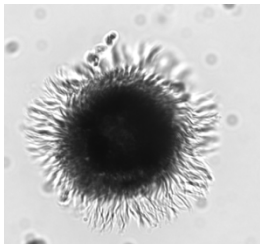
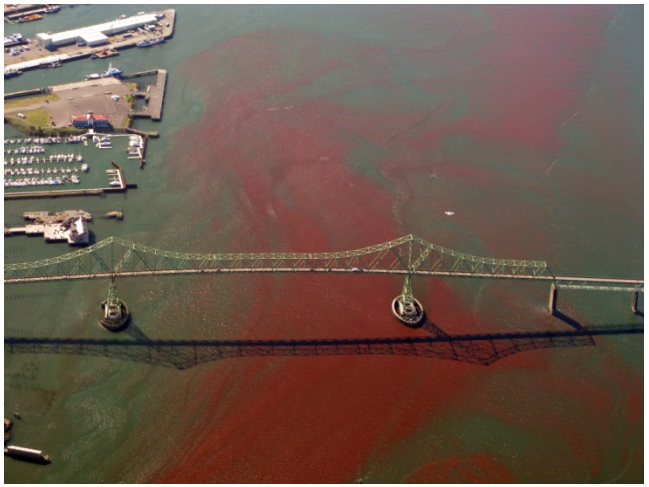
Myrionecta rubra blooms in the Columbia River estuary

Lydie Herfort



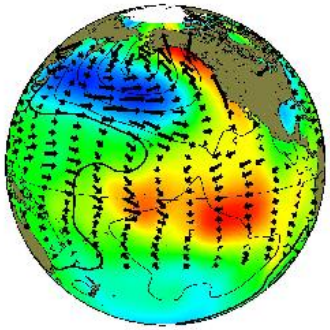


CMOP
Center For Coastal Margin Observation & Prediction

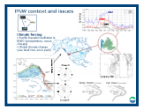
OHSU OREGON HEALTH & SCIENCE UNIVERSITY

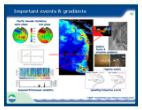
II. Coastal margin science

Opening the all-important microbial "black box", in context of prevalent physical and chemical events and gradients



Human activity & climate



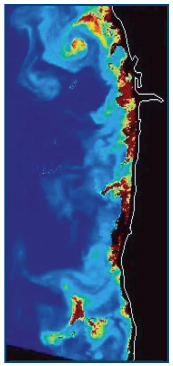


Physical circulation and transport

Chemical stressors

Biological mediation

Microbes



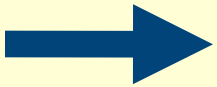
Ecosystem & human health

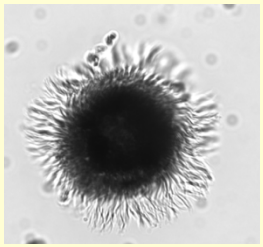
Microbial environmental sentinels

Hypothesis H-3a.

Phytoplankton blooms (e.g., *M. rubra) exhibit defined, repeatable responses to environmental forcings.*

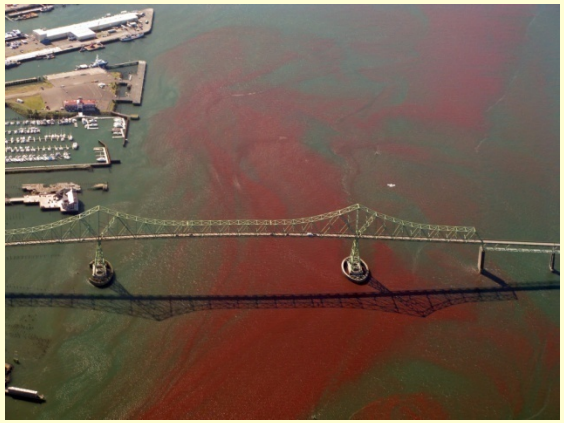
Predictable changes in the occurrence, timing and duration of blooms will give **advance warning** of environmental shifts (physical, chemical, biological) that will guide effective resource management practices.



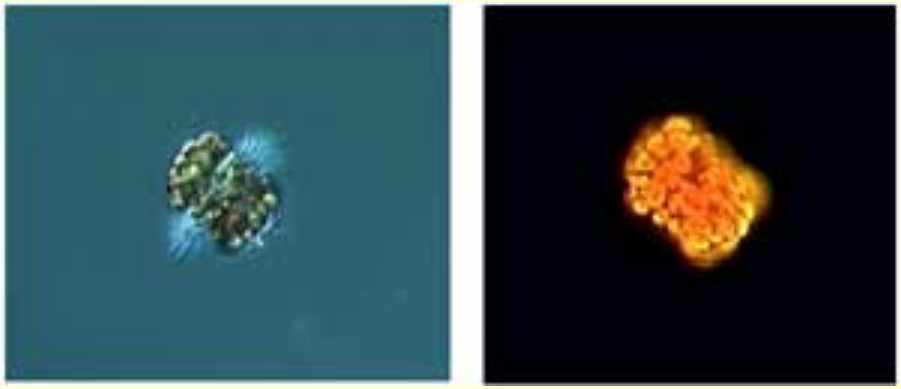


Myrionecta rubra = *Mesodinium rubrum*

Aerial photograph of *M. rubra* bloom by A. Derr



M. rubra under transmitted light (left) & epiflourescence microscopy (right) by D. Stoecker

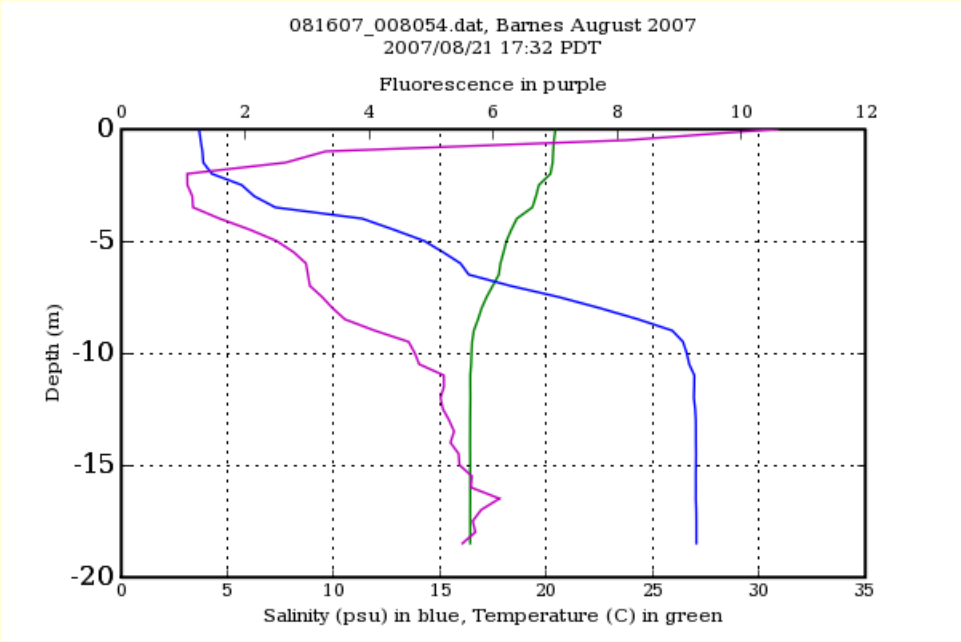


- **Ciliate**, ø=20-40 μm
- Form **non-toxic** red water blooms in estuary, fiord, upwelling area & lakes
- Possesses chloroplasts & nucleus from **cryptophyte** algae
- Red because of photosynthetic pigment: **phycoerythrin**
- Both heterotroph & **photoautotroph** (highest rates of primary production)
- Fastest **jumping** ciliate (1.2 cm/s), can move 40m in water column
- Phototactic **aggregation** to surface & downward migration or dispersion at night

Smith & Barber 1979; Lindholm 1985; Dale 1987; Crawford et al 1997; Fenchel & Hansen 2006; Johnson et al. 2007

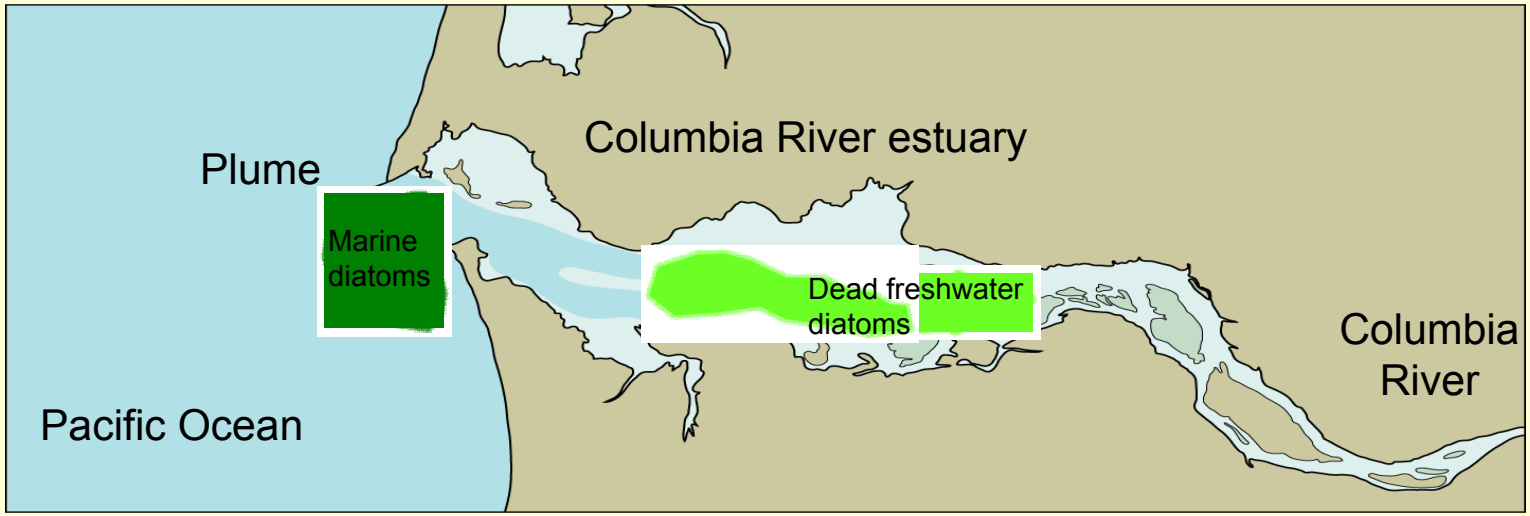
M. rubra cells aggregation to the surface

Patchiness



Cast 54: 0m (left), 1m (right)

M. rubra blooms are atypical of CR estuary



- **Low primary productivity in Columbia River estuary because of**
  - **short estuarine residence time**
  - **light limitation of photosynthesis**

- **Detritus-based system characterised by**
  - **high riverborne particulate organic carbon**
  - **high bacterial production**

Haertel et al. 1969; Frey et al. 1984; Lara-Lara et al. 1990; Small et al. 1990; Crump & Baross 2000; Sullivan et al. 2001

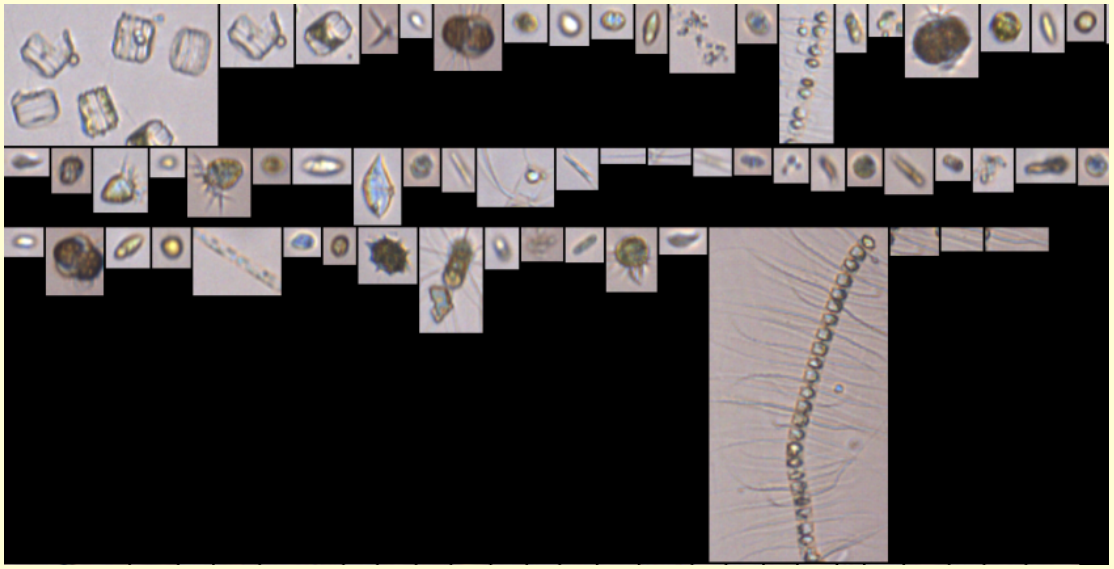
The main question is

why is *M. rubra* so successful in the Columbia River estuary?

- *M. rubra* bloom initiation ?
- *M. rubra* population genetic diversity ?
- *M. rubra* cryptophyte chloroplast specificity ?
- Towards monitoring *M. rubra*…

M. rubra has a oceanic origin

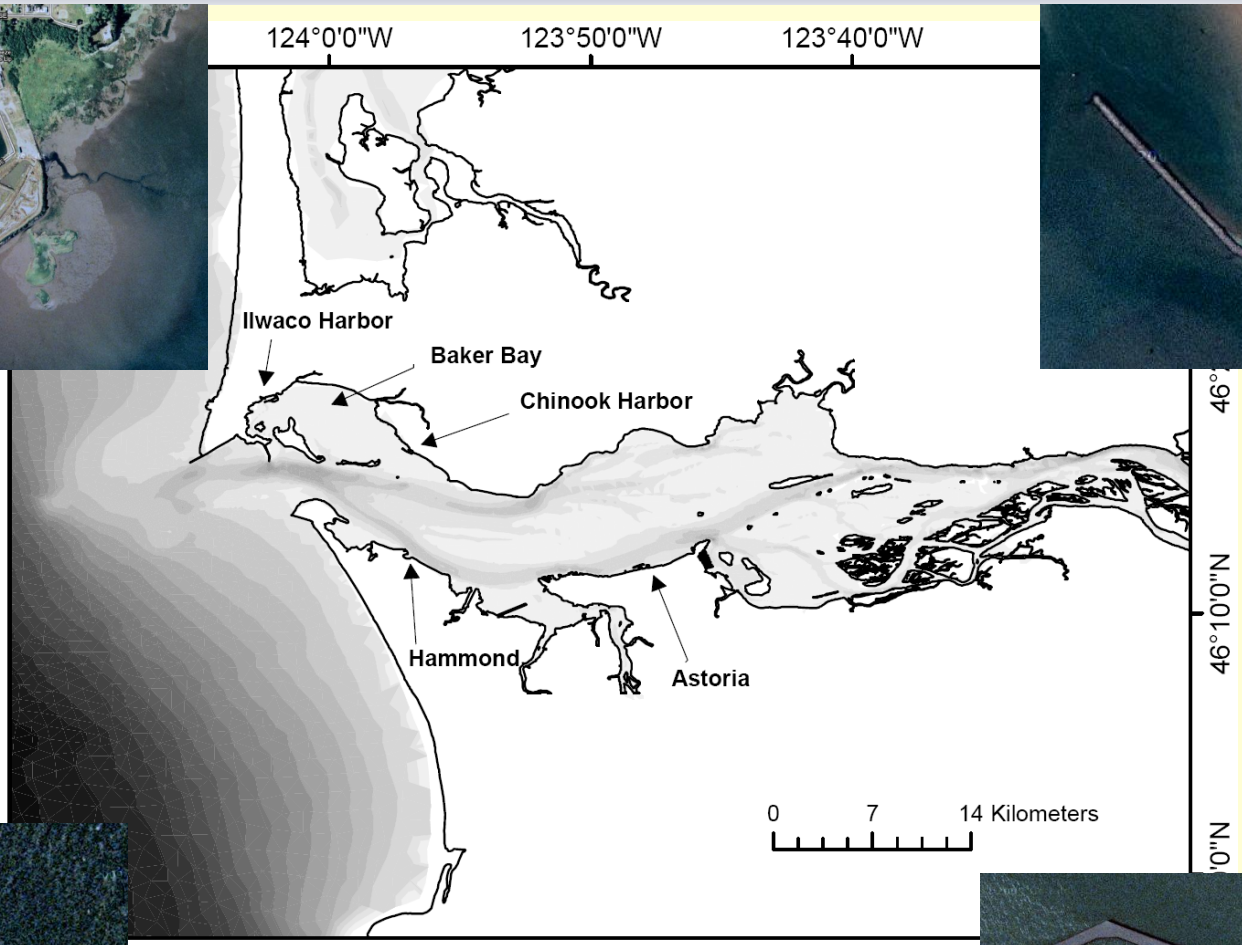
- *M. rubra* was detected in '07-'08 spring coastal & estuarine 18S rDNA clone libraries, but never in freshwater
- In spring '09-'10, *M. rubra* detected by FlowCAM off the Washington coast
- *M. rubra* first detected in '10 in Baker Bay during neap tide of July (increased salinity intrusion: 4 psu June to 8-9 psu July)



FlowCAM images
by T. Peterson

Sampling sites

Ilwaco harbor



Chinook harbor

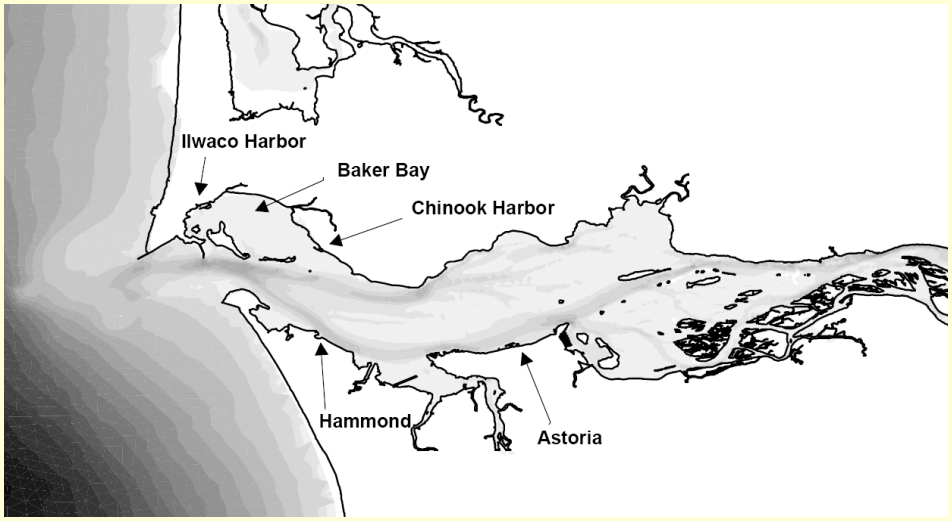
Hammond

Astoria

Bloom starts in Baker Bay in Ilwaco harbor

M. rubra abundance (cells/mL; s.e. in bracket; n.d. = not determined)

		Estuary main channels		Baker Bay				
		Astoria	Hammond	Chinook harbor	Ilwaco harbor			Salinity at high tide
		0 m	0 m	0 m	0 m	1 m	3 m	
2009	30-Jun	n.d.	0 (0)	n.d.	0 (0)	n.d.	n.d.	n.d.
	21-Jul	0 (0) [a]	n.d.	n.d.	220 (12)	n.d.	n.d.	n.d.
	6-Aug	n.d.	42 (4)	178 (15)	304 (16)	n.d.	n.d.	n.d.



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		0 m	0 m	0 m	0 m	1 m	3 m	
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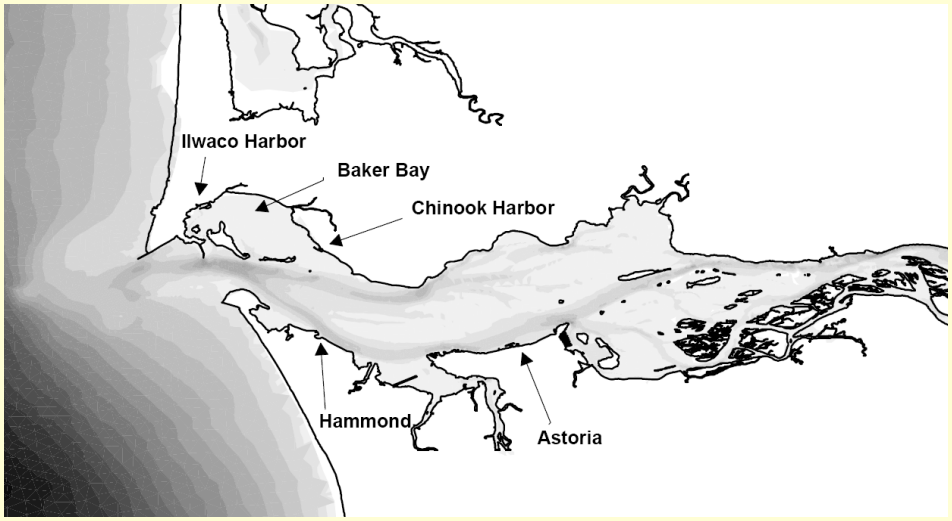
	21-Jul	0 (0) [a]	n.d.	n.d.	220 (12)	n.d.	n.d.	n.d.
	6-Aug	n.d.	42 (4)	178 (15)	304 (16)	n.d.	n.d.	n.d.
2010	10-Jun	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.1
	24-Jun	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.3
	8-Jul	0 (0)	0 (0)	0 (0)	0 (0)	5 (1)	1 (0)	8.3
	22-Jul	0 (0)	0 (0)	0 (0)	15 (2)	213 (9)	18 (4)	9.3
	29-Jul	0 (0)	1 (0)	1 (0)	598 (39)	259 (16)	49 (3)	n.d.



Bloom starts in Baker Bay in Ilwaco harbor

M. rubra abundance (cells/mL; s.e. in bracket; n.d. = not determined)

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		0 m	0 m	0 m	0 m	1 m	3 m	
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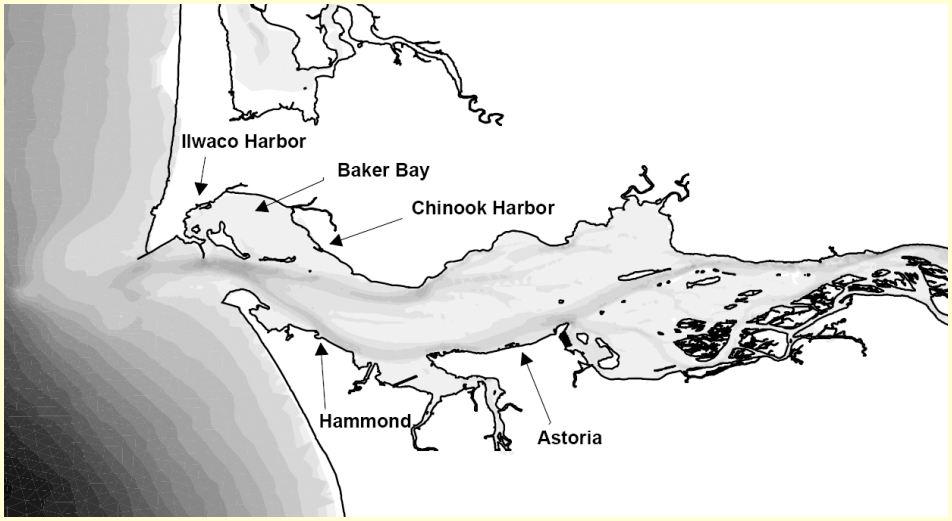
	21-Jul	0 (0) [a]	n.d.	n.d.	220 (12)	n.d.	n.d.	n.d.
	6-Aug	n.d.	42 (4)	178 (15)	304 (16)	n.d.	n.d.	n.d.
2010	10-Jun	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.1
	24-Jun	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.3
	8-Jul	0 (0)	0 (0)	0 (0)	0 (0)	5 (1)	1 (0)	8.3
	22-Jul	0 (0)	0 (0)	0 (0)	15 (2)	213 (9)	18 (4)	9.3
	29-Jul	0 (0)	1 (0)	1 (0)	598 (39)	259 (16)	49 (3)	n.d.



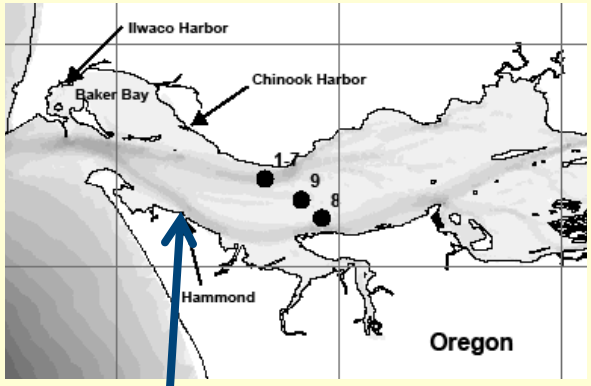
M. rubra was not detected in North Channel on 01-03 August 2010 over two full tidal cycles (Cyclops-7 phycoerythrin sensor profiles & cell counts at 1, 5 & 10m).

Detected a month later in main channels

- Neap tide of Aug 2010
- Saturn03: 18 August

Phycoerythrin sensor
Phycoerythrin = pigment giving *M. rubra* red color

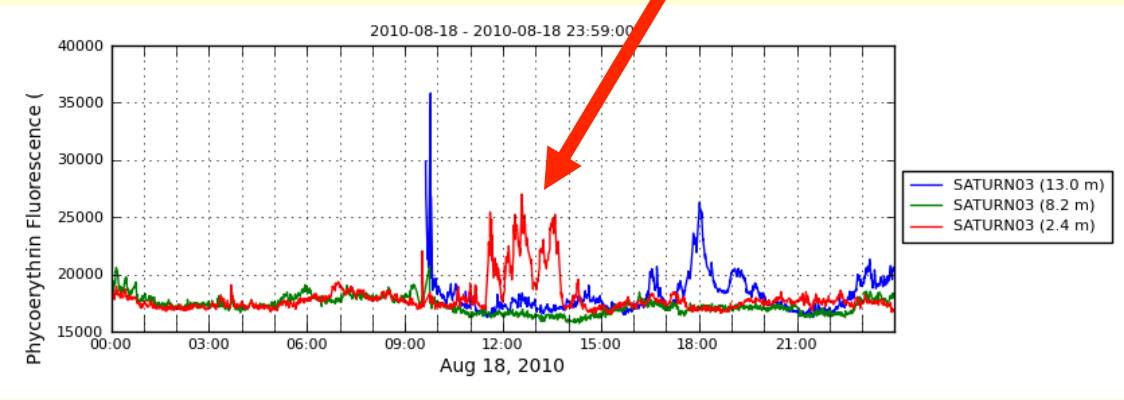
- Red water: 23 August



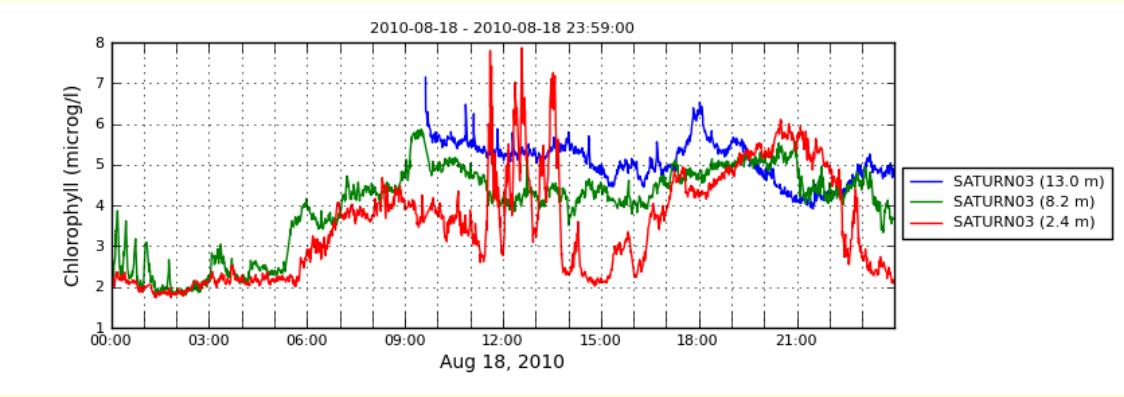
Saturn03

~400 *M. rubra* cells mL^{-1}

Phycoerythrin

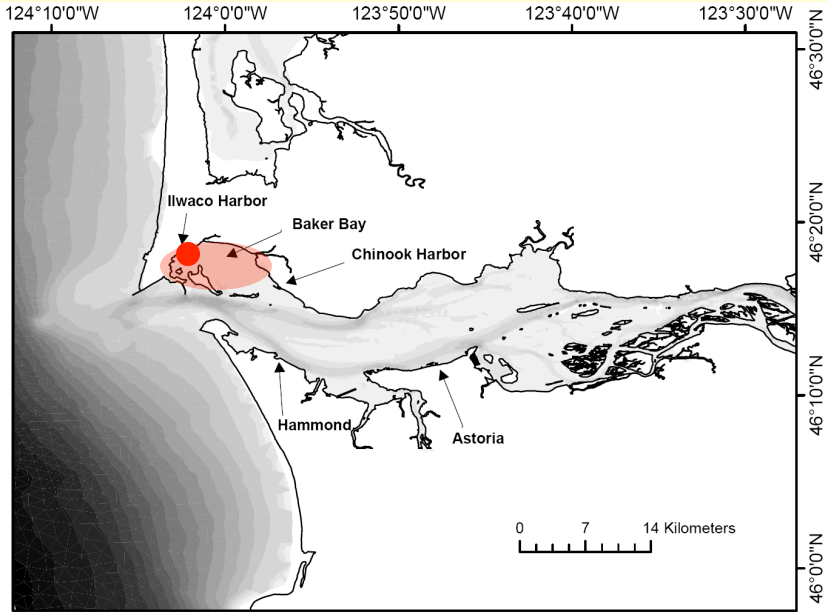


Chlorophyll



M. rubra bloom development : 2 phases

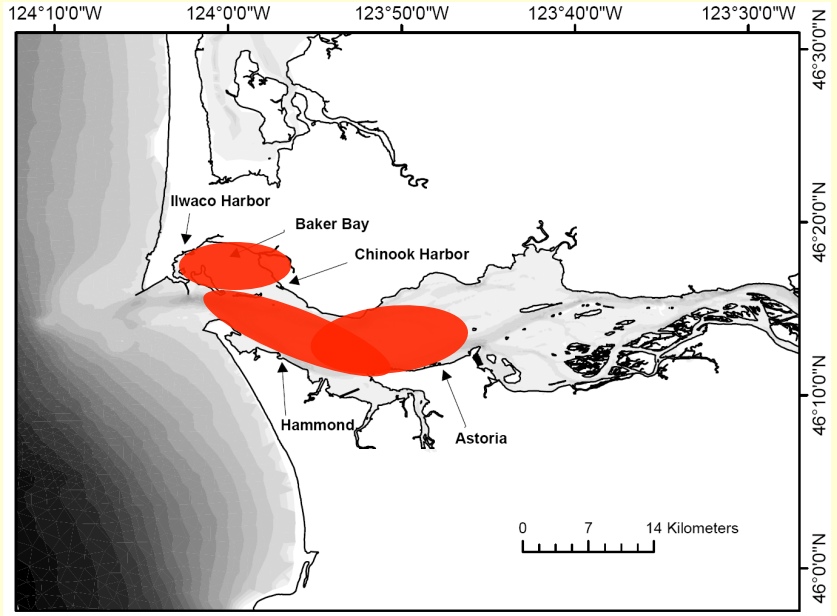
Bloom initiation



Appearance of *M. rubra* in Ilwaco harbor during 1st neap tide of July 2010

Advection into main channels

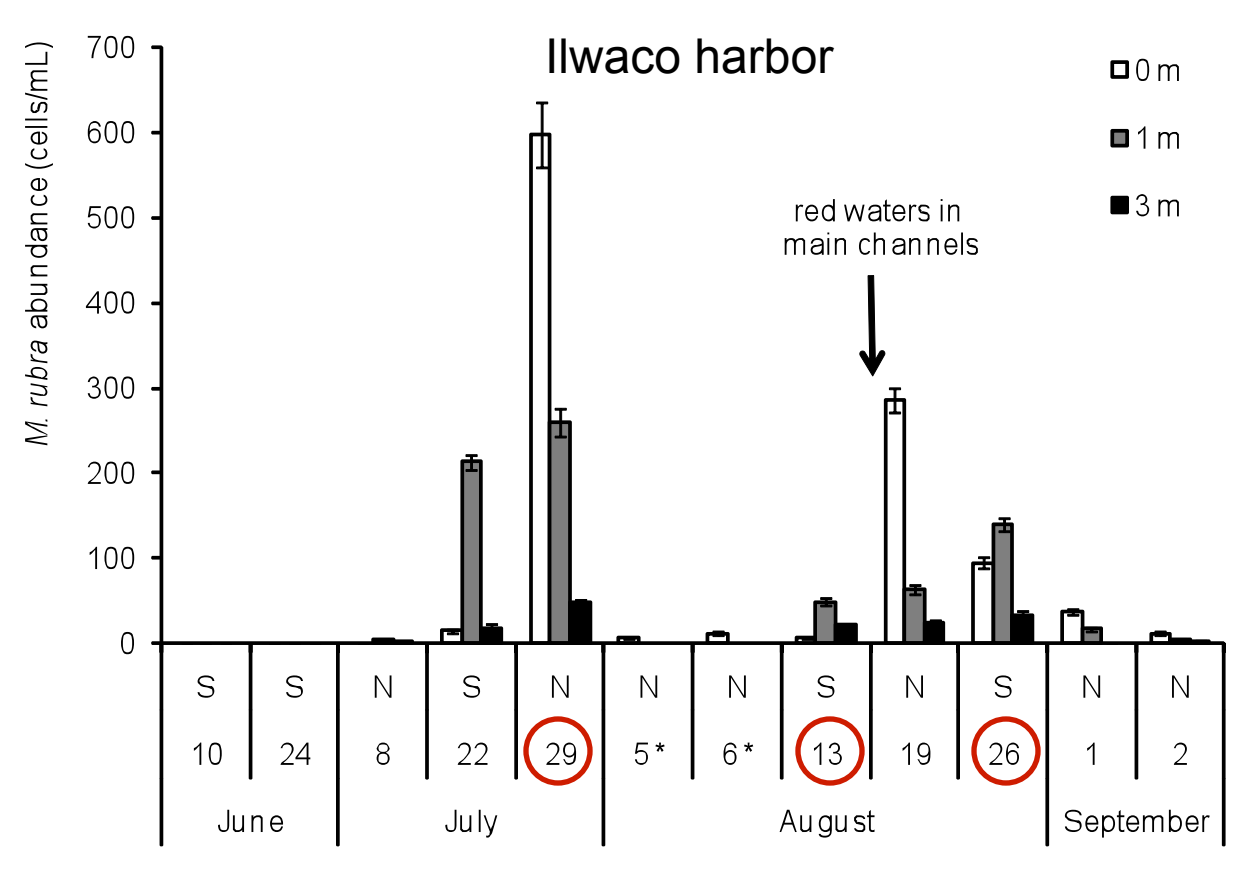
Established bloom



Appearance of red water in main channels during 2nd neap tide of August 2010

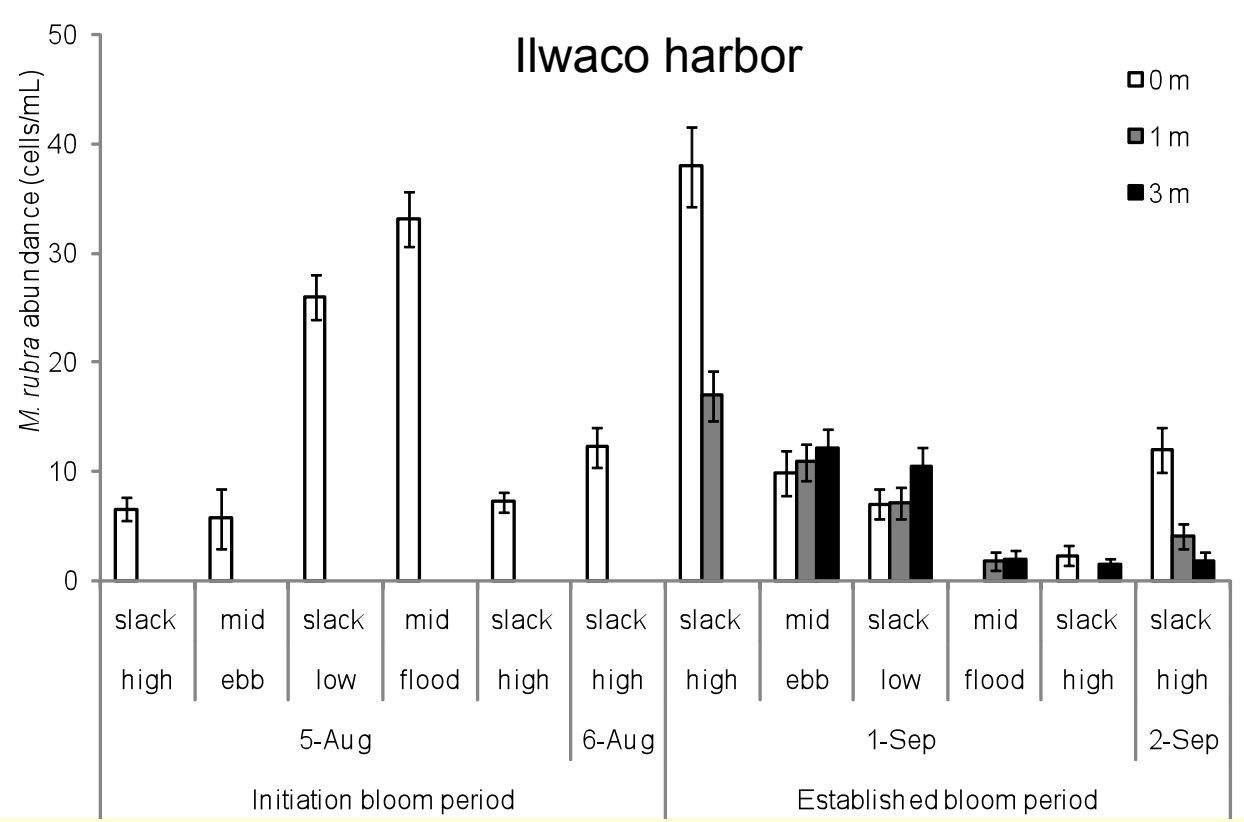
M. rubra abundances fluctuate with time

◯ = not samples at high tide



All waters were collected during daytime at slack high tide, except for samples obtained on 29 July and 26 August and on 13 August that were gathered during mid-flood and slack low tide, respectively.

Tidal forcing not linked with low abundances



05-06 Aug = only surface water (0m) collected.
Bars = error bars.

Fast growing *M. rubra* during initiation period

Location	Water type	Date	Tide	Incubation depth (m)	Incubation period	*M. rubra* (cells mL^{-1}) at onset of incubation	Growth rate (d^{-1})
Ilwaco	Non-red water	5-Aug	high slack	0	23 hrs	7	1.2
Ilwaco	Non-red water	5-Aug	high slack	1	23 hrs	7	3.1

- Photoinhibition at 0m
- High growth rates in Ilwaco harbor during the initiation period

No growth in Ilwaco during established bloom

Location	Water type	Date	Tide	Incubation depth (m)	Incubation period	*M. rubra* (cells mL^{-1}) at onset of incubation	Growth rate (d^{-1})
Ilwaco	Non-red water	5-Aug	high slack	0	23 hrs	7	1.2
Ilwaco	Non-red water	5-Aug	high slack	1	23 hrs	7	3.1
Ilwaco	Non-red water	2-Sep	high slack	1	24 hrs	38	0.0
Ilwaco	Non-red water	2-Sep	high slack	1*	24 hrs	17	0.0
Ilwaco	Non-red water	2-Sep	low slack	1	23 hrs	7	0.0

* denotes a sample collected at 1 m instead of 0 m.

- No *M. rubra* growth in Ilwaco harbor during the established period.

Fast growing *M. rubra* in main channels

Location	Water type	Date	Tide	Incubation depth (m)	Incubation period	*M. rubra* (cells mL^{-1}) at onset of incubation	Growth rate (d^{-1})
Ilwaco	Non-red water	5-Aug	high slack	0	23 hrs	7	1.2
Ilwaco	Non-red water	5-Aug	high slack	1	23 hrs	7	3.1
Ilwaco	Non-red water	2-Sep	high slack	1	24 hrs	38	0.0
Ilwaco	Non-red water	2-Sep	high slack	1*	24 hrs	17	0.0
Ilwaco	Non-red water	2-Sep	low slack	1	23 hrs	7	0.0
Hammond	Red water	2-Sep	low slack	1	7 hrs	1484	1.4
Hammond	Red water	2-Sep	low slack	1	24 hrs	1484	0.7
Hammond	Non-red water	2-Sep	low slack	1	7 hrs	2	4.2
Hammond	Non-red water	2-Sep	low slack	1	24 hrs	2	1.0

- Self-shading in red water compared to non-red water
- More *M. rubra* growth during daylight
- High growth rates in estuary main channels (red and non-red waters) during the established phase

M. rubra bloom initiation: importance of Ilwaco harbor

- *M. rubra* is detected in **Ilwaco harbor first** before Chinook harbor or estuary main channels.
- Bloom establishment during **neap tide** when river flow is reduced (higher salinity intrusion)
- Following bloom establishment in Baker Bay, **advection** into the main channels of the estuary.
- *M. rubra* bloom development : **initial colonization phase** with *M. rubra* in Baker Bay, and **established phase** with red waters throughout the lower estuary.
- **High growth** rates
  - in **Ilwaco harbor** during the **initiation period**
  - in **estuary main channels** (red and non-red waters) during the **established phase**
- **No growth** in **Ilwaco harbor** during the **established period**.
- Ilwaco harbor act as a refugium area, enabling a **seeding *M. rubra* population to form during the initiation period**.

The main question is

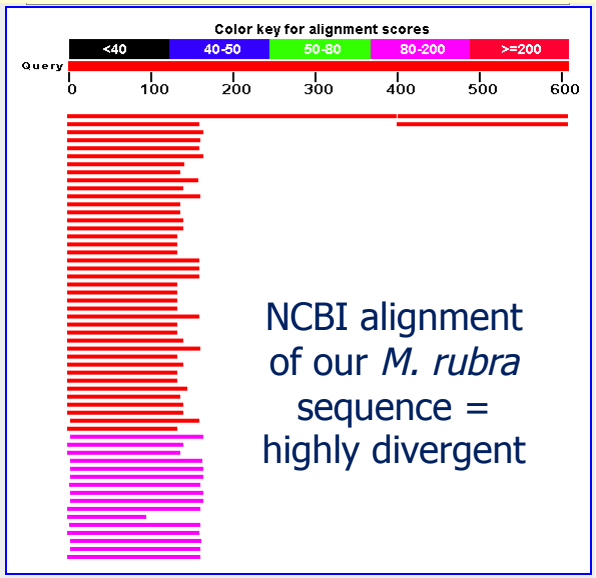
why is *M. rubra* so successful in the Columbia River estuary?

- *M. rubra* bloom initiation ?
- *M. rubra* population genetic diversity ?
- *M. rubra* cryptophyte chloroplast specificity ?

M. rubra population genetic diversity

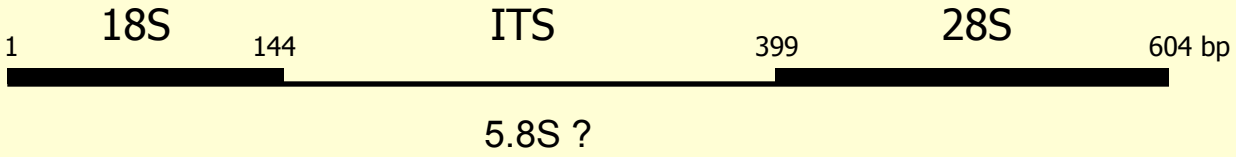
M. rubra Internal Transcribed Spacer:

M. rubra specific 18S and 28S primers (NCBI Mesodiniidae sequences; Marande et al. 2009)



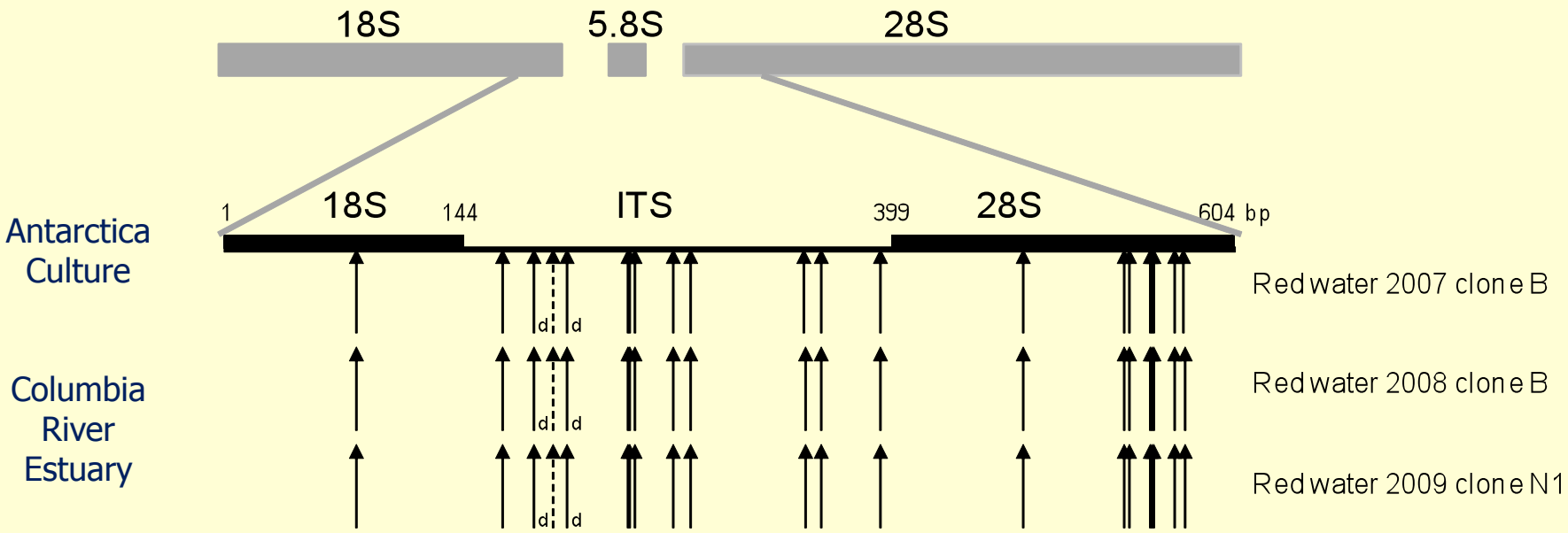
NCBI alignment of our *M. rubra* sequence = highly divergent

Our *M. rubra* partial rDNA sequence :



Identical *M. rubra* sequences in '07-'09 blooms

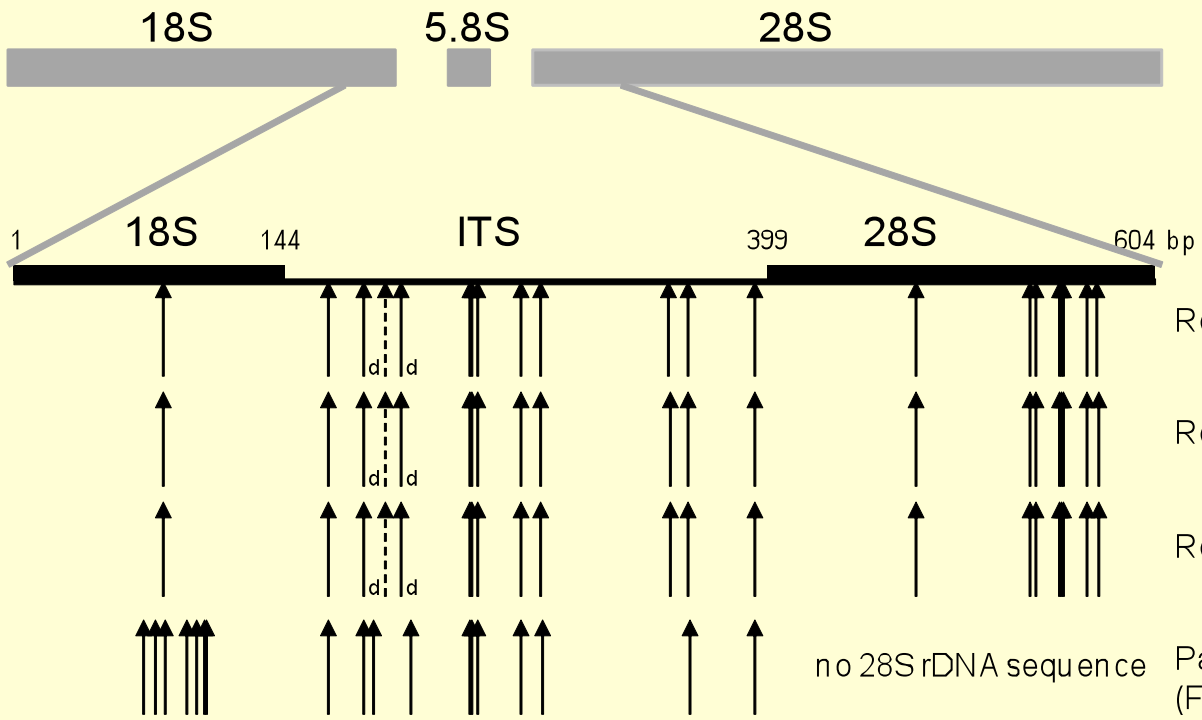
Sites of sequence polymorphisms



d= deletion. Dashed arrow = insertion.
Base pair numbering corresponds to the Columbia River red water sequences.

3 variants of *M. rubra*

Sites of sequence polymorphisms



Antarctica Culture

Columbia River Estuary

S. Atlantic NCBI

Red water 2007 clone B

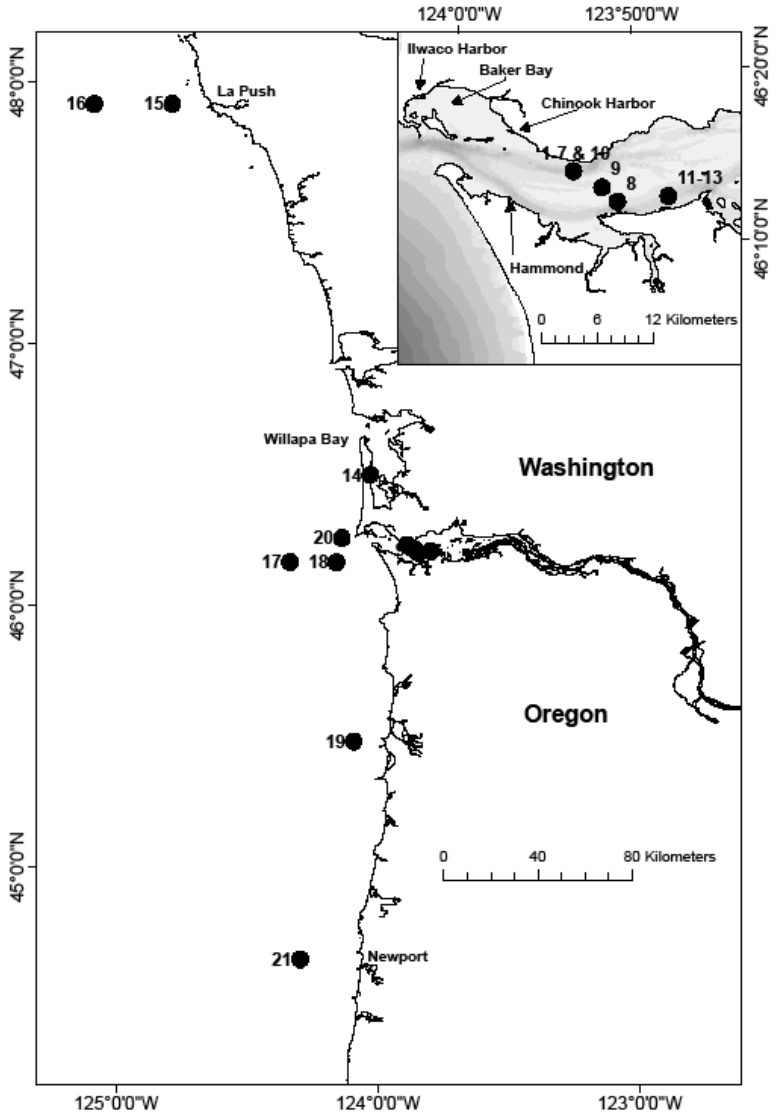
Red water 2008 clone B

Red water 2009 clone N1

Partial 18S rDNA-ITS sequence (FJ032688; Marande et al. 2010)

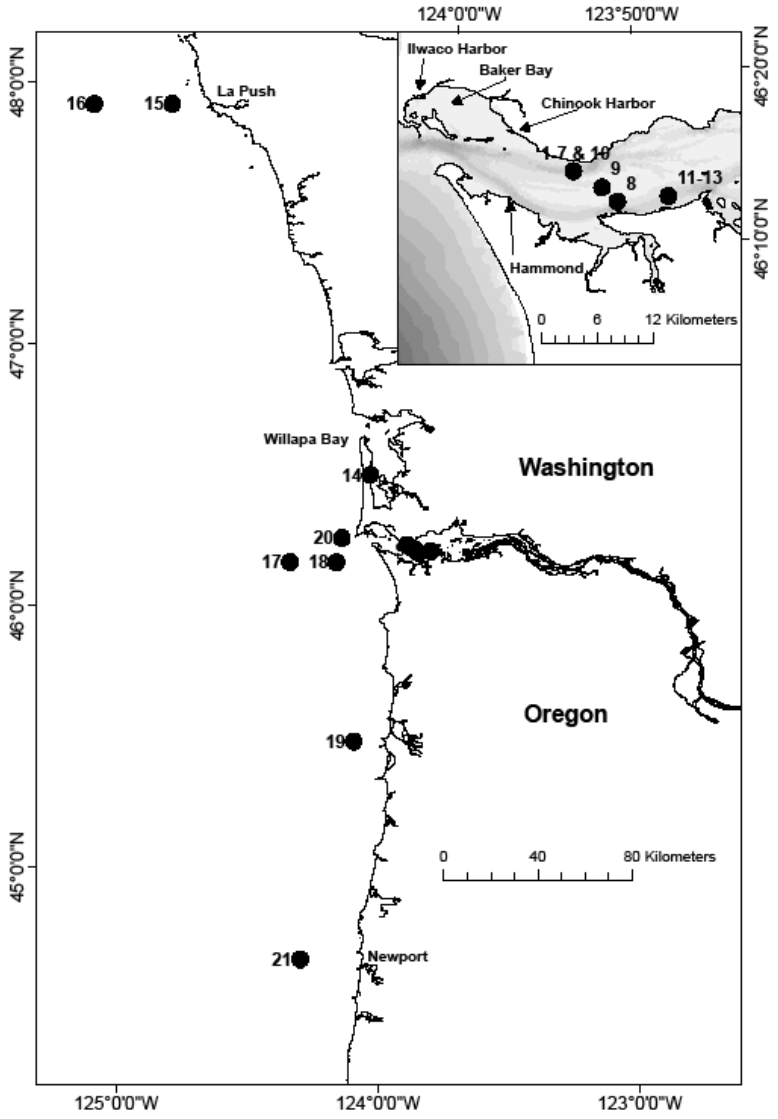
d= deletion. Dashed arrow = insertion.
Base pair numbering corresponds to the Columbia River red water sequences.

Variant analysis throughout CR coastal margin



- 24 samples collected at different times throughout the Columbia River coastal margin
- 154 *M. rubra* partial '18S-28S' rDNA sequences

5 variants of *M. rubra* detected on the coast



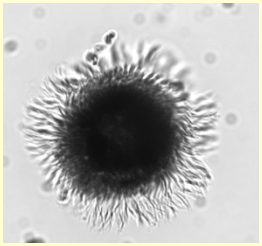
		M. rubra variants				
	Nuc. position	A	B	C	D	E
18S rDNA partial region	69	T	T	A	T	A
	75	G	G	A	G	A
	80	C	T	T	T	T
	92	A	A	T	A	A
	97	C	C	T	C	C
	101	A	A	G	A	A
	102	G	G	A	G	G
	GAP	GAP	GAP	GAP	GAP	A or G
Internal Transcribed Spacer	167	T	A	A	A	A
	177	A	A	A	A	A or G
	183	T	T	T	A	A
	186	C	T	T	T	T
	190	C	C	C	T	C
	191	G	GAP	A	GAP	A
	192	T	T	T	T	T
	GAP	GAP	G	GAP	G	G
	199	T	T	T	G	G
	206	C	T	T	T	T
	211	T	GAP	A	GAP	A
	242	G	A	A	A	A
	243	T	C	C	C	C
	246	G	A	A	A	A
	269	A	C	C	C	C
	280	G	A	A	A	A
	348	T	C	T	C	C
	358	A	C	C	C	C
	393	C	A	A	A	A
28S rDNA partial region	419	A	A	G	A	A
	469	C	C	T	C	C
	479	C	T	T	T	T
	539	G	T	T	T	T
	542	G	T	G	G	G
	555	T	C	C	C	C
	556	C	T	A	T	A
	557	A	G	A	A	A
	558	C	C	T	C	C
	567	G	G	A	G	A
	569	G	A	T	A	A
	574	G	G	A	G	A
	575	T	C	T	C	C

Only one variant leads to bloom in estuary

Several variants of *M. rubra* exist in the Columbia River Coastal Margin, but only one leads to bloom in the estuary

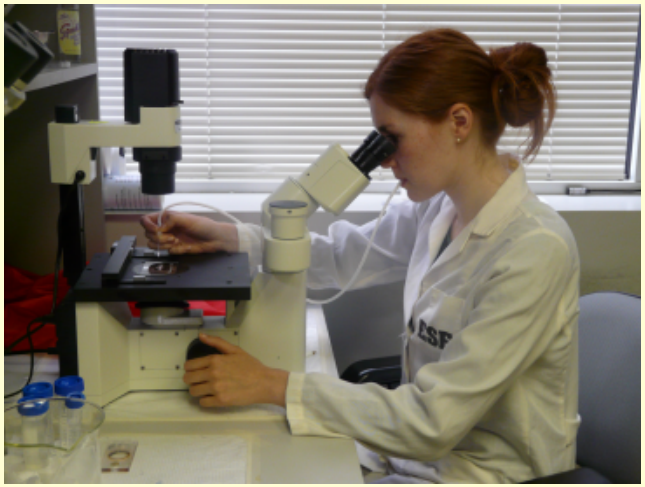
						M. rubra variants				
					Salinity	A	B	C	D	E
Estuary	Main channels Summer '07-'09	Surface	Red Water patch 2007 (#7)	27-Aug-07	9.0		9			
			Red Water patch 2008 (#8)	03-Oct-08	10.0		2			
			Red Water patch 2009 (#10)	07-Sep-09	n.d		11			
			Hammond	06-Aug-09	8.0		2			
			Hammond	20-Aug-09	10.0		2			
		Bottom	South Channel (#11)	26-Aug-07	5.9		2			
			South Channel (#12)	26-Aug-07	10.3		2			
			South Channel (#13)	26-Aug-07	13.1		2			
	Baker Bay Spring Summer Fall '09	Surface	Ilwaco harbor	30-Jun-09	n.d.		10			
			Ilwaco harbor	21-Jul-09	3.4		10			
			Ilwaco harbor	06-Aug-09	15.0		10			
			Baker Bay mud flat *	06-Aug-09	13.0		10			
			Chinook harbor	06-Aug-09	16.0		8	1		
			Ilwaco harbor	20-Aug-09	20.0		10			
			Ilwaco harbor	25-Sep-09	15.0		2			
Willapa	Spring '09	Surface	Willapa Bay (#14)	23-Jun-09	28.0		5	1	1	
Coast	Spring '09	Surface	LaPush line (#15)	18-May-09	27.8			2	2	3
			LaPush line (#16)	18-May-09	31.9			4	2	
			Plume (#17)	14-May-09	29.8			2	5	
	Summer '09	Surface	Columbia River line (#18)	01-Sep-09	31.0		1	3	2	
			Cape Meares line (#19)	30-Aug-09	32.4		1	1	5	
			Plume (#20)	03-Sep-09	23.6	6	1			
			Newport Hydoline (#21)	29-Aug-09	32.6	2	4	1	1	
Culture			Culture of *M. rubra* (Antarctica)		n.d.	6				
NCBI			Mesodiniidae (S. Atlantic - FJ032688)		n.d.			1		

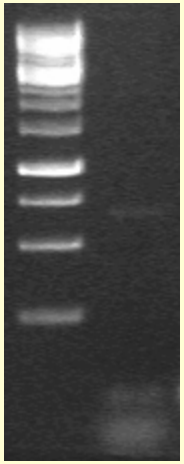
Existence of variants confirmed by SC-PCR



Single Cell PCR

M. rubra partial '18S-28S' rDNA sequences



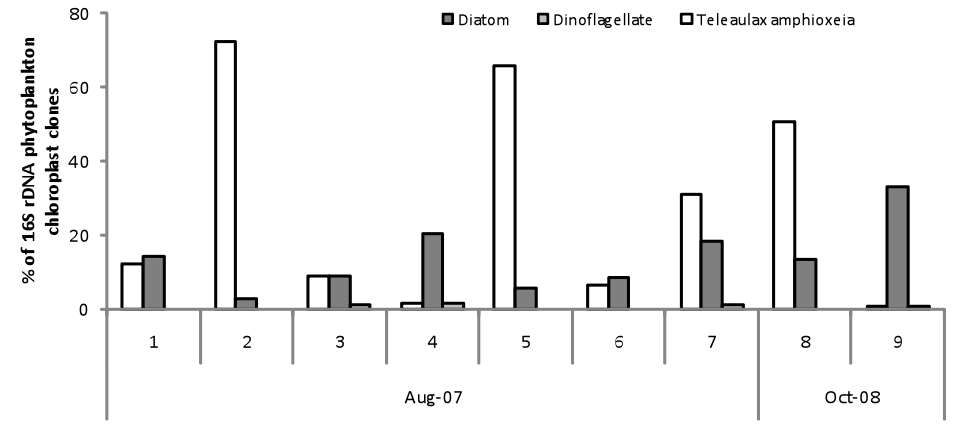


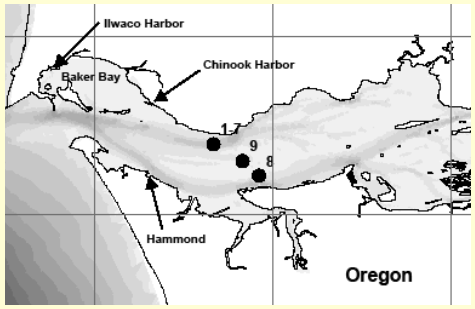
Ilwaco harbor - September 2009
variant B

Oregon coast - April 2010
variant A
Variant C

A single cryptophyte chloroplast

Chloroplast 16S rDNA



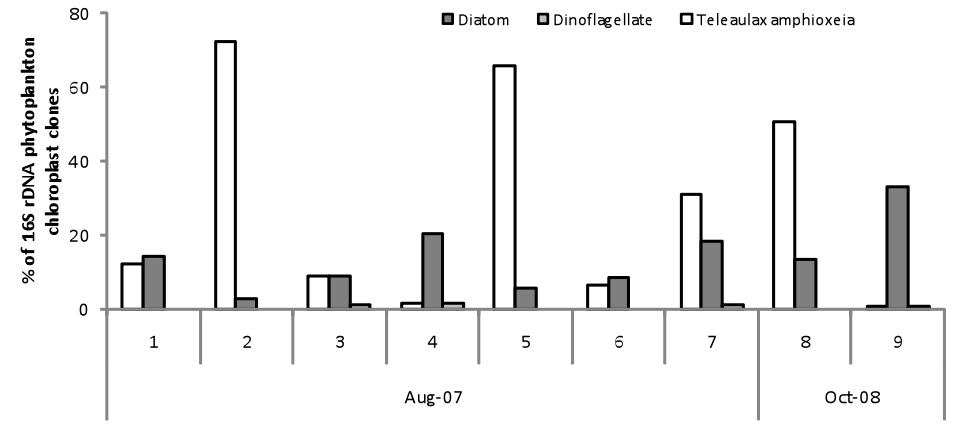


- **Each year, *M. rubra* prey on a single cryptophyte** :

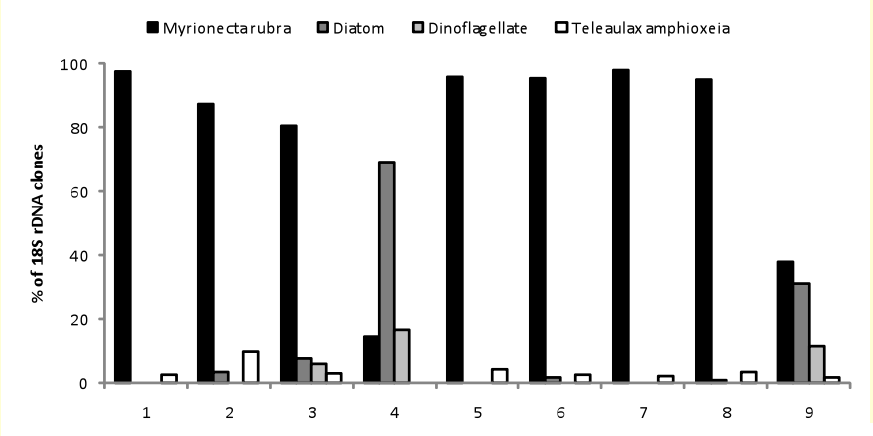
Teleaulax amphioxeia was detected by chloroplast 16S rDNA, RuBisCO gene & PsbA gene analyses in estuary during '07, '08 & '09 blooms

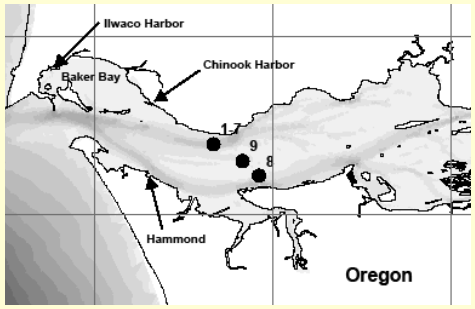
A single cryptophyte chloroplast

Chloroplast 16S rDNA



18S rDNA





- **Each year, *M. rubra* prey on a single cryptophyte** :

Teleaulax amphioxeia was detected by chloroplast 16S rDNA, RuBisCO gene & PsbA gene analyses in estuary during '07, '08 & '09 blooms

- **No free-living cryptophyte in estuary main channels during bloom** :

few 18S rDNA cryptophyte sequences in estuary during '07 & '08 blooms

M. rubra bloom=1 variant & 1 cryptophyte chloroplast

- **Several variants of *M. rubra*** exist in Columbia River Coastal Margin, but only **one variant forms blooms** in estuary
- Each year, the bloom forming *M. rubra* preys on a **single cryptophyte** (*Teleaulax amphioxeia*)
- *M. rubra* in the main estuary channels during established bloom periods **lose their cryptophyte nuclei .**
- **free-living cryptophytes were not in high abundance** in the Columbia River estuary main channels in our 2007 and 2008 samples during the established bloom periods
- the **genetic diversity in *M. rubra* populations** is likely not limited to the Columbia River coastal margin and thus the controversy in the literature regarding the exact nature of the cryptophyte chloroplast/*M. rubra* association (**endosymbiosis vs. kleptoplastidy**) might be at least in part attributed to the genetic variability of *M. rubra* uncovered in this study

Herfort et al. 2011 AME 62:85-97

More questions….

- Where do *M. rubra* acquire cryptophytes ?

- Which cryptophyte chloroplast is present in the different variants of coastal *M. rubra* cells ?

- Is the bloom-forming variant of *M. rubra* generating blooms because of its inherent (genetic) characteristics or it is the nature of the association between *T. amphioxeia* chloroplast and the bloom-forming *M. rubra* variant that drives proliferation and bloom formation or both ?

To bloom in the CRE a phytoplankton needs to:

1. withstand large **salinity gradients** (prevent lyses)

2. develop a **population**

4. achieve **efficient primary production** despite ambient low light

4. avoid being flushed out of the estuary by surface currents and ebb tides

To bloom in the CRE a phytoplankton needs to:

1. withstand large **salinity gradients** (prevent lyses)

→ *2010 summer-fall dataset: healthy-looking* M. rubra *detected in estuary in 1-32 PSU water*

2. develop a **population**

→ *for bloom development: importance of refugium area & high growth rates*

4. achieve **efficient primary production** despite ambient low light

→ *cryptophyte chloroplasts are adapted to low light and* M. rubra *possess Microsporine-like amino acids that protect against UV inhibition*

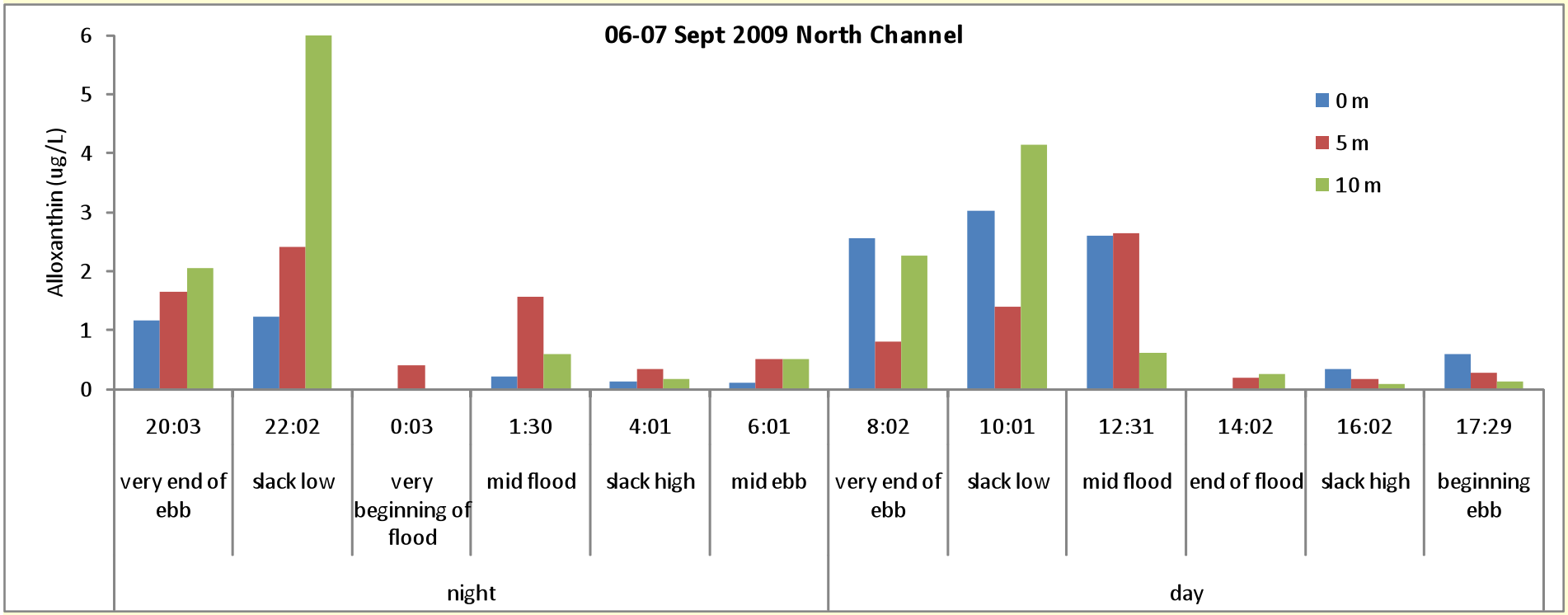
4. avoid being flushed out of the estuary by surface currents and ebb tides

→ *motility*

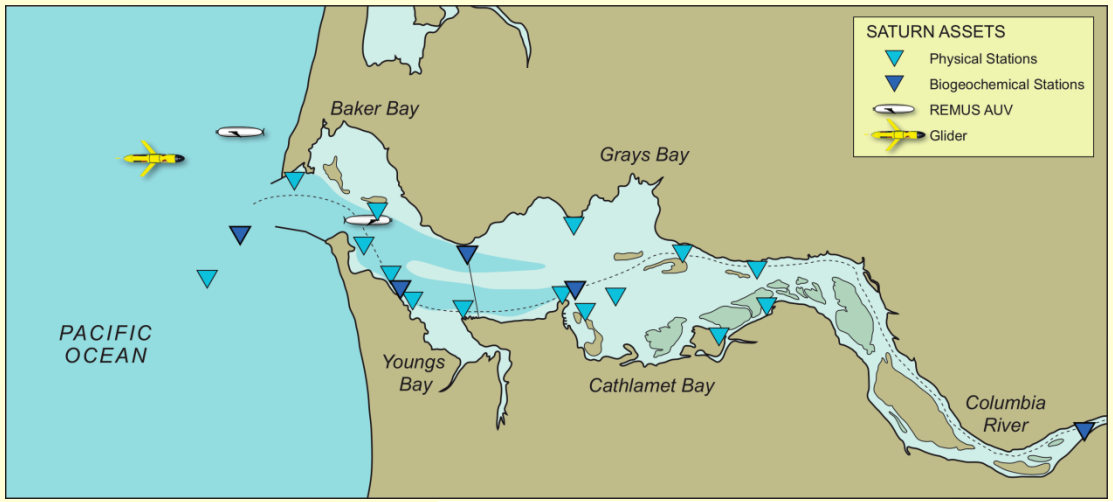
- *biological modeling (Yvette Spitz, OSU)*
- *monitoring via SATURN network*

Understanding *M. rubra* vertical dynamics

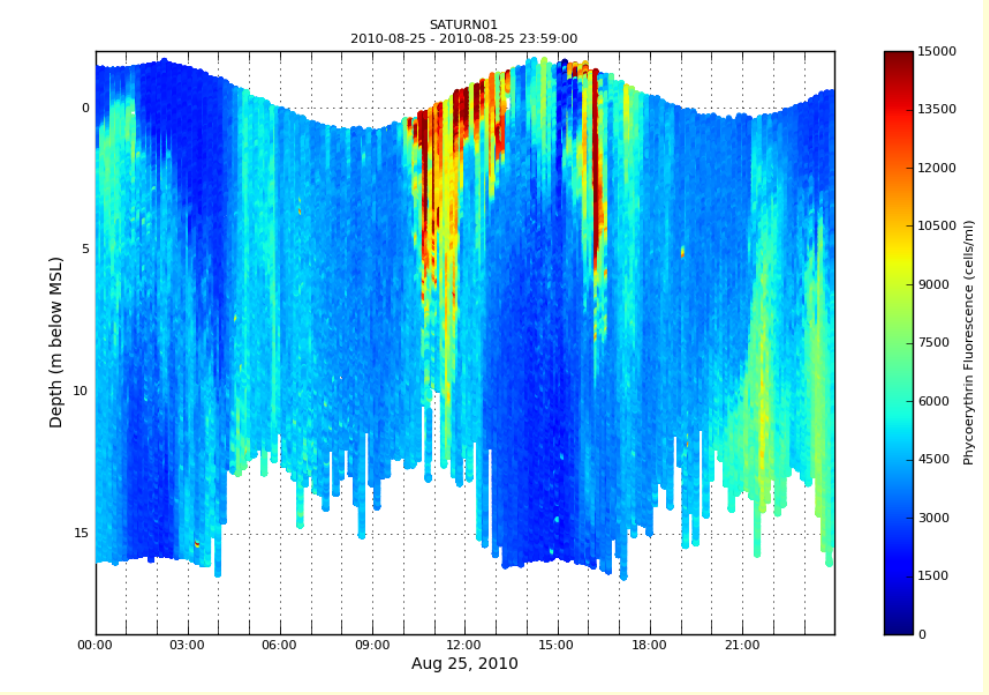
Shipboard Eularian sampling



Monitor *M. rubra* bloom via SATURN network

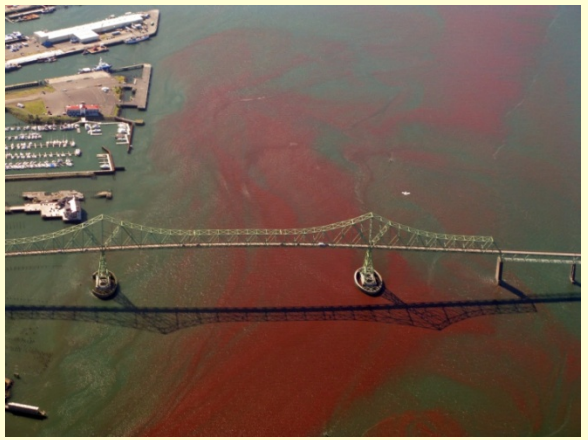


Cyclop-7 Phycoerythrin Turner Design at Saturn01



Understanding *M. rubra* involvement in biogechemistry

Shipboard sampling in & out of red water patches



		21-Aug-07						27-Aug-07	3-Oct-08	
		1	2	3	4	5	6	7	8	9
In situ measurements	Salinity (PSU)	5.0	8.0	3.9	2.7	3.6	5.2	9.0	10.0	13.0
	Temperature (°C)	19.6	19.2	19.9	20.6	20.5	19.8	18.5	15.5	15.0
	Turbidity (NTU)	1.40	1.54	1.46	1.11	2.03	1.71	1.52	ND	ND
	Dissolved oxygen (%)	92	94	99	97	129	109	90	ND	ND
	Fluorescence (V)	3.0	5.1	3.9	0.8	8.1	2.1	2.1	ND	ND
Bacterial production rates	of whole water ($\mu g\ C\ L^{-1}\ h^{-1}$)	0.52	0.84	0.82	0.40	1.67	0.55	4.55	ND	ND
	of free-living bacteria ($\mu g\ C\ L^{-1}\ h^{-1}$)	0.09	0.14	0.13	0.11	0.05	0.24	0.58	ND	ND
Silicate	Dissolved silicate ($\mu mol\ L^{-1}$)	120	110	118	122	124	122	108	132	117
Phosphorus	Dissolved inorganic phosphorus ($\mu mol\ L^{-1}$)	0.36	0.29	0.32	0.40	0.55	0.20	0.94	1.37	0.65
	Dissolved organic phosphorus ($\mu mol\ L^{-1}$)	0.39	0.66	0.29	0.06	1.51	0.41	2.69	ND	ND
Nitrogen	Ammonium ($\mu mol\ L^{-1}$)	0.57	0.42	0.46	1.02	0.41	0.45	51.36	1.33	8.17
	Nitrate ($\mu mol\ L^{-1}$)	6.7	4.9	5.4	7.9	1.2	3.3	4.1	5.1	9.0
	Nitrite ($\mu mol\ L^{-1}$)	0.20	0.13	0.10	0.13	0.14	0.14	0.21	0.29	0.22
	Dissolved organic nitrogen ($\mu mol\ L^{-1}$)	8.6	9.6	9.1	5.3	19.0	8.7	28.8	ND	ND
	Particulate organic nitrogen ($mg\ L^{-1}$)	ND	0.31	0.12	0.05	0.51	0.11	0.27	ND	ND
	Nitrate: Phosphorus	18.5	16.8	17.1	19.9	2.1	16.4	4.4	3.7	14.0
	Nitrate/Nitrite/Ammonium: Phosphorus	20.7	18.7	18.8	22.7	3.1	19.4	59.3	4.9	27.0
Carbon	Dissolved organic carbon ($\mu g\ L^{-1}$)	1.3	1.4	1.3	1.1	1.6	1.5	2.3	ND	ND
	Particulate organic carbon ($mg\ L^{-1}$)	ND	1.54	0.68	0.33	3.91	0.75	2.16	ND	ND
	Chlorophyll *a* / Particulate organic carbon	ND	16.9	11.0	5.5	29.2	8.6	58.6	ND	ND

Monitor *M. rubra* bloom via SATURN network

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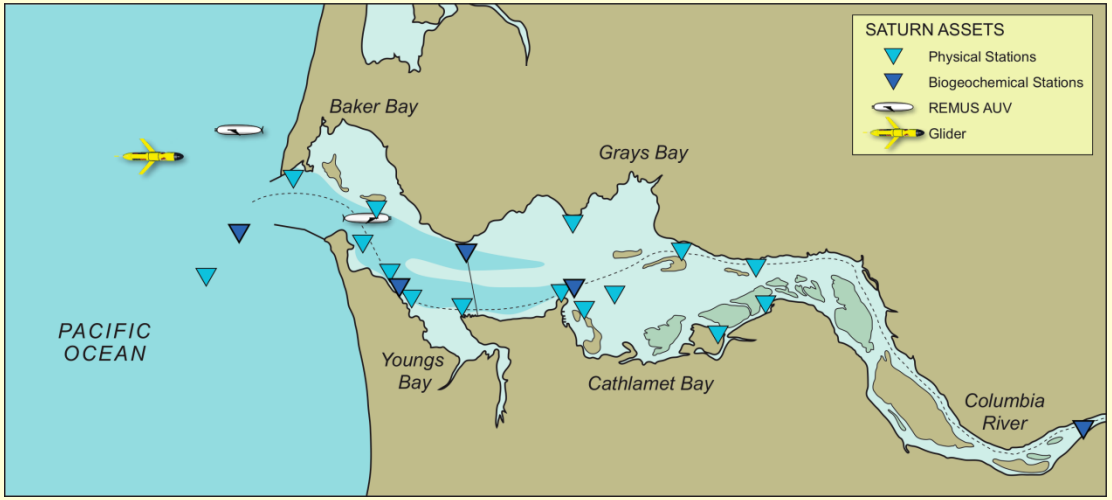
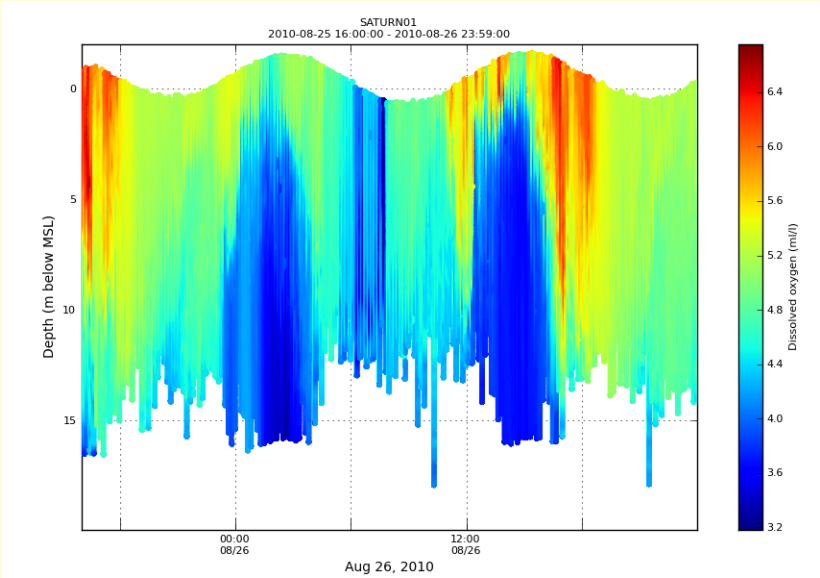


SATURN 01 & 03
Phycoerythrin
Salinity
Temperature
SUNA nitrate
CDOM
Chlorophyll *a* fluorescence
Turbidity
Dissolved oxygen
Variable fluorescence of chlorophyll *a*
Sediment concentration & size
Velocity
APNA & CycleP (Sat03 only)



Dissolved oxygen

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National Science Foundation
WHERE DISCOVERIES BEGIN

U.S. DEPARTMENT OF ENERGY