



# Bloom formation potential in the harmful dinoflagellate *Akashiwo sanguinea*: Clues from movement behaviors and growth characteristics



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## ARTICLE INFO

### Article history:

Received 18 March 2015

Received in revised form 1 June 2015

Accepted 1 June 2015

Available online

### Keywords:

Dinoflagellates

Motility

Plankton production

Growth

Turbulence

Bloom formation

## ABSTRACT

We measured the growth rates and swimming behaviors of recently isolated strains of the dinoflagellate *Akashiwo sanguinea* to investigate to what degree growth and motility could contribute to the formation of in situ blooms. To quantify the effect of variation in in situ conditions on population growth rate, we applied two temperature treatments (10 °C and 20 °C) and measured growth in still conditions and on a shaker table, to emulate mild turbulence. To quantify the importance of intra-strain variability and trait variation in the species growth potential and vertical distribution, we included six strains isolated from a spatially and temporally extensive bloom on the US West Coast. Overall, as reported previously, *A. sanguinea* was observed to tolerate conditions amounting to a broad ecological niche with intra-specific variability further broadening tolerable conditions. In agreement with prior observations of slow growth rates of the species, average growth rates across all strains increased significantly from 0.12 d<sup>-1</sup> (±0.03) at 10 °C to 0.28 d<sup>-1</sup> (±0.13) at 20 °C in still conditions. Contrary to prior reports, mild turbulence had neutral or positive effects on most strains' growth rates, with one strain only able to grow on the shaker table. Growth rates in mild turbulence were higher than in still conditions and increased from 0.15 d<sup>-1</sup> (±0.01) at 10 °C to 0.43 d<sup>-1</sup> (±0.04) at 20 °C. There was significant intra-strain variation in growth rates (>50% coefficient of variation) and movement behaviors. All strains had both up and down swimming fractions, leading to predictions of vertically patchy distributions, rather than surface aggregations. Slow growth rates and dispersive swimming behaviors suggest in situ mortality must be low and tolerance of seasonally varying water temperatures lead to accumulation and persistence of cells over months and kilometers. Estimates of in situ loss rates are a critical but missing component of identifying the bloom formation mechanisms of this species.

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## 1. Introduction

Harmful algal blooms (HAB) are broadly disruptive to aquatic ecosystems, fisheries and tourism in many coastal areas. Economic damages from HABs average \$50 million annually in the United States alone (Anderson et al., 2000). Understanding the mechanisms behind these large biomass aggregations of HAB species is key to anticipating their formation and potentially mitigating their effects. Hypothesized bloom formation mechanisms include growth, superior competitive nutrient uptake, cyst formation, biology-independent aggregation due to advection, interactions of fluid flow and swimming behaviors as well as vertical migration behaviors that exploit nutrient gradients (Cullen and Horrigan, 1981; Imai et al., 1986; Trigueros and

Orive, 2000; Lennert-Cody and Franks, 2002; Smayda, 2010; Katano et al., 2011; Peacock and Kudela, 2014). Finally, algal behaviors, including predator induced size changes (Selander et al., 2011) and fleeing behaviors (Harvey and Menden-Deuer, 2012) have been described as being potentially influential in bloom formation.

The vast majority, 75% of the approximately 300 known HAB forming species are dinoflagellates (Smayda, 1997). Of these dinoflagellates *Akashiwo sanguinea* is a conspicuous harmful, but not toxic species (Kudela et al., 2005). In the literature, the species currently known as *A. sanguinea* is treated under several names, some of which reflect renaming of the species, others are synonyms, including most recently *Gymnodinium sanguineum* Hirasaka, *Gymnodinium splendens* (Lebour) and *Gymnodinium nelsonii* (Martin) (Daugbjerg et al., 2000).

*Akashiwo sanguinea* is a prominent and easily identifiable species that is well known to form large, noticeable blooms (Horner et al., 1997). *A. sanguinea* forms blooms infrequently, but

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globally, including the California Current System (Horner et al., 1997) and the South China Sea (Lu and Hodgkiss, 2004). Occurrence of *A. sanguinea* blooms have been linked to upwelling systems and correlated to climate anomalies that result in reduced mixing, indicated by enhanced stratification, reduced mixed layer depth and decreased upwelling (Kudela et al., 2010). Although the connectivity among individual bloom events remains unknown, recurring observations of large *A. sanguinea* blooms have been reported off the west coast of the United States in the last decade. Reported events occurred as far south as Monterey Bay, as early as 2004 (Kudela et al., 2008) with the largest bloom on record reported off the central Oregon coast in autumn 2009 (Du et al., 2011). Recently, White et al. (2014) expanded the report of this bloom to cover a wider spatial and temporal extent, supported by cell counts and satellite observation. The cell isolates for this study were derived from the 2009 bloom event and were collected in Washington state (see methods).

These large scale *Akashiwo sanguinea* blooms have received attention recently because of the resultant death of seabirds through hypothermia caused by the saponification of *A. sanguinea* exudates (Jessup et al., 2009). Laboratory experiments have shown that *A. sanguinea* can induce death in microzooplankton grazers (Jeong and Latz, 1994) and are deleterious to abalone larvae (Botes et al., 2003). On the other hand, the species has been shown to be a suitable food in raising finfish (Lasker et al., 1970; Rodriguez and Hirayama, 1997) and reports from the field suggest that *A. sanguinea* is critical for the survival of larval fish (Lasker, 1975).

*Akashiwo sanguinea* has been described as a eurythermal and euryhaline species, with broad tolerance to salinity ranging from 5 to 40 psu and positive growth rates measured between 10 and 30 °C (Matsubara et al., 2007) although Boyd et al. (2013) suggested a narrower temperature and reported no growth at 10 °C. *A. sanguinea* growth rates are strongly temperature dependent and can achieve up to 1 division per day at intermediate salinity (20 psu) and high temperatures (25 °C). However, growth rates at temperatures <20 °C are typically low (<0.2 divisions d<sup>-1</sup>). Such low growth rates are not uncommon and dinoflagellates are thought to be slow growers compared to similar sized microplankton (Hansen et al., 1997; Strom and Morello, 1998). Given these relatively slow growth rates and higher growth rate at higher temperatures, it is unclear how this species could form recurrent, massive blooms in the conditions typical for the U.S. west coast waters.

Dinoflagellate blooms have been suggested to occur preferentially in calm conditions without turbulence interfering with many processes, inhibiting division, morphology, and movement (reviewed in Berdalet et al., 2007). Even turbulence at rates of 1 cm<sup>2</sup> s<sup>-3</sup> could lead to cell death (Berdalet, 1992). Countering such generalizations, (Smayda and Reynolds, 2000) point to the considerable diversity of life-forms, adaptive strategies and habitat preferences observed for dinoflagellate species and question such generalizations. If the laboratory results summarized in Berdalet et al. (2007) are applicable to in situ growth responses by *Akashiwo sanguinea*, it is a conundrum, how this species could achieve high, in situ densities at conditions not known to favor this species growth.

The goal of this research was to use many strains isolated from the largest reported bloom event of *Akashiwo sanguinea* (Du et al., 2011; White et al., 2014) to determine the potential mechanisms of bloom formation by (1) measuring growth rates of multiple strains of *A. sanguinea* as a function of temperature, (2) quantifying the effect of mild turbulence on growth rate magnitude, and (3) measure the species swimming behaviors to predict vertical population distributions and dispersal rates of *A. sanguinea*. In combination, we wanted to determine if the behavioral and physiological characteristics of this species were suitable to support the observed buildup of *A. sanguinea* biomass in situ.

## 2. Methods

### 2.1. Culturing and growth rate estimates

Isolates of *Akashiwo sanguinea* (Hirasaka) G. Hansen and Moestrup were obtained from a single water sample taken in Grays Harbor, Washington State, USA on November 19th, 2009. According to White et al. (2014) this location only had a relatively minor peak in *A. sanguinea* abundance of 14 cells mL<sup>-1</sup>, which coincided with the time of our sampling. Water temperature at the collection site was 9.8 °C. Clonal cultures were established from single-cell isolates, subjected to multiple rinsing steps in filtered seawater. Thus, each isolate represents an independent, clonal strain of the species. All cultures were grown in autoclaved, 0.2 µm sterile-filtered amended seawater with f/2 medium without silica (Guillard, 1975). These cultures were not axenic. All cultures were maintained at 30 psu. Cells were maintained and growth experiments conducted at a light level of 100–115 µmol photon m<sup>-2</sup> s<sup>-1</sup> and a 12:12 h light:dark cycle. The same source water was used for culture maintenance and experiments. Stock cultures for the experiments were monitored from an initial lag phase to a stationary phase and were exponentially growing at population densities between 300 and 1800 cells mL<sup>-1</sup>. Only cells from exponentially growing cultures were used for behavior or growth experiments.

To examine growth rate responses to mild turbulence, strains were grown on an orbital shaker (Thermo Scientific MaxQ2000) with an orbital range of 1.9 cm, rotated at 75 rpm, and the volume of the cultures in 250 mL flasks was maintained at 100 mL. The rotation frequency and all other aspects of this set up followed Zirbel et al. (2000), who quantified fluid velocity as a function of shaker settings for a range of rotation frequencies. Note that because of wall effects, the level of induced turbulence within the flask at any one rotation frequency varies significantly as a function of distance from the center (Zirbel et al., 2000). Therefore, we report turbulence over a range, rather than an absolute value. Moreover, for consistency with the literature review by Berdalet et al. (2007) we converted the shear stress and velocity reported by Zirbel et al. (2000) to turbulence values ( $\varepsilon$ ). Under the given settings in our shaker growth experiments the induced turbulence is estimated to range from  $\varepsilon = 0.1$  to 1 cm<sup>2</sup> s<sup>-3</sup>. This range is on the lower end of the spectrum used in previous turbulence experiments, but comparable to maximum measurements in a coastal fjord (Sullivan et al., 2003). Thus, although our turbulence treatment is considered mild in comparison to laboratory studies, it may be reflective of more intense turbulence compared to in situ conditions. Moreover, turbulence in these experiments is continuous, whereas in situ conditions likely expose organisms to intermittent turbulence. Lastly, cells were not acclimated to the turbulence treatment and this transition may have been a shock and could have reduced population growth rates. All other growth conditions for the turbulence treatments were identical to the still treatments.

Triplicate cultures were grown at 10 °C and 20 °C for each strain of *Akashiwo sanguinea* (D, K and O) on and off the orbital shaker, so that strains were acclimated to target temperatures for at least one week. Single cultures of additional strains (J, P, and E) were grown for growth rate measurements in still (non-shaker) conditions only. All other cultures used for movement behavior measurements were grown in still conditions at 20 °C, except strain G, which was maintained on the shaker table. Note that the differences in swimming speed between strain G and the other strains may have arisen due to these differences in growth conditions. However, no comparisons of swimming speed as a function of growth conditions in still water or mild turbulence were made.

Culture concentrations were determined both through a Multisizer 3 Beckman Coulter counter and microscopy (Kim and Menden-Deuer, 2013). Cultures were initially counted after

fixation in 1% acid Lugol's solution on a Sedgwick–Rafter but because of adverse effects of fixation on the cells, counting was subsequently done on 10 mL of live cultures using the Coulter counter.

## 2.2. Swimming behaviors

To quantify movement behaviors, a 30 cm tall, 5.5 cm wide 800 mL octagonal, acrylic observational chamber was used. The chamber was filled with FSW using a peristaltic pump; this method allows for the creation of a defined salinity structure with a range of 1–3 ppt salinity gradient over the height of the tank, while also eliminating convection in the chamber (Bearon et al., 2006). Two infrared sensitive cameras (Pixelink) with Nikon 60 mm Micro Nikkor lenses monitored an area of 1 cm × 1 cm × 2 cm, an approximate volume of 2 mL of water in the center of the tank. The cameras were mounted at a 45° angle with maximally overlapping fields of view to enable reconstruction of three-dimensional (3D) movement behaviors. All filming was conducted in the dark, to eliminate the potential for light-mediated behavioral responses. In order to view organisms, the chamber was illuminated with infrared (960 nm) light-emitting diodes (LED). Filming occurred at 4–8, initially random, horizons approximately 2–3 cm apart. Each horizon was filmed for 1–3 min every hour for up to 11 h. Video was captured at 15–30 frames s<sup>-1</sup>.

Using a syringe, organisms were introduced to the bottom of the tank through silicone tubing with an internal diameter of 1 mm. Cells were introduced slowly at a rate of 10 mL min<sup>-1</sup> to reduce stress to cells as well as disturbance of the water column. To determine swimming behaviors, each video was analyzed using the same protocol. The 2D position of each individual organism in each frame of the stereo cameras was determined using automated ImageJ image-processing software based on user defined settings for size and threshold to remove stationary background objects. All analyses of all treatments and videos used the same settings. Three-dimensional swimming paths were determined by first assembling 2D trajectories from Cartesian coordinates of each organism in each stereo frame and then joining 2D tracks based on matching space–time occurrence in the two 2D segments. Trajectories from all treatments were determined using the exact same video analysis and trajectory assembly parameters (Menden-Deuer and Grünbaum, 2006). Swimming metrics, including speed ( $\mu\text{m s}^{-1}$ ), the x, y, and z velocity vectors, direction (°) and turning rates (° s<sup>-1</sup>) were calculated from 3D paths, subsampled at 0.2 s intervals. Movement behavior measurements were based on time points that had more than 30 individuals in each replicate, and cells tracked for a minimum duration of 5 s or longer. Predictions of vertical distributions of different strains were made by extrapolating the net vertical velocity over a 10 h time period, taking the standard deviation in movement behaviors into account to reflect the upper and lower limit of the resulting population distribution.

Boundary conditions were reflective such that cells remained at the surface or bottom and were not removed from the system. For more details, see Menden-Deuer (2010).

## 2.3. Statistical analysis

All statistical analyses were made using Matlab R 7.10. Differences in growth rates ( $\mu$ ) presented in Table 1 were calculated as % difference =  $\mu_1/\mu_2$  with subscripts indicating the treatment factors temperature and turbulence exposure. Among strain variation was estimated by calculating the coefficient of variation CV = standard deviation/mean \* 100, expressed as %. Growth rates for strains D, G, K, and O were compared using a 3-factor ANOVA with replication, with factors: strain, turbulence exposure and temperature. This ANOVA was only performed on the subset of available growth rate measurements made at both temperature and turbulence exposure treatments because only these strains were tested across the different treatment factor combinations. The Kolmogorov–Smirnov test (KS test) was used to determine significant differences among strain specific swimming behaviors. All errors provided are standard deviations of the mean, unless otherwise indicated. All analyses were considered significant when  $p \leq 0.05$ .

## 3. Results

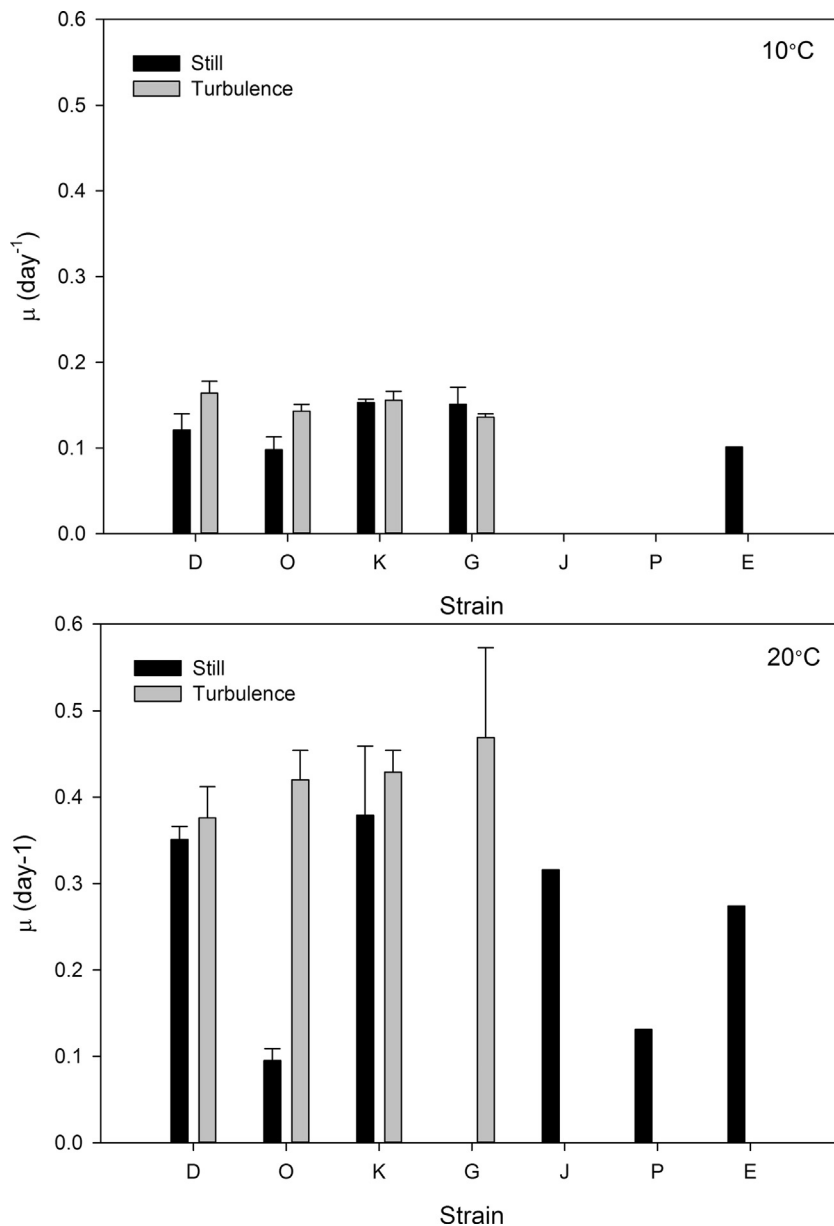
### 3.1. Growth response

All strains showed positive growth at 10 °C and 20 °C on and off the shaker table, except strains J & P that did not grow at 10 °C (Fig. 1). Of the three additional strains (J, P, and E) grown only in still conditions, only strain E was capable of growth at 10 °C. Average growth rate at 10 °C, was 0.12 d<sup>-1</sup> ( $\pm 0.03$ ) in still conditions and 0.15 d<sup>-1</sup> ( $\pm 0.01$ ) exposed to mild turbulence (Fig. 1, Table 1). The among strain variation in these growth rates was greatest for the 10 °C still treatment with 20% variation among strains. In contrast, among strain variation in growth rate was 10% in the mild turbulence treatment. Growth rate increased significantly ( $p < 0.0001$ ) up to almost 3-fold, when incubation temperature was increased to 20 °C. An increase in growth rate was observed for all strains except for strain O, which had no difference in growth rate between growth temperatures of 10 °C or 20 °C in still conditions. The across strain average growth rate in still conditions at 20 °C was 0.28 d<sup>-1</sup> ( $\pm 0.13$ ) and 0.43 ( $\pm 0.04$ ) when grown exposed to mild turbulence (Fig. 1, Table 1). As was observed in the cooler, 10 °C treatment, the variation among strains was much lower when cells were grown exposed to mild turbulence (8.5%). At 20 °C in still conditions, CV among growth rates could exceed 50% variation. This was due to the fact that strains D and K both grew at  $>0.3 \text{ d}^{-1}$  in still conditions at 20 °C, but strain O did not show the temperature dependent increase in growth rate observed for the other strains and grew at 0.09 d<sup>-1</sup>, despite the higher temperature. Strains grown exposed to mild turbulence showed a

**Table 1**

Growth rates ( $\mu$ , d<sup>-1</sup>) and fold change (%) in growth rate for 5 strains of *Akashiwo sanguinea*, measured at low incubation temperature (L) of 10 °C or a high incubation temperature of 20 °C (H) in still (S) and mild turbulence (T) conditions. Note that not all data comparisons were possible because not all strains were evaluated under all conditions. Errors are one standard deviation of the mean.

Strain	$\mu$ (d <sup>-1</sup> ) $\pm$ std				$\mu_{\text{HS}}/\mu_{\text{LS}}$	$\mu_{\text{HT}}/\mu_{\text{LT}}$	$\mu_{\text{LT}}/\mu_{\text{LS}}$	$\mu_{\text{HT}}/\mu_{\text{HS}}$
	10 °C		20 °C					
	S	T	S	T				
D	0.12 $\pm$ 0.02	0.16 $\pm$ 0.01	0.35 $\pm$ 0.02	0.38 $\pm$ 0.04	292%	238%	133%	109%
O	0.10 $\pm$ 0.015	0.14 $\pm$ 8 $\times 10^{-3}$	0.10 $\pm$ 0.01	0.42 $\pm$ 0.03	100%	300%	140%	440%
K	0.15 $\pm$ 4 $\times 10^{-3}$	0.16 $\pm$ 0.01	0.38 $\pm$ 0.08	0.43 $\pm$ 0.03	253%	269%	107%	113%
G	0.15 $\pm$ 0.02	0.14 $\pm$ 4 $\times 10^{-3}$		0.47 $\pm$ 0.10	–	336%	93%	–
E	0.10		0.27		270%	–	–	–
Average	0.12 $\pm$ 0.03	0.15 $\pm$ 0.01	0.28 $\pm$ 0.13	0.43 $\pm$ 0.04				



**Fig. 1.** Growth rate of *Akashiwo sanguinea* strains at 10 °C (top) and 20 °C (bottom), grown at identical light levels in still (control) and in mild turbulence. Error bars indicate 1 standard deviation of triplicate measurements. Strains J, P & E were only grown in still conditions.

similar, though slightly greater enhancement of growth rate. In the mild turbulence treatment, even strain O showed the nearly 3-fold increase in growth rate that had not been observed in still conditions for this strain but was characteristic for the other strains (Fig. 2). Mild turbulence appears to be critical for this strain to attain higher growth rates.

The effect of both temperature and turbulence was strain-specific. It is noteworthy that neither temperature nor mild turbulence had a uniform effect on growth rate on all strains. A 3-factor ANOVA with replication, testing the importance of temperature and presence of turbulence on the different strains' growth rates, revealed that all factors had significant (max  $p = 0.02$ ) effects on *Akashiwo sanguinea* growth rates (Table 2). Temperature dominated among these three factors, overall growth rates at 20 °C were 2.4× greater than at 10 °C ( $p < 0.001$ ). Incubations exposed to turbulence conditions enhanced average growth rates, irrespective of temperature, with the across strain average growth rate increasing from 0.19 d<sup>-1</sup> (±0.11) to 0.29 d<sup>-1</sup> (±0.15) ( $p = 0.002$ ).

Remarkably, strain variation was the least significant factor among the three treatments ( $p = 0.02$ ) and strains. While three strains (D, K, and G) responded quite similarly, the fourth strain O showed the overall lowest growth rate and did not display the otherwise observed strong enhancement of growth due to increased incubation temperature in still conditions. There was a significant, strain-specific interaction effect (max  $p < 0.002$ ), exemplified by the observation that growth rate of strain O only increased when growth conditions combined turbulence and elevation of incubation temperature.

### 3.2. Movement behaviors

Movement behaviors were highly variable among the six strains investigated, with speed varying over 3-fold and turning rate nearly 2-fold (Fig. 3). Swimming speed of strain G was significantly higher than that of all other strains ( $p < 0.001$ ). Statistically, turning rate of the different strains varied significantly, with strains D, G, and P having similar turning rates, and strains

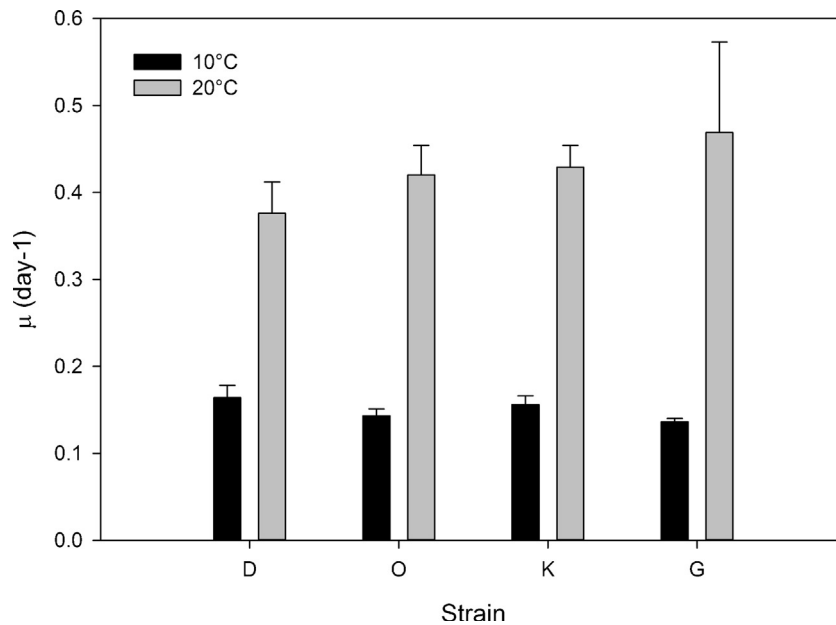


Fig. 2. As in Fig. 1, contrasting growth rates for the same strain across temperatures for strains grown in mild turbulence treatments.

J, K and O falling into a second, statistically similar group ( $p \ll 0.001$ ). Vertical velocity was the most variable movement parameter and ranged from upward velocities of  $50 \mu\text{m s}^{-1}$  to nearly  $250 \mu\text{m s}^{-1}$  between strains (Fig. 3). Remarkably downward velocity was relatively more similar among strains, ranging from 100 to  $200 \mu\text{m s}^{-1}$ . For all but strain G, the upward velocity was lower than the downward velocity. Thus, strain G's significantly higher upward velocity ( $p < 0.02$ ) was rather unusual, as typically it is assumed that sinking and downward swimming speeds are additive and thus result in greater downward velocity. Since for the other strains downward velocity was typically twice or more greater than upward velocity, it has to be assumed that a behavioral component in addition to sinking was in effect.

Direction of cell swimming, particularly upward and downward swimming was restricted to  $\sim 60^\circ$  relative to the upward and downward direction (Fig. 4). Variance among strains was similar in the upward and downward direction, with overall, a relatively tight coherence among strains. The greater variance in upward swimming speeds is reflected in the combined effects of directionality and vertical velocity as well (Fig. 4), with strain G outpacing the other strains significantly in upward but not in downward swimming. Eliminating light from the stimulus repertoire demonstrated that even in the absence of light, a strong vertical bias exists in the swimming behavior.

### 3.3. Predicted distributions

A 1D model was used to predict *A. sanguinea* vertical distribution over time based on the empirically measured

Table 2

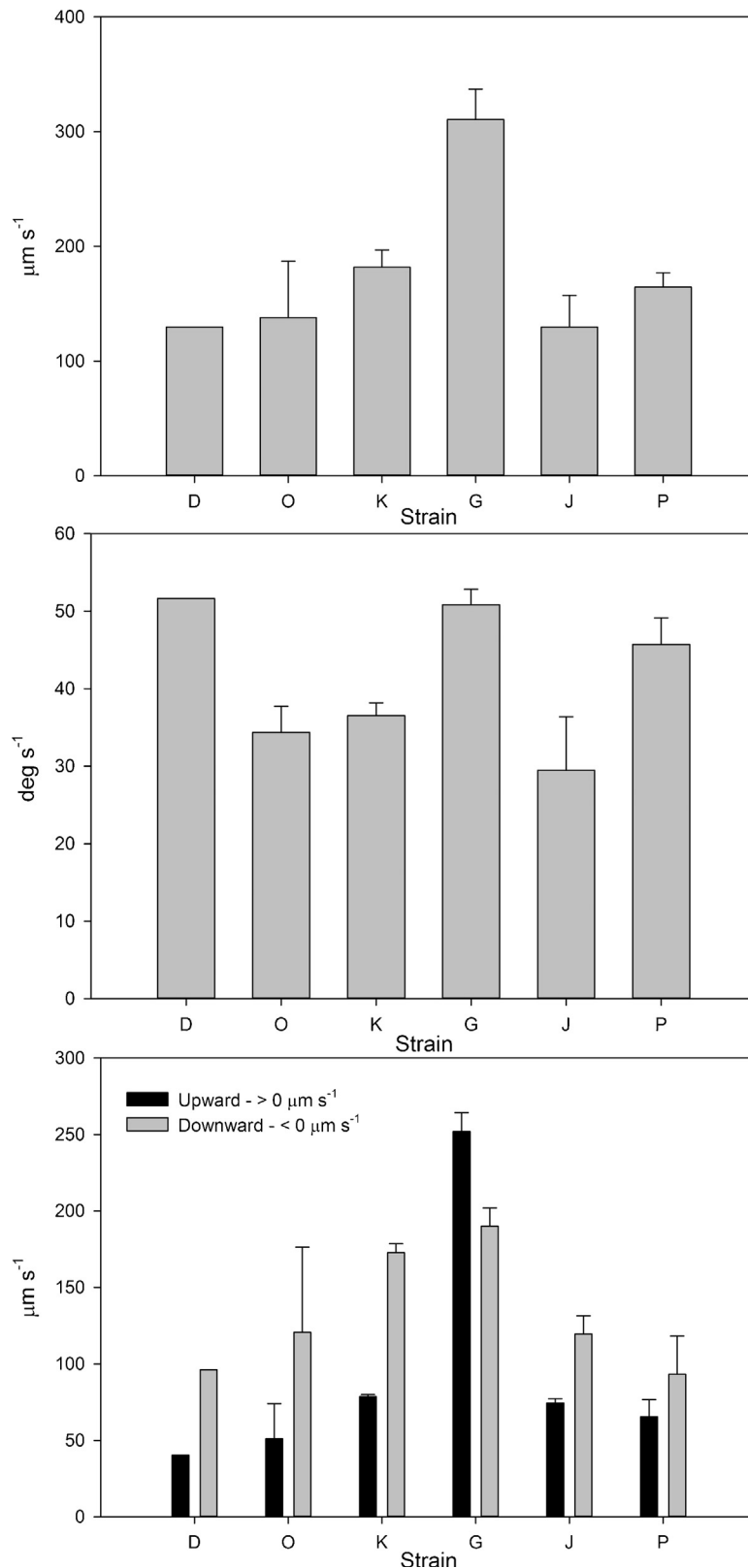
Analysis of variance (3-factor (strain, temperature and turbulence) with replication) examining the relative effects of temperature and mild turbulence on triplicate *Akashiwo sanguinea* growth rates for strains D, G, K, and O.

Source of variation	SS	df	MS	F	p-value
Temperature	0.36952	1	0.36952	81.05	<0.0001
Turbulence	0.04844	1	0.04844	10.62	0.0024
Strain	0.05175	3	0.01725	3.78	0.0186
Error	0.16413	36	0.00456		
Total	0.68231	41			

swimming behaviors, simulating swimming over 10 h and assuming that organisms were initially located at 5 m water depth. The vertical bias in the movement behaviors, and the intra-strain variability in vertical velocity, with a CV of up to 46%, is reflected in the predicted vertical distributions of the six strains, each of which spread both upward and downward in the water column (Fig. 5). It is noteworthy that the strain specific differences persisted and resulted in different vertical distributions. Upward swimming velocity, except for strain G, was generally slower than downward swimming, reflected in a relatively deeper distribution of cells with depth. The high upward swimming speed of strain G resulted in an accumulation of cells at the surface and this strain could have migrated further, if the initial starting point had been chosen deeper. The upper cohort was separated into two distinct distributions, one (strain G) at the surface and the remainder ranging from slightly lower than 2 m to 4 m depth. Lower in the water column, all cells formed one cohort, with cells ranging from a depth of slightly less than 8 m all the way to over 11 m depth. It is noteworthy that some strains had a very broad distribution, reflecting the high variance in their vertical velocity (e.g. strain O), particularly the downward swimming fraction, whereas some strains had very narrow vertical distributions, reflective of the very low variance in vertical velocity (e.g. strain K, 2% coefficient of variation).

## 4. Discussion

The reasons for the occurrence of the largest bloom to date of *Akashiwo sanguinea* off the northwest coast of the USA, both in terms of cell abundances and spatio-temporal extent (Du et al., 2011; White et al., 2014), remain elusive. Our goal here was to quantify the growth potential and motility of several strains of monoclonal isolates from a single water sample of this bloom to estimate whether growth or aggregation could explain the in situ observations. We deliberately included multiple strains of *A. sanguinea* to assess the importance of intra-specific variability in expanding the species' ecological niche, which ultimately enhances potential for persistence in the broad range of environmental conditions encountered over several months and along a several hundred kilometer stretch of the US west coast. Although we could not replicate many of the factors potentially

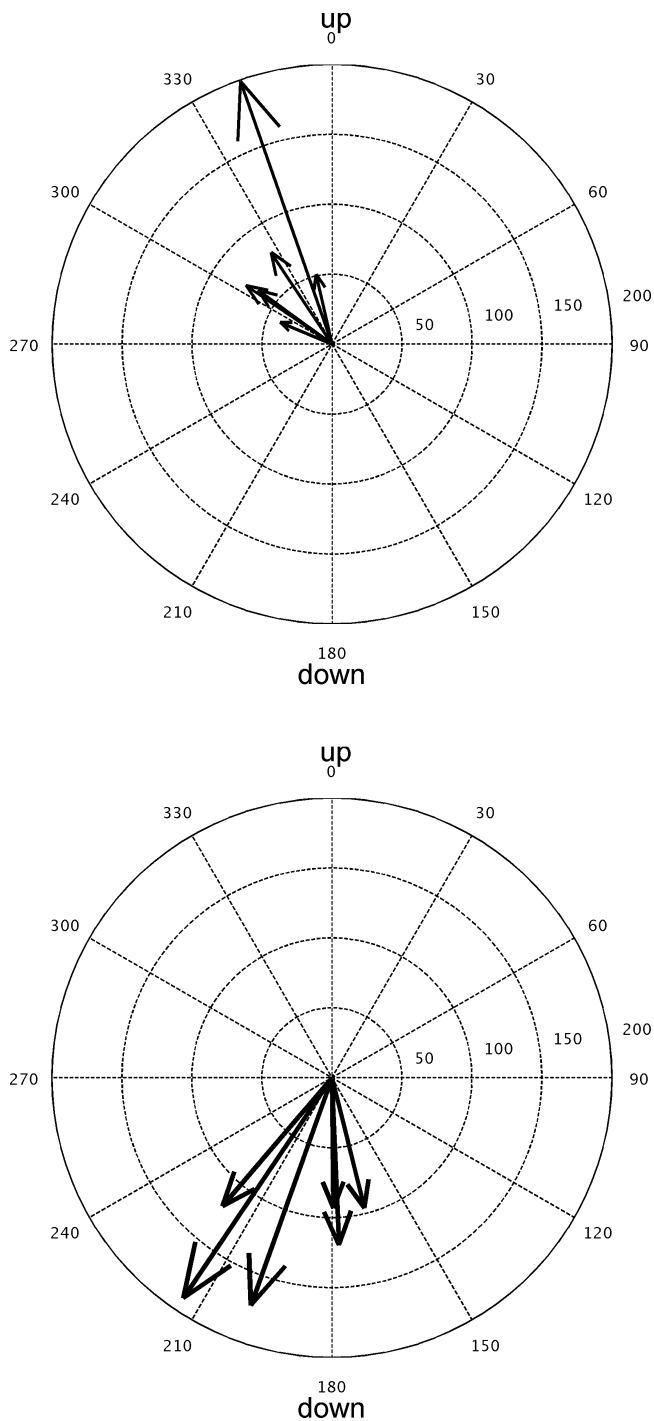


**Fig. 3.** Top: speed ( $\mu\text{m s}^{-1}$ ), Middle: turning rate ( $\text{deg s}^{-1}$ ). Bottom: upward and downward vertical velocity ( $\mu\text{m s}^{-1}$ ) of six different strains of free swimming *Akashiwo sanguinea*. Error bars indicate one standard error of the mean.

important for the growth and survival of *A. sanguinea* in situ, we did include a turbulence treatment. We anticipated that mild turbulence due to water motion would be a frequently encountered condition in situ and this factor has been shown to negatively affect *A. sanguinea* population growth rate and induce cell death

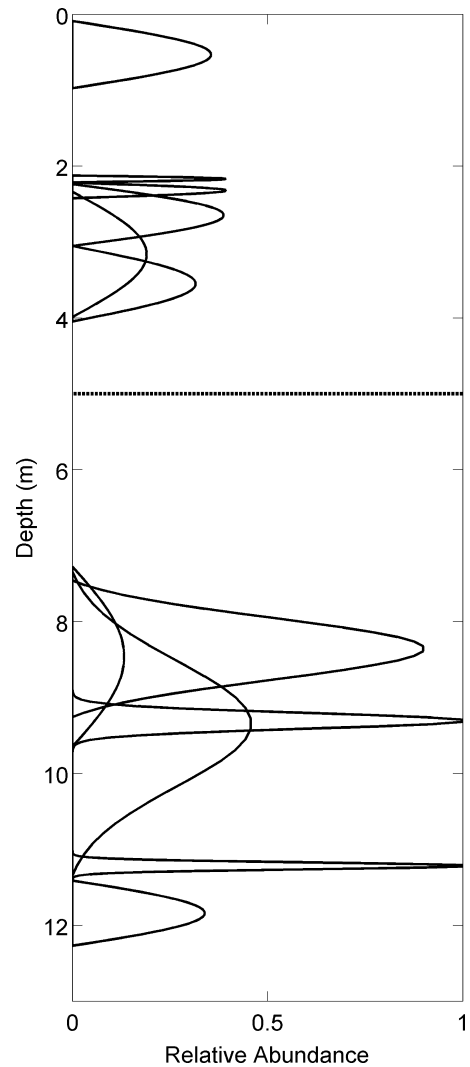
(Berdalet, 1992; Thomas and Gibson, 1992; Berdalet et al., 2007 and references therein).

Our results are in general agreement with prior studies that show that *Akashiwo sanguinea* is a slow grower with significant increases in growth rates observed at higher incubation



**Fig. 4.** Directionality and upward or downward velocity, of up-swimming (top) and down-swimming (bottom) fractions of *Akashiwo sanguinea* strains. The general swimming direction is upward (up) indicated by 0° and downward is indicated by 180°. Vertical velocity is shown by the length of the vector. The grid lines give the scale of the vertical velocity between 50 and 200  $\mu\text{m s}^{-1}$ . Note that for one strain (G) for the upward swimming strains, the maximum velocity was 250  $\mu\text{m s}^{-1}$  but for the data shown the maximum velocity was set to 200  $\mu\text{m s}^{-1}$  to allow better display of the slower upward swimming strains.

temperatures. Even at 20 °C, the maximum growth rates achieved by any of the strains examined here was  $\sim 0.4 \text{ d}^{-1}$ , which is slower than the 0.5–0.6  $\text{d}^{-1}$  growth rate observed by Matsubara et al. (2007). The slower growth rate observed here was likely due to the fact that our study used a lower irradiance. In a 4-strain comparison involving *A. sanguinea* also isolated from the US west



**Fig. 5.** Predicted vertical distribution of the six different *Akashiwo sanguinea* strains, initially introduced at 5 m depth, after 10 h of simulated swimming based on the empirically measured motility parameters. Each curve represents the location (depth) and abundance (height of the peak) of the population. The width of each distribution indicates the strain-specific, relatively smaller effect of diffusion and the much larger contribution of intra-strain variability in vertical velocity, with a CV of up to 46%. It is noteworthy that upward motility had much lower variability in swimming speeds and thus lower variability in predicted vertical range. The high upward velocity of strain G resulted in a separation into 2 near surface distributions, whereas the deeper located cells from multiple strains combined into a continuous distribution at depth.

coast, and two isolates stemming from the same bloom event, a maximum growth rate of  $0.4 \text{ d}^{-1}$  had been measured at 25 °C, with survival at 10 °C but failure to observe growth at 10 °C and above 33 °C (Boyd et al., 2013). It is noteworthy that the strains examined here had the ability to grow at 10 °C, which was the approximate temperature the cells were collected at, but their prior temperature exposure is not known. Thus, all of the strains examined here grew according to previously reported patterns of generally slow growth, and a significant increase was only observed at higher temperatures. There was considerable variation in growth rates among strains, but all followed this general, temperature dependent pattern.

The population growth rate at in situ temperature was  $< 0.2 \text{ d}^{-1}$  and thus, it would require 3–4 days for cell abundance to double. Du et al. (2011) reported *Akashiwo sanguinea* concentrations of up to 800 cells  $\text{mL}^{-1}$  and White et al. (2014) counted peak abundances

of  $>1500$  cells  $\text{mL}^{-1}$ . Assuming an initial, below detection concentration, the maximum concentrations reported could be reached in less than one month of continuous growth at the rates measured here at in situ temperature. That population growth rate, however, is highly theoretical, as it assumes no losses and constant supply of nutrients.

In situ population growth rate was likely much lower. A limitation in our attempt to estimate the suitability of the growth characteristics of this species for the formation of the observed bloom is the lack of measurements of the in situ loss rates. To our knowledge, measurements of in situ mortality rates of *Akashiwo sanguinea* have not been published from this bloom event. *A. sanguinea* has clearly been identified as an important prey type, including for, among others, anchovetta (Lasker, 1975; Lasker and Zweifel, 1978). High predator induced mortality rates for *A. sanguinea* have been reported due to predation by ciliates and other microplankton, which due to the protistan predators often high abundance can significantly reduce population abundance of *A. sanguinea* (Jeong et al., 1999; Yoo et al., 2013). Thus, in situ mortality due to predation must be a factor in the population dynamics of this species. We only had the opportunity to sample the source water but could not estimate predator induced mortality rates, or the intra-strain variation therein, but recognize the importance of this loss term. Thus estimates of the required growth rates to form the observed bloom have to be considered minimum estimates, with any predation incurred losses requiring additional production.

*Akashiwo sanguinea*'s remarkable tolerance of broad temperature ranges makes it likely that cells could grow or survive for long periods, thus permitting a very slow accumulation to form the observed bloom. It has previously been noted, that *A. sanguinea* is able to show positive growth over a considerable temperature range from  $\geq 10$  to  $40$  °C (Matsubara et al., 2007; Boyd et al., 2013). This large temperature range suggests that this species can survive and grow over a large spatial and temporal (seasonal) range, which could support accumulation of cells, or at least persistence, over a long period of time.

It has been reported that for many phytoplankton species, laboratory experiments typically suggest higher temperature optima than temperatures encountered in situ and that bloom formation can occur at much lower than optimum temperature conditions (Karentz and Smayda, 1984; Matsubara et al., 2007 and references therein). This discrepancy between in situ growth temperatures and laboratory based temperature optima is partially rooted in providing cells a refuge from exceeding upper temperature limits, as the latter would be detrimental for cell growth, whereas cooler than optimal temperatures may only result in a slowed growth rate or cells survived without growing actively. Thus, *Akashiwo sanguinea* cells could have accumulated for a long period of time, before a recognizable bloom formed. In fact, Du et al. (2011) suggested that the bloom off Oregon was advected in from Washington coast waters, vastly expanding the period over which cells could have accumulate to form the recorded bloom, while White et al. (2014) drawing on cell counts and oceanographic data from multiple locations along the Oregon and Washington coast suggest multiple, separate initiation events. These multiple initiation events are supported by satellite records of  $5$  °C higher than normal sea surface temperature anomalies that coincided with the period of bloom initiation (White et al., 2014). The reported time elapsed between bloom detection and peak in many cases was several weeks, and often in excess of a month (White et al., 2014), providing ample time for cell accumulation although a question remains regarding the lower detection limit of cells sampled in situ, which typically have to reach several cells per mL before they can be recognized with typical techniques. The most parsimonious explanation seems to be that the positive

temperature anomalies reported by White et al. (2014) provided an additional boost for in situ growth and that the considerable temperature tolerance of *A. sanguinea* allowed for cells to persist, irrespective of temperature throughout the expansive region covered by the bloom.

Traditionally, dinoflagellate biology is thought to be particularly susceptible to turbulence and elevated dinoflagellate abundance, growth enhancing physiology and ultimately bloom formation was broadly linked to calm hydrodynamic regimes because dinoflagellates were thought to be intolerant of elevated turbulence associated with turbulence induced by mixing and tidal exchange, particularly in coastal areas (reviewed in Margalef, 1978, 1997; Smayda and Reynolds, 2000). However, this view has been amply challenged, based both on theoretical grounds as well as empirical observations of tolerance of turbulence by some dinoflagellate species (Smayda, 2000, 2002; Sullivan and Swift, 2003). It is noteworthy that in dinoflagellates the widely distributed trait motility appears adaptive to the tolerance of turbulence (Smayda, 2002). Our observations support the notion that assumptions of uniformly negative effects of turbulence on dinoflagellate biology are not warranted and that broad generalizations about competitive advantages or disadvantages of this group in relation to hydro-dynamic regimes are not warranted. The inclusion of mild turbulence in the incubation conditions had uniformly neutral or positive effects on the growth rates of all strains of *Akashiwo sanguinea*. This contradicts prior studies that have found exclusively negative effects of turbulence on the growth and survival of this species. Of 48 studies included in a review and analysis by Berdalet et al. (2007), over 70% indicated adverse effects of turbulence on dinoflagellate growth rate, including cell death. All studies cited for *A. sanguinea* reported negative and detrimental effects on population growth. Note however, that some of the cited studies showed growth enhancing effects at lower turbulence levels for species other than *A. sanguinea* (e.g. Sullivan et al., 2003). Juhl et al. (2000) reported that turbulence effects were not only species-specific but physiological conditions and growth stage of the culture could affect the magnitude of the turbulence effects. For *A. sanguinea*, turbulence at rates ranging from  $\varepsilon = 0.011$  to  $4.6 \text{ cm}^2 \text{ s}^{-3}$  had uniformly negative effects, including decreased and inhibited cell growth and cell death (Berdalet, 1992; Thomas and Gibson, 1992). The turbulence induced in this study ( $\varepsilon = 0.1\text{--}1 \text{ cm}^2 \text{ s}^{-3}$ ) was on the lower end of the ranges included in Berdalet et al.'s (2007) review, and was an order of magnitude higher than the lowest turbulence treatment reported but yet none of the negative effects reported were reproduced in our study. To the contrary, in our study turbulence had neutral or positive effects on *A. sanguinea* growth rates. Several strains showed no difference in growth rate as a function of turbulence, whereas for a few strains, mild turbulence was essential for survival or growth (e.g. strain O). Moreover, the effect size of turbulence-enhanced growth was much reduced at the lower incubation temperature and only readily apparent at the higher incubation temperature. These observations posit the empirically challenging indication that treatment factor effects have both strain-specificity and are subject to interactive effects.

In this context it is also noteworthy that *Akashiwo sanguinea* is recognized as a type III species (Smayda and Reynolds, 2000) that is characteristic of upwelling systems (Smayda, 2010). Interestingly, upwelling systems tend to be associated with lower water temperatures, which is not conducive to high growth rates for *A. sanguinea*. These observations suggest that, in situ there may be at least a tolerance, if not even a benefit of mild turbulence to the growth of *A. sanguinea*. Furthermore, we also did not observe deleterious effects of mild turbulence on morphology or deformation of cells, as previously reported for this species



(Berdalet et al., 2007) or other dinoflagellate species analyzed using the same turbulence generating technique (Zirbel et al., 2000). Stoecker et al. (2006) posed the question whether trophic mode may affect sensitivity to hydrodynamic regime through observation that *Pfiesteria piscicida* showed a greater sensitivity to small-scale shear than non-heterotrophic dinoflagellates. Finally, Smayda (2002) suggested that mucus formation may be an adaptation by dinoflagellates to dampen increase water viscosity and ultimately reduce turbulence levels. While Jessup et al. (2009) reported mucus formation in *A. sanguinea* which was the ultimate cause of sea bird deaths, we could not detect mucus formation and no measurable effects on seawater viscosity were recordable in any of the strains included here (data not shown). Overall, we could not confirm any deleterious effect of the presence of mild turbulence on these strains of *A. sanguinea* and conclude that in situ, presence of mild turbulence can enhance the growth of some strains of this species and may suggest that turbulence can play a positive role in the population dynamics of some dinoflagellates.

On the other hand, how is an enhancement of growth rate due to mild turbulence explicable? The most obvious suggestion would be an enhanced delivery of nutrients to cells, overcoming limitations due to diffusion (Karp-Boss et al., 1996). However, cultures were kept in nutrient replete conditions, with excess nutrients available throughout the culturing cycle. Juhl et al. (2000) formally examined this line of inquiry and determined that nutrient additions to late stage cultures did not ameliorate negative effects due to turbulence exposure. Thus, although the mechanism of turbulence enhanced growth rates is unknown, it is unlikely due to enhanced nutrient delivery. Since cells are motile (e.g. Kamykowski, 1981), it is unlikely that light exposure was significantly lower in still conditions due to cell shading.

*Akashiwo sanguinea* is a well-known vertical migrator, observed both in the lab and field (Kiefer and Lasker, 1975; Cullen and Horrigan, 1981; Kamykowski, 1981). Field observations and laboratory experiments suggest that these migrations aid in the exploitation of vertical light and nutrient gradients (Rines et al., 2010; Peacock and Kudela, 2014) and the magnitude of vertical migration has been observed to be enhanced by the presence of copepod predators (Bollens et al., 2012).

The vertical distributions predicted based on our measurements of motility however did not show an aggregation of this species at a specific depth, or coherence of several strains, which in situ could be interpreted as a growth independent bloom formation. Thus, based on the swimming behaviors observed here, we can exclude motility as a factor in aggregating cells at the surface. Indeed, the swimming behaviors of *Akashiwo sanguinea* measured here suggest a dispersive effect that would counter accumulation of cells locally. It is of course possible that external stimuli or internal factors not included in this study could alter *A. sanguinea's* swimming behavior in such a way as to support surface aggregations. For example, infection with the parasite Amoebophrya was found to be disruptive to *A. sanguinea's* diel vertical migration (Park et al., 2002). However we found no obvious suggestions that the cultures used here were infected with the parasite.

The spreading of cells based on the swimming speed observations would result in sub-surface abundance peaks. Such sub-surface maxima have been recognized early in the California current system and their importance as possible feeding grounds for larval fish (Lasker, 1975). In the absence of vertical mixing, the observed strain-specific swimming behaviors would lead to considerable vertical separation of some strains. It is noteworthy that studies measuring treatment effects on swimming speed in *Akashiwo sanguinea* have found a reduction in swimming speed of up to 50% in response to turbulence (Berdalet et al., 2007) and a doubling of swimming speeds at higher temperatures (Forward et al., 1986).

Of course, internal and external factors have been shown to alter movement behaviors for dinoflagellates and non-dinoflagellate species, including changes in water carbonate chemistry or scents of predators and prey (Menden-Deuer and Grünbaum, 2006; Harvey and Menden-Deuer, 2012; Harvey et al., 2013; Kim et al., 2013). Thus, influenced by these internal and external factors, in situ swimming speeds may be slower or faster than those reported here. This potential variation in swimming speed, and by implication other motility metrics, further emphasizes that these model predictions provide insight into the potential of cell swimming to affect population vertical distributions and should not be interpreted as predictions for the ocean, because of the much more complex conditions and the many more stimulating factors, such as the presence of light, predators, potential nutrient limitations and other drivers that could alter cell responses and motility patterns as well as mixing and advection that can alter the distributions of cells. These external stimuli, alongside physical aggregation mechanisms could very well promote surface aggregations of cells.

Our results reveal that these vertical migration behaviors do not imply that all strains move synchronously, but rather that fractions can move in opposite directions. The upward and downward moving fractions have previously been estimated at 70% and 30% respectively (Schuech and Menden-Deuer, 2014). These observations imply that *Akashiwo sanguinea* could form multiple discrete layers at high density and that vertically, several peaks in abundance are to be expected with high resolution sampling. In a high-resolution sampling effort however, Rines et al. (2010) found *A. sanguinea* constrained to 2 out of 6 depths sampled, forming a single, coherent layer. Interestingly, discrete layers of *A. sanguinea* in Monterey Bay observed in situ persisted, despite turbulence induced by weak mixing; the same layers were dissipated however by stronger mixing and water mass exchange (Wang and Goodman, 2010).

The predictions of the vertical distribution of *Akashiwo sanguinea* made based on the swimming behaviors suggest that in addition to satellite signals of surface cell accumulations, strain-specific differences in swimming behavior alone can result in substantial cell densities at depth that can even exceed surface concentrations. Thus reports of blooms of the species based solely on satellite measurements may underestimate or even miss subsurface bloom events.

There was considerable among strain variation with respect to all parameters measured. Trait based approaches in plankton ecology (e.g. Litchman and Klausmeier, 2008) are increasingly utilized to characterize phytoplankton functional groups to help categorize the immense species diversity into a comprehensible set of descriptive biogeochemical footprints, ecological roles or factors structuring plankton communities. Similarities in traits (e.g. cell size) are used to establish allometric relationships used to predict cumbersome to measure features from more accessible ones (e.g. Menden-Deuer and Lessard, 2000; Edwards et al., 2012). Genetic markers hold promise to link genetic diversity to trait diversity quantitatively. For example, microsatellite markers have revealed considerable genetic variability in field populations of phytoplankton (e.g. Ryneerson and Armbrust, 2005; Frommlet and Iglesias-Rodriguez, 2008) and this clonal diversity has been linked to differences in traits such as genome size (Whittaker et al., 2012), morphology (Saravanan and Godhe, 2010), and growth rate (Ryneerson and Armbrust, 2004; Campbell et al., 2003). While trait based approaches typically emerge over order of magnitude ranges (e.g. Barton et al., 2013) the considerable variation in growth rates and behavior observed here demonstrate considerable variation at the intra-specific level. Assessment of species' traits based on single strains may not reveal the physiological, morphological, behavioral and genetic diversity necessary to characterize planktonic species. A concerted effort at characterizing both intra-specific variability relative to among

species and group characteristics will be necessary to determine to what degree traits and their ecological consequences vary with abiotic and biotic conditions (Litchman and Klausmeier, 2008) and if those relationships exceed intra-strain variability sufficiently to distinguish among functional groups.

It is noteworthy though that we do not know how frequent each of the strains examined here was represented in the population sampled in situ, as we did not profile molecular diversity. Since all strains were isolated from a single water sample, intra-strain variation may have been even greater in situ, had more strains been sampled at a greater number of locations and over time. Even if the intra-specific variability estimated here is an underestimate, it appears that this strain diversity along with the broad temperature tolerance of *Akashiwo sanguinea* provides a broad ecological niche to the species, and the measured growth parameters, despite slow growth rates, would permit accumulation of cells over several weeks potentially resulting in the observed bloom. Swimming behaviors, particularly the directionality and speeds do not suggest a surface aggregation of cells, but rather a vertically differentiated population, both by abundance and strain. As has been suggested based on theoretical grounds (Menden-Deuer and Rowlett, 2014), phenotypic plasticity enhances species survival and ultimately promotes biodiversity in the plankton. The degree of intra-specific variability observed here provides empirical support for this notion. Tolerance of a considerable range of environmental conditions can serve as a contributing factor to broadening *Akashiwo sanguinea's* niche and provides an adaptive advantage over other species with lower intra-specific variation in physiology or behavior and likely contributed to the formation and persistence of the high abundance of *Akashiwo sanguinea* observed in 2009 along the U.S. West Coast (Du et al., 2011; White et al., 2014).

## Acknowledgements

We thank Harry Nelson from Fluid Imaging for sharing his seawater sample which provided the source cultures for the experiments presented here. We thank students Henry Wladkowski and Samantha DeCuollo for measurements of swimming behaviors and growth rates for some strains and Rudi Schuech for processing some movement data. An earlier version of the manuscript was improved by comments from Elizabeth Harvey and three anonymous reviewers. This research was conducted at the Rhode Island NSF EPSCoR supported Marine Life Science Center, supported in part by the National Science Foundation EPSCoR Cooperative Agreement #EPS-1004057. This work was supported through funding from the National Science Foundation (Bio OCE 0826205) and through a Rhode Island Science and Technology Collaborative Research Grant award.[SS]

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