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The goal of this project was to acquire basic biological and ecological information regarding the Giant Pink Barnacle, *Megabalanus coccopoma,* in coastal Georgia and to provide a means of distributing information regarding marine invasive species through public exhibition and public involvement. This project included research activities and objectives and education and outreach activities and objectives.

The overall purpose of the project was to initiate a research and education program at the Tybee Island Marine Science Center to increase awareness and to educate the public about marine invasive species and Georgia coastal resources.

Ecological and distribution research objectives of the project included the following:

1. Determine the spatial distribution of the Giant Pink Barnacle upriver and landward by sampling intertidal zone hard substrata in the vicinity of Tybee Island and the mouth of the Savannah River.

2. Expand knowledge of the distribution of the Giant Pink Barnacle throughout coastal Georgia by creating a means for the public to report sightings of it, recording those sightings, and publicizing this expanding knowledge through an exhibit at the Tybee Island Marine Science Center and through its outreach resources and activities.

Biological research objectives of the project included the following:

3. Determine the growth rate of the Giant Pink Barnacle in coastal Georgia by monitoring growth rates (height and base width) of specimens growing on the North Beach jetty at Tybee Island.

4. Determine the settlement period for new individuals in coastal Georgia by conducting monthly settlement monitoring surveys of specific areas/surfaces on the North Beach jetty at Tybee Island.

5. Determine the lower salinity tolerance limit (LD-50) for the Giant Pink Barnacle in coastal Georgia by conducting 96-hour LD-50 experiments in a series of differing salinity, closed-system aquariums.

Summary of Research Findings

The Giant Pink Barnacle, *Megabalanus coccopoma*, has established a population in the mid and lower intertidal zone on the Tybee Island North Beach jetty. From settlement/colonization studies, it appears that new individuals settle onto the jetty during May-June. Growth is rapid during the spring and summer and is slower through the winter, but individuals do live more than a single year. In controlled laboratory conditions, members of this population can withstand low salinity (20 ppt), and if allowed a few days of acclimation to this lower salinity, they can withstand salinity as low as 16 ppt. Although this low salinity tolerance range suggests that individuals could survive in coastal estuaries, no intertidal pink barnacles were observed during surveys upriver along the Savannah River channel jetty. Possibly the

larval and young stages of this population have more restricted salinity tolerance ranges. This species appears to be a well established component of the mid and low intertidal zone on the North Beach jetty and competes for space with native oysters, ivory barnacles and striped barnacles.

Research Findings/Results

1. Distribution study/surveys and spreadsheet for data entry

Throughout the project period, numerous locations on and around Tybee Island were visited and surveyed to look for the presence of intertidal pink barnacles. Locations included the south jetty of the Savannah River Chanel, public and private floating docks and pilings, intertidal rocks and riprap, and any other noted hard substrata. In most cases, GPS latitude and longitude coordinates of the visited locations were recorded. This spatial distribution information was recorded in an electronic spreadsheet.

To date, the Giant Pink Barnacle appears to be predominately confined and most evident in the Tybee Island vicinity to the North Beach area. Although the salinity tolerance studies indicate that the species (at least its sessile life-stage) is tolerant of lower salinity, no individuals were found or have yet been reported during this study beyond the mouth of the Savannah River.

2. Growth studies

Winter season growth

The initial study of growth rates of Giant Pink Barnacles in the lower intertidal zone on the North Beach jetty began on December 8, 2008, and ran through May 22, 2009. During this winter/spring period, monthly measurements of specifically identified barnacles were made and recorded during low tide. Barnacle height and diameter were measured in the field, recorded, and throughout the period, 26 individual barnacles were included. During this period and using this method, mean Pink Barnacle diameter growth was 0.9 mm per month, and mean height growth was 1.8 mm per month (Table 3-1). Many of the individuals, however, revealed no net measurable size increase (14 of 26 having no net diameter increase; and 9 of 26 having no net height increase).

Table 2-1. Growth rates during winter and spring of established Pink Barnacles.

Growth rates of established pink barnacles during period December 2008 through May 2009 using direct measurements of 26 individual barnacles

Mean diameter growth = 0.87 mm per month (n=26)

Mean height increase = 1.8 mm per month (n=26)

Mean height increase = 1.16 mm per month (n=25 and excluding a single 18mm measurement increase of one barnacle during one month)

Spring/summer growth

During the following 2009 spring/summer period, a different method for measuring growth was employed. During this period, the growth of small, newly settled pink barnacles were measured monthly from June 22, through September 15, 2009. Rather than direct field measurements of individual barnacles, photographs (using digital macro-photography) of specific clusters were made. Each photograph included a millimeter-scale ruler positioned flat against the barnacle cluster.

The digital photographs were printed, and the section of the picture of the scale was cut off, and the scale-picture was used to measure the greatest dimension of each barnacle in the picture. This method was employed to measure the size of specific barnacles, identified and photographed each month, as well as for measuring the sizes of non-specific barnacles within clusters. This method allowed many more barnacles to be measured each month than did the initial method. Mean barnacle sizes were calculated each month for each of these two groups of barnacles (specific individuals, and clusters) (Table 3-2).

Table 2-2. Spring/summer 2009 mean size of newly settled pink barnacles through a three-month period.

Date	Cum. Days	Mean size (mm) of Individuals	Number measured (n)	Mean size (mm) from clusters	Number measured (n)
6/22	0	7.4	13	9	47
7/17	24	9.6	21	10.6	60
8/5	43	11.8	21	13.5	77
9/15	84			18.1	29

As indicated in Table 2-2 above, these newly settled pink barnacles doubled their size (from 9mm to 18mm) during the three months from late June through mid September.

Table 2-3. Using the mean sizes recorded in Table 2-2, and normalizing each month's sampling date based on a 30-day month (eg. from June 22nd to July 17th = 0.8 month), the following growth rates for each month were calculated for these young pink barnacles.

Date	Cum. Days	Calculated No. months	Individuals Mean Growth (mm) per 30-day month	Clusters Mean Growth (mm) per 30-day month
6/22	0			
7/17	24	24/30 = 0.8	2.19/0.8 = 2.7	1.57/0.8 = 1.96
8/5	43	14/30 = 0.63	2.24/0.63 = 3.6	2.88/0.63 = 4.57
9/15	84	41/30 = 1.37		4.64/1.37 = 3.4

As indicated in Tables 2-2 and 2-3, growth during the summer period was rapid. By the mid-September measuring date, barnacles were growing against each other, perhaps slowing diameter size increase due to

crowding. By this date, because so many of the barnacles had grown against each other, the group of "individual" barnacles photographed previously could not be positively identified (and thus data for the "individuals" for Sept. 15th is missing).

By mid September, this mean barnacle size of 18mm was comparable to the size (diameter or height) of most of the barnacles that were measured and followed during the previous winter growth study. Thus it appears that for this species, settlement occurs during the May-June period, and it is followed by 3-4 months of rapid growth during the summer. During the winter, however, growth is slow. This would suggest that the very large (2 inch range) pink barnacles have grown through at least two summer growth periods.

3. Settlement studies

On December 8, 2008, two areas in the lower intertidal zone of the North Beach jetty were cleared of all sessile organisms (barnacles, oysters, hydroids, mussels). Each of these areas was approximately 0.5m^2. The areas were cleared with a steel pry-bar. Both areas were in the vicinity of the pink barnacles that were being measured monthly and were typical of surrounding lower intertidal zone rock surfaces prior to being cleared. Each surface was a rather smooth, rounded area. One of the surfaces faced eastward toward the ocean, and the other surface faced westward toward the land. Each of these cleared areas was monitored and photographed monthly for evidence of settlement of pink barnacles.

The cleared areas remained clean of settling/fouling organisms until March. During the March 23, 2009, field visit, the cleared areas supported small individuals of *Porphyra* (red alga common on jetty during late winter and spring). During April, only the bases of the small *Porphyra* were present in the cleared area. When visited on May 22, 2009, both cleared areas were 100% covered with small individuals of the Fragile Barnacle (*Chthamalus fragilis*). The formerly cleared areas were smothered and covered by accumulated sand against the jetty during mid June. Throughout the summer, the cleared areas eventually became uncovered again, but as of late September, 2009, no pink barnacles were observed to have settled in these cleared areas.

Although the cleared areas in the lower intertidal zone were covered with accumulated sand in mid June, newly settled, baby pink barnacles were noticed on low intertidal rocks near the study area. These clusters of newly settled pink barnacle were subsequently measured monthly as described in the "Growth Studies" section of this report. At this time, mid June, these newly settled pink barnacles had a mean diameter of only 7mm. Throughout the summer of 2009, as these clusters of newly settled pink barnacles were measured on a monthly basis, the surrounding jetty region was examined to see if subsequent settlement of new barnacles occurred during the summer. Although occasional small pink barnacles were observed, most all of the additional pink barnacles were in the same size range as those that were being measured. Exceptions were larger pink barnacles that had apparently settled during previous years. It thus appears that the primary colonization and settlement period for the pink barnacles is during May-June.

4. Lower Salinity Tolerance Limit of Invasive Giant Pink Barnacle, *Megabalanus coccopoma*

Materials and Method

Approximately five gallons of seawater were collected in buckets from the boat ramp on the Back River of Tybee Island at high tide on Sept. 8, 2009. The salinity of this water was 32 ppt. This water was used for maintaining the barnacles used during the salinity tolerance studies, and it was used as the stock water to make all salinity variations. This water was stored in closed buckets. Pink barnacles were collected from the low intertidal zone of the North Beach jetty on September 8, 2009, during low tide by using a pry-bar to remove clusters from the rocks. The clusters were transported in a bucket of seawater (salinity of 27 ppt) collected from shore at the same time the barnacles were collected. The clusters were cleaned of sand/silt and epibionts before being placed in a holding tank of 32 ppt salinity water for a (two-day) acclimation period and to be sure that only live, healthy barnacles would be used for experiments. Approximately 40 live pink barnacles were collected, cleaned and maintained.

As the barnacles acclimated to lab conditions, three experimental tanks were prepared with two liters of seawater. The seawater for each tank was prepared by mixing the stock seawater with distilled water to yield a specific, pre-determined salinity. For the first series of salinity tolerance LD-50 determinations, the prepared salinity values were: 32 ppt, 20 ppt, and 15 ppt. Air lines with air stones were added to each tank to provide constant water mixing. Ten pink barnacles were added to each tank and arranged so that each barnacle could be observed for feeding activity. All barnacles were observed periodically for 96 hours and the number of live barnacles, indicated by feeding activity, were recorded during each observation period. These counts are recorded in Table 4-1.

Table 4-1. Number of live pink barnacles observed feeding in various salinities during 96 hour LD-50 study. Dates of trial: 9/9/2009 through 9/13/2009.

Elapsed Hours	32 ppt	20 ppt	15 ppt
0	10	10	10
14	10	10	0
22	10	9	1 (slow, short motion)
24	10	9	1 (slow, short motion)
30	9	8	0
49	10	10	1 (slow) 9 dead; discontinued tank
75	10	10	
96	10	10	

Based on the previous study of salinity tolerance that indicated that the LD-50 for the pink barnacles appears to be within the 15 ppt to 20 ppt range, a second series of tanks were prepared. The stock 32 ppt seawater was used for dilutions to prepare two liter tanks having the following salinities: 20 ppt, 18 ppt, 16 ppt. These salinity values were prepared to attempt to narrow down the LD-50 salinity tolerance range for the pink barnacles. Air lines with air stones were added to each tank to provide constant water mixing.

To each tank was added at least six pink barnacles that had been maintained in 32 ppt salinity water since their collection from the jetty. These barnacles were arranged so that each barnacle could be observed for feeding activity. Also added to the 18 ppt and 16 ppt tank were barnacles that had survived in the previous 96 hour study in the 20 ppt salinity. This allowed for the investigation of possible affects on salinity tolerance due to previous acclimation to lower salinity. All barnacles were observed periodically

for 96 hours and the number of live barnacles, indicated by feeding activity, were recorded during each observation period. These counts are recorded in Table 4-2.

Table 4-2. Number of live pink barnacles observed feeding in various salinities during second round of 96 hour LD-50 study. Barnacles transferred directly from 32 ppt to lower salinity indicated by "from 32." Barnacles previously surviving 20 ppt before transfer to lower salinity indicated by "from 20." Dates of trial: 9/13/2009 through 9/17/2009.

Elapsed Hours	20 ppt	18 ppt (from 32)	18 ppt (from 20)	16 ppt (from 32)	16 ppt (from 20)
0	8	6	2	6	8
25	8	1	2	0	8
50	7	2	2	1	8
75	7	0 (6 dead)	2	0 (6 dead)	8
96	7	0	2	0	8

Results and Discussion of salinity tolerance studies

Based on the survivability of the Giant Pink Barnacles during the initial 96 hour salinity series study, it appears that the *Megabalanus coccopoma* that have been introduced to the southeastern US shore are capable of surviving coastal euryhaline conditions. They appear, therefore, to possess lower salinity tolerance capabilities that would allow them to spread inland into the higher salinity zones of coastal estuaries. This information is important because it suggests that this invasive species could indeed become a threat to low intertidal zone oysters that share this same habitat. It should be noted, however, that these salinity tolerance results pertain to sessile, established pink barnacles. Spatial distribution of the species depends on larval transport, and successful colonization and settlement into lower salinity areas, eg. estuaries, would depend on the larval stages also being tolerant of lower salinity. Future research should investigate such larval salinity tolerance limits.

Additional evidence of the Giant Pink Barnacle's ability to deal with salinity variations typical of southeastern US estuaries was revealed by the second series of salinity tolerance tests. During this series of survivability investigations, it was revealed that this invasive species is capable of acclimating to lower salinity and then extending its survivability tolerance range to even lower values. These findings suggest that this species is capable of rather rapid adjustments to lowered salinity, and further indicates its ability to survive, once established, in coastal estuaries.