Pike Spector
Coastal and Marine Institute
San Diego State University
310.880.9311
mspector.ecology@gmail.com

Review of "Photosynthesis of *Laminaria digitata* over the immersion and emersion alternation of spring tides under sunny and hot weather*"* – Milgné et al. *in review*

General Impression

Overall, I was very intrigued by this paper's novel experimental design. They correctly assert that, "…multifactorial experiments have rarely been conducted. Generalization to field conditions remained thus hazardous.". Given this claim, and proof of concept, this paper merits publication. However, I think the authors would benefit from proofreading this manuscript more carefully, and synthesizing their findings in a more contextual manner.

Milgné et al. provide a very clear understanding of the "phytophysiology" of an intertidal alga during stressful tidal emersion. The multiple stressors, or synergistic effects, of prolonged tidal emersion have profound implications for intertidal species zonation. However, the authors did not address this in the context of their findings, nor did they provide a clear understanding of how their findings fit in to the general theme of ecology. They would be well served by extrapolating on the range expansion of *L. digitata*, and what this might mean for intertidal zonation in the English Channel under a changing climate.

After reviewing this manuscript, I recommend it for publication after minor revisions. At the very least, the authors should spend some time addressing spelling and grammatical errors. However, I recommend that the authors revisit their model, and consider restructuring both the Introduction and the Discussion.

Introduction

Based on the abstract, this paper provides an exciting look into the parameters affecting the photosynthetic efficiency of an intertidal alga under inherently stressful conditions. The authors do a relatively reasonable job of introducing kelps into the landscape of marine macrophytes, and the previous studies that have addressed their photosynthetic efficiency. However, the authors jump abruptly from previous studies to the novelty of their study in the first paragraph. While this ambitious study provides exciting context for *Laminaria digitata*'s productivity under tidal immersion/emersion cycles, I think the authors should spend more time introducing the study species, the study location, and the motivation behind this study. The sentence "This boreal species approaches there its trailing edge which is expected to shift northward in the context of the global warming" is the only argument for the implications of this study under a broader ecological context. I recommend that the authors condense the novelty of their study, and make their claim at the end of the introduction, after providing more context regarding the natural history of *L. digitata* (such as the zonation of the "kelp belt"), and the broader significance of this study.

Further, there are several spelling errors and inconsistencies throughout. The authors should devote some time to correcting grammatical spelling errors, and consult the primary literature for appropriate terminology (e.g. "*L. digitata* germlings" might be better phrased as "L. *digitata* juvenile sporophytes").

Methods

The "Materials and Methods" section was hard to follow. I recommend that the authors follow their section breaks more closely. For example, the authors spend considerable time describing the sampling protocol under the "Study Site and Measurement Schedule" subheading. These descriptions would be better placed under their appropriate subheadings, which will facilitate space for a more detailed description of the study site. Further, the logic behind the site selection for this study was lacking under the first subsection; why was Roscoff chosen, what properties about this site make it appropriate for this study? This first subsection might be better expressed in a table (similar to Table 1).

The subsequent subsections are well described, and mostly free of grammatical inconsistencies. However, and most importantly, the details regarding Net Carbon Production (NP) are not well described. There are a lot of papers that claim to quantify the *net* productivity of an individual or a community; the authors should pay special attention to both their description of NP, and their use of acronyms ("NCP" is used at least once in the methods, although they define Net Carbon Production at NP). Further, I am not convinced by the authors' quantification of NP in their methods. "…[NP is] the balance between gross primary production and respiration"; typically *net* production is given as the difference between gross primary production (GPP) and respiration (R). Perhaps this is a phrasing issue and not a quantitative one.

Regardless, I would definitely like to see a schematic or picture of the "benthic chambers". Specifically, I would like to see how the chambers are sealed to the benthos (or sealed from the environment and anchored in place), and how the pumps work to displace the internal volume of the chamber during immersion and emersion. I would also like to know how the chambers behaved during tidal emersion; did the authors control for possible "greenhouse effects" potentially caused by the Perspex material? Did the authors physically remove *L. digitata* individuals from the benthos and place them in the chambers? The sentence, "Sporophytes were left standing in the field over night to be investigated again the following day" implies that individuals were removed and placed in chambers. What impact does this have on the health and productivity of an *L. digitata* individual?

Again, there are several, relatively minor, spelling and grammatical errors throughout. However, the methods seem very appropriate for this study. Clearly, the authors are well versed in the laboratory protocols detailed throughout the Methods. However, I am uncertain about the validity of this procedure: "…Taken back to the laboratory, it was rehydrated for one night to assess its fresh weight (FW) and then dried for 48 h at 60°C to assess its dry weight (DW)." How does this affect estimates of FW and DW?

Results

Overall, I was very surprised with the lack of statistics in the Results section. While the authors do a good job of outlining the variables they are working with, I would have liked to have seen tables with data, and the equations used to calculate NP. At the very least, I would have liked to have seen the equations used to calculate the fluorescent properties and the de-epoxidation ratio for *L. digitata*. These equations are given in the methods section, but they would be better served (along with a better explanation) in the Results section.

Further, the authors conducted this study over three two-day sampling periods (one in 2010 and two in 2011), which means that the authors can then compare the abiotic factors affecting *L. digitata* productivity using time as a fixed factor. A series of simple mixed model

ANOVAS can easily help explain why the performance of *L. digitata* varied with factors such as immersion/emersion, daylight and ambient air temperature.

Discussion

The authors do a good job of linking their study, which is inherently novel, to a general understanding of algal physiology. They were relatively conservative in their assertions, but this is not to their detriment. However, as in the Introduction, I would have liked to have seen conclusions drawn to our understanding of patterns of zonation in the intertidal, and the potential implications for a northward migration of *L. digitata*. In fact, only the last paragraph addresses this pertinent observation. Will *L. digitata* competitively outcompete aother intertidal algae as it makes it way northward? The authors appear to assert that *L. digitata* might benefit from a warming climate. Yet, the final sentence of their abstract, "…this reinforces the expectation of detrimental effect of warming events on this marginal population of *L. digitata*" suggests otherwise.

While the merit of this study is implicit, the discussion reads as merely an explanation of phenomena observed in the lab and in the field. The authors should consider synthesizing their results with a little more creativity, especially so that this study's results can be interpretable at a larger scale.

Only a few instances of grammatical/spelling errors were found. However, the authors should pay close attention to their use of acronyms. For example, "…ETR remained ~~to~~ at a relatively high value…"

Figures

My main comment about the figures is the order of days in Figures 1 - 4. The authors present data from 2011 ahead of two consecutive days in 2010. This reads as: Time 3, Time 1, Time 2 but is erroneously presented as Time 1, 2 and 3. I was further confused by the data presented in Table 2. This Table is not presented in context, and uses uncited data from 2008.

Again, a schematic and/or picture of the benthic chambers would have been very useful here, as well as perhaps a contextual image of *L. digitata in situ*.

Suggested Edits

<u>Introduction</u>

•Page 3, paragraph 2

"This boreal species approaches there its trailing edge which is expected to shift northward in the context of the global warming". This phrasing is confusing, and contradicts statements later in the manuscript that suggest a reduction in *L. digitata*'s range due to changing climatic conditions.

"In the south- western English Channel, low spring tides occur around noon and *L. digitata* can then be exposed to over-saturating irradiances." Do spring tides *always* occur around noon? Is it *always* sunny at noon? Consider rephrasing.

"A previous survey allowed to relate the pattern of photosynthetic performance of *L. digitata* sporophytes to changes in underwater light during spring tides in the mid part of the kelp belt which remained underwater at low tide". Awkward, consider rephrasing.

Consider replacing "germlings" with "juvenile sporophytes"

"Furthermore, at emersion light stress combines to water and nutrient depletion as well as to rapid changes in temperature." Very awkward, consider rephrasing

"To date, …, has been notified in intertidal *L. digitata* populations …" "Notified" should be "noted".

•Page 4 paragraph 3

"Pigments involved in the xanthophyll cycle, the main mechanism of photoprotection of this species (Rodrigues et al. 2002), were also measured out." What does "measured out" mean? Does that mean the pigments were extracted?

"…very sharp environmental changes." Consider rephrasing, "…extreme environmental changes"

Methods

•Page 5 Paragraph 1

"…and measurements were performed from 11am to 3pm; NP was measured during 5 successive incubations (3 under immersion and 2 under emersion) …" Numbers less than 10 (not related to units) should be spelled out (i.e. …was measured during five successive incubations…)

"…NCP was measured during 9 successive incubations (2 under immersion, 2 under emersion and 5 under immersion) …" Same comment as above; NP is reported as NCP here.

"Sporophytes were left standing in the field over night to be investigated again the following day" What does this mean; were sporophytes removed from the benthos entirely? Chamber methods unclear.

•Page 6 Paragraph 4

"A *Laminaria digitata* adult sporophyte (frond length of about 1 m) from the upper part of the kelp belt was placed by divers inside a benthic chamber, on the shore at the collection site, to measure its net carbon production (i.e. the balance between gross primary production and respiration)" Same comment as above, chamber methods unclear; how were the chambers attached to the benthos? Were *L. digitata* individuals removed from the benthos and placed inside a chamber or was the chamber placed around an *L. digitata* individual?

Laminaria digitata should be consistently abbreviated as *L. digitata*.

"i.e. the balance between gross primary production and respiration" This may be stylistic, but this is unclear. NCP should be reflected as the *difference* between GPP (*always* positive and respiration (*always* negative).

"During emersion, inorganic carbon fluxes were measured in the benthic chamber using a closed air circuit for CO2 analysis" Please expand on the chamber methods; a diagram or picture would be very helpful. What happened to the chambers during tidal emersion? Was there a greenhouse effect?

"The sporophyte was weighted between two consecutive…" I believe "weighted" should be "weighed", otherwise the authors need to explain this.

•Page 7 Paragraph 5

"The effective quantum yield of photosystem II (ΦPSII) was measured under ambient light." Ambient light in the lab? Are these light levels reflected in field measurements? If not, is this ecologically relevant?

•Page 8 Paragraph 6

"…small drops of methylene chloride under dim light." Same comment as above. Was the light dimmed to reduce phototypic effects in the lab?

Results

•Pages 8 and 9 Paragraph 1

"The carbon flux inside the benthic chamber containing a *Laminaria digitata* sporophyte was negative, indicating ~~a~~ carbon uptake (i.e. gross primary production greater than respiration) …"

Again, should keep reference to *L. digitata* consistent.

This phrasing is a little misleading, the authors should consider rephrasing "We saw a drawdown of carbon inside the chamber, which is indicating of productivity, thus GPP was greater than respiration…"

"Under emersion, the carbon flux was positive, indicating a carbon release (i.e. respiration greater than gross primary production)" Same comment as above, and the "a" before "carbon" should be removed.

Discussion

•Page 10 Paragraph 1

"…but ETR remained to a relatively high value (about 17 μmol e- m-2 s-1) under emersion" "To" should be changed to "at"

"At the sporophyte scale, the net primary production…" This is the first instance net *primary* production is mentioned; if the authors would like to discuss NPP they should introduce it sooner and in greater detail.