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Myrionecta rubra (*Mesodinium rubrum*) bloom initiation in the Columbia River estuary

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ABSTRACT

To better understand the development of the annually recurring late summer red water blooms of the phototrophic ciliate *Myrionecta rubra* in the Columbia River estuary we examined its standing stocks and measured its growth rates both in the estuary main channels and in Baker Bay, a peripheral embayment situated near the river mouth. Data collected during two summers show a biphasic development of *M. rubra* blooms, with an initial phase when the protist was only detected in Baker Bay, followed by an established phase when red waters were observed throughout the lower estuary. Ilwaco harbor (Baker Bay's seaward-end) is at least one of the locations where the bloom starts since *M. rubra* was detected there at concentrations >100s cell L^{-1} before Chinook harbor (Baker Bay's upriver-end) or the estuary main channels. In 2010, this initial phase lasted about 1.5 months, spanning the neap tide of early July to the beginning of the neap tide of mid-August. While high growth rates were measured in Ilwaco harbor during the initial phase (1.2–3.1 d^{-1}) and in the estuary main channels in both surface red (0.7 d^{-1}) and adjacent non-red (1.1 d^{-1}) waters during the established period, growth of the ciliate was not detected in Ilwaco harbor during this second phase. Growth rate data obtained during the established bloom phase also suggest that *M. rubra* cells in the estuary mostly divide during the daytime and that red water patches might experience self-shading.

1. Introduction

In contrast to most large North American estuaries, the Columbia River estuary is characterized by low total primary productivity because of short water residence time (2–5 days, Neal, 1972) and low light (Haertel et al., 1969; Frey et al., 1984, Small et al., 1990, Lara-Lara et al., 1990). It is therefore intriguing that for several decades massive red water blooms of the photosynthetic ciliate *Myrionecta rubra* (Jankowski, 1976), formerly *Mesodinium rubrum* (Lohmann, 1908), have been observed in the Columbia River every summer or early autumn (Herfort et al., 2011). *M. rubra* is a neritic, planktonic ciliate distributed in marine and brackish waters throughout the globe and is well-known for generating impressive non-toxic blooms in estuaries, fjords, and upwelling areas of the coastal ocean (Lindholm, 1985). It is a fast moving ciliate, reaching velocities of up to 1.2 cm s^{-1} (Fenchel and Hansen, 2006) and exhibits phototactic behavior, by aggregating at the surface/subsurface during the day (Lindholm 1985; Dale, 1987). The intense red color of the blooms is the result of surface aggregation of a large number of *M. rubra* cells (>10^4 cells mL^{-1}, Taylor et al., 1971), each containing several phycoerythrin-rich chloroplasts that originate from cryptophyte algae. Consequently, this ciliate is considered a mixotroph being both a heterotroph and functionally a photoautotroph. Recently we have shown that there are at least five *M. rubra* haplotypes present with the Columbia River coastal margin, and yet only one haplotype (haplotype B) is found in the red water bloom population (Herfort et al., 2011). Haplotype B possesses a chloroplast originating from the cryptophyte *Teleaulax amphioxeia* (Herfort et al., 2011), which is also found in dinoflagellates of the genus, *Dinophysis* (Janson, 2004).

It is unclear what conditions generally trigger *Myrionecta rubra* bloom formation. Hypotheses include the combined effect of high light intensity, low turbulence, and perhaps elevated concentrations of dissolved organic compounds produced by preceding diatom blooms or released during rainfall events (Crawford et al., 1997). Indeed, this ciliate rarely blooms in fall or winter; *M. rubra* blooms start during neap tide in San Francisco Bay when vertical density gradients are steep and turbulent mixing is weak (Cloern et al., 1994); and *M. rubra* blooms in San Francisco Bay and Southampton estuary often follow either the spring diatom bloom or heavy rainfalls (Cloern et al., 1994; Crawford et al., 1997). In the

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Columbia River estuary, anecdotal evidence suggested that *M. rubra* blooms are usually first noticeable in Ilwaco harbor of Baker Bay on the seaward-end of the Washington bank of the Columbia River estuary (Fig. 1) before being later visible in the estuary main channels (G.C. Roegner and several local fishermen, pers. comm.).

Therefore, to improve our knowledge of the *Myrionecta rubra* B haplotype bloom development in the Columbia River estuary, we collected surface water (0 m) in Baker Bay at Ilwaco (seaward-end of the Bay) and Chinook harbors (upriver-end of the Bay) and in the estuary main channels before and during the 2009 and 2010 *M. rubra* red water blooms, and determined the ciliate's abundance and specific growth rates. Because the ciliate is highly motile and phototactic, we sought to determine if these properties were reflected in its vertical distribution by conducting sampling at multiple depths in Ilwaco harbor.

2. Materials and methods

2.1. Study area

The Columbia River is characterized by a drainage basin that covers 670,000 km^2 (including seven U.S. states and two Canadian provinces) and by an average annual freshwater discharge of 2×10^{11} m^3, making it the second largest river in North America (Frey et al., 1984; Sullivan et al., 2001). Its narrow estuary (relative to its length), which is bordered by Washington State to the North and Oregon State to the South (Fig. 1), is strongly influenced by this large river inputs and by the mixed semi-diurnal tides that are typical of the northeast Pacific Ocean (Jay, 1984). This oceanic and freshwater inputs create a turbulent estuarine environment characterized by short residence times (0.5–5 days; Neal, 1972) and stratification in terms of salinity and temperature, which varies in strength both tidally and seasonally. The maximum river discharge period occurs in late spring due to the melting of snowpack built-up in the drainage basin throughout the winter, while the summer is characterized typically by the lowest annual discharge levels. Since the volume of freshwater discharge from the Columbia River essentially modulate the distance that saltwater reaches upstream into the estuary (Chawla et al., 2008), the salt wedge intrusion is longer in summer. For more than a decade, the water masses in the estuary have been under intense study via modeling of the physical data obtained from numerous observatory stations localized throughout the estuary (Baptista et al., 2005) and data are readily accessible at http://www.stccmop.org/datamart/virtualcolumbiariver. The estuary has a few shallow and wide-mouthed bays, lacks important tributary rivers, and has two narrow main channels (0.5–3 km) separated by broad shoals (Fig. 1). The south channel, with an average and maximum depth of 10 and 20 m, respectively, is dredged to enable shipping activities up to Portland (Oregon), while the north channel is dredged moderately and shoals near the freshwater-brackish water interface.

Baker Bay is located on the seaward-end of the Washington bank of the Columbia River estuary (Fig. 1). This bay is characterized by heavy deposition of silt particles, and is thus essentially less than 1.5 m deep and predominately above water at low tide. The opening of the Bay is partially obstructed by two small islands with the largest, Sand Island, situated on the west side of the opening (river mile 3). Ilwaco and Chinook harbors are both in Baker Bay at the seaward-ends (river mile 3) and upriver-ends (river mile 6), respectively (Fig. 1). Since both ports are essential for local commercial and recreational fishing activities, accessibility is maintained by regular dredging of their channels. Ilwaco channel (also called Baker Bay channel) is the deeper water channel of Baker Bay through which the main current flows. Ilwaco harbor has the largest marina with 800 slips, while Chinook harbor marina has 358 slips.

2.2. Sample acquisition for microscopy and growth rate incubations

Water for cell counts was collected in the Columbia River estuary in the Spring and Summer of 2009 and 2010 in Baker

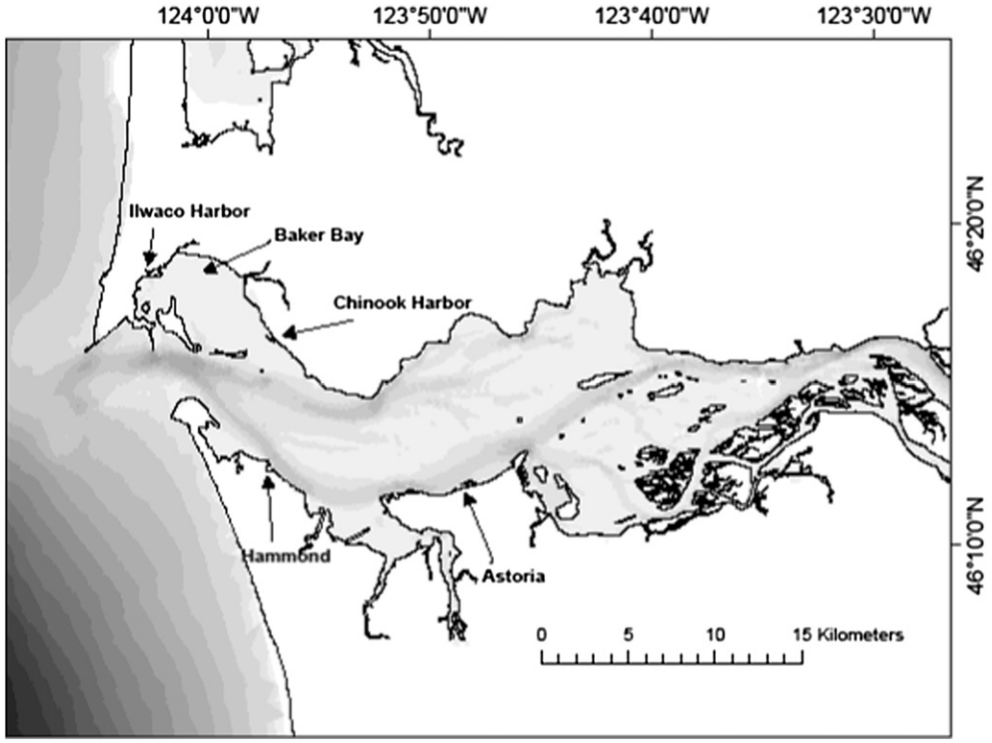

Fig. 1. Map showing locations of Ilwaco and Chinook harbors in Baker Bay, as well as Hammond and Astoria on the bank of the Columbia River estuary south Channel.

Bay at Ilwaco and Chinook harbors and on the Oregon bank of the estuary south channel at Hammond and Astoria (Fig. 1). Samples were obtained at Hammond off a small beach with direct exposure to the estuary south channel, while at Astoria, Ilwaco harbor and Chinook harbor they were acquired from a floating pier. At all sites, a 7.6 L polycarbonate carboy was used to collect surface water (0 m). A 1 L Van Dorn bottle (Lab Safety Supply, Janesville, WI, USA) was also used to collect additional water in Ilwaco harbor at 1 m and 3 m depths. Water temperature was recorded immediately after collection, while salinity was measured in the laboratory using a refractometer and values are reported using the Practical Salinity Scale. For growth rate incubations, water was collected in Ilwaco harbor or in the south channel of the estuary off Hammond beach (nearby Hammond harbor), and incubations were conducted in Ilwaco or Hammond harbors, respectively (see growth rate incubation details below).

2.3. Light microscopy

For cell counts, water samples (50 mL) were immediately fixed with Lugol's iodine (final concentration: 1%) and stored in the dark at 4 °C. The sample (25 mL) was allowed to settle overnight according to the Utermöhl (1958) method. Using an inverted microscope (Leica), a minimum of 200 *Myrionecta rubra* cells were counted in a minimum of six fields of view. Cell counts reported as zero indicated that *M. rubra* were not detectable in the 25 mL sample. In other environments *M. rubra* is commonly present in abundances of 100s cells L^{-1} or less, which would correspond to only 2–3 cells in a 25 mL settling chamber. Therefore, to reliably estimate these low ciliate abundances most of the field of view was examined (up to 132 distinct fields of view equivalent to 85% of the surface area). Importantly, the limit of detection of this approach is about 300 *M. rubra* cells L^{-1}, so that abundances of the protist reported here as 0 cells mL^{-1} represent samples with absolutely no *M. rubra* cells or with low (and commonly observed) numbers in the 100s cells L^{-1}.

2.4. Growth rates

Water collected in the estuary main channels and in Ilwaco harbor on 05 August and 02 September 2010 at 0 m or 1 m was incubated *in situ* at 0 m or 1 m. Sampling and incubation depths as well as incubation times for each location are given in Table 2. Since growth rates of protists are often not even throughout the day, incubations were started during daytime and carried out for a full diel cycle (23–24 h). In addition, to obtain better insight into *Myrionecta rubra* cell division dependence on diel cycle phases, a diurnal incubation of 7 h was also done on 02 September for water collected in the main channel. To extrapolate these 7 h diurnal growth rates to fictive 24 h diurnal rates, values were multiply by a factor of 3.4. Henceforth these values will be referred to as 'extrapolated 7 h diurnal growth rates'. While light was not measured during these *in situ* incubations, note that conditions were overcast on 05 August but sunny on 02 September. Completely filled transparent 1L wide-mouth field sample bottles (Fisher Scientific, Pittsburgh, PA, USA) were attached to a nearby floating pier at either Ilwaco or Hammond harbors and lowered to the appropriate depth. Sub-samples for determining *M. rubra* abundance were collected before and after incubations by fixing 50 mL of water with Lugol's iodine (final concentration: 1%) and storing it in dark at 4 °C. Results are reported as an average of triplicate incubations and standard error.

3. Results

3.1. Myrionecta rubra abundance in Columbia River estuary during the initial phase of bloom development

2009 – A delay was observed between the time *Myrionecta rubra* cells were first detected at concentrations >100s cell L^{-1} in Ilwaco harbor and when they were first observed in Chinook harbor and the estuary main channels (Table 1). On 30 June 2009, *M. rubra* was not detected in Ilwaco harbor or Hammond. On 21 July, high numbers of *M. rubra* cells were identified in Ilwaco harbor (200 cell mL^{-1}) but they were not yet observed in the estuary main channels (below detection limit of 100s cell L^{-1} in the north channel at the Astoria-Megler Bridge). Red water was observed by our field team for the first time that year in the estuary main channels on 05 August (Katie Rathmell, pers. comm.) and microscopic observations of surface water collected on 06 August confirmed that *M. rubra* was then present in Ilwaco and Chinook harbors as well as in the Columbia River estuary south channel at Hammond (304, 178 and 42 cell mL^{-1}, respectively) (Table 1).

2010 – The detection of *Myrionecta rubra* cells in Baker Bay at concentrations >100s cell L^{-1} on 08 July (e.g. 5 cell mL^{-1} at 1 m depth in Ilwaco harbor) (Table 1) coincided with the first neap tide of July 2010. That year the peak water discharge of the Columbia River at Beaver Army Terminal (Quincy, OR, USA) as recorded by the United States Geological Survey occurred in June. Relative to the preceding spring tide in June, the first neap tide of July 2010 was a time of reduced river flow and increased stratification, and thus increased salinity intrusion into the estuary, including into Baker Bay. This was indicated by a doubling in the salinity values of the water collected in Baker Bay at slack high tide between 24 June and 08 July (Table 1 and Table S1). Within 2 weeks (22 July) of the appearance of *M. rubra* in Baker Bay at concentrations >100s cell L^{-1}, cell numbers were 2- to 3-fold higher in Ilwaco harbor (up to 213 cell mL^{-1} at 1 m depth) compared to Chinook harbor, and the ciliate remained undetected in the estuary main channels (Table 1). A week later, almost 600 *M. rubra* cell mL^{-1} were enumerated in surface waters (0 m) in Ilwaco harbor, but for the first time low *M. rubra* numbers (1 cell mL^{-1}) were also detected in Chinook harbor and in the estuary south channel at Hammond (Table 1).

To verify that the absence of detectable *Myrionecta rubra* at concentrations >100s cell L^{-1} in the estuary main channel before the appearance of red water was not related to our discrete sampling scheme, a time series study was conducted on board of the R/V *Wecoma* in the Columbia River estuary north channel (46.235 N, 123.90 W) on 01–03 August 2010 over two full tidal cycles. Water column profiling with a Cyclops-7 phycoerythrin sensor (Turner Designs, Sunnyvale, CA, USA) was conducted every 2 h. This sensor detects the red pigment in *M. rubra*, cryptophytes and cyanobacteria chloroplasts. At the same times Lugol's iodine-fixed water samples were also collected at various depths in the water column (1, 5 and 10 m). The phycoerythrin sensor did not show any relevant peak in fluorescence in the water column during this time series, and none or extremely low *M. rubra* cells numbers (<8 cells mL^{-1}) were observed by microscopy in water samples collected over the initial flood and ebb tides of this time series, therefore demonstrating an absence of a significant number of *M. rubra* cells in the estuary proper (data not shown).

3.2. Myrionecta rubra abundance in Ilwaco harbor during the initial and established phases of the 2010 bloom

June–September 2010 time series – although *Myrionecta rubra* cell counts in our samples varied throughout this time span, with

Table 1
M. rubra abundance (cells mL^{-1}) during the initial phase of the 2009 and 2010 *M. rubra* blooms in the Columbia River estuary in water collected in Baker Bay at Ilwaco and Chinook harbors and on the bank of the south channel at Hammond and Astoria. Average salinity values are also listed for Baker Bay samples that were collected at slack high tide (detailed salinity and temperature data are given in Table S1).

		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 (12.4)	n.d.	n.d.	n.d.
	6-Aug	n.d.	42 (4.3)	178 (15.5)	304 (15.9)	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 (0.3)	0 (0)	5 (1.1)	1 (0.5)	8.3
	22-Jul	0 (0)	0 (0)	0 (0)	15 (1.9)	213 (8.8)	18 (4.5)	9.3
	29-Jul	0 (0)	1 (0.3)	1 (0.4)	598 (39.2)	259 (15.7)	49 (3.3)	n.d.

[a] Sample was collected in Astoria but at the Astoria-Megler Bridge in the middle of the north channel. n.d., not determined. Locations are given in map of Fig. 1. Standard errors are given in brackets.

the highest values measured on 29 July and 19 August (598 and 287 cells mL^{-1}, respectively; still well below red water abundances of 1000 cells mL^{-1}), the ciliate was almost always more abundant in water collected at 0 and 1 m than at 3 m (Fig. 2). Low *M. rubra* abundance was observed in Ilwaco harbor in surface water at the end of the first neap tide of August (7 and 12 cells mL^{-1} for 05 and 06 August, respectively) (Fig. 2). This was unexpected since, as mentioned above, sampling a week before (29 July) during the beginning of the same neap tide cycle returned the highest numbers of this dataset. Interestingly, during the following neap tide, on 18 August, based on a calibration between phycoerythrin sensor signal and *M. rubra* cell counts (made on water collected on 02 September 2010 at SATURN-03) an estimation of up to 400 *M. rubra* cell mL^{-1} were for the first time detected in the estuary main channels at 2.4 m depth using a Cyclops-7 phycoerythrin sensor (Turner Designs, Sunnyvale, CA, USA) at an observatory station (SATURN-03) located in the south channel at Hammond (data not shown). On 23 August large red water patches were observed for the first time that year at mid-day throughout the lower Columbia River estuary by our field team (Katie Rathmell, pers. comm.).

3.3. Tidal cycle time series in Ilwaco harbor

To determine if tidal forcing had an important impact on *Myrionecta rubra* abundance in Ilwaco harbor (i.e. if sampling at slack high tide – as presented on Fig. 2 – is representative of *M. rubra* abundance throughout a tidal cycle), water was collected in Ilwaco harbor over a neap tide tidal cycle during the initial (05–06 August) and established (01–02 September) phases of the 2010 *M. rubra* bloom and results are reported on Fig. 3. Both sampling events were characterized by low *M. rubra* cell counts in the waters collected at

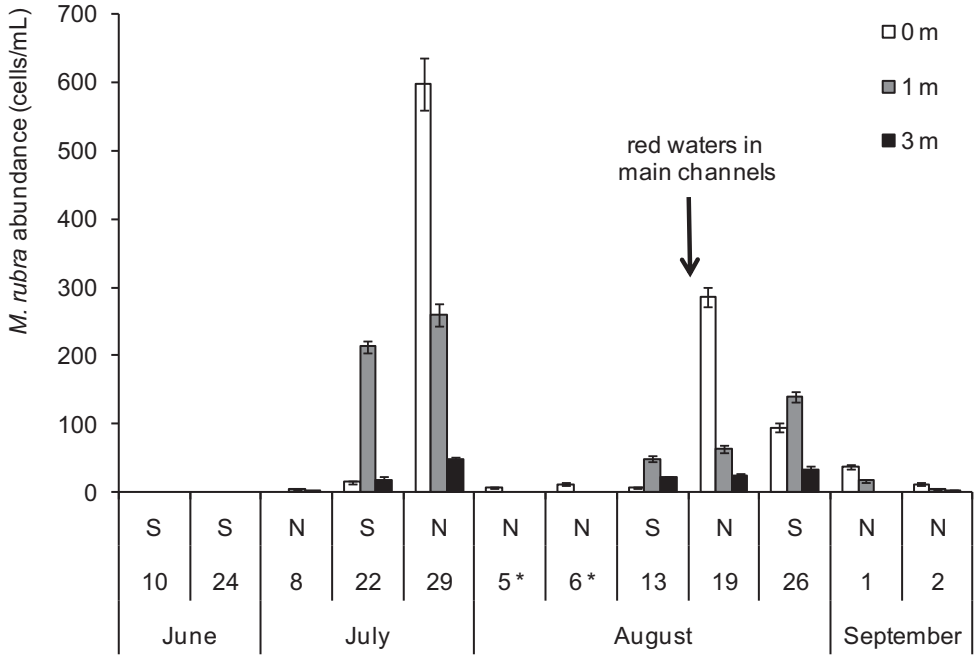

Fig. 2. *M. rubra* abundance in water collected at different depths (0, 1 and 3 m) in Ilwaco harbor between 10 June and 02 September 2010. All waters were collected during daytime at slack high tide, except for samples obtained on 29 July and 26 August and on 13 August that were gathered during mid-flood and slack low tide, respectively. Arrow indicates that *M. rubra* red water blooms were detected in the Columbia River estuary main channels from that date on. *, Only surface water (0 m) collected. S, Spring tide and N, neap tide. Location of Ilwaco harbor in the Columbia River estuary is given in map of Fig. 1. Bars error bars.

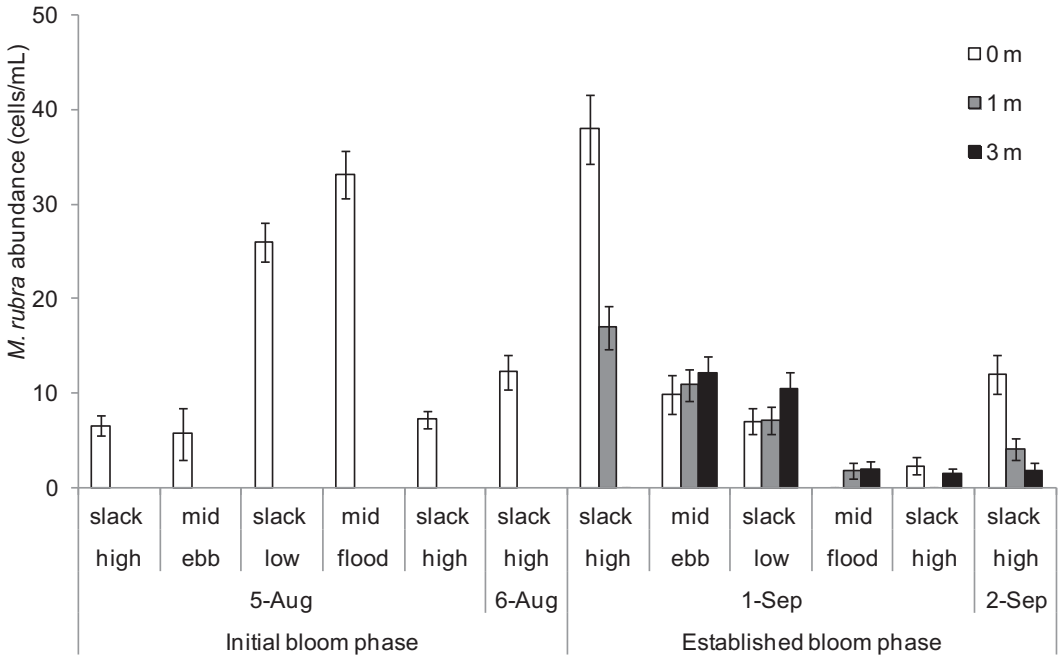

Fig. 3. *M. rubra* abundance in water collected in Ilwaco harbor over a neap tide tidal cycle during the initial (05–06 August) and established (01–02 September) phases of the 2010 *M. rubra* bloom. Water was only collected at the surface (0 m) on 05–06 August, but at three depths (0, 1 and 3 m) on 01–02 September. Location of Ilwaco Harbor in the Columbia River estuary is given in map of Fig. 1. Bars error bars.

slack high tide with a maximum of 40 cells mL^{-1}, and values remained below this amount at all other sampling times throughout the tidal cycles.

3.4. *Myrionecta rubra* growth rates in Ilwaco harbor and the estuary main channels during the initial and established phases of the 2010 bloom

On 05 August, despite low *Myrionecta rubra* abundance in Ilwaco harbor surface water (7 cells mL^{-1}), the high growth rates measured (1.2–3.1 d^{-1}) indicate that the ciliate was actively dividing (Table 2). Interestingly, cells collected at the surface (0 m) had a growth rate almost 3 times higher when incubated at 1 m than 0 m, suggesting that at the very surface *M. rubra* growth may be photo-inhibited. Subsequent incubations were thus carried out at 1 m. Although *M. rubra* cells were present on 02 September, growth of the ciliate was not detected in any of the incubations from Ilwaco harbor, whether samples were collected at slack high or low tide (Table 2). High growth rates were measured in the estuary south channel in both red water (0.7 d^{-1}) and adjacent non-red water (1.1 d^{-1}) patches (Table 2). Two sets of incubations of differing length (7 h mostly daytime – and 24 h) were carried out for these south channel samples collected at slack low tide. The 2–4 times higher growth rates measured during the shorter incubations (7 h) for both red and non-red waters suggest that *M. rubra* cells mostly divide during the daytime (Table 2). Growth rates were also 3 times higher in non-red water compared to red water patches for incubations carried out for 7 h (mostly daytime) (Table 2).

4. Discussion

For several decades massive red water blooms of *Myrionecta rubra* have been observed in the Columbia River estuary every summer or early autumn (Herfort et al., 2011), and although anecdotal evidence suggested that *M. rubra* blooms are usually first

Table 2
Growth rates of *M. rubra* from red and non-red surface waters collected and incubated in the Columbia River estuary at either Baker Bay (Ilwaco harbor) or the south channel (Hammond) during the initial (05 August) or established (02 September) *M. rubra* bloom period of 2010.

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 h	7 (1.1)	1.2 (0.2)
Ilwaco	Non-red water	5-Aug	High slack	1	23 h	7 (1.1)	3.1
Ilwaco	Non-red water	2-Sep	High slack	1	24 h	38 (3.6)	0.0 (0.0)
Ilwaco	Non-red water	2-Sep	High slack	1[a]	24 h	17 (2.3)	0.0 (0.0)
Ilwaco	Non-red water	2-Sep	Low slack	1	23 h	7 (1.3)	0.0 (0.0)
Hammond	Red water	2-Sep	Low slack	1	7 h	1484 (81.5)	1.4 (0.4)
Hammond	Red water	2-Sep	Low slack	1	24 h	1484 (81.5)	0.7 (0.6)
Hammond	Non-red water	2-Sep	Low slack	1	7 h	2 (0.5)	4.2 (0.2)
Hammond	Non-red water	2-Sep	Low slack	1	24 h	2 (0.5)	1.0

[a] A sample collected at 1 m instead of 0 m. Locations are given in map of Fig. 1. Standard errors are given in brackets, with missing error values indicating that one of the triplicate bottles was lost during *in situ* incubations.

noticeable in Ilwaco harbor (seaward-end of Baker Bay) before being later visible in the estuary main channels (G. C. Roegner and several local fishermen, pers. comm.), the time course of bloom development had not been established. Our 2009 and 2010 dataset corroborates the anecdotal observations and suggests that Ilwaco harbor is at least one of the locations where the bloom first develops since in both years *M. rubra* was detected at concentrations >100s cell L^{-1} in Ilwaco harbor before being observed in Chinook harbor (upriver-end of Baker Bay) or the estuary main channels (Table 1). *M. rubra* bloom development in the Columbia River estuary is, therefore, biphasic, with an initial phase when *M. rubra* is mostly present in Baker Bay – i.e. in the estuary but outside the main channels – and an established phase when red waters are observed throughout the lower estuary. The initial phase of 2010 lasted about 1.5 months, spanning the neap tide of early July to the beginning of the neap tide of mid-August. The appearance of red waters in the main channels during a neap tide agrees well with previous reports from the San Francisco Bay or the Southampton estuary (Cloern et al., 1994; Crawford et al., 1997) and thus lend further support to the idea that a decrease in water column turbulence plays an important role in the initial surface aggregation of large numbers of bloom-forming *M. rubra* cells.

Since the limit of detection of the method used here to assess *Myrionecta rubra* abundance does not allow for distinction between no or low cell numbers in the 100s per L, one may argue that this early detection in Ilwaco harbor might be the result of a sudden growth of *M. rubra* cells in this locale. However, taken together, several lines of circumstantial evidence suggest an oceanic origin of the estuarine bloom-forming ciliate: (1) the presence of the bloom-forming *M. rubra* haplotype B on the Oregon and Washington coast during the spring (i.e. when the protist was not detectable in the estuary) (Herfort et al., 2011); (2) the retentive nature of Ilwaco harbor that was demonstrated by the succession of protist blooms (e.g. *Skeletonema*, Euglena, dinoflagellates) that occurred between the spring and fall of 2010 in but at no other sampling location in the estuary (Peterson et al. in prep.); (3) the timing of the appearance of *M. rubra* at concentrations >100s cell L^{-1} in Baker Bay in early July 2010 during a time of increased salt wedge intrusion into the estuary (neap tide/reduction of river flow compared to the spring). Notably, during the initial phase, spatial and temporal variations in *M. rubra* abundance were apparent in Ilwaco harbor. Elevated surface *M. rubra* cell numbers (Fig. 2) were likely linked to its motile and phototactic behavior (Lindholm 1985; Dale 1987, Fenchel and Hansen, 2006), while, at later times during the bloom period, temporal fluctuations in cell counts were not linked to tidal forcing since few *M. rubra* were observed in Ilwaco harbor surface waters throughout a tidal cycle on 05–06 August 2010 (Fig. 3).

Interestingly, high growth rates were measured during the initial phase in Ilwaco harbor (1.2–3.1 d^{-1}) and during the established bloom period in the estuary south channel in both surface red water (0.7 d^{-1}) and adjacent non-red water (1.1 d^{-1}) patches (Table 2). These values are much higher than the highest growth rate ever recorded for a *Myrionecta rubra* culture (0.52 d^{-1}; Yih et al., 2004) or even compared to that of the only other field estimate (0.55 d^{-1}; Crawford et al., 1997). This *in situ* growth rate measurement by Crawford and co-workers was obtained in June 1986 by determining the increase in *M. rubra* cell numbers within the water column for a few days before red water was first detected in the Southampton estuary (UK). By the authors' own admission, this is a "crude" approach for estimating growth rates. The large differences in *M. rubra* growth rates that exist between our study and others might be attributed to a disparity in optimum growth conditions between cultures and field, and/or to the existence of different *M. rubra* haplotypes with possibly varying physiological characteristics (Herfort et al., 2011). Furthermore, although our *in situ M. rubra* growth rates, which were determined using the traditional incubation approach, appear high compared to those recorded in other *M. rubra* studies, they nonetheless fall within reported values for ciliates (up to 3.5 d^{-1}, Strom and Morello, 1998). This indicates that the high growth rates measured in the Columbia River estuary likely reflect a fast growing *M. rubra* population. Taylor and Shuter (1981) argued that the high growth rates achieved by ciliates might be due to the complexity of their genomes that contain both a germ-like micronucleus that functions in sexual or asexual nuclear fusion to create a zygote, and a somatic macronucleus that is the site of gene copy number amplification and expression (Jahn and Klobutcher, 2002). This separation between sexual and somatic function, which directs high rates of macromolecular synthesis, might confer a reproductive advantage upon ciliates leading to high growth rates.

While, as mentioned above, *Myrionecta rubra* was actively growing in Ilwaco harbor during the initial phase of the bloom, growth of the ciliate was not detected in any of the incubations from this locale during the established bloom period, suggesting that *M. rubra* blooms in the estuary main channels do not depend on a constant supply of *M. rubra* cells from Ilwaco harbor and instead rely, at least in part, on high *in situ M. rubra* growth rates. Growth rate data also suggest that *M. rubra* cells in the estuary during the established bloom phase mostly divide during the daytime because the extrapolated 7 h diurnal growth rates were at least twice as high as those calculated from diel cycle incubations (Table 2). Furthermore, red water patches might experience self-shading since growth rates were 3 times higher in non-red water compared to red water patches for incubations carried out for these 7 h (Table 2).

The observed biphasic development of Columbia River estuary *M. rubra* blooms may be derived from a temporal sequence of growth of a *M. rubra* B haplotype population throughout the estuary or may be the result of the advection into the main channels of a seeding *M. rubra* B haplotype population from refugium embayment areas such as Ilwaco harbor. Presently, it is impossible to determine which of these two hypotheses is correct (if either), but Baker Bay, and particularly Ilwaco harbor, appear to be an unusually productive area for protist proliferation compared to the rest of the Columbia River estuary since (1) benthic algae assemblages found in this embayment are more diverse than those of Young, Gray and Cathalamet Bays (Small et al., 1990); (2) Baker Bay is the most productive site for benthic autotrophy in the Columbia River estuary (Small et al., 1990); and (3) in the spring and fall of 2010 a succession of protist blooms was observed in Ilwaco harbor but not in Chinook harbor or in the Columbia River estuary south channel (Peterson et al., in prep). In the future, using ecogenomic sensors (Greenfield et al., 2008; Scholin, 2010) equipped with probes specific for this protist and attached to profiling stations located at key water transport points between Baker Bay and the estuary north channel, it will likely be possible to ascertain the underpinning dynamics of the biphasic development of the Columbia River estuary *M. rubra* blooms uncovered in the work reported herein.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ecss.2011.10.015.

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