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Advanced Aquarist's Online Magazine

How to Make Corals More Colorful
Quantification of Skimmer Performance
Reflector Analysis
Tips for Healthy Aquarium Inhabitants

photographs by Mike Maddox

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Cover Photo

Periclimenes yucatanicus shrimp engaged in a commensal relationship with a *Condylactys gigantae* anemone in the drowned cayes belize.

Photograph by Mike Maddox

Publication Information

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ADVANCED AQUARIST'S ONLINE MAGAZINE

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EDITORIAL

JANUARY 2009

By Terry Siegel

Terry discusses the magazine and the articles in this issue.

Published January 2009, Advanced Aquarist's Online Magazine.

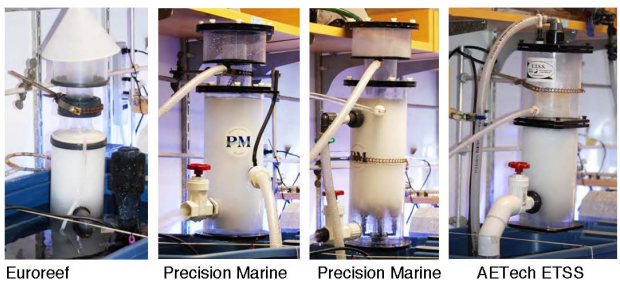
Keywords (AdvancedAquarist.com Search Enabled): Editorial, Terry Siegel
Link to original article: http://www.advancedaquarist.com/2009/1/aaeditorial

As we begin a new year I thought I would talk about the editorial position of *Advanced Aquarist*. As I've said in the past this electronic publication tries to position itself, in terms of content, somewhere between a hobbyist magazine and a vetted science publication. However, exactly what does that mean.

Anecdotal Information: Information based on personal observation, case study reports, or random investigations rather than systematic, quantifiable, scientific evaluation. In my opinion, most aquarists share anecdotal information, which I should add is often useful. However, one of the difficulties with anecdotal information is that it is often unreliable. It is certainly true that an advanced aquarist, with a science background, is more likely to offer anecdotal advice that is more valuable than the observations of a beginner, but without careful scientific testing even that information is suspect

Scientific Information: This is information derived from what is known as the scientific method; that is, principles and procedures for the systematic pursuit of knowledge involving the recognition and formulation of a problem, the collection of data through observation and experiment, and the formulation and testing of hypotheses. Also, the scientific method is the disinterested pursuit of the truth. The key word here is "disinterested." To put it in another way, the likes, dislikes, or prejudices of the researcher are irrelevant. This is not to suggest that science does not come to erroneous conclusions, which it sometimes does, but most importantly it is self correcting - new data, new conclusions/hypotheses. And, in that sense science is not dogmatic, unlike religious faith.

In this issue of *Advanced Aquarist* we have three examples of science at work, by Dana Riddle, Sanjay Joshi, and Ken Feldman. Experienced reef keepers usually have a variety of anecdotally based opinions regarding which type of protein skimmer downdraft, venturi, counter-current air stone, beckett, needle wheel, etc. is best. Ken's carefully researched article on skimmers will, I have little doubt, surprise many reef keepers. Check it out yourself. Here is an illustration from the article to whet your appetite,



Euroreef CS80 needlewheel V_s = 0.62 gallons | Precision Marine ES100 venturi V_s = 1.12 gallons | Precision Marine AP624 airstone V_s = 1.26 gallons | AETech ETSS Evolution 500 downdraft V_s = 0.87 gallons

FEATURE ARTICLE

How to Make Corals Colorful, Part One: New Information, With Particular Attention to Blue-Green Fluorescent Pigments

By Dana Riddle

Light intensity and its spectral characteristics play important parts in promotion of coral coloration.

Published January 2009, Advanced Aquarist's Online Magazine.

Keywords (AdvancedAquarist.com Search Enabled): Coloration, Coral, Dana Riddle, Feature Article, Intensity, Pigment, UV
Link to original article: http://www.advancedaquarist.com/2009/1/aafeature1

A coral crab jealously guards its cyan-colored Pocillopora damicornis host. Note the red fluorescence of the algae growing on the live rock to the right, and how the coral's blue-green and the red fluorescence of the algae combine to make the coral appear orange. Photo by the author, 10-second exposure at f/32 using a black light as an excitation source.

This month we'll begin to examine brand new information on effects of lighting (both intensity and spectral quality) and how it affects coral coloration. In addition, we'll piece together some information from various research papers, and parts of the coral pigmentation puzzle will begin to fall in place.

It is very unusual for important research papers on coral coloration to appear simultaneously, yet this happened in the later portion of 2008. Together, results of efforts by researchers (Alieva et al., 2008, D'Angelo et al., 2008, Feldman et al., 2008, Gruber et al., 2008, Vogt et al., 2008), along with others' (Oswald et al., 2007, Schlacher et al., 2007), offer fascinating glimpses into the mysterious world of coral coloration, with insights offered on types of pigments and how we, as hobbyists, can manipulate the environments of captive corals and induce coloration.

The goals of these articles are to enable the hobbyist to visually identify the color bandwidth of a particular pigment and, once

identified, categorize the pigment and apply information provided on light intensity and light color to induce, or maintain, coral coloration.

We should note that a number of factors are involved when making corals colorful. Light, of course, is critical, but the importance of this parameter should not diminish the criticality of other co-factors such as water motion, pH, alkalinity, nutrient loadings, etc. However, it can not be denied that, when all other conditions are correct, light can literally make or break colorful pigmentation.

In situations where the pigment can gain or lose coloration, light energy can bend the shape of the pigment molecule (called the '*hula twist*'), sometimes resulting in coloration (photoconversion) or coloration loss (photobleaching). When the light energy falls below a certain threshold level (either intensity or spectral quality), the pigment molecule 'relaxes' to its normal state.

In some cases, light energy breaks the molecular bond(s) completely, leading to an irreversible change in apparent coloration.

This month, we'll examine pigment clades, and effects of light quantity and quality on the blue-green (cyan) fluorescent pigments. But first, a review of terminology used in this and following articles is in order.

Glossary

The following terms will be used in this article:

Absorbance:
Ability of a solution or layer of a substance to retain light without reflection or transmission.

Absorption:
The process in which incident radiation is retained without reflection or transmission.

Brightness:
The intensity of a fluorescent emission. Extinction coefficient times Quantum Yield = Brightness.

Clade:
For our purposes in this article, a grouping of pigments based on similar features inherited from a common ancestor. Pigments from corals includes Clades A, B, C, and D. Clades can refer to living organisms as well (clades of *Symbiodinium* - zooxanthellae - are a good example.)

Chromophore:
The colorful portion of a pigment molecule. In some cases, chromophore refers to a granular packet containing many pigment molecules.

Chromoprotein pigment:
A non-fluorescent but colorful pigment. These pigments appear colorful because they reflect light. For example, a chromoprotein with a maximum absorption at 580nm might appear purple because it preferentially reflects blue and red wavelengths.

Chromo-Red Pigment:
A newly described type of pigment possessing characteristics of both chromoproteins and Ds-Red fluorescent proteins. Peak fluorescence is at 609nm (super red).

Cyan Fluorescent Protein (CFP):
Blue-green pigments with fluorescent emissions in the range of ~477-500nm. Cyan and green pigments share a similar chromophore structure. Cyan pigments are expressed at lower light levels than green, red or non-fluorescent pigments.

Emission:
That light which is fluoresced by a fluorescent pigment.

Extinction Coefficient:
The quantity of light absorbed by a protein under a specific set of circumstances.

Excitation:
That light absorbed by a fluorescent pigment. Some of the excitation light is fluoresced or emitted at a less energetic wavelength (color).

Ds-Red type pigment:
A type of red fluorescent pigment with a single primary emission bandwidth at 574-620nm. Originally found in the false coral *Discosoma*.

Fluorescence:
Absorption of radiation at one wavelength (or color) and *emission* at another wavelength (color). Absorption is also called *excitation.* Fluorescence ends very soon after the excitation source is removed (on the order of ~2-3 nanoseconds: Salih and Cox, 2006).

Green Fluorescent Protein (GFP):
Fluorescent pigments with emissions of 500-525nm.

'Hula Twist':
A bending of a pigment molecule resulting in a change of apparent color. Molecular bonds are not broken; therefore the pigment can shift back and forth, with movements reminiscent of a hula dancer.

Kaede-type pigment:
A type of red fluorescent pigment with a characteristic primary emission at ~574-580nm *and* a secondary (shoulder) emission at ~630nm. Originally found in the stony coral *Trachyphyllia geoffroyi*, but common in corals of suborder Faviina.

Kindling Protein:
A protein capable of being converted from a non-fluorescent chromoprotein to a fluorescent protein. Sometimes called a 'Kindling Fluorescent Protein', or KFP.

Quantum Yield:
Amount of that energy absorbed which is fluoresced. If 100 photons are absorbed, and 50 are fluoresced, the quantum yield is 0.50.

Photobleaching:
Some pigments, such as *Dronpa*, loss fluorescence if exposed to strong light (in this case, initially appearing green and bleaching to a non-fluorescent state when exposed to blue-green light). Photobleaching can obviously cause drastic changes in apparent fluorescence. In cases where multiple pigments are involved, the loss of fluorescence (or energy transfer from a donor pigment to an acceptor pigment) could also result in dramatic shifts in apparent color.

Photoconversion:
A rearrangement of the chemical structure of a colorful protein by light. Depending upon the protein, photoconversion can increase or decrease fluorescence (in processes called photoactivation and photobleaching, respectively). Photoconversion can break proteins' molecular bonds (as with *Kaede* and *Eos* fluorescent pigments) resulting in an irreversible color shift, or the molecule can be 'twisted' by light energy (a 'hula twist') where coloration reversal are possible depending upon the quality or quantity of light available. This process is known as photoswitching).

Red Fluorescent Protein (RFP):
Those pigments with an emission of ~570nm and above. Includes Ds-Red, Kaede and Chromo-Red pigments.

Stokes Shift:
The difference in the maximum wavelength of fluorescent pigment excitation light and the maximum wavelength of the fluoresced light (emission). For example, a pigment with an excitation wavelength of 508nm and an emission wavelength of 535nm would have a Stokes Shift of 27nm.

Threshold or Coloration Threshold:
The point at which pigment production is sufficient to make its fluorescence (or in the case of non-fluorescent chromoproteins, it absorption) visually apparent. The term threshold generally refers pigment production, although, in some cases, it could apply to a light level where a pigment disappears (as in the cases of photobleaching, or photoconversion).

Yellow Fluorescent Protein:
An uncommon group of fluorescent proteins with emissions in the 525-570 nm range (Alieva et al., 2008).

Types of Pigments

There are at least 9 described types of coral pigments. Note: Pigment types, such as green or red *might* not be structurally similar to another green or red pigment in a different clade - see below). These include:

- Cyan Fluorescent Proteins (CFP) - Cyan pigments are blue-green pigments with a maximum emission of up to ~500 nm. The chromophore structure of a cyan pigment is very similar to that of a green fluorescent pigment.
- Green Fluorescent Proteins (GFP) - This group, by far, is the most numerous of the fluorescent proteins. The structure of green fluorescent chromophores is very similar to that of cyan fluorescent chromophores.
- Yellow Fluorescent Proteins (YFP) - An unusual type of fluorescent protein with maximum emission in the yellow portion of the spectrum. Rare in its biological distribution, YFP is found in a zoanthid and some specimens of the stony coral *Agaricia*. Personally, I've noted yellow fluorescence in a very few stony corals (*Porites* specimens) here in Hawaii while on night dives using specialized equipment to observe such colorations (see www.nightsea.com for details on this equipment).
- Orange Fluorescent Protein (OFP) - I've included this protein 'type' in an attempt to avoid confusion. OFP is used to describe a pigment found in stony coral *Lobophyllia hemprichii* and its name suggests a rather unique sort of protein. In fact, OFP is simply a variant of the Kaede-type fluorescent proteins.
- Red Fluorescent Proteins (RFP) - A group of proteins including several different subtypes (Kaede, Ds-Red and Chromo-Red). Typically, fluorescent emission is in the range of ~580 nm to slightly over 600 nm.
- Dronpa - A green fluorescent protein that loses fluorescent when exposed to blue-green light (~490 nm) but returns when irradiated with violet light at ~400 nm.
- Kindling Proteins - A protein (notably from the anemone *Anemonia sculata*) that changes from a non-fluorescent pigment to one demonstrating fluorescence. This change is switchable/reversible and its state depends upon the spectral quality of light striking it.
- Chromo-Red Proteins - A new classification (Alieva et al., 2008) of a single fluorescent pigment found in the stony coral *Echinophyllia*. This chromo-red pigment has qualities of a non-fluorescent chromoprotein, but fluoresces at a maximum of 609 nm.

- Chromoproteins (CP) - This group of pigments is non-fluorescent, or has minimal fluorescence (where the quantum yield is essentially zero). Instead of relying upon fluorescence for coloration, these pigments instead absorb light most strongly in a relatively narrow portion of the visible spectrum. Most coral chromoproteins absorb light maximally at 560-593 nm. There are reports of anemones absorbing light at a maximum wavelength of 610nm, and a couple of reports of stony corals absorbing wavelengths in the 480-500nm range. Some chromoproteins are very similar in structure to the fluorescent Ds-Red proteins. In fact, genetic engineers have found that a single amino acid substitution in a protein can make the difference between non-fluorescence and fluorescence. Chromoproteins do not get much attention by researchers (relative to that of fluorescence proteins) and there are only about 40 described.

Pigment Clades

Examination of pigment clades might seem a fruitless endeavor, but, to the contrary, there is some interesting information to be garnered. Working on the hypothesis that pigment clades have evolved over the millennia and could have specific within-clade properties, we can possibly use cladal data, along with other information, to form loose categories as to how pigments respond to various stimuli, including light and perhaps other factors. It is interesting to note that some pigments seem to have evolved within certain groups of animals (Clade A in anemones, or Clade D's Kaede-type pigments found mostly in stony corals of suborder Faviina).

Pigment Names

I've attempted to list as much of the shorthand names for coral pigments within the following Tables 1 through 4. It is becoming common for researchers to use a pigment's given name (such as *mcav4*, *Eos*, or *r1.2*) without providing further information, and this information was included in the following Tables for my convenience, although any serious student not familiar with these names can also benefit.

There are currently 4 major pigment clades, with Clade C containing several subgroups. We begin our study with:

Clade A

Consists *entirely* (at present) of anemones (Actinaria genera) and contains *green* and *red* fluorescent variants, along with a number of *non-fluorescent* chromoproteins. No cyan or yellow fluorescent pigments are included. See Table 1. Evidence suggests that photoconversion is possible in at least some fluorescent pigments of Clade A, and a transition from a non-fluorescent state to one of fluorescence is possible in the 'kindling' protein from *Anemonia sculata* (that transforms from a non-fluorescent protein with maximum absorption at 562 nm to a fluorescence state with maximum emission at 595 nm upon exposure to strong light).

Clade B

Clade B is currently populated by scleractinian *non-fluorescent* chromoproteins (including those found in *Acropora*, *Montipora*, *Pocillopora*, etc.), three *red* fluorescent pigments (from the stony corals *Porites*, *Montipora*, and the false coral*Discosoma*), along with one *cyan* fluorescent pigment. At present, Clade B does not contain any green or yellow fluorescent pigments. See Table 2. Photoconversion occurs in many Clade B pigments, but is not common in the cyan pigments discussed in this article.

Clade C, including Subclades C1, C2, and C3

This clade is the most diverse of the 4 clades currently described, and includes some of the cyan, green, yellow and red fluorescent proteins plus one non-fluorescent chromoprotein (a 'red' pocilloporan) from the stony coral *Stylophora pistillata*. Green fluorescent proteins are mostly from *Acropora* and *Montipora* species but also several from orders/suborders Meandriina, Fungiina and Faviina. See Table 3.

Taxon Pigment Name Type of Pigment Order/Suborder Excitation Emission Clade

Clade D

All pigments within this clade are fluorescent to some extent, and includes Ds-Red and Kaede-type orange/red fluorescent proteins (see Glossary). Clade D contains pigments found in at least two soft corals (*Clavularia* and *Dendronephthya* species), false corals (*Discosoma* and *Ricordea*), and many stony corals. Photoconversion is possible in many Clade D pigments. One green fluorescent pigment, *Dronpa*, bleaches upon exposure to 'strong' blue-green light. Interestingly, the majority of these pigments are from the stony coral suborder Faviina. See Table 4.

Experimental Evidence of Light Intensity and Quality on Expression of Pigmentation by the Coral Host

A recent paper (D'Angelo et al., 2008) reports the results of experiments designed to investigate the effects of light intensity and light quality on expression of fluorescent pigments and non-fluorescent chromoproteins by various corals. The small-polyped stony corals used in these experiments were *Acropora millepora*, *Acropora pulchra*, *Hydnophora grandis*, *Montipora digitata* and *Seriatopora hystrix*. It appears that typographical errors mislabeled some of the pigments, but it seems that at least 11 coral pigments were examined (4 cyan fluorescent pigments, 3 green fluorescent pigments, 1 orange fluorescent

pigment and 1 red fluorescent pigment. Two non-fluorescent chromoproteins were also studied).

Expression of coral coloration was examined with varying photon flux densities (PAR values classified as 'very low' (80 µmol·m²·sec), 'low' (100 µmol·m²·sec) 'moderate' (400 µmol·m²·sec), and 'high' (700 µmol·m²·sec). Photoperiod was 12 hours. Metal halide lamps producing ~50% of its visible radiation in the blue portion of the spectrum were used. See the Discussion section below for comments concerning how these categories relate to those seen in aquaria.

The effects of spectral quality were estimated by using commercially-available filters to separate the light generated by a metal halide lamp into 3 general categories. See Figure 1 for the approximate transmission qualities of these filters.

Unfortunately, we do not know the exact type of metal halide lamps used. We are told it is an 'Aqua Light' but no information is provided other than they 'emit ~50% of the photons in the blue spectral region.' The lamps' brand, kelvin rating, etc. would be of great use since it would allow us to better estimate the quality of light used to illuminate the corals. I'm tempted to suggest the lamps were Ushio's 'AquaLite' metal halide lamp series, but lack any evidence other than similar names. Since metal halide lamps produce 'spiky' spectra we might expect to see these filters transmitting generous portions of relatively narrow bandwidths, while transmitting lesser, if any, amounts at other wavelengths. An email inquiry to the primary investigator has remained unanswered.

When using the blue, green and red filters, these researchers standardized the photon flux density to 200 µmol·m²·sec, but be aware that the green filter transmitted blue light as well (estimated to be 25% of the total transmitted light. In other words, the green filter transmitted at least 50 µmol·m²·sec of blue light). The red filter transmitted red wavelengths almost exclusively.

The experimental results of D'Angelo et al. offer fascinating insights on the effects of light intensity and spectral qualities on coral pigmentation. Some pigments require relatively little light while others are not expressed until light intensity reached a certain threshold. At least one pigment examined decreased in concentration upon exposure to increasing light intensity. By the same token, production of coral pigments by the coral animal can be a response to different colors of light and each pigment class seems to have a different reaction to 'colored' light.

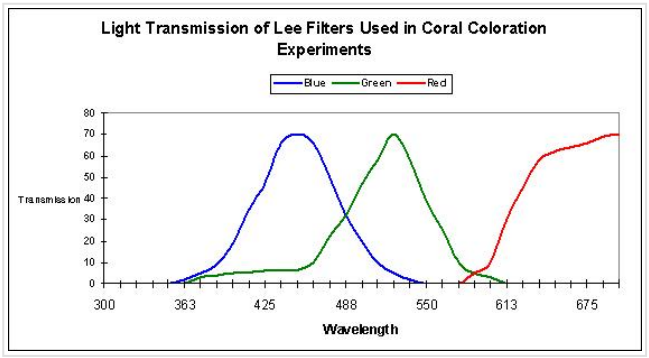


Figure 1. Approximate transmission qualities of three colored filters used by D'Angelo et al. (2008).

Cyan Fluorescent Pigments

Cyan (from the Greek work *kanos* meaning dark blue) pigments are, for our purposes, a group of pigments with a fluorescent emission of up to a wavelength of 500 nanometers.

Cyan Fluorescent Pigment 483

This pigment from *Acropora pulchra* was found by D'Angelo et al., (2008) to be most notable at their 'moderate' light intensity

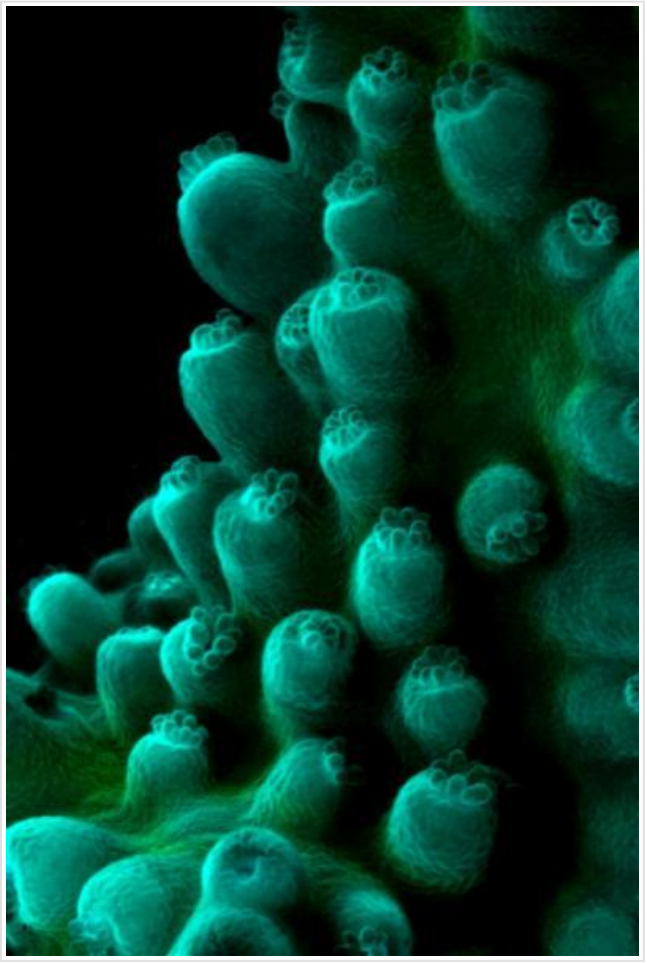


Figure 2. An unidentified Acropora specimen demonstrating blue-green fluorescence. The pigment is most likely of Clade C2. Photo by the author.

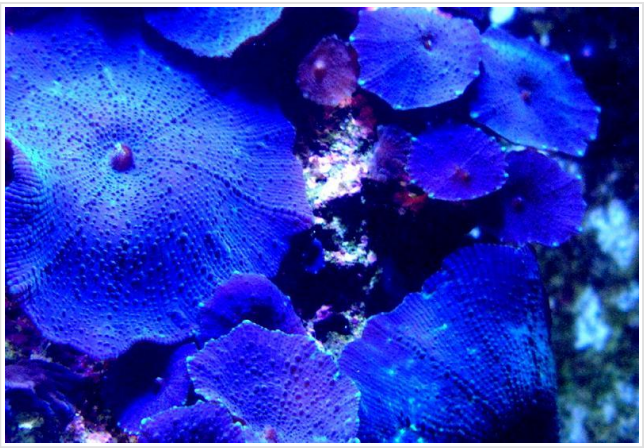


Figure 3. The beautiful blue-shifted cyan fluorescence of Discosoma specimens. This pigment is likely of Clade B.

category of 400 µmol·m²·sec. Increasing light intensity did not increase cyan fluorescence. CFP483 was also noted at lesser but significant concentrations at lower light intensities of 80 µmol·m²·sec and 100 µmol·m²·sec (see Figure 5). In their spectral quality experiments, these researchers found red light alone could promote the expression of this particular cyan pigment, although 'green' and blue light were more efficient in making the coral animal produce this coloration (see Figure 6).

Cyan Fluorescent Pigment 484

This pigment, also found in an *Acropora* species (specifically *A. millepora* - see Figure 7) responds in a different fashion to light intensity and spectrum than CFP-483 (described above).

D'Angelo and her research partners found this particular pigment was found in increasing concentrations as light intensity rose from 80 µmol·m²·sec to 100 µmol·m²·sec and finally 400 µmol·m²·sec. Fluorescent intensity decreased when light intensity was increased to 700 µmol·m²·sec (see Figure 8). Red

Figure 4. Blue-shifted cyan fluorescence of Montipora danae (the 'Superman' Montipora). This pigment probably belongs to Clade C3. The orange-red polyp fluorescence is peaking at 611 nm, and its classification is currently unknown. Photo by the author.

light was least efficient in promoting the production of Pigment 484 (see Figure 9).

Cyan Fluorescent Pigment 486

As most hobbyists know, *Montipora digitata* specimens are available in quite a number of colors, ranging the common orange morph to the exotic multi-colored specimens. Figures 10 and 11 demonstrate this pigments production in relation to light intensity and light quality, respectively.

Cyan Fluorescent Pigment 492

The stony coral *Hydnophora grandis* contains this pigment (see Figure 12). Expression of this fluorescent pigment is seen a low light intensities of 80 and 100 µmol·m²·sec, with fluorescent pigmentation increasing to a maximum at 400 µmol·m²·sec, and then slightly falling when the coral is exposed to 700 µmol·m²·sec (see Figure 13). When examined for response to blue, green and red light, the least expression was noted under red light (although there is a considerable amount of fluorescence), while green and blue light were better at promoting fluorescent pigment production (see Figure 14).

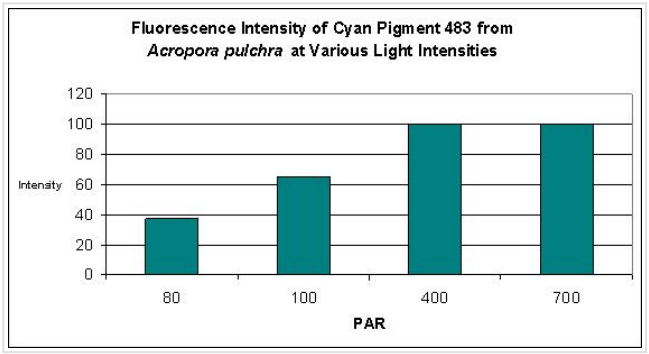


Figure 5. Effects of light intensity (generated by a metal halide lamp) on the generation of fluorescent Pigment 483.

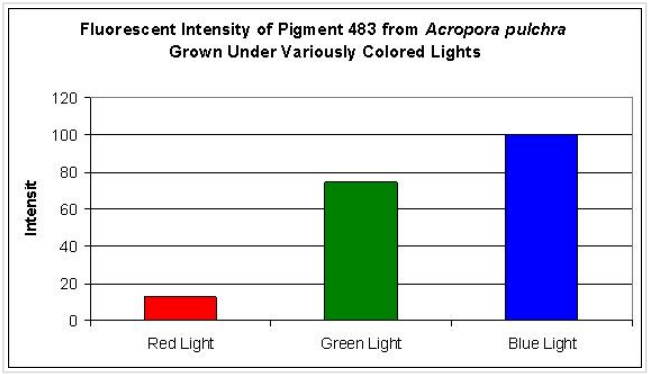


Figure 6. Effects of different colored lighting on expression of fluorescence of Pigment 483. Light intensity was 200 µmol·m²·sec for each treatment.

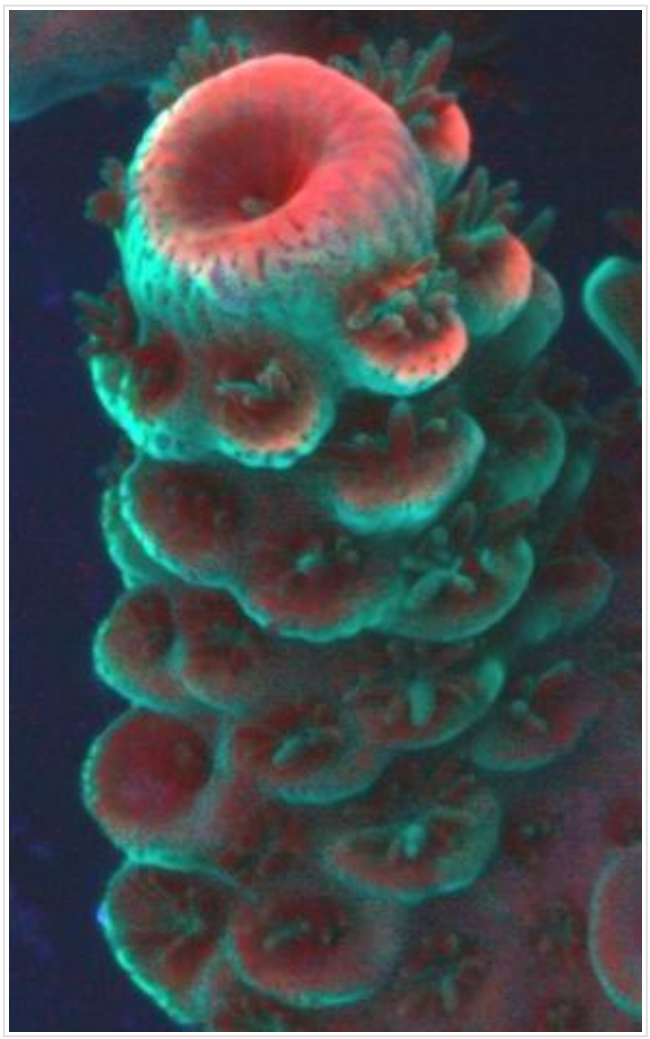


Figure 7. The cyan fluorescence (along with red fluorescence) of Acropora millepora. Photo by the author.

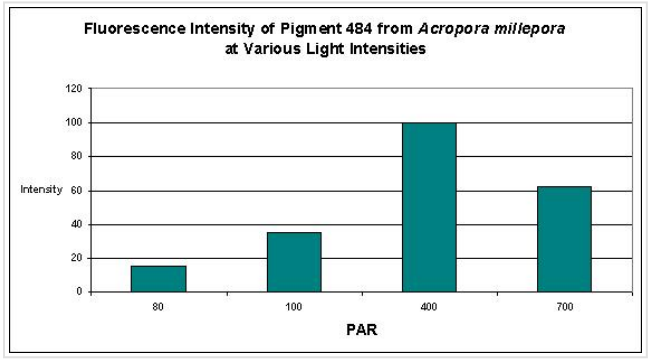


Figure 8. Effects of light intensity (generated by a metal halide lamp) on the generation of fluorescent Pigment 484.

Cyan Fluorescent Pigment 497

Pigment 497 is a bit different than other cyan pigments in its responses to both light intensity and spectral qualities. Production of this particular pigment in *Acropora millepora* was found to be most efficient at 100 µmol·m²·sec and only slightly less so at 400 µmol·m²·sec. Interestingly expression of this pigment was found to be practically non-existent at 80

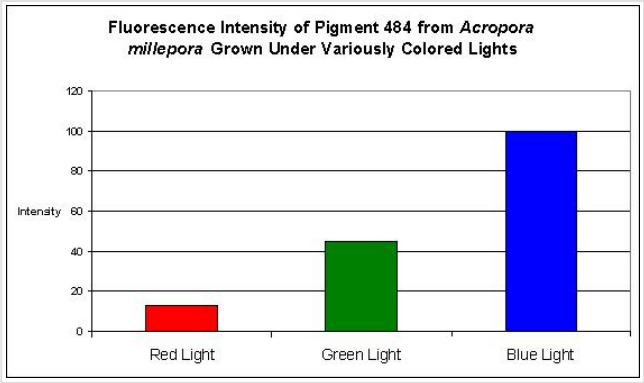


Figure 9. Effects of different colored lighting on expression of fluorescence of Pigment 484. Light intensity was 200 µmol·m²·sec for each treatment.

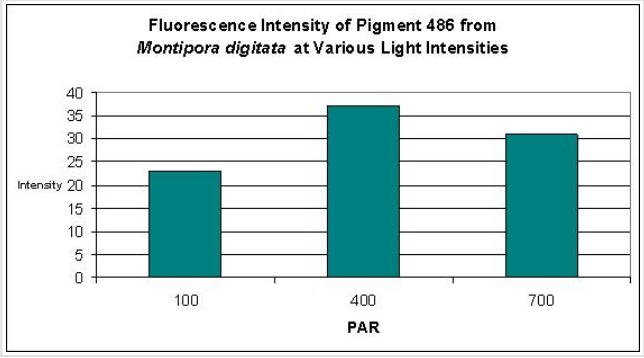


Figure 10. Effects of light intensity (generated by a metal halide lamp) on the generation of fluorescent Pigment 486.

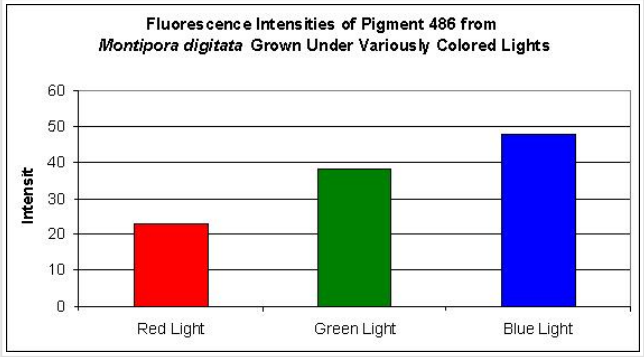


Figure 11. Effects of different colored lighting on expression of fluorescence of Pigment 486. Light intensity was 200 µmol·m²·sec for each treatment.

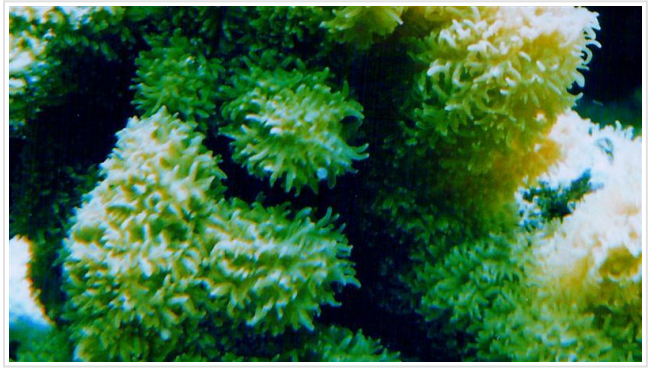


Figure 12. The greenish fluorescence of a Hydnophora specimen. Photo by the author.

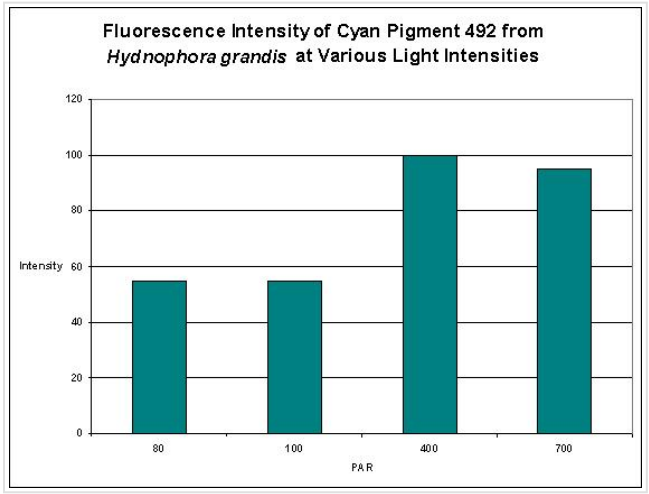


Figure 13. Effects of light intensity (generated by a metal halide lamp) on the generation of fluorescent Pigment 492.

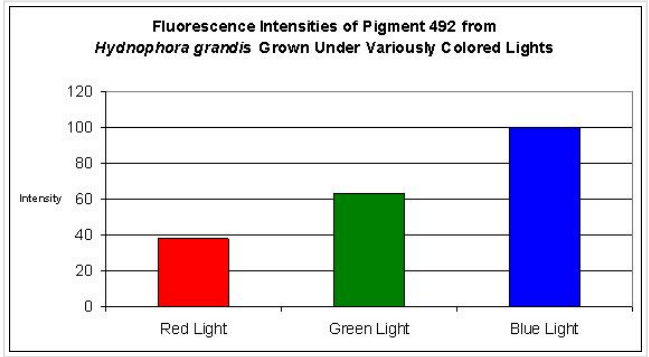


Figure 14. Effects of different colored lighting on expression of fluorescence of Pigment 492. Light intensity was 200 µmol·m²·sec for each treatment.

µmol·m²·sec, and even less so at 700 µmol·m²·sec (see Figure 15).

Although red light promoted pigment production to a slight degree, green light and blue light were much better in making this coral colorful (see Figure 16).

DISCUSSION

Before beginning our discussion of various cyan pigments and their reactions to light intensity and spectral quality, we should put the light intensities used by D'Angelo et al. in the perspective of reef aquarium hobbyists.

Recall that these researchers used these categories of light: 'Very low' (80 µmol·m²·sec), 'low' (100 µmol·m²·sec) 'moderate' (400 µmol·m²·sec), and 'high' (700 µmol·m²·sec) and used a photoperiod of 12 hours. Since a 12-hour photoperiod (or close to it) is fairly common among the hundreds of reef tanks I've had the pleasure of viewing, we will not concern ourselves with this any further. It is the light intensities that should be scrutinized. If light intensity plays an important part, this question begs to be asked:

How common is a light intensity of, say, 400 µmol·m²·sec in reef tanks?

To try to answer this question, I present PAR measurements made in aquaria across the country. Various lighting systems were used (i.e., metal halides, fluorescents, PCs, etc. or combinations of any of these). Measurements were taken at or about the vicinity of corals that were thriving in captivity, and it give us a good idea of general light intensities found in home aquaria. See Figure 17.

As we can see from Figure 17, light intensities of 80 µmol·m²·sec and 100 µmol·m²·sec are easily achievable in home aquaria. However, the same cannot be said for the category of 400 µmol·m²·sec since only 13% (approximately) of the light measurements matched or exceeded that category. Very few aquariums (~1%) with thriving corals reached D'Angelo's highest category (700 µmol·m²·sec). We should bear this in mind while evaluating following information.

In the same vein, we should review the spectral transmission qualities of the filters used in these experiments. Recall that we do not know the exact lamps used (although we know they were 'blue' metal halides). It would be much better to have the spectral signature of the light transmitted through the blue, green and red filters, but we do not.

Most of the cyan fluorescent pigments were expressed most strongly by the coral animals at 400 µmol·m²·sec (remember, the lamps used in these experiments produced ~50% of their output in the 'blue' region of the spectrum, suggesting they were high kelvin lamps. Unfortunately, email to the primary investigator requesting clarification has gone unanswered). These pigments include CFP483 (from *Acropora pulchra*), CFP484 (from *Acropora millepora*), CFP486 (from *Montipora digitata*), and CFP492 (from *Hydnophora grandis*).

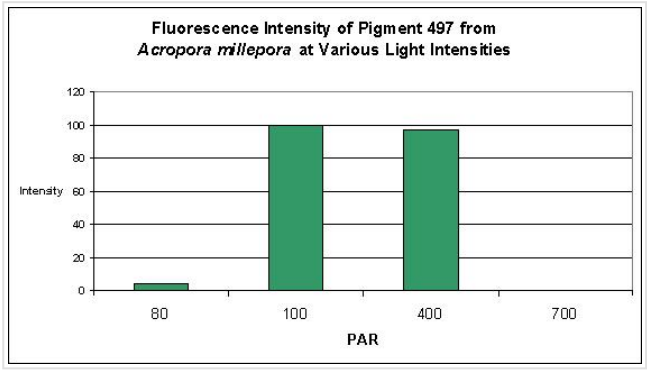


Figure 15. Effects of light intensity (generated by a metal halide lamp) on the generation of fluorescent Pigment 497.

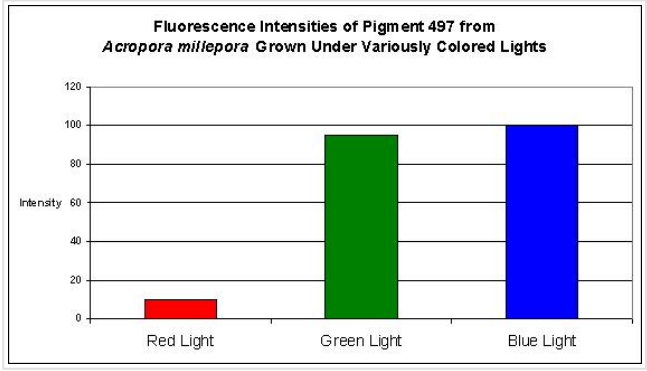


Figure 16. Effects of different colored lighting on expression of fluorescence of Pigment 497. Light intensity was 200 µmol·m²·sec for each treatment.

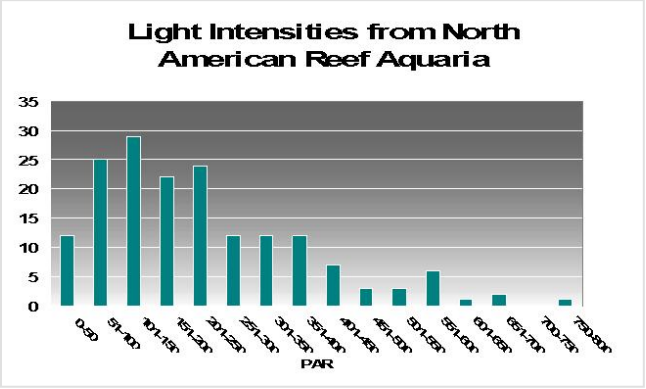


Figure 17. Light intensities as measured around corals thriving in home aquaria.

The cyan fluorescent pigment 497 from *Acropora millepora* behaved differently - it was expressed most strongly at a light intensity of only 100 µmol·m²·sec, and was not expressed at all at 700 µmol·m²·sec.

In every case, red light promoted the least amount of colorful pigmentation although there were considerable differences in the level of expressions at the given light intensity of 200 µmol·m²·sec used in these experiments. For example, the levels of cyan pigment 486 found in the 'red light' *Montipora digitata* specimens were found to be at a level of 50% of that found in the specimens exposed to blue light. Similarly, the cyan pigment 492 levels in the *Hydnophora grandis* specimens were ~40% of those 'blue light' specimens. In all other cases, pigment expression in the animals exposed to red light was only 10-15% of that seen in the corals subjected to blue light.

Photoconversion, Pigment Bleaching and Color Mixing

It is common knowledge that corals often contain at more than one fluorescent pigment (a *Favia* specimen was found to have at least one dozen). The combined fluorescence of these pigments can and likely will influence our visual perception of the animal's color in a phenomenon called 'color mixing'. The addition (increase in expression or photoconversion), alternation (photoconversion) or subtraction (photobleaching or decrease in expression) of a pigment can have a profound affect on the animal's appearance. Figure 18 demonstrates the possible but hypothetical increase or decrease in pigments according to light *intensity* alone. Imagine the possibilities we might observe when we add the effects of *spectral quality* on pigment expression. The exercise becomes mind boggling, and this is a relatively simple example!

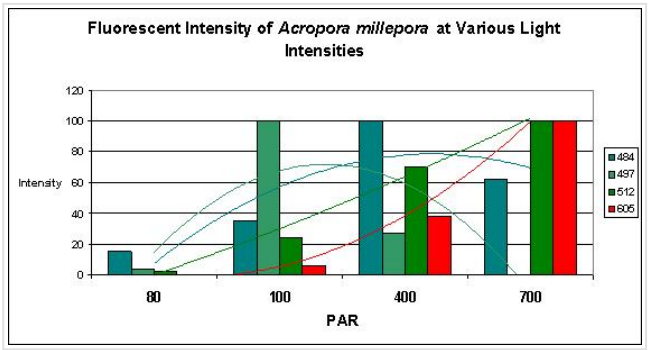


Figure 18. Trend lines show that Pigments 484 and 497 decrease in fluorescent intensity as PAR increases, while Pigments 512 and 605 increase in intensity. At 80 µmol·m²·sec, this coral would appear blue-green with apparent color becoming progressively greener until red and green pigments are dominant at 700 µmol·m²·sec. Is this a case of photoconversion, where light intensity alters the chemical makeup of pigments and causes them to shift from one fluorescent color to another? Or is this a case of pigment bleaching (P-484 and P-497) and light intensity-induced fluorescence (Pigments 512 and 605), or both?

There are only a few described cases of photoconversion in cyan pigments. Two are from Clade A (496 nm to an undetermined wavelength in *Condylactis gigantea* - Labas et al., 2002); 499 nm to 522 nm *Anemonia sculata* - Wiedenmann, 2002; and one case involves *Acropora secale*'s conversion from cyan (484 nm) to green (515 nm) - Papina et al., 2002. See Figures 18-20 for additional information.

Conclusions

Light intensity and its spectral characteristics play important parts in promotion of coral coloration. Certainly, these are not the only requirements and become deciding factors only when other conditions such as water motion and water chemistry are correct.

The light intensity categories used by D'Angelo et al. are apparently relative to amounts of radiation received by natural reefs. Surprisingly, these categories were fairly close to those seen in aquaria when I divided the number of light measurements made in aquaria and divided the total number into 4 categories

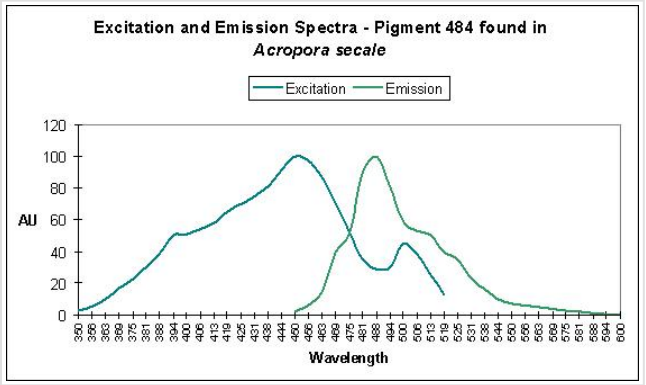


Figure 18. Light (mostly likely blue light) induces a shift in fluorescence from blue-green (484 nm) to green (with a peak emission at 515 nm). After Papina et al., 2002.

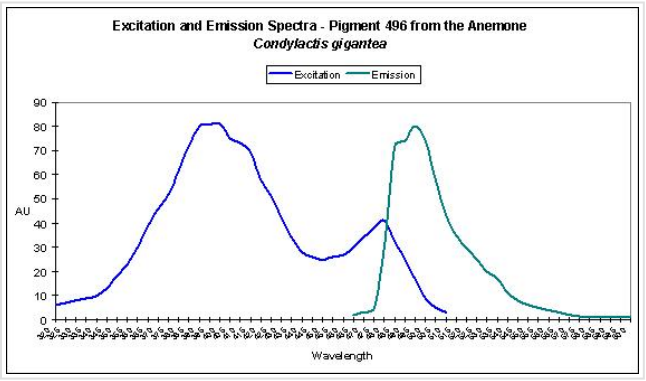


Figure 19. A pigment from Clade A, the spectral signature suggests photoconversion is possible. After Labas et al., 2002.

containing an equal number (see Figure 17). These were my classifications:

1. Very Low: 15-100 µmol·m²·sec
2. Low: 101-200 µmol·m²·sec
3. Moderate: 201-400 µmol·m²·sec
4. High: 401-700 µmol·m²·sec

Note that light intensities fitting into the moderate/high categories are often found directly beneath high intensity lamps and are comparatively small in area. Hence, some sort of light-measuring device, whether a lux meter or quantum meter, would be most helpful in determining proper placement of corals containing cyan fluorescent pigments. See here for comments on lux-to-PAR conversions: (www.advancedaquarist.com/2008/2/review) and a Product Review on a relatively inexpensive PAR meter: (www.advancedaquarist.com/2005/7/review).

It will take some time for me to review 11 years of lighting data I've collected, but I will try to refine these categories before this series is completed.

Most of the cyan pigments' maximum fluorescent was noted in light fields of around 400 µmol·m²·sec (with the exception of *Acropora millepora*'s pigment 497 that peaked at 100 µmol·m²·sec).

When we examine the effects of spectral quality on pigmentation, we find in all cases that blue light promoted the expression of fluorescent pigmentation most effectively. Green light ran second best in promoting fluorescent pigment production, although the filter used by these researchers transmitted a fair amount of blue light. The red filter effectively, and almost exclusively, transmitted red wavelengths and was least effective in coaxing the coral animal to produce fluorescent pigments. Lamp spectral quality is another issue that should be investigated further.

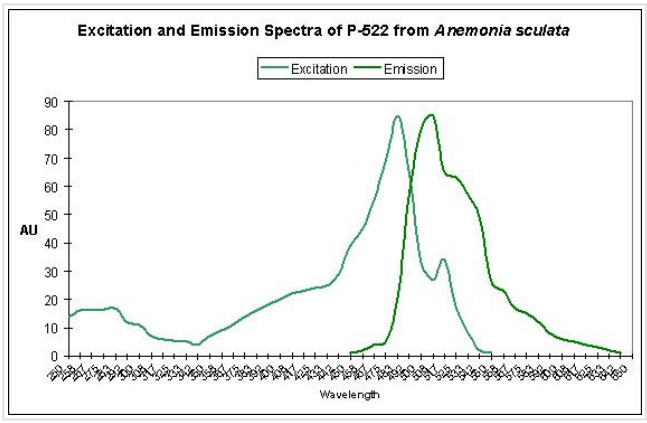


Figure 20. The double-peaked emission (and excitation, for that matter) suggests we are observing a pigment in transmission from one peak emission to another (499 nm to 522 nm, which is blue-green to green, respectively). From Wiedenmann, 2002.

In conclusion, the amount of light required to make corals 'color up' is not particularly difficult to achieve, although the low and high coloration thresholds can be fairly narrow (as in the case of Pigment 486 found in *Montipora digitata*). Perhaps more importantly, the production and/or maintenance of cyan pigments are not as sensitive to spectral quality (especially to red light) as some of the pigments we'll examine in future articles. Simple visual observations of corals within our aquaria support the experimental evidence that blue-green pigments are not all that difficult for corals to produce with these artificial environments.

The negative effects of exposure to red light for prolonged periods (exceeding ~30 days or so) are becoming more apparent. In this article, we see that red light apparently fails to promote coral coloration as efficiently as either green or blue bandwidths. We discussed the effects of red light on coral growth in the December 2008 edition of Advanced Aquarist. See here for details: www.advancedaquarist.com/2008/12/aafeature1

The concept and practice of classifying pigments by clade is another important step. Previously, our best method of categorizing pigments was by fluorescence excitation and, more particularly, emission. While we can not say that pigments within a certain clade will react in roughly the same manner as another pigment within the same clade, we can begin to form generalizations. With time, the clade categories will probably prove to be an important tool in our understanding of fluorescent pigments and their production and maintenance.

I'm presently working on a project with the goal of finding an inexpensive method of classifying the particular fluorescent pigment(s) within corals. If successful, I'll present this information in a future article.

With that, our examination of fluorescent pigments will end for this time. Next month, we'll look at new information on green fluorescent pigments and how light intensity and quality promotes or discourages pigmentation. Future articles will focus on orange and red fluorescent proteins. This series will close with a look at the non-fluorescent chromoproteins found in so many corals. A complete reference list will appear in that article.

Table 1. Clade A includes only anemones with green or red fluorescent pigments along with a number of non-fluorescent chromoproteins. Also included is the 'kindling' protein from *Anemonia sculata*. Key: Non-fluorescent protein (Chromoprotein); DsRed-type protein (DsRed); Green Fluorescent Protein (GFP).

Species	Name	Type	Order	Excitation	Em/Abs	Clade
Anemonia equina	CP-597	Chromoprotein	Actinaria	n/a	597	A
Anemonia sculata	CP-568	Chromoprotein	Actinaria	n/a	568	A
Anemonia sculata	CP-575	Chromoprotein	Actinaria	n/a	575	A
Anemonia sculata	CP-595	Chromoprotein (KPF1)	Actinaria	n/a	595	A
Anemonia sculata	CP-562	Chromoprotein	Actinaria	n/a	562	A
Cnidopis japonica	CP-610	Chromoprotein	Actinaria	n/a	610	A
Condylactis gigantea	CP-571	Chromoprotein	Actinaria	n/a	571	A
Condylactis passiflora	CP-571	Chromoprotein	Actinaria	n/a	571	A
Heteractis crisperi	hmagnCP	Chromoprotein	Actinaria	n/a	578	A
Rhizostoma anemone	CP-588	Chromoprotein	Actinaria	n/a	588	A
Entacmaea quadricolor	eqFP611	Ds-Red	Actinaria	559	611	A
Anemonia sculata	asFP499	GFP	Actinaria	?	499	A
Condylactis gigantea	cgigGFP	GFP	Actinaria	399/482	496	A
Heteractis crisperi	hcriGFP	GFP	Actinaria	405/481	500	A

Table 2. Clade B includes all *Acropora* and *Montipora* non-fluorescent proteins (along with those of other genera) and several Ds-Red type fluorescent pigments. Key: Non-fluorescent protein (Chromoprotein); DsRed-type protein (DsRed); Cyan Fluorescent Protein (CFP).

Taxon	Pigment Name	Type of Pigment	Order/Suborder	Excitation	Emission	Clade
Discosoma striata	P-583	CFP	Corallimorpharia	443	483	B
Acanthastrea	CP-579.5	Chromoprotein	Faviina	n/a	579.5	B
Acropora	CP-500	Chromoprotein	Astrocoeiina	n/a	500	B
Acropora	CP-586	Chromoprotein	Astrocoeiina	n/a	586	B
Acropora aculeus	aacuCP	Chromoprotein	Astrocoeiina	n/a	580	B
Acropora aculeus	CP-580	Chromoprotein	Astrocoeiina	n/a	580	B
Acropora digitifera	CP-578	Chromoprotein	Astrocoeiina	n/a	578	B
Acropora formosa	CP-588	Chromoprotein	Astrocoeiina	n/a	588	B
Acropora hyacinthus	ahyaCP	Chromoprotein	Astrocoeiina	n/a	580	B
Acropora millepora	amilCP	Chromoprotein	Astrocoeiina	n/a	588	B
Acropora nobilis	CP-579	Chromoprotein	Astrocoeiina	n/a	579	B
Acropora nobilis	CP-583.5	Chromoprotein	Astrocoeiina	n/a	583.5	B
Acropora nobilis	CP-591	Chromoprotein	Astrocoeiina	n/a	591	B
Caulastrea sp.	CP-591.5	Chromoprotein	Faviina	n/a	591.5	B
Galaxea fascicularis	gfasCP	Chromoprotein	Meandriina	n/a	577	B
Goniopora djiboutiensis	gdjiCP	Chromoprotein	Fungiina	n/a	583	B
Goniopora tenuidens	gten	Chromoprotein	Fungiina	n/a	580	B
Millepora (hydrocoral)	CP-579	Chromoprotein	Capitata	n/a	579	B
Montipora efflorescens	meffCP	Chromoprotein	Astrocoeiina	n/a	574	B
Montipora efflorescens	CP-590	Chromoprotein	Astrocoeiina	n/a	590	B
Montipora sp.	CP-579.5	Chromoprotein	Astrocoeiina	n/a	579.5	B
Montipora sp.	CP-584	Chromoprotein	Astrocoeiina	n/a	584	B
Montipora sp.	CP-592	Chromoprotein	Astrocoeiina	n/a	592	B
Pocillopora sp.	CP-480	Chromoprotein	Astrocoeiina	n/a	480	B
Pocillopora damicornis	CP-560	Chromoprotein	Astrocoeiina	n/a	560	B
Pocillopora sp.	CP-593	Chromoprotein	Astrocoeiina	n/a	593	B
Porites murrayensis	CP-579.5	Chromoprotein	Faviina	n/a	579.5	B
Seriatopora hystrix	CP-560	Chromoprotein	Astrocoeiina	n/a	560	B
Stylocoeniella sp.	stylCP	Chromoprotein	Astrocoeiina	n/a	574	B
Stylophora pistillata	spisCP	Chromoprotein	Astrocoeiina	n/a	560	B
Discosoma sp.	Ds-Red	Ds-Red	Corallimorpharia	558	583	B
Montipora sp.	Keima-Red	Ds-Red	Astrocoeiina	440	620	B
Porites porites	pPorRFP	Ds-Red	Faviina	578	595	B

Table 3. Clade C consists of several sub-clades (C1, C2 and C3) collectively consisting of 4 major types - all are fluorescent and can be cyan, green, yellow or red. Key: DsRed-type protein (DsRed); Cyan Fluorescent Protein (CFP); Green Fluorescent Protein (GFP); Yellow Fluorescent Protein (YFP).

Taxon	Pigment Name	Type of Pigment	Order/Suborder	Excitation	Emission	Clade
Acropora millepora	amilCFP	CFP	Astrocoeiina	441	489	C2
Acropora nobilis	anobCFP1	CFP	Astrocoeiina	462	490	C2
Acropora nobilis	anobCFP2	CFP	Astrocoeiina	477	495	C2
Anemonia majano	P-486	CFP	Actinaria	458	486	C2
Eusmilia fastigata	efasCFP	CFP	Meandriina	466	490	C1
Montipora efflorescens	meffCFP	CFP	Astrocoeiina	467	492	C3
Montipora millepora	mmilCFP	CFP	Astrocoeiina	404	492	C3
Psammocora sp.	psamCFP	CFP	Faviina	404	492	C3
Acropora aculeus	aacuGFP1	GFP	Astrocoeiina	478	502	C2
Acropora aculeus	aacuGFP2	GFP	Astrocoeiina	502	513	C2
Acropora eurostoma	AeurGFP	GFP	Astrocoeiina	504	515	C2
Acropora millepora	amilGFP	GFP	Astrocoeiina	503	512	C2
Acropora nobilis	anobGFP	GFP	Astrocoeiina	502	511	C2
Eusmilia fastigata	efasGFP	GFP	Meandriina	496	507	C1
Meandrites meandrina	mmeanGFP	GFP	Faviina	487	515	C1
Montipora efflorescens	meffGFP	GFP	Astrocoeiina	492	506	C3
Porites porites	pporGFP	GFP	Faviina	495	507	C3
Stylocoeniella sp.	stylGFP	GFP	Astrocoeiina	485	500	C2
Acropora millepora	amilRFP	Ds-Red	Astrocoeiina	560	593	C3
Fungia concinna	Kusabira-Orange	Ds-Red	Fungiina	548	561	C1
Montipora digitata	mdigFP572	Ds-Red	Astrocoeiina	556	572	C3
Montipora efflorescens	meffRFP	Ds-Red	Astrocoeiina	560	576	C3
Zoanthid sp.	Zoan2RFP	Ds-Red	Zoanthidae	506	574	C3
Zoanthid sp.	Zoan2RFP	Ds-Red	Zoanthidae	552	576	C*
Zoanthus sp.	zoanGFP	Ds-Red	Zoanthidae	496	506	C3
Zoanthid sp.	zoanYFP	YFP	Zoanthidea	528	538	C3

Table 4. Clade D has only fluorescent proteins. They are cyan, green and two types of red (Kaede and Ds-Red) pigments. Key: Cyan Fluorescent Protein (CFP); DsRed-type protein (DsRed); Green Fluorescent Protein (GFP); Kaede-type Fluorescent Protein (Kaede).

Taxon	Pigment Name	Type of Pigment	Order/Suborder	Excitation	Emission	Clade
Clavularia sp.	P-484	CFP	Alcyonaria	456	484	D
Montastraea cavernosa	P-486	CFP	Faviina	440	486	D
Mycedium elephantotus	meleCFP	CFP	Faviina	454	485	D
Scolymia cubensis	scubCFP1	CFP	Faviina	?	483	D
Scolymia cubensis	scubCFP2	CFP	Faviina	?	484	D
Montastrea cavernosa	CP-486	CFP	Faviina	440	486	D
Montastrea cavernosa	mcav5	CFP	Faviina	435	495	D
Montastrea cavernosa	r5	CFP	Faviina	?	495	D
Montastrea cavernosa	g5.1	CFP	Faviina	?	495	D
Montastrea cavernosa	g5.2	CFP	Faviina	?	495	D
Discosoma sp.	dis2RFP	Ds-Red	Corallimorpharia	558	593	D
Agaricia fragilis	afraGFP	GFP	Fungiina	494	503	D
Catalaphyllia jardineri	cjar	GFP	Faviina	509	517	D
Dendronephthya sp.	dendGFP	GFP	Alcyonaria	494	508	D
Discosoma sp.	dis3GFP	GFP	Corallimorpharia	503	512	D
Echinophyllia echinata	eechGFP1	GFP	Faviina	497	510	D
Echinophyllia echinata	eechGFP2	GFP	Faviina	506	520	D
Echinophyllia echinata	eechGFP3	GFP	Faviina	512	524	D
Favites abdita	fabdGFP	GFP	Faviina	508	520	D
Galaxea fascicularis	gfasGFP	GFP	Meandriina	492	506	D
Galaxea fascicularis	Azami-Green	GFP	Meandriina	492	505	D
Lobophyllia hemprichii	Eos	GFP	Faviina	506	516	D
Lobophyllia hemprichii	lhemOFP	GFP	Faviina	507	517	D
Montastraea annularis	monannGFP	GFP	Faviina	?	510	D
Montastraea cavernosa	mcavRFP	GFP	Faviina	504	517	D
Montastraea cavernosa	mcavGFP	GFP	Faviina	506	516	D
Montastraea cavernosa	P-510	GFP	Faviina	440	510	D
Montastraea cavernosa	mcav2	GFP	Faviina	505±3	515±3	D
Montastraea cavernosa	mcav3	GFP	Faviina	505±3	515±3	D
Montastraea cavernosa	mcav4	GFP	Faviina	505±3	515±3	D
Montastraea cavernosa	mcav6	GFP	Faviina	495	507	D

Taxon	Pigment Name	Type of Pigment	Order/Suborder	Excitation	Emission	Clade
Montastraea cavernosa	r2	GFP	Faviina	?	522	D
Montastraea cavernosa	r3	GFP	Faviina	?	514	D
Montastraea cavernosa	r4	GFP	Faviina	?	519	D
Montastraea cavernosa	r7	GFP	Faviina	?	505	D
Montastraea cavernosa	g1.2	GFP	Faviina	?	518	D
Montastraea cavernosa	g4	GFP	Faviina	?	518	D
Montastraea cavernosa	g6	GFP	Faviina	?	507	D
Montastraea faveolata	monfavGFP1	GFP	Faviina	440	510-520	D
Pectiniidae	Dronpa	GFP	Faviina	503	518	D
Platygyra lamellina	plamGFP	GFP	Faviina	502	514	D
Ricordea florida	P-510	GFP	Corallimorpharia	?	510	D
Ricordea florida	P-513	GFP	Corallimorpharia	?	513	D
Ricordea florida	P-517	GFP	Corallimorpharia	506	517	D
Ricordea florida	rfloGFP	GFP	Corallimorpharia	508	518	D
Ricordea florida	P-520	GFP	Corallimorpharia	?	520	D
Ricordea florida	P-573	GFP	Corallimorpharia	?	573	D
Ricordea florida	rfloRFP	GFP	Corallimorpharia	506	574	D
Sarcophyton sp.	sarcGFP	GFP	Octocorallia	483	500	D
Scolymia cubensis	scubGFP1	GFP	Faviina	497	506	D
Scolymia cubensis	scubGFP2	GFP	Faviina	497	506	D
Trachyphyllia geoffroyi	P-518	GFP	Faviina	508	518	D
Catalaphyllia jardineri	cjar	Kaede	Faviina	573	582	D
Dendronephthya sp.	Dendra	Kaede	Alcyonaria	557	575	D
Echinophyllia echinata	eechRFP	Kaede	Faviina	574	582	D
Favia favus	P-582	Kaede	Faviina	583	582	D
Favia speciosa	P-582	Kaede	Faviina	422	582	D
Lobophyllia hemprichii	EOS	Kaede	Faviina	571	581	D
Lobophyllia hemprichii	lhemOFP	Kaede	Faviina	543	574	D
Montastraea cavernosa	P-575	Kaede	Faviina	525	575	D
Montastraea cavernosa	mcav1	Kaede	Faviina	508	582	D
Montastraea cavernosa	r1.1	Kaede	Faviina	?	582	D
Montastraea cavernosa	r1.2	Kaede	Faviina	?	582	D
Montastraea cavernosa	g1.1	Kaede	Faviina	?	582	D
Montastraea cavernosa	mcavRFP	Kaede	Faviina	573	582	D
Montastraea cavernosa	mcavRFP	Kaede	Faviina	508	520/580	D

Taxon	Pigment Name	Type of Pigment	Order/Suborder	Excitation	Emission	Clade
Mycedium elephantotus	meleRFP	Kaede	Faviina	573	579	D
Plesiastrea verispora	P-574	Kaede	Faviina	560	574	D
Ricordea florida	rfloRFP	Kaede	Corallimorpharia	?	576	D
Scolymia cubensis	scubRFP	Kaede	Faviina	570	578	D
Trachyphyllia geoffroyi	Kaede	Kaede	Faviina	558	582	D

Table 5. A listing of cyan pigments (by species), along with information on host order/suborder, genera/species, excitation/emission and, if known, the pigments' clade. At this point, it seems fairly certain that cyan *Acropora* and *Montipora* pigments are Clades C2 and C3, respectively.

Species	Pigment Name	Type	Suborder	Excit.	Emission	Clade
Acropora millepora	amilCFP	CFP	Astrocoeiina	441	489	C2
Acropora millepora	amilFP484	CFP	Astrocoeiina	420	484	?
Acropora millepora	amilFP497	CFP	Astrocoeiina	477	497	?
Acropora nobilis	anobCFP1	CFP	Astrocoeiina	462	490	C2
Acropora nobilis	anobCFP2	CFP	Astrocoeiina	477	495	C2
Acropora pulchra	apulFP486	CFP	Astrocoeiina	420	483	?
Anemonia majano	P-486	CFP	Actinaria	458	486	C2
Clavularia sp.	P-484	CFP	Alcyonaria	456	484	D
Discosoma striata	P-483	CFP	Corallimorpharia	443	483	B
Eusmilia fastigata	efasCFP	CFP	Meandriina	466	490	C1
Hydnophora grandis	hgraFP492	CFP	Faviina	443	492	?
Montastraea cavernosa	P-486	CFP	Faviina	440	486	D
Montipora digitata	mdigFP486	CFP	Astrocoeiina	470	486	?
Montipora efflorescens	meffCFP	CFP	Astrocoeiina	467	492	C3
Montipora millepora	mmilCFP	CFP	Astrocoeiina	404	492	C3
Mycedium elephantotus	meleCFP	CFP	Faviina	454	485	D
Pocillopora damicornis	P-499	CFP	Astrocoeiina	484	499	C2
Psammocora sp.	psamCFP	CFP	Faviina	404	492	C3
Scolymia cubensis	scubCFP1	CFP	Faviina	?	483	D
Scolymia cubensis	scubCFP2	CFP	Faviina	?	484	D
Montastrea cavernosa	CP-486	CFP	Faviina	440	486	D
Montastrea cavernosa	mcav5	CFP	Faviina	435	495	D
Montastrea cavernosa	r5	CFP	Faviina	?	495	D
Montastrea cavernosa	g5.1	CFP	Faviina	?	495	D
Montastrea cavernosa	g5.2	CFP	Faviina	?	495	D

FEATURE ARTICLE

The Development of a Method for the Quantitative Evaluation of Protein Skimmer Performance

By Ken S. Feldman, Kelly M. Maers, Lauren F. Vernese, Elizabeth A. Huber, Matthew R. Test

Department of Chemistry, The Pennsylvania State University, University Park, PA 16802. *Protein skimmers have become indispensable for many aquarists who strive to maintain the high water quality necessary to keep stony corals.*

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Purifying aquarium water with a protein skimmer represents one of the major strategies for maintaining water quality. This singular piece of equipment can often constitute the major set-up capital expense after the tank itself. With its presumably pivotal role in aquarium husbandry and its high price tag, it is no surprise that skimmer manufacturers strive to outdo one another with their exhortations and grandiose claims. For example, what aquarist can resist statements like:

***Euroreef*:**

> *"Custom modified pumps, "Euro-AirTM" venturis, and "Euro-WheelTM" pinwheel style impellers are combined to create that incredible volume of micro bubbles that provide the immense surface area resulting in unrivaled quick removal or organics resulting in a healthy aquarium."*
>
> *"The efficiency achieved with this design translates into higher performance at a lower operating cost to you!"*

***Precision Marine*:**

> *"These skimmers will outperform anything in their class."*
>
> *"Our fractionators strip organics from the system quickly due to the high flow rates they are designed to operate."*

***AETech (ETSS)*:**

> *"This provides certain unique operational properties set it apart from the rest of the ETSS line. Giving your tank gentle but highly effective waste removal that will never over skim your aquarium. It creates the finest bubble size that totally fills the skimmer body with so much air that it turns the water to a solid milk color. The countercurrent action allows for far greater air water contact time and reduces the amount of wet foaming to a minimum."*

These statements, which are representative and certainly not unique amongst skimmer manufacturers, raise a series of questions:

- What is the factual basis for these claims?
- How do the skimmer manufacturers assay skimmer "performance"?
- Are there any metrics at all available to quantify skimmer performance?
- What does the concept of performance even mean when discussing skimmers?

In the article to follow, we present a new approach to addressing these questions. We will introduce concrete definitions for skimmer performance that focus on both the *rate* of organic contaminant removal and the *extent* of organic contaminant removal from saltwater. We then will introduce an experimental procedure, based on a model system, to measure these performance metrics for four representative skimmers. Finally, we will show how this model system accurately replicates skimmer performance with authentic aquarium water. We hope that these advances will prove valuable in the hands of forward thinking skimmer designers by providing the means to optimize skimmer performance as a function of both design variables and operational variables. Thus, it actually might be possible to provide, for the first time, unequivocal evidence in support of the claims of "best" so indiscriminately dispensed today.

What is Protein Skimming?

Water purification through skimming has been thoroughly reviewed in the aquarium literature (Holmes-Farley, 2006) and so the description of the basics will be brief. In essence, skimming involves introducing air bubbles into aquarium water, and then physically removing those bubbles after they have absorbed organic impurities. The mechanisms by which the bubbles are introduced, how they are mixed with the water, and how they are removed from the bubble/water mixture can be addressed by distinctly different engineering approaches, and these differences presumably constitute the basis for claims of "better" (or at least different) amongst the various skimmer manufacturers. The bubbles themselves are the key, as they provide an air-water interface that is particularly favorable for adsorption of certain kinds of organic molecules. Not all organic molecules are susceptible to this type of capture; in fact, only those molecules that have distinct hydrophilic (= water loving) and hydrophobic (= water hating) sub-regions attach tightly to this surface. The hydrophilic portion of the molecule remains in the water, but the hydrophobic portion is forced out of the water and into the air space of the bubble. The process is illustrated for the generic skimmer pictured in Fig. 1. This skimmer is fed aquarium water via a pump, and that water is mixed vigorously with small air bubbles at the inlet. These bubbles pass through the water in the skimmer body and absorb susceptible organic molecules as they transit up the column. The water in the skimmer body then exits back to the aquarium, less the removed organics. The bubbles, on the other hand, coalesce into a foam at the top of the water column, and their continuous replenishment at the bottom of the foam forces the top of the foam out over a riser tube and into a collection annulus. This foam carries with it an organic load, and physical removal of this organic-rich foam/residue/sludge completes the water purification process. In addition to some dissolved organics, small particulates and microbes (bacterioplankton, phytoplankton) can be removed at the air/water interface of the bubble as well (Suzuki, 2008). The skimming process does not remove atoms/molecules that are strictly polar and readily dissolve in water, such as some organics, salts, inorganic phosphate, carbonate, etc.

History of Protein Skimming

The first documented examples of using foam generation to remove organic compounds from water can be traced to Ostwald, and independently, Schutz in 1937 (Ostwald, 1937; Schutz, 1937). Thiel claims that water purification via skimming was introduced into the aquarium hobby in the 1960's by Huckstedt (Thiel, 1997; Huckstedt, 1972), but the practice did not gain much traction until a resurgence of interest in keeping corals brought it to the fore again in the 1990's. Another early notable advance in using foam flotation technology for saltwater purification was described by Wallace (Wallace, 1969). The early developments in water purification then led to advances in two disparate venues; wastewater remediation, and protein purification (Lemlich, 1972; Okamoto, 1979; Clark, 1983; Caballero, 1990). The application of skimming in aquarium husbandry was an outgrowth of successful implementation of foam fractionation techniques in these areas, and the development of modern skimmers owes much to these pioneering efforts. Foam fractionation in particular proved to be a valuable asset in enabling the isolation/recovery of desirable proteins from dilute solutions in many areas of food and pharmaceutical science. In this context, the goal was just the opposite of protein skimming in aquaria; recovery of valuable proteins in the foam with discharge of the depleted water phase. In contrast, of course, protein skimming in aquaria is used to remove undesirable organics from the (valuable) tank water.

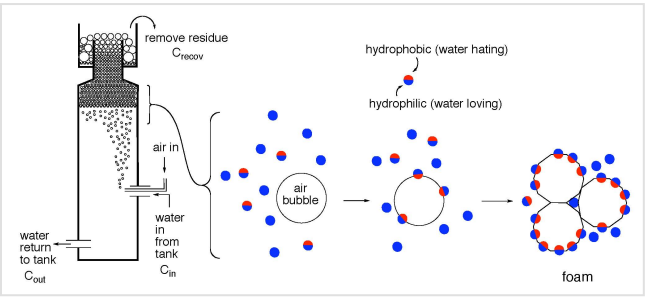

Figure 1. Schematic of the skimming process.

Nevertheless, the processes are identical, a conceptual convergence that becomes important in assessing the influence of various input parameters on skimmer performance. Specifically, the pivotal role of foam fractionation-based purification in protein recovery has prompted many research groups to conduct studies designed to optimize protein purification by tweaking input variables. It is possible that these studies can inform the aquarium skimming area as well. Much effort has been directed to measuring how changes in (a) gas flow rate, (b) liquid flow rate, and (c) bubble size influence two important figures-of-merit in the protein purification (and by inference, aquarium skimming) area; enrichment (E) and recovery (R). Enrichment (E) is defined slightly differently by different authors. Some authors describe E as the ratio of the protein concentration in the (collapsed and removed) foam head relative to the protein concentration in the skimmer feed solution ($E = C_{recov}/C_{in}$ in Fig. 1) (Uraizee, 1996; Brown, 1990), whereas other authors define this quantity as the ratio of protein concentration in the foam head compared to the protein concentration in the output solution of the skimmer ($E = C_{recov}/C_{out}$ in Fig. 1) (Ahmed, 1975; Schnepf, 1959). The numbers obtained by these two definitions do not differ greatly, and so this distinction is not critical. A second figure-of-merit often cited in these skimmer performance studies is recovery (R), which is defined as the amount of protein removed from the solution by the skimmer relative to the amount of protein fed into the skimmer. The recovery R can be expressed as a percent of protein removed after a specified time: i.e., 50% of the protein has been recovered after 90 minutes. These two measurable quantities typically run in opposite directions; that is, those changes that increase the enrichment typically decrease the recovery, and vice versa.

Both enrichment and recovery have counterparts in the aquarium skimming area. Dry skimming implies very little water hold-up in the foam, and this scenario is more closely aligned with enrichment. Thus, maximizing foam enrichment while dry skimming should maximize impurity removal from aquarium water. In contrast, wet skimming, with its proportionally larger liquid hold-up in the foam, falls more under the aegis of the "recovery" manifold of skimmer operation. That is, the removal of organic-rich foam *and* entrained aquarium water that contains organics (= wet skimming) should lead to a greater overall removal of the organic impurities in the aquarium water. In this case, maximizing recovery R should lead to maximizing water purification. To the extent that an aquarist aligns their skimming technique with one or the other extreme, then the lessons learned about optimizing either enrichment or recovery might prove insightful. Wet skimming bears the added burden of introducing possible salinity fluctuations, as the aquarium water removed in the foam phase must be replaced by water of equivalent salinity in order to maintain the overall tank's salinity. To the extent that this match is not maintained, the tank's overall salinity may vary. Thus, a compromise between wet and dry skimming often is sought.

In the final analysis, any operational parameter changes that increase the amount of organic material removed from the aquarium water is equivalent to achieving better water purification. Protein purification studies typically focus on enrichment E, as the purity of the recovered protein, which may find use in food and/or biomedical applications, often is paramount. Thus, biomedical and food science researchers are willing to sacrifice removal capacity (higher R) for product purity (higher E). Nevertheless, the reciprocal relationship between the two skimmer benchmarks suggests that lessons learned in the protein purification area can provide insight into aquarium skimming as well. Typical results from the protein purification area serve to illustrate the connection, Figs. 2, 3, and 4. In these studies, a model protein, Bovine Serum Albumin (BSA) in high ionic strength water (~ 5.8 ppt of NaCl; compare saltwater ~ 35 ppt of all salts) is circulated through a model skimmer with concomitant introduction of air through a bubble-making porous frit. The collected and collapsed foam is removed and assayed for protein concentration, as are the skimmer's input and output streams. Fig. 2 illustrates how E and R run in opposition when gas flow rate is the experimental variable. Thus, higher gas flow rates decrease E but increase R, at least over the flow ranges tested.

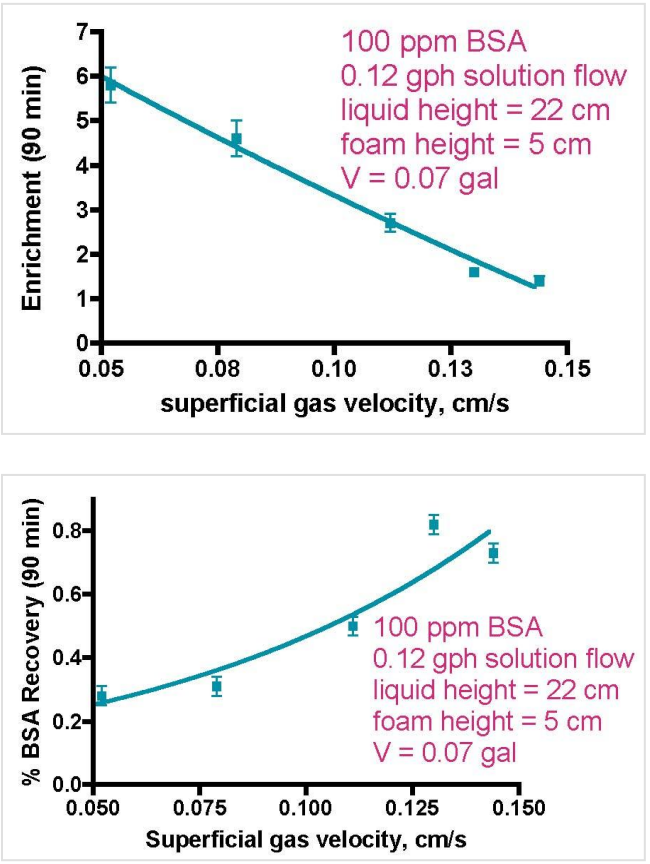

Figure 2. Skimmer performance as a function of gas flow rate (from Brown, 1990).

A comprehensive and quantitative explanation for these trends (E or R vs. gas flow rate) is lacking, but a qualitative rationale for these observations has been developed (Brown, 1996; Uraizee, 1996; Du, 2000; Wong, 2001; Rosa, 2007). The key is the behavior of the bubbles, both as they transverse the solution absorbing BSA, and then in the foam, where they coalesce. There are several different properties of bubbles in these different environments that impact the overall E or R, and some of these properties run in opposition. Thus, the aggregate observed behavior (increasing or decreasing E and R) reflects the competition between these opposing phenomena. Specifically,

1. The surface area of the bubbles is a key parameter, as that is where protein absorption occurs. Smaller bubbles have a greater surface area per unit volume. In solution, the bubbles are nearly spherical, and the surface area, A, = 6/d (d = diameter of the bubble). In the foam, the bubbles actually adopt a dodecahedral shape (= 12 sided) as a consequence of its six nearest neighbor interactions, and A = 6.6/d (Du, 2001). So, *in the liquid phase*, smaller bubbles will lead to greater bubble surface area for a given volume, which in turn enhances both enrichment and recovery.

2. Liquid entrained in the foam drains back down from the foam phase into the bulk solution. For a variety of complicated reasons, larger bubbles result in faster foam drainage. In general, this drainage serves to increase enrichment, E, as it removes from the foam some liquid which is not as rich in protein as are the bubbles themselves. By default, the remaining foam then is more highly enriched in protein, leading to a larger measured E. For these reasons, larger bubbles *in the foam phase* increase enrichment.

But, what factors influence the bubble size?

The relationship between superficial gas flow rate and bubble size is complex and appears to depend on the details of the bubble generation process. In the Brown work cited above, faster gas velocities lead to marginally smaller bubbles for the 4-10 µM frit size employed (Brown, 1990). Using frits with larger pore sizes for bubble formation than the one Brown et al. employed in generating the data of Fig. 2, Rosa and, independently, Wong, and Tanner report that the bubble size slightly increases with increasing superficial gas velocity (Wong, 2001; Du, 2002; Rosa, 2007). Enhanced bubble coalescence due to more bubble-bubble collisions at the faster gas flow rates is cited as the rationale (Wong, 2001). In no case are the changes in bubble size very large as the gas flow rate varies over the range examined.

As far as enrichment vs. gas flow rate goes, drainage from larger bubbles in the foam (point 2 above) appears to predominate over the greater protein binding surface area of the smaller bubbles in the liquid (point 1 above) under Brown's experimental conditions, and so the enrichment data in Fig. 2 result. In actuality, slower gas velocities result in complementary effects that both influence E in the same direction; (a) the aforementioned greater foam drainage as a consequence of the larger bubbles, which increases E (Gehle, 1984), and (b) greater protein absorption due to greater bubble residence time in the liquid phase (Bhattacharjee, 1997), which should increase E also (Uraizee, 1996), thus providing a physical explanation for the E vs. gas flow trend shown in Fig. 2. Of course, at the other extreme, faster gas velocities entrain more liquid into the foam. This dilution with relatively protein-poor water diminishes the relative contribution of the protein absorbed on the bubble surface to the overall protein present in the foam, thus contributing to a decrease in enrichment at these faster gas flow rates.

Recovery vs. gas velocity is a different story; in this instance, the lesser amount of foam drainage resulting from the smaller bubbles that are generated at faster gas velocities actually should increase R, the recovery. That is, since the amount of protein residing in the foam is the sum of the (concentrated) protein on the bubble surface layer *and* the protein dissolved in the interstitial hold-up liquid, then anything that *decreases* foam drainage will increase the *overall* amount of protein present and hence recovered from the collapsed foam, and R will increase (Fig. 2, second graph). Enhancing this effect is the fact that more liquid is entrained into the foam at higher gas velocities (Uraizee, 1996; Wong, 2001). The complementary conclusion holds as well; larger bubbles from slower gas velocities lead to more foam drainage and a decrease in recovery.

In a separate series of experiments, Brown also has shown that increasing the flow rate of the liquid through the model skimmer leads to a measurable decrease in recovery, but not much change, except at very low flow rates, in the enrichment, Fig. 3. Of course, the liquid flow rates examined in these experiments are orders-of-magnitude less that the flow rates used in aquarium skimmers, but that disconnect is balanced out somewhat by the fact that the model skimmer used in these studies is just a bit larger than a toilet paper tube. An explanation for these trends is provided below.

Protein recovery as a function of liquid flow rate is determined once again by the intersection of two opposing effects. Faster liquid flow rates lead to smaller bubbles both in the liquid phase and in the foam phase (Brown, 1990; Wong, 2001; Du, 2002). The former observation is attributed to less opportunity for bubble-enlarging coalescence, whereas the latter result is explained by citing diminished coalescence of bubbles in the foam as a consequence of protein concentration/bubble surface tension effects (Wong, 2001). If this bubble size effect was dominant, we might expect that recovery would increase as liquid flow increased (= smaller bubbles), since foam drainage would be diminished. However, that expectation is not met experimentally. Therefore, another phenomenon must be in play, and Wong theorizes that faster liquid flow leads to less contact

time between the rising bubbles and the protein-containing liquid phase. In this scenario, less protein will be captured on the bubble surface, and overall recovery suffers (Wong, 2001).

Bubble size occupies a central role in aquarium skimmer performance discussions, and not surprisingly, this topic clearly retains its importance in the protein purification literature as well. Unfortunately, hard data on the direct effect of bubble size on either enrichment or recovery are scarce, and Aksay's report of enrichment and recovery as a function of bubble size represents perhaps the best information to date (Aksay, 2007; see also Uraizee, 1996). In this report, Aksay and Mazza document that the enrichment increases and the recovery decreases as bubble size increases, Fig. 4. Explanations for these trends have been discussed previously; larger bubbles lead to more foam drainage, which increases enrichment. On the other hand, these larger bubbles (a) trap less protein per-unit-volume and of course (b) enhance foam drainage, leading to a decrease in recovery. As an aside, the whole topic of measuring bubble sizes during a skimmer run is fraught with controversy; most authors use photography to characterize bubbles at the skimmer wall only (Brown, 1990; Uraizee, 1996; Wong, 2001; Aksay, 2007), but that approach has been criticized by Tanner, who has developed an indirect technique to measure bubble sizes at any position in the foam (Du, 2001; Du, 2003). He found that wall effects do indeed exist, and foam interior bubbles appear to be ~ 1.5 times larger than the wall bubbles.

The relationship of the Figs. 2-4 model system data to authentic skimmer performance in an aquarium setting remains to be established, of course, but these experimental results do serve as an alert that skimmer performance studies are possible when the criteria for success can be defined and measured. For example, one of the implications of these studies is that there may be an "optimum" bubble size for maximizing enrichment, as discussed by Uraizee (Uraizee, 1995). This provocative suggestion adds a new dimension to typical skimmer operational

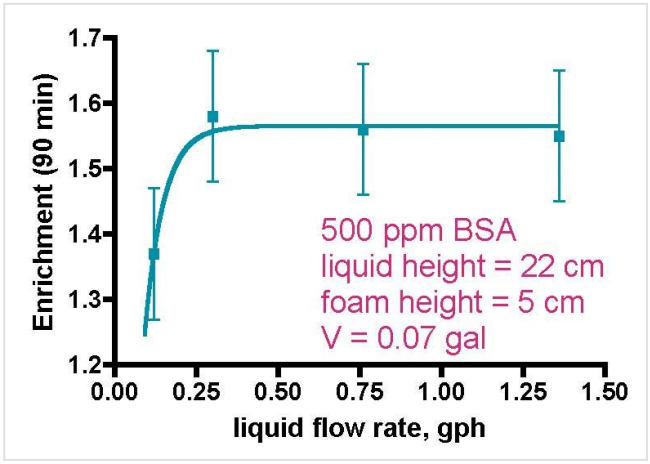

Figure 3. Skimmer performance as a function of water flow rate (from Brown, 1990).

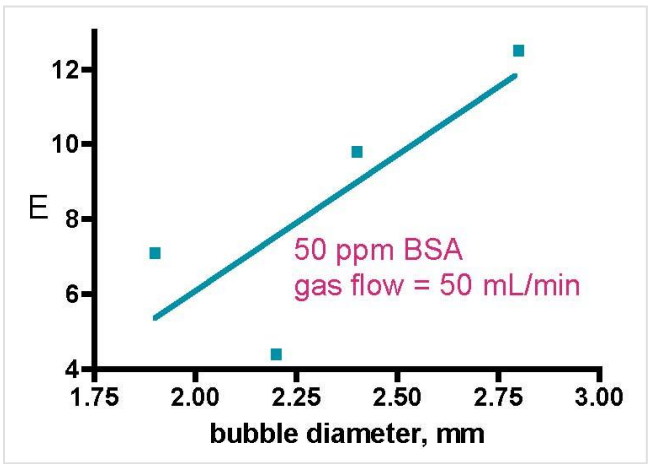

Figure 4. Skimmer performance as a function of bubble size (Aksay, 2007). These experiments were run in batch mode.

descriptions in the aquarium literature that emphasize only maximizing bubble surface area (i.e., more smaller bubbles per unit volume) in order to maximize organic removal. An aquarist using the information on enrichment and recovery discussed above might be inclined to conclude that the best skimmer performance can be achieved by operating with a lower liquid flow rate and a higher gas velocity. We will see later that when other criteria perhaps more relevant to aquarium skimming are included in the discussion, this conclusion does not necessarily hold.

In addition to the three operating variables described above (liquid flow rate, gas flow rate, and bubble size), several other aspects of protein skimmer performance have been examined. For example, the effect of (1) pH of the liquid phase, (2) feed flow concentration of BSA, and (3) liquid pool height all have been benchmarked vs. enrichment and recovery. Furthermore, much effort has been directed to developing mathematical models with predictive value for E and R as a function of various input variables. Unfortunately, that modeling has not lent any greater insight into protein skimming than could be derived by examining the data directly. The interested reader is referred to the articles cited above for details on all of these points.

The Goals of Our Research

The research described above on protein enrichment and recovery with model skimmers does not directly address the needs of aquarists, although it does provide some insight into the relationship between operational parameters and some aspects of skimmer performance. We sought to develop more useful metrics for measuring skimmer performance in an authentic aquarium setting. Further, we sought to apply those metrics to comparing directly the performance of (a) four different but representative skimmers, and (b) a given skimmer under different operating conditions, to illustrate how this methodology might be employed.

Defining Useful Skimmer Performance Metrics

Two measures of skimmer performance that we postulate will be of interests to aquarists are (1) the rate (speed) by which the skimmer removes organic contaminants, and (2) the amount of organic contaminants removed relative to the total amount present in the tank water. From here on, we will refer to the organic contaminants as Total Organic Carbon (TOC). For a review of TOC values and related issues in marine aquaria, see our earlier articles (Feldman, 2008c; Feldman, 2008d). The first of these metrics, the rate of TOC removal, is a kinetic quantity; the second (how much TOC is removed) is a thermodynamic quantity. They both report on different properties of skimming, and there is no conceptual reason why they must run in parallel. That is, high rates of TOC removal do not necessarily correlate with large amounts of TOC removal, and vice versa. These criteria for skimmer performance are no different that the criteria we invoked in our earlier GAC (granular activated carbon) studies, and the interested reader can obtain further background information from those articles (Feldman, 2008a; Feldman, 2008b).

The Rate of TOC Removal - Mathematical Formulae

It is essential to devise a robust mathematical model in order to extract useful quantitative information about the rate of organic impurity removal by a skimmer. The recirculating reservoir/skimmer system maps quite closely onto a fundamental textbook problem in mass transfer/fluid flow encountered in introductory chemical engineering courses called the "well-stirred reactor" problem. In this instance, both the skimmer and also the reservoir can be modeled as "well-stirred reactors" with a given flow Q between them. A component of the water is depleted in the skimmer by bubble-mediated removal. Since both "reactors" are interconnected, the level of the component will drop in the reservoir as well, and our task will be to develop a mathematical model that relates the removal in the skimmer with the measured depletion in the reservoir. In our experimental setup, a liquid volume V_r (saltwater in a Rubbermaid tub) has an input stream and an output stream, and some organic impurities in the liquid become depleted over time via bubble-mediated removal in a skimmer with water volume V_s. In actuality, V_s is just that volume in the skimmer reaction chamber where bubble-mediated organic extraction occurs. Inspection of the skimmers in action (see Fig. 7) permits estimation of this "active" skimmer volume, which is the value that we will use for V_s. For the purposes of this analysis, we will assume that all of the active volume is water; that is, we will ignore the void volume of the bubbles, as we cannot independently assess the relative contributions of bubbles and water. This assumption will introduce an error into the calculations, but that error should be systematic for all skimmers, and since we are interested in relative and not absolute skimmer performance, this error should not affect the conclusions. Knowledge of the precise mechanism by which the skimmer's bubbles removes the water component(s) is not required; all that we need to know is that the concentration of the measured water component (the organic impurity in this case) is diminishing with time in the reservoir. We have examined skimmer performance with both Bovine Serum Albumin (BSA) in freshly prepared saltwater as a model for TOC in aquarium water, and authentic TOC in reef tank water. The mathematical derivation uses TOC for convenience, but the formulae are identical with BSA as well (i.e., substitute BSA for TOC in the equations below).

It is essential for solving this problem that both the reservoir and the skimmer water volumes are well mixed to avoid concentration gradients (Felder, 2005). The reservoir water mixing in the experiments described below is provided by the skimmer return flow and by two powerheads in the reservoir. We independently tested the "well mixed" assumption in the reservoir

by sampling TOC levels at a given time point at different locations (i.e., top, bottom, left side, right side) during a skimmer run. We observed that the site-to-site variation in TOC levels at different locations was no greater than the sample-to-sample variation at one location (both ~ 10%), suggesting that there is no reason to suspect that the "well mixed" assumption is not applicable. The mixing in the skimmer reaction chamber is provided by both rapid water movement and the agitation caused by the motion of the bubble stream. We have no independent experimental measurement/confirmation of mixing behavior in the skimmer. A related but simpler mathematical approach recently has been applied to skimmer performance analysis in a different context by Rosa (Rosa, 2007, cf. Eq. 4).

The fundamental physical property of the system that we will rely on to develop a mathematical model for skimmer performance is called *mass balance*; conservation of mass dictates that mass (matter, in this case TOC) cannot be created or destroyed, and so the amount of TOC depleted from the reservoir must be equal to the amount of TOC that is removed by the skimmer's bubbles. The fundamental mass balance equation is given in Eq. (1). Details can be found in the Wikipedia entry for "Mass Balance" and "Continuously Stirred-Tank Reactor".

(1) input + generation = output + accumulation

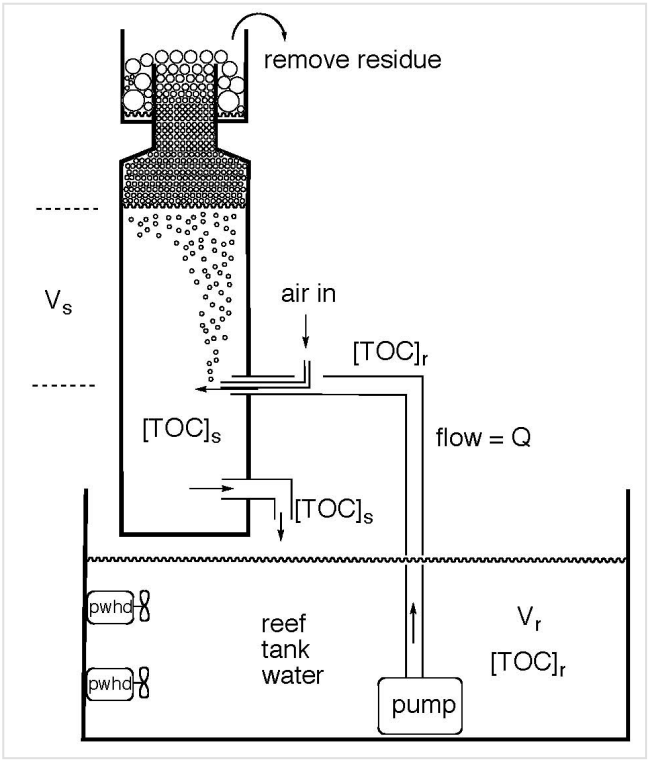

Figure 5. The skimmer/reservoir system used for derivation of the math that leads to k, the rate constant for TOC removal.

For the problem in hand, there is no generation of TOC; rather, TOC is removed by the skimmer's bubbles, so we will replace the "generation" term with "removal" (actually, just the negative of generation). In addition, since there is no TOC generation, there will be no TOC accumulation; rather, TOC is depleted in the system over time, so we will replace the "accumulation" term with "depletion" (just the negative of accumulation). So, the new mass balance expression applicable to both the reservoir, and independently, the skimmer is

(2) input + removal = output + depletion

We will focus on the reservoir first. There is no explicit TOC removal in the reservoir, so "removal" in Eq. (2) = 0. The input is equivalent to the mass of TOC added over time, say milligrams-per-minute (mg/min). In fact, since one term of Eq. (2) has units of mg/min (or more generally, mass/time), then all of the terms in this equation must be expressed in these units. The mass (amount) of TOC added in the input stream is the concentration of TOC, $[TOC]_s$ (in mg/gal) times the volumetric flow rate of the input stream, Q (Q in gal/min). That is,

(3) mass of TOC added over time in input stream = $Q \bullet [TOC]_s$

Note how the units of these terms are consistent: Q (in gal/min) • $[TOC]_s$ (in mg/gal) = mg/min units, which is just what the "mass of TOC added over time" requires. The output stream of the reservoir can be treated in a similar manner:

(4) mass of TOC removed over time in output stream = $Q \bullet [TOC]_r$

So, referring back to Eq. (2), with removal = 0, we have

(5) $Q \bullet [TOC]_s = Q \bullet [TOC]_r$ + depletion

What mathematical expression can we use for "depletion"? The amount of TOC in the reservoir volume V_r is simply the product of the TOC concentration, $[TOC]_r$, and the volume:

(6) amount of TOC in the reservoir = $V_r \bullet [TOC]_r$

We can simply define "depletion" to mean the rate of change (decrease) in the TOC amount in the reservoir over time. Note that once again, the units of this term are in mass/time ("mg/min").

(7) depletion = $V_r \bullet d[TOC]_r/dt$

Now, returning to Eq. (5), we can insert the depletion term and generate the full mass balance expression for the reservoir:

(8) $V_r \bullet d[TOC]_r/dt = Q \bullet ([TOC]_s - [TOC]_r)$, where

V_r = the total volume of the reservoir water, in gal

$[TOC]_r$ = the concentration of TOC in the reservoir at any time t and also the concentration of TOC in the stream leaving the reservoir and entering the skimmer

Q = the flow through the system, in gpm

$[TOC]_s$ = the concentration of TOC in the stream leaving the skimmer and entering the reservoir

Eq. (8) says that the change in the amount of TOC in the reservoir (the left hand side) is equal to the difference between the reservoir input and output TOC concentrations ($[TOC]_s$ - $[TOC]_r$) times the flow rate (the right hand side). Note that this expression includes information about TOC concentrations in both the reservoir and the skimmer.

A similar expression can be developed for the fate of the TOC concentration just in the skimmer. However, in this case, "removal" does not equal 0, as the bubbles in the skimmer actively remove the organic impurity. So, from Eq. (2),

(9) $V_s \bullet d[TOC]_s/dt = Q \bullet ([TOC]_r - [TOC]_s)$ + removal

We must include another term (removal) in Eq. (9) that explicitly accounts for this TOC removal in order to maintain the required mass balance. This TOC removal term must take into account the function of the bubbles. The fundamental chemical equation for TOC removal by the bubbles is:

(10) TOC + bubbles → TOC • bubbles

This simple equation undergirds the assumption that allows the mathematical analysis to proceed: *the key assumption is that the rate of TOC removed by the skimmer's bubbles is proportional to the amount of TOC present in solution.* This assumption permits us to connect the [TOC] changes that happen in the skimmer with the [TOC] changes that happen in the reservoir. Since the bubbles are continually being introduced in large excess compared to the TOC concentration in solution, the "concentration" of the bubbles remains for all intents and purposes constant, and so we do not have to worry about how changes in bubble concentration might influence the rate of TOC removal. This model (and its underlying assumptions) greatly simplifies the mathematical analysis. In terms of the discipline of chemical kinetics, this approach is called the "pseudo-first order" approximation. So, the amount of TOC in the active skimmer reaction chamber volume V_s is given by the concentration times the volume:

(11) amount of TOC in the active water volume of the skimmer = $V_s \bullet [TOC]_s$

By the assumption discussed above, the rate of TOC removal by the bubbles is proportional to the amount of TOC present:

(12) rate of TOC removal by the bubbles $\propto -V_s \bullet [TOC]_s$

Note that we must include a "-" sign in front of $V_s \bullet [TOC]_s$ because the amount is *decreasing* with time. We can introduce a proportionality constant, k, to convert Eq. (12) into an equality.

(13) rate of TOC removal by the bubbles = $-k \bullet V_s \bullet [TOC]_s$

The term $k \bullet V_s \bullet [TOC]_s$ must have units of mass/time (i.e., mg/min) in order to "fit" into Eq. (9). Since $Vs \bullet [TOC]_s$ has units of mass (mg), then k must have units of /time (i.e., per min). As such, k is often referred to as a *rate constant*; it expresses how something changes over time ("per min"). This proportionality constant is not just a mathematical convenience. It will report on a fundamentally important property of a skimmer. The constant k can be viewed as a measure of how efficiently the bubbles remove TOC. The efficiency of TOC removal is a complex function of many factors (bubble size/density, bubble residence time, solution diffusion, mass transfer to the bubble, foam coalescence, binding to the bubble surface, etc.). *In fact, it is just this value k that we are after; k is actually a singular measure of skimmer efficiency in removing TOC.* The larger that k is, the faster that the skimmer will remove TOC. Thus, k is an intrinsic measure for TOC removal, and it reflects, in the aggregate, all of the parameters that contribute to the efficiency of TOC removal for a given skimmer. However, k does not offer any insight into which parameters, in particular, are more or less important in determining skimmer performance. We can measure experimentally k for different skimmers, and compare these values. Skimmers with larger k values will be more efficient (i.e., faster) at removing TOC from aquarium water. So, by including the information in Eq. (13) in Eq. (9), we have

(14) $V_s \bullet d[TOC]_s/dt = Q \bullet ([TOC]_r - [TOC]_s) - V_s \bullet k \bullet [TOC]_s$

This rather complex equation is important because, for the first time, we have mathematically linked the experimentally measurable quantity, the concentration of TOC in the reservoir, $[TOC]_r$, with quantities specific to the skimmer (V_s, $[TOC]_s$). However, Eq. (14) as written is difficult to manipulate, so we will define a new mathematical quantity that will help us simply the math and arrive at a very useful expression. We define a dimensionless time $\tau = Q \bullet t/V_r$. Applying this definition to Eq. (14), we can generate the following expression:

(15) $(V_s/V_r) \bullet d[TOC]_s/d\tau = [TOC]_r - [TOC]_s - V_s \bullet k \bullet [TOC]_s/Q$

The purpose for this seemingly arbitrary definition/substitution exercise now can be revealed. The use of τ allows us to isolate the dimensionless quantity V_s/V_r, which is the ratio of the active skimmer volume to the reservoir volume. We can inject a dose of physical reality into the mathematics at this point. The reservoir volume (30 or 35 gallons) is much larger than the skimmers' volumes (0.62 - 1.26 gallons, see below) and so the ratio V_s/V_r hovers in the 0.02 - 0.04 range. If the second term in the left-hand part of Eq. (15), $d[TOC]_s/d\tau$ is not too large, then the V_s/V_r term will dominate, and we can neglect the entire left-hand term of Eq. (15); that is, we can, to a first approximation, set

$(V_S/V_r) \bullet d[TOC]_S/d\tau = 0$. In the Results section, we will provide experimental justification for the claim that $d[TOC]_S/d\tau$, is not large. By applying this approximation, we can relate the TOC concentration in the skimmer, $[TOC]_S$, to the TOC concentration in the reservoir, $[TOC]_r$:

(16) $0 = [TOC]_t - [TOC]_S - k \bullet [TOC]_S \bullet V_S/Q$, or

(17) $[TOC]_S = [TOC]_r/(1 + k \bullet V_S/Q)$

We now can plug this $[TOC]_S$ value back into Eq. (8) and divide both sides by V_r:

(18) $d[TOC]_r/dt = (Q/V_r) \bullet ([TOC]_r/(1 + k \bullet V_S/Q) - [TOC]_r)$

Rearranging the various terms yields a differential equation:

(19) $d[TOC]_r/[TOC]_r = -[k \bullet Q/(V_r \bullet (k + Q/V_S))] \bullet dt$,

and solving the differential equation yields

(20) $Ln([TOC]_r/[TOC]_0) = -[k \bullet Q/(V_r \bullet (k + Q/V_S))] \bullet t$

Eq. (20) provides the means to extract the desired quantity, the rate constant k, from the experimental data. Eq. (20) predicts that the graph of $-Ln([TOC]_r/[TOC]_0)$ vs. time t will be linear, with a slope of $k \bullet Q/(V_r \bullet (k + Q/V_S))$. By plotting $-Ln([TOC]_r/[TOC]_0)$ vs. t, the desired value k can be calculated for any input flow rate Q, reservoir solution volume V_r and active skimmer volume V_S. Note that $[TOC]_0$ = the concentration of TOC at t = 0, the beginning of the experiment. Remember that the rate constant k for TOC removal has the units min^{-1}; that is, k is expressed as "per minute".

Equation (18) expresses a subtle but important aspect of this modeling; the difference between a *rate* and a *rate constant*. Our interest in comparing different skimmers focuses our attention on the rate constant k. As discussed above, this single value is an aggregate measure of the efficiency of a given skimmer in removing TOC; it reflects all of the intimate structural and molecular details that impact on organics removal (see above). However, the overall *rate* of TOC removal (different than the rate constant!) is expressed by Eq. (18). That is, the rate is defined as the depletion of TOC over time, or d[TOC]/dt (the left hand part of Eq. (18)). Eq. (18) indicates that the *rate* of TOC removal is a complex function of k, the flow rate Q, and the system volumes V_S and V_r. If fact, the quantity V_r/Q (the inverse of Q/V_r in front of the right-hand term) is really just the turnover of the system; V_r/Q = the time that it takes for one reservoir volume to pass through the skimmer. Thus, increasing k, or increasing Q, or decreasing V_r will all increase the overall *rate* of TOC removal. There is nothing in the mathematical derivation to indicate whether k itself is a function of Q or not. That point will have to be tested through experiment, as described below.

The Amount of TOC Removed

Obtaining a reliable measure of the amount of organic impurity removed from the aquarium water requires a little more analysis and a bit more math. The mathematical expressions in Eqs. (19) and (20) above speak to a particular type of TOC depletion process called "exponential decay". That process is characteristic of a scenario where the rate of TOC depletion is proportional to the amount of TOC present (remember our assumption). It is possible to use a computational technique called curve fitting to derive a new mathematical expression, different than (but related to) those discussed above, to tease out one critical number; the level of TOC remaining when the skimmer just does not remove any more material no matter how long the experiment is run. The expression is illustrated in Eq. (21). The key number is called the "plateau", and simply subtracting the plateau value from the starting [TOC] value, $[TOC]_0$, will give the desired quantity; the amount of TOC removed (see Fig. 6). In principle, we can report this quantity as a concentration (i.e., ppm of TOC removed), or as an absolute amount by multiplying the concentration by the system volume; in practice, we will simply describe it as the % of TOC depletion during the skimmer run. The implication of this analysis is that the skimmer will not remove all of the TOC. Referring back to Fig. 1, the molecules that do not have pronounced hydrophobic sections are not good candidates for removal by skimming, and so they should remain at this plateau level after all of the "skimmable" molecules have been removed. In reality (see Experimental Results below), this model provides a reasonable approximation of the data; it gives us a means to estimate how much TOC is remaining when the skimmer has exhausted the supply of removable TOC.

(21) $[TOC]_r = ([TOC]_0 - plateau)e^{-Jt} + plateau$,

J = a constant which makes the curve fit the data; it has no direct physical meaning

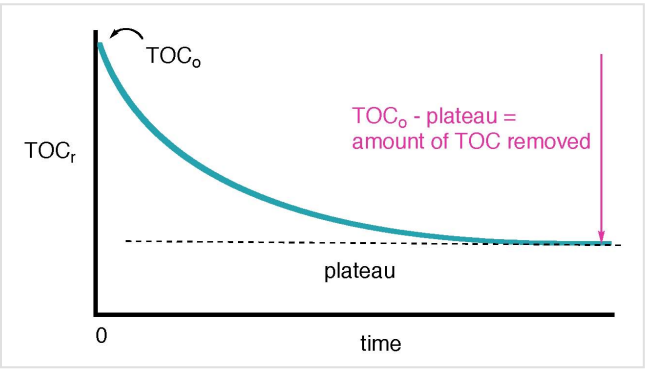

Figure 6. An illustration of how the amount of TOC removed can be determined from a hypothetical TOC exponential decay curve.

Experimental Design

The experimental setup illustrated in Fig. 5 was used. A 40-gallon Rubbermaid tub served as the reservoir, and the various skimmers (see Fig. 7 below) were positioned above the reservoir so that the exit water returned to the reservoir about 3-5 inches above the water surface. The skimmers were fed by pumps that were on the manufacturers' recommendation list. The pumps resided at the bottom of the reservoir and were attached to the skimmers via a ball valve and appropriately sized tygon tubing. The reservoir was equipped with a heater set at 77 °F and two powerheads to aid in circulation. In practice, the pump and powerheads generated enough heat so that the heater rarely turned on; the reservoir temperature fluctuated between 75 and 79 °F during the course of the runs. Prior to running a skimmer test, the reservoir was filled with distilled water, and this pure water was run through the skimmer, pump, powerheads, etc for 24 hours and then emptied. This cleaning procedure was repeated at least five times before an actual skimmer run was attempted. Control measurements of TOC for this wash water revealed that by five washings, the TOC level in the reservoir was indistinguishable from that of pure distilled water. Thus, at that point, the equipment was no longer leaching organics into the water. Bovine Serum Albumin (1.33 gm, 96% pure from Sigma Chemical Company) was dissolved in 35 gallons of 35 ppt freshly prepared saltwater (Coralife brand salt mix) for the model system runs. For the skimmer runs with authentic TOC measurement, 30 gallons of aquarium water was removed at 60 ± 15 min after tank feeding (tank skimmer off) and transferred to the reservoir. The basis for the choice of this particular time point is discussed below. In all experiments, the skimmer pump was turned on (t = 0 min), and three independent 25 - 35 mL water samples were removed from the reservoir at fixed 10-min time points typically for 70 - 120 min. These samples were removed from a well-mixed portion of the reservoir distant from the skimmer effluent entry point. They were collected in specially prepared TOC analysis vials held by tongs to avoid contamination (see Feldman, 2008c for details) and immediately placed in a -23 °C freezer pending analysis. Control experiments determined that there was no difference in results between fresh and frozen samples. The BSA-containing samples from the model system runs were analyzed using a Pierce Micro BCA Protein assay kit following the manufacturer's instructions with the addition of a brief sample centrifugation step prior to UV/VIS analysis. The assay was calibrated with BSA in saltwater. All of the authentic reef tank water samples were analyzed on a Shimadzu 5000 TOC Analyzer in one batch per experiment. The Analyzer was calibrated with potassium hydrogen phthalate as per instrument instructions. See our earlier work (Feldman, 2008c; Feldman, 2008d) for details of the TOC analysis. Data workup followed from the mathematical formulae described above.

The skimmers examined are shown in Fig. 7. These skimmers were chosen to have about the same manufacturers' rating for tank size. The active volume of the reaction chamber for each skimmer is given in Fig. 7. In each case, the manufacturer's recommendations were followed with respect to liquid height in the skimmer column and choice of feed pump. The Euroreef CS80 came with its own Sedra pump, whereas the remaining three skimmers were run from a Mag 9 pump. The water flow rates through the Precision Marine and ETSS skimmers were controlled by adjusting a ball value at the Mag 9 output and a gate valve at the skimmer output, as indicated in the manufacturers' instructions, in order to keep the liquid level at the suggested height. The water flow rate through the Euroreef CS80 skimmer was adjusted by varying the height of the exit tube, again to keep the water level at the suggested height. In all cases, the water flow rate was explicitly measured by timing the filling of a calibrated receptacle.

These skimmers were chosen based on their similar tank size ratings, but they are not identically sized (see the volume values in Fig. 7). Thus, these four skimmers differ in two potentially significant variables; the physical dimensions of the active reaction chamber and the method of bubble production. As discussed earlier, the mathematical analysis of this system explicitly includes the difference in physical dimensions of the different skimmer's reaction chambers by incorporation of the active skimmer volume, V_S, in the formulae. In addition, adhering to the manufacturers' pump/foam height recommendations will lead to different volumetric flow rates for the different skimmers. We will have to take this variation into account as we analyze the data, and it will constrain the conclusions that we can make regarding skimmer performance. Finally, we will not be able to extrapolate any conclusion we draw from these specific skimmers, for example, to all needlewheel skimmers vs. all downdraft skimmers or even to all needlewheel skimmers from the same manufacturer.

Experimental Results

Our solution to the overarching goal of developing a useful and exportable skimmer performance evaluation methodology owes much to the contributions of the researchers discussed in the History of Protein Skimming section. All of those studies used the test protein Bovine Serum Albumin (BSA) as an

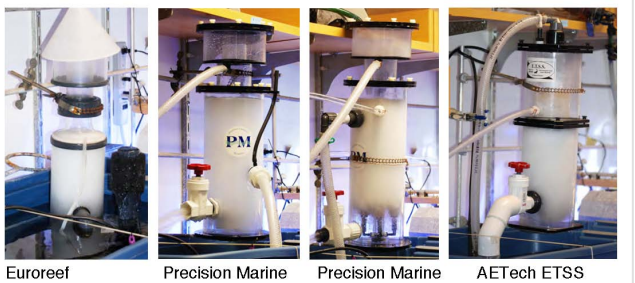

Figure 7. The skimmers examined in this study.

experimental tool. BSA offers some unique advantages as a model protein in skimmer studies, not the least of which is the fact that it is virtually the only commercially available protein that can be acquired in quite pure form (~ 96% pure) for little cost; about 5$ per skimmer run. BSA is soluble in saltwater, stable under skimming conditions, and easily measured down to the ~ 1 ppm level using the commercially available Pierce micro-protein assay kit. Use of this kit requires access to a UV/VIS spectrophotometer ($5 - 10K new, $500 used!), a constant temperature (60 °C) water bath, and a Microcentrifuge (~ $350 new). This procedure removes the need for really hi-tech instrumentation like the Shimadzu TOC Analyzer; with the above equipment, the simple assays can be conducted over the course of a few hours.

Thus, we initiated our studies of skimmer performance using BSA in freshly prepared saltwater. It will, of course, be necessary to demonstrate that this simple model system accurately reflects the operation of a skimmer with authentic aquarium water, and those studies will be described later in this report. We used the experimental set-up detailed in Fig. 5 and the accompanying text. The data workup utilized the mathematical formalisms derived earlier; we sought the two metrics, k (rate constant for BSA removal) and % of BSA removed. Data from an exemplary skimmer run with BSA in saltwater are illustrated in Fig. 8, and the mathematical work-up using Eq. (20) to obtain k is shown in Fig. 9. There is little-to-no refractory fraction that resists skimming; in fact, most of the BSA is removed over the experimental time course. Furthermore, the depletion in BSA concentration fits the experimental decay model (Eq. (21)) quite nicely over the entire time course; r^2 for this curve = 0.97! These observations are consistent with the premise that BSA is a well-behaved material for skimming studies in saltwater.

One of the assumptions that underpinned the mathematical derivation of Eq. (16) was that $d[TOC]_S/d\tau$ was not large, so the product $(V_S/V_r) \bullet d[TOC]_S/d\tau$ was very small (since V_S/V_r was small) and could effectively be set to 0. This assumption is equivalent to claiming that the skimmer did not remove a large amount of the organic impurity (BSA or TOC) per passage of one skimmer water volume through the skimmer. The data exhibited in Fig. 8 support that view. At the flow rate of the experiment (174 gph), the active skimmer volume (~ 0.62 gallons) is exchanged every 0.2 min. From inspection of the curve in Fig. 8, it appears that less than 1% of the BSA is removed per each 0.2 min increment. Thus, the assumption that only small amounts of organic impurities (BSA or TOC) are removed in each pass is validated.

The mathematical manipulation of these data in order to extract the rate constant k provided further encouragement that BSA in saltwater is a useful model for measuring skimmer performance. The $-Ln([BSA]_t/[BSA]_0)$ vs. t plot of Fig. 9 is linear throughout the entire data collection range (r^2 = 0.97), which is completely consistent with one of the major assumptions of the mathematical model (e.g., the rate of BSA removal is proportional to the amount of BSA present). The rate constant k can be calculated from the slope of this line; k = 0.95 min^{-1}. This number has no intrinsic meaning with respect to skimmer performance. Only upon comparison of this value to the k's calculated for other skimmers or for other operational parameters can meaningful conclusions be drawn. As indicated earlier, comparatively larger k values are indicative of faster BSA removal. Of course, the volumetric flow rates for the various skimmers are different, and we will see later that the rate constant k has a complex relationship with the flow rate Q (see Table 2) that ultimately limits the breadth of the conclusions that we can draw from the data.

The analysis of BSA removal rate constants (k's) and % BSA removed can be extended to the other skimmers as well, Table 1. The water flow rates are given also. A comparison of the average k values for all four skimmers reveals no statistically significant difference amongst these skimmers' rate constants

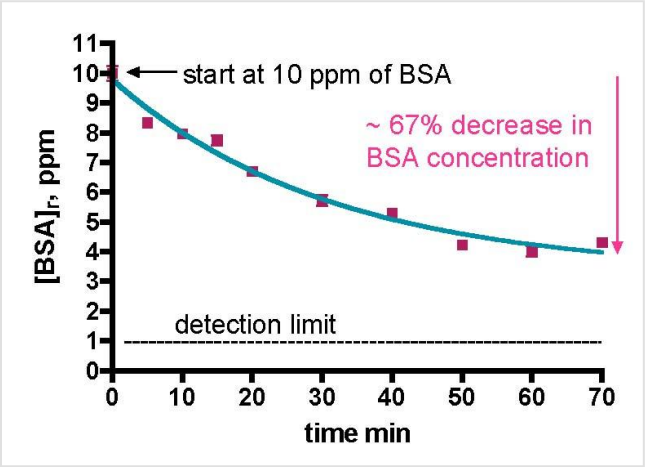

Figure 8. BSA depletion in freshly prepared saltwater using the Euroreef CS80 skimmer. The green line is the best fit using Eq. (21).

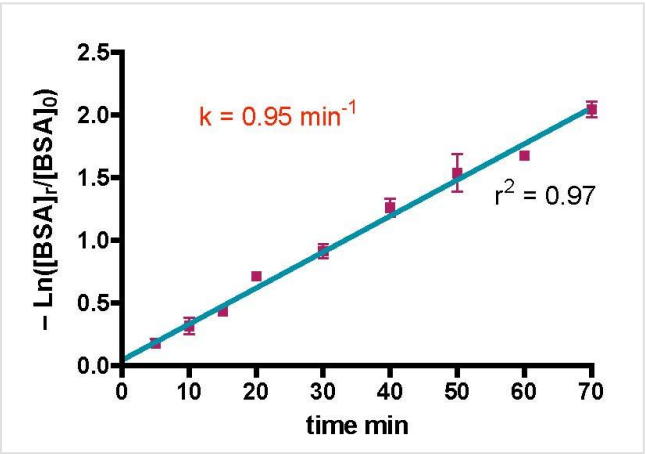

Figure 9. Mathematical treatment of the Euroreef CS80 data in Fig. 8 using Eq. (20) with Q = 2.9 gpm, V_S = 0.62 gallons, and V_r = 35 gallons.

for BSA removal from saltwater under the specified experimental conditions. Since the mathematical modeling already takes into account the differences in active reaction chamber volume between the skimmers, any remaining differences in rate constant k for BSA removal would have to be attributable to the other significant differences between these four skimmers; the method of bubble generation and the flow rate Q. The influence of Q is difficult to tease out (see below). Nevertheless, we can say that when running all four skimmers under the manufacturers' recommended operating conditions, there was, in fact, no difference between the rate constants k. In this instance, the fundamental lesson learned from these studies can be summarized as "bubbles is bubbles"; the method of bubble generation (venturi vs. needlewheel vs. airstone vs. downdraft) does not have any demonstrable impact on the rate of BSA removal under these operating conditions.

Table 1 details the % BSA removal values for the different skimmers in addition to the rate-of-removal (k) studies. For this figure-of-merit as well, there is not much variation amongst the 4 skimmers. The Precision Marine ES100, Precision Marine AP624, and the ETSS evolution 500 all remove BSA at the ~ 80% or greater level. The Euroreef CS80 stands out for its slightly lower BSA clearance. The large amount of scatter in these data (large error bars) tends to diminish the significance of any apparent difference amongst all four skimmers' % BSA removal values. BSA contains both hydrophilic and hydrophobic portions, and so it is an excellent candidate for bubble binding and hence removal. This point, of course, was recognized by all of the previous workers who used BSA as a test case molecule in the protein purification studies discussed earlier.

The role of superficial gas velocity, and independently, liquid flow velocity, occupied the attention of many of the researchers studying protein purification by foam fractionation. These operational parameters can be varied in aquarium skimming as well, and so it seemed worthwhile to determine if the BSA-based experimental methodology could expose any correlation between gas or liquid flow rates and skimmer performance. Many of the more recently introduced needlewheel-based skimmers have an explicit means to vary input air velocity via an independent bubble-generating pump with a valve at the air input line. Unfortunately, neither the Euroreef CS80, nor the Precision Marine ES100 or ETSS evolution 500 skimmers offered any airflow regulation option, and so we did not have an opportunity to deliberately vary superficial air velocity as part of our studies with these skimmers. Fortunately, the airstone-based Precision Marine AP624 did have an air input system that was independent of the water flow system, and so it was possible to examine the question of air flow vs. performance, as indicated by the rate constant k, for this skimmer. Unfortunately, we did not have access to an air flow meter to directly measure this quantity. We could, however, adjust the pressure of the air source that feeds the airstones. This experimental approach is based upon the presumption that an increase in air pressure feeding the airstone translates into a higher superficial air velocity, but the precise relationship between these two quantities is unknown. In any event, the k value for BSA removal using the Precision Marine AP624 skimmer with a feed air pressure of 3 psi is k = 0.97 ± 0.26 min^{-1} (from Table 1). Increasing the air pressure to 6 psi led to k = 3.1 ± 0.2 min^{-1}. Thus, doubling the air pressure approximately tripled the rate constant for BSA removal for the AP624 skimmer under the specified set of reaction conditions (~ 156/157 gph water flow in each case).

Table 1a. Accumulated data for measuring skimmer performance with BSA/saltwater for the four skimmers illustrated in Fig. 7.

Trial	EuroReef CS80 needlewheel			ETSS Evolution 500 downdraft		
	k min^{-1}	% BSA removed	Flow gph	k min^{-1}	% BSA removed	Flow gph
1	0.67	70	114	0.51	92	382
2	0.66	56	114	1.22	100	364
3	0.95	67	102	1.09	81	364
4	0.63	56	129	0.61	60	364
5	0.79	22	109	1.01	61	364
6	1.20	64	108			
7	1.20	55	108			
Ave	0.87±0.23	56±15	112±8	0.89±0.31	79±16	368±7

Table 1b. Accumulated data for measuring skimmer performance with BSA/saltwater for the four skimmers illustrated in Fig. 7.

Trial	Precision Marine ES100 venturi			Precision Marine AP624 airstone		
	k min^{-1}	% BSA removed	Flow gph	k min^{-1}	% BSA removed	Flow gph
1	1.00	90	231	1.49	100	157
2	0.79	96	227	0.82	100	157
3	0.68	68	227	0.84	100	157
4	0.98	78	218	0.81	82	156
5	0.92	70	218	0.90	94	156
6	0.81	60	227			
7	0.83	79	216			
Ave	0.86±0.11	77±12	223±5	0.97±0.26	95±5	157±0.5

The final skimmer operational parameter tested was water flow rate. As discussed earlier, the mathematical derivation of the rate constant formula (Eq. 20) does not speak to the possibility that the water flow rate might influence the rate constant k, and so that relationship will have to be revealed through experiment. In general, simple adjustments in the ball valve at the pump exit and the gate valve at the skimmer exit port should suffice to keep the skimmer water level at the manufacturer's suggested height. Nevertheless, we had some concerns about problematical experimental aspects for these trials, and these concerns constrained our choices of flow rate and skimmer. For example, we would have preferred to vary the flow rate by a significant amount, say 10x, in order to push the concept to its limits. However, the need to keep the water/foam boundary at the manufacturer's recommended height limited the range that we could usefully examine. In addition, the Euroreef CS80 and the Precision Marine ES100 skimmers, which generate air flow (and hence bubbles) via the Venturi effect, might suffer from a materially diminished bubble stream at too low of a water velocity. In fact, preliminary scouting experiments with the Precision Marine ES100 venturi skimmer supported this view. The reaction chamber was filled with dense bubbles at 223 gph (Table 1 and Fig. 7), but dropping the flow rate to 180 gph led to a noticeable decrease in bubble density, and lowering the flow further to 144 gph almost abolished bubble generation. These observations underscore the difficulty with probing k vs. Q. In principle, we could adjust each skimmer's flow to be equal to some predetermined "standard" value in an effort to remove this variable from the simmer runs. However, those adjustments would inevitably move at least one skimmer off of the manufacturer's recommendations, and the bubble stream could suffer. Thus, equalizing the water flow rates is likely to skew rather than clarify the Q vs. k data. Therefore, we are faced with the prospect of trusting the manufacturers' pump recommendations and their derived flows as being optimal, or at least adequate, for satisfactory bubble generation.

With these thoughts in mind, we examined the consequences of varying the water flow rate with the Precision Marine AP624 airstone skimmer, since this skimmer, uniquely, uncouples bubble generation from water flow. That is, the superficial gas velocity is set independently of water flow velocity, and so we could expand our range of flow rates without the concerns indicated above for Venturi-effect skimmers. The results are tabulated in Table 2. The k values do change as volumetric flow rate increases, but a trend is difficult to extract from these limited data. Initial doubling of flow Q led to an approximate doubling of k, but further increase of Q to 3.4x the initial flow resulted in a *decrease* in k to below the original value. The high r^2 values for the mathematical analyses reflect the high quality of the data, but it is not possible to draw any compelling conclusions from this information. In addition, it is not prudent to extrapolate from these data to the behavior of the other three skimmers under varying flow rates. Overall, this uncertainty leaves us in a bit of a quandary with respect to assessing the impact of the differing flow rates on the k values obtained for the different skimmers in Table 1. In the final analysis, we have to trust the manufacturers' pump recommendations as adequate for supplying sufficient water velocity to drive satisfactory bubble generation, and the strongest conclusion that we can draw regarding the k performance metric amongst the four skimmers is that *under the manufacturers' recommended operating conditions*, all four skimmers exhibit indistinguishable rate constants for BSA removal.

Table 2. Flow rate vs. k for the Precision Marine AP624 skimmer run at 6 psi gas pressure.

Trial	Flow Q gph	k min^{-1}	r^2
1	156	2.5	0.98
2	156	3.2	0.99
3	156	3.6	0.97
4	318	7.7	0.98
5	318	7.9	0.96
6	318	7.3	0.97
7	540	2.5	0.98

The acquisition of internally consistent BSA k values for the different skimmers supports the premise that BSA can serve as an effective surrogate for TOC in reef tank water. Nevertheless, the relevance of these BSA studies to authentic aquarium water has not been established. In order to forge this link, we must demonstrate that the skimmers will also remove TOC from aquarium water with a similar trend.

As indicated in the Experimental section, the skimmer runs focusing on TOC removal from authentic reef aquarium water utilized tank water removed about 1 hour after feeding, with the system skimmer (H&S A200-1260) off. We arrived at this time point after a little experimentation. We had examined TOC levels in this system over the course of a week's time (Feldman, 2008c), and those data indicated that there was a spike in TOC values a few hours after feeding, but that the TOC levels returned to baseline after about 24 hours or so. The system's skimmer was turned off for ~ 1-2 hours after feeding but otherwise was operational. These observations prompted an initial decision to remove tank water 24 hours after feeding to minimize the possibility of contamination by food residue. In addition, we anticipated that once we removed the tank water from the TOC-producing corals, then little additional TOC would be added to the water and so the test skimmer would simply deplete the 30-gallon water sample of existing TOC over time. Data from this first run using the Euroreef CS80 skimmer are shown in Fig 10. It is clear that our expectation was not met. In fact, the TOC levels did not change much beyond random scatter in the data. The Euroreef CS80 skimmer did not do anything! We surmised that the problem lay in the fact that the aquarium water already had been depleted of its "removable" TOC by both the H&S skimmer and by bacterial consumption (Ferrier-Pagès, 1998; Ferrier-Pagès, 2000; Kuntz, 2005; Kline, 2006; Feldman, 2008c) over the 24-hr post-feeding period. Thus, apparently there was almost nothing left for the Euroreef skimmer to remove. To avoid this problem, the aquarium water removal time had to be changed, and after some experimentation, we

determined that water removal at 1 hour ± 15 min after feeding (H&S skimmer off during this time) provided a sample of water that was amenable to TOC removal via skimming. Justification for this choice can be found in Figs. 5 and 6 of Feldman, 2008c. By simple observation, there was no evidence for particulate matter in the aquarium water at that point in time; the visible food had been consumed long before. Is this a legitimate procedure for assaying aquarium water? We argue that in the normal course of tank husbandry, an aquarist would, of course, feed the tank (skimmer on or off) and then at some point would rely on the skimmer to remove "excess" TOC from the water. That excess TOC might be food residue directly, but also it would likely contain components originating from the consequences of food addition; fish poop and coral mucus. It is just this uncharacterized mixture that we are assaying when we remove aquarium water at the 1-hour mark, and so we suggest that our skimmer tests do probe the issue of aquarium water purification under realistic conditions.

The implementation of the 1-hour-after-feeding water removal protocol with the Euroreef CS80 skimmer led to more favorable results, Fig. 11. The data points (in purple) can be fit by Eq. (21) (in green) fairly well; r^2 = 0.86. The TOC removal profile using the Euroreef CS80 skimmer (Fig. 11) is slightly different than the profile obtained from BSA removal from aquarium water (Fig. 8; r^2 = 0.97). These differences reside largely at the longer time data points, and they will impact the mathematical analysis, as described below.

The data points (purple) of Fig. 11 can be utilized as input for Eq. (20) with Q = 2.5 gpm, V_S = 0.62 gallons and V_r = 30 gallons. The derived plot of -Ln($[TOC]_r/[TOC]_0$) vs. t is illustrated in Fig. 12. According to our mathematical modeling, this graph should be linear just as it was in the BSA data analysis (see Fig. 9). Clearly it is not. What went wrong? A closer examination of the data in Fig. 12 reveals that, in fact, a very well defined straight line can be drawn through the 0 - 50 min data range (r^2 = 0.91). If we confine our analysis to this region of the graph, then using Eq. (20) permits calculation of the rate constant, k, based on the straight line indicated in blue on Fig. 12; k = 0.26 min^{-1}. What about the discarded data at 60 - 120 min of Fig. 12? Is it legitimate to ignore these values? In this case yes; the skimmer pulls out all of the TOC that it is going to remove by the 50-minute mark. Beyond that time point, nothing much is happening and the TOC level doesn't change much. This leveling of the TOC value probably accounts for the r^2 value of less than 0.9 for the exponential decay curve fit in Fig 11, as the lack of TOC removal after 50 min is not consistent with one of the tenets of the mathematical model (rate of TOC removal is proportional to the amount of TOC present). Nevertheless, the fit is not too bad, and that correspondence allows us to arrive at an amount of TOC depletion for this run of ~ 21% (see Fig. 11). The derived k value, 0.26 min^{-1}, is directly comparable to the k value for BSA removal with this skimmer (0.87 min^{-1}), and it is apparent that the Euroreef CS80 is approximately 3.3 times better (faster) at removing BSA than it is at removing TOC.

This procedure was repeated for the Euroreef CS80 skimmer five more times, and the accumulated data are displayed in Table 3. Every attempt was made to keep the water flow rate Q constant between the runs; the measured flow values are given as well. The average figures of merit for these six independent runs are: rate constant k = 0.24 ± 0.07 min^{-1} and amount of TOC depletion = 20 ± 5%. Similar TOC removal experiments were performed for the other three skimmers illustrated in Fig. 7. These data are tallied in Table 3 as well. For the two cases where % TOC removed values are not reported, the data did not fit the exponential decay of Eq. (21) closely enough to get a legitimate plateau value. The collection of r^2 values obtained for the various curves span the following ranges: Euroreef CS80 k r^2 values: 0.81 - 0.95, % TOC removal r^2 values: 0.76 - 0.96; PM ES100 k r^2 values: 0.52 - 0.93, % TOC removal r^2 values: 0.65 - 0.82; PM AP624 k r^2 values: 0.85 - 0.94, % TOC removal r^2 values: 0.84 - 0.9; and ETSS evolution 500 k r^2 values: 0.67 - 0.93, % TOC removal r^2 values: 0.69 - 0.92. The lower end r^2 values are indicative of lower quality data, which likely reflects our imperfect attempts to hold all non-varying parameters constant. Some of

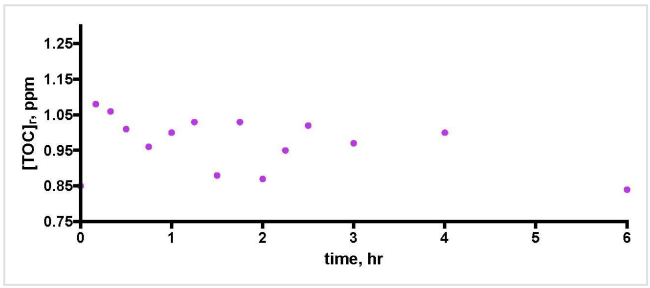

Figure 10. A first attempt to measure TOC depletion in authentic aquarium water using the Euroreef CS80 skimmer. The water sample was removed from the aquarium 24 hours after feeding.

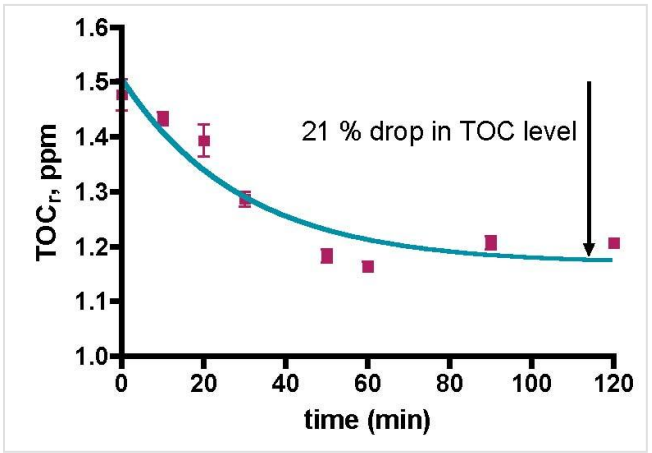

Figure 11. A second attempt to measure TOC depletion in authentic aquarium water using the Euroreef CS80 skimmer. The water sample was removed from the aquarium 1 hour after feeding. The green line is the best fit using Eq. (21).

the data inconsistency also may arise from the inevitable differences that attend unrecognized variability in both the food composition and the fate of that food on any given day during the hour that it is consumed in the aquarium prior to water removal.

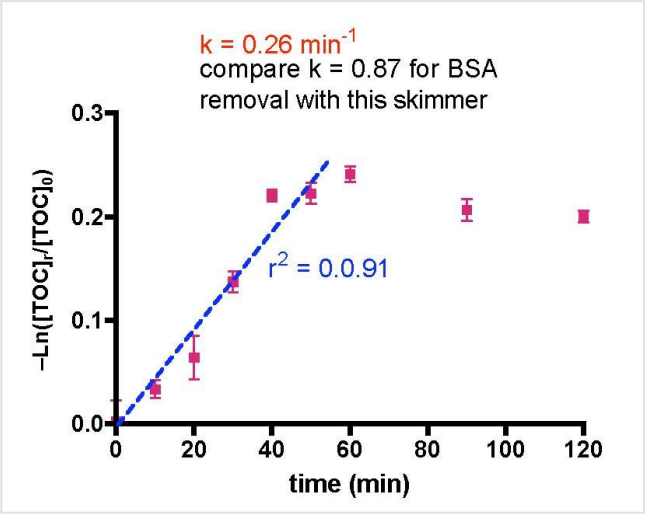

Figure 12. Mathematical treatment of the data in Fig. 12 using Eq. (20) with Q = 2.9 gpm, V_s = 0.62 gallons, and V_r = 30 gallons.

There is no statistical difference between the k values for the Euroreef CS80, Precision Marine ES100, Precision Marine AP624, and ETSS evolution 500 skimmers; all of the k value data sets fall within the same range. This near equivalency between the rate constants for TOC removal mirrors the same convergence of k values with the model BSA system (Table 1). Thus, there is a welcome consistency between the "real" system, TOC in authentic reef tank water, and the model system, BSA in freshly prepared saltwater, that raises confidence in using the latter as a surrogate for the former.

The %TOC removal data also evinces a very consistent picture of skimmer operation. The rather large scatter in the data as indicated by the error bars (from 16 - 44% of the mean value) does not permit any statistically significant distinction to be made between any two skimmers in their overall capacity to remove TOC. The source of this scatter is likely to be a combination of the approximations used in applying Eq. (21) to determining the % TOC removed, and the seemingly unavoidable fluctuations of the data at longer time points, as discussed earlier. Nevertheless, the data are robust enough to glean general trends. *Thus, all skimmers tested remove around 20 - 30% of the TOC in the aquarium water, and that's it; 70 - 80% of the measurable TOC is left behind unperturbed by the skimming process.* It may be possible to develop a rationalization for this unexpected behavior by referring back to Fig. 1. Perhaps only 20 - 30% of the organic species in the aquarium water meet the hydrophobic requirements for bubble capture, whereas the remaining 70-80%, for whatever reason, don't. Since the chemical structures of the

Table 3a. Accumulated data for measuring skimmer performance with TOC removal from authentic reef tank water for the four skimmers illustrated in Fig. 7.

Trial	EuroReef CS80 needlewheel			ETSS Evolution 500 downdraft		
	k min^{-1}	% TOC removed	Flow gph	k min^{-1}	% TOC removed	Flow gph
1	0.26	21	172	0.33	38	398
2	0.11	18	168	0.16	16	398
3	0.20	30	178	0.20	31	350
4	0.23	17	174	0.11	16	372
5	0.26	15	174	0.11	17	367
6	0.35	19	166			
Ave	0.24±0.07	20±5	172±4	0.18±0.08	24±9	377±19

Table 3b. Accumulated data for measuring skimmer performance with TOC removal from authentic reef tank water for the four skimmers illustrated in Fig. 7.

Trial	Precision Marine ES100 venturi			Precision Marine AP624 airstone		
	k min^{-1}	% TOC removed	Flow gph	k min^{-1}	% TOC removed	Flow gph
1	0.14	34	222	0.11	23	205
2	0.20	18	215	0.16	34	209
3	0.23	27	224	0.16	31	216
4	0.15	---	228	0.17	34	213
5	0.21	21	215	0.23	---	202
6						
Ave	0.19±0.03	25±11	221±5	0.17±0.04	31±5	209±5

TOC components are not known, any further speculation along these lines must await chemical structural analysis for verification.

One final observation about skimmer performance can be gleaned from these experiments. Skimmer manufacturers often describe a "break-in" period at start-up during which the skimmer does not collect much skimmate but the riser tube gets coated with a biocompatible film (?) that then favors skimmate deposition. In all of the skimmer runs described above, the skimmer was scrupulously cleaned and dried before each use. Thus, each skimmer run proceeded from the equivalent of initial start-up conditions. All skimmers in all runs exhibited TOC depletion in the reservoir water by the 20-minute time point. Fifteen out of the 21 total skimmer runs exhibited marked TOC depletion by the 10-min mark, the earliest time point in the experiments. There did not seem to be any preference amongst the four skimmers; three of the skimmers generated two of the "slow start" runs each. Thus, it appears, based upon these results, that a perfectly clean skimmer will begin to extract organic residue from aquarium water before 20 minutes of operation. For the six skimmer runs that did not display a diminished TOC level at the 10-min mark, the mathematical determination of k excluded the 10-min data point.

Conclusions

Protein skimmers have become indispensable for many aquarists who strive to maintain the high water quality necessary to keep stony corals. This singular piece of equipment is typically the second largest expense after the tank itself, and as of this writing, there are at least 23 different skimmer vendors in operation. The advertising that accompanies these products often boasts of superlative performance, but no skimmer manufacturer has offered any quantitative support for their claims, in part because no useful metric for skimmer performance exists. In this article, we introduce two different measures for skimmer performance; (1) how fast the skimmer removes organic matter from salt water, and (2) how much of the existing organic material actually is removed. We demonstrate how these quantities can be obtained via experimental measurement and mathematical modeling on a model system consisting of Bovine Serum Albumin (BSA) in freshly prepared saltwater. In addition, we demonstrate that the trends among the numerical values obtained in the BSA model system also are observed with TOC removal from authentic aquarium (reef tank) water. The correlation between the BSA model system results and the TOC reef tank water results supports the hypothesis that BSA in saltwater can be used as an effective surrogate for TOC in aquarium water.

Four skimmers having four different reaction chamber volumes and representing four distinct types of bubble generation (needlewheel, venturi, airstone, and downdraft) were tested under the manufacturers' specified conditions. These tests revealed that there was no demonstrable difference between the Euroreef CS80 needlewheel skimmer, the Precision Marine ES100 venturi skimmer, the Precision Marine AP624 airstone skimmer, and the ETSS evolution 500 downdraft skimmer with respect to the rate constant for either TOC or BSA removal. Thus it is fair to conclude that, at least for the skimmers tested under the specified conditions, the individual manufacturers' claims of superior performance are without merit. Whether this conclusion can be extended to other skimmers remains to be seen.

Two operational parameters were examined; airflow rate and water flow rate. For the one skimmer tested at different airflow rates (Precision Marine AP624), the rate constant k for BSA removal did increase significantly as air velocity increased. The performance response upon varying water flow rate was not unequivocal. Using the Precision Marine AP624 airstone skimmer as a test system, increasing the water flow rate at first increased, and then decreased, the rate constant k. The basis for these divergent results has not yet been elucidated.

All four skimmers were quite similar in the second performance figure-of-merit, the total amount of organics removed. The skimmers typically removed greater than 80% of the BSA. In contrast, perhaps one of the more interesting observations to emerge from these studies is the fact that all four skimmers tested removed only 20 - 30% of the total organics present in authentic reef tank water.

Several critical questions that cannot yet be answered concern the generality of the conclusions drawn above. Can any skimmer beat the 30% organic removal level, or is that an intrinsic property of the TOC (cf. Fig. 1)? What are the effects of either gas flow rates or water flow rates that are far outside of the examined range, on k? Are there other unrecognized factors buried in the "k" term involved in determining skimmer performance? These questions can only be answered by examining more skimmers under a wider range of conditions.

From a different perspective, the methodology introduced in this article, in particular the BSA model system, presents a real opportunity for skimmer designers/manufacturers actually to optimize skimmer design/operational parameters in a deliberate and rational manner. Through these types of product development studies, skimmer manufacturers finally might be able to include descriptors in their advertising such as "best", "fastest" etc. that really mean something.

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References

1. Aksay, S.; Mazza, G. 2007. "Optimization of Protein Recovery by Foam Separation Using Response Surface Methodology." J. Food Eng. 79, 598-606.
2. Ahmed, S. I. 1975. "Laws of Foam Formation and Foam Fractionation." Sep. Sci. 10, 673-688.
3. Bhattacharjee, S.; Kumar, R.; Gandhi, K. S. 1997. "Prediction of Separation Factor in Foam Separation of Proteins." Chem. Eng. Sci. 52, 4625-4636.
4. Brown, L.; Narsimhan, G.; Wankat, P. C. 1990. "Foam Fractionation of Globular Proteins." Biotechnol. Bioeng. 36, 947-959.
5. Caballero, M.; Cela, R.; Perez-Bustamante, J. A. 1990. "Analytical Applications of Some Flotation Techniques - A Review." Talanta 37, 275-300.
6. Clark, A. N.; Wilson, D. J. 1983. *Foam Flotation: Theory and Applications*. Marcel Dekker, Inc. New York.
7. Du, L.; Loha, V.; Tanner, R. D. 2000. "Modeling a Protein Foam Fractionation Process." Appl. Biochem. Biotechnol. 84-86, 1087-1099.
8. Du, L.; Ding, Y.; Prokop, A.; Tanner, R. D. 2001. "Measurement of Bubble Size Distribution in Protein Foam Fractionation Column Capillary Probe with Photoelectric Sensors." Appl. Biochem. Biotechnol. 91-93, 387-404.
9. Du, L.; Prokop, A.; Tanner, R. D. 2002. "Effect of Bubble Size on Foam Fractionation of Ovalbumin." Appl. Biochem. Biotechnol. 98-100, 1075-1091.
10. Du, L.; Prokop, A.; Tanner, R. D. 2003. "Variation of Bubble Size Distribution in a Protein Foam Fractionation Column Measured Using a Capillary Probe with Photoelectric Sensors." J. Colloid Interface Sci. 259, 180-185.
11. Felder, R. M.; Rousseau, R. W. 2005. *Elementary Principles of Chemical Processes, 3rd Ed.*, John Wiley and Sons, New York.
12. Feldman, K. S.; Vernese, L. F.; Mueller, K. T.; Maers, K. 2008a. "Granular Activated Carbon: Modeling of Operational Parameters for Dissolved Organic Carbon Removal from Marine Aquaria, Part 1." Advanced Aquarist, 7, http://www.advancedaquarist.com/2008/1/aafeature1/.
13. Feldman, K. S.; Vernese, L. F.; Mueller, K. T.; Maers, K. 2008b. "Granular Activated Carbon: Modeling of Operational Parameters for Dissolved Organic Carbon Removal from Marine Aquaria, Part 2." Advanced Aquarist, 7, http://www.advancedaquarist.com/2008/2/aafeature1/.
14. Feldman, K. S.; Maers, K. M. 2008c. "Total Organic Carbon (TOC) and the Reef Aquarium; an Initial Survey. Part 1." Advanced Aquarist, 7, http://www.advancedaquarist.com/2008/8/aafeature3/.
15. Feldman, K. S.; Maers, K. M. 2008d. "Total Organic Carbon (TOC) and the Reef Aquarium; an Initial Survey. Part 2." Advanced Aquarist, 7, http://www.advancedaquarist.com/2008/9/aafeature2/.
16. Ferrier-Pagès, C.; Gattuso, J.-P.; Cauwet, G.; Jaubert, J.; Allemand, D. 1998. "Release of Dissolved Organic Carbon and Nitrogen by the Zooxanthellate Coral *Galaxea fascicularis*." Mar. Ecol. Prog. Ser., 172, 265-274.
17. Ferrier-Pagès, C.; Leclercq, N.; Jaubert, J.; Pelegri, S. P. 2000. "Enhancement of Pico- and nanoplankton Growth by Coral Exudates." Aquat. Microb. Ecol., 21, 203-209.
18. Gehle, R. D.; Schügerl, K. 1894. "Protein Recovery by Continuous Flotation." Appl. Microbiol. Biotechnol. 20, 133-138.
19. Holmes-Farley, R. 2006. "What is Skimming?" Reefkeeping, 5, http://www.reefkeeping.com/issues/2006-08/rhf/index.php.
20. Kuntz, N. M.; Kline, D. I.; Sandin, S. A.; Rohwer, F. 2005. "Pathologies and Mortality Rates Caused by Organic Carbon and Nutrient Stressors in Three Caribbean Coral Species." Mar. Ecol. Prog. Ser., 294, 173-180.
21. Huckstedt, G. 1972. "Foam Flotation Separation System Particularly Suitable for Separating Dissolved Protein Compounds and Toxic Metallic Ions from Aquarium Water." US Patn. 3,669,883.
22. Kline, D. I.; Kuntz, N. M.; Breitbart, M.; Knowlton, N.; Rohwer, F. 2006. "Role of Elevated Organic Carbon Levels and Microbial Activity in Coral Mortality. Mar. Ecol. Prog. Ser., 314, 119-125.
23. Lemlich, R., Ed. 1972. *Adsorptive Bubble Separation Techniques*. Academic Press, New York.
24. Lockwood, C. E.; Bummer, P. M.; Jay, M. 1997. "Purification of Proteins Using Foam Fractionation." Pharm. Res. 14, 1511-1515.

25. Okamoto, Y.; Chou, E. J. 1979. "Foam Separation Processes" in *Handbook of Separation Techniques for Chemical Engineers*, Schweitzer, P. A., Ed. Wiley, New York.

26. Ostwald, W.; Siehr, A. 1937. "Analysis of Foams and Solution by Foaming." Chem. Ztg. 61, 649-660.

27. Rosa, P. T. V.; Santana, C. C.; Carbonell, R. G. 2007. "Determination of the Liquid Pool Surfactant and Protein Concentration for Semi-Batch Foam Fractionation Columns." Brazilian J. Chem. Eng. 24, 1-14.

28. Thiel, A. J. 1997. "Trends in Foam Fractionation." http://www.netpets.com/fish/reference/saltref/foam.html.

29. Schnepf, R. W.; Gaden, E. L. 1959. "Foam Fractionation of Proteins: Concentration of Aqueous Solutions of BSA." J. Biochem. Microbiol. Technol. Eng. 1, 1-11.

30. Schutz, F. 1937. "Adsorption on Foams." Nature 139, 629-630.

31. Suzuki, Y.; Hanagasaki, N.; Furukawa, T.; Yoshida, T. 2008. "Removal of Bacteria from Coastal Seawater by Foam Separation Using Dispersed Bubbles and Surface-Active Substances." J. Biosci. Bioeng. 105, 383-388.

32. Uraizee, F.; Narsimhan, G. 1995. "A Model for Continuous Foam Concentration of Proteins: Effects of Kinetics of Absorption of Proteins and Coalescence of Foam." Sep. Sci. Technol. 30, 847-881.

33. Uraizee, F.; Narsimhan, G. 1996. "Effects of Kinetics of Adsorption and Coalescence on Continuous Foam Concentration of Proteins: Comparison of Experimental Results with Model Predictions." Biotech. Bioeng. 51, 384-398.

34. Wallace, G. T., Jr.; Wilson, D. F. 1969. "Foam Separation as a Tool in Chemical Oceanography." Naval Research Laboratory, Accession number: AD 0697924.

35. Wong, C. H.; Hossain, M. M.; Davies, C. E. 2001. "Performance of a Continuous Foam Separation Column as a Function of Process Variables." Bioprosess Biosys. Eng. 24, 73-81.

PRODUCT REVIEW

Analyzing Reflectors: Lumenbrite III, Lumenmax Elite, Lumenmax, and Lumenarc III

By Sanjay Joshi

Reflector design for reef aquariums has improved considerably allowing for more efficient use of light. With these reflectors we can now light reef aquarium with fewer lamps, get deeper light penetration over larger areas.

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Link to original article: http://www.advancedaquarist.com/2009/1/review

Since the last set of reflector tests a few more new reflectors have been introduced in the market. These new reflectors all carry the "Lumen" name - Lumenbrite, Lumenmax Elite, Lumenmax, similar to their predecessor the Lumenarc III, even though they are manufactured and marketed by different companies. This article presents test results on these new fixtures/reflectors. The reflectors were tested using the same setup and methodology as used for the tests on other reflectors, described in [1]. Previous reflector tests had data for light dispersions at 6",9" and 12". While this data allowed for a meaningful comparison between the reflectors, it could be argued these are not practical distances at which corals would be placed in the tank and data further from the lamp would be more useful. Further, there was speculation among hobbyists that some reflectors were better at projecting light further away from the reflector and hence making them more suitable for deeper tanks. To accommodate some of these concerns, measurements in this study have been made at 24" and 30' from the center of the lamp in the reflector. This article continues in a similar vein as the previous reflector articles and presents the data and analysis of these new reflectors in my inventory, and compares them to the Lumenarc III reflector.

Table 1: Listing of the Reflectors Tested

Reflector	Ballast	Lamp
Lumenmax Elite	400W Icecap Electronic Ballast	Ushio 14000K 400W Mogul
Lumenbrite III		
Lumenarc III		
Lumenmax (Large)		

Table 1, presents the information on the ballasts and lamp used to test all the reflectors. Since the same ballast and lamp is used, direct comparisons can be made between the reflectors. Table 2 lists the physical specifications of the reflectors, manufacturer, pictures and some comments on these reflectors.

Reflector Data and Analysis

The data plots for each reflector at the distances 24" and 30" are plotted as a surface graph, top view graph, and a % distribution graph to illustrate the intensity and spread at different points on the 36"X36" measuring grid, with data recorded at 3" intervals. The lamps are oriented so that they are parallel to the X-axis in the plots, with the center of the lamp aligned with

Table 2: Specifications of the Reflectors tested

Reflector	Size	Manufacturer	Comments
Lumenarc III	19.5" x 19.5" x 8.5" tall	Pacific Garden Supply	Bulb position can be adjusted to center bulb; Air Cooled option available; Accepts glass shield.
Lumenbrite III	20" x 20" x 9" tall	Coralvue	Octagonal Shape; Does not accept glass shield.
Lumenmax (large)	22¼" x 22¼" x 8¾" tall	Sunlight Supply	Combination of textured and smooth facets; Cannot adjust for bulb length; Does not accept glass shield.
Lumenmax Elite	15" x 15" x 5" tall	Sunlight Supply	Textured reflective surface

(0,0). All measurements of distances are taken from the centerline of the lamp. Table 3 below shows the list of figures associated with each reflector.

One of the measures of a reflector performance could be its ability to direct light into the aquarium. A reflector's total incident light upon a surface of a given area is representative of the performance of a reflector. It is computed by adding up all the measurements taken at the discrete points within the region. It

Photo 1: Lumenarc III Reflector

Photo 2: Lumenbrite III Reflector

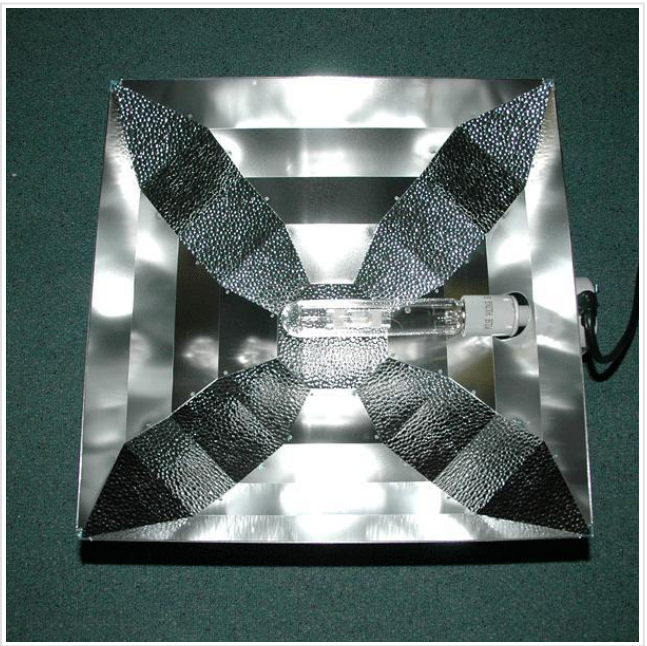

Photo 3: Lumenmax (large) Reflector

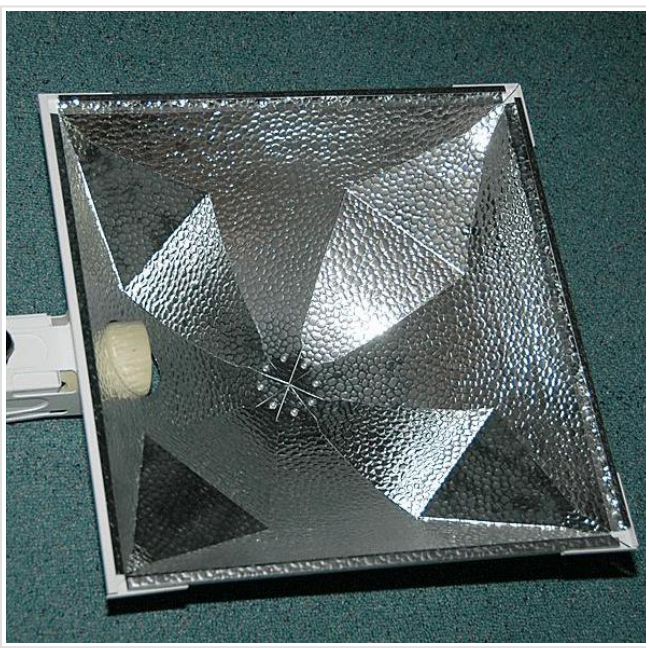

Photo 4: Lumenmax Elite Reflector

Table 3: List of Figures associated with each reflector

Reflector	Figures
LumenMax Elite	Figs. 1-3
LumenBrite III	Figs. 4-6
LumenArc III	Figs. 7-9
LumenMax (Large)	Figs. 10-12

demonstrates how much light the reflector is able to focus downward when compared to other reflectors with similar operating conditions (same ballast and lamp). While it can be argued that adding all the PPFD values is technically not a valid measure as per the definition of PPFD (since PPFD is defined as microEinstiens/m^2/sec), it can be used to provide a metric for reflector performance. Further summing over the data points on a given area can easily be used to compute the average, if so desired. Since the area under consideration is the same for all reflectors, we can just as well use the sum of the PPFD values distributed over this area (169 data points) instead of an average as a performance metric.

Table 4 presents this data for the reflectors in this article. Note that the initial PPFD values of the lamp/ballast combo used to test the reflector influences this data. For these tests, the Ushio 14000K 400W lamp used with the Icecap Electronic ballast has a PPFD reading of 151.7 at 18". For any other ballast/lamp combo the PPFD data at 18" can be obtained from the website http://reeflightinginfo.arvixe.com and the reflector data can be scaled appropriately. For example, if using a magnetic ballast with Ushio Blue 20000K lamp with a PPFD of 109 (from the data on the website), the reflector data can be scaled by 0.718 (109/151.7) to get a sense of the light values obtained with this combination.

In addition to knowing how much light is incident on a given area, we could also look at how much loss of light occurs on a given area when moving the lamp and reflector higher. Table 5, presents the % of light lost on a specified area as one moves the lamp/reflector from 24" to 30" above the surface. A higher % loss would indicate that the reflector is creating a larger spread with an increase in distance.

Discussion

Reflectors are typically designed taking into account 2 conflicting design criteria - focus and spread. Given that a lamp is only emanating light at a certain fixed rate, the light from the lamp is gathered by the reflector and can either be focused to give a higher intensity over an area or spread out to give a large coverage albeit at lower light intensity. Light levels at any given point can also be influenced by changing the distance from the light source by raising or lowering the reflector. So saying one reflector is better than the other is not a trivial task and may even be meaningless, without taking into account the need of the user and the designer's intent along with the physical constraints of the application. Choosing a reflector often requires taking into considerations the tank dimensions, aquascaping within the tank, the type of corals in the tank and their light requirements, cost budget, space available for mounting, etc.

The reflectors seem to fall into 2 separate groups, Group 1, the Lumenarc and the Lumenmax and Group 2 comprising Lumen Brite and Lumenmax Elite. The Group 1 reflectors seem to be better at providing a wider coverage, whereas the group 2 reflectors tend to focus the light slightly more creating higher peak values and smaller spread.

The lumenarc III and the Lumenmax reflectors provide a large area of light coverage and hence are more suitable for tanks wider than 3 ft. At similar distances they both provide a large coverage of area where the PPFD values reach between 100-200 micromoles/m^2/sec. In my experience, if a user can achieve PPFD values of 75-100 at the bottom of the tank, then they will have enough gradation of light in the tank to find places to keep most photosynthetic corals happy in the tank. The Lumenmax reflector at 30" provided a larger area where the PPFD values were greater than 200, as compared to the

Table 5: Percent of PPFD lost from 24" to 30" from the lamp

	3x3' Area	2x2' Area	1x1' Area
Lumenmax Elite	12.48	16.8	24.6
LumenBrite	11.5	21.4	30.5
Lumenarc III	19.5	26.3	33.0
Lumenmax (large)	15.9	21.6	26.6

Table 4: Total Incident PPFD on a given Surface area

		Total Incident Light			
Reflector	Distance:	3x3 Area	2x2 Area	1x1 Area	Maximum PPFD:
LumenMax Elite	24"	33229	26138	13571	711
	30"	29080	21735	10219	519
LumenBrite	24"	35700	28214	13473	669
	30"	31586	22171	9361	444
LumenArc III	24"	28147	18544	7299	349
	30"	22645	13660	4890	222
LumenMax (Large)	24"	32886	21954	8391	383
	30"	27630	17202	6159	270

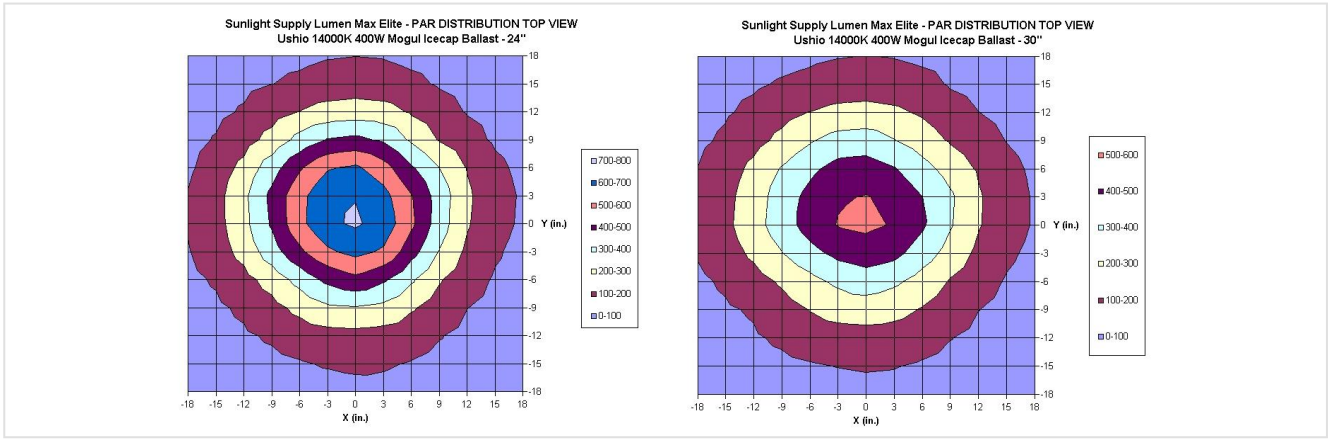

Figure 1: LumenMax Elite: Par distribution top view, 24" and 30".

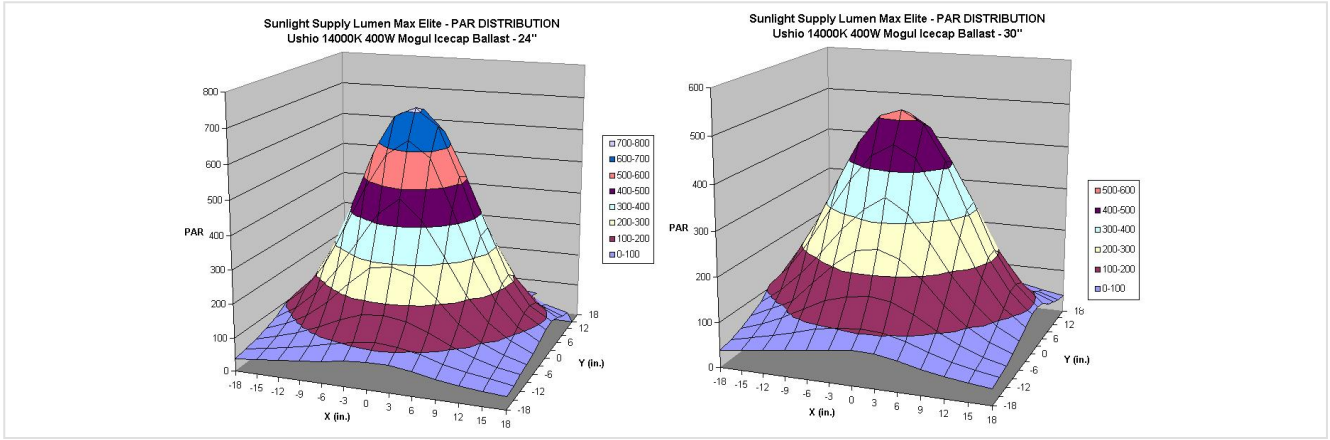

Figure 2: LumenMax Elite: Par distribution side view, 24" and 30".

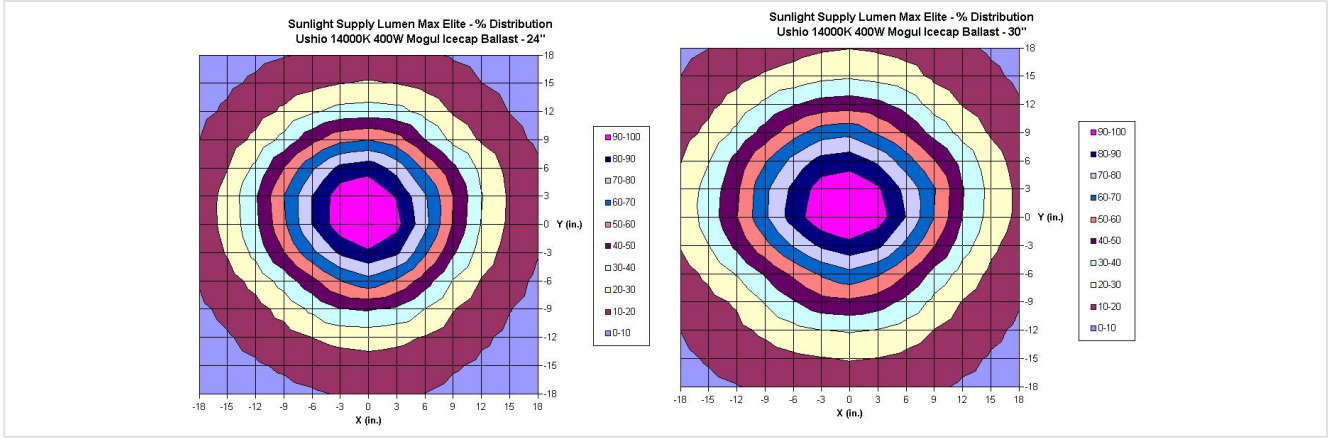

Figure 3: LumenMax Elite: Par distribution percent, 24" and 30".

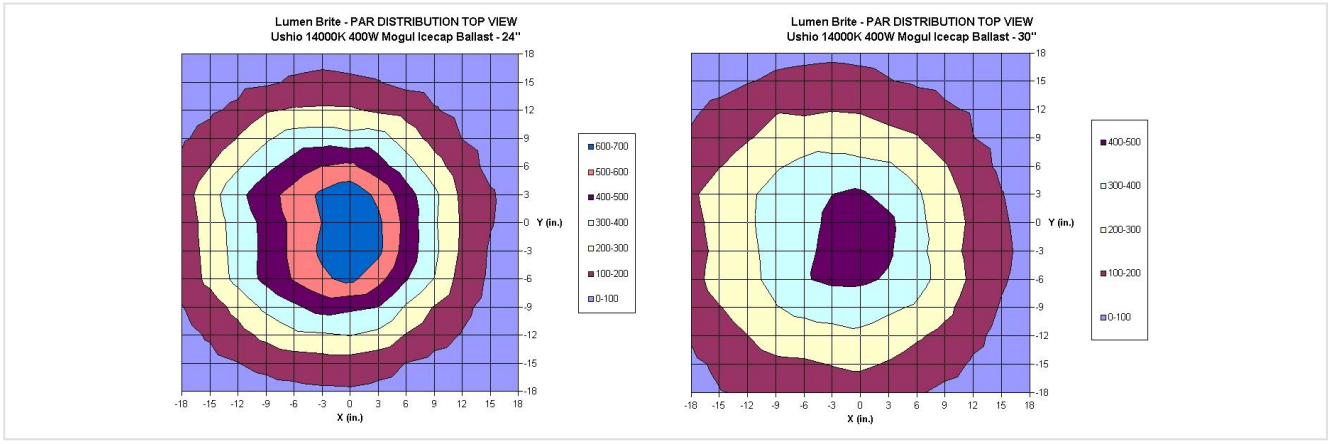

Figure 4: LumenBrite III: Par distribution top view, 24" and 30".

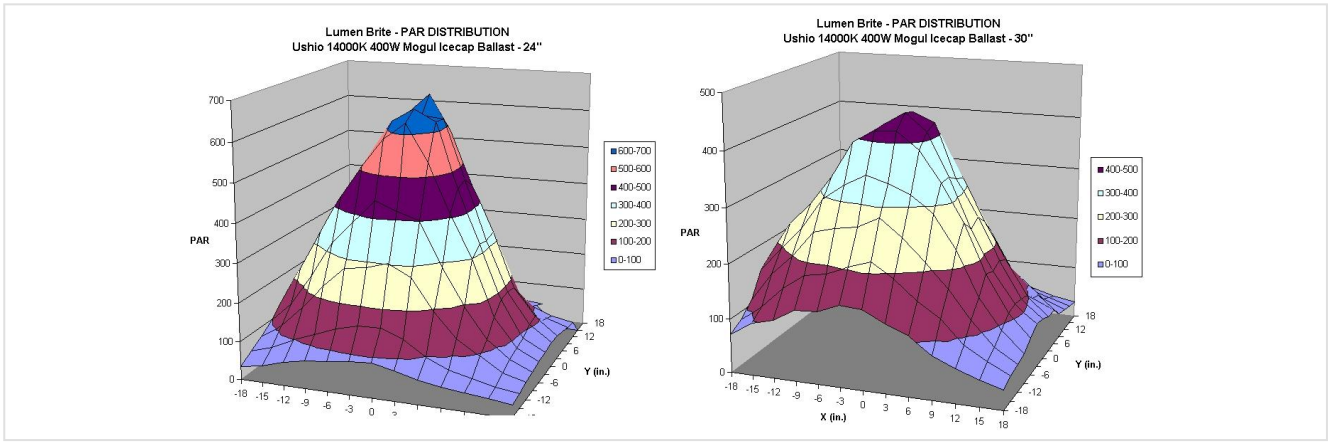

Figure 5: LumenBrite III: Par distribution side view, 24" and 30".

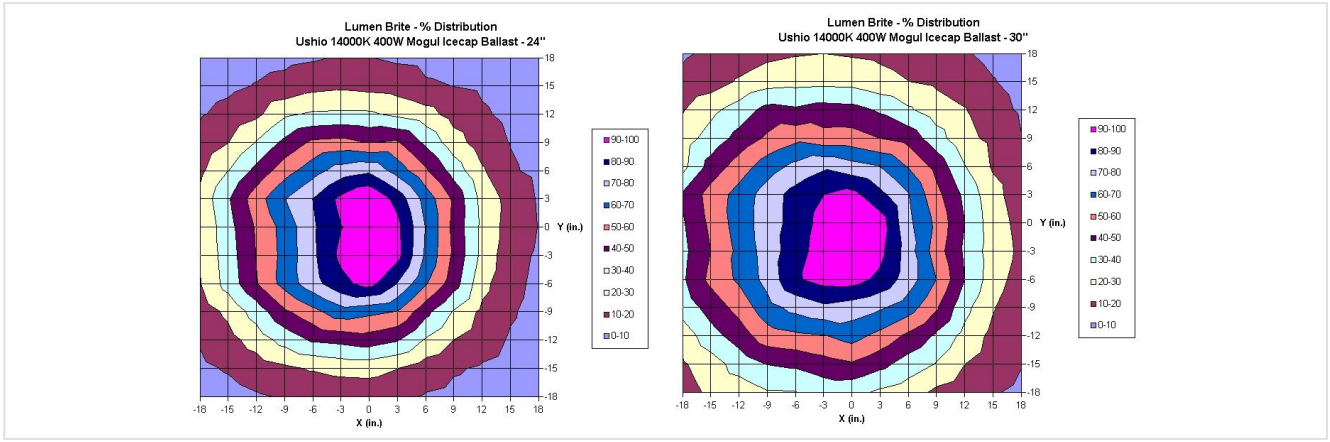

Figure 6: LumenBrite III: Par distribution percent, 24" and 30".

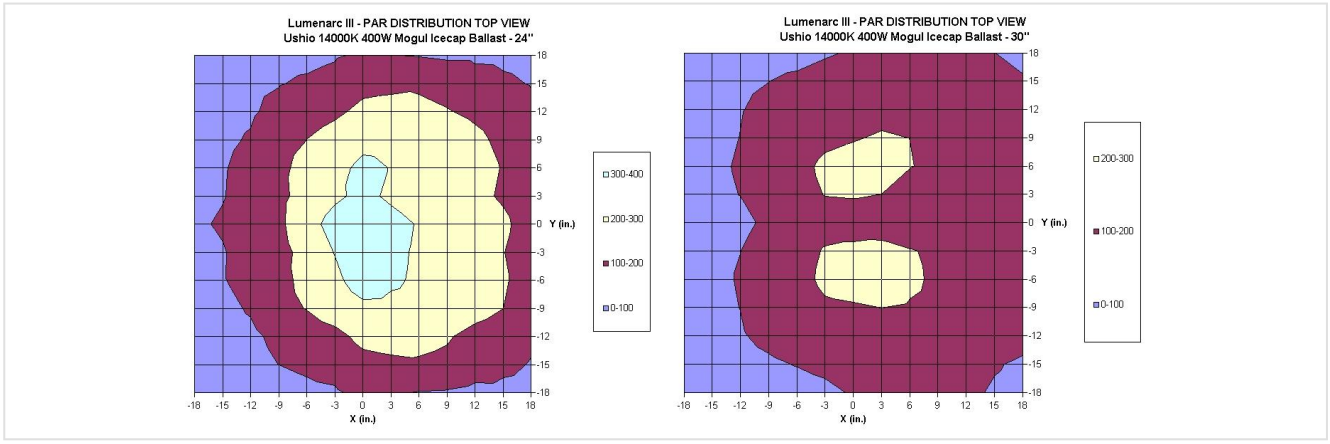

Figure 7: LumenArc III: Par distribution top view, 24" and 30".

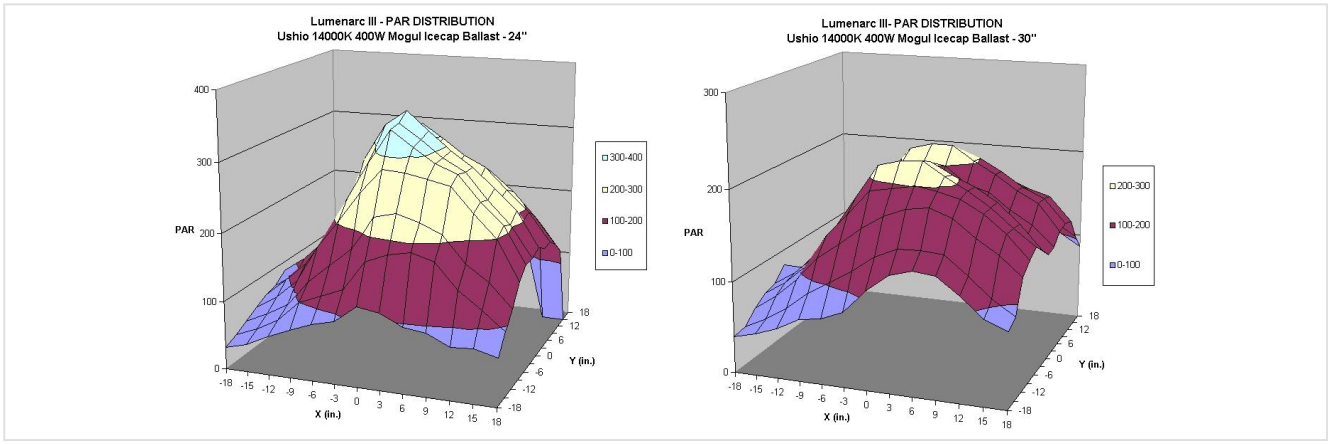

Figure 8: LumenArc III: Par distribution side view, 24" and 30".

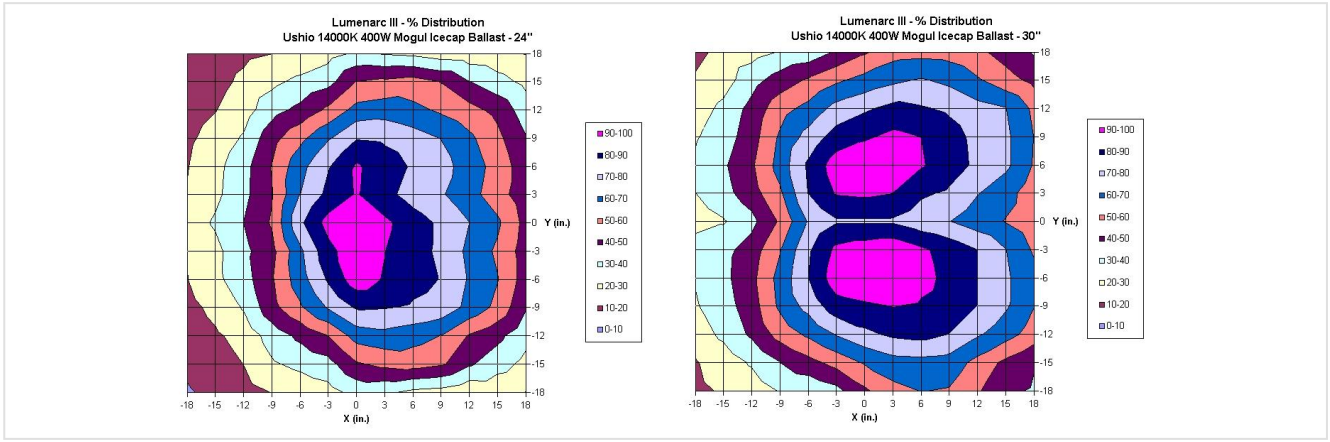

Figure 9: LumenArc III: Par distribution percent, 24" and 30".

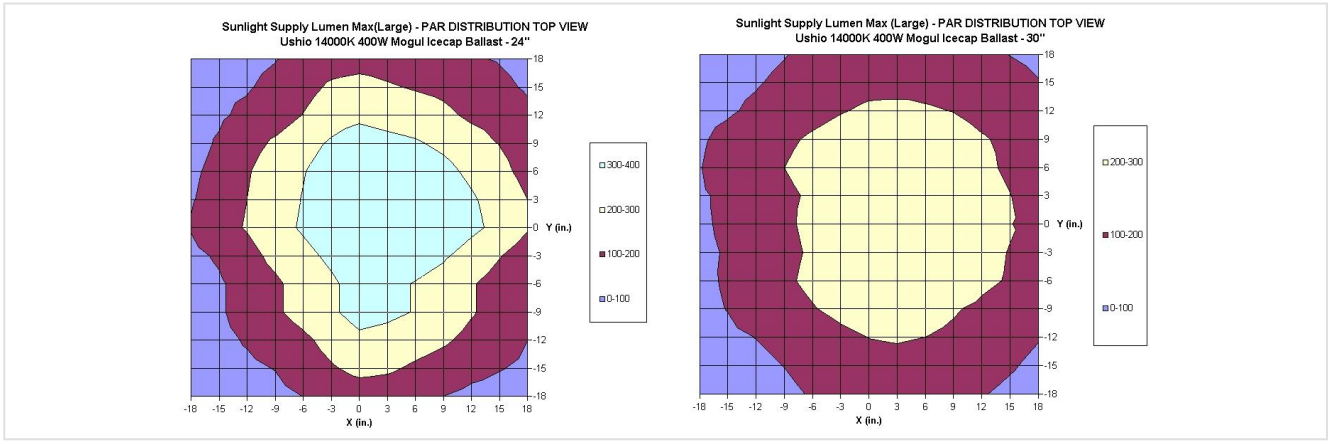

Figure 10: LumenMax (large): Par distribution top view, 24" and 30".

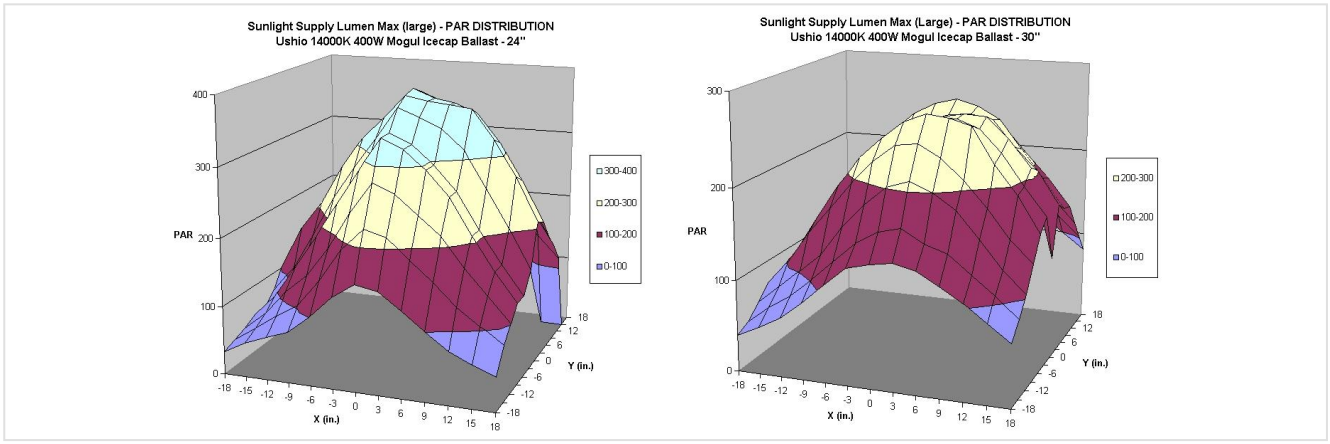

Figure 11: LumenMax (large): Par distribution side view, 24" and 30".

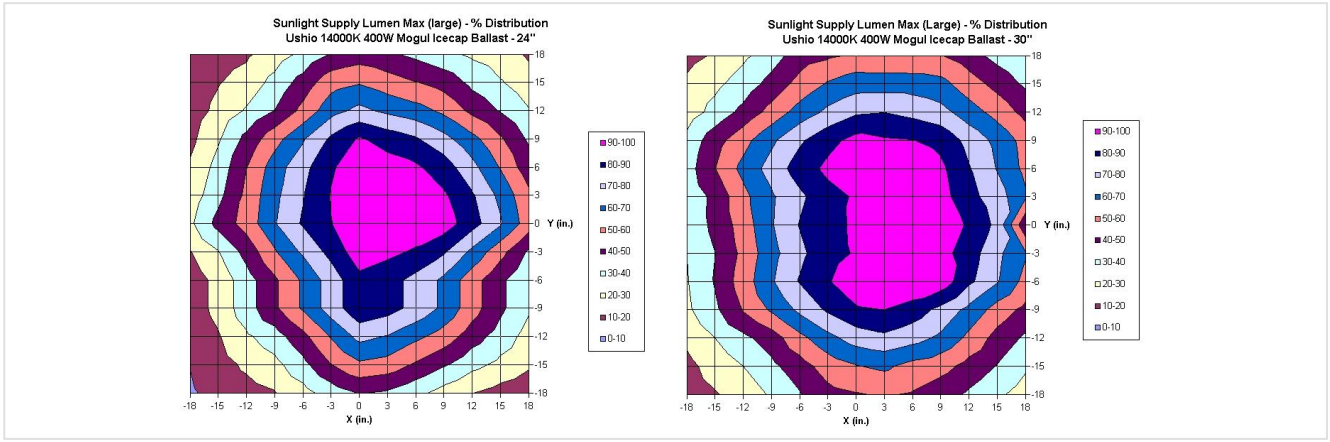

Figure 12: LumenMax (large): Par distribution percent, 24" and 30".

Lumenarc reflector. Hence, the reflector will be able to provide a higher intensity deeper in the tank, thus making it a better choice for someone looking to light a wide tank that is deeper than 30", or for providing the ability to grow higher light corals lower in the tank.

The Lumenbrite and Lumenmax elite are more suitable for tanks 3ft or less in width. They tend to provide a more focused coverage and higher peak values than the Lumenmax and Lumenarc reflectors. This can allow for better use in taller tanks, as more light can reach the bottom, or allow for higher placement of the lights thus reducing the heat dissipating in the water, or allow for higher light values to be obtained lower in the tank. This would allow the user to create a reef with a much lower rock profile. On shallower tanks 24" or less these reflectors would easily allow the user to move from a 400W lamp to a 250W lamp, resulting in savings in electrical costs.

Conclusions

Reflector design for reef aquariums has improved considerably allowing for more efficient use of light. With these reflectors we can now light reef aquarium with fewer lamps, get deeper light penetration over larger areas. As an example, I am currently lighting my 500G reef aquarium (84"LX48"WX30"D) with just 3 400W metal halide lamps in Lumenarc reflectors with no supplemental lighting. All of these reflectors help shatter the age old paradigm of having one metal halide lamp for every 2 sq feet of surface area. At the time my aquarium was set up, there was no other choice for reflectors. The new reflectors Lumenmax, Lumenmax Elite, and Lumenbrite have added to the choices available. They are all excellent choices, given that these reflectors are fairly close in performance to each other within their designed scope the selection would have to come down to other factors as well - such as construction quality, size constraints, space available for mounting, aesthetics, cost and availability. It is my hope, that the readers would use the data to choose the reflector that best suits their needs, rather than to promote reflector A as being better than reflector B.

Acknowledgements

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References

1. Joshi, S. and Marks, Timothy. 2003. Analyzing Reflectors: Part I - Mogul Reflectors http://www.advancedaquarist.com/issues/mar2003/feature.htm

SHORT TAKE

Tips for Healthy Aquarium Inhabitants: Preventive Measures for Aquarists, Part One

By Terry D. Bartelme

Practicing these good habits will minimize stress, prevent injury and insure that your stock will be well nourished.

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One of the hallmarks of a good aquarist is practicing preventive measures. Experienced hobbyists usually realize how important these habits are to their long-term success. Prevention consists of more than merely quarantining newly acquired fish. It also includes researching animals prior to purchase, making wise choices and being prepared in the event of an emergency. Practicing these good habits will minimize stress, prevent injury and insure that your stock will be well nourished.

Quarantine

Good things take time, but bad things can happen quickly in this hobby. That is why patience is such an important virtue for aquarists. While preventive measures do take some time and effort they also save time in the long run. One may think that it is easier to skip quarantine so they can get fish into the display aquarium quicker, but taking such risks often results in lengthy setbacks. This can mean losing stock to contagious disease or parasites. How much time is actually saved if all the fish have to be removed to another tank for treatment, because the proper steps were not taken to prevent an infectious disease from entering the display aquarium?

Six weeks of quarantine is recommended for recently captured reef fish. This allows enough time for them to regain their health, recover from the stress, adapt to captivity and learn to eat new foods. All newly acquired fish should be quarantined in isolation from other animals for a minimum of thirty days, regardless of the conditions that they came from. Exceptions can be made for mated pairs or small groups of shoaling fish to share a quarantine tank.

A quarantine system may be the best investment that an aquarist can make. Not only will it save money in the long run, it will save time and help you to avoid heartbreaking situations. Medicating a display aquarium may destroy the biological filtration and the invertebrates, as well as the fish, may not survive the treatment. Using a quarantine system will help you to steer clear of many situations that may make it tempting to medicate the display aquarium.

An efficient quarantine system does not require a sizable financial investment. All that is really necessary is a tank or food grade Rubbermaid™ container, a heater, some PVC pipe for hiding places and a something to provide biological filtration. An outside filter with a biowheel, or a sponge filter driven by an air pump will suffice. Don't forget to use a lid or screen to keep the fish from ending up on the floor. Overhead lighting is optional for fish, while some invertebrates require intense illumination. Place the isolation tank in a quiet location away from the main traffic flow of the house.

Do not keep rock, substrate or other calcareous materials in a quarantine system. It is better to rely on a filter that can easily be removed or replaced for biological filtration. Some medications may destroy or inhibit the bacteria that perform nitrification and calcareous materials will react with copper to take it out of solution.

Stable water conditions are important to the health of all fish, especially those that have been recently exposed to stressors. Keep a cycled quarantine tank up and running at all times so you won't have to deal with ammonia poisoning or unstable water parameters.

At times it may be necessary to sterilize the quarantine tank after finishing treatment for a contagious disease or parasite. This can be accomplished by adding a little chlorine to the water for a few hours. Then remove the chlorine and change the water. Keep a spare sponge filter or biowheel in the sump of your display aquarium so you always have a backup biological filter ready for use in your quarantine system.

The water parameters should be monitored closely in a quarantine system. Keep test kits for ammonia, pH, alkalinity, copper, oxygen, nitrate and nitrite near the aquarium. Regular, weekly water tests will help you keep track of the water parameters. Record a log of the results so any trends, such as a falling pH, will become evident. Don't forget to check the expiration dates of your test reagents to insure accurate readings.

An easy to read thermometer should placed in or on the aquarium. Use two small heaters rather than one that is powerful enough to overheat the aquarium. This is safer in the event of a malfunction. One small heater can't overheat the aquarium while it will prevent the water temperature from falling quickly if the second small heater stops working.

Do not use the same nets, cleaning equipment, etc., for your quarantine and display aquarium. It is not worth the risk of spreading a contagious disease or parasite. Use a food-grade Rubbermaid container when you give your fish a freshwater or medicated dip. Don't forget to use a ground fault interrupter and to turn off the power whenever working on your aquarium. Just in case you might forget, a timer can be used to automatically turn the power back on after you have finished.

Plastic swing-arm type hydrometers will work well enough provided you don't need to measure the salinity precisely. If you ever need to treat your fish with hyposalinity therapy then an accurate means of measuring the salinity is essential. A refractometer is a good investment and more accurate than most hydrometers.

Observe the animals in quarantine daily for any changes in behavior or visible symptoms of disease. Check the respiration rate to see if it is accelerated, normal, or abnormally slow. Are the eyes clear? Are they eating and showing an interest in their surroundings? Do they rub against objects in the aquarium? Is the fish swimming normally and using all of the fins? Is their coloration bright or faded? Do they react to your approach? While it is essential to closely observe the specimens in quarantine don't do it several times a day, because this may cause stress for the animals.

To get a good look at a fish in the aquarium trap it against the glass with a specimen container or net. Then look them over closely using a magnifying glass and a flashlight. This should make it easier to identify any potential visible symptoms. Have at least one book that you can refer to that can help you identify fish diseases and infections: "Manual of Fish Health" by Andrews, Exell and Carrington is a good example. Keep notes each day about what is happening with your quarantine tank and the inhabitants. This will help you to remember your experiences and assist you in the future.

Four factors in successful quarantine

1. Sufficient quarantine length
2. Monitoring the water parameters closely
3. Carefully observing the animals each day
4. Keeping accurate notes

Medications

When fish are sick administering treatment without delay can mean the difference between success and failure. There are a few medications that should be kept on hand for some of the more common infections. Keep a good copper-based medication such as Cupramine™, some praziquantel, formalin, and at least one good antibiotic handy. Maracyn-Two™ (minocycline), nitrofurazone, Furanase™ (nifurpirinol), kanamycin and neomycin are some good choices for antibiotics. Do not overstock with medications as they may expire before you use them.

Catching the Fish

Netting and removing fish from the water causes stress and frequently leads to injuries. This can be avoided by herding the fish into clear plastic bags or specimen containers with a net. Then the fish can be lifted from the aquarium in water. Clear plastic bags and specimen containers are more difficult for fish to see in the water than nets. This should make it easier to catch them and minimize the stress of chasing them around the aquarium.

Spare Equipment

You never know when you may be faced with a power outage or equipment failure. It may not happen when it is a convenient time for you to run to a store and purchase what you need. Stock an extra water pump, a heater, some airstones, batteries and at least one battery operated airpump for just such an occasion. Make sure that you have some firewood around for your fireplace in case you need to keep the house and the aquarium warm. It is also a good idea to make arrangements with someone that can take care of your aquarium in an emergency, or when you are away on vacation.

Research the Animals Prior to Purchase

Research may be the most important tool an aquarist can use. Knowing the needs, requirements and compatibility of each species prior to purchase will help you avoid making mistakes and greatly improves the animal's chance of living a long healthy life in your care. You may learn that a species that you were considering has special dietary needs, requires a larger tank or simply is not compatible with another fish or invertebrate in your aquarium.

Selecting a Specimen

Once you have thoroughly researched a particular species of fish it is time to closely scrutinize each potential acquisition. Starting with a healthy specimen to begin with greatly increases their odds of long-term survival. Look for signals that indicate good health: such as eating, vibrant colors, a rounded appearance, clear eyes, fully extended fins, swimming normally and showing interest in the environment around them. Always ask to see the fish eat before you purchase it. Buying any fish that is eating less than two types of food is a risky proposition. If the specimen you are interested in is not eating then go back to the dealer in a day or two for another try.

Avoid specimens that exhibit labored or rapid respiration, are lethargic, have injuries, torn fins, are emaciated, or are obviously diseased. It is not uncommon for recently transported fish to be a bit thin in the stomach area. They will usually recover from this once they begin eating again, provided they receive an adequate diet. Fish suffering from long term or severe starvation will be thin behind the head and above the lateral line. These specimens may not recover.

Bringing them Home

Check the pH and water temperature in the tank of the specimen that you are interested in at the store. Then prepare your quarantine tank before you bring them home by matching those parameters as closely as possible (provided those parameters are safe).

Take a few precautions when transporting newly acquired specimens to reduce the risk of injury and stress on the animals. Start with enough water. A half gallon of water per three inches of fish is a good guideline. The rest of the transport bag should be filled with oxygen. Double bag the fish as leak insurance and to help insulate the water from temperature fluctuations. Keep the fish in the dark during transport to decrease their awareness of their surroundings and help reduce stress. Then get the fish into the quarantine tank as quickly as possible.

Stress

Any discussion of prevention and health in fish should include the topic of stress. Stress causes a myriad of physical, chemical and behavioral changes in fish. Stress can interfere with eating behaviors and digestion, cause difficulty in maintaining normal homeostasis (such as osmotic balance) and reduce immune function among other things. Behavioral changes are often the first indicators that some sort of stress is ongoing. Be aware of any potential sources of stress and immediately eliminate or reduce them whenever possible.

Stress factors can be classified into four loosely fitting categories: human interference, water pollution, animal interactions and extreme changes in the physical environment. Many of the stress factors mentioned below belong in more than one of these categories. Some examples of stress caused by human interference are: handling, toxins, photo shock, transport, electrical shock, crowding, malnutrition, noise or vibrations and netting or removing the fish from water. Water pollution would include: high ammonia, nitrite, or nitrate levels, heavy metals, high organic or suspended solids levels and other chemical toxins. Stressors that fall into the animal interactions category are: aggression, pathogens such as parasites and injury, hunger or nutritional problems related to aggressive behaviors. Stressors that fit into the extreme changes in the physical environment include: high carbon dioxide, low oxygen, improper hardness, turbidity, gas super-saturation, sudden changes in temperature or salinity, excessive water velocity, photo shock and low pH.

Next Installment

Part two of this two-part series will continue with an updated method for acclimation, ideas for providing a nutritious diet, and other tips for avoiding pitfalls so you can keep your stock in the best possible health.

References

1. Wedemeyer, G.A. "*Handling and Transportation of Salmonids*," Principals of Salmonid Aquaculture. Pennel, W. & Barton, B., eds., Elsevier Publishing, Netherlands, 1996.

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