Algal Pigments as Biomarkers Linking Fish and Benthic Organisms With Type E Botulism

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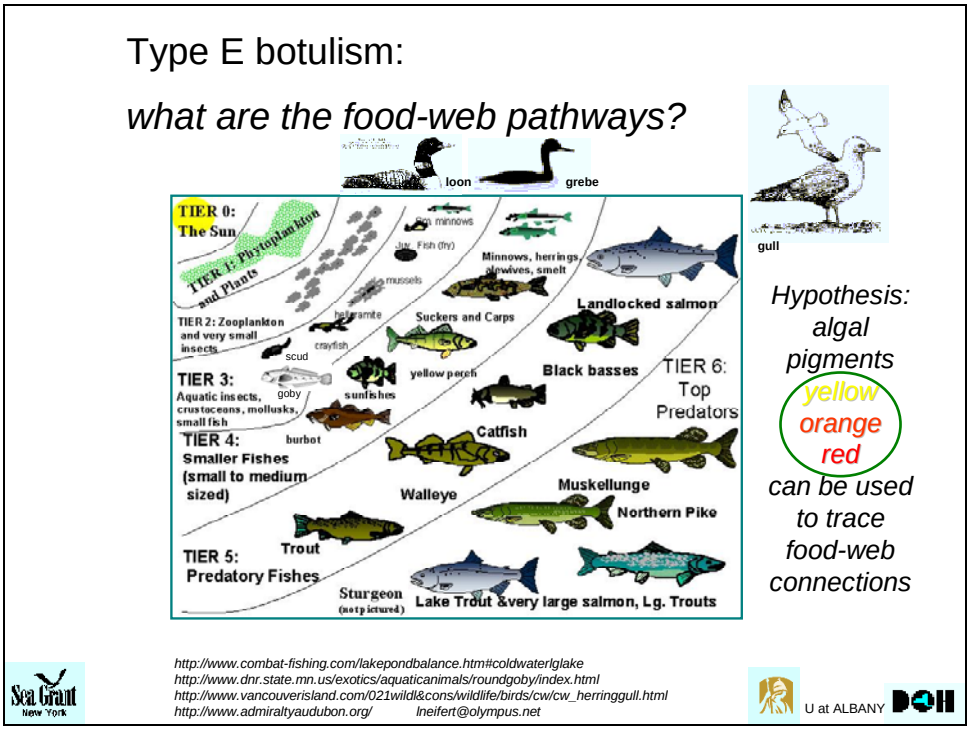
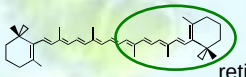


Common to all photosynthetic algae

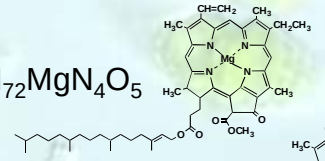
β-carotene $C_{40}H_{56}$



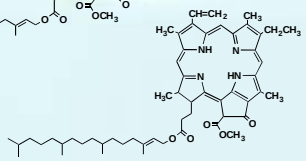
+ retained from diet

retinol (vitamin A) $C_{20}H_{29}OH$

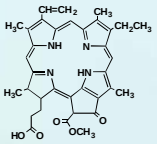
chlorophyll a $C_{55}H_{72}MgN_4O_5$



pheophytin a $C_{55}H_{74}N_4O_5$



pheophorbide a $C_{35}H_{36}N_4O_5$
(chl a degradation)



- degraded, excreted; fecal pellets

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Y. Tsukii June 2001 http://protist.i.hosei.ac.jp/

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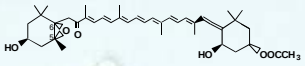
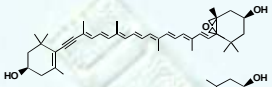
Diatoms

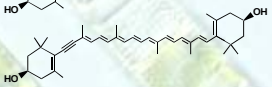
fucoxanthin $C_{42}H_{60}O_6$



diadinoxanthin $C_{40}H_{54}O_3$



diatoxanthin $C_{40}H_{54}O_2$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	93.%	*Aulacoseira islandica* (centric)
spring Lake Ontario	78.%	*Aulacoseira islandica* (centric)
summer Lake Erie	32.%	*Fragilaria crotonensis* (pennate)
summer Lake Ontario	7.%	*Fragilaria crotonensis* (pennate)

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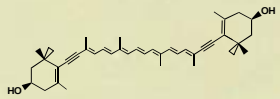
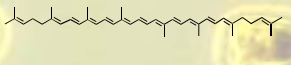
Cryptophytes

alloxanthin $C_{40}H_{52}O_2$



lycopene $C_{40}H_{56}$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	5.2%	*Rhodomonas minutae*
spring Lake Ontario	13.%	*Rhodomonas minutae*
summer Lake Erie	17.%	*Rhodomonas minutae*
summer Lake Ontario	23.%	*Cryptomonas erosa*

http://huey.colorado.edu/LTER/images/pictures/lakes/plankton/rhodomonas/Rhodomonas_891204_T2D.html

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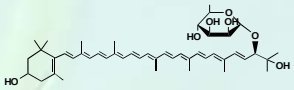
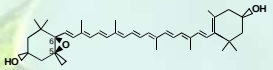
Chlorophytes

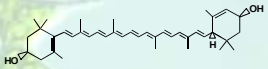
violaxanthin $C_{40}H_{56}O_4$



antheraxanthin $C_{40}H_{56}O_3$



lutein $C_{40}H_{56}O_2$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	not abundant	
spring Lake Ontario	not abundant	
summer Lake Erie	36%	*Pediastrum simplex, Pediastrum spp*.
summer Lake Ontario	19%	*Staurastrum gracile, Oocystis borgei, tetraedron min*.
Epilithic chlorophytes	*??%*	*Cladophora glomerata, C. vagabonda*

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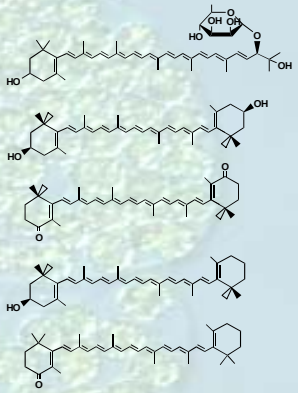
Cyanobacteria

myxoxanthophyll	$C_{46}H_{66}O_7$
zeaxanthin	$C_{40}H_{56}O_2$
canthaxanthin	$C_{40}H_{52}O_2$
β-cryptoxanthin	$C_{40}H_{56}O$
echinenone	$C_{40}H_{54}O$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	1%	*Anacystis montana*
summer Lake Erie	10%	*Microcystis spp*

Yuuji Tsukii: Zenko-ji Nagano 2003 http://protist.i.hosei.ac.jp/

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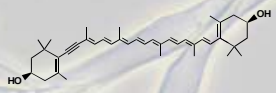
Chrysophytes

diatoxanthin $C_{40}H_{54}O_2$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	not abundant
spring Lake Ontario	not abundant
summer Lake Erie	not abundant
summer Lake Ontario	5.5% *Dinobryon divergens*

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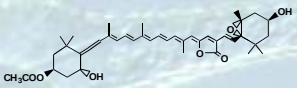
Dinoflagellates

peridinin $C_{39}H_{52}O_7$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	not abundant	
spring Lake Ontario	8.9%	*Gymnodinium helveticum*
summer Lake Erie	4.9%	*Ceratium hirundinella*
summer Lake Ontario	46.%	*Ceratium hirundinella*

Yuuji Tsukii: Daidarabotchi-ike, Iizuna-kogen, Nagano 2003
http://protist.i.hosei.ac.jp/

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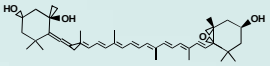
Euglenophytes

neoxanthin $C_{40}H_{56}O_4$



Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

spring Lake Erie	not abundant
spring Lake Ontario	not abundant
summer Lake Erie	not abundant
summer Lake Ontario	not abundant

Yuuji Tsukii: Sugadaira, Nagano, 2001 http://protist.i.hosei.ac.jp/

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Seasonal changes: Lake Erie

Phytoplankton	spring*	summer*	pigments expected in diet	
diatoms	93%	32%	fucoxanthin	$C_{42}H_{60}O_6$
			diadinoxanthin	$C_{40}H_{54}O_3$
			diatoxanthin	$C_{40}H_{54}O_2$
cryptophytes	5%	17%	alloxanthin	$C_{40}H_{52}O_2$
			lycopene	$C_{40}H_{56}$
chlorophytes	-	36%	violaxanthin	$C_{40}H_{56}O_4$
			antheraxanthin	$C_{40}H_{56}O_3$
			lutein	$C_{40}H_{56}O_2$
cyanophytes	1%	10%	myxoxanthophyll	$C_{46}H_{66}O_7$
			zeaxanthin	$C_{40}H_{56}O_2$
			canthaxanthin	$C_{40}H_{52}O_2$
			β-cryptoxanthin	$C_{40}H_{56}O$
			echinenone	$C_{40}H_{54}O$

* Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

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Seasonal changes: Lake Ontario

Phytoplankton	spring*	summer*	pigments expected in diet	
diatoms	78%	7%	fucoxanthin	$C_{42}H_{60}O_6$
			diadinoxanthin	$C_{40}H_{54}O_3$
			diatoxanthin	$C_{40}H_{54}O_2$
cryptophytes	13%	23%	alloxanthin	$C_{40}H_{52}O_2$
			lycopene	$C_{40}H_{56}$
chlorophytes	-	19%	violaxanthin	$C_{40}H_{56}O_4$
			antheraxanthin	$C_{40}H_{56}O_3$
			lutein	$C_{40}H_{56}O_2$
chrysophytes	-	6%	diatoxanthin	$C_{40}H_{54}O_2$
dinoflagellates	-	46%	peridinin	$C_{39}H_{52}O_7$

* Lake Guardian phytoplankton survey 1998 (Barbiero, Tuchman 2000)

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Carotenoids found in the food web

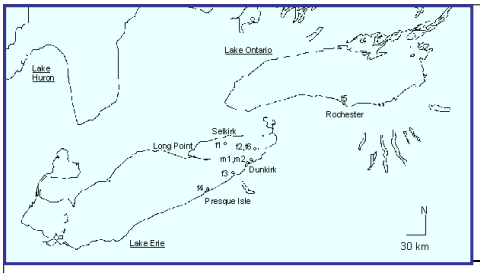
diatoms &	fucoxanthin	$C_{42}H_{60}O_6$	✲	✲		
chrysophytes	diatoxanthin	$C_{40}H_{54}O_2$		✲	✲	
cryptophytes	alloxanthin	$C_{40}H_{52}O_2$		✲	✲	
chlorophytes	lutein	$C_{40}H_{56}O_2$	(✲)	✲	✲	✲
cyanobacteria	zeaxanthin	$C_{40}H_{56}O_2$	✲	✲	✲	✲
	cantha-	$C_{40}H_{52}O_2$			✲	✲
	β-crypto-	$C_{40}H_{56}O$			✲	✲
	echinenone	$C_{40}H_{54}O$	✲		✲	✲
euglenophytes	neoxanthin	$C_{40}H_{56}O_4$			✲	
dinoflagellates	peridinin	$C_{39}H_{52}O_7$	NF	NF	NF	NF
crustacean metabolism	astaxanthin	$C_{40}H_{52}O_4$	✲	✲	✲	✲

Biomarkers and time: prey vs predator profiles

	food resources: present biomarkers of prey	**past and future biomarkers of predator *and* past ingestion**
tissue analyzed:	recent ingestion	long-term accumulation
	stomach	liver
	intestines	muscle
	(spleen)	integument (skin)
		eye
		brain
		gonads, eggs

http://www.dnr.state.wi.us/org/water/fhp/fish/species/index.shtml
http://www.dnr.state.mn.us/exotics/aquaticanimals/roundgoby/index.html

Sample collection: Lake Erie (Lake Ontario)



EPA GLNPO: late 2003

Culligan (NYDEC)
Getchell, Bowser (Cornell)
Fynn-Aikins, Trometer, Goehle (FWS)

Fish	2003
smelt	Oct
round gobies	Sept - Oct
smallmouth bass	Sept
rock bass	Sept - Oct
black crappie	Sept
sheepshead	
white perch	Sept - Oct
yellow perch	Sept
spottail shiner	Sept

A. Perez Fuentetaja (SUNY-Fredonia)

	Macroinvertebrates
	Sept - Oct
diptera	weekly (Aug)
oligochaeta	weekly (Aug)
dreissenids	June, Aug (6)

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Sample preparation: pigments in tissue specimens

Precautions

* Specimen storage: - 80 °C, dark (use dry ice in field)
** Biological safety cabinet (level II) including chemical fume hood

** protect pigments* *** protect analyst - C. b. type E toxin, pathogens*

Procedures

i dissect specimen
ii homogenize tissue
iii freeze dry and determine percent dry weight
(mussels ~ 5% dw; fish ~ 20% dw)
iv weigh specimen to be extracted: 75 - 300 mg dw (1500 mg ww)
v solvent extract in acetone, sonnicate in dark, cold
vi saponify KOH, methanol; solvent exchange, cleanup, dry
vii concentrate extract: dry N_2
viii filter extract, 0.2 μm, into screw-cap vial

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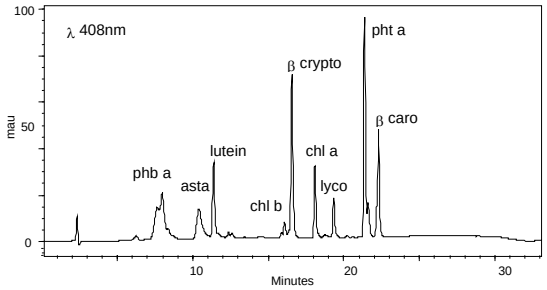
HPLC-photodiode array analysis

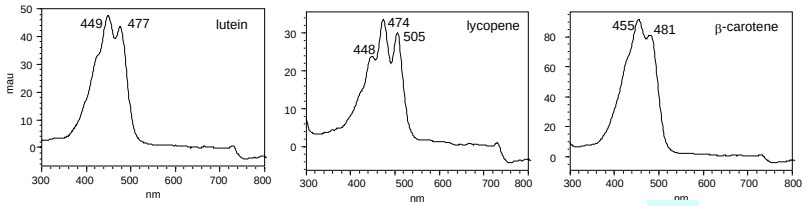
Chromatograms: retention times, peak areas



Mixed standards
21 carotenoids
6 chlorophylls

Concentration ranges
0.05 - 1.0 µg/mL
1.2 - 20 µg/g dw mussels
0.5 - 5 µg/g dw fish

Spectra (characteristic wavelengths for pigment identification)



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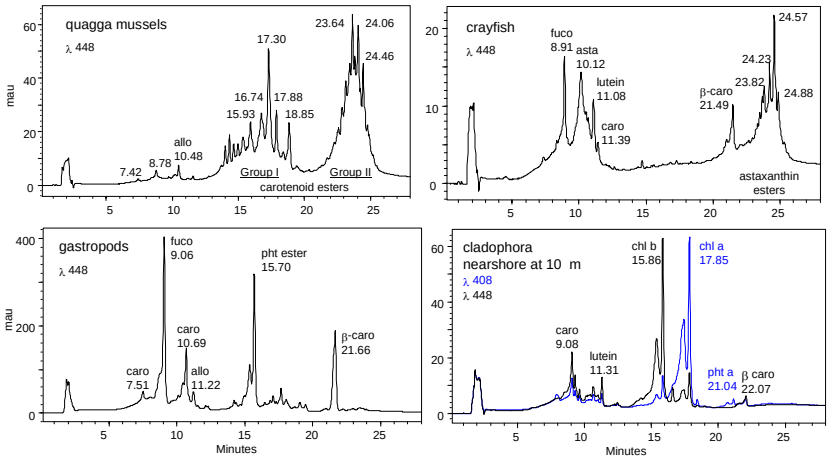
Lake Ontario samples, Aug 2002*



NY GLPF small grant: Alben, Makarewicz 2002-3

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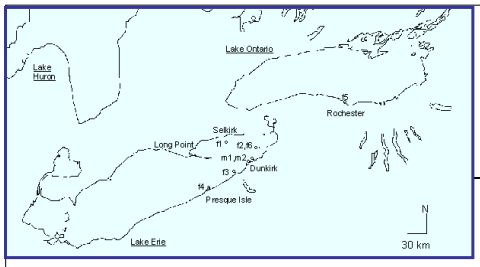
NY Sea Grant 2004-6: Lake Erie (Lake Ontario)



Culligan (NYDEC)
Getchell, Bowser (Cornell)
Fynn-Aikins, Trometer, Goehle (FWS)

Fish	collection
smelt	summer, fall
round gobies	summer, fall
smallmouth bass	summer, fall
sheepshead	summer, fall
yellow perch	fall
walleye	fall
white perch	fall
trout	????

A. Perez Fuentetaja (SUNY-Fredonia)

Macroinvertebrates	collection
diptera	weekly
oligochaetes	weekly
quagga mussels	weekly

Others of interest	Birds
crayfish	gulls
mudpuppies	long-tailed ducks
	loons
	grebes

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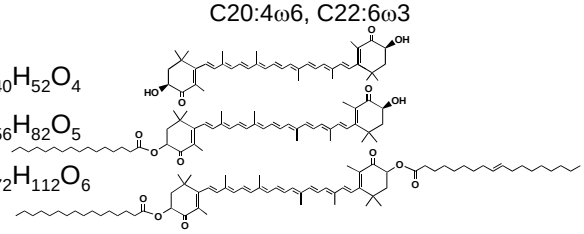
Crustaceans: astaxanthin; fatty acid (FA) esters

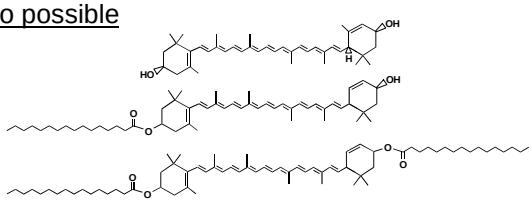
FAs	*saturated*	*monounsaturated*	*PUFAs*
	C14:0, C16:0, C18:0, C20:0	C18:1n9	C18:3ω3, C18:2ω6, C20:5ω3 C20:4ω6, C22:6ω3

'free' astaxanthin	$C_{40}H_{52}O_4$
C16:0 monoester	$C_{56}H_{82}O_5$
C16:0-C18:1 diester	$C_{72}H_{112}O_6$



mollusks, fish, birds - also possible

'free' lutein	$C_{40}H_{56}O_2$
C16:0 mono-	$C_{56}H_{86}O_3$
C16:0 diester *(helenien)*	$C_{72}H_{116}O_4$



Whitney Stocker: http://webby.cc.denison.edu/~stocker/limosus.html

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Acknowledgements

Students - insights

Jamie Woodall*xanthin*
Justin George*xanthin*
Wellington Guzman*tein*
Abdulabasit Luke*xanthin*
Kaitlin Harrington*xanthin*
Christine Simmons*anthin*
Jamie Iannacone*none*
Yuliana De los Santos*anthin*
Ikenna Anaka*xanthin*
Jessica Reiner*xanthin*

Collaborators - field sites, samples

Joe Makarewicz, SUNY Brockport
Ted Lewis, SUNY Brockport
Alicia Perez Fuentetaja, SUNY Fredonia
Chris Mayer, University of Toledo
Bill Culligan, NY DEC Region 9
Paul Bowser, Cornell
Rod Getchell, Cornell
Kofi Fynn-Aikins, USFWS
Betsy Trometer, USFWS
Mike Goehle, USFWS

Sponsors - exploratory research projects and foodweb ventures

Hudson River Foundation - Polgar Fellowship Program
McNair Scholars Progam and CSTEP, SUNY Albany
Great Lakes Protection Fund Small Grant Program
US EPA Great Lakes National Program Office
New York Sea Grant