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Final Report to the NOAA Chesapeake Bay Office: Influence of Oyster Reef Elevation on the Health of *Crassostrea virginica*

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Introduction

Diseases caused by protistan parasites *Perkinsus marinus* (dermo) and *Haplosporidium nelsoni* (MSX) continue to be key sources of natural mortality in the oyster *C. virginica* in Chesapeake Bay. Infection pressure on oysters is historically high (Carnegie and Burreson 2009; Carnegie and Burreson 2011). Despite the intense disease pressure, and likely *because* of it, promising signs of resistance to disease are beginning to emerge. Accumulations of larger, older oysters in high-disease areas like the lower Rappahannock River that had been long closed to harvesting have captured the attention of the harvest community. Oysters in Maryland are showing decreasing levels of mortality despite continuing high levels of parasitism (e.g., MD DNR 2010). A profusion of oysters in disease-intense tributaries like the Great Wicomico and Lynnhaven Rivers has captivated the public. We have argued in a recent paper integrating years of research results (Carnegie and Burreson 2011) that these oysters cannot simply be the products of recruitment derived from low-salinity refuges from parasitism, which we know from experimental work would rapidly die of disease; rather, they must largely be the products of recruitment by relatively resistant oysters residing in the areas of high disease. Intensification of MSX and dermo diseases in Chesapeake Bay since 1986 (Burreson and Andrews 1988; Carnegie and Burreson 2009) may paradoxically have been beneficial in purging more susceptible individuals from oyster populations more rapidly. Theoretically, it should have increased the rate at which resistance developed.

In the last few years, agencies and non-governmental organizations promoting and effecting oyster restoration in the region have followed this research and embraced the concept of locating sanctuaries from harvest in high-disease areas, expressly for the purpose of giving more resistant oysters more opportunities to pass on their genes. Sanctuary creation has thus come to represent an essential nexus between disease management and restoration science. A potential second connection has been promoted but it is more controversial, and was the focus of this project. Progress in oyster restoration in the Great Wicomico River has been attributed to the contribution of reef elevation, with taller reef constructions purportedly showing more accretion, a function of increased recruitment, growth, and/or survival (Schulte et al. 2009). The importance of three-dimensionality in reef architecture has long been recognized (e.g., Chesapeake Research Consortium 1999; Hargis and Haven 1999). Among numerous other benefits, a larger reef causes currents to accelerate over and around it (Breitburg et al. 1995; Lenihan 1999), with faster currents promoting oyster growth and reducing sediment deposition, both of which favor oyster recruitment. What is not clear is whether reef elevation will actually convey any benefits with respect to oyster health.

The only study relevant to the effects of reef position on oyster health was conducted by Lenihan et al. (1999), who evaluated *P. marinus* parasitism (among other factors) at the bases and crests of tall (2-

m) and short (1-m) oyster reefs at 3- and 6-m depths in the Neuse River estuary, North Carolina. They found *P. marinus* prevalences and intensities to be greater, often significantly, at the bases of reefs than at crests, and on short reefs rather than on tall ones. They attributed this to poorer food quality at short reefs and the base of tall reefs contributing to physiological stress, and to proximity of oysters in these positions to *P. marinus* cells suspended near the benthos. The oyster mortality pattern they observed was similar to that of *P. marinus* infection, but hypoxia probably contributed to this (Lenihan and Peterson 1998; Lenihan et al. 1999). Schulte et al. (2009) relied upon the North Carolina observations in arguing in favor of high relief reefs, reasoning that they not only "maximize growth and survival" and prevent sedimentation, but also "minimize disease influence". In Stokstad (2009), a Schulte et al. (2009) co-author suggested that "a population of healthy adults living in good habitat will resist disease better than other populations have". We might ask, however, whether any conclusions concerning dermo disease—or MSX disease, which was not evaluated in the North Carolina studies—may actually be drawn from the Lenihan et al. (1999) work.

The Lenihan et al. (1999) study of *P. marinus* was conducted between December 1994 and June 1995 using reefs constructed in July 1993. This is problematic for three reasons. First, it covered the time of year when *P. marinus* levels are at their *lowest* in the region (Burreson and Ragone Calvo 1996). At Wreck Shoal in the James River, Virginia, for example, *P. marinus* weighted prevalence decreased from 2.44 in November 1994—indicative of serious dermo disease—to 0.24 in May 2005, before increasing to 1.04 in June (Ragone Calvo and Burreson 1994, 1995). Weighted prevalences this low indicate little or no actual dermo disease (Andrews and Hewatt 1957). The highest weighted prevalence measured in June 1995 by Lenihan et al. (1999) was 0.89. While these authors may have detected significant differences among *P. marinus* levels among their treatments, they did not capture a dermo disease epizootic in their sampling. Their results make no statement regarding elevation-specific differences with respect to dermo disease. Second, their study did not address MSX disease, which is active in North Carolina and which is very important in areas of Chesapeake Bay where resistance is not well developed, like the Great Wicomico River. Record levels of MSX disease—prevalences exceeding 60%, with abundant serious infections (Carnegie and Burreson 2011)—were observed only two years ago in the Great Wicomico. And third, fully epizootic dermo disease probably does not develop on newly constructed reefs until more than two years post-construction anyway. The Lenihan et al. (1999) reefs were sampled after the second season of dermo activity, and before the third. Oysters we obtained at peak dermo season in 2009 from a constructed oyster reef in its second year of exposure in the Lynnhaven River, where dermo disease levels are so high that weighted prevalences can exceed 3.00 (levels over 2.00 indicate serious dermo disease and mortality), revealed a weighted prevalence of only 0.97 (Carnegie and Burreson, unpublished data). For all these reasons, we must conclude that questions regarding the influence of reef position on interactions with key parasites were not satisfactorily answered by Lenihan et al. (1999).

The question is still important, however. As construction of higher-relief, three-dimensional reefs remains a focus in the Chesapeake region, we need to know what suitable restoration goals should be. Should the goal be a stable, accreting structure that, by virtue of its rate of accretion relative to burial, can maintain a positive shell budget (Powell et al. 2006; Mann and Powell 2007; Powell and Klinck 2007)? Or a reef constructed to such a height that oysters at its crest are significantly healthier than oysters at its base? If oysters in the most optimal positions are not significantly healthier than those in marginal areas of the reef, then pathogen considerations would be moot, and restoration planners may be able to model the influences of water depth, reef height, and energy inputs from currents and waves on oyster growth and recruitment more generically to design for positive shell budgets. If there is a difference in oyster health vis-à-vis position, then we must ask whether the cost of building to yield health benefits in a given system exceeds the cost of building for a positive shell budget—the most fundamental objective—and whether the additional expense is worthwhile.

The overall goal of this project was to provide the first quantitative determination of the impact of oyster reef position on oyster health at times and in places where oyster diseases are most active and intense. We pursued three primary objectives:

Objective 1: To determine the influence of position on an oyster reef on dermo disease, caused by *P. marinus*, in *C. virginica*;
Objective 2: To determine the influence of position on an oyster reef on MSX disease, caused by *H. nelsoni*, in *C. virginica*; and
Objective 3: To communicate results to stakeholders through the Virginia Sea Grant Marine Advisory Program and the VIMS Shellfish Pathology Laboratory web site.

Methods

Working with the team of Dr. Kennedy Paynter from the University of Maryland, we sampled Bland Point Reef in the Piankatank River and Shell Bar Reef in the Great Wicomico at peak dermo disease season in autumn 2011 (26-27 September) and peak MSX season in spring 2012 (24 May). Both these reefs have oysters distributed across vertical elevations of at least 1.5 m (Fig. 1), exceeding the 0.5-0.6 m elevation difference that has been suggested to allow oysters at the higher elevation to better resist disease. Collection was by divers swimming either along the crest (~ 0.9 m depth at Shell Bar, and ~ 1.2 m at Bland Point) or the base, the latter defined as the lowest elevation with substantial numbers of live oysters (~ 2.4-3.4 m depth at Shell Bar, 2.7 m at Bland Point). Four samples of ≥ 20 oysters (sub-market-sized to market-sized, generally greater than 65 mm in shell height) were collected at each elevation, on each reef, on each date. For the September 2011 sample, disease analyses were conducted using two methods: Ray's fluid thioglycollate method (RFTM, Ray 1966) for *P. marinus* detection, and paraffin histology for *H. nelsoni* (Carnegie and Burreson 2011) and generally pathology. For the May 2012 sample, only histology was performed because *H. nelsoni* was targeted, although perspective on early-season levels of *P. marinus* was also acquired because *P. marinus* can also be detected histologically.

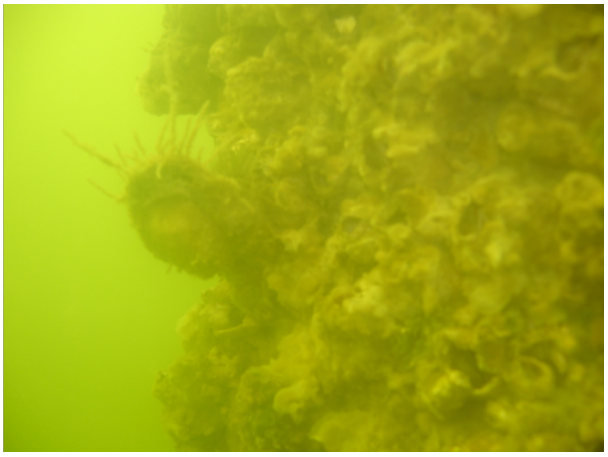

Fig. 1. Vertical relief at Shell Bar Reef. Hillary Lane, Paynter Lab photo.

Results

In September 2011, *P. marinus* levels were slightly higher at Shell Bar Reef than at Bland Point, although they were typical for autumn. Mean dermo prevalence among all oysters at Shell Bar Reef was 79.3 ± 29.1% at the crest (here and below, expressed as the mean with a 95% confidence interval), and 85.0 ± 18.3% at the base (Fig. 2A). At Bland Point, dermo prevalence among all oysters was 72.0 ± 18.7% at the crest, and 67.0 ± 8.0% at the base (Fig. 2B). The 95% confidence intervals indicate that differences between crest and base were not statistically significant at either reef. Analyses of market-sized oysters alone, conducted because the samples contained varying proportions of smaller oysters, produced similar results (not shown). Mean intensities of infection among all oysters ranged narrowly from 2.02-2.12 (indicating infections that were light-moderate on average), with differences also statistically insignificant (Figs. 2C,D). Again, analyses of market-sized oysters alone produced similar results (not shown). Histopathology revealed no MSX in the autumn samples.

In May 2012, *H. nelsoni* was again not detected at either site. *P. marinus* was detected in all crest and base samples from Shell Bar Reef, at 5-25% prevalence, and in half the samples from Bland Point Reef at 10-20% prevalence. Most of these infections were light. Of 32 total *P. marinus* infections observed, 6 reached *moderate* intensity, a typically low level of intense early-season dermo disease. Three of these

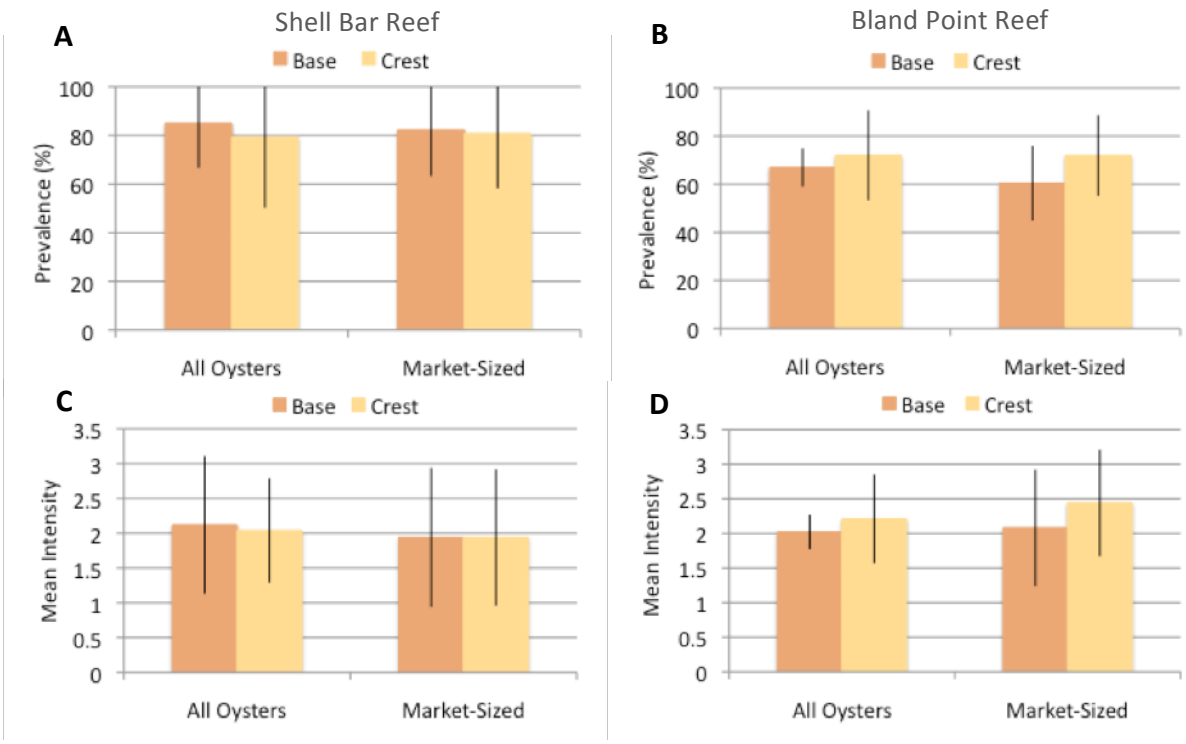

Fig. 2. *Perkinsus marinus* observations, September 2011. **A.** Percent prevalence at Shell Bar Reef. **B.** Percent prevalence at Bland Point Reef. **C.** Mean infection intensity at Shell Bar Reef. **D.** Mean infection intensity at Bland Point Reef. Error bars represent 95% confidence intervals for each mean.

cases were at the crest of Shell Bar Reef, two were at the crest of Bland Point Reef, and one was at the Bland Point base. Histopathology yielded additional perspective on May samples, and most oysters from both elevations at both sites were nearing full ripeness and beginning to spawn.

Nineteen other conditions were noted in the histological examinations, ranging in prevalence from > 97% (infection with the gregarine apicomplexan *Nematopsis* sp.) to < 1% (as in the presence of cysts and edema at the mantle)(Table 1). None of these conditions displayed clear patterns with respect to reef elevation, although at least one appeared to vary with location (mantle coccidians) and another with season (viral gametocytic hypertrophy). In general, most of these conditions were not very prevalent or intense and they were not detected consistently, which produced substantial variability about the means for prevalence. Most of these conditions are typically innocuous, such as infection by *Nematopsis*; colonization of gills and gut, respectively, by *Sphenophrya* and *Stegotricha* ciliates; and the presence of *Rickettsia*-like organisms (bacterial), viral gametocytic hypertrophy, mantle coccidians (protistan), oocyte microsporidians (protistan), and nematodes and other metazoans. Infection by the trematode *Bucephalus* is typically castrating to infected hosts, but this was not very prevalent. Gut and gill disruption or erosion are pathological conditions, and hemocytosis (elevated levels of circulating hemocytes), digestive tubule atrophy, and reduced fecundity can be indicative of underlying diseases, but these were again not very prevalent, and not obviously variable between reef elevations.

Table 1.
Percent prevalence of other observed conditions. SB = Shell Bar Reef. BP = Bland Point Reef. RLOs = *Rickettsia*-like organisms. Ranges represent 95% confidence intervals about means.

	Nematopsis	*Sphenophrya*	*Stegotricha*	Chlamydia / RLOs	Viral Gametocytic Hypertrophy	Mantle Coccidian	Reduced Fecundity
Sept. 2011							
SB crest	98.9 ± 3.5	16.9 ± 12.5	32.2 ± 22.7	8.6 ± 6.3	1.1 ± 3.6	20.0 ± 10.6	0.0 ± 0.0
SB base	100.0 ± 0.0	14.8 ± 6.0	32.3 ± 8.3	8.5 ± 9.1	0.0 ± 0.0	10.0 ± 19.1	0.0 ± 0.0
BP crest	99.0 ± 3.2	30.0 ± 27.7	31.0 ± 15.9	10.0 ± 8.2	1.0 ± 3.2	0.0 ± 0.0	0.0 ± 0.0
BP base	99.0 ± 3.2	23.0 ± 21.0	15.0 ± 14.1	7.0 ± 6.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
May 2012							
SB crest	100.0 ± 0.0	13.8 ± 7.6	3.8 ± 4.0	12.5 ± 8.0	11.3 ± 4.0	10.0 ± 11.3	17.5 ± 23.9
SB base	98.8 ± 4.0	10.0 ± 11.3	7.5 ± 10.3	6.3 ± 11.9	12.5 ± 8.0	7.5 ± 4.3	3.8 ± 4.0
BP crest	100.0 ± 0.0	8.8 ± 4.0	1.3 ± 4.0	6.3 ± 11.9	7.5 ± 10.3	0.0 ± 0.0	8.8 ± 13.6
BP base	97.5 ± 4.6	8.8 ± 7.6	1.3 ± 4.0	10.0 ± 6.5	16.3 ± 22.9	0.0 ± 0.0	15.0 ± 13.0

	Mod-Heavy Hemocytosis	Oocyte Microsporidian	Digestive Tubule Atrophy	Gut Epithelial Disruption	*Bucephalus*	Other Metazoans
Sept. 2011						
SB crest	8.2 ± 13.8	2.0 ± 3.7	0.0 ± 0.0	1.1 ± 3.5	3.2 ± 6.6	1.0 ± 3.2
SB base	4.0 ± 9.0	2.3 ± 4.2	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 4.2	2.3 ± 4.2
BP crest	2.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 3.2
BP base	8.0 ± 9.0	0.0 ± 0.0	1.0 ± 3.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
May 2012						
SB crest	1.3 ± 4.0	1.3 ± 4.0	8.8 ± 10.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SB base	2.5 ± 4.6	6.3 ± 10.0	1.3 ± 4.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BP crest	0.0 ± 0.0	1.3 ± 4.0	1.3 ± 4.0	2.5 ± 8.0	0.0 ± 0.0	1.3 ± 4.0
BP base	2.5 ± 4.6	2.5 ± 4.6	0.0 ± 0.0	5.0 ± 6.5	0.0 ± 0.0	0.0 ± 0.0

	Nematodes	Gill Erosion	Emaciation	Coccidia	Mantle Edema	Mantle Cysts
Sept. 2011						
SB crest	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SB base	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0
BP crest	3.0 ± 6.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 3.2	0.0 ± 0.0
BP base	1.0 ± 3.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 3.2	0.0 ± 0.0	1.0 ± 3.2
May 2012						
SB crest	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 4.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SB base	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BP crest	0.0 ± 0.0	1.3 ± 4.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BP base	0.0 ± 0.0	2.5 ± 4.6	2.5 ± 4.6	0.0 ± 0.0	1.3 ± 4.0	0.0 ± 0.0

Discussion

Significant vertical relief (of magnitude appropriate to the geomorphology and hydrodynamics of, and reef position in, a given system) has long been recognized as a key to oyster reef growth and persistence; it allows recruitment and growth to outpace sedimentation. There has been no evidence yet, however, that it provides significant benefits with respect to oyster health, despite the assertions of Lenihan et al. (1999) and Schulte et al. (2009). Our analyses of oysters on reefs of substantial vertical relief makes a strong case that *P. marinus* levels are unaffected by greater vertical relief. Some caution is warranted, however, in drawing broader conclusions. First, questions with regard to the effect of vertical relief on *H. nelsoni* levels remain unresolved. The last few years have seen a decreasing trend in *H. nelsoni* levels in Chesapeake Bay with the parasite now largely undetectable in most of the Maryland part of Chesapeake Bay and in the upper reaches of the large Virginia rivers. *H. nelsoni* was not detected at Shell Bar Reef in the Great Wicomico River, and it is possible that the parasite is mostly or entirely

absent from that system. Shell Bar Reef, therefore, may not have received an MSX challenge during the course of the study. While *H. nelsoni* was not detected at Bland Point in the Piankatank either, we know from work in the York River and Mobjack Bay that the parasite is present at those locations, and the Piankatank is similar in salinity and—as long-term monitoring demonstrates—in parasite levels to those systems. So *H. nelsoni* may not have been absent from the Piankatank, but present at levels below detection. If this plausible scenario is true, the absence of infections in the sampled oysters could represent resistance to MSX as much as a decreased abundance of the organism. The "Spring Imports" leg of the VIMS Shellfish Pathology Laboratory monitoring program makes clear that a system characterized by very low *H. nelsoni* levels in oysters can still be associated with an intense MSX disease challenge on non-resistant individuals or stocks (Carnegie and Burreson 2011).

The other reason why caution is required is that our study did not address the matter of disease *tolerance*. There is a distinction between resistance and tolerance that dates back at least to 1894, when Cobb wrote of "rust-enduring" wheat:

> *A rust-enduring wheat is one which, though liable to rust, is able, notwithstanding the attack of the rust, to mature a fair crop of grain under ordinary circumstances* (Cobb 1894).

Orton (1909) similarly wrote of "disease endurance" ("the ability of the plant to grow in spite of an attack", possibly owing to "exceptional vigor"), and Caldwell (1958) of "tolerance" ("enabling a susceptible plant to endure severe attack. . .without sustaining severe losses in yield or quality." Roy and Kirchner (2000) considered host-pathogen relationships more generally and described the resistance-tolerance distinction succinctly:

> *Thus we use "resistance" to refer to host strategies that limit infection, and we note that any such strategies necessarily limit the pathogen's fitness. By contrast, tolerance traits do not limit the infection itself, but reduce its fitness consequences for the host* (Roy and Kirchner 2000).

There is no basis for restricting the concepts of either disease resistance or tolerance to the plant realm, and we have reason to believe that both resistance and tolerance dynamics are at work with regard to oyster-*P. marinus* interactions. While *P. marinus* prevalences in naïve sentinel deployments to high-disease systems like the York River (e.g., the Spring Imports deployments of the VIMS Shellfish Pathology Laboratory, which date to 1960) may not be significantly higher than in oysters like those at Shell Bar and Bland Point Reefs, since prevalences in most Virginia oyster populations approach 100% in fall, infection intensities in the sentinels tend to be much higher. In September 2011, the average intensities at Shell Bar and Bland Point Reefs were ≤ 2.12, indicating an average infection that was *light-moderate* in intensity. Wild oysters at Aberdeen Rock in the York River sampled on 11 October 2011 had an average intensity of infection that was only slightly higher, 2.34. The average intensity in the naïve sentinel Spring Imports in the York River was 4.04, indicating an average infection that was *moderate-heavy* (Carnegie, unpublished). This indicates relative resistance in the wild oysters that is apparent in comparisons with the sentinels. At the same time, there is evidence for tolerance as well. *P. marinus* levels continue to be very high in lower Chesapeake Bay by historical standards, yet oyster mortality appears to have declined. An ongoing retrospective analysis of archival histological materials indicates that a given level of *P. marinus* infection today is less disruptive to gametogenesis than it was two decades ago. There is the possibility, therefore, that while parasite levels may have been similar between elevations, these equivalent parasite levels may nonetheless have produced dissimilar physiological consequences due to differences in inherent tolerance or to differences in food quantity or quality. Future research should include evaluation not only of parasite levels but of mortality and of reproductive and physiological measures as well to provide better perspective on whether tolerance mechanisms may be at work.

To conclude, while questions regarding MSX and tolerance dynamics remain, our results suggest that there is no effect of reef elevation on the most significant disease in our region. There is presently no

scientific justification for engineering reefs to meet disease-related goals. Building reefs of some vertical relief clearly has merits, but these relate to deposition profiles, recruitment, and reef accretion, not to interactions with the major parasites.

Output

An advisory presenting these results in the larger context of regional shellfish restoration strategies has been produced for dissemination through the VIMS Marine Advisory Program and the VIMS Shellfish Pathology Laboratory web site. A manuscript is in preparation for submission to the Journal of Shellfish Research.

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