10 EXOTIC SPECIES

Convenors: Dr. Ronald Klauda and Dr. Daniel Terlizzi

Toward a Sustainable Coastal Watershed:
The Chesapeake Experiment. Proceedings of a Conference
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Non-Indigenous Species of Chesapeake Bay: Past Invasions and Future Risks

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Abstract: Chesapeake Bay has been a site for past invasions of nonindigenous species and may be at particular risk for future introductions. Major mechanisms for past invasions of Chesapeake Bay have included transport of fouling organisms on the bottom of ships (mostly before 1900) and introductions associated with the importation of oysters from the Gulf of Mexico (mostly 1950s-1970s). Today, the global movement of ballast water by ships is the primary mechanism for estuarine invasions, and Chesapeake Bay receives more foreign ballast water (> 15 million metric tons annually) than other eastern U.S. ports. To understand better the impact of biotic invasions in Chesapeake Bay, we are compiling a history of invasions for the Chesapeake similar to those available for Pacific coast estuaries, the Hudson River estuary, and the Great Lakes. We are also assessing the risk of local invasions by nonindigenous species associated with discharged ballast water. Our analysis of ships' ballast water indicates a diverse assemblage of plant and animal species is arriving alive at Baltimore and Norfolk from foreign ports. We have begun to examine the fate of these organisms by (1) experimentally testing their viability under local conditions and (2) testing for successful invasions at the sites of ballast release. Taken together, our analyses of past invasions and the fate of organisms currently released in ballast water will provide a measure of the relative susceptibility of Chesapeake Bay to invasions.

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THE IMPACTS OF THE EXOTIC SPECIES *HYDRILLA VERTICILLATA* ON THE SHALLOWS IN CHESAPEAKE BAY

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Abstract: In the early 1980s *Hydrilla verticillata* was introduced to Chesapeake Bay in both the Potomac River and at Susquehanna Flats near Havre de Grace. *Hydrilla* is an effective competitor with native submersed aquatic vegetation (SAV) and initially invaded the landward margins of existing beds as soon as water temperatures warmed in early summer. As the growing season progressed, *Hydrilla* invaded into deeper areas by sending out lateral shoots along the bottom. Eventually, vertical shoots emerged from the bottom shoots form to dense canopies that limited the light to competitors. Not only does *Hydrilla* have higher productivity (5.4 mg 02gzdw-1h-1) in shallow waters than native SAV, but it can more effectively use bicarbonate ions as an inorganic carbon source than competitors. This physiological adaptation is especially important in that the pH in the surface waters of dense *Hydrilla* canopies often reach a pH of 9.0 - 10.5 in late afternoon, making CO_2 unavailable as a carbon source. In deeper waters where pH is lower. native SAV, such as Vallisneria, have slightly higher productivity (3.8 mg 0_2 gzdw^{-1}h^{-1}) than *Hydrilla* (2.8 mg 0_2 gdw^{-1}h^{-1}) and may be able to uptake phosphorus more efficiently which is often the limiting nutrient. *Hydrilla* was found to have little tolerance to salinity in mesocosms and only maintains itself in tidal freshwater sections of the Bay. However, in these areas *Hydrilla* has a very marked impact in the shallows. Not only is the dense vegetation important habitat for a variety of consumers (e.g., fish), *Hydrilla* can sequester large amounts of nutrients in its biomass (500-1000 g m^{-2}) as the season progresses. Transects indicate that nutrients in the overlying waters may be lowered in the afternoon, when beds are most productive, and diel sampling of nutrients every 2 hours suggests *Hydrilla* beds can attenuate nutrient pulses from the riverine and land sources.

INTRODUCTION

Over the last 25 years, extensive documentation by a variety of survey techniques (see Stevenson and Confer 1978, Orth et al. 198y) in the shallows of upper Chesapeake Bay indicate there have been significant changes in the abundance and dominance patterns of submersed aquatic vegetation (SAV). For example, *Myriophyllum spicatum*, an introduced species, became widespread in the Bay in the late 1950s, displacing many native species, only to decline and itself be replaced by natives by the 1970s (Bayley et al. 1978). An extensive, Bay-wide decline in SAV during the 1970s (Orth and Moore 1983) preceded the accidental introduction of another exotic species, *Hydrilla verticillata*, at two sites on the Bay, the Potomac River just south of Washington, D.C. (Steward et al. 1984) and at the mouth of the Susquehanna River near Havre de Grace, Maryland (Staver 1986). *Hydrilla* is an opportunistic species with multiple modes of reproduction and a keen competitor in warm freshwater environments (Pieterse 1981), and was initially viewed as a threat to the recovery of native SAV in Chesapeake Bay.

In previous studies in Florida, Van et al. (1976) found that *Hydrilla* has the ability to form dense canopies, thereby reducing the availability of light to competing species. In addition, several studies have shown that *Hydrilla* utilizes bicarbonate as a carbon source for photosynthesis more efficiently than other submersed aquatics (Bowes et al. 1977, Van et al. 1977, Holaday and Bowes 1980), enabling it to outcompete other species when pH is

high (> 8) and the CO_2 concentration is very low (Haller and Sutton 1975). These studies, however, have been carried out using a tetraploid strain of *Hydrilla* which is common in Florida lakes (Pieterse 1981). The biotype introduced into Chesapeake Bay is a diploid strain that had not been reported elsewhere on this continent (Steward et al. 1984). Also, unlike the lacustrine environments, the estuarine environment presents a salinity barrier to species that have a low salinity tolerance. Thus, a study of the competitive potential of this biotype under salinity and nutrient conditions found in upper Chesapeake Bay and of the effects of *Hydrilla* on its environment was warranted in order to predict its invasive potential in Chesapeake Bay.

Several aspects of the competitive nature of *Hydrilla* were the focus of this research, especially its growth strategy and productivity in comparison to other SAV species found in Chesapeake Bay. Also, the high rates of production associated with *Hydrilla* beds reported in other studies coupled with preliminary findings of high pH in Chesapeake Bay *Hydrilla* beds led to speculation that *Hydrilla* may play an important role in local nutrient dynamics through uptake, sequestering, and release. These concerns were addressed through field investigations carried out near Havre de Grace, Maryland, and a decomposition experiment at the Horn Point Laboratory. Finally, the potential for *Hydrilla* to expand its range beyond the freshwater areas of the Bay was addressed in a salinity tolerance experiment at Horn Point.

METHODS

Mesocosm Studies

An experiment designed to examine biomass production of *Hydrilla, Vallisneria americana, and Potamogeton perfoliatus* under ambient and low-light conditions at a range of salinities typical of the upper estuary was conducted outdoors, in paired, temperature-ontrolled, recirculating, 1 m^3 fiberglass tanks. The plants were grown in a 10 cm sediment layer at a temperature of approximately 28½C under 90% and 10% ambient light at salinities of 0, 2 , and 4 ppt. After 12 weeks, total above-ground (AG) and below-ground(BG) biomass, the number of lateral branches (a measure of spreading), and stem height (a measure of canopy formation) were determined for each species.

The release of nutrients sequestered in *Hydrilla* biomass during decomposition was examined under aerated and nonaerated conditions in dark environmental chambers maintained at 15½C. Aproximately 10 g (fresh weight) Live *Hydrilla* shoots were placed in litter bags constructed of fiberglass screening. The bags were held in glass vessels containing 3.45 l ambient Potomac River water and 2 cc of sieved, homogenized sediment (used as an innoculant of decomposing organisms). Wet weight of the litter bags, water column nutrients (nitrate, nitrite, ammonium, phosphate, total nitrogen, and total phosphorus), and particulate and plant nutrients (carbon, nitrogen, and phosphorus) were determined periodically over 18 weeks. Aqueous nutrient samples were analyzed on a Technicon Auto Analyzer II; particulate samples were analyzed for carbon and nitrogen on a Control Equipment CHN Analyzer, while phosphorus concentrations were determined on a Technicon Auto Analyzer II following acid digestion.

Field Studies

The primary field site was a dense SAV bed consisting of *Hydrilla, V americana, Ceratophyllum demersum* and *M spicatum* located at the mouth of the Susquehanna River on the north shore of Havre de Grace, Maryland. Three permanent 50 m transects established perpendicular to the shoreline at this site were used to monitor changes in the percentage of cover of each species over the 1985 and 1986 growing seasons. Data were collected bimonthly from July to November 1985 and monthly from June to October 1986.

During each visit, temperature and pH (Beckman model 31 pH meter) were recorded at several undisturbed points along the middle transect through the center of the bed. Filtered water samples collected along this transect were immediately refrigerated for later analysis of dissolved nutrients (Technicon Auto Analyzer II). In addition unfiltered samples were collected for determination of total suspended solids, alkalinity (modified Gran titration), and conductance (YSI Model 34 Conductance-Resistance Meter). During the 1986 growing season, water samples were also collected every 2 hours over a 24-hour period at two locations (one within, one outside the bed) with ISCO model 2700 automatic samplers. Dissolved oxygen, pH, temperature, and conductance were simultaneously measured within the bed with a Hydrolab Surveyor II.

Triplicate or quadruplicate (AG) plant biomass samples were collected in September 1985 and monthly from June to October 1986 using a 0.25 m^2

frame at three locations at average depths of 0.5, 1.0 and 1.5 m. BG biomass was sampled with a 9 cm plexiglas core. All biomass samples were placed in nylon mesh bags, rinsed free of sediment on site, and dried in a forced draft oven at 60½C. For the purpose of comparison, AG and BG biomass samples were also collected in August 1986 at a site southeast of Havre de Grace consisting of a very robust *V. americana* population with little *Hydrilla*.

Net production of *Hydrilla*, *Vallisneria*, and *Myriophyllum* at ambient pH (pH 9.10) was determined under a range of light levels by measuring changes in dissolved oxygen concentrations produced by plant sprigs in duplicate 300 m*l* BOD bottles as described in Twilley et al. (1985). Incubations were carried out in situ, with shading provided by neutral-density nylon screening. The ability to use bicarbonate as a carbon source at high pH (pH 9.18) was tested using the same technique for measuring production, with the addition of bicarbonate ($NaHCO_3$) directly to the BOD bottles. Triplicate bottles were incubated for each species at each treatment level.

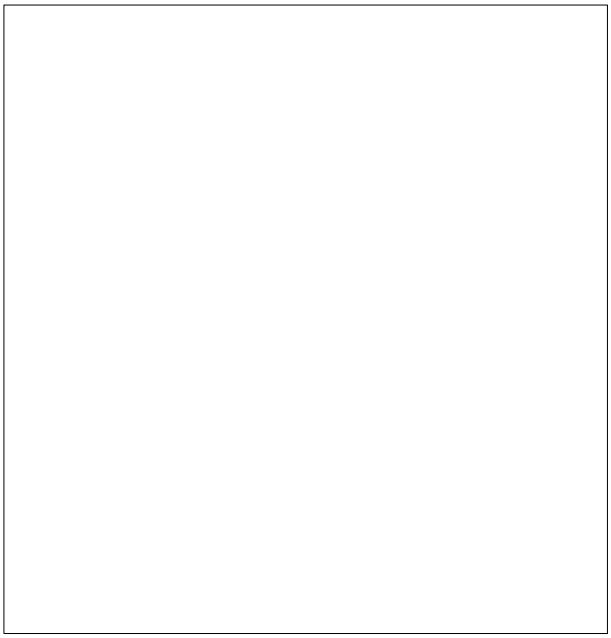

Figure 1. Biomass accumulation through 1986 growing season in the *Hydrilla* bed at Havre de Grace, MD. Bar height equals mean total biomass; error bar equals one standard error; shaded portion shows mean *Hydrilla* biomass.

DISCUSSION

Observations of the growth habits of *Hydrilla* in Chesapeake Bay indicate that it behaves similarly to other biotypes found elsewhere (Pieterse 1981), although the growing season is highly compressed. Lateral stems emerge from tubers in June and spread out along the sediment surface, rooting at each node. While the terminal ends of the lateral branches continue to grow horizontally, secondary branches originating at the nodes of the lateral branches begin growing vertically, elongating toward the water surface. Branching of the lateral stems allows rapid areal coverage, while emergence of vertical stems begins the process of canopy formation. By the end of September *Hydrilla* has virtually filled the water column with biomass.

In fresh water, *Hydrilla* appears to be an efficient competitor with both native and other introduced species. Biomass data along three permanent transects in the *Hydrilla* bed during 1985 and 1986 indicate that, although Hydrilla is slower to begin growth early in the season, rapid growth in midsummer allows it to overtake other species (figure 1). Suppression of *Hydrilla* tuber germination or growth by low temperatures (< 20 ½C) may give other species a head start; however, with a maximum productivity rate (15 mg O_2 gdw^{-1} h^{-1}) exceeding those of the other species present (figure 2a), *Hydrilla* quickly caught up at our research site. Maximum biomass ranged from 457 gdw m^{-2} in 1986 to 710 gdw m^{-2} in 1985. *Hydrilla* (158.8 gdw m^2) was a significant component of biomass samples from the landward edge of the *Vallisneria* bed in 1986, far exceeding *Vallisneria* (12.5 gdw m^2) on a weight basis in the shallows (< 5 m depth). Toward the offshore side of both beds, however, other species continued to dominate, suggesting that although *Hydrilla* is an efficient competitor in terms of photosynthesis, its fragile stems render it less tolerant of turbulence than the straplike leaves of *Vallisneria* or the relatively strong, elastic stems of *Myriophyllum*. Hence SAV located in protected embayments appears to be most susceptible to displacement by *Hydrilla*.

The development of dense vegetation has numerous implications for the SAV community. Initially, the reduction in light availability may be the primary factor affecting other species of SAV, especially those with a basal growth form like *Vallisneria*. However, as the season progresses, daytime increases in pH associated with productivity in the *Hydrilla* bed (figure 3) reduce the gaseous CO_2 concentration to very low levels, inhibiting productivity in species unable to use

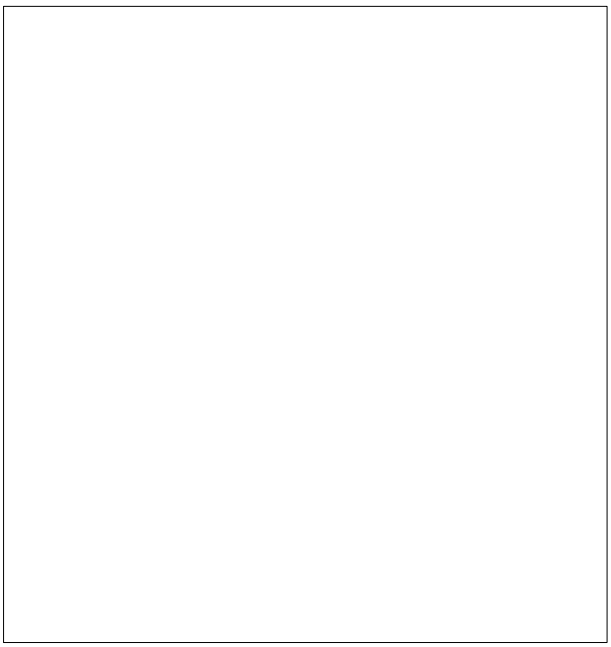

Figure 2. Net productivity of *Hydrilla, Vallisneria,* and *Myriophyllum* from the *Hydrilla* bed. (a): mean net productivity (n = 2) versus light intensity; (b): mean net productivity (n = 3) versus $NaHCO_3$ concentration at pH 9.18 (error bar equals 2 standard errors).

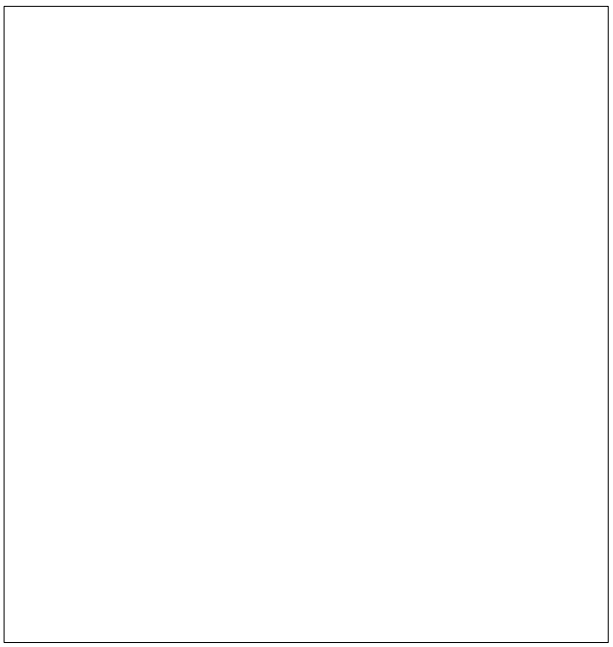

Figure 3. Dissolved oxygen concentration and pH over a diel period in the *Hydrilla* bed on 12-13 August 1986.

bicarbonate as efficiently as a source of carbon for photosynthesis (Stevensonand Comfer 1978). Increases in production in response to bicarbonate additions to ambient water with pH 9.18 (figure 2b) indicate that *Hydrilla* is better able to cope with the CO_2 depletion resulting from high productivity than other species present at this site (ie., *Vallisneria, Myriophyllum,* and *Ceratophyllum*).

The demand for nutrients created by standing stock of the magnitude found in this *Hydrilla* bed creates a distinct pattern of dissolved nutrient depletion toward the center of the bed, coinciding with the region of high pH. Studies in the Potomac River (Carter et al. 1988, Stevenson et al. 1989) show that this zone of high pH, carbon and nutrient-depleted water extends down about 0.5 m or less and is heavily influenced by tidal action. In the nitrogen enriched environments where *Hydrilla* is currently found in upper Chesapeake Bay, nitrogen availability is high (> 50 µM) and never becomes a factor in either interspecific competition or in competition between rooted aquatic plants and algae. However, typical of many freshwater areas of the Bay, water column phosphorus concentrations in the Susquehanna Flats *Hydrilla* bed were generally low (< 0.5 µM). Yet, tissue phosphorus concentrations of the species growing here (table 1) are high compared to the critical level (1.3 mg g^{-1}) established by Gerloff and Krombholz (1966) for *Vallisneria* (with close agreement among several other species), indicating that uptake from the sediment or from the water column during input pulses is meeting growth requirements.

On a seasonal basis an extensive *Hydrilla* bed can have a significant impact on the local nutrient regime, acting as an important sink. As standing stock increases throughout the growing season, nitrogen and phosphorus are sequestered in plant tissue. While some nutrients remain stored in underground tubers until the following spring, much is returned to the water column upon senescence in the fall. Plant storage may not represent a large component of the overall nutrient budget of Susquehanna Flats, where the area occupied by *Hydrilla* is limited and water column nitrogen concentrations are high (50-100 µM). However, Stevenson et al. (1989) estimate that in the Potomac River in 1985, when SAV (primarily *Hydrilla*) covered 1,673 hectares, approximately 2.5% and 40% of annual point -source inputs of nitrogen and phosphorus, respectively, were stored in SAV biomass during the summer months.

Table 1. Mineral nutrient content (mg gdw^{-1}±SE) of *Hydrilla* and *Myriophyllum* shoots collected on 4 September 1985 from the *Hydrilla* bed in Havre de Grace, Maryland.

Genus	Depth (m)	N	P	K	Ca	Mg	N:P[1]
		(mg gdw^{-1})					
Hydrilla	0.5	19.7 ± 0.3	3.6 ± 0.1	25.7 ± 0.3	74.0 ± 1.0	8.0 ± 0.5	13.00
	1.5	30.7 ± 0.3	4.2 ± 0.1	40.0 ± 0.7	38.0 ± 2.5	5.7 ± 0.2	17.00
Myriophyllum	1.0	20.3 ± 1.2	3.6 ± 0.1	33.0 ± 1.5	120.0 ± 6.2	8.2 ± 0.4	14.27
	1.5	27.7 ± 0.3	3.2 ± 0.1	15.7 ± 0.3	52.7 ± 2.3	7.5 ± 0.3	20.00

[1]Adjusted for atomic weights.

Figure 4. Nitrogen and phosphorus release from decomposing *Hydrilla* under aerated and nonaerated conditions.

The release of nitrogen and phosphorus from senescent aquatic plant biomass can occur quickly in autumn, on the order of days to weeks. The form in which it is released is dependent upon oxygen availability, with dissolved forms predominating under anaerobic or hypoxic conditions (figure 4). Nitrogen loss, presumably via denitrification, occurs under hypoxic conditions (figure 4) where anaerobic microzones can support denitifiying organisms. Similar losses may occur in the field when the dense standing stock that accrues in *Hydrilla* beds during the growing season turns into floating rafts of decomposing plant material in late October. Although well-oxygenated water may surround the rafts, oxygen depletion within them may result in hypoxic conditions where dissolved forms of nitrogen and phosphorus are released and denitrification occurs. In either case, the timing of the release in the fall after water temperatures drop and the growing season is over means that the potential for burial or transport out of the system is greater, uptake by algae is less. Thus, *Hydrilla* may serve a useful function in helping to suppress summer algal blooms and improving water quality.

Hydrilla also provides protection for small fauna seeking food and shelter from predators by creating structural complexity in the water column (Serafy et al. 198, Killgore et al. 1989). However, the effectiveness of an SAV bed as a habitat for fish is density related (Colle and Shireman 1980), with very high biomass density impairing the movement of fish through the bed. *Hydrilla*, therefore, may not be as desirable as other SAV species. While few species actually reside within the dense biomass, many species frequent the periphery of the beds or are found underneath or above (at high tide) the dense canopy (Killgore et al. 1989). In addition, Serafy et al. (1988) found that midday numbers of fish were lower in the *Hydrilla* bed than in the nearby *Vallisneria* bed at our study sites, possibly as a result of high pH.

The intolerance of *Hydrilla* to even small increases in salinity is evident in the marked reduction in biomass accumulated at 2 and 4 ppt salinity compared to that produced in fresh water in the mesocosm study (table 2). *Perfoliatus* and *V. americana*, both native to the estuary, show greater tolerance to increased salinity, with the former exhibiting little effect at all. Thus *Hydrilla* does not compete effectively with species that are adapted to a large degree to the variable salinity regime found in tributaries of Chesapeake Bay, and is limited by saltwater incursions during dry years. Evidence of such limitation has been observed in the Potomac River (Orth et al. 1987). Furthermore, Carter et al. (1987) report reductions in tuber and propagule germination at salinities > 3 ppt.

CONCLUSION

Although *Hydrilla* is an aggressive species that was initially viewed with disdain, it has not overtaken the native SAV in brackish environments of Chesapeake Bay. Despite very high plant densities in the tidal freshwater regions of the Potomac and Susquehanna Rivers, it provides significant habitat for fish, provides food for migratory waterfowl, and sequesters nutrients during the summer. The extent to which *Hydrilla* will outcompete native SAV in the long term will depend upon natural biological factors such as disease and grazing pressure, which function via negative feedback to bring "rogue" species into balance in ecosystems. As such, the *Hydrilla* "invasion" appears to be like the *Myriophyllum* outbreak in the late 1950s. Although at first both were candidates for eradication, we now view both as part of the ecologically valuable SAV compliment in the Bay, although less desirable than their native counterparts.

Table 2. Above-ground biomass per tank (gdw ± SE) of three species of SAV after 12 weeks of growth. Light is expressed as percentage of ambient.

Salinity	2 ppt		4 ppt		6 ppt	
Light	90%	10%	90%	10%	90%	10%
Potamogeton	20.4 ± 3.0	11.7 ± 0.8	20.0 ± 1.2	12.1 ± 2.8	18.7 ± 4.5	8.7 ± 1.1
Vallisneria	10.0 ± 7.2	4.6 ± 0.1	4.1 ± 0.2	4.0 ± 0.5	5.1 ±0 .1	4.0 ± 1.8
Hydrilla	12.7 ± 7.37.1 ± 0.4	1.0 ± 0.2	0.7 ± 0.7	0.8 ± 0.7	0.2 ± 0.0	

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TEMPERATURE/SALINITY TOLERANCE IN LARVAE OF ZEBRA MUSSELS AND THEIR POTENTIAL IMPACT IN BRACKISH WATER ESTUARIES

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Abstract: We have investigated the temperature/salinity tolerance of larvae of the exotic zebra mussel *Dreissena polymorpha* using a temperature/salinity matrix that encompasses late spring and early summer conditions in Chesapeake Bay and its tidal tributaries. We determined growth and survival of larvae from 72hr posthatch (D-hinge stage) through 11 days posthatch at temperatures of 18½C, 22½C, and 26½C and salinities of 0, 2, 4, and 8 ‰. Preliminary results indicate that after 48 hrs at temperatures of 18½C and 22½C, survival of zebra mussel larvae ranged from 65% to 87% and was not significantly different from that of controls. At 26½C however, survival of larvae was only 1.4% at 8‰. After 8 days, control survival at 18½C and 22½C dropped to 48% and 42%; survival of larvae ranged from 20% at 18½C and 4‰ to 0.2% at 18½C and 8‰. Survival of larvae at 26½C was much lower, ranging from 13% at 2‰ to 0% at 4 ‰ and 8‰. Preliminary results from ion manipulation experiments indicate good survival of larvae after 48 hrs at both high Ca^{++} and high K^{+} (2.3 mM each) and low Ca^{++} and high K^{+} (0.2 mM and 2.3 mM, respectively) and lesser survival at high Ca^{++} and intermediate and low K^{+} concentrations. Our preliminary results and a review of zebra mussel habitat in Europe and elsewhere suggest that the potential for zebra mussel colonization in brackish water estuaries is probably limited to waters with salinities of less than 3-4 ‰.

INTRODUCTION

Since its discovery in Lake St. Clair in the summer 1988 and subsequent colonization of large portions of the Laurentian Great Lakes, the anticipated southward spread of the nonindigenous zebra mussel, *(Dreissena polymorpha)*, into the major rivers of the United States has proceeded rapidly. Zebra mussels are now found in eight major river systems: the St. Lawrence, Hudson, Mississippi, Ohio, Illinois, Tennessee, and Susquehanna, Arkansas (Ludyanskiy et al. 1993). Although measures taken to curtail its spread will undoubtedly be locally effective and will slow its advance in several areas, the ultimate distribution of the species will depend largely upon climatic and water chemistry characteristics. Information on these boundary conditions can be either obtained experimentally or may be inferred from the species' distribution in other parts of the world.

Temperature

In Europe, *D. polymorpha* extends as far west as the British Isles in a band that reaches as far north as southern Sweden and to the northern shores of the Mediterranean in the south. It has not colonized southern Italy or the Iberian Peninsula. Based upon temperature considerations alone, its European distribution would imply a tolerance range of about 0 - 30½C. For fertilization, however, the temperature must be > 12½C (Neumann et al. 1993 Sprung 1993) and spawning will occur only at water temperature > 12-16½C (Nichols 1993). The reproductive cycle and early larval survival probably represent the major temperature limiting steps in the life cycle. For example, egg viability between $2^{1}/_{2}$ and $4^{3}/_{4}$ hr is seen between 12 - 24½C, although sperm mobility at these temperatures may be up to 22 hr (Sprung 1993).

Temperature tolerance of the veliger larvae remains uncertain, although field studies from the eastern European literature also suggest an upper temperature tolerance limit of 24½C (Shevtsova 1968, Walz 1975). The current southern extension of the mussel's North American range into parts of Arkansas and Alabama suggests that *D. polymorpha* may be capable of tolerating even higher maximum summer temperatures. McMahon et al. (1993) and Strayer (1991) concluded that zebra mussels would be unlikely to colonize parts of the southern United States where water temperatures exceed 30½C during the summer months. Recent temperature tolerance studies conducted in the laboratory have demonstrated adult survival above 30½C over limited time periods (Iwanyzki and McCauley 1993, McMahon et al. 1993), although experiments were short-term. In a field situation, it is likely that species survival may have as much to do with the timing and longevity of periods of higher water temperatures as with maximum summer temperature. Water temperatures at the outset of spawning, fertilization, and larval dispersal in particular are likely to be critical.

Salinity

The toleration of salinities > 10‰ by *D. polymorpha* in the Caspian and Aral Seas compared with other habitats in Europe has been attributed to the higher calcium and sulphate levels in these waters (Mordukhai-Boltovskoi 1960). Caspian Sea water has calcium and sulphate concentration that are respectively 31 and 45 times their respective levels in eastern Atlantic waters supporting zebra mussel populations (Strayer and Smith 1993). Higher salinity tolerance may be apparent in areas subject to little short-term fluctuation in salinity (Reshöft 1961, Wolff 1969). Based upon European data, Barber (1991) hypothesized some future colonization of Chesapeake Bay.

Because of such concerns, we began our 1993 study of temperature/salinity tolerance in *D. polymorpha* veliger larvae reared in the laboratory. Strayer and Smith (1993) hypothesized that the likely seaward limit of *D. polymorpha* in North American estuaries will be 0.4-2 ‰ (currently the Hudson River distribution suggests ca. 2‰) with a possible 6‰ limit in more chemically stable estuaries such as nontidal lagoons. owing to its size and semi-enclosed nature, Chesapeake Bay maintains a high degree of chemical stability with a salinity profile influenced more by seasonal run-off than by diurnal tides.

Experimental Rationale

Models relating zebra mussel distribution to environmental variables such as salinity and temperature tend to concentrate on extremes, that is, the maximum or minimum value of a particular variable that is compatible with survival. However, it is likely that the most sensitive life stage of the species, the larvae, may impose more subtle constraints on its ultimate distribution. For example, salinity and temperature variations encountered during the spawning season may represent more realistic limiting factors than the more extreme values reached later in the year. Accordingly, it is most important to ascertain the effects of environmental variables on survival of early life stages.

Based on the projections and comments of Strayer and Smith (1993) and the relative stability of the Chesapeake Bay salinity profile, we tested larval survival between 0‰ and 8‰ at temperatures of 18½C, 22½C, and 26½C. Experimental temperatures and salinities were suggested by summer temperature and salinity profiles of the Bay (Seitz 1971). Here we report preliminary results from our first summer (1993) of investigations on salinity/ temperature tolerance of zebra mussel larvae. This research continued in our laboratory during 1994.

METHODS

Culture

Adult broodstock were collected from Put-In-Bay, Lake Erie, (Ohio) and were transported to Maryland in sealed, labeled containers according to protocols required by the Maryland Department of Natural Resources governing their transport, handling, and disposal. (Protocols are available from the authors upon request).

Adult mussels were held in culture water made by adding 8.18 g $CaCl_2$, 10.23 g $MgSO_4$, 20.2 g $NaHCO_3$, and 0.9 g $MgCl_2$ to 114l of Chesapeake Biological Laboratory well water, at either 8½C or 17½C according to the spawning regime to be adopted. Culture water contained ³ 0.42 mM Ca^{++}, 2.2 mM Na^{+}, and 0.45 mM Mg^{++}. Mussels used in the 26½C experiments were held in culture water made by using Sprung's recipe (Sprung 1987). Mussels were fed daily 1 g dried *Chlorella* sp. for every 5,000 individuals of 0.75-2 cm length, added to 500 ml of culture water and blended for 10 min prior to adding to the holding tanks.

Spawning

Approximately 25 adults were cleaned of debris and transferred to 1,500 ml glass beakers with 800 ml of 17½C

culture water. The temperature was raised to 30-32½C by the addition of warm (50-60½C) culture water. If no spawning occurred within 30 min, a slurry made from ripe gonads homogenized in culture water sufficient to produce a slightly cloudy suspension was added. Every 45 min after spawning began, the water in the beakers was gently poured into a holding container and replaced with fresh culture water at 30-32½C. Spawning continued for 2-4 hrs although not all individuals spawned during that time.

Counting

Larvae were filtered through a 33 µm nonmetallic sieve and concentrated in a 100 ml beaker. After mixing with a plastic stirrer, three 1ml aliquots were counted using a Sedwick-Rafter counting chamber. We differentiated among live, recently dead, and long dead larvae in the 18½C and 22½C experiments. Larvae at 26½C were fixed in 10% buffered formalin and counted later. Live larvae swam actively or their velum was beating. Recently dead larvae were defined as those that had not yet decayed and did not move. Long-dead larvae were distinguished by the presence of only the two empty valves of the D-hinge stage.

Salinity Tolerance Tests

Tests were undertaken at 18½C, 22½C, and 26½C in plastic bags containing 5l of culture water at each test salinity: 0 ‰ culture water, 2‰, 4‰, and 8‰, with triplicate bags for each different temperature/salinity combination. Filtered (1 µm) Patuxent River water was diluted with culture water to obtain experimental salinities. Three day-old larvae (D-hinge stage) were concentrated, counted, and 5,000 larvae added to each bag (1 larva/ml) with an Eppendorf hand-held pipetter.

Four ml of 0.21M Streptomycin sulfate solution was added to each bag to reduce bacterial contamination. Larvae were fed dried *Chlorella* sp. blended in culture water daily at a concentration of 4.5×10^4 cells/ml.

Larval survival was assessed after 48 and 192 hrs. Each bag was gently poured through a 32 µm sieve and the contents of the sieve diluted to 50 ml. Larvae were counted in triplicated 1 ml samples (with replacement in the concentrated sample after each count) and then returned to the bags with new culture water of appropriate salinity. Half of the culture water in each bag was replaced three times a week. Salinity, pH, and ammonia concentration were determined for each bag when the water was changed, and 2.0 ml. Streptomycin were added at each water change.

Ion Manipulation Assay

Experimental culture water was made by adding 107.5 mM NaCl, 6.4 mM $MgSO_4$, 5.81 mM $MgCl_2$, and 0.54 $NaHCO_3$ to deionized water resulting in Na^+, Mg^{++}, and Cl^- concentrations equivalent to 8 ‰ salinity. Calcium and potassium concentrations were manipulated against this background salinity as follows. Treatment no.1 contained "high" Ca^{++}, 2.29 mM Ca^{++} and "high" K^+, 2.29 mM K^+; treatment no.2 contained 2.29 mM Ca^{++} and "mid" K^+, 0.6 mM K^+; treatment no.3 contained 2.29 mM Ca^{++} and "low" K^+, 0.2 mM K^+; and treatment no.4 contained "low" Ca^{++}, 0.2 mM Ca^{++} and 2.29 mM K^+. Larval survival was assessed after 48 and 192 hrs. Methods were the same as for the salinity tolerance tests.

DISCUSSION

Temperature/Salinity Tolerance

Survival of D-hinge stage larvae after 48 hrs at 18½C was greater at 4‰ than at 8‰, although there were no differences in percentage survival between the control mussels and those at 2‰, 4‰, and 8‰ (table 1). At 22½C, survival at 48 hrs at 4‰ and 8‰ was significantly less than at 0‰ and 2‰. At 26½C, survival of zebra mussel larvae was only 1.4% at 8 ‰.

After 192 hrs, survival of control larvae dropped to 48% and 42% at 18½C and 22½C respectively, and after 168 hrs was only 6% at 26½C. We were unable to rear larvae to metamorphosis under experimental conditions and feel the lack of adequate nutrition (algae were not observed in the guts of the larvae) compromised the interpretation of our results after 168 and 192 hrs. However the additional stress of salinity is evident as survival at 18½C ranged from 21% at 4‰ to 0.1% at 8‰ (table 1), and survival at 22½C decreased from 42% in the controls to between 3% at 8‰ and 6% at 4‰. The compounding stresses of lack of food and elevated temperature are evident at 26½C; control survival was only 6% and survival at 2‰ was 13%. No larvae survived to 168 hrs in either 4‰ or 8‰. The greater percentage survival at 18½C after 192 hrs at 4‰ versus 2‰ (21% versus 2%), may have resulted from the presence of various ciliated protozoans that were abundant at 2‰, but were absent from higher salinities. The effects of these protozoans on the veliger larvae can only be speculated, although we saw no sign of active predation. Competition for food is likely.

We stress these are preliminary results. We continued this work during 1994, using more appropriate algal diets, feeding at higher algal densities, and

Table 1. Survival of D-hing stage zebra mussel larvae in 0‰, 2‰, 4‰, and 8 ‰ at 18½C, 22½C, and 26½C after 48 and 192 hrs. Table presents mean percentage survival ± two standard errors of the mean; n = 3.

Percent Survival-48 Hours

Temperature	0 ‰	2 ‰	4 ‰	8 ‰
18½C	72.0 ± 13.0	83.6 ± 11.0	86.6 ± 3.3	70.1 ± 3.8
22½C	78.2 ± 1.2	83.6 ± 5.0	70.6 ± 4.5	65.1 ± 2.5
26½C	88.3 ± 3.8	83.0 ± 5.2	64.7[1] (n=2)	1.4 ± 1.3

Percent Survival - 192 Hours

	0 ‰	2 ‰	4 ‰	8 ‰
18½C	47.9 ± 3.1	2.0 ± 2.8	21.0 ± 10.2	0.1
22½C	42.4[2] (n=1)	4.6 ± 2.7	5.8 ± 4.7	2.7 ± 0.6
26½C[3]	6.4 ± 4.3	12.6 ± 12.8	0.0	0.0

[1] Sample spilled.
[2] Two samples with much protozoan and bacterial contamination. It was impossible to accurately count sample.
[3] Percent survival after 168 hrs.

acclimating larvae to both changes in temperature and salinity. These results will be reported later.

Ion Manipulation Assays

Although there were no significant differences in survival after 48 hrs in the high Ca^{++}, high K^{+}; high Ca^{++}, mid K^{+}; and low Ca^{++}, high K^{+} treatments (table 2), there was less variance in survival among the three replicates in the high Ca^{++}, high K^{+} treatment. Survival was significantly less in the high Ca^{++}, low K^{+} treatment. Some larvae in both the mid (0.6 mM K^{+}) and low (0.2 mM K^{+}) cultures were "shell-less," although alive, or although still attached to the shell, were abnormally protruding from the shell. Many of the larvae in these two experiments that were classified as "freshly dead" (larvae that were not yet decayed, but did not move or possess beating cilia) also were "shell-less." No larvae were alive after 192 hrs in any of these ion manipulation assays.

We anticipated decreased survival in calcium-deficient media in light of published minimal calcium requirements for this species (Sprung 1987, Ramcharan et al. 1992). Although our preliminary results after 48 hrs suggest no differences in percentage survival, there is little overlap in the mean percentage survival ± 2 standard errors in the high Ca^{++}, high K^{+} treatment (94.0%; 91.0 - 97.0) and the low Ca^{++}, high K^{+} treatment (83.2%; 75.0 - 91.4). Future investigations will examine survival in the intermediate time periods between 48 and 192 hrs.

The decreased survival in potassium-deficient media was surprising since K^{+} ions are toxic in freshwater environments (Bailey et al. 1993). We feel that ionic ratios e.g. Ca^{++}: Na^{+}, or K^{+} : Na^{+} probably play a role in determining survivability, in addition to their absolute concentration. This topic also will be an area of future investigations.

Potential Impact in Chesapeake Bay and Other Temperate Estuaries

A review of current literature indicates that the primary environmental requirements of zebra mussels are salinities of 0-4‰, average summer water temperatures of 17½-23½C, pH level of 7.4 - 9.0, calcium concentration of 20-125 ppm, water clarity of

Table 2. Survival of D-hing stage zebra mussel larvae at 48 hrs in culture with Na^+, Mg^{++}, and Cl^- concentrations equivalent to 8 ‰ salinity and containing varying concentrations of Ca^{++} and K^+. Table presents mean percentage survival ± two standard errors of the mean; n = 3. Test conducted at 22½C.

Ca^{++} Concentration	K^+ Concentration	Percent Survival
2.3 mM	2.3 mM	94.0 ± 3.0
2.3 mM	0.6 mM	75.4 ± 17.8
2.3 mM	0.2 mM	37.1 ± 5.5
0.2 mM	2.3 mM	83.2 ± 8.2

40-200 cm (measured by Secchi disk) and dissolved oxygen concentrations of 8-10 ppm (Ludyanskiy et al. 1993). Both salinity regimes and short term variations in these salinities seem to be important in determining which habitats may be colonized by zebra mussels (Strayer and Smith 1993). In the Netherlands, zebra mussels are apparently restricted to waters with salinities less than 0.4‰ to 2.0‰ in areas with considerable short-term variation in salinity owing to either tidal fluctuations or freshwater input whereas they are found in salinities of 1-4‰ in nontidal habitats. Zebra mussels have colonized the Hudson River downstream to West Haverstraw Bay, an area with salinities of 2-3 ‰. In more chemically stable estuaries and nontidal lagoons, zebra mussels may be found in salinities up to 6‰. Zebra mussels are found in the northern part of the Caspian Sea at 6-9 ‰, and prior to the recent dramatic increases in salinities in the Aral Sea, were found up to 10.2‰. Strayer and Smith (1993) have hypothesized that zebra mussels will tolerate salinities up to 10-14 ‰ in brackish lakes and other sulfate-rich waters i.e., the brackish lakes of the Dakotas and the Canadian Prairie Provinces.

Based on our preliminary results, we suggest that if zebra mussels invade Chesapeake Bay, they will be restricted to salinities < 3-4‰. However, zebra mussels have demonstrated their ability to quickly colonize many habitats, and the plasticity of the gene pool of mussels that have recently invaded North American waters remains unknown.

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Toward a Sustainable Coastal Watershed:
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FIELD EXPOSURE OF TRIPLOID *CRASSOSTREA GIGAS* TO *HAPLOSPORIDIUM NELSONI* (MSX) AND *PERKINSUS MARINUS* (DERMO) IN THE LOWER CHESAPEAKE BAY

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Abstract: To determine the disease susceptibility of the Pacific oyster, *(Crassostrea gigas)*, under natural conditions in the lower Chesapeake Bay, 200 individually–typed triploid individuals from Rutgers University were placed in mesh bags in four replicate trays in the lower York River at theVirginia Institute of Marine Science (VIMS). Control oysters consisted of 200 diploid *C. virginica* from the upper Rappahannock River, Virginia, and 200 diploid *C. virginica* from the Wye River, Maryland, placed in separate mesh bags in the same trays as the *C. gigas*. Mean shell height of the *C. gigas* and Maryland controls was approximately 45 mm; Virginia controls were slightly larger. All oysters were deployed on 29 June 1993. Salinity averaged 20±2 ppt. and temperature was greater than 25°C during the sampling period. Samples of 25 oysters from each group were removed for disease diagnoses on 10 August, 8 September, and 14 October. Maximum prevalence of *H. nelsoni* was 84% in the Virginia controls and 92% in the Maryland controls, with a high proportion of heavy and moderate infection intensities; no *C. gigas* was infected with *H. nelsoni*. Maximum prevalence of *P. marinus* was 96% in the Virginia controls, 100% in the Maryland controls, and 24% in the *C. gigas*. A high proportion of heavy and moderate infection intensities occurred in both control groups, but all *P. marinus* infections in *C. gigas* were low intensity. Mortality was greater than 90% in both control groups by 1 November; mortality was 25% in *C. gigas* and was not attributable to disease. Shells of *C. gigas* were heavily infested with the polychaete *Polydora* sp. Results suggest that *C. gigas* of the size range tested are not susceptible to the major oyster diseases of Chesapeake Bay.

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ECOLOGICAL RISK ASSESMENT OF THE EFFECTS OF GRASS CARP ON SUBMERGED AQUATIC VEGETATION IN CHESAPEAKE BAY

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Abstract: Triploid grass carp, *Ctenopharyngodon idella*, are stocked in impoundments within the Chesapeake Bay drainage basin for control of nuisance aquatic vegetation. Some resource managers consider triploid grass carp a safe and effective alternative to chemical and mechanical control of nuisance vegetation; others conclude that grass carp pose an unacceptable risk to submerged aquatic vegetation (SAV) beds in Chesapeake Bay and its tributaries. This paper describes the rationale, methods, and preliminary results of an ongoing effort to quantitatively assess the risk that stocking of triploid grass carp in impoundments within the drainage basin poses to SAV restoration efforts in Chesapeake Bay and its tributaries. A key element of the risk posed by stocking of triploid grass carp is the incidence of diploid (reproductively competent) fish among fish certified as triploid by the U.S. Fish and Wildlife Service (FWS). An important factor controlling the incidence of diploidy in FWS-certified fish is the effectiveness of prescreening for diploidy by grass carp dealers. The triploid certification program has no direct control over the effectiveness of prescreening by grass carp dealers because the program requires retesting of only 120 fish per lot (unlimited lot size) in the presence of the FWS inspector. Preliminary results indicate that current FWS certification procedures may be inadequate to ensure that the incidence of diploidy in stocked fish is acceptably low.

INTRODUCTION AND DISCUSSION

Submerged aquatic vegetation (SAV) is an important component of the Chesapeake Bay ecosystem. Historically, SAV played a major role in maintaining water quality and providing food and habitat for fish and wildlife; however, the abundance of this important component of the ecosystem is severely reduced from historical values (Funderburk et al. 1991). The Chesapeake Bay Program (CBP) has long recognized the importance of SAV to the functioning of the Bay. Restoration of SAV beds in Chesapeake Bay and its tributaries is a focal point of the CBP.

Nuisance infestations of SAV occur in many impoundments within the Chesapeake Bay watershed, especially where exotic hydrilla (*Hydrilla verticillata*) and Eurasian watermilfoil (*Myriophyllum spicatum*) have become established. Chemicals and mechanical harvesters are often used to control nuisance vegetation; however, these approaches can be costly, and use of chemicals is problematic in some circumstances because of concerns about toxicity and carcinogenicity in nontarget organisms, including humans.

Triploid grass carp offer an inexpensive and effective alternative to chemical and mechanical control. The grass carp is a long-lived (15 years or more), voracious herbivore native to China and Russia (Chilton and Muoneke 1992). This exotic fish gained acceptance as a biological control agent following the development of methods for inducing triploidy that render grass carp functionally sterile. The U.S. Fish and Wildlife Service (FWS) has implemented a voluntary grass carp certification program that ensures a low incidence of diploidy among certified fish.

The acceptability of triploid grass carp as a tool for controlling nuisance aquatic vegetation is based on the premise that the chance of reproduction is virtually zero. The FWS certification program is designed to provide a high degree of confidence that reproduction will not occur; however, the effectiveness of prescreening for diploids by grass carp dealers is a potentially important component of the certification process over which the FWS exerts no direct control. The standards of the triploid grass carp inspection program are as follows (Griffin and Mitchell 1992):

1. Producers must have a fully operational particle sizer (such as a Coulter counter with channelyzer) and trained personnel available for the FWS inspector's use in performing the inspection. All grass carp in an identified lot offered for sale are expected to have been individually tested by Coulter counter techniques before a FWS triploid grass carp inspection will be performed. This is not a requirement, but to our knowledge no producer has yet risked offering an untested lot of fish for a FWS inspection.
2. The FWS inspection consists of a retesting, in the presence of the inspector, of 120 individuals randomly selected from the identified lot of alleged 100% triploid grass carp. (Note: The FWS certification program does not specify a maximum lot size.)
3. If a diploid is found in the course of testing of the 120-fish sample, all fish in that lot of fish are to be retested individually by the dealer before another inspection of that lot of fish is scheduled.
4. A three-working-day limit is imposed between the time of the FWS inspection of a lot of fish and arrival of fish from that lot into the state of destination. Beyond that time limit, fish must be reinspected, unless the receiving state makes special arrangements.
5. No additions can be made to an identified lot of fish after a FWS inspection has been done on that lot of fish.
6. Officials in states where fish are scheduled for delivery are notified by phone within 24 hours and told the number of fish involved in a shipment, the source of the fish, and the name of the dealer or hauler of the fish. Written documentation is then sent by mail.

Use of FWS certified fish substantially reduces the chance of successful reproduction and establishment of self-sustaining populations; however, the chance of reproduction is non-zero. The triploidy induction process is not 100% effective (McCarter 1988) and screening procedures are subject to human error. Furthermore, grass carp are capable of dispersing to nontarget waters, including Chesapeake Bay and its tributaries. Some resource managers within the Chesapeake Bay watershed are concerned that grass carp could escape from impoundments and wreak havoc on recovering SAV beds.

There are large differences among individuals and institutions in the perceived level of risk associated with stocking of triploid grass carp in impoundments. Quantitative analysis cannot eliminate all of the sources of uncertainty that contribute to conflicting perceptions of risk; however, quantitative analysis can provide a synthesis of available information. A quantitative analysis can provide an explicit representation of relevant knowledge and assumptions thereby permitting technical review. Furthermore, a quantitative risk assessment can reduce the scope of uncertainty, thereby facilitating consensus on this contentious issue.

Following is a description of the ecological risk assessment framework used.

Problem Formulation

The biology of the grass carp makes it a potent biological control agent, and contributes to the risk posed by stocking of triploid grass carp in the Chesapeake Bay drainage basin Important biological characteristics include (Chilton and Muoneke 1992):

- Life span: up to 20+ years
- Weight: up to 45 kg
- Salinity tolerance: up to 17 ppt (feeding declines above 6 ppt)
- Diet: all aquatic vegetation (hydrilla is a preferred item)
- Daily consumption: >100% body weight per day
- Spawning requirements: similar to striped bass
- Dispersal: 33 km in four months (triploids), up to 500 km in 2 years (diploids)

Ecological Effects

When introduced to a system, grass carp can affect the abundance and distribution of the native aquatic plant species. Typically, grass carp are introduced to a system in which a nonnative, nuisance species of SAV has become established. If grass carp prefer the introduced species as a food source, then grass carp can be initially beneficial at

restoring the natural SAV species composition. Once preferred species such as *Hydrilla* are removed, however, grass carp may begin targeting other species, including emergent and floating macrophytes (Fowler and Robson 1978, Baker et al. 1974, Thompson et al. 1988), which can result in decreased abundance and diversity of desirable native species. The magnitude of the ecological effects depends, among other factors, upon the seasonal abundance of various plant species and grass carp population densities.

Effects on fish community structure are somewhat predictable. Because SAV beds provide important habitat for certain species (centrarchids and cyprinodontids) and life stages of fish, removal of SAV affects their abundance and distribution. Baur et al. (1979) reported declines in bluegill and age 0 largemouth bass populations. Likewise, in Texas, an overall decrease in sunfish species accompanied SAV removal by grass carp, although first-year growth of largemouth bass increased, presumably owing to lower population densities (Klussmann et al. 1988). Aliyev (1976) reported population declines of pike (Esocidae), another group of fish normally found in close association with SAV. Fish not associated with vegetation, such as channel catfish, apparently are unaffected (at least directly) by vegetation removal. In Lake Marion, South Carolina, long-term electrofishing surveys document fish community changes from pelagic species to littoral species assemblages and back over a 6-year period during which grass carp altered SAV abundance and distribution (Foltz, Clemson University, pers. comm.).

Ecosystem Potentially at Risk

All portions of the Chesapeake Bay and its tributaries in which salinity during at least part of the SAV growing season is less than 6 ppt are potentially at risk. By way of example figure 1 shows the average surface salinity in the Potomac River during the month of May. Additional areas of the lower Potomac River could be affected if exposed to grazing by grass carp that was of sufficient frequency, duration, and intensity.

Endpoint Selection

Abundance of SAV is the natural endpoint for this assessment. SAV abundance is the measurement, assessment, and management endpoint for the Chesapeake Bay Program; consequently, it is meaningful to resource managers and the public. Change in SAV abundance can be assessed given available information on grass carp biology, estimates of grass carp abundance, and information on SAV production in the Bay.

Figure 1. Average salinities in the Potomac River during May. From Lippson et al. 1976.

Conceptual Model

Figure 2 depicts the conceptual model for this assessment. Brood stock are the ultimate source of grass carp in the system. Artificial propagation of grass carp is assumed to occur only outside of the Chesapeake Bay drainage basin. Legal grass carp are imported and stocked in impoundments following triploidy induction and certification by FWS. Illegal grass carp are assumed to be diploids. A small portion of stocked individuals escape and enter the pool of free-ranging fish. The pool of free-ranging fish consists of both triploid and diploid individuals, both of which undergo natural mortality. Free-ranging diploids can spawn, provided mates are available and located, and survivors of successful spawning events constitute recruitment of wild fish to the free-ranging population of grass carp. Free-ranging grass carp consume SAV, potentially altering the abundance of SAV in the Bay and its tributaries.

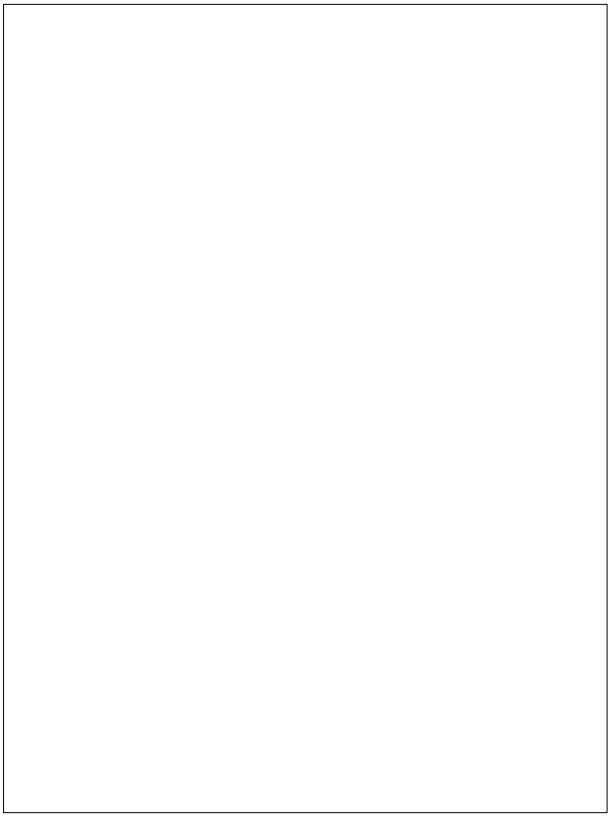

Figure 2. Conceptual model of the grass carp risk assessment.

Preliminary Analysis

A model of annual grass carp certification and stocking is depicted in figure 3. Annual survival of stocked grass carp was modeled as a binomial process. Stochastic projections of the potential number of diploids in the Potomac River basin in the absence of reproduction were made using the parameter values listed in table 1. The level of annual stocking represents an intensive stocking scenario in which 60% of the pond acreage in the Potomac River basin is stocked at an intermediate density of 12 fish per acre. Figure 4 depicts preliminary results of the simulations. Figure 4a depicts results from a representative sample of five model runs. Figure 4b summarizes the results of 1,000 model runs in each of 5 years. The model indicates that, in the absence of reproduction, the abundance of diploid grass carp in the basin reaches equilibrium within 25 years; there is a high probability (approximately 90%) of having 50 or more diploids in the basin at equilibrium.

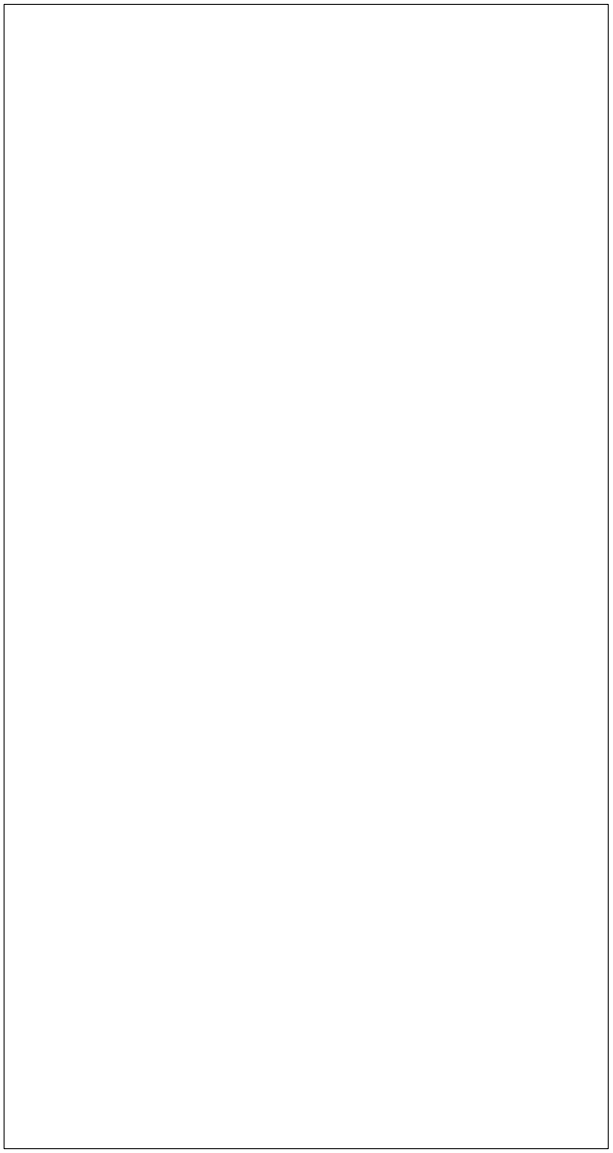

Figure 3. Model of annual triploid grass carp certification and stocking.

CONCLUSION

The USFWS triploid grass carp certification program is successful in ensuring a low incidence of diploids among triploid-certified fish; however, under an intensive stocking scenario within the Potomac River basin, use of triploid-certified grass carp may be insufficient to prevent natural reproduction in the river. Additional conditions may include effective containment in impoundments and inability of the diploids that escape from impoundments to locate mates in the river. A more extensive assessment of the risk posed by stocking of triploid certified grass carp in the Potomac River watershed is being prepared for the Chesapeake Bay Program

Table 1. Constants and parameter values used in the triploid certification and stocking model see (figure 3)

Constant/Parameter	Symbol	Value	Source
Lot size	n	1,500[1]	G. Looney, FWS, pers. comm.
Annual Stocking intensity. (m=number of lots/year)	n*m	25,500[2]	Back calculated from pond acreage and stocking
Annual Survival	s	0.8	Phil Kirk, Clemson Univ., pers. comm.
Triploidy Induction Efficiency	t	0.95	McCarter 1988
Dealer Screening Efficiency	E	0.99	Estimated from FWS data on inspection failure rate.

[1] Representative value used in model. Actual range 120 - 5,000.
[2] Stocking level required to achieve 12 fish/treated acre in approximately 60% of the total pond area in the Potomac River basin (total acreage in ponds ≥ acre in size = 18,200 acres) at equilibrium.

Figure 4. Projection of the total number of diploid grass carp in the Potomac River basin. 4a: five representative realizations of the stochastic model. 4b: Compliment distribution functions (survival functions) for five different model years.

and the U.S. Environmental Protection Agency's Office of Research and Development, Office of Environmental Processes and Effects Research. That analysis will examine the likelihood of establishing a self-sustaining population given alternative escape and reproductive success rates.

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TRANSGENIC FISH AND AQUACULTURE

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Abstract: A wide range of transgenic animal species, including fish, can be produced by transferring foreign DNA into developing embryos by microinjection or electroporation. This technology offers an excellent opportunity for modifying the genetic traits of commercially important finfish, shellfish and crustacean species for aquaculture. Studies conducted in our laboratory and others showed that administration of recombinant fish or mammalian growth hormone (GH) to juvenile fish or oysters resulted in a significant growth enhancement. These results point to the possibility of improving growth rates of finfish and shellfish by manipulating GH or its gene. In this paper, we review results of our own studies as well as those of many others to determine the efficacy of improving the growth rates of finfish, mollusks, and crustaceans by administering recombinant fish GH or producing fast-growing transgenic animals by means of the gene transfer technology.

INTRODUCTION AND DISCUSSION

The worldwide harvest of fishery products traditionally depends upon the natural population of finfish, shellfish, and crustaceans and macroalgae of both freshwater and marine sources. Owing to a rapid increase in consumption of fishery products as a consequence of population growth, as well as poor management and overfishing activity, the level of total worldwide annual harvest of fish products has already approached the maximal potential level of about 150 million metric tons as forecasted by the U.S. Department of Commerce and National Oceanic and Atmospheric Administration. In addition, accumulation of chemical pollutants in aquatic environments as a consequence of increasing industrial activities has detrimental effects. A number of regions have recently experienced a significant decline in the catches of important fish species such as salmon, striped bass, sturgeon, eel, jack, mullet, mackerel, kris, abalone, oysters, and crabs. Fishery fleets now travel great distances to exploit more productive areas. They have switched to alternative species, and have begun to employ a variety of sophisticated technologies. These recent developments have caused a significant increase in international fish prices.

In the past several decades, many countries have turned to aquaculture/mariculture for increasing production of fishery products. In 1985, for instance, the world production of finfish, crustaceans, shellfish, and macroalgae by aquaculture/mariculture reached 10.6 million metric tons, an amount equal to 12.3% of the worldwide tonnage of international fishery catches. Thus, aquaculture/mariculture has the potential to resolve the pressing problem of meeting the world demand for fishery products. Production of fish by intensive culture depends on: (1) complete control of the reproductive cycle of the fish species in culture; (2) excellent genetic background of the broodstock; (3) efficient detection and effective prevention of disease infection; (4) thorough understanding of the optimal physiological, environmental, and nutritional conditions for growth and development; (5) sufficient supply of good quality water; and (6) application of innovative management techniques. By improving some of these factors, the aquaculture industry has made impressive progress over the last several years. The application of molecular biology and biotechnology will further accelerate the expansion of this industry. These applications include enhancing

growth rates, controlling reproductive cycles, improving feed composition, producing new vaccines, and developing disease-resistant and hardier genetic stocks. Over the last several years, our laboratory and others have been searching for innovative strategies to increase fish production by applying the contemporary technologies of molecular biology and biotechnology. In this paper, we summarize the results of our studies as well as those of many others to demonstrate the efficacy of applying techniques of modern biology, including transgenic fish technology, to increasing the production of cultured finfish, shellfish, and crustaceans.

Enhancement of Recombinant Fish Growth Hormone on Somatic Growth in Fish

In recent years, cDNA and the genomic sequence of growth hormone (GH) have been isolated and characterized for several fish species. Expression of rainbow trout (rt) GH1 cDNA in *E. coli* cells has resulted in the production of a large quantity of biologically active recombinant GH polypeptide. Agellon et al. (1986) showed in a series of studies that application of this recombinant hormone to yearling rainbow trout resulted in significant growth enhancement. After treatment of yearling rainbow trout with the recombinant rtGH for four weeks at a dose of 1 mg/g body weight/week, the weight gain among the individuals of the hormone-treated group was two times greater than that of the controls. Significant length increase was also evident in hormone-treated animals. When the same recombinant hormone was administered to rainbow trout fry (table 1) or small juveniles by immersing the fish in a GH-containing solution, the same growth-promoting effect was also observed (Agellon et al. 1986, Leong and Chen unpublished results). These results are in agreement with those reported by Sekine et al. (1985), Gill et al. (1985), and many others. However, it is important to mention that the growth enhancement effect of the biosynthetic hormone was markedly reduced when more than 2 mg/g body weight of the hormone was applied to the test animals (Agellson et al. 1986). These results suggest that when the total amount of GH exceeds the maximal threshold level, the homeostasis of the hormone will be disturbed, consequently affecting the growth performance of the animals.

Several years ago, Morse (1984) reported that bovine insulin and bovine GH enhanced the growth rate of California red abalone. Recently, Paynter and Chen (1991) observed that administration of recombinant rtGH polypeptide to juvenile oysters (*Crassostrea virginica)* by the "dipping method" referred to above also resulted in significant increases in shell height, shell weight, wet weight, and dry weight (table 2). Furthermore, they also showed that oysters treated with recombinant rtGH, native bovine GH, or bovine insulin consumed more oxygen per unit time than controls. These findings suggest that recombinant fish GH can be used to enhance the growth rate of shellfish under intensive culture conditions. The study further suggests that growth in shellfish may also be regulated by hormonal factors similar to mammalian GH and insulin-like growth factors (IGFs).

Table 1. Effect of growth hormone treatment on the growth of rainbow trout fry. Groups of rainbow trout fry (n = 15) were subjected to osmotic shock in the presence or absence of trout recombinant growth hormone. Weight was measured prior to (initial) and 5 weeks post-treatment (final). Differences between mean weights of GH-treated and control groups were evaluated using Student's t-test. Mean weights were considered to be significantly different if $P < 0.01$. (From Agellon et al. (1986), with permission)

	Mean Weight (gm ± SD)		
Treatment	Initial	Final	% Gain
Saline control	1.33+0.6[2]	3.94+1.8[1]	196
GH (50 mg/l)	1.29+0.7[2]	5.51+1.6[3]	327
GH (500 mg/l)	1.35+0.7[2]	5.30+1.3[3]	293

[1]Significantly different between these groups ($P < 0.01$); [2] Significant difference from the GH-treated group ($P < 0.01$). [3]No significant difference between these groups.

Table 2. Effect of exogenously applied recombinant rainbow trout growth hormone on oyster growth Initial height represents the mean size at the begining of the experiment. Final height, total weight, shell weight, and dry weight are mean values determined after the 5-week treatment cycle. Height (ht) was measured in mm from the umbo to the ventral shell margin; weight (wet) was measured in mg. Standard errors of the mean are given in parentheses. (2, with permission)

Treatment	Initial ht (mm)	Final ht (mm)	Total wt (mg)	Shell wt (mg)	Dry wt (mg)
Control	8.14 (0.25)	11.68 (0.27)	206 (11)	136 (8)	6.10 (0.66)
10-9 M	8.04 (0.27)	11.74 (0.23)	199 (9)	131 (6)	6.87 (0.66)
10-8 M	8.72 (0.18)	12.79 (0.27)[1,2]	244 (20)	171 (11)[2]	9.42 (0.41)[1,2]
10-7 M	8.65 (0,32)	13.00 (0.36)[1,2]	252 (13)[2]	189 (13)[1,2]	9.41 (0.74)[1,2]

[1] Significantly larger than the control group (t-test; $P < 0.05$).
[2] Significantly larger than 10-9 M treatment group (t-test; P , 0.05).

Growth Hormone Transgenic Fish

Although exogenous application of recombinant GH results in significant growth enhancement in fish, it may not be cost-effective. If new strains of fish producing elevated but optimal levels of GH can be produced, it would bypass many of those problems associated with exogenous GH treatment. Moreover, once these fish strains have been generated, they would be far more cost-effective than their ordinary counterparts because these fish would have their own means of producing and delivering the hormone, and could transmit their enhanced growth characteristics to their offspring.

Gene Transfer Methodology

Animals into which a segment of foreign DNA has been introduced and stably integrated into the host genome are called transgenic. Since 1982, many transgenic animal species, including fish, have been constructed. These animals play important roles both in basic research as well as in biotechnology application. Although various methods such as direct microinjection, retrovirus infection, electroporation, calcium phosphate precipitation, and particle gun bombardment have been used to introduce foreign DNA into somatic cells as well as germlines of mammals and other higher vertebrates, direct microinjection of DNA into the male pronuclei of the fertilized eggs has been the most prevalent method. This method has resulted in successful production of transgenic mice and domestic animals including rabbits, sheep and pigs.

The microinjection method has also been employed to introduce foreign genes into several fish species in recent years. These include common carp, catfish, goldfish, medaka, rainbow trout, salmon, tilapia, and zebrafish. In general, gene transfer in fish by microinjection is carried out as follows. Eggs and sperm are collected in separate dry containers. Fertilization is initiated by adding water and sperm to eggs, with gentle stirring to enhance fertilization. Eggs are water hardened for various periods of time and then rinsed. Microinjection is done within the first 2 hours after fertilization, using a setup that consists of a dissecting stereo microscope and two micromanipulators, one with a microneedle for injection and the other with a micropipette to hold the egg in position during the injection. Because the male pronuclei of the fish embryos studied to date are not visible, the foreign genes are usually injected into the egg cytoplasm and the amount of the DNA injected into each embryo is in the range of 1 million copies or higher. Following injection, the embryos are incubated in water until hatching. Because natural spawning can be induced by adjusting photoperiod and water temperature in zebrafish and medaka, precisely staged newly fertilized embryos can be readily collected from the rearing aquaria for microinjection. Within the first 2 hours after fertilization in medaka and zebrafish, the micropyl on the embryos is still visible under the microscope. Hence the DNA solution can be easily delivered into the embryos by a microinjection needle through this opening.

Although the microinjection method is successful in transferring foreign DNA into fish embryos, it is a very laborious and time-consuming procedure. There is a genuine interest in developing convenient mass gene transfer technologies for use in fish transgenesis studies. Among many of the mass gene transfer methods such as retrovirus-mediated gene transfer, liposome-mediated or sperm-mediated gene transfer, particle gun bombardment, and electroporation, the method of electroporation has been shown to be the most effective means of transferring foreign genes into fish embryos. This method utilizes a series of short electrical pulses to permeate the cell membrane, thereby permitting the entry of DNA molecules into the embryos. Studies conducted by Lu et al. (1992) showed that the rate of foreign gene integration in transgenic medaka produced by electroporation was in the order of 20% or higher. Powers et al. (1992) has recently reported a much higher rate of gene transfer in common carp and channel catfsh by using the same electroporator. Although the overall rate of transgene integration in transgenic medaka produced by electroporation was slightly higher than that of microinjection, the actual amount of time required for producing the same numbers of transgenic fish by this method is orders of magnitude shorter than by microinjection.

Transgenic Fish Harboring Growth Hormone Gene

Zhu et al. (1985) reported the first successful transfer of human GH gene fused to a mouse metallothionein (MT) gene promoter into goldfish and loach. According to Zhu (pers. comm.), the F1 offspring of these transgenic fish grew twice as large as their nontransgenic siblings. Unfortunately, Zhu and his colleagues failed to present compelling evidence for integration and expression of the foreign genes in their transgenic fish studies. Recently, many laboratories throughout the world have successfully confirmed Zhu's work by demonstrating that human or fish GH and many other genes can be readily transferred into embryos of a number of fish species and integrated into the genome of the host fish. While a few groups have demonstrated expression of foreign genes in transgenic fish, only Zhang et al. (1990), Du et al., (1992) and Lu et al., (1992) have documented that a foreign GH gene could be: (1) transferred to the target fish species; (2) integrated into the fish genome; and (3) genetically transmitted to the subsequent generations. Furthermore, the expression of the foreign GH gene may result in enhancement of growth rates of both P1 and F1 generations of transgenic fish.

In gene transfer studies conducted in common carp and channel catfish, about 106 molecules of a linearized recombinant plasmid containing the long terminal repeat (LTR) sequence of avian Rous sarcoma virus (RSV) and the rainbow trout GH1 or GH2 cDNA were injected into the cytoplasm of one-cell, two-cell, and four-cell embryos. Genomic DNA samples extracted from the pectoral fins of presumptive transgenic fish were analyzed for the presence of RSVLTR-rtGH1-cDNA by PCR amplification and followed by Southern blot hybridization of the amplified DNA samples, using radiolabeled LTR of RSV and/or trout GH1 cDNA as hybridization probes. In the case of transgenic carp studies, about 35% of the injected embryos survived at hatching, of which about 10% of the survivors had stably integrated the RSVLTR-rtGH1-cDNA sequence. A similar percentage of transgenic fish was also obtained when RSVLTR-csGH-cDNA construct was injected into catfish embryos. Southern blot analysis of genomic DNA samples of several transgenic carp revealed that a single copy of the RSVLTR-rtGH1-cDNA sequence was integrated at multiple chromosomal sites.

In the microinjection studies conducted in medaka by Lu et al. (1992), a much higher rate of foreign gene integration (20-30% of the hatched individuals) than that in common carp or channel catfish was observed. This results suggest that DNA microinjected into the embryos via the micropyle may have better access to the nucleus since the nucleus is situated beneath the micropyle.

Inheritance and Expression of Foreign Growth Hormone Gene in Transgeic Fish

The patterns of inheritance of RSVLTR-rtGH1 cDNA in the transgenic common carp were studied by fertilizing eggs collected from non/transgenic females or P1 transgenic females, with sperm samples collected from several sexually mature P1 male transgenic fish. DNA samples extracted from the resulting F1 progeny were assayed for the presence of RSVLTR-rtGH1-cDNA sequence by PCR amplification and dot blot hybridization. The percentage of the transgenic progeny resulting from nine matings were, 0, 32, 26, 100 (4 progeny only), 25, 17, 31, 30 and 23%, respectively. If each of the transgenic parents in these nine matings carries at least one copy of the transgene in the gonad cell, about 50 %to 75 %

transgenic progeny would have been expected in each pairing. Out of these nine matings, two siblots, both control x P1, gave transgenic progeny numbers as large or larger than expected ($P < 0.05$), and the remaining had lower than expected numbers of transgenic progeny. These results indicate that, though most of these P1 transgenic fish had RSVLTR-rtGH1 cDNA in their germline, they might be mosaics. Similar patterns of mosaicism in the germline of P1 transgenic fish have been observed in many fish species studied to date(19,21,22,24,28,34).

If the transgene carries a functional promoter, some of the transgenic individuals are expected to express the transgene activity. According to Zhang et al. (1990) and Chen et al. (1993), many of the P1 and F1 transgenic common carp produced rtGH, and the levels of rtGH produced by the transgenic individuals varied about 10-fold. Chen et al. (1993) recently confirmed these results by detecting the presence of rtGH mRNA in the F1 transgenic carp using an assay involving reverse transcription (RT)/PCR amplification and RNA dot blot hybridization. Different levels of rtGH mRNA were detected in liver, eyes, gonads, intestine, and muscle of the F1 transgenic individuals.

Growth Performance of Growth Hormone Transgenic Fish

Because the site of transgene gene integration differs among the individuals in any population of P1 transgenic fish, they should be considered as totally different transgenic individuals and thus inappropriate for direct comparison of growth performance among themselves. Instead, the growth performance studies should be conducted in F1 transgenic and nontransgenic siblings derived from the same family. Recently Chen et al. (1993) conducted studies to evaluate the growth performance of F1 transgenic carp in seven families. In these experiments, transgenic and nontransgenic full siblings were spawned, hatched, and reared communally under the same environment. Results of these studies showed that growth response by families of F1 transgenic individuals in response to the presence of rtGH1 cDNA varied widely. When compared to the nontransgenic full siblings, the results of these seven growth trials showed 20, 40, -27, 59, 22, -15, and -2% increase in growth. In three of the four families where F1 transgenics grew faster than their nontransgenic full siblings, the maximum and minimum body weights of the transgenics were larger than those of the nontransgenics. In the fourth family, the minimum, but not the maximum, body weight of the transgenics was larger than that of the nontransgenics. In two of those three transgenic families in which transgenics did not grow faster than their nontransgenic full siblings, the maximum and minimum body weights of the transgenics were smaller than those of the nontransgenics. In the third family, however, one of the F1 transgenics was the largest fish in the family. Since the response of the transgenic fish to the insertion of the RSVLTR-rtGH1 cDNA appears to be variable, as a result of random integration of the transgene, the fastest-growing genotype will likely be developed by utilizing a combination of family selection and mass selection of transgenic individuals following the insertion of the foreign gene. In the studies of transgenic medaka carrying chicken b-actin gene promoter human GH gene construct, the F1 transgenic individuals also grew significantly faster than the nontransgenic siblings Lu et al. (1992).

In an effort to study the biological effect of elevated levels of IGF I on somatic growth, transgenic medaka harboring trout IGF cDNA driven by carp b-actin gene promoter have been produced in our laboratory. Both P1 and F1 IGF I transgenic medaka hatched2 days earlier than their nontransgenic controls. Furthermore, the P1 transgenic individuals also grew faster than their nontransgenic controls.

CONCLUSION

Transgenic fish technology has a great potential in the aquaculture/mariculture industry. By introducing desirable genetic traits into finfish or shellfish, superior transgenic strains can be produced for aquaculture. These traits may include elevated growth enhancement, improved food conversion efficiency, resistance to some known diseases, tolerance to low oxygen concentrations, and tolerance to sub-zero temperatures. Recent progress in our laboratory and those of others has shown that the transfer, expression, and inheritance of fish growth hormone transgenes can be achieved in several finfish species and that the resulting animals grow substantially faster than their control siblings. This is a vivid example of the potential application of the gene transfer technology to aquaculture However, in order to realize the full potential of the transgenic fish

technology in aquaculture or other biotechnological applications, several important scientific breakthroughs are required. These are:

1. Developing more efficent mass gene transfer technologies.
2. Identifying genes of desirable traits for aquaculture and other application.
3. Developing targeted gene transfer technologies such as embryonic stem cell gene transfer method or ribozyme gene inactivation methods.
4. Identifying suitable promoters to direct the expression of transgenes at optimal levels during the desired developmental stages.
5. Determining physiological, nutritional, immunological and environmental factors that will maximize the performance of the transgenic individuals.
6. Assessing safety and environmental impacts of transgenic fish.

Once these problems are resolved, the commercial application of the transgenic fish technology will be readily attained.

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ASSESMENT OF POTENTIAL ZEBRA MUSSEL HABITAT IN TWO CHESAPEAKE BAY TRIBUTARIES

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Abstract: Salinity, pH, calcium concentration, and water temperature were mapped in order to predict potential habitat in two Maryland tributaries for zebra mussels, which have not yet been found in Maryland waters. Five consecutive years of data (1988-92) were evaluated for 105 sites in the Maryland portion of the Potomac River and 3 sites in the Maryland portion of the Susquehanna River. In the Potomac River, 496 km were predicted to be vulnerable to zebra mussel colonization, and in the Susquehanna River, 23 km were predicted to be vulnerable, based on the parameters evaluated. In the Potomac River, zebra mussel habitat appears to be limited primarily by salinity. However, pH may be a limiting factor in the Anacostia River and immediately downstream of its confluence with the Potomac River. In the Susquehanna River, pH appears to be the primary limiting factor for zebra mussel proliferation.

INTRODUCTION

The economic and ecological effects of zebra mussel, *Dreissena polymorpha*, infestations have been substantial in North America since they were first detected in the Great Lakes in 1988 (Weigmann et al. 1991). Because of the propensity for zebra mussels to attach in thick layers to hard substrates such as water intake pipes, extensive control efforts have been required at numerous power generating, public water supply, and industrial facilities (Lepage 1993). From 1990 to 1999, the estimated cost of controlling zebra mussels at water intake facilities in the Great Lakes region alone will exceed $2 billion (O'Neill and MacNeill 1990). The economic, and ecological effects of zebra mussel, *Dreissena polymorpha*, infestations have been substantial in North America since they were first detected in the Great Lakes in 1988 (Weigmann et al. 1991). Because of the propensity for zebra mussels to attach in thick layers to hard substrates such as water intake pipes, extensive control efforts have been required at numerous power generating, public water supply, and industrial facilities, at considerable expense (LePage 1993). From 1990 to 1999, the estimated cost of controlling zebra mussels at water intake facilities in the Great Lake region alone will exceed $2 billion (O'Neill and MacNeill 1990). The ecological consequences of zebra mussel infestations are more difficult to ascertain. Most notable are the adverse effects that zebra mussel colonization has had on populations of native unionid mussels (Schloesser and Kovalak 1991, Schloesser 1993). High mortalities of unionids in Lake Erie and Lake St. Clair in recent years have been attributed to heavy fouling by zebra mussels (Ricciardi 1994), and it is projected that zebra mussels will have a continuing deleterious effect on other unionid populations as their range expands throughout North America.

As a result of the problems associated with such zebra mussel infestations, much effort has been directed to determining those water bodies that are most vulnerable to invasion and colonization. The approaches most often taken include comparing available water quality data with known environmental tolerances of zebra mussels and comparatively evaluating possible transport vectors in different areas to develop dispersion

indices (Ramcharan et al. 1992, New York City Department of Environmental Protection 1992). Additionally, geographic information system (GIS) mapping is sometimes used to graphically depict those areas where water quality values fall within the range of the environmental tolerances of zebra mussels for particular parameters (Neary and Leach 1992).

The most common approach used to assess the vulnerability of a water body to invasion and colonization is a comparison of the water quality of the area of concern with the environmental tolerances of zebra mussels. The general environmental tolerances of zebra mussels are relatively well known for many parameters such as salinity, pH, calcium, and water temperature (Yount 1990), which are among the most commonly used environmental parameters in such risk assessments. This information has been used to develop multi-parameter probability tables for use in risk assessment, based on the known susceptibility of surface waters to colonization (O'Neill 1992).

Several investigators have used GIS mapping to graphically depict those areas that should be most vulnerable to zebra mussel colonization, based on a comparison of environmental tolerances with physical and chemical water quality data. Neary and Leach (1992) incorporated water quality information relating to lakes throughout Ontario, Canada, into multi-parameter maps that evaluated calcium concentration, pH, and the buffering capacity of soils and bedrock. Ignacio et al. (1994) mapped potential habitat available for zebra mussel colonization in a portion of Green Bay, Lake Michigan, based upon water temperature, substrate availability, and water depth.

Although zebra mussels have not yet been detected in Maryland's waters, their proximity in the Ohio and upper Susquehanna Rivers suggest that infestation is imminent. Colonies are present in the Ohio River near Wheeling, West Virginia. Veligers were reported from the Susquehanna River near Johnson City, New York, in 1991, 1992, and 1993, although no adults have been detected. The purpose of this paper is to use GIS-based mapping of water quality data to evaluate the relative probability of zebra mussel invasion and colonization in the Potomac and Susquehanna Rivers, which are potentially two of Maryland's most vulnerable river systems (Christmas et al. 1994).

METHODS

The Potomac and Susquehanna Rivers are the largest rivers whose confluences with Chesapeake Bay are within Maryland's borders. The Potomac River is 616 km long (North Branch headwaters to mouth), with a drainage basin of 37995 km^2, and contributes 20% of the freshwater inflow to Chesapeake Bay. The Susquehanna River is 714 km long, with a drainage basin of 71,432 km^2, and contributes 45% of the freshwater inflow to Chesapeake Bay.

The water quality parameters evaluated in this mapping effort were salinity, pH, water temperature, and calcium concentration. Data sets from 1988 to 1992 were evaluated for 105 sites in the Maryland portion of the Potomac River and two sites in the Maryland portion of the Susquehanna River. Data were also obtained from a third Susquehanna River site in Pennsylvania, at Holtwood Dam (river mile 24), because of the paucity of data available in the Maryland portion. The statistics evaluated at each site were the arithmetic means of each selected parameter during the period of interest, which was defined as the period between the first and last occurrence of a water temperature of 12°C (April-November), averaged over the 5-year period. This interval approximates the period within which zebra mussel spawning could potentially occur in this region.

Calcium data were not available for all sites. Therefore, at those fresh-water sites in the Potomac and Susquehanna Rivers, for which calcium data was lacking, a linear regression model (Neary and Leach 1992) was used to predict calcium values based on conductivity measurements [Potomac River, $Y=(X)(0.138)-3.93$ ($r=0.83$); Susquehanna River, $Y=(X)(0.091)+5.27$ ($r=0.83$)]. For brackish water sites, adequate calcium was assumed, even though few data were available.

Three classifications of potential zebra mussel colonization were defined based primarily upon known environmental tolerances compiled by New York Sea Grant (table 1). After classifying each site based on these criteria, the classifications corresponding to each site were mapped separately for each parameter. The appropriate classification of each site was then plotted using GIS with Map and Image Processing software. Each site was then assigned an overall classification based on the most limiting parameter (i.e, the lowest classification) at that site, and a series of more elaborate maps were then produced, using CORELDRAW software, based upon the GIS-generated maps. The classification scheme is shown in(figure 1).

Table 1. Environmental tolerances of zebra mussels to selected water quality parameters.

Water Quality Parameter	Colonization Potential High	Moderate	Low
Salinity (ppt)	0 - 1	1 - 10	10 - 35
Calcium (mg/l)	25 - 125	9 - 25	< 9
pH	7.4 - 8.5	7.0 - 7.4 8.5 - 9.0	<7.0 >9.0
Temperature °C	17 - 25	15 - 17 20 - 30	>30

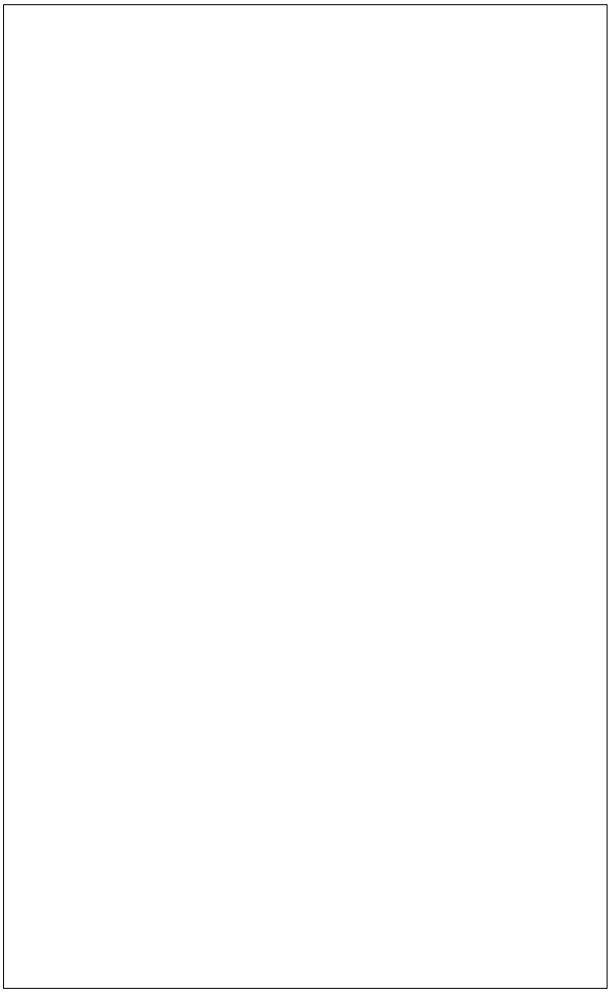

Figure 1. Classification scheme used in mapping procedure to evaluate vulnerabilty of the Potomac and Susquehanna Rivers to colonization by zebra mussels.

RESULTS

Potomac River

Considerable portions of the Potomac River appear to be vulnerable to zebra mussel colonization, based on the water quality parameters that were evaluated. A total of 120 km have a low vulnerabilty to zebra mussel colonization, 68 km have a moderate vulnerability, and 428 km have a high vulnerability. In the Potomac River, zebra mussel habitat appears to be limited primarily by salinity; the colonization potential decreased downstream as salinity increase . However, in the Anacostia River, a tributary of the Potomac River, and immediately downstream of its confluence with the Potomac River it appears that pH may be the most limiting factor. Temperature values at all sites were within the optimal range for zebra mussels. Similarly, calcium levels were in the optimal range for zebra mussels at all but two of the 105 sites: Town Creek and the upstream portion of Mattawoman Creek.

Susquehanna River

Much of the Maryland portion of the Susquehanna River also appears to be vulnerable to zebra mussel colonization **(Figure 7)**. About 15 km have a moderate vulnerability to zebra mussels colonization while 8 km have a high vulnerability. In the Susquehanna River, pH appears to be the primary limiting factor. Calcium concentration and water temperature were within the optimal range for colonization in the entire area which was evaluated.