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Biogeosciences Discussions

Interactive comment on "Modulation of ecdysal cyst and toxin dynamics of two *Alexandrium* (Dinophyceae) species under small-scale turbulence" *by* L. Bolli et al.

L. Bolli et al.

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Before proceeding with my responses, I must say that the somewhat aggressive tone and, we think, unfair statements of the referee suggest prejudices in front of this kind of studies, and turns us off. We do not hide nor selectively choose biased information. The brevity of the paper responds to the following reasons: - we do not show what does not add information to the text - detailed information on the turbulence measurements by ADV are in a paper accepted since last fall in the Journal of Phycology (however, see complete description below) - detailed discussion about the implications of this kind of studies for the comprehension of natural phenomena is also given in this paper in press in the Journal of Phycology; we do not want to repeat our previously published information - page charges are also an issue

General comments: Referee comment: "The results are not unique" (S435). There are previous data in net growth and cell size on Alexandrium catenella (Sullivan et al. 2003) and Alexandrium minutum (Berdalet et al. in press). The two references are already indicated in the text. However, there are not at all previous data on the interference with ecdysal cyst and toxin production in those species, and those aspects are the focus of the present paper.

Referee comment: misleading terms "low" and "high turbulence" (S436). They are relative terms, that should be read in the context of the present study, not concerning high or low levels in nature: "low" refers to the intensity generated by vertically oscillating grids, "high" to the orbital ones.

Referee comment: the experimental design "may have little or no ecological significance" (S436). This is essentially a physiological paper. Physiological experiments often force the cells to unveil the capacities of their metabolism, which otherwise would be difficult to note. This applies to most photosynthetic, respiratory, reproduction or nutritional studies, and many papers are published in reputed journals as Nature or Science. We do not hide this, as indicated in the first paragraph of the discussion: "The much higher epsilon generated in the orbital shaker (27 cm2s-3) would be even higher than those associated with intense wind conditions (>20 m s-1, ... Although our experimental values of epsilon are very high, both in intensity and persistence (Guadayol and Peters, 2006), these conditions may help to ascertain the underlying mechanisms of cell adaptations."

Referee comment: "I challenge the authors to document an epsilon of 27 cm2s-3 (S436)." We do not need to be challenged for this, as we know sustained turbulence of this intensity is a rare event. We cite Guadayol & Peters 2006, which estimate natural levels of epsilon in the NW Mediterranean. We are a multidisciplinary research team that share our experiences in the study of turbulence events both in the laboratory and the field, and are very concerned about the estimation of epsilon in our experimental setups, as our publishing record shows.

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Referee comment: "the results may simply represent laboratory artifacts that never occur in nature (S436)." We are careful in not making grandieuse inferences. In the discussion we say: "(Concerning toxins) In consequence, it is too soon to draw general conclusions and the question about the possible effect of turbulence on toxin production is an open one for future research." and "On the contrary, ecdysal cysts must be essential phases of the life cycles of these organisms playing a major role in population dynamics of certain dinoflagellates and requiring stability of the water column to proceed. Certainly, careful studies focussing on the link between small-scale turbulence and the different aspects of the life cycle of dinoflagellates will shed further light to understand the dynamics of this phytoplankton group in nature."

Referee comment: "This is an example of the subjective nature ... the context of the older paradigm ... turbulence is detrimental to ... dinoflagellates ... chosen to only highlight negative results ... contrary to their dogma (S437)". Definitely, the referee and us have opposite ways of looking at our data. We do not believe in any older paradigm that turbulence is detrimental to dinoflagellates, otherwise they would not exist. This is not "our dogma". Otherwise, we could have avoided mentioning that they grew better in the vertically shaken grids. Our idea is that dinoflagellates have the capacity to respond with their behaviour to turbulence. To show this capacity we expose them to high turbulence levels in the lab. We never said in this paper that "turbulence is detrimental".

Abstract Referee comment: turbulence units. It would not be any problem to indicate both units, because they are used in the literature: 1 Watt kg-1 = 1m2s-3 Materials and Methods Referee comment: "£Why didn't you use one method at different speeds to generate the needed turbulence levels (S438)?" On one side, using the vertically oscillating grids we can not generate levels higher than 1 cm2s-3. On the other, with an orbital speed lower than 100 rpm the fluid motion is not turbulent, but homogeneous. The two systems have been previously used by us, we have controlled carefully the physiological state of the mother cultures too and the rest of environmental factors, so

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that at least, we can compare with our previous experiments. We indicate it in the text: " This study was done using the same experimental devices and designs of previous studies (Berdalet, 1992; Berdalet and Estrada, 1993; Havskum et al. 2005; Berdalet et al., in press) to allow direct comparison between results. (p. 896)"

Referee comment: "questions about page 896, lines 16-19 (S438)". Detailed information on the turbulence measurements by ADV are in a paper accepted since last fall in Journal of Phycology, but that unfortunately will not be published until October 2007. The explanations are as follow: "Turbulence was measured with a customized side-looking acoustic Doppler velocimeter probe (NDVlab from Nortek AS, Norway). The stem was detached from the signal conditioning module and held in place vertically with a hydraulic articulated arm (Hoffmann Group, Germany) (Fig. 1 of J. Phycol). A Florence flask was customized widening its mouth gap to allow the insertion of the probe. The flask was firmly held in place on the shaker table with bars and clamps. The articulated arm holding the probe was also firmly secured on the shaker table. Cabling reached loosely to the conditioning module about 0.5 m away from the shaker table. From there, an standard connection went to the data acquisition board in a personal computer. The turbulent kinetic energy dissipation rate (epsilon) was estimated from the -5/3 slope of the energy spectrum plot of each of the 3D velocity time series. We used the linear regression method developed by Stiansen and Sundby 2001 that allows for filtering out the instrumental white noise. A total of 47 measurements were done at different points in the container and for rotation speeds from 60 to 125 rpm. Turbulence at a particular point in space was considered isotropic since dissipation rates calculated for each velocity component were similar, at least up to the minimum distance from the wall that was measured (1.5 cm). A multiple regression model with rotation frequency (omega, in Hz), distance of the probe to both, the wall (d, in cm) and the water surface (s , in cm) as explanatory variables was fitted to epsilon log (epsilon) = -8.08 + 6.36omega -0.26d -0.24s; n = 47; adjusted multiple r2 = 0.89). Rotation frequency was the most important explanatory variable (80% of the variance) while and accounted for the spatial variability within the flask. At 100 rpm, turbulence ranged

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from 0.27 cm2ůs-3 in the middle of the container to 24 cm2ůs-3 near the walls. At this rotation frequency, a bulk value of 2.01 cm2ůs-3 was estimated by applying the above model to the equation of a truncated sphere, from the bottom of the flask to the height of the water level, at 1 mm spatial increments, and averaging the data."

In additon, much more and detailed information is part of a paper submitted to Limnology and Oceanography Methods entitled "Evaluation of oscillating grids and orbital shakers as means to generate isotropic and homogeneous small-scale turbulence in laboratory enclosures commonly used in plankton studies", by Guadayol, Peters, Stiansen, Marrasé and Lohrman.

Referee comment. "page 898, line 21: Why is this data not shown?" As I said at the very beginning, this is mainly an economic reason. The data can be summarized in a table (similar to Table 1) as also suggested below.

Referee comment. "page 898, lines 22-24". Sorry, we do not understand what the referee misses. The statistics is both in the text and Table 1. There were not significant differences between the growth rate in the cultures shaken only during the exponential pahse and those always shaken (p=0.704). For simplicity, we do not repeat this in the text. Of course, we will add it if required, no problem.

Referee comment. "page 899, lines 1-3". We do not selectively report data. At the beginning of the results section, we have indicated that "The Alexandrium minutum cultures exposed to the high epsilon intensities generated by the orbital shaker for more than 4 days (Always treatment), had a significantly ($p < 0.0001$) lower exponential growth rate compared to that of the unshaken ones (Table 1, Fig. 1A). page 898, lines 22-24" In Table 1, the reader can see that the cell numbers at the end of the experiment in the turbulent treatment was the 96.5% of the unshaken ones. For simplicity, we do not repeat this in the text. Of course, we will add it if required, no problem.

Referee comment. "page 899, lines 4-11, you really need to provide a second table that statistically summarizes all of your cyst and toxin results (similar to Table 1)." I will

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make the required table and the editor can decide whether it is necessary to be added in the fianl version, if accepted.

Referee comment. "page 899, lines 12-18". OK. See my response above.

Table 1 Referee comment: "a) request for 2 decimal instead of 3". Growth rate is estimated as the slope of the regression line of ln(N) versus time (t), where N is the estimated cell concentration in the portions of the growth curve showing exponential increase. The 3 decimals respond to this mathematical calculation. It can be done as required. No problem.

Referee comment: "b) reporting epsilon in the table". It can be done as required. No problem.

Discussion Referee comment: page 900, lines 2-3 (S441). Well, we say "Interestingly, ...". Is this a way to say that we underestimate our results?

Referee comment: "This is an incredibly important result." (S441). We are perfectly aware that dinoflagellates may develop in well mixed waters, we cite Smayda and Reynolds 2001, who specifically describe this situation. But also, investigators of our Institution reported it as early as 1979: Estrada, M. & Blasco, D. 1979. Two phases of the phytoplankton community in the Baja California upwelling. Limnol. Oceanogr. 24: 1065-80. The discussion in the present paper is not at all whether turbulence can enhance or hamper the outbreak of dinoflagellates red tides. We study the physiological processes that may eventually be sensitive to high turbulence levels.

Referee comment: "speculating about how "high biomass" results could be artifacts" (S441). We do not treat them as artifacts. We explain possible ways to explain a better growth, so that turbulence benefits dinoflagellates. We do not see a problem there.

Referee comment: "even if challenges your preconceptions" (S441). We do not have preconceptions. We are sorry that the referee seems to be looking at the paper with his/her own preconceptions about the usefulness of laboratory experiments with turbu-

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lence. In the introduction, although very synthesized we indicate that either positive, indifferent or negative responses to turbulence have been observed in dinoflagellates (Berdalet and Estrada, 1993; Sullivan and Swift, 2003; Havskum et al., 2005; Havskum and Hansen, 2006). We are perfectly aware and not biased at all. What is true, is that dinoflagellates seem to be the phytoplankton life-form with the highest capacity to respond to turbulence.

ELISA BERDALET Barcelona, May 2007

Interactive comment on Biogeosciences Discuss., 4, 893, 2007.