

Seagrass Workshop
Everglades National Park

Michael Durako

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Video Transcript

00 minutes :19 seconds

Note: This video begins with a conversation in progress.

Michael Durako: This is the year after, and what it shows is basically autocorrelation. I will change it to autocorrelate. And, what we see in ranking that what we start out with is low densities that are basically contiguous increases. However, this record ends in '97 unfortunately. [indecipherable] that were severely affected by die-off and they are on a trajectory. Penny will show later that trajectory continued basically it reached a very high density whereas where you see these kind of cyclical patterns basically at Twin and Rabbit you got oscillations. What we notice is like in Madeira and Blackwater that is interesting is that we have an increase early in the record and something happened around '99 or 2000 then there is a decline in both of these systems. Basically a decline in density which correlates to some sort of phase shift. At Crane, where there was just a relatively small oscillation and high cover and an event happened. And, now there is a small oscillation and a low cover so something happened in '99-00. I'm going to talk about how there was actually a die-off back then. And, then we have these sort of spirals at Twin, I'm going to talk about that also. This sort of works a correlation through time that gives us sort of a sense of this oscillation. This is all Thalassia. This is an autocorrelation down at Johnson Key and at Rankin. And, this is a lag of years and how autocorrelated the densities are and that there is a long term cycle going on with Thalassia. I bring this up all the time, it takes decades, you know, for this plant to recover from a major perturbation. One of the questions we always get is are we in equilibrium? Do we have a baseline for places that had die-off or none. We haven't reached that place yet, we haven't reached full circle. And, now for something completely different. I just wanted to throw that out about the discussion we'll have this afternoon.

Participant: Did you say that Thalassia has not recovered?

Michael Durako: It's not that it hasn't recovered. It's that basically there is still succession going on, it's still coming back in those two bases.

Participant: Did I understand that the last graph that you had suggested that there's an upwards of a 6 year temporal lag?

Michael Durako: Actually it looks like it's longer, basically it's positively correlated and then it gets negatively correlated. And, we go back through the cycle we got twelve years. After twelve years and it hasn't come full circle. When it's seasonal you'd expect to go like that in a twelve month, January to January. And, so for this we are seeing a really long term process. This is like the redwood tree, we have to think about changes like that when we are talking about *Thalassia*. And, now we'll talk about things that happen in millisecond time frame.

We've been looking at *Thalassia* using chlorophyll fluorescence since about 2000. So why do we measure photosynthesis? It is very sensitive to stress. It's sensitive to light, salinity, sulfide, stress and it's well known. And, one of the reasons we wanted to apply it to our FHAP program is that photosynthetic changes will precede mortality and morphometric changes. It should give us an early warning indicator of something actually happening in the system before we actually have to have mortality. Density can only change when you kill shoots. Photosynthetic changes can occur in the short term. In PAM fluorescence, pulse amplitude modulated fluorescence, we use it to measure photosynthetic efficiency, capacity, and acclimation, that measured in situ using an optical system is very rapid and nondestructive. Before we were taking cores and describing density, that's destructive.

PAM fluorometry is well established as a method for looking at stress in seagrasses. You can see just a few of the many references. Oxidative stress, UV, pathogens and herbicides, high/low irradiances, desiccation and salinity stress. All have been well documented to have changes related to measures of PAM fluorometry, which I will describe right now.

So what PAM fluorescence measures is fluorescence before and after a short saturating burst. And, it's based on the theory that basically when light hits a photosynthetic system it has three fates.

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Photosynthesis, the energy used in the electrophotosynthetic transport system. It can generate heat, excess energy that is not used in photochemistry. Or, it comes back as fluorescence as the electrons come back to the ground state. In the PAM world, it is such a short burst that heat is negligible. So basically you have light that comes back as photochemicals or as fluorescence. And you measure a base yield and then the yield after the saturating pulse under dark acclimated conditions. And under dark acclimated conditions when everything's relaxed you get the maximum quantum efficiency. In the light where some of the reaction centers are closed and where you have some of these dissipation processes going on you get an effective yield which is always well within maximum yield. If you have the effective yield, you know what the light level is, and you have the absorption of light you can generate something called electron transport rate, which is a proxy for actual photosynthetic rate and fairly highly correlated to oxygen and so it is a measure of photosynthesis and is nondestructive.

So I have to put this slide up. We are talking about a recent die-off event. And it started in 1988. Paul Carlson and I jumped in the water in 1988. That was the original die-off. For a couple of years, Paul and Jay came up with a sort of conceptual model of the die-off as we understood it up to that point. And, the point here is that photosynthesis, low photosynthesis, high respiration, were central to both of these photosynthetic stress different driving factors, salinity stress, sulfide stress. Things you are going to hear

about later, but photosynthesis was central in the original die-off. Here's that [indecipherable] field that appears in the original die-off. I don't know that you saw that last time.

So back then, this is pre-PAM, we used an oxygen and electrode system to actually look at shoots that had been infected by a brine slime mold, which sort of dropped of discussion over the last decade. But, during the original die-off and this red area shows the distribution of this original die-off, it had a contiguous distribution. Paul and I got out of the water on our first dive and we said this looks like a disease. It was a very distinct disease and it was associated with lesions that were associated with the green slime, *Labyrinthula*, and we verified post postulates, like Marguerite's. Post postulates is a traditional microbiological technique where you have an infectin, isolate the organism, reinfect an uninfected tissue, which is shown right here where we had shoots that we put *Labyrinthula* on, grew live seedlings and got lesions every time. Using the optical electrode system we got photosynthetic oxygen against the gradients. What you might not be able to see is that these are healthy shoots, these are shoots with *Labyrinthulian* lesions, this is the zero line. Efficiency decreases with the increased lesion coverage and respiration increases. *Labyrinthula* can kill these shoots or make them more susceptible to sulfide toxicity, which is probably moving toward the mode of operation.

So in 2000 there was another die-off event that was observed in summer. It was fairly significant in June and July. And, I just got a PAM barometer so we looked at some die-off patches in Sunset, Cross Bank, and in Barnes Key. This is showing the leaf lesions. This is the effective yield. This is a clean leaf segment and a lesioned leaf segment. Not surprising a decrease. And, here is a transect into a healthy bed, ecotone of a die-off patch and in the die-off patch a decreasing yield. Showing that these plants were photosynthetically stressed, lower quantum yields.

Barnes Key is where the most significant patches were occurring. We use an meaning f-hap, we used a mini f-hap to do some mapping. The distribution of the die-off patches, which are shown here, broadwalk k data, the greener it is the greener it is. Brown indicates die-off. Very sharp ectones and we looked at yield. Green short shoots at these sites versus die-off short-shoots versus ecotone short shoots. Again you can see a decrease in yield. We also found, we did some measurements on some of Paul's bucket experiments and he added acetate and glucose which spikes up the sulfide. We also did see a decrease in yield. So both synthesis is sensitive to stress.

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So since early 2001 we have been looking at this instrument as an early warning sensor of a condition. I'm going to start talking about the classic. This is a map of FHAP in South Florida as it was in its greatest extent, but I'm just going to talk about the Florida Bay stations today.

And, what we use is this tessellated hexagonal system. We have 30 hexes per basin, a random point within each basin. It gives us a systematic sampling design that is good for interpolation and maps. The important point for the PAM work is that we sample each point in the course of a day, we have fifteen minutes per station, which is a very rapid sampling technique where you run along [indecipherable] sampling it and do PAM sampling at the same time, and we take a route that minimizes our traveling

time so we hit banks and each sampling station randomly which becomes more important during the day, and we do it no matter which the weather which becomes important.

So the PAM methods that we use we measure the middle of the rank of the 2nd blade. Which is the youngest fully formed blade, which is robust and probably hasn't started to senesce, doesn't have epiphytes usually on it. And, we did a lot of statistical work determining that. We know have a custom made leaf acrylic tip that we use. It's just a distance clip we use to minimize light reduction. We really wanted to not have quasi-dark adapted which changes the yields quite quickly. We measure six shoots per station at least 2 meters apart to try to get individual plants but these are clonal plants. And, then we measured bottom PAR with a scalar sensor so we have an idea of the light environment when we take these measurements.

So this is from the spring of 2001, the first time we applied the instrument to the first yield, first effective yield mat. These are effective yields, from low to high yield, green means really health to robust yield, yellow is lower and red is bad. And, you know if this was an early warning system everyone in the park would be jumping all over eagle key basin saying oh my god, the seagrasses are dying, the seagrasses are dying. There is something weird with this data. If we look at the data more closely what we realize is that any station that we sampled later in the day tended to have lower yields and we were cautious. So we said there is something wrong with these data. So when we plotted the data as a function of time of day, and these are effective maximum and minimum yields or irradiance, we see the significant time of day effect, which is driven by the irradiance. So there is an irradiance signal and this is what we have been having to work with. Throughout the day we have the light yield changing. We know that as irradiance increases the yield decreases because there is an increase of thermal dissipation, [indecipherable] cycle basically as you increase light you lose efficiency. Because you are filling up the electron transport system and more energy is going into alternative pathways. These are called photoprotection. There are a number of mechanisms plants use to photoprotect so they don't have damage. And in extreme conditions you can actually get D1 protein damage. This is the protein in photosystem 2 that surrounds the chlorophyll that gives you the fluorescence and that's true photoinhibition. These are different terms. Photoprotection is dynamic, photoinhibition is actually destruction. So what is known now, more easily is that the efficiency of this dissipation process, in other words, you get a negative slope as you increase irradiance, it tells you a little about the photo acclimation state and if they are stressed. If you are stressed, you are going to have a much steeper slope than if you are not stressed. If you are shade acclimated you are going to have a much steeper slope than if you are sun acclimated. You are going to be more efficient at dissipating this excess energy. And, this is light and this is yield. So we know that from experimental work.

So, this is the data from the last four years of work, 2006, 2007, 2008, 2009. This is PAR, the irradiance level and these are the effective yields. The stars in the middle, which you might not be able to see, this is a big part of the PAR dataset, is the average yield and the average PAR for the whole bay. So it's just the grand mean of this dataset. And, what we can see, what I can see, you might not be able to see it back there, is that both the mean bottom irradiance has increased in the last four years and the effective yield has decreased, which one would expect. It's a pretty pattern and I'll show you something that shows it even more clearly. What's interesting is that when I look at the regressions, you can also

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see that the slopes have declined, indicating a better ability to deal with excess energy, reflective of they were sun acclimated or more healthy. The intercepts have also declined giving an indication that the community at the bay level were sun acclimated through time.

These are just the stars of 2006, 2007, 2008, 2009. So you can see, this is the bottom irradiance, the water is clear. This is the sun. This is where there is more sun. The sun is brighter where the water is clearer. It's more shallow. So there is a pretty clear distinction between, and this is when the phytoplankton was growing, if anyone was worried about this. Things look like they are getting better.

We looked at the intercepts, we see a similar pattern. Higher intercepts early, lower intercepts later. And, if we put the slopes in here, shallow slopes, er, steep slopes, shallow slopes. And, what this shows is that basically at the bay scale there was a shade acclimated or a turbid acclimated population going to a more sun acclimated population at a more recent time.

If we look at the SERC FIU chlorophyll data for the basins in Florida Bay I know this is kind of noisy but I think you can tell that clearly in 2006 and 2007 there was more chlorophyll than in 2008 from what we know in 2009. So as the water's clear the physiology of the *Thalassia* responded accordingly in a way we would expect. So at the bay scale there is a lot of noise and there is a lot of basins.

So I picked out three basins. Crane Key which is really been sort of a stable basin and away from a lot of the events, Whipray which is another area of interest, it had some blooms and then it was clear, and Rankin. That's the bay. So basically we have Crane, Whipray and Rankin, PAR, and the effective yield for the four years. What you see is the slopes really don't change much at Crane, pretty stable with slight shifts up and down but not a whole lot of difference. What you see at Whipray is really quite different. The first three years we have a fairly steep slope, and then in 2009 the slope dramatically decreases. We know just from our eyes that it was turbid the first three years and then it was much clearer in 2009. Rankin is rather noisy. It was shallow, then it was steep. It was shallow, then it was steep. It's pretty noisy data for the four years.

This is just a summary of all the slopes for all the basins. The dashed line is the grand mean slope. We interpret that as slopes above this are steep and you are shade acclimated. Below this indicates that you are sun adapted and less of decrease with higher light. Crane didn't really change much in the slopes. Whipray, you could have, the slopes are quite negative shade acclimated. For the most recent year when we know the water was clear and it grew significantly and we did ancovas on this Rankin is just bouncing all around. So it indicates that Rankin, the physiology is kinda going all over the place. Which may indicate a potential affect on the variables as a stresser of a more variable system, which we know it isn't more variable. The intercepts, the grand mean, again above it the shade, below it the sun. Not much with Crane. A very dramatic shift with Whipray, and then noisy with Rankin so Rankin's been much more variable.

This is basin-scale chlorophyll this is 2006 to 2008. These are when we were doing our PAM measurements. You can see Crane here, one little blip here, but pretty low, stable chlorophyll. Whipray

and Rankin are much more noisy, Whipray being green and Rankin being maroon. As you can see Whipray had more chlorophyll then it goes down to baseline.

So we think that looking at this slope function gives us a photophysiology state. The thing is we don't know if it is sun acclimated, shade acclimated, or stress. We really need to drill more into the data and actually set up more specific sites, which might be something that we can do is actually look at some specific areas of interest like we did in 2000. So at a bay scale it looks like things are getting much better, waters are clearing, and getting much more sun acclimated. So you probably noticed that the data was

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somewhat noisy. And, some of that is because of the fact that we do this shallow, deep, shallow, deep, shallow deep. The effective yield is in black and PAR that we measure in blue during the day. And, it's not a real clean hyperbola you'd expect, a bell shaped curve because we going to shallow areas early on, deeper areas later on. It just bounces all over.

What we need, and, this is my plea if you want to focus on research minds. This is the prototype a-channel PAM fluorometer that I had a chance to work with while I was in Australia. John Ronsee [indecipherable] designed this instrument who now the director of the company that commercialized it. We call it an Octo PAM. What the OctoPAM allows you to do is deploy the instrument and log fluorescence during any kind of period and you get replicate measurements throughout time so you can really look at stress, you can shade part of the plot, you can do some manipulations, put some in Paul's buckets, get some inside the buckets or outside the ecotone. You can really look at get some close interval measurements.

And, it's an open clip. It just holds the leaf down with an open clip so don't affect the light dramatically it shouldn't affect the flow field.

When we deployed this instrument in a bay south of Sydney, this is the light, basically light, this is a smooth light curve we have a much cleaner response. With getting maximum yields later at night, down regulation during the day with electron transport rate. An insitu instrument will allow us to directly measure maximum yields and then look at that slope function to see if it actually does estimate maximum yields. We can look at down regulation. There is hysteresis, which is important. Hysteresis is the slope going down in the morning is the same as the slope going up in recovery. When it is not you sort of infer photoprotection versus photodegradation, photoregulation [indecipherable] and it directly measures variability which is another measure of photophysiology among shoots where we can deploy this in a die-off area or an area where we want to test. Maybe some of your experimental tests, maybe inside and outside the plots as well.

So I will finish there. Are there any questions?

Participant: How much do the Octo devices cost?

Michael Durako: The 3 channel is around 40, the 6 channel is about 60, the 9 channel is about 90, but each diopanel is about 20. So it is about half as much as getting as many diving PAMs. The marketing of

the system is very sophisticated. It has far red. It allows you to rapid light curves, which is another thing I did not talk about. There are other things you can do with the instrument. I think we are looking at some acute events and test some hypotheses and get some answers. It has a really high physiological resolution.

Participant: How long can you deploy for? For two days?

Michael Durako: Yeah. It depends on the program. How many times you fire. It has a fairly large internal battery pack and has the capability for an external battery pack. It depends on the program. If you do rapid light curves where you are using the [indcipherable] halogen light source that draws the battery down a lot more than if just than if you are just doing yields. If you are doing measurements every 5 minutes, yield measurements, you can leave it out there at least 24, 48 hours.

Participant: Can you do phytoplankton?

Michael Durako: This is not a, if you want to do phytoplankton you really need a fast repetition radio barometer. Now PAMS [indcipherable] aren't really sensitive enough to get fluorescence. [indcipherable]

Participant: When you do your, I'm trying to just get a comfort level, when you do these yearly measurements it could be like the week before was cloudy and then you go out. You go out what, one day, two days? a week? How many days does it take you to do the entire bay?

Michael Durako: We basically sample a basin a day, we do 20 basins in 10 days with two boats running. And, we're doing them at the same time. There is all kinds of preceding [indcipherable].

Participant: But, you still feel comfortable with that given any variability?

Michael Durako: The slope function is what we can deal with with diurnal variability. I think what we need to do now is focus down and get some close interval. Right now I just saw the data and the chlorophyll I said this is pretty compelling on a large scale. Definitely short term variability. One of the

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things where a logging instrument would be helpful with FHAP is that if we just put a multi channel PAM in the middle of the basin that we are going to sample, set it up, then we do our random sampling while it's sitting in place without all the variable light and location, compare what we see in our mapping sampling versus what you see in the basin. Is it representative? But I also see the value of this instrument when someone is doing acute events or you are doing your glucose or iron accumulation or something like that.

Participant: Mike you know I've been trying to use PAM in our monitoring for a long time as well. It's difficult because of all these ambient light incidents in these sun adapted and shade adapted plants. How are you controlling these differences in plant densities, which has a huge affect on ambient light?

Michael Durako: It does. In fact one of the ecotone effect that we see is that you get more down regulation because you get more light.

Participant: It does. If we had really high slopes and low densities [indecipherable] for instance?

Michael Durako: Exactly.

Participant: How do you disentangle?

Michael Durako: You look at it within the basin?

Participant: How do you tell the differences across the basin?

Michael Durako: And, we haven't been able to look at [indecipherable] to do that. This is the point I'm at now. It looks like the dissipation aspect of irradiance versus yield, that slope function should give us some indicator of the condition, that's another reason to put it in place. I'd love to be able to just sit our two or three PAMS we have in place, but I'm not willing to do that with this data set now. I want to continue this data set but also give it some additional data along with that. There is a density effect in the preceding, you know, couple of days. We do know there is black water during the phytoplankton blooms we are getting some really steep slopes. And, the mean, if you take just the bay mean, it goes down before we see a loss of cover at the bay scale. So to answer your question, we are hitting so many types of densities we are kinda getting a representation of the bay, but there are all types of small things. It's a great instrument in experimental [indecipherable].

Participant: One more question. You are measuring whether or not the plants are sun adapted or shade adapted but you were not relating it directly to stress? So how are you going to do that or is not part of this?

Michael Durako: As the slope increases increasing irradiance, which indicates shade acclimation or a function of stress under the same light conditions. I'm just saying do we have a data set, and I haven't had a chance to mine the data, where the light conditions are the same and there's a shift in the slope that's an indication of stress. Now that I've looked at Rankin and see how bouncy it is and Rankin is pretty shallow and the water has been fairly clear there. So if we can get good light data, you know, look at the data there and see if the light was the same between years and that we've seen a change in slope that's where you can separate it. At this point I haven't drilled into the data.

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[credits]