Bird Use of *Phragmites australis* in Coastal Marshes of Northern Massachusetts

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ABSTRACT: One of the major management concerns regarding salt marshes on the east coast is the replacement of existing salt and brackish vegetation by common reed (*Phragmites australis*). This invasive grass thrives in areas where tidal flushing has been reduced by dikes, embankments, and undersized culverts. *P. australis* habitats are thought to be of substantially less wildlife value than the marsh vegetation they replace. There is, however, little documentation of this assertion. We carried out three seasons of quantitative bird censuses in *P. australis*, *Spartina* spp., and *Typha angustifolia* marshes in northern Massachusetts. Censuses methodologies included visual observation, passive listening, and playback techniques in 50-m radius point count circles. Circles were selected that contained various amounts of forest, *P. australis*, *Spartina* spp., or *T. angustifolia*. The significance of correlations between the abundance of bird species detected anywhere within the circle and the plant communities present there were tested using Spearman's rank correlation coefficients. The significance of habitat preferences was also tested by comparing the availability of plant communities present in counting circles and the bird abundances detected in those plant communities. Ivlev deviations were used to test the significance of these preferences. Based on data from 1997, the number of bird species commonly encountered in each habitat differed, with *Spartina* spp. and *P. australis* marshes having the most and coastal *T. angustifolia* marshes the least number of species. The amount of *P. australis* present within each point-count circle had a positive effect on Redwing Blackbirds and had little impact on the numbers of Marsh Wrens observed in a circle. Marsh Wrens were negatively impacted by increases in the amount of either salt marsh or forest present. Saltmarsh Sharp-tailed Sparrows occurred more frequently in *Spartina* spp. marshes, and *Phragmites* had a non-significant negative effect on Virginia Rails, while cattails had a highly significant positive effect on them. Our study suggests that the most common birds in these northern Massachusetts marshes are unaffected by the presence of *P. australis*; however, future research that targets other seasons and a landscape analysis is needed.

Key words: *Phragmites australis*, common reed, *Spartina*, *Typha angustifolia*, salt marsh, invasive plant, habitat value, bird survey, marsh bird, Ivlev deviation

INTRODUCTION

One of the major problems facing salt marshes on the east coast of the United States is the replacement of existing salt and brackish marsh vegetation by the common reed (*Phragmites australis* Cav.). This invasive grass thrives in areas where tidal flushing has been reduced by dikes, embankments, and undersized culverts. Researchers and resource managers often claim that *P. australis* habitats are of substantially less wildlife value than the marsh vegetation they replace (Roman et al. 1984; Jones and Lehman 1987; Hauser et al. 1991; Marks et al. 1994; Tiner 1995), however, data in support of this claim are limited. Apart from a recently published study of Connecticut salt marshes by Benoit and Askins (1999), we are aware of no quantitative work on the impact of *P. australis* invasions on marsh birds. In this paper, we report preliminary results of a study on how the presence of *P. australis* impacts the avian ecology of salt marshes in northeastern Massachusetts.

We have two goals: (1) to determine how *P. australis*-dominated habitats differ from other major vegetated coastal marsh habitats in terms of bird species composition and abundance, and (2) to predict what the effects on birds will be if this plant continues to spread. Our approach has been to compare bird abundances in coastal marsh study plots differing in their percent cover of *P. australis* and other major vegetation types. Our primary aim has been to document differences in bird abundances in four of the
major habitats of the coastal zone; salt marsh, 
P. australis marshes, Typha angustifolia marshes, and
adjoining woods. We have used three research strate-
gies: (1) correlation of individual bird species' abun-
dances with the percent cover of each of the four veg-
etation communities potentially found at each site,
(2) use of an electivity index based on the relation-
ship between the relative abundance of birds (by
species) found within a specific plant community at
each site and the availability of that plant community
at that site, and (3) a comparison of the frequencies
with which various stereotypical behaviors are record-
ed in the different plant communities.

Our study area encompassed coastal marshes on
the east coast of the United States from southern
New Hampshire (42° N, 71° W), through Boston
Harbor (43° N, 71° W). Some of the marshes, such as
Neooset Marsh near Boston, are in urban areas,
often with relatively large areas of P. australis. Others,
particularly the Parker River and Essex Bay salt
marshes, the largest contiguous acreage of salt march
north of Long Island, are still relatively undisturbed
by human activity. P. australis occurs to some extent
in all marsh systems investigated. Since P. australis
typically invades and spreads from the oligohaline
transition zone between salt marshes and upland,
many of our observations were within vegetation
communities at or adjacent to this transition zone.

Since increased abundance does not necessarily
indicate that the habitat is of higher quality (van
Horne 1983, Vickery et al. 1992a, b), we also carried
out a preliminary assessment of those stereotypical
behaviors that might indicate breeding in Red-winged
Blackbirds, the only bird in our study area occurring
in sufficient numbers to allow quantification of
behaviors. We assume that abundance indicates the
degree to which a habitat is important to the bird,
but not whether that area is a source, a sink, or a
"reservoir" of potential replacement breeders.

METHODS

SELECTION OF VEGETATION COMMUNITIES

The vegetation communities of New England salt
marshes are relatively simple and easy to characterize.
They are dominated by a few abundant species (pri-
marily graminoids) that grow in dense patches
(Niering and Warren 1980). The location of these
communities is determined by marsh elevation in
relation to tidal inundation. The four vegetation
communities we compared for their avian species
were P. australis marsh, salt marsh, cattail marsh, and
adjacent forests. The first three were chosen because
they are the dominant communities in terms of cover
in coastal marshes in the region. The latter was chosen
because many marsh birds make use of adjacent
woodlands for part of their life cycle. The four com-
unities are defined as follows:

Phragmites australis marsh contains close to 100%
cover of P. australis. Canopy height in our study sites
ranged from 1 to 2.5 m. In most cases P. australis
grows as a monoculture with no understory; in oth-
ers, some salt marsh or upland vegetation co-occurs.

Salt marsh is dominated by three salt marsh grass-
es, Spartina patens (salt marsh hay) and Distichlis spica-
ta (spike grass) in high marsh (higher elevations), and
Spartina alterniflora (salt marsh cordgrass) in low
marsh. High marsh occurs immediately seaward of P.
australis and cattail marshes and is flooded several
days each month during spring tides. Juncus gerardi
(black grass, actually a rush) grows where elevations
are slightly higher. Slight depressions in the high
marsh surface that are poorly drained contain a short
growth form of S. alterniflora. Tall form S. alterniflora
grows in areas regularly flooded during high tide,
such as the banks of salt marsh creeks.

Cattail marsh in the coastal zone is almost a
monoculture of Typha angustifolia. Since this species
is restricted to lower salinities than plant species
growing in the salt marsh, it occurs in higher eleva-
tions and where there are large freshwater inputs.
Since the salinity range of cattails is similar to that of
P. australis, it may be the habitat most vulnerable to
P. australis invasion.

Forest consists of a variety of trees and shrubs
growing immediately adjacent to the marsh. Major
species include oak, especially Quercus rubra (red
oak), hickories (e.g., Carya glabra), and Amelanchier
spp. (shadbush). This plant community was included
because a number of the bird species in this study
move readily between the marsh and bordering
upland.

COUNTING CIRCLES

The use of counting circles is a standard way to
census birds in grassland habitats (Järvinen 1978;
1992; Sliwa and Sherry 1992; Ralph et al. 1995;
Dettmers et al. 1999). It allows the observer to use
both visual and auditory cues to determine the num-
ber of birds of each species present while also allow-
ing quantitative analysis of the habitat enclosed by each circle. The assumption is that, over a short observation period (10-30 min), a census of a circle represents the number of birds present at one point in time — hence the reference to a point count. Replication comes from periodic visits at predetermined intervals to the counting circle and by having replicate circles.

Birds were censused in 68-point count circles of 50-m radius each, with varying amounts of *P. australis*, cattails, salt marsh, and forest vegetation in each circle. Circles were selected to reflect a variety of conditions with respect to vegetation, marsh size, and soil moisture. Sites were selected without knowledge of the bird communities present, but were not selected randomly. Most salt marsh sites selected were relatively close to habitat edges, the zone of maximum potential *P. australis* invasion. No circles containing 100% forest were selected.

**Bird Censuses**

Each counting circle was censused every 2-3 wk (6-7 times/yr) from April to September in 1997 and 1998. Sites were surveyed from 0.5 hour before sunrise to 12 noon, and at all tides. Tides and times of day were distributed evenly among sites. Censuses of birds were carried out by a single observer from the middle of each counting circle. During each census, the observer recorded all birds seen or heard and the plant community in which they were first seen. We also recorded behaviors observed (e.g., carrying nesting material or carrying food to young), distance from the observer, and the time of observation.

Because some birds are quite cryptic and the vegetation dense, playing tapes of bird songs and listening for auditory responses was included in each census. Bird songs of eight species were broadcast for a total 16 min using a Johnny Stewart Bird and Animal Caller with booster. An initial 3 min without song broadcast was followed by 30 sec of bird song and 30 sec of silence. This sequence was repeated twice for each species. Songs were broadcast in the following sequence: Song Sparrow (scientific names appear in Table 1), Clapper Rail, Swamp Sparrow, Sora, American Bittern, Virginia Rail, Least Bittern, and Marsh Wren. Song tapes were obtained from the Cornell Library of Natural Sounds, *A Field Guide to Bird Songs* (Peterson 1990), and Birding by Ear (Walton and Lawson 1989). Birds were counted as the observer approached the center of the circle, for the 3

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Total/Yr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Starling</td>
<td><em>Sturnus vulgaris</em></td>
<td>1090</td>
</tr>
<tr>
<td>Tree Swallow</td>
<td><em>Tachycineta bicolor</em></td>
<td>1024</td>
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<tr>
<td>Red-winged Blackbird</td>
<td><em>Agelaius phoeniceus</em></td>
<td>909</td>
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<tr>
<td>Common Grackle</td>
<td><em>Quiscalus quiscula</em></td>
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</tr>
<tr>
<td>Least Sandpiper</td>
<td><em>Caldida minuta</em></td>
<td>155</td>
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<tr>
<td>Song Sparrow</td>
<td><em>Melospiza melodia</em></td>
<td>129</td>
</tr>
<tr>
<td>Yellow legs sp.</td>
<td><em>Tinga sp.</em></td>
<td>121</td>
</tr>
<tr>
<td>Saltmarsh</td>
<td><em>Amdromodus caudatus</em></td>
<td>105</td>
</tr>
<tr>
<td>Sharp-tailed Sparrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsh Wren</td>
<td><em>Cistothorus palustris</em></td>
<td>104</td>
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<tr>
<td>Common Yellowthroat</td>
<td><em>Geothlypis trichas</em></td>
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<tr>
<td>Virginia Rail</td>
<td><em>Rallus limicola</em></td>
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<tr>
<td>American Goldfinch</td>
<td><em>Carduelis tristis</em></td>
<td>43</td>
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<td>Eastern Kingbird</td>
<td><em>Tyrannus tyrannus</em></td>
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<tr>
<td>Barri Swallow</td>
<td><em>Hirundo rustica</em></td>
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<td><em>Plegadis falcinellus</em></td>
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<tr>
<td>American Robin</td>
<td><em>Turdus migratorius</em></td>
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</tr>
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<td>Cedar Waxwing</td>
<td><em>Bombycilla cedrorum</em></td>
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<td><em>Parus atricapillus</em></td>
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<tr>
<td>Eastern Towhee</td>
<td><em>Pipilo erythrophthalmus</em></td>
<td>19</td>
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<tr>
<td>American Black Duck</td>
<td><em>Anas rubripes</em></td>
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<td>Baltimore Oriole</td>
<td><em>Icterus galbula</em></td>
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<tr>
<td>Golden Oriole</td>
<td><em>Icterus auricollis</em></td>
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<tr>
<td>Northern Mockingbird</td>
<td><em>Mimus polyglottos</em></td>
<td>13</td>
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<tr>
<td>Semipalmated Sandpiper</td>
<td><em>Caldris pusilla</em></td>
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<tr>
<td>Chipping Sparrow</td>
<td><em>Spizello passerina</em></td>
<td>10</td>
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<tr>
<td>Killdeer</td>
<td><em>Charadrius vociferus</em></td>
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<tr>
<td>Northern Flicker</td>
<td><em>Colaptes auratus</em></td>
<td>8</td>
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<tr>
<td>American Crow</td>
<td><em>Corvus brachyrhynchos</em></td>
<td>7</td>
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<tr>
<td>Mallard</td>
<td><em>Anas platyrhynchos</em></td>
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<tr>
<td>Mourning Dove</td>
<td><em>Zenaida macroura</em></td>
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<tr>
<td>Snowy Egret</td>
<td><em>Egretta thula</em></td>
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</tr>
<tr>
<td>Tufted Titmouse</td>
<td><em>Parus bicolor</em></td>
<td>6</td>
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<tr>
<td>Blue Jay</td>
<td><em>Cyanocitta cristata</em></td>
<td>5</td>
</tr>
<tr>
<td>Canada Goose</td>
<td><em>Branta canadensis</em></td>
<td>5</td>
</tr>
<tr>
<td>House Sparrow</td>
<td><em>Passer domesticus</em></td>
<td>5</td>
</tr>
<tr>
<td>Purple Finch</td>
<td><em>Carpodacus purpurus</em></td>
<td>5</td>
</tr>
<tr>
<td>Yellow Warbler</td>
<td><em>Dendroica petechia</em></td>
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<tr>
<td>Northern Cardinal</td>
<td><em>Cardinalis cardinalis</em></td>
<td>4</td>
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<tr>
<td>Willet</td>
<td><em>Catoptrophorus semipalatus</em></td>
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<tr>
<td>Brown Thrasher</td>
<td><em>Toxostoma rufum</em></td>
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<tr>
<td>Great Egret</td>
<td><em>Ardea alba</em></td>
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</tr>
<tr>
<td>Green Heron</td>
<td><em>Butorides virescens</em></td>
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</tr>
<tr>
<td>Semipalmated Plover</td>
<td><em>Charadrius semipalatus</em></td>
<td>3</td>
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<tr>
<td>Eastern Bluebird</td>
<td><em>Sialis sialis</em></td>
<td>2</td>
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<tr>
<td>Seaside Sparrow</td>
<td><em>Ammodramus malacrus</em></td>
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</tr>
<tr>
<td>Spotted Sandpiper</td>
<td><em>Actitis macularia</em></td>
<td>2</td>
</tr>
<tr>
<td>Belted Kingfisher</td>
<td><em>Ceryle alcyon</em></td>
<td>1</td>
</tr>
<tr>
<td>Black-crowned Night-Heron</td>
<td><em>Nycticorax nycticorax</em></td>
<td>1</td>
</tr>
<tr>
<td>Bobolink</td>
<td><em>Dolichonyx oryzivorus</em></td>
<td>1</td>
</tr>
<tr>
<td>Connecticut Warbler</td>
<td><em>Opisramis agilis</em></td>
<td>1</td>
</tr>
<tr>
<td>Red-breasted Nuthatch</td>
<td><em>Sitta canadensis</em></td>
<td>1</td>
</tr>
<tr>
<td>Ring-necked Pheasant</td>
<td><em>Phasianus colchicus</em></td>
<td>1</td>
</tr>
<tr>
<td>Scarlet Tanager</td>
<td><em>Pranga olivacea</em></td>
<td>1</td>
</tr>
<tr>
<td>Swamp Sparrow</td>
<td><em>Melospiza georgiana</em></td>
<td>1</td>
</tr>
<tr>
<td>American Bittern</td>
<td><em>Botaurus lentiginosus</em></td>
<td>0*</td>
</tr>
<tr>
<td>Clapper Rail</td>
<td><em>Rallus longirostris</em></td>
<td>0*</td>
</tr>
<tr>
<td>Least Bittern</td>
<td><em>Isbruchy exilis</em></td>
<td>0*</td>
</tr>
<tr>
<td>Sora</td>
<td><em>Porzana carolina</em></td>
<td>0*</td>
</tr>
</tbody>
</table>

* Spp. song broadcast during playback. No individuals observed.
Table 2. Spearman Rank Correlation of bird abundance and plant communities — selected bird species. N=number/visit, over all sample points; r_s=Spearman’s r; p are corrected for ties.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>r_s</th>
<th>Phragmites p</th>
<th>Cattails r_s</th>
<th>Salt Marsh p</th>
<th>Forest r_s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-wing Blackbird</td>
<td>132</td>
<td>0.47</td>
<td>0.001</td>
<td>**</td>
<td>0.21</td>
<td>0.122</td>
</tr>
<tr>
<td>Song Sparrow</td>
<td>20</td>
<td>0.02</td>
<td>0.862</td>
<td>ns</td>
<td>0.03</td>
<td>0.837</td>
</tr>
<tr>
<td>Saltmarsh</td>
<td>14</td>
<td>-0.01</td>
<td>0.920</td>
<td>ns</td>
<td>-0.05</td>
<td>0.724</td>
</tr>
<tr>
<td>Sharp-tailed Sparrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsh Wren</td>
<td>14</td>
<td>0.14</td>
<td>0.325</td>
<td>ns</td>
<td>0.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Common Yellowthroat</td>
<td>11</td>
<td>0.17</td>
<td>0.223</td>
<td>ns</td>
<td>0.11</td>
<td>0.413</td>
</tr>
<tr>
<td>Virginia Rail</td>
<td>7</td>
<td>-0.04</td>
<td>0.801</td>
<td>ns</td>
<td>0.48</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Vegetation analysis was carried out from mid-September to mid-November, using two separate techniques. The major plant communities (P. australis, salt marsh, cattail, forest) were directly mapped using a compass and a Bushnell Yardage Pro to measure distances (accuracy = ±1 m). Distances and bearings were measured to the central stake and the resulting coordinate system was used to draw the resulting polygons in their respective places within the study plot. Polygon areas, and thus the percent of each site covered by each plant community, were calculated from these drawings. In addition to the maps, ten 0.25-m² quadrat frames were located randomly within each plant community using the same coordinate system as for the maps. Percent cover of all plants within the frame, stem densities, plant heights, and percent reproductives of P. australis and Typha, when present, were measured. Only the results of the mapping studies are used here.

Statistical Procedures

Bird Abundances

The effect of differences in the percent cover of each of the four plant communities within a counting circle on the abundance of birds detected in that counting circle was evaluated by the magnitude and sign of the Spearman’s rank correlation coefficient (r_s), and its significance tested with the associated p-value (both corrected for ties) (Sokal and Rohlf 1981). Bivariate plots were used to assess the adequacy of a linear correlation. Both means and maxima were used to characterize species abundances (N) at each site. Means tended to be low, especially when several of the visits detected few to no birds of a given species. An alternative is to use the maximum number detected over all visits to a given site during each year (Benot and Askins 1999; Greg Shriver, pers. comm.)—this leads to substantially higher N’s. Both methods were used.

Bird Species Richness

We related bird species richness (i.e., the number of species recorded) within individual counting circles to the presence of the four different vegetation communities in two ways: (1) by comparing the number of bird species present within each counting circle to the percent cover values of each vegetation community within that circle, and (2) by characterizing each counting circle as belonging to a single “site plant community” based on the extent of dominance of that community. Two definitions of dominance were tested. Sites were considered as belonging to a particular plant community if either >50% or >75% of the site was covered by that plant community. The effect on species richness was analyzed separately for each definition.

Electivity

In addition to correlating the abundance of birds detected anywhere in the circle to plant communities found there, we quantified “preference” and “avoidance” by comparing the percent of each bird species detected within each plant community to the percent of the study circle occupied by that plant community. The expectation under the null hypothesis is that the proportion of a given bird species detected in a given plant community will not differ significantly from the proportions of that plant community available in the study circle. The significance of deviations
Table 3. Spearman Rank Correlation of bird species richness and plant communities. $r_s$ = Spearman's $r$; $p$ and $p$ are corrected for ties

<table>
<thead>
<tr>
<th></th>
<th>Phragmites</th>
<th>Cattails</th>
<th>Salt Marsh</th>
<th>Forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_s$</td>
<td>0.06</td>
<td>0.21</td>
<td>-0.30</td>
<td>0.56</td>
</tr>
<tr>
<td>$p$</td>
<td>0.639</td>
<td>0.126</td>
<td>0.028</td>
<td>&gt;0.000</td>
</tr>
</tbody>
</table>

**Correlation Studies — Bird Abundance**

The Spearman rank correlation coefficients indicate a strong and positive association between *P. australis* and Red-winged Blackbirds and no association with the abundances of Song Sparrows, Saltmarsh Sharp-tailed Sparrows, Marsh Wrens, Common Yellowthroats, or Virginia Rails (Table 2). Using maxima rather than means produced the same results. Shorebird species, while among the most abundant species encountered (Table 1), were dependent on the presence of salt marsh pannes, and not on the percent cover of any plant community within the circle. As the amount of *P. australis* present was uniformly low for all locations where shorebirds were encountered, the percent of variation in shorebird abundance explained by the percent cover of *P. australis* was also low. The correlation of shorebirds with percent *P. australis* was therefore poor. This conclusion is an artifact of using univariate correlations. (Multivariate analyses are in preparation.)

**Correlation Studies — Bird Species Richness**

Species richness of birds was not significantly affected by the amount of *P. australis* or cattails within counting circles (Table 3). Bird species richness was slightly reduced as the percent of salt marsh increased. In contrast, species richness was dramatically increased as the percent of forest within a counting circle increased. When each counting circle was assigned to a single “site plant community”, forests remained the only plant community that had a significant effect on species richness (Figure 1). This remained true whether > 50%, or >75% was used to determine dominance.

**Electivity Indices**

Preference for, or avoidance of, a plant community by Marsh Wrens as measured by the weighted Ivlev deviation, is illustrated using 1997 data.
P. australis marshes had significantly fewer Virginia Rails than were encountered in cattail or brackish marshes, and significantly fewer Saltmarsh Sharp-tailed Sparrows than were encountered in short-grass salt marsh. All Willets were found in salt marsh. In Connecticut, Seaside Sparrows, Snowy Egrets, Green Herons, Least and Semipalmated Sandpipers, Mallards, and American Black Ducks were entirely absent from P. australis marshes. Species found in P. australis marshes were more often habitat generalists, while species absent from P. australis marshes were dependent on salt marsh. Species richness, especially of those species listed by the state of Connecticut as being of management concern, was also significantly lower in P. australis marshes.

In north coastal Massachusetts, Saltmarsh Sharp-tailed Sparrow abundance does not appear to be significantly affected by percent cover of P. australis, but the number of individuals encountered was low. Only four Willets were encountered in our study area. Breeding populations of Seaside Sparrows are rare in north coastal Massachusetts (Marshall and Reinert 1990); only two individuals were encountered. Egrets and Herons may be underrepresented in both studies as the methodology used in both studies

![Graph showing bird preferences and deviations](image)

Figure 2. Marsh Wren preferences as detected by weighted Ivlev deviations (error bars=±2 SE).

(Figure 2). All error bars enclose “0”, indicating that no significant plant community preferences were detected for this species.

**Behavioral Observations**

Behavioral observations on Red-winged Blackbirds revealed a preference for P. australis. In most classes of behavior except foraging, frequencies of occurrence were far greater in P. australis than in most other plant communities (Table 4).

**Discussion**

The goal of this paper is to quantify the effect of P. australis on the avian ecology of the coastal zone in northern Massachusetts. Using playback techniques in point count circles and bivariate correlations, we conclude that changes in percent cover of P. australis has a positive effect on the abundance of Redwinged Blackbirds and no effect on Song Sparrows, Saltmarsh Sharp-tailed Sparrows, Marsh Wrens, Common Yellowthroats or Virginia Rails. No significant negative effects on abundance were detected. Only the percent cover of forests in the counting circle had a significant effect on species richness. Benoit and Akins (1999), on the other hand, report that in coastal Connecticut the bird communities encountered in P. australis marshes were distinctly different from those found in short-grass salt marsh meadows (i.e., salt marsh exclusive of tall-form S. alterniflora).

<table>
<thead>
<tr>
<th>A. evidence of pairing (N=34)</th>
<th>plant community observed in</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattail</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Phragmites</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Salt Marsh</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. food or nesting material in bill (N=25)</th>
<th>plant community observed in</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattail</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Phragmites</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Salt Marsh</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. aggressive behaviors* (N=68)</th>
<th>plant community observed in</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattail</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Phragmites</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Salt Marsh</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* displacement, chase, sexual chase, song spread (Driars and Christman 1968), attacks on observer

---

Table 4. Relationship of Red Winged Blackbird behaviors to plant species dominant in counting circles.
may have seriously biased estimates of Heron and Egret abundance (Benoit and Askins 1999, and below). Even when our circles contained shallow pannes, the preferred feeding area for these birds (Brush et al. 1986), nearly all Herons and Egrets were observed considerably outside of our study circles. These birds appear more sensitive than passerines to disturbance by the observer. Censusing in full view, within 50-m circles, while broadcasting bird song appears to seriously underestimate the abundance of these birds. Least and Semipalmated Sandpipers, Mallards, and American Black Ducks depend on the presence of shallow pannes or deep pools. Benoit and Askins (1999) report significantly higher species richness of state-listed species at sites in Connecticut that have pools. We did not encounter pannes or pools in P. australis marshes with sufficient frequency to test their effect. The effect of P. australis on the abundance of pannes and pools, and on their usefulness to panne- and pool-dependent species, remains to be studied.

Fribil (1998) argues that the correlation of abundance data with environmental features such as percent cover, without regard to the availability of that feature in the habitat, can lead to false conclusions of "no effect" when these features are not limiting. We have used a weighted Ivlev deviation to test the effect of resource availability on habitat selection. For Marsh Wrens, the only species tested to date, our conclusions of "no effect" of P. australis on abundance remains unchanged.

Van Horne (1983) argues that abundance alone is a poor indicator of habitat quality. Vickery et al. (1992b) suggest the use of stereotypical behaviors associated with breeding, when direct measures of breeding success are not available. We attempted to do this, but were successful only in collecting sufficient data for Red-winged Blackbirds. Among the plant communities surveyed, the behavior data indicated a preference of Red-winged Blackbirds for P. australis habitats for breeding. This is in agreement with the abundance data.

Our point count circles were not selected randomly; however, given the diversity of treatments and analytic techniques described, we are confident of the robustness of our results. The 68 sites surveyed contained a wide range of percent cover of each of the plant communities studied, and were situated in a variety of matrices. P. australis sites were located in P. australis stands of various sizes and at various distances from the habitat edge. Most sites of all plant types were in moderate-density suburban communities, but several were within high-density urban areas. Agreement between different methods of calculating correlations between species abundance and the percent of a plant community present also demonstrate the robustness of these conclusions. Whether birds were counted anywhere in the circle, as in the correlation studies, or, as with the electivity index, associated only with the plant community in which they were observed produced the same results. Likewise, our results were unaffected by whether species richness was calculated as a continuous or a categorical variable, or whether >50% or >75% cover was used to characterize study circles.

Conclusions of "no effect" are always hard to demonstrate. When the number (N) is low, "no effect" can easily be due to the low power of the test. Using maxima rather than means dramatically increases N, appearing to increase the power of the test. However, given the long period of bird observations in our study, from April to September, we did not think that the use of maxima was appropriate. Low N may explain why two marsh species, Saltmarsh Sharp-tailed Sparrow and Virginia Rail, demonstrated no significant negative relationship to the amount of forest present. Failure of bird abundances to respond to changes in the percent cover of a plant community can also be due to the scale of measurement. Fifty-meter study circles enclose only about two acres. The robustness of our conclusions is supported, however, by the number of strong patterns detected. Despite low sample sizes, Virginia Rails had a strong, positive, and highly significant correlation with cattails. Common Yellowthroats, also detected in low numbers, had a highly significant Spearman rank correlation coefficient with percent forest. It would be inconsistent to accept findings where there was strong association and reject those findings where there was none.

Management

The impact of P. australis on birds has management implications. Ongoing salt marsh restoration projects in New England will likely result in the loss of significant areas of P. australis marshes. Benoit and Askins (1999) found no bird species of management concern significantly associated with P. australis habitats and therefore found no impediments to its removal. They did indicate that its removal would
benefit several species of management concern in Connecticut. While we found no reason for its removal, we found no critical use by species of management concern that would present impediments to its removal in north coastal Massachusetts. Both our study and the study of Benoit and Askins, however, only censused within the breeding season or immediately before or after it. Unpublished data indicate that American Bitterns are making regular use of *P. australis* stands in both Massachusetts and New Hampshire during November and December, sometimes in urban environments (R. Donovan, R. Kleiman, and T. Diers, pers. comm.). The American Bittern is a state-listed bird in Massachusetts. We need to know if American Bittern population size is limited by non-breeding habitat loss, and what alternatives exist in the region. Salt marsh restoration is going on in a changed, and often urbanized, context. Before we remove degraded salt marsh habitats, we need to know that alternatives to the habitats we remove still exist in the landscape. We have no quantitative information on this at this time.

**Future Work**

Several topics for future work remain. First, *P. australis* invasions may lead to increased elevation and drying of the marsh surface through peat and sediment accumulation, and possibly lead to the filling-in of pannes or ponds. Such marshes are likely less suitable habitat for waders, shorebirds, and waterfowl. Second, dense, tall *P. australis* stands may also make ponds and pannes less accessible to those species that depend on them. Quantitative work on these issues is needed. Third, while we cited study circles in a variety of matrices, an explicitly landscape-level study is still needed. The effect of differences in size, shape, and isolation of *P. australis* stands is unknown. Likewise, the effect of the landscape context is also unknown. Buchsbaum (1994), for example, suggested that *P. australis* marshes surrounded by urban development may function differently from those in other contexts, providing local refuge, or acting as buffers from human disturbance. Fourth, long-range research is needed on how *P. australis* invasions affect marsh processes and bird populations over longer time scales than the two years of the present study. Fifth, *P. australis* in the Massachusetts coastal zone may be playing an important stabilizing role in Red-winged Blackbird populations during the breeding season. We observed flocks of several thousand non-breeding Red-winged Blackbird males in *P. australis* during September 1997 and April 1998. When lost to predation, Red-winged Blackbird territory-holding males are quickly replaced from such flocks (Yasukawa 1981; Yasukawa et al. 1982; Beletsky and Orians 1993). The role of *P. australis* in stabilizing these populations needs further investigation. Finally, and most critically however, work is needed on the role of *P. australis* in non-breeding avian ecology. Large numbers of Tree Swallows use *P. australis* for pre-migration roosts, and American Bitterns may have come to depend on it during fall migration. The effect of changes in the availability and location of *P. australis* stands on the conservation of American Bitterns is especially important.

**Acknowledgments**

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**Sources of Unpublished Material**

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Donovan, R., Boston, MA.

Kleinein, R., Boston Harbor Basin Team leader, Office of Watershed Protection, Boston, MA.

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Microsatellite DNA Analysis of Native and Invading Populations of European Green Crabs

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Key words: Carcinus, population bottleneck, population genetics, microsatellite DNA

INTRODUCTION

The analysis of molecular genetic variation can potentially provide important insights into the invasion process, including identification of source populations, estimation of the number of founding individuals contributing to an invasion, and assessment of population dynamics following the initial introduction. To date, this potential has not been adequately explored as the majority of genetic studies have utilized molecular marker systems with relatively low levels of polymorphism (e.g., allozymes) or single locus systems with large associated sampling error (e.g., mitochondrial DNA). More recently developed molecular markers such as microsatellite DNA appear to be better suited for analysis of invading populations. Microsatellite DNA tends to be hypervariable, thus even introduced populations that undergo severe bottlenecks are expected to be polymorphic. In addition, microsatellite DNA is ubiquitous in eukaryotic genomes, so many independent markers can be assessed.

The worldwide invasions of green crabs in the genus Carcinus provide an excellent model system to evaluate the utility of a molecular genetic approach for the study of marine invasions. Green crabs native to Europe have invaded six regions in the last two centuries: Australia, Tasmania, eastern North America, western North America, South Africa, and Japan. Recent assessments of mitochondrial DNA variation for green crabs (Geller et al. 1997; McElligott and Geller, this volume) indicated the presence of two sibling species in Europe, Carcinus maenas on the Atlantic coast and C. aestuarii in the Mediterranean Sea. Interestingly, introduced populations in Japan and South Africa possessed mitochondrial haplotypes characteristic of both C. maenas and C. aestuarii, suggesting that these sites had been multiply invaded.

RESULTS AND DISCUSSION

We developed five microsatellite DNA markers for green crabs to complement and extend the mitochondrial DNA analyses. Our objectives for the microsatellite analysis were several fold: (1) to evaluate the hypothesis of cryptic Carcinus invasions posed by Geller et al. (1997) using an independent set of molecular markers; (2) to determine whether successful invasions were accompanied by a significant loss of genetic variability, and (3) to refine estimates of the geographic sources of each invasion.

Despite large genetic variability in native crabs (Figure 1), we were unable to detect significant genetic differentiation between sites on the Atlantic coast of Europe (western Spain and Netherlands) nor between sites in the Mediterranean Sea (southern France and Italy). Large allelic frequency differences were observed between native populations in the Atlantic Ocean and the Mediterranean Sea for most loci, and nearly fixed differences in the sizes of microsatellite alleles were observed at locus CM9 (Table 1). These microsatellite results supported earlier conclusions based on mitochondrial DNA analysis that Mediterranean and Atlantic populations repre-
sent sibling species, but did not provide further resolution of the native source regions for invading populations.

Invasions were accompanied by large reductions in average heterozygosity and the average number of segregating alleles per locus (Figure 1). Average heterozygosity for introduced populations was 7 to 31% less than for native populations, indicating that most introduced populations underwent relatively severe genetic bottlenecks. The population in South Africa was exceptional in that it retained much more diversity than other introduced populations (Figure 1). The number of segregating alleles in introduced populations was drastically reduced, and similarities in the distributions of remaining alleles indicated that western North America and Tasmania, the two regions that were most recently invaded, had been invaded by green crabs originating from introduced populations in eastern North America and Australia, respectively.

Introduced populations in Australia, Tasmania, eastern North America, and western North America possessed allelic distributions that were characteristic of C. maenas, the Atlantic form of green crab. Allelic distributions for the highly diagnostic locus CM9 revealed no evidence of C. maenas influence in the Japanese samples; however, 14% of the alleles were of a unique size class not observed previously in either form of native crab. Since Japanese crabs also demonstrated very little genetic variability relative to native populations (Figure 1), it appears unlikely that Japan was multiply invaded by both C. maenas and C. australis, as suggested by Geller et al. (1997). The presence of mitochondrial haplotypes characteristic of both C. maenas and C. australis in the Japanese population was likely a consequence of invasion from a single source population possessing both mitochondrial haplotypes. The South African population, which was also found to possess mitochondrial haplotypes of both Carcinus species (Geller et al. 1997), possessed microsatellite alleles that were characteristic of both sibling species (Table 1). In addition, the South African population was much more genetically diverse than other introduced populations (Figure 1), which is consistent with an hypothesis of multiple invasions from distinct sources. Thus, the microsatellite data appear to support previous mitochondrial data in suggesting that South Africa has been invaded by green crabs more than once. There was no evidence for cosegregation of microsatellite alleles and mitochondrial haplotypes in South Africa, suggesting that the two forms have interbred.

These results demonstrate the power of microsatellite DNA analysis for studying marine bioinvasions. Our data indicate that green crabs were able to successfully invade several regions despite losing large amounts of genetic variation and suggest that a new trend is emerging in which prior introductions have become stepping stones for new invasions.

<table>
<thead>
<tr>
<th>Locale</th>
<th>Allele Size Range (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>198-272</td>
</tr>
<tr>
<td></td>
<td>275-280</td>
</tr>
<tr>
<td></td>
<td>300-360</td>
</tr>
<tr>
<td>Netherlands</td>
<td>0.99</td>
</tr>
<tr>
<td>Western Spain</td>
<td>1.00</td>
</tr>
<tr>
<td>Southern France</td>
<td>0.06</td>
</tr>
<tr>
<td>Italy</td>
<td>0.07</td>
</tr>
<tr>
<td>Australia</td>
<td>1.00</td>
</tr>
<tr>
<td>Tasmania</td>
<td>1.00</td>
</tr>
<tr>
<td>Eastern North America</td>
<td>1.00</td>
</tr>
<tr>
<td>Western North America</td>
<td>1.00</td>
</tr>
<tr>
<td>Japan</td>
<td>0.14</td>
</tr>
<tr>
<td>South Africa</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Genetic characterization of native and introduced populations of additional marine bioinvading species is likely to be a fruitful research approach and will allow assessment of the generality of our results.

**Literature Cited**

The Use of Molecular Genetics to Investigate the Geographic Origin and Vector of an Invasive Red Alga

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Abstract: In 1996, a large red alga, Grateloupia doryphora, was recorded for the first time in Narragansett Bay, Rhode Island. Since its arrival, it has continued to spread and will likely have an effect on native biota. In an effort to identify the geographic origin and vector of the Rhode Island G. doryphora population, RAPD (randomly amplified polymorphic DNA) analyses and sequences of the nuclear ribosomal DNA internal transcribed spacer (ITS) region were used to compare G. doryphora individuals from Rhode Island with specimens from locations around the world. The RAPD and ITS data sets were highly congruent. These genetic markers revealed low levels of genetic variation within the Rhode Island G. doryphora population as well as within the populations located in Brittany, France, and Galicia, Spain. However, there was considerable genetic variation partitioned among populations. G. doryphora individuals in the Rhode Island population were genetically similar to specimens from Brittany, France; Portsmouth, England; and to some specimens from the Mediterranean, suggesting that one of these locations could be the origin of the Rhode Island population. Individuals in the Rhode Island population were genetically distinct from the Galicia, Spain; Oregon, USA; and other of the Mediterranean specimens. Additional specimens from other geographic locations are currently being screened.

Keywords: Grateloupia doryphora, red alga, origin of invasive species, ITS sequences, RAPDs

Introduction

Until recently, marine biological invasions by algal species have received little attention (e.g., Walker and Kendrick 1998; Baskin 1996; Ribera and Boudouresque 1995; Farnham 1980). Algal invasions are of great concern because introduced species can have serious ecological consequences by reducing or replacing native macroalgae, thus leading to changes in community structure and food webs (Walker and Kendrick 1998). Efforts to stop or reduce marine bioinvasions are facilitated by a knowledge of the origin and vectors responsible for the introduction. Although these have often been difficult to identify by traditional methods (Farnham 1980), molecular techniques that use DNA characters as genetic markers may make it easier to identify and trace the origin of invasive species.

In 1996, Grateloupia doryphora, a large red alga (Figure 1), was found attached to rocks, pebbles, and shells in the southern portion of Narragansett Bay, Rhode Island (Villalard-Bohnsack and Harlin 1997).

Figure 1. Grateloupia doryphora herbarium specimens. (A) Thalli showing foliose, undivided habit. (B) Single specimen showing basal division and marginal proliferations.

This was the first time this species had been recorded on the northeast coast of North America. Since 1996, the population has spread to new locations within Narragansett Bay and to several sites along the open ocean (Villalard-Bohnsack and Harlin submitted). The present distribution of G. doryphora includes the Pacific Ocean, Mediterranean Sea, and Atlantic
Table 1. Sources of Grateloupia doryphora specimens used in the present study.

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Code</th>
<th>Collector (Date)</th>
<th># Individuals Screened</th>
<th>RAPDS</th>
<th>ITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narragansett Bay, Rhode Island, USA</td>
<td>R</td>
<td>M. Viela-Kohrsack, M. Marston (1996-99)</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Brittany, France</td>
<td>F</td>
<td>J. Cabioch (1997-98)</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Galicia, Spain</td>
<td>S</td>
<td>J. Pérez Cirera López-Niño (1997)</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Seal Rock, Oregon, USA</td>
<td>O</td>
<td>G. Hansen (1998)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Basin of Thau, (Mediterranean) France</td>
<td>M</td>
<td>M. Verlaque (1998)</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Ocean from the English Channel to Angola and from Florida to Uruguay (Farnham and Irvine 1973; Dawson et al. 1964; André and Gayral 1961). This species has repeatedly been reported to be invasive. In 1969, specimens were observed in Portsmouth, England (Farnham and Irvine 1973); in 1982 it was recorded in the Mediterranean Sea (Ribera and Boudouresque 1995; Maiz et al. 1986); and, more recently, the species has been reported on the coast of Brittany (Cabioch et al. 1997) and in the Netherlands (Stegenga and Otten 1997). It is unclear how long G. doryphora may have been present in the other locations along the coasts of Europe and Africa.

Considerable morphological variation in blade shape and size exists within and among populations of G. doryphora. Blades, growing singly or in clumps, can range from 2 to 40 cm across and up to 2 m in length. Few morphological or anatomical diagnostic characteristics are available to distinguish specimens from different geographical locations. The origin of the Rhode Island population, therefore, would be almost impossible to determine based solely on morphology and anatomy. Nevertheless, morphologically indistinguishable populations may be genetically very divergent (van Oppen et al. 1996a; van Oppen et al. 1995). Migrations (e.g., van Oppen et al. 1995b), and recently to the study of the origin of introduced species (e.g., Jousson et al. 1998; Olsen et al. 1998). In this study, we examine whether molecular genetic techniques could be used to identify the geographic origin of the recently established G. doryphora population in Rhode Island. A knowledge of the geographic origin may allow us to identify the vector responsible for the introduction and aid efforts to prevent future introductions.

**Material and Methods**

**Specimens and DNA Isolation**

Sources of material used in our analyses are summarized in Table 1. DNA was extracted from 3-cm² sections of thalli. Tissue was carefully examined for the presence of epiphytes prior to use. DNA was extracted from fresh tissue, herbarium specimens, or silica-gel dried material using the CTAB method (Doyle and Doyle 1987). Tissue was ground to a powder in liquid nitrogen using a mortar and pestle and then mixed with 6 mls of hot (65°C) CTAB isolation buffer [50 mM Tris-HCL (pH 8.0), 700 mM NaCl, 10 mM EDTA, 3% (w/v) CTAB (SIGMA)]. After an hour incubation at 65°C, samples were cooled at room temperature for 10 min and then extracted with 5 ml of chloroform-isooamyl alcohol (24:1; v/v) for 10 min and centrifuged in a table-top clinical centrifuge to separate phases. After centrifugation, the aqueous phase was removed with a wide-bore pipet and DNA was precipitated by adding 2/3 volume of cold isopropanol. To assist in precipitation, samples were sometimes placed in a -20°C freezer for up to 2 hr. DNA was collected via centrifugation. The DNA pellet was washed twice with 76% ethanol containing 10 mM ammonium acetate to remove the CTAB. After air drying, the pellets were resuspended in either ddH₂O or 10 mM Tris-HCL (pH 8.0), 1 mM EDTA. DNA was analyzed on a 1% agarose gel.

**RAPDs**

Oligonucleotide primers of 10 bases were used to prime PCR reactions. Amplifications were performed in 50-µl volumes containing Taq reaction buffer (BRL), 1.9 mM MgCl₂, 100 µM dNTPs (BRL), 0.2 µM primer, 1.25 units Taq DNA polymerase (BRL), and 100-200 ng of template DNA. Mineral oil was added to each reaction tube prior to amplification. Reactions were carried out in a Thermolyne Temp*Tronic thermocycler with an initial denature
Table 2. Average within- and between-population RAPD genetic similarities based on Nei & Li's similarity coefficient. Bold numerals represent within-in population averages. A dash represents that only a single individual was examined.

<table>
<thead>
<tr>
<th>Population</th>
<th>Rhode Island</th>
<th>France</th>
<th>England</th>
<th>Mediterranean</th>
<th>Spain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(M4)</td>
<td>(M1,M2,M3)</td>
<td></td>
<td>(M1,M2,M3)</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>France</td>
<td>0.89</td>
<td>1.00</td>
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<tr>
<td>England</td>
<td>0.67</td>
<td>0.74</td>
<td>0.75</td>
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<tr>
<td>Mediterranean</td>
<td>(M4)</td>
<td>0.78</td>
<td>0.93</td>
<td>0.67</td>
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<tr>
<td>(M1,M2,M3)</td>
<td>0.23</td>
<td>0.26</td>
<td>0.23</td>
<td>0.27</td>
<td>1.00</td>
</tr>
<tr>
<td>Spain</td>
<td>0.24</td>
<td>0.27</td>
<td>0.16</td>
<td>0.29</td>
<td>0.28</td>
</tr>
</tbody>
</table>

* Two distinct banding patterns were observed in the Mediterranean population. Specimen M4 was genetically very distinct from specimens M1, M2, and M3.

step of 10 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 35°C, 3 min at 25°C and a final extension for 7 min at 72°C. All RAPD reactions were repeated at least once for each template/primer combination. Reaction mixes without DNA template were run as negative controls to check for contamination. After amplification, reaction products were separated by electrophoresis through a 1.0 or 1.2% TBE agarose gel stained with ethidium bromide and visualized and photographed under UV light. Presence or absence of bands was scored for all major bands. Minor or weak bands can be variable in expression, perhaps because they are products of nonspecific binding (Patwary et al. 1993); thus, they were excluded from the analyses. A data matrix containing the presence or absence of bands was generated and used to calculate pairwise RAPD similarities between each of the specimens using the Nei and Li's similarity coefficient: 

\[ S = 2N_{ab}(N_a + N_b) \]

where \( N_a \) is the number of amplified bands from specimen a, \( N_b \) is the number of amplified bands from specimen b, and \( N_{ab} \) is the number of matched bands between the two specimens.

**ITS sequences**

The primer pair TW81 (5-GGGATCCTTTTGCTAGGGTAAACCTCGG) and AB28 (5-GGGATCATATGCCTTAAGTTCAGGCGG) was used to amplify the ITS1, 5.8s ribosomal DNA (rDNA), and ITS2 region (Goff et al. 1994). TW81 anneals to the 3' end of the 18s rDNA, while AB28 anneals to the 5' end of the 25S rDNA. Amplifications were carried out in 50-μl volumes as described in Goff and Moon (1993). The reaction mix contained Taq reaction buffer (BRL), 1.5 mM MgCl2, 100 μM dNTPs (BRL), 0.2 μM of the TW81 primer, 0.2 μM of the AB28 primer, 1.25 units Taq DNA polymerase (BRL), and 50 - 250 ng of template DNA. Each reaction contained DNA from one individual. For each set of reactions, a control sample containing all reagents but lacking template DNA was included. The following cycling parameters were used: denaturation for 10 minutes at 97°C, 37 cycles of 95°C for 1.25 min, 68°C for 2 min, 72°C for 4 min, and a final 10-min extension at 72°C.

Products of all PCR reactions were visualized on a 1% agarose gel and the products from three different PCR reactions all containing the same primer/template combination were pooled prior to cloning. Products were cloned using a TOPO TA cloning kit (Invitrogen) following the vendor's instructions. Plasmid DNA was isolated using Qiagen-tip 20 or GFX Micro Plasmid Prep (Pharmacia Biotech). For each individual, two to three clones were sequenced using an ABI Prism 377 automated sequencer. Sequences were aligned using ClustalW version 1.7 (Thompson et al. 1994). Using the aligned sequences, nucleotide distances were calculated following the Kimura two-parameter model (Kimura 1980) and parsimony analysis was performed using the PAUP software package (Version 4.0b4a; Swofford 1991).

**RESULTS**

**RAPDs**

Thus far, four arbitrary primers have been used to compare banding patterns among G. doryphora individuals from Rhode Island, Spain, France, England, and the Mediterranean. The major banding pattern for a particular combination of primer and template DNA was reproducible in repeated amplifications. The four primers amplified a total of 38 loci, 36 of which were polymorphic. Bands ranged in size from 500 to 2000 bp.

The RAPD banding patterns of individuals from the same population were very similar to one another, with similarity coefficients ranging from 0.75 to 1.00, where 1.00 indicates that the patterns are identical and 0 indicates that there are no bands in common (Figure 2, Table 2). The one exception was the Mediterranean population where two distinct banding patterns with a similarity coefficient of 0.27 were observed (Figure 2).

Even though there was little genetic variability
within most of the populations, RAPD markers did reveal that there is substantial genetic diversity partitioned among the populations, with the specimens falling into three distinct groupings (Table 2). The first group includes individuals in the Rhode Island, USA; Brittany, France; and Portsmouth, England, populations and one of the specimens from the Mediterranean population (M4). These individuals are genetically similar to one another, with pairwise similarity coefficients ranging from 0.67 to 0.93 (Table 2). This first group is genetically distinct from the second group, which consists of individuals collected in Spain, and from the third group, which includes Mediterranean specimens M1, M2 and M3. The average similarity coefficients between groups ranges from 0.16 to 0.29 (Table 2). We are continuing to test different primers as well as more individuals from each of these populations and additional geographic locations.

**ITS Sequences**

The ITS2 region from selected individuals in each population was sequenced (Marston and Villalard-Bohnsack, in prep.). This region ranges from 338 to 373 nucleotides for these *G. doryphora* individuals. All of the sequences were easily aligned. Parsimony analysis produced a single most parsimonious tree of 140 steps with a consistency index (CI) of 0.97 and a retention index (RI) of 0.97. Individuals from the Rhode Island, French, and English populations, as
Table 3. Pairwise distance comparison of sequence variation in *Graetlearia doryphora* individuals from aligned ITS2 sequences.

<table>
<thead>
<tr>
<th>Specimen</th>
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<th>M3</th>
<th>E2</th>
<th>F3</th>
<th>F2</th>
<th>R6</th>
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<th>F1</th>
<th>M4</th>
<th>O1</th>
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well as one individual from the Mediterranean (M4), have exactly the same ITS2 sequence and thus form a distinct clade on the tree (Figure 3). The specimen from Oregon is closely related to this group (Table 3; Figure 3). However, the two individuals from Spain form another distant clade, as do two of the individuals from the Mediterranean (M1 and M3) (Figure 3). Currently, we are analyzing sequences obtained from *G. doryphora* individuals collected in additional locations.

**DISCUSSION**

In this study, we are using RAPD analyses and ITS sequences both to examine the genetic variation within *Graetlearia* populations and to help identify the geographic origin of the newly established *G. doryphora* population in Rhode Island. To this end, we have thus far examined specimens from Rhode Island, USA; Portsmouth, England; Brittany, France; Galicia, Spain; and the Mediterranean. Although herbarium specimens from these locations are morphologically indistinguishable, substantial genetic variation is revealed by RAPD and ITS sequence analyses, and these two data sets are highly congruent.

RAPD analysis is based on the polymerase chain reaction (PCR). It uses short, single oligonucleotide primers of arbitrary sequence to amplify regions of genomic DNA, although the identity of the amplification products usually is not known (Williams *et al.* 1990). These products are separated by gel electrophoresis and then scored based on the presence or absence of bands. The analysis of RAPD data is not always straightforward, due to either technical limitations (*e.g.*, reproducibility of banding patterns) or assumptions made during the analysis (*e.g.*, bands at the same position on the gel are homologous) (see van Oppen *et al.* 1996b; Weising *et al.* 1995). Nevertheless, while results based on RAPDs should be interpreted with caution, they can provide a useful estimate of genetic variation among closely related taxa.

![Tree diagram](image)

**Figure 3.** The single most parsimonious tree of ITS2 sequences constructed using the heuristic search option in PAUP with 100 replications of random addition sequences. Numbers above the branches are bootstrap values (500 reps). M, specimens from the Mediterranean; F, Brittany, France; E, Portsmouth, England; R, Rhode Island, USA; O, Oregon, USA.
Our RAPD analyses indicate that there is very little genetic diversity present within the Rhode Island G. doryphora population. Although we are limited by the small number of individuals available to us from the other populations, low levels of variation were also observed within the French, English, and Spanish populations. Our preliminary data suggest that there is more genetic variation partitioned among populations than within a population or geographic area. The one exception is the Mediterranean population where two genetically distinct groups were detected.

Both ITS and RAPD data sets reveal the same relationships among the populations we have sampled. Individuals in the Rhode Island population are genetically similar to specimens collected in Brittany, France; Portsmouth, England; and to some specimens from the Mediterranean, suggesting that one of these locations could be the origin of the Rhode Island population. The Rhode Island population is genetically distinct from other Mediterranean samples and from specimens collected at Galicia, Spain, and Oregon, USA. These data have allowed us to eliminate possible geographic origins (i.e., Spain, Oregon), but cannot be used to positively identify the origin. Apparently, there has been too little time since the separation of some of these populations for variation to arise in the ITS region. It is possible that by using more RAPD primers and a larger sample size or another genetic marker (e.g., microsatellites), we may be able to distinguish these genetically similar populations. This research is ongoing and we are beginning to screen G. doryphora individuals from other geographic locations (e.g., California, Alaska, and Japan).

At this point, the vector responsible for the introduction is not known. However, based on the pattern of ship traffic in Narragansett Bay, the direction of water movement in the Bay, and the locations of the first G. doryphora populations, it seems likely that either hulls of ships or ballast water dumping was responsible for the introduction. Using RAPD markers and ITS sequences, we are establishing a genetic baseline of the distribution and level of genetic variation present in different geographic populations. This information can be used to monitor not only the Rhode Island G. doryphora population but any future introductions of this species.

Acknowledgments

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**SOURCES OF UNPUBLISHED MATERIAL**

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Determining the Pathways of Marine Bioinvasion: Genetical and Statistical Approaches

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ABSTRACT: Advances in genetic technology have enabled biologists to reconstruct the history of populations, their evolutionary relationships, and geographical origins. Such information is essential in understanding the biology of invasions and in designing successful management responses. Unfortunately, the wholesale transfer of “traditional” population genetic methodology to identify the origins of marine bioinvasions is inappropriate. By definition, invading populations are characterized by rapid and recent range expansion. This has two important genetic consequences: (1) genetic diversity is often very low due to the bottlenecks in population size associated with the founding of new populations, and (2) evolutionary relationships among genes may bear no relation to the history of populations. These characteristics of invading populations limit our ability to reconstruct their geographic history. The situation is further complicated by the fact that many bioinvasions occur as a dynamic series of sequential or overlapping invasion events, the totality of which can be termed a metainvasion. Here, we evaluate the genetic markers and statistical methods currently being used to determine invasion pathways. Analyses of molecular genetic data fall into two categories: those based on phylogenies, and those based on frequency differences of genetic markers. We describe these two approaches and outline the conditions under which they are appropriate and useful in marine bioinvasions. We also outline recent technical and analytical developments that may assist in the study of marine bioinvasions.

Key words: multilocus, genetic, DNA, population, origin of invasive species

INTRODUCTION

Following the discovery of an alien species, nearly all attempts at eradication or control (including sterile male release, biological control, and the use of transgenic plants) have a higher probability of success when source regions and the demographic parameters of invading and source populations are known. For example, very different management measures are required when invasions involve a species with panmictic population structure, compared to those that involve a species with sharply defined and genetically isolated populations (Carey 1991). Here we review genetic methods of determining the source of a bioinvasion. We begin with a brief description of the problem and evaluate the various genetic tools that are available for reconstructing invasion pathways. Next, we review the statistical developments that are needed to make sense of the new genetic data. Finally, we propose some future developments that might help to elucidate the invasion process.

MAPPING A METAINVASION

When an invading species is recognized in a particular location, assigning the invading individuals to a source population is an important task (Davies et al. 1999a; Roderick et al. 1998). Unfortunately, many bioinvasions are hierarchical, consisting of several sequential or overlapping invasion events, which together constitute a “metainvasion” (Davies et al. 1999b). In many cases metainvasions are global in nature and any single invasion will often have multiple, genetically similar potential sources, many of which are likely to have been recently established themselves. Species that spread in association with human activity, especially invasive species, are likely to be characterized by newly established populations
and these represent a severe obstacle for genetic analysis. First, new populations are often genetically impoverished due to the population bottleneck associated with colonization (Nei et al. 1975), making it hard to find sufficiently variable markers. Second, what little variation is present tends to be ancestral, rendering phylogenetic methods less appropriate or meaningless. These are the principal challenges faced by those seeking to identify the source of marine bioinvasions and to reconstruct their invasion pathways.

Allozyme electrophoresis is a useful technique for rapidly assessing the similarity of various populations; however, only a small amount of the underlying genetic variation at any given locus can be discerned using protein electrophoresis. Consequently, allozymes have provided only very limited resolution in invading populations of species such as the medfly, Ceratitis capitata (Huettel et al. 1980; Roderick 1996a). A number of workers have identified heritable markers that reveal genetic variation at the DNA sequence level for reviews see (Geller 1996; Palumbi and Baker 1996; Roderick 1996b), with mitochondrial DNA being the most commonly used genetic marker in population studies (Avise 1994).

Unfortunately, the reduction in diversity associated with colonization bottlenecks is exacerbated for mitochondrial genes because they have only a quarter of the effective population size (Ne) of nuclear genes (Moore 1995). In the medfly, C. capitata, for example, most New World populations are less than 100 years old and have a single high frequency and a single low frequency haplotype. By contrast, ancestral C. capitata populations in Africa display up to six haplotypes (Gasparich et al. 1997). Fortunately, new markers, such as microsatellites (Queller et al. 1993; Weber and May 1989), introns (Palumbi and Baker 1994), randomly amplified polymorphic DNA (RAPDs) (Welsh and McClelland 1990; Williams et al. 1990), and restriction length polymorphisms (RFLPs) (Aquadro et al. 1992) assay nuclear DNA variation. These markers have revealed high levels of diversity in ancestral and invading populations, even when mtDNA and allozymes are relatively impoverished (Baruffi et al. 1995; Villablanka et al. 1998). The greater variability of these markers can reveal population structure over a much finer scale. For example, genetic analysis of DNA sequence variation at four intron loci revealed significant population structure among previously indistinguishable (Gasparich et al. 1997) C. capitata populations in California, Central America and eastern South America (Davies et al. 1999b).

STATISTICAL DEVELOPMENTS IN THE ANALYSIS OF BIOINVASIONS

As the practical difficulties of finding suitable genetic markers have been overcome, severe theoretical problems became apparent. First, new populations violate the assumptions of equilibrium integral to most population genetic theory (e.g., methods of estimating gene flow (Slatkin and Barton 1989). Second, phylogenetic methods that reconstruct the historical biogeography of relatively well established populations (Avise 1994; Roderick 1996b) seem inappropriate in the latter phases of a metapopulation where events are all very recent. For example, in the case of a secondary invasion event (a new invasion originating from another invading population), the likely sources are populations that were themselves only recently established as part of the primary invasion. Phylogeographic structure (Avise 1989; Avise 1994; Roderick and Gillespie 1998; Roderick and Villablanka 1996) is not expected in such recently founded populations because there has been little time for mutations to occur, and the relationships among alleles mostly reflect evolutionary events in the ancestral range of the species rather than their history in newly occupied areas (Davies et al. 1999a; Villablanka et al. 1998). For example, McGuigan et al. (1998) reported significant differences in haplotype frequencies over a fine geographic scale among Australian frog, Litoria pearsoniana, populations; however, a smaller and insignificant F-statistic was obtained when the genetic distance among alleles was considered.

With the traditional approaches being of only limited use for invasion biologists, it is fortunate that a new generation of statistical analyses have been developed based on multilocus genotype data. One of the first applications was in fisheries management, where mixed stock analysis (MSA) was developed to determine fish catch composition, mainly using allozyme data. In MSA, maximum likelihood is used to estimate the combination of potential source populations that best explain the observed allele frequencies in a catch (see Utter and Ryman 1993). Methods, such as MSA, that focus on populations are limited by the need to define those populations a priori and in doing so they risk missing individuals that have an unusual origin. An alternative approach focuses on
the most likely origin of an individual, or rather its multilocus genotype, and is known as an assignment test (Paetkau et al. 1995).

Assignment tests use maximum likelihood to assign individual genotypes to potential sources based on the allele frequencies of the source populations. These tests are particularly useful for determining the origin of an individual when there are multiple, genetically similar candidate sources. For example, chinook salmon, Oncorhyncus tshawytscha, occur as different temporal populations (runs) that spawn in the same river but at different times of the year. Runs are genetically very similar but have such distinct life histories that some are considered separate (and endangered) species. Microsatellites have been used to assign chinook salmon, O. tsawytscha, to particular runs (Banks et al. 1996).

There are two major sources of error associated with the population level data used in an assignment test. First, observed allele frequencies are estimates, so sampling error must be considered. Second, differences in genetic diversity among potential source populations can cause a bias because the likelihood of drawing any genotype is inversely proportional to the diversity of the population from which it is drawn. Rannala and Mountain's (1997) assignment test takes into account the sampling error associated with estimating allele frequencies and the differences in diversity among two potential sources (Davies et al. 1999a). Although Rannala and Mountain's (1997) test applied much needed statistical rigor to source estimation, further modifications of the test are still needed. First, laboratory scoring mistakes must be taken into account. For example, scoring errors occur in allozyme studies with a frequency of about 1% (Lathrop et al. 1983). One can attempt to correct for scoring mistakes prior to analysis, as we did for the errors associated with sequencing cloned polymerase chain reaction (PCR) products. Alternatively, an error rate factor may be incorporated into the analysis; such an approach was used by Marshall et al. (1998) in their multilocus paternity test. A second source of error that should be considered is the implications of not sampling all the potential sources. Again with a focus on paternity testing, Marshall et al. (1998) presented a simulation method to assess the likelihood that a more probable source remains unsampled. Finally, assignment tests focus on the origin of single multilocus genotypes, although bioinvasions usually consist of multiple invading individuals, each of which will have their own associated likelihood of being from one source or another. With such multiple assignments, one will be able to plot a distribution of likelihood statistics for the invading population as a whole, adding a new level of complexity to source estimation. For example, one interpretation of a bimodal distribution is that the invading population had two sources, but how can one assess the significance of such a conclusion, and how should one correct for multiple comparisons? Such issues are currently being examined in our laboratory using computer simulation (Bohonak et al., in prep.).

Multilocus genotyping is a powerful technique and many different markers, including allozymes, mtDNA, and microsatellites can be analyzed simultaneously. In most cases additional markers will increase the power of these tests; however, some markers may be incompatible with this approach. RAPDs, for example, are very useful in providing high levels of genetic variation and their main advantage over introns and microsatellites is that they can be applied with very little prior genetic knowledge of a species (Williams et al. 1990). Unfortunately, it is not clear how RAPD data can be incorporated into the same statistical framework as introns and other markers, where genotypes can be identified at each locus.

Although we have stated that phylogenetic approaches are less likely to be useful in many bioinvasions (Davies et al. 1999a; Roderick et al. 1998; Villablanca et al. 1998), recent data have suggested that phylogenetic data can still be used to help determine sources (Davies et al. 1999b). Somewhat to our surprise, we found that including an estimate of the genetic distance among alleles revealed a higher level of population structure among American C. capitata than estimates based solely on allele frequencies (Davies et al. 1999b). This was surprising because C. capitata only colonized the Americas this century, providing little time for the evolutionary divergence of alleles among American populations. It is possible that phylogeographic structure might reflect multiple colonizations—one of several possibilities we are exploring using computer simulations (Bohonak et al., in prep.)

It is possible, therefore, that phylogenetic information might turn out to be useful even in the later stages of a metainvasion. This is especially likely if genetic markers are found with very high evolutionary rates. We have proposed (Davies et al. 1999a) that extragenomic markers (EGMs), such as viruses, be
used to reconstruct invasion pathways. The RNA genomes of many viruses evolve extremely quickly (Holland et al. 1982) and can be used to reconstruct the geographic history of their hosts (Ho et al. 1993) (Yanagihara 1994). Another possible set of markers that provide phylogenetic information and evolve rapidly are transposable element polymorphisms (TEPs). We are currently working with groups in Greece and Italy to explore the utility of TEPs for the C. capitata metainvasion. Transposable elements occur widely in nature (Li 1997) and could be applied to marine invasive species should they prove successful in model terrestrial systems such as the medfly.

If EGMs or TEPs do become widely used, approaches that utilize phylogenetic data to detect gene flow (Slatkin 1994) might be applicable. Assignment tests might be modified to consider the relationships among alleles in addition to their frequencies. Currently, assignment tests do not consider the relatedness of alleles because most multilocus markers such as microsatellites (Glenn 1998) do not easily permit the phylogenetic analysis of alleles. However, sequence data is much more phylogenetically informative, and with introns, EGMs, and TEPs becoming more widely used, restricting analysis to mere allele frequencies may waste useful information. A new test should assess the multilocus likelihood of sampling a given set of alleles from a potential source population based on the distance between alleles as well as their frequencies.

To conclude, the new genetic markers and statistical methods briefly described here will reveal much about the spread of invasive species. Bioinvasion genetics will not provide all the answers, but combined with ecological data, we might eventually be able to determine the common characteristics of invasive species and to identify the pathways that allow them to spread around the globe. Ultimately, invasion biology needs to become a predictive science, identifying species that are likely to invade, and those that will become established should they reach a new area. Such information would finally enable managers to adopt truly proactive policies.

Acknowledgments

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Atlantic Salmon (Salmo salar) in British Columbia

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Abstract: Farmed salmon is British Columbia's largest agri-food export product. Approximately 80% of production is Atlantic salmon (Salmo salar), an exotic species on the Pacific coast. Large scale escapes and small scale "leakage" of adults from marine net pens are not uncommon and lead to over 2600 marine and 150 freshwater reports of Atlantic salmon in B.C. waters in 1997 alone. The continuous addition of free ranging adult Atlantic salmon into the coastal environment combined with the weak state of many native Pacific salmon (Oncorhynchus spp.) stocks has been suggested to enhance the likelihood of colonisation. We present preliminary results of our work conducted to delineate what, if any, ecological or genetic impacts are associated with aquaculture escapee Atlantic salmon on native Pacific salmon species. We also present evidence that suggests colonisation may be occurring on a Vancouver Island river, an event which would mark the first anadromous expansion of the species beyond its native range.

Keywords: Atlantic salmon, aquaculture, Pacific Northwest, niche competition, steelhead

Introduction

Atlantic salmon (Salmo salar) account for approximately 80% of finfish aquaculture production in British Columbia (B.C.) and is the largest agri-food export of the province. The increasing frequency of S. salar observed in coastal marine waters and rivers has generated a lively debate regarding the eventual fate of this species along the Pacific coast of North America. At present, empirical data regarding potential genetic and ecological impacts of escaped Atlantic salmon in this region are nonexistent. Thus, any current predictions regarding the persistence of free-ranging S. salar and what effects may result are not robust.

S. salar is endemic to most countries with drainage into the North Atlantic Ocean and Baltic Sea (Mills 1989). This encompasses an area roughly from Portugal to the Arctic Circle and from Iceland to southern Greenland to Labrador south to the Connecticut River. Introductions of S. salar have been documented in every continent save Antarctica; the vast majority of these introductions have ended in failure (MacCrimmon and Gots 1979; Alverson and Ruggerone 1997; McKinnel et al. 1997). Indeed, a self-supporting anadromous population of S. salar has never been established outside the species' native range.

Only two notable successful introductions outside the native range have been recorded—one in Argentine Patagonia, the other in New Zealand—both are nonanadromous (MacCrimmon and Gots 1979). In North America, success of S. salar introductions has followed a similar pattern and has been limited to a number of small oligotrophic lakes in eastern Canada and northeastern United States (MacCrimmon and Gots 1979).

The first transfer of S. salar to western Canada occurred in 1905 when 90,000 Atlantic salmon eggs from New Brunswick were transferred to the Fraser River Hatchery and the Cowichan Hatchery on Vancouver Island (Prince 1905 reviewed by MacCrimmon and Gots 1979; Carl and Guiguet 1958). The fry were released into three lower mainland rivers and six Vancouver Island systems (Carl and Guiguet 1958). Importation of eggs from New Brunswick and later Scotland continued until 1933. Estimates of total number stocked (mostly eyed eggs and alevins) are not consistent, having been reported as 5.5 million (Castledine 1991), 6 million (Needham 1995),
>7.5 million (Burt et al. 1992 in Alverson and Ruggereone 1997), and 13.2 million (McKinnell et al. 1997). Although some returning adults were recovered in the Cowichan River system (MacCrimmon and Gots 1979), these introductions failed to establish a permanent population.

The seeming inability of Atlantic salmon to establish viable populations when deliberately planted suggests that the potential to do so via farmed escapées is remote. However, failure of these early attempts is likely attributable to causes that would have undermined the potential success of any species’ introduction. Historical introductions of *S. salar* were made into mature, stable environments that were saturated with predators and niche-equivalents competitors (*i.e.*, Pacific salmonids) and as a result tended to be more resistant to a biotic invasion (reviewed in Pinn 1991). Predation and interspecific resource competition would have been much more severe than is likely today, when, because of severely depressed native stocks, progeny of present-day *S. salar* grow in underutilized habitat, which is more accessible to colonization. Historically, Atlantic salmon were released at early life history stages (egg and fry) that experience naturally high mortality. Large, healthy, immunized adults are the norm today. The two scenarios, historical and present-day, are clearly different, and using the outcome of one to predict the fate of the other is not valid.

**Aquaculture escapes**

Escapes of *S. salar* from marine net-pens in the eastern Pacific often occur as large-scale escapées, typically due to weather events, human error, and predators (Alverson and Ruggereone 1997). Unaccounted-for losses (leakage, predation, unrecovered mortalitites, etc.) have been estimated to be between 10 and 30% of the cage population (Moring 1989). From 1991 to 1997, 28 escape events involving 162,453 salmon were reported at B.C. marine net-pens (Thomson and Candy 1998). Reporting of escape events is a mandatory condition of a farm license; however, there are no mechanisms to evaluate compliance and therefore reported escape numbers should be considered as minimum values.

In British Columbia, the first free-ranging *S. salar* was caught in 1987 (one year before the first reported escape) (McKinnell et al. 1997). Reported marine captures in B.C. waters peaked in 1993 at 4,543 fish (Thomson and McKinnel 1994). Capture data are compiled opportunistically from various management databases and voluntary reports by commercial and sports fishers. As such, these data, like escape data, are considered to be minimum values only and likely do not reflect the actual number of captures. *S. salar* often go unreported; commercial crews and to a lesser extent sport fishers no longer consider the capture of a *S. salar* noteworthy and often do not go through the trouble of reporting it to authorities (Volpe, pers. obs.). *S. salar* landed commercially may be disposed of through unofficial channels or are frozen and used for halibut bait (Volpe, unpublished data).

**Evidence of Natural Reproduction of Escapées**

In 1997, Atlantic salmon were reported in 40 freshwater systems in British Columbia (34 on Vancouver Island). Again, because most of these data are collected opportunistically, this figure should be considered as a minimum only. To date, three river systems, all on the northeast coast of Vancouver Island, have been identified as supporting juvenile Atlantic salmon: the Tsitika River (Volpe et al., 2000), Amor de Cosmos Creek (Volpe, pers. obs.), and the Adam River (Volpe, pers. obs.). At least two age-classes are present in both the Tsitika River and Amor de Cosmos Creek. Parr estimated to be one-year old have been observed in the Adam River. To date, scales and otoliths from only the Tsitika River fish have been examined and were consistent with these fish being wild reared (Volpe et al., 2000). Further, there are no commercial aquaculture activities of any kind on or around any of these systems, therefore, the only logical explanation for the presence of these fish is as products of natural spawning events of aquaculture escapées. The presence of multiple age- and size-classes raises the possibility of previous year-classes having successfully reared and smolted undetected. At present, there is no way to discriminate between wild-reared adult *S. salar* and escaped adult *S. salar* in the field. Therefore, there is no way to easily discern if feral progeny are successfully completing their life cycle.

**Ecological Concerns**

Domesticated *S. salar* may not be behaviorally adapted for interspecific competition and other challenges of a natural environment (Olla et al. 1994). Dickson and MacCrimmon (1982) noted differences
in behavioral patterns between hatchery and wild-reared salmon, which they suggested may account for poor survival of planted *S. salar*. Many studies have reported similar results (reviewed by Hindar 1994). Due to intense artificial selection to repress wild traits (in contrast to supplemental hatchery programs), it is possible that behavioral deficiencies may be even more pronounced in aquaculture escapee salmon (Gausen and Moen 1991; Olla et al. 1994). However, each farm generation undergoes selection for traits appropriate to the B.C. coastal environment. As long as *S. salar* are reared in open net-pens, selection for a “Pacific strain” of Atlantic salmon will continue. Further, typical aquaculture production fish are already proven capable of spawning in a controlled west coast stream channel (Volpe; unpublished data) and in the wild (Volpe et al. 2000, Volpe, per. obs.). If spawning events continue to occur, adaptation will be hastened by natural selection altering the behavioral or phenotypic profile of the feral population. Released from old constraints and under a radically different selection regime, adaptive changes can occur rapidly in invading populations (Carrol and Dingle 1996). For instance, American shad (*Alosa sapidissima*), when introduced from the Atlantic to the Pacific, evolved novel, locally adapted life histories in less than a century (Shobrige 1977, reviewed by Dingle 1980).

Steelhead (*Oncorhynchus mykiss*) and Atlantic salmon share similar environmental requirements, habitat preferences, growth rates, and life histories (Biewy and Moring 1988). Therefore, we predict that any negative effects resulting from the presence of *S. salar* in B.C. will manifest first among sympatric *O. mykiss*. We are aware of only three studies that directly compare competitive ability of juvenile *S. salar* and *O. mykiss* (Gibson 1981; Hearn and Kynard 1986; Jones and Stanfield 1993). All three concluded that *O. mykiss* were more aggressive than Atlantic salmon. However, these results are confounded by their experimental design. All three investigations did not quantify intra- versus interspecific competition, therefore, defining unambiguous interaction terms is not possible (Underwood 1986; Fausch 1998). Our data, generated in the laboratory and the field, suggest that *O. mykiss* individuals are indeed more aggressive than *S. salar* individuals. However, *O. mykiss* individuals are much more likely to attack a conspecific than an Atlantic salmon (Volpe, unpublished data). Thus, while *O. mykiss* individuals are aggressive, agonism is unlikely to result in *S. salar* being competitively excluded.

**Genetic Concerns**

Potential genetic concerns of *S. salar* on the west coast center on the possibility of *S. salar* x *Oncorhynchus* spp. hybridization events. Laboratory trials of all possible crosses involving *S. salar* and *Oncorhynchus* spp. in coastal British Columbia demonstrated that the most successful combinations in terms of the survival of F1 to hatching would involve steelhead females (6.07%) and pink salmon (*O. gorbuscha*) males (0.36%) (reviewed in Alverson and Ruggerone 1997). Therefore, although hybrid progeny can be produced in a laboratory environment, survivorship is poor in all cases and reproductive viability of the few F1s produced remains unknown. It should be noted, however, that these data were extracted from a small, unpublished pilot study and this issue awaits rigorous examination.

**Conclusions**

Current methodologies used to estimate *S. salar* abundance in the wild provide minimum values only. The actual numbers of individuals remain undefined. Invasion potential of escaped *S. salar* cannot be assessed until reliable estimates of escapes and captures are generated or until colonization occurs, at which point the issue is moot.

Historical anecdotes cannot be used in a predictive context. The failure of sporadic attempts to introduce fry and eggs over half a century ago bears no relationship to the present continuous introduction of robust adults.

When a species colonizes novel territory, adaptive modification of the phenotype to a more appropriate state is possible. Selection pressure and thus rate of change is likely to be greatest during the initial generations of the colonization. Predicting the fate of *S. salar* in the Pacific based on static models (derived from analyses of domestic fish only) is likely to be increasingly valid as colonization continues.

**Acknowledgments**

We would like to thank the organizers of the First National Conference on Marine Bioinvasions for their exemplary efforts and for allowing us to bring this issue to the attention of our colleagues. This work was supported by a grant to JPV from the B.C. Habitat Conservation Trust Fund.
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Biomonitoring of an Aquacultured Introduced Seaweed, *Porphyra yezoensis* (Rhodophyta, Bangiophycidae) in Cobscook Bay, Maine, USA

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Abstract: The intentional introduction of organisms for the purpose of mariculture requires a balance between minimizing ecological impact and maximizing economic gain. Phycogen Inc. (formerly Coastal Plantations International) has commercially farmed an introduced species of nori, *Porphyra yezoensis*, in Cobscook Bay, Maine, for the past eight years. Permits were granted based on the presumed inability of this seaweed to sexually reproduce under Gulf of Maine temperature regimes. *P. yezoensis*, a cultivar from Japan, has been grown by Phycogen at both a nursery and a grow-out farm site since 1991. A monitoring program was begun in 1996 to examine the potential dispersal and establishment of *P. yezoensis* around the sites. This report describes our monitoring program’s results for the nursery farm site at Huckins Ledge from 1997 and 1999. *Porphyra* samples were collected from 10-m intertidal transects and artificial substrates constructed of Japanese netting. *Porphyra* species were identified using morphological characteristics and isoenzyme electrophoretic markers. Collections from transects and artificial substrates at this site suggested that *P. yezoensis* only recruited ephemerally during Phycogen’s summer/autumn growing season and did not overwinter. The overwintering potential of *P. yezoensis* was further examined by deploying established blades attached to Japanese netting in the field from December until March. *P. yezoensis* was not observed on the netting the following spring. Based upon the results at Huckins Ledge, *P. yezoensis* does not appear able to establish a permanent population. However, data from Phycogen’s grow-out site at Mathews Island is still being examined and will be reported in a future publication.

Key words: *Porphyra*, monitoring, Maine, aquaculture

Introduction

*Porphyra* is the second most widely cultivated seaweed in the world. Currently, over 900,000 mt worth over $1.5 billion dollars are cultivated in Japan, China and Korea annually (Hanisak 1998). *Porphyra* is grown for the production of “nori”, which is eaten as dried sheets in soups and sushi. The first attempt to farm a nonindigenous *Porphyra* species in North America occurred in Washington state in the 1980s. Although cultivation attempts were successful, this early effort ultimately failed due to permitting difficulties (Mumford 1990). In 1990, Phycogen Inc., of Portland, Maine (formerly Coastal Plantations International), received state, federal, and international (International Council for the Exploration of the Sea) permits to introduce and farm *P. yezoensis*, a species native to Japan, in the Cobscook Bay region of Maine (Levine 1998).

Permits were granted for the aquaculture of *P. yezoensis* in the Gulf of Maine based on temperature restrictions in its reproductive cycle. *P. yezoensis* demonstrates a heteromorphic alternation of generations typical of the genus, with a haploid foliose blade phase alternating with a diploid filamentous conchoecis phase. The diploid conchoecis phase requires temperatures exceeding 28°C to mature sexually and release conchoecides, which give rise to the...
haploid blade phase (Melvin et al. 1986). P. yezoensis was thought to be incapable of sexual reproduction in the Gulf of Maine due to this temperature requirement. Haploid blades, however, can also reproduce asexually by releasing monosporangia during the Gulf of Maine’s summer temperatures (12-16°C), which coincide with Phycogen’s summer/fall growing season. This raised concerns regarding the potential establishment of this seaweed in the local intertidal via asexual reproduction, especially in the vicinity of Phycogen’s nursery farm site where juvenile blades would be producing the greatest numbers of monosporangia. The present study was undertaken to monitor the establishment of P. yezoensis near Phycogen’s farming sites and is one of the few studies to examine an aquacultured introduced seaweed.

Phycogen initially utilized two sites for its nori farming operation: a grow-out site at Mathews Island, northwest of Eastport, Maine, and a nursery site at Huckins Ledge, southwest of Eastport (Figure 1). Farming was conducted at Mathews Island from 1991 to 1997, until the grow-out nets were moved and combined with nursery nets at the Huckins Ledge site in August 1997. Farming at Huckins Ledge was continuous until May 1998. P. yezoensis was aquacultured seasonally during favorable conditions, usually in the late spring/early summer and in the autumn/early winter. A monitoring study was initiated at Phycogen’s sites in August 1996. The ability of P. yezoensis to recruit at the nursery farm site was evaluated by sampling Porphyra blades from intertidal transects and artificial substrata we constructed of Japanese netting strung between poles. Porphyra blades have simple morphologies and species can be difficult to discern. In order to identify and distinguish P. yezoensis from the six species described for the North Atlantic we used both morphological traits (Bird and McLachlan 1992) and isoenzyme electrophoretic markers. Isozyme markers have proven useful for distinguishing Porphyra species in previous studies (Lindstrom and Cole 1990, 1992a,b). Data from our monitoring program at the Huckins Ledge nursery farm site from August 1997 until July 1999 will be addressed in this paper.
settlement during nori farming. The netting is constructed of synthetic fibers to which Porphyra spores readily attach (Mumford and Miura 1988). Netting pieces (2 m x 1 m) were suspended vertically between 3-m metal posts, approximately 0.5 m off the ground (Figure 2). Eight artificial substrata were placed at low- and high-tide marks at four locations (A, B, C, and D) in the vicinity of the farm site (Figure 1). Artificial substrata and transect samples were collected seasonally from August, 1997 to July 1999.

Porphyra species identification was first attempted using classical features (thallus shape, size, cell height, number of cell layers and patterns of reproductive cells when available) described in the key of Bird and McLachlan (1992). After initial examination, isozyme electrophoresis was used to identify a set of random samples and any questionable samples to support visual identification. A modified starch-polyacrylamide isoenzyme electrophoresis gel system was used with a Tris-citrate buffer (Cheney and Babbel 1978; Cheney 1985). Although several enzymes were initially tested, including malate dehydrogenase (MDH) and phosphoglucone mutase (PGM), we found that phosphoglucone isomerase (PGI) gave reproducible banding patterns that could distinguish *P. yezoensis* from the local *Porphyra* species in question. PGI has been used to distinguish Pacific coast *Porphyra* species (Lindstrom and Cole 1990, 1992a, b; Brostoff and Gordon 1997) and is currently being used in phylogenetic studies of Atlantic coast species (C. Neefus, pers. comm.). *P. yezoensis* farmed by Phycogen is from a single conchocelis stock culture and as expected showed the same allele for the PGI locus throughout this study. *P. yezoensis* PGI migrates more slowly (*i.e.,* has lower bands) than that of the local species (Figure 3) and electrophoresed in a consistent pattern. Samples from our monitoring study were always run against stock laboratory cultures of *P. yezoensis* and the two most frequently encountered local species, *P. umbilicalis* and *P. purpurea*, as controls on each gel (Figure 3). *P. umbilicalis* and *P. purpurea* samples occasionally showed polymorphism at the PGI locus, but samples always corresponded to at least one of the control bands when screened.

The ability of *P. yezoensis* blades to overwinter in the field was tested by subjecting blades to winter conditions *in situ*. Individual strands of netting seeded with established *P. yezoensis* blades were affixed by cable ties to the middle of new artificial substrata at
high-and low-intertidal locations at the Huckins Ledge site at the end of Phycogen's growing season, in the autumns of 1997 and 1998. These strands were grown and supplied by Phycogen, and had blades ranging from 1 mm to 10 cm long, in densities >100 blades/strand. Both seeded strands and nets were analyzed in the spring of 1998 and 1999 for survival of the original blades and for potential recruitment and establishment on the surrounding netting. Strands recollected in the spring were cultured under laboratory conditions (aerated, sterile, enriched seawater media, 15°C, 12:12 light-dark cycle) to permit regeneration of any *P. yezoensis* blades from spores that might be present but not visible. Similarly seeded strands were deployed in May 1999 and collected in July 1999 to test the ability of *P. yezoensis* to persist in the intertidal under favorable conditions. Recollected strands were cultured as above and *Porphyra* recruits on the surrounding artificial substrata were screened electrophoretically.

**RESULTS**

**Monitoring Study**

The abundance of *Porphyra* species from transects at Huckins Ledge varied with location and season. Greater numbers of individual blades were found epilithically on small cobble in muddy substrata near sampling location A and sparser populations were found on sandy cobble substratum near sampling locations B, C, and D along the rocky shoreline (Figure 1). The performance of the artificial substrata exceeded expectations and recruited *Porphyra* plants in large numbers. A total of 653 individual *Porphyra* blades were collected from artificial substrata at Huckins Ledge during the course of this study and 334 individual blades were collected during transect sampling. Approximately 26% of the *Porphyra* collected on artificial substrata and 48% collected in transects were analyzed electrophoretically. The most common *Porphyra* species identified from both artificial substrate and transect collections taken throughout the year was *P. purpurea*. The second most commonly encountered species was *P. umbilicalis*. The seasonal and spatial distribution of these species between the two years sampled was consistent.

None of the *Porphyra* blades collected in transect surveys between August 1997 and July 1999 were identified as *P. yezoensis*, of a total of 163 individuals tested. However, five blades from the artificial substrata collected in November 1997 were identified as *P. yezoensis*. *P. yezoensis* was not identified in subsequent sampling from March 1998 to July 1999 in either transect or artificial substrate surveys (Figure 4).

**Overwintering Study**

Six *P. yezoensis*-seeded strands of netting used in the overwintering study at Huckins Ledge in December 1997, were collected and analyzed in March 1998. *P. yezoensis* was not found on the original pieces or on the surrounding netting. The netting pieces were overgrown with *Enteromorpha* spp. and *P. yezoensis* blades did not regenerate after a month in laboratory culture. This study was repeated in December 1998, with 11 seeded strands. Of the 11, 9 strands were recovered in May 1999. One strand had two *Porphyra* blades that were identified morphologically and electrophoretically as *P. umbilicalis*. All recovered strands were cultured in the lab for in excess of 1 mo to allow for any spores that might be present to grow into blades; however, no blades were observed. Strands deployed in May 1999 and recovered in July 1999 were also bare of *P. yezoensis* blades and did not regenerate after lab culture. However, the netting surrounding the strands was well recruited with *P. purpurea* blades.

**Discussion**

Monitoring of the nursery farm site at Huckins Ledge was initiated in 1997 because this is where we believed monospores would have been released in the greatest numbers and was therefore the most likely area for *P. yezoensis* to recruit and establish a population. During the two years of this study, only limited and ephemeral recruitment of *P. yezoensis* was
observed at Huckins Ledge. This recruitment was observed only on artificial substrata, immediately following a farming season and in small numbers compared to the presence of local Porphyra species. The P. yezoensis blades collected from artificial substrata in November 1997 represented only 8% of the total Porphyra blades collected during that sampling event and P. yezoensis was not found in either transect or artificial substrate collections taken the following spring. Thus, it appears that P. yezoensis was able to release asexual monosporas during Phycogon’s farming season, but new blades survived only ephemeral in the intertidal surrounding the farm site. Overwintering experiments conducted in 1998 and 1999 at Huckins Ledge also suggest that P. yezoensis is not capable of overwintering in the field. The local species of Porphyra produce blades in the spring/summer, generally die off over the winter, and then return again in the spring when conchospores are released by subtidal conchoecilis populations.

However, P. yezoensis was not identified in any spring transect or artificial substrate sampling when the local species blades had reappeared, most likely due to the temperature restriction in its life cycle upon which permitting was granted. The inability of P. yezoensis to overwinter is further supported by the fact that an intertidal population of P. yezoensis was never discovered during our transect sampling or that of another researcher (A. Mathieson, pers. comm.), at the Huckins Ledge site.

It must be acknowledged that this paper reports only results for the Huckins Ledge nursery farm site and that differences in site locations may play a vital role in the ability of P. yezoensis to recruit and survive in Cobscook Bay. Data from Phycogon’s former grow-out site (Mathews Island), currently under investigation, will be described in a forthcoming publication. Together, our monitoring studies at Huckins Ledge and Mathews Island should be able to assess whether P. yezoensis can become established in Cobscook Bay and demonstrates the usefulness of monitoring species introduced for aquaculture.

Acknowledgements

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Mathieson, A., University of New Hampshire, Durham, NH
The “Silver Lining”—The Economic Impact of Red Sea Species in the Mediterranean

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Key words: economic impact, invasion, Red Sea, Mediterranean, fishery, prawn, jellyfish

INTRODUCTION

Biological invasions threaten natural ecosystems with impacts at genetic, population, and ecosystem levels (Ruiz et al. 1997). The Mediterranean, with its particular geological history and nearly landlocked geography, has been exceptionally susceptible to biological invasions. Ecologically, environmentally, and economically, the invasions into the Mediterranean Sea have had serious consequences (Boudouresque 1994). The major pathway of anthropogenic introduction into the Mediterranean Sea is the Suez Canal (Zibrowius 1994). Despite physical and hydrological impediments, hundreds of Red Sea species traversed the Suez Canal and settled in the Mediterranean, forming thriving populations along the Levant coasts, some invaders spreading as far west as Malta, Sicily, and Tunis (Galil 1994). Some abundant invaders constitute a nuisance or an economic burden, others outcompete local species, and yet others are exploited commercially.

DISCUSSION

Each summer since the mid-1980s huge swarms of the invading jellyfish, Rhopilema nomadica appear along the southern Levantine coast. In 1995 the jellyfish was recorded off the southeastern coast of Turkey (Kideys and Güçü 1995), and in 1998 a specimen was collected near Izmir (A. Karatas, pers. comm.). These massive swarms of voracious planktотrophs must play havoc with the meager resources of this oligotrophic sea, and when the shoals draw nearer shore, they impact fisheries, coastal installations and tourism. Local municipalities report a decrease in

holiday makers frequenting the beaches because of the public’s concern over the painful stings inflicted by the jellyfish. Coastal trawling and purse-seine fishing is disrupted for the duration of the swarming due to net clogging and inability to sort yield. Jellyfish-blocked water intake pipes pose a threat to cooling systems of port-bound vessels and coastal power plants: in the summer of 1996 Israel Electric removed 25 tons of jellyfish daily from its scawter intake pipes at the Hadera power plant and has since installed a “jellyfish barrier” at the entrance to the cooling pond. Yet, that same jellyfish, R. nomadica, known to shelter among its tentacles the juveniles of a Red Sea carangid fish, Alepes djedaba, (Galil et al. 1990), may have precipitated the sudden population increase of this commercially valuable fish (Grofit 1987).

A native penaeid prawn, Melicertus kerathurus, was “very commonly caught by trawlers on Israel coastal shelf especially on sandy or sandy mud bottoms” (Holthuis and Gottlieb 1958) and supported a commercial fishery throughout the 1950s. It has since nearly disappeared and its habitat overrun by the Red Sea penaeid prawns. Geldiay and Kocatas (1972) reported that Marsupenaeus japonicus has also replaced M. kerathurus off the southern coast of Turkey and the rapid advent of another Red Sea prawn, Metapenaeus monoceros, into the Gulf of Gabes, Tunisia, has raised concerns over the fate of M. kerathurus fisheries there (Chaouachi et al. 1998).

Red Sea fish constitute nearly half of the trawl catches along the Israeli coast (Golani and Ben Tuvia 1995). In the late 1940s the invading goldband goatfish, Upeneus moluccensis, made up 10-15% of the total mulloid catches off the Israeli coast. Following the exceptionally warm winter of 1954-55, its percentages

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increased to 83% of the catch, then dropped to 30% of the catch (Ben Tuvia 1973). In the 1990s, both invading mullids, *U. moluccensis* and *U. porti*, formed 87% of the mulloid catch off the coast of Israel at depths of 20 m, and 50% at 55 m, whereas the native mullids are more abundant at greater depths (Golani and Ben Tuvia 1995). Following the winter of 1954-55, the brushtooth lizardfish, *Saurida undosquamosis*, has become a commercially important fish and its share in trawl fisheries catches rose to 25% in 1979 (Groit 1987). The population then diminished and catches have stabilized at about 5% of the total trawl catch (Ben Yami and Glaser 1974; Snovsky and Shapiro 1999). The Red Sea orbute barracuda, *Sphyraena chrysotailia*, has outnumbered the native sphyraenids in inshore trawl and purse-seine catches along the Israeli coast (Groit 1987). In addition, two of the four species of Red Sea cichlids that established populations in the Levant—*Dissumieria acuta* and *Herklotisichthys punctatus*—are of importance in the inshore-pelagic fishery.

Red Sea species make up most of the commercially valuable crustacean catch along both Egyptian and Israeli coasts (Galil 1986). An early invader, the swimming crab, *Portunus pelagicus*, was recorded from Port Said in 1898 (Calman 1927), where it soon became abundant, and by the beginning of the century was offered in the markets of Port Said, Alexandria, and Haifa (Fox 1924). Red Sea penaeid prawns are highly prized and a small fleet of Israeli coastal “mini” trawlers has specialized, since the mid 1980s, in shrimpning, bringing in a quarter of the total trawl catch volume and a third of the trawl gross income (Snovsky and Shapiro 1999). *Marsupenaeus japonicus*, *Metapenaeus monoceros*, and *M. stebbingi* compose most of the prawn catch off the Mediterranean coast of Egypt and in the Nile delta lagoons (Dowidar and Ramadan 1976; Bishara 1976).

The sizable assemblage of Red Sea species that has taken up residence along the Levantine infralittoral, modifying the composition and structure of the biota, and enhancing its tropical affinities, has disrupted the biogeographic unity of the Mediterranean, and turned the Levant into a “quasi-tropical” province. The unique history of the easternmost Mediterranean that left it warm, salty, and impoverished is at the base of a singular synergy between anthropogenic and environmental factors. Though the expected outcome of invasion is reduction in diversity, we witness an invasion that increases faunal diversity, and augments the local fisheries—every cloud has a silver lining.

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**Source of Unpublished Material**

Karatis, A., Izmir University, Turkey
How and When to Protect Native Species from Exotic Invaders: Lessons from a Predictive Model

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Key words: Batillaria, bottom-up effect, Cerithidea, coexistence, control, estuary, exploitative competition, individual based models, local extinction, monitoring, mudsnail, prediction, top-down effect

INTRODUCTION

The exotic mudsnail, Batillaria attramentaria, was introduced to the west coast of North America in the early part of this century with aquaculture imports of the Pacific oyster, Crassostrea gigas, and has been displacing the native mudsnail, Cerithidea californica (Byers 1999). Both species produce demersal egg sacs with directly developing larvae and exhibit limited movement as adults, and thus comprise essentially closed populations within bays. Both snails also are susceptible to infection by trophically transmitted trematode parasites that typically infect C. californica at a higher rate than B. attramentaria (Sousa 1983; LaFerty 1993; McDermott 1996; Byers 2000). The trematode species substantially affect these snail species by castrating infected individuals, eliminating future reproduction. Byers (2000) demonstrated that these snail species compete for shared, limited diatom food resources and that the introduced snail, B. attramentaria, is superior in exploitative competition due to its higher resource conversion efficiency. Predictions of individual-level interspecific effects of each species upon the other based on consumer-resource data were highly accurate at an individual level (Byers 2000).

RESULTS AND DISCUSSION

Here we use an individual-based model to expand these predictions and project the population-level impact on the native snail, C. californica, caused by the nonindigenous species. By using empirical data to parameterize the model, we can project times of local extinction for the native snail, and also focus on two areas of primary importance to invasion biology not directly amenable to field manipulations: (1) the relative importance of mechanisms responsible for the exclusion of Cerithidea by Batillaria, and (2) identification of the metrics and biological measurements within a system that provide useful information about the likely course of the invasion in a given location. Identification of the metrics most sensitive to invasion impact would greatly aid resource managers in making decisions about which characteristics of a native species or system to monitor to provide earliest detection of problematic exotic species.

Our model tracked the species, sex, age, size, and infection status of each snail species through time. We set most demographic rates and interaction coefficients with empirical data derived from populations of the snails in Bolinas Lagoon, California (Byers 2000; Byers unpublished data). In summary, the model parameters and operations allow the number, sizes, and species of snails to determine the amount of resource available. Snail growth rates vary with resource level. Both species exhibit similar per capita rates of resource consumption; however, Batillaria is superior to Cerithidea in terms of how it responds to resource level (resource conversion efficiency). Egg production also is dependent on resource level, since no reproduction occurs if the level falls below 0.5 mm² diatom surface area/mm² sediment surface area. Egg mortality is constant and equal between the species. Infected and immature snails (<14 mm) cannot reproduce; otherwise, reproductive output increases with snail size. Monthly mortality is density independent and Cerithidea dies at a higher rate than
Table 1. State variables and parameters and their associated mathematical relationships used within the model. For the first three state variables, empirical relationships were determined experimentally from plots with snails of single species (Cerithidea or Batillaria) and sizes (10 mm or 20 mm); hence, corresponding values are denoted by the first initial of each species with size class in mm as a subscript. For the first three state variables, snails of intermediate sizes were calculated by linear interpolation from the 10- and 20-mm snails. Snails below this range were treated as 10 mm and snails above this range were treated as 20 mm. Parameter values appear at bottom of table. Parasite infection rates were derived from field data; egg survival and shell erosion are informed estimates.

<table>
<thead>
<tr>
<th>Model component</th>
<th>Relationship/Values</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. State Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diatom level ( \frac{\mu m^2}{mm} )</td>
<td>( a \times \exp (b \times \text{snail density}) )</td>
<td>When snails of mixed species and sizes were present, the coefficients ( a ) and ( b ) were the means of their separate values.</td>
</tr>
<tr>
<td>Diatom level ( \frac{\mu m^2}{mm} )</td>
<td>4.3 (-0.0044)  C(_{10})</td>
<td></td>
</tr>
<tr>
<td>Diatom level ( \frac{\mu m^2}{mm} )</td>
<td>4.02 (-0.0067)  C(_{20})</td>
<td></td>
</tr>
<tr>
<td>Diatom level ( \frac{\mu m^2}{mm} )</td>
<td>4.46 (-0.0024)  B(_{10})</td>
<td></td>
</tr>
<tr>
<td>Diatom level ( \frac{\mu m^2}{mm} )</td>
<td>4.22 (-0.0067)  B(_{20})</td>
<td></td>
</tr>
<tr>
<td>Resource-dependent individual growth ( \frac{\mu m^2}{mm} )</td>
<td>( m \times \text{diatom level} + b )</td>
<td>Growth was constrained to be positive; a small decrease due to erosion or basic metabolism was applied separately. (In the field, large Cerithidea at low resource levels exhibit negative tissue growth).</td>
</tr>
<tr>
<td>Resource-dependent individual growth ( \frac{\mu m^2}{mm} )</td>
<td>0.36 (1.73)  C(_{10})</td>
<td></td>
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<tr>
<td>Resource-dependent individual growth ( \frac{\mu m^2}{mm} )</td>
<td>0.40 (-0.26)  C(_{20})</td>
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<tr>
<td>Resource-dependent individual growth ( \frac{\mu m^2}{mm} )</td>
<td>0.03 (2.85)  B(_{10})</td>
<td></td>
</tr>
<tr>
<td>Resource-dependent individual growth ( \frac{\mu m^2}{mm} )</td>
<td>0.26 (1.48)  B(_{20})</td>
<td></td>
</tr>
<tr>
<td>Density-independent mortality* ( \frac{\mu m^2}{mm} )</td>
<td>( m \times \text{length} + b )</td>
<td>For infected snails, the calculated value was increased 10%; The maximum age for all snails = 20 years.</td>
</tr>
<tr>
<td>Density-independent mortality* ( \frac{\mu m^2}{mm} )</td>
<td>0.24 (-2.07)  C (_{0.093})</td>
<td></td>
</tr>
<tr>
<td>Density-independent mortality* ( \frac{\mu m^2}{mm} )</td>
<td>0.0093 (0.23)  B (_{0.093})</td>
<td></td>
</tr>
<tr>
<td>Reproduction ( \frac{\mu m^2}{mm} )</td>
<td>( 0.079 \times (\text{snail length})^{2.7} )</td>
<td>None if snail infected, if &lt;14 mm, or if diatom level &lt;0.5</td>
</tr>
<tr>
<td><strong>B. Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg survival ( \frac{\mu m^2}{mm} )</td>
<td>1/300</td>
<td></td>
</tr>
<tr>
<td>Parasitic infection rate* ( \frac{\mu m^2}{mm} )</td>
<td>( p(Batillaria) = 0.3 \times p(Cerithidea) )</td>
<td>Probability was applied once during spring and once during summer; Snails &lt;14 mm not susceptible to infection</td>
</tr>
<tr>
<td>Shell erosion ( \frac{\mu m^2}{mm} )</td>
<td>(-0.1 mm/yr)</td>
<td></td>
</tr>
</tbody>
</table>

*For one or more of the state variables and parameters that differed between the species, some runs set the value for Batillaria equal to the value for Cerithidea, to determine which differences may be most important in promoting the displacement of the latter by the former. 
†Both a species' effect on the diatom resource and its growth response to diatom levels combined represent its exploitative competitive abilities. In simulations that removed Batillaria's competitive advantage, both state variables were equalized to Cerithidea's values. Since the species impact the diatom level similarly, the majority of the effect of equalizing competition derives from changes in the efficiency of Batillaria's conversion of resources to growth.

Batillaria. Maximum longevity for both species is capped at 20 years. Finally, parasitic infection rates, which are naturally lower in Batillaria, reflect the probability of uninfected individuals becoming infected at a given time step within the model, with infection increasing the typical monthly mortality rate for an infected snail by 10% (Table 1). The basic flow of the model is depicted in Figure 1.

We first tested the relative importance of Batillaria's demonstrated advantages in parasite resistance (top-down effect), exploitative competition (bottom-up effect), and mortality rate (demographic advantage) in driving its successful invasion and displacement of Cerithidea. Such an analysis pinpoints the key pathway through which the exotic species derives the majority of its success, and also suggests pathways of intervention that may more successfully control or delay the impact of the exotic species. Model results indicate that displacement and ultimate local exclusion of Cerithidea by Batillaria takes
between 55 and 70 years. Furthermore, exploitative competition and susceptibility to parasitic infection are relatively weak mechanisms in driving the overall success of Batillaria. Although these interactions provide the mechanism for Batillaria to exert an influence on Cerithidea, Batillaria's lower density-independent mortality rate plays the key role governing Batillaria's displacement of Cerithidea. Management techniques can therefore focus particular attention on alleviating species-specific differences of this mechanism, for example, through physical removal of Batillaria, to most effectively neutralize the invasion.

To determine the earliest point that a monitoring program could detect the impact of the invader in the native system, we tested the sensitivity of many response variables of Cerithidea at the population and individual level, including density, population biomass, egg production, mean size, proportion of infected individuals, and individual growth rate. We also tracked the overall level of diatom resource in the marsh. For these model simulations, we chose an initial number of Batillaria invaders to inoculate into Cerithidea populations that previous results showed would guarantee extinction of the native snail in 100% of the runs within 90 years. In this manner, we could identify which biological responses of the native snail gave the earliest signal that a detrimental invader had entered the system. Despite almost immediate detection of increasing invader populations, all metrics for Cerithidea were slow to exhibit evidence that this observed increase in invader density impacted the native species. Most metrics took at least 25 years from the beginning of the invasion to show significant declines. By this point, the presence of the exotic species and its effect on the native species was essentially irreversible. Native snail egg production and marsh-wide diatom abundance reflected impact from the invader the quickest of all metrics—within 15-20 years. Difficulty in finding reliable, early-warning metrics has crucial implications for how we should view and conduct monitoring programs and risk assessment analyses.

**Literature Cited**


