of persistence of symptoms (observed for months and years)
- Difficult diagnostic from other NSP
- First aid
  - Mannitol – diuretic
  - Control of life functions
  - No antidote
  - Treatment of long-termed symptoms
    - Amitriptiline, gabapentine



Gambierdiscus toxicus Adachi et Fukuyo

Domoic acid
- *Nitzchia pungens*
- Amnesic shellfish poisoning (ASP)
  - Intoxication accompanied by neurologic disorders
    - Hallucination, time-space disorientation
    - Loss of short-term memory
- Symptoms of intoxication
  - Vomiting, stomach convulsions, diarrhea, headache
  - ASP
- Accumulation of toxin in hepatopancreas, branchiae, so called siphon of pelecypods
- Pelecypods resistant, meat becomes toxic
- New Zealand, coast of Canada, Mexico
- Red tide
- Structure:
  - Tricarboxylic acid
  - Derivative of proline
  - Structural similarity with excitation aminoacids (cainate, glutamate)
- Mechanism of effect:
  - Excitation AMA
  - 100times higher effect then glutamate
    - Rigidity of ring
  - Binding to a NMDA receptor
    - Influence on Ca2+ channels, entry of calcium into cell
      - » Stimulation of many proceses → damage of neurons
    - Mediation of loss of memory


http://www.regione.emilia-romagna.it/laguna/immagine_dettaglio.asp?id_img=1002



kyselina domoová kyselina kainová kyselina glutamová

Obr. 1: Strukturální podobnost neurotoxinů ze skupiny excitačních aminokyselin (domoové a kainové) s kyselinou glutamovou, přirozeným agonistou NMDA-glutamátových receptorů.

KYSELINA DOMOOVÁ, NEBEZPEČNÝ NEUROTOXIN

Plk. v zál. prof. MUDr. Vratislav HRDINA, CSc.,[1,2] prof. RNDr. Jiří PATOČKA, DrSc.,
plk. v zál. doc. RNDr. Vladimír MĚRKA, CSc.,[2] doc. MUDr. Radomír HRDINA, CSc.

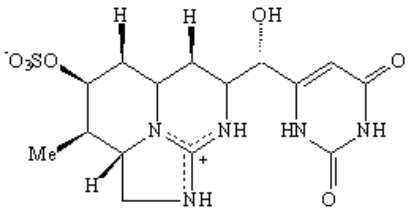
- Doses:
  - 0.9-1.9 mg/kg GIT disorders
  - 1.9-4.2 mg/kg neurotoxic to lethal
- Clinical symptoms:
  - neurotoxic symptoms predominating
    - Headache, vertigo, confusion, time-space distortions
    - Disorders of motoric coordination, hallucinations, loss of short termed memory
  - gastrointestinal difficulties
  - excessive secretion of mucus into respiratory tract
  - tachycardia, peripheral vasodilatation and hypotension
  - cardiac arrhythmia and coma.
  - Intoxication can terminated sudden death 12 to 14 hours caused by respiratory paralysis
- Therapy:
  - antagonists of NMDA
  - prophylactic administration of melatonin

Hepatotoxins

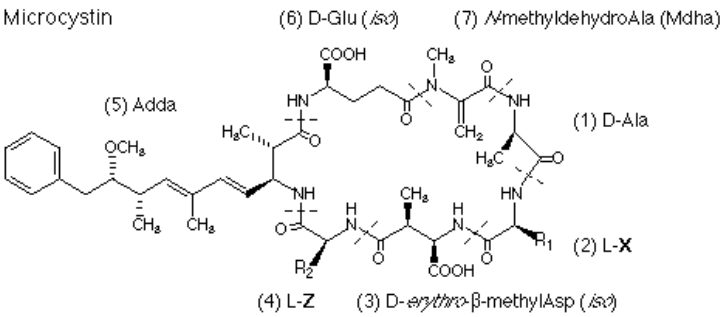
- **Representatives of compounds**
  - **Alkaloid:**
    - Cylindrospermopsine
  - **Protein:**
    - Microcystins (cyanoginosin) and nodularins
      - cyclic heptapeptides
- **Sources:**
  - *Trichodesmium, Umezakia, Cylindrospermopsis, Aphanizomenon,* ***Microcystis****, Anabaena, Planktothrix, Nostoc, Anabaenopsis,* ***Nodularia***

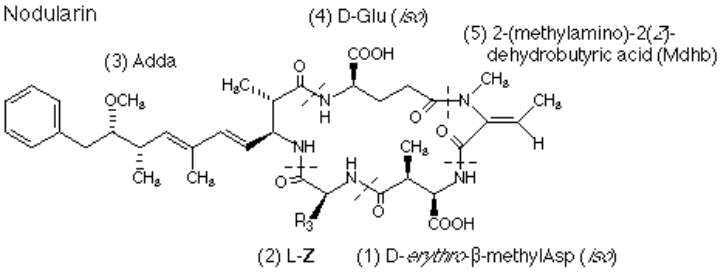
- **Mechanism of action:**
  - Inhibitors of proteosynthesis and synthesis of glutathione
  - Active inhibitor of eukaryota protein serine/threonine phosphatases 1 and 2A.
    - Uncontrolled phosphorylation of target proteins leads to cell proliferation, posttranslational modification of proteins, errorneous transmission of signals nad to cellular transformation to a cancer type cell

- Cylindospermopsine
  - *Cylindrospermopsis raciborskii*, *Umezakia natans, Aphanizomenon ovalisporum*
  - Alkaloid
    - tricyclic guanidine derivative bridged with hydroxymethyluracil
    - OH group of the bridge necessary for toxicity
  - Liver damage
    - Inhibition of proteosynthesis, proliferation of smooth endoplasmatic reticulum
    - Fat degeneration
    - Centrilobular necrosis
  - Common in tropical waters, especially Australia
  - Health problems:
    - Water contamination, strong need of purification
    - Degradation products also toxic



- Mycrocystins (cyanoginosins)
  - Seven AMA
  - To date more than 50 compounds known
- Nodularins
  - 5 AMA
  - amino-9-methoxy-10-fenyl-2,6,8 trimethyl deca-4,6-dienooic acid (ADDA) essential for pharmocologic/toxic activity
  - Mechanism of effect
    - Hepatotoxicity
    - Inhibitors of serine/threonine protein phosphateses
      - Inhibition of dephosphorylation, switchers of function
    - Good penetration into liver cells
      - High concentration, extracted together with bile acids
      - Influence of cytoskeletar filaments reparation
        - » Collapse of cytoskelet
        - » Implosion of cell
        - » If cells neighboring with vein – penetration of blood to liver tissue, gathering of blood in liver
        - » Inflammation, death
    - Symptoms of intoxication
      - Vomiting, nausea
      - Pale skin, cyanosis, breath difficulties, hepatic coma, death
    - Promotors of cancerogenesis of liver
      - Epidemiological studies in China
      - Brasil
  - Contamination of drinkable water
    - In danger both human and animals





http://www.abo.fi/~jmeriluo/Resources/structure.gif

Tumor Promoting Factors, Genotoxins and Mutagenes

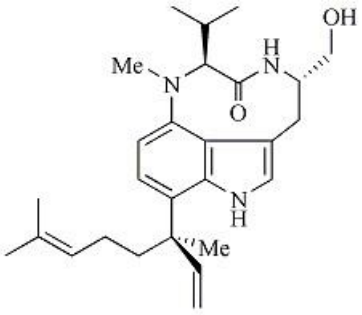
- **Compounds representatives:**
  - Microcystins, nodularin
  - Okadaiic acid
  - Sources:
    - *Microcystis, Nodularia*
- **Principples of effect:**
  - Inhibitors of proteinphosphatases (PP) type 1, 2A and 3
  - Elevated activity of phosphorylation of proteins caused by inhibition of proteinphosphatases
  - Uncontrolled phosphorylation of target proteins lead to cellular proliferation, posttranslational modification of proteins, errorneous signal transmission and cell transformation to a cancer cell type.
- **Nodularin more active than microcystins**
  - Nodularin not only tumor promoting factor, but can also initiate.
    - Lower molecular weight – higher penetration to hepatocytes
- **Promotors of liver cancerogenesis**
  - Epidemiologic studies in China
  - Brazil

Cytotoxins, prymnetoxins

- **Compounds representatives:**
  - Tubercidine
  - Proteolipids
  - Macrolides
    - Amphidinolid B
    - Caribenolid
    - Goniodomin
  - Polycyclic ethers
    - Prymnesins
- **Mechanism of primnetoxins effect:**
  - Decay of blood elements
  - Cytotoxicity - integrity of cell membranes
  - Ichtyotoxicity (jen na žábry - obojživelník po metamorfóze není intoxikován, zatím co pulec umírá do 5 min)
- **Cytotoxic and cytostatic effects**
  - Biotechnologic promising organisms
  - Freshly isolated species (from natural enviroment) higher producing ability than „pure“ laboratory species
    - Cyanobacteria *Spirulina subsalsa*
    - Alga *Chlorella pyrenoidosa*
      - Cytostatics stopping development of S-180 cancer type

Dermatotoxic compounds

- Lyngbyatoxins A, B, C
  - *Lyngbya majuscula*
    - Bentic marine cyanobacteria
- Ebromoaplysiatoxin
  - Proteinkinase C activator
  - Dermatitis
  - Puchýřnatění pokožky
  - Tumor promotor



Lyngbyatoxin A

Lipopolysaccharides

- **Mechanism of effect:**
  - Increased capillary permeability for proteins, effect on non-specific immune response, part of LPS complex so-called O-antigenic region posses several antigenic dominants with receptor site for lysogenic bacteriophages.
  - Chemical composition of LPS is not very different fo cell wall of *Salmonella* species.
  - Difficult to predict physiologic activity of single species without performance of tests
- **Symptoms**
  - Pustules, nausea, vomiting, diarrhea

Imunotoxiny a alergeny

- Vodnatá rýma, ekzémy, slzení očí, spasmus bronchů
- Kosmopolitní organismy
  - Žijí všude, identifikovány i domácím prachu
- Vysoký obsah proteinů
- Počet alergiků vzrůstá
  - ?změna alergenů nebo zvýšená citlivost?
  - Přesun od vláknitých ke koloniálním sinicím
  - Narůstající kontaminace stojatých vod