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***Zostera japonica* Eradication Project
Annual Report: 2010**

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Introduction

This report summarizes the activities and accomplishments of the *Zostera japonica* Eradication Project conducted under Humboldt Bay Harbor Recreation and Conservation District Permit No. 03-03 during the period October 21, 2009 to December 31, 2010. The *Z. japonica* Eradication Project is a cooperative effort involving University of California Sea Grant Extension staff, California Department of Fish and Game staff, and Humboldt State University student interns.

Project Background

This ongoing eradication project represents the front line of defense in California against a non-native eelgrass that rapidly colonizes areas of unvegetated mudflat. Native to Asia, *Z. japonica* has become established on tidal flats from Boundary Bay, British Columbia to Coos Bay, Oregon. The detection of dwarf eelgrass (*Zostera japonica* Aschers and Graebn) on Indian Island in Humboldt Bay, California, in June 2002 represents the southern extent of its range in the Eastern Pacific and it is the first time this introduced species has been encountered in California.

After this initial discovery, a team of state and local scientists, including representatives from NOAA Fisheries, United States Fish and Wildlife Service, California Department of Fish and Game (DFG), UC Sea Grant Extension (Sea Grant), Humboldt State University, and UC Davis, was assembled to assess the available information on *Z. japonica* and the extent of its introduction into Humboldt Bay. It was the consensus of the team and additional marine scientists who were consulted that an eradication program should be undertaken. No previous attempts have been made to eradicate this species.

In April 2003, under Humboldt Bay Harbor, Recreation, and Conservation District (HBHRCD) Emergency Permit No. E-2003-1, a team of volunteers excavated all known *Z. japonica* in Humboldt Bay. Sea Grant, in partnership with DFG, later acquired grant funding to address *Z. japonica* eradication. On October 21, 2003, HBHRCD Permit No. 03-03 was issued to allow continued eradication work for one year, and since then, one-year extensions for the permit have been issued annually. A permit amendment was granted on July 3, 2009 to include additional methods of eradication; including covering, hot water, flame heat, and infrared heat. On May 27, 2010, the permit was again amended to include the use of small cartridge heaters as an additional method of control.

For the work period October 21, 2009 through December 31, 2010, our objectives were:

1. Detect new occurrences of *Z. japonica* in Humboldt Bay as early as possible.
2. Eradicate all *Z. japonica* found as quickly as possible.
3. Gather data that will further our understanding of the species and help assess the effectiveness of our eradication methods.
4. Restore affected areas to pre-invasion conditions.

Methods

Shoreline Surveys

In Humboldt Bay, DFG staff, Sea Grant staff, HSU students, and volunteers (Team) have conducted shoreline surveys every year since the first detection of *Z. japonica* in 2002. For the work period October 21, 2009 through December 31, 2010, the Team intensively surveyed all known infestation sites along the shoreline of Indian Island, the Arcata Wastewater Treatment Plant (AWTP), the Arcata Marsh, Mad River Wildlife Area, and the shoreline of Manila. Additionally, the Team searched other nearby areas of suitable intertidal mudflat habitat in Humboldt Bay.

There were no patches discovered along the shoreline of Indian Island this past year, which is extremely encouraging for our control efforts. However, *Z. japonica* was discovered at one additional location in Arcata Bay, near Bracut. In May of last year two new locations were discovered; Wallace Ranch and Mad River Wildlife Area. Six patches were discovered in August at Wallace Ranch and a survey in June along Mad River Wildlife Area did not find any *Z. japonica*. Several patches were observed at Manila, the AWTP, the Arcata Marsh, and the mudflat located about 0.25 miles offshore of the marsh, referred to as Arcata Marsh Outer (Figure 1).

Shore surveys and control efforts were synchronized this year to eliminate the need to mark all *Z. japonica* patches with PVC piping. In situations where the Team was unable to quickly return (within a day) and treat the infestation, the patches were temporarily marked with staking. All shore survey tracks were recorded using a handheld GPS and a waypoint was captured for all *Z. japonica* patches. All GPS data was entered into the existing spatial database.

Prior to treating any *Z. japonica* plants, data was collected on patch meristics, including diameter (based on the longest measurement of the patch) and percent cover (based on an ocular estimate of cover within the circle defined by the diameter). To determine the density of vegetative and reproductive shoots and the biomass, a set of six core samples were collected from each location. One set consists of a core sample collected from inside the patch, outside the patch, and on the inner edge of the patch. Each core sample was collected using a 3-inch deep by 3.5-inch diameter cup.

In the laboratory, all core samples were rinsed using a 2mm sieve and again using a .25mm sieve to separate the plant material based on vegetative shoots, reproductive shoots, and rhizomes. All vegetative shoots and reproductive shoots were counted and weighed to determine aboveground biomass. Rhizomes plus roots were weighed to determine belowground biomass. The material was dried at 50°C for approximately 14 days and re-weighed to calculate percentage dry matter. Finally, the amount of material removed was compared to previous years.

Seed Viability Study

In August, 2010, a member of the Team conducted a seed viability study to determine a correlation between temperature and germination. Ten core samples, measuring eight inches in diameter, were collected during low tide along the shoreline of Manila.

Approximately 350 *Z. japonica* seeds were collected, which were separated into two test groups. Twenty-five seeds were tested for viability using Tetrazolium Chloride resulting in 72% viability. The remaining seeds were used to test germination success at varying temperatures. Three 20-gallon aquarium tanks were set-up at Humboldt State University's Telonicher Marine Laboratory with heaters and coolers to establish constant water temperatures. Each tank contained four Petri dishes that held ten *Z. japonica* seeds each for a total of 40 seeds per tank. Trial one investigated germination success of *Z. japonica* seeds at 30, 25, and 15 degrees Celsius. Trial two tested germination at 20, 10, and 5 degrees Celsius. The duration of the experiment was for 11 days after the first seed germinated. The number of seeds that germinated were observed and recorded daily.

Germination was observed at all six temperature groups. Germination rates at the end of the test period were 48% at 5°C, 45% at 10°C, 35% at 15°C, 53% at 20°C, 30% at 25°C, and 15% at 30°C. The analysis of variance results of the study indicate that there were no significant differences in germination rates at the varying temperatures. Within its native habitat, the optimal water temperature for seed germination is in the range 15-20°C (Abe et al. 2009). This study agreed with the results from Abe et al. (2009) which concluded that *Zostera japonica* seeds are able to germinate between 5-25°C, and that some of the highest germination rates are at 15-20°C.

Removal Methods

Excavation

Using the standard methodology of manual excavation, 48 patches of dwarf eelgrass were removed at the Manila site and one patch was removed from Bracut. In 2009, approximately 20 patches were located at the Arcata Wastewater Treatment Plant, but were not eradicated last year due to inaccessibility of the site. The Team excavated these 20 patches plus an additional 66 more patches that were discovered at this location. Finally, the Team excavated approximately 245 patches of *Z. japonica* from Arcata Marsh.

Flame Heat Treatment

After the discovery in 2009 of a large infestation of *Z. japonica* offshore, referred to as Arcata Marsh Outer, approximately 30 patches were established as experimental plots to test repeated burning as an eradication method. The burning experiment extended through mid-summer 2010, but was determined to not be an effective control for *Z. japonica*. The Team decided to use the covering method to treat this large infestation, which is discussed in the next section.

Covering

At Wallace Ranch, the Team implemented the covering method to eradicate the six patches located there. Burlap fabric was used in place of carpet squares as a more environmentally friendly material. The burlap fabric was installed on top of the patches in late August and monitored during each tidal cycle. The fabric remained in place until early December and the Team collected core samples that did not show any evidence of *Z. japonica* rhizomes. It was determined that the material must remain in place for

several months to successfully eradicate the above ground and below ground biomass.

The large infestation at Arcata Marsh Outer posed a large challenge to control after the burning treatment was ineffective. The Team began using the covering method in early November and by the end of December had covered 130 patches of *Z. japonica*. Patches were monitored during available tidal cycles to ensure all covering materials remained in place. The control efforts for this large infestation extended into 2011 and will be discussed in the 2011 *Zostera japonica* Eradication Project Annual Report.

Heater Cartridges

A new heat treatment technique, suggested by Dr. Paul Rusanowski of the University Utah, was experimented with in October this year by the Team. The method uses small cylindrical heater cartridges that are wired to a cable that is plugged into a generator. The cartridges were inserted into the substrate in areas infested with *Z. japonica*. Thermocouples were inserted in line from the cartridge radiating out at 1 cm increments. The heater cartridge operated until the temperature at the nearest thermocouple read 100°C, and then was shut down. Biomass cores collected at the site are being processed to determine the density of plant material after the experiment. We will continue to experiment with this method to assess how far to space the heater cartridges in an array. This is a promising method to treat large infested areas in shorter times, with less labor and waste.

Results and Discussion

An “early detection, rapid response” strategy is becoming widely recognized as the most effective way to combat invasive species. Early detection of *Z. japonica* is challenging because the habitat it occupies is only exposed at tides of 2.0 ft MLLW or lower, these intertidal mudflats are not easily traversed, and the very narrow blades of the eelgrass make it easy to miss. The surveys are therefore quite labor-intensive. The most recent challenge encountered is the presence of *Z. japonica* in the vast expanse of mudflat that extends offshore. This area has not been carefully surveyed previously due to the difficulty of access. The Team is still evaluating how to approach this new challenge.

The amount of *Z. japonica* discovered at Arcata Marsh, Manila, and Arcata Wastewater Treatment Plant was a large increase from what was discovered last year (Table 1). Arcata Marsh had the largest number of patches discovered (Figure 2); however only covered approximately 75 m² (Figure 3). The *Z. japonica* population at Arcata Marsh Outer covered a much greater area (2,271 m²) that was grouped into 130 patches (Figure 4). This indicates that the Arcata Marsh population consisted of hundreds of patches that were significantly smaller in size compared to Arcata Marsh Outer.

One priority for eradication efforts is to remove material prior to the reproductive season. Figure 5 depicts the amount of *Z. japonica* vegetative, reproductive, and below-ground material that was removed in 2010. A very small amount of reproductive material was observed which indicates the Team was successful in eradicating the plants prior to flowering.

It is believed that the increase in the amount of material observed in 2010 was the result of unsuccessful removal efforts in 2009. The large infestation located at Arcata Marsh Outer was able to expand greatly after the unsuccessful flame heat experiment in late 2009, early 2010. The Team is hopeful that the extensive eradication efforts completed this year will result in a decline in the amount of material found in Humboldt Bay in the future. Despite the setback at other locations, we are still encouraged by the fact that we did not discover any *Z. japonica* at Indian Island in 2010 (Figure 6).

The benefits of our work include removal of an introduced, intertidal eelgrass; passive restoration of native eelgrass habitat; detailed mapping of *Z. japonica* removed; and monitoring of revegetation in affected areas. The mud and sand flats that we are restoring are important feeding grounds for resident and migrating shorebirds such as whimbrel, long-billed curlews, willets, and marbled godwits. We plan to continue to monitor the shoreline of Humboldt Bay to enable early detection of any new occurrences of *Z. japonica* should they arise. The project will be determined successful when no *Z. japonica* can be found anywhere in Humboldt Bay.

Proposed Scope of Work for 2011-2012

Macro Invertebrate Study

Per the condition of the Coastal Development Permit for the project, the Team began an investigation into how the macro invertebrate community responds to the covering method of *Z. japonica* eradication and to determine the rate of recovery of the macro invertebrate community. The study consists of 32 cover treatment plots and 32 control plots that are each separated into four groups with 8 replicates per group. The study will continue over a 24-week period that is separated into four time periods. The sampling regime is described in Table 2. Results of the study will be discussed in the 2011 *Zostera japonica* Eradication Project Annual Report.

Our objectives remain the same as previously stated and we will continue to evaluate the effectiveness of different eradication methods, including the heater cartridge experiment that began in October.

All locations where *Z. japonica* was removed previously will be intensively monitored. Other areas of suitable habitat in the bay will be surveyed as well for early detection of any new occurrences. Wherever we find *Z. japonica*, we plan to collect data and remove all material. We will continue to notify resource managers of any new occurrences that are found and whether each new site presents any new challenges or concerns.

Zostera japonica in North Humboldt Bay, 2010

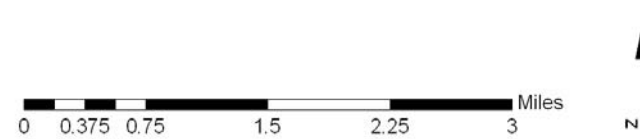
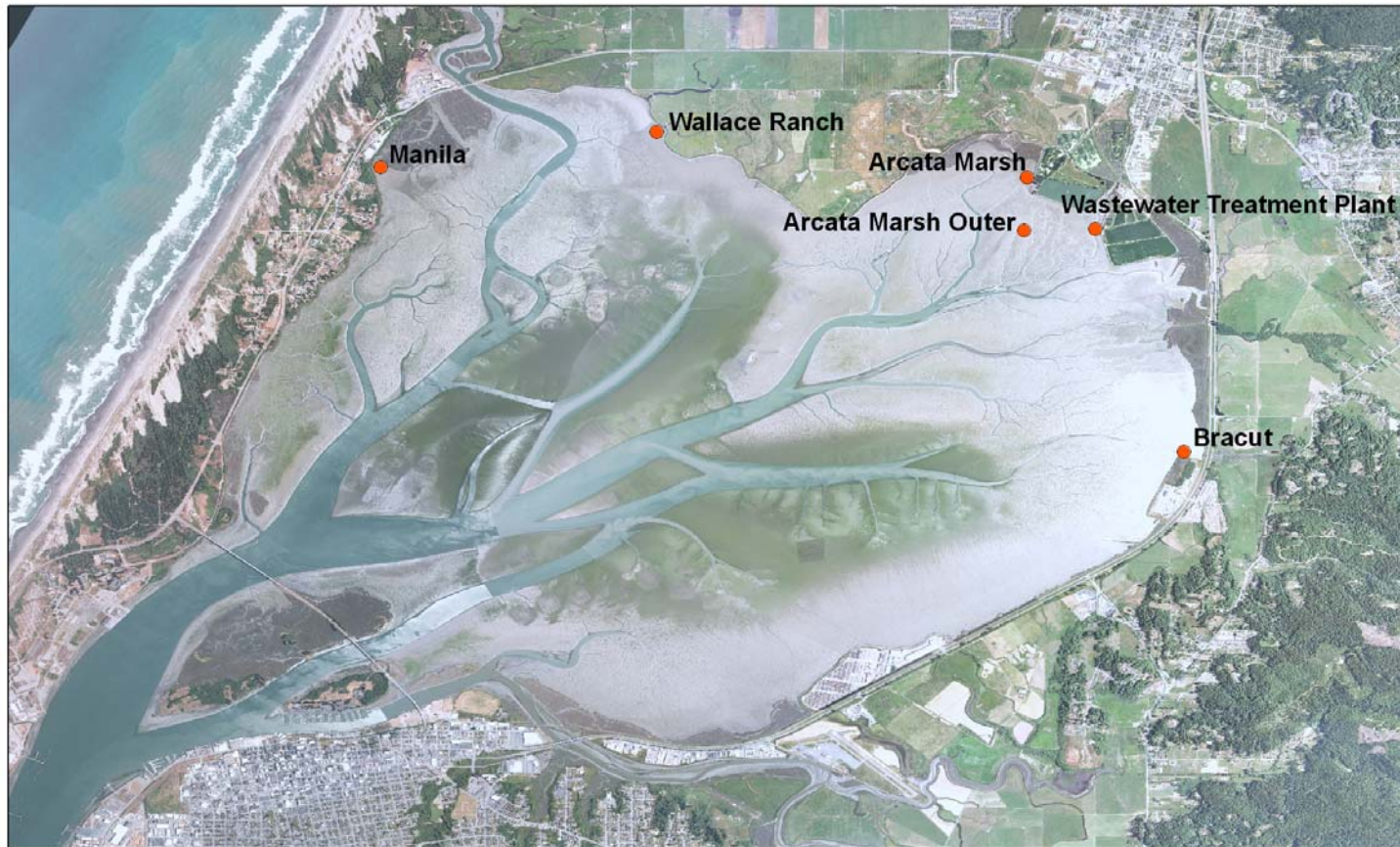


Figure 1. Six locations in North Humboldt Bay where *Zostera japonica* was removed in 2010.

Table 1. Quantitative summary of the amount of *Zostera japonica* removed from Humboldt Bay 2004 to 2010.

		Number of Patches	Area (m ²)	Dry Weight of Aboveground Biomass, Vegetative (kg)	Dry Weight of Aboveground Biomass, Reproductive (kg)	Dry Weight of Belowground Biomass (kg)	Total Biomass Dry Weight (kg)	
Indian Island	2004	188	186.86	5.24	4.13	8.50	17.87	
	2005	149	26.06	0.71	0.15	2.53	3.39	
	2006	23	6.62	0.23	0.12	0.23	0.58	
	2007	19	5.92	0.33	0.11	0.16	0.60	
	2008	4	1.93	0.03	0.01	0.01	0.05	
	2009	1	<i>Plant material has not been removed from this location yet</i>					
	2010	0	0.00	0.00	0.00	0.00	0.00	
AWTP	2007	4	1.02	< 0.01	0.00	0.03	0.04	
	2008	0	0.00	0.00	0.00	0.00	0.00	
	2009	20	<i>Plant material was included in the experimental burning treatment</i>					
	2010	86	8.79	3.11	0.00	2.53	5.64	
Arcata Marsh	2007	33	10.56	0.34	< 0.01	0.14	0.49	
	2008	36	1.87	0.25	0.05	0.25	0.55	
	2009	24	4.25	0.36	0.03	0.36	0.76	
	*2010	245	74.56	26.36	0.01	21.47	47.84	
Manila	2007	60	6.73	0.07	< 0.01	0.04	0.12	

2008	15	1.10	0.02	0.00	0.06	0.08
2009	27	5.36	0.49	0.12	0.23	0.84
2010	48	30.65	1.33	0.28	1.61	3.23
Mad R. Slough 2009	4	6.07	0.41	0.00	0.37	0.78
Wallace Ranch 2010	6	17.37	0.31	0.05	0.47	0.83
Arcata Marsh Outer 2010	130	2271.32	802.98	4.01	662.44	1469.43

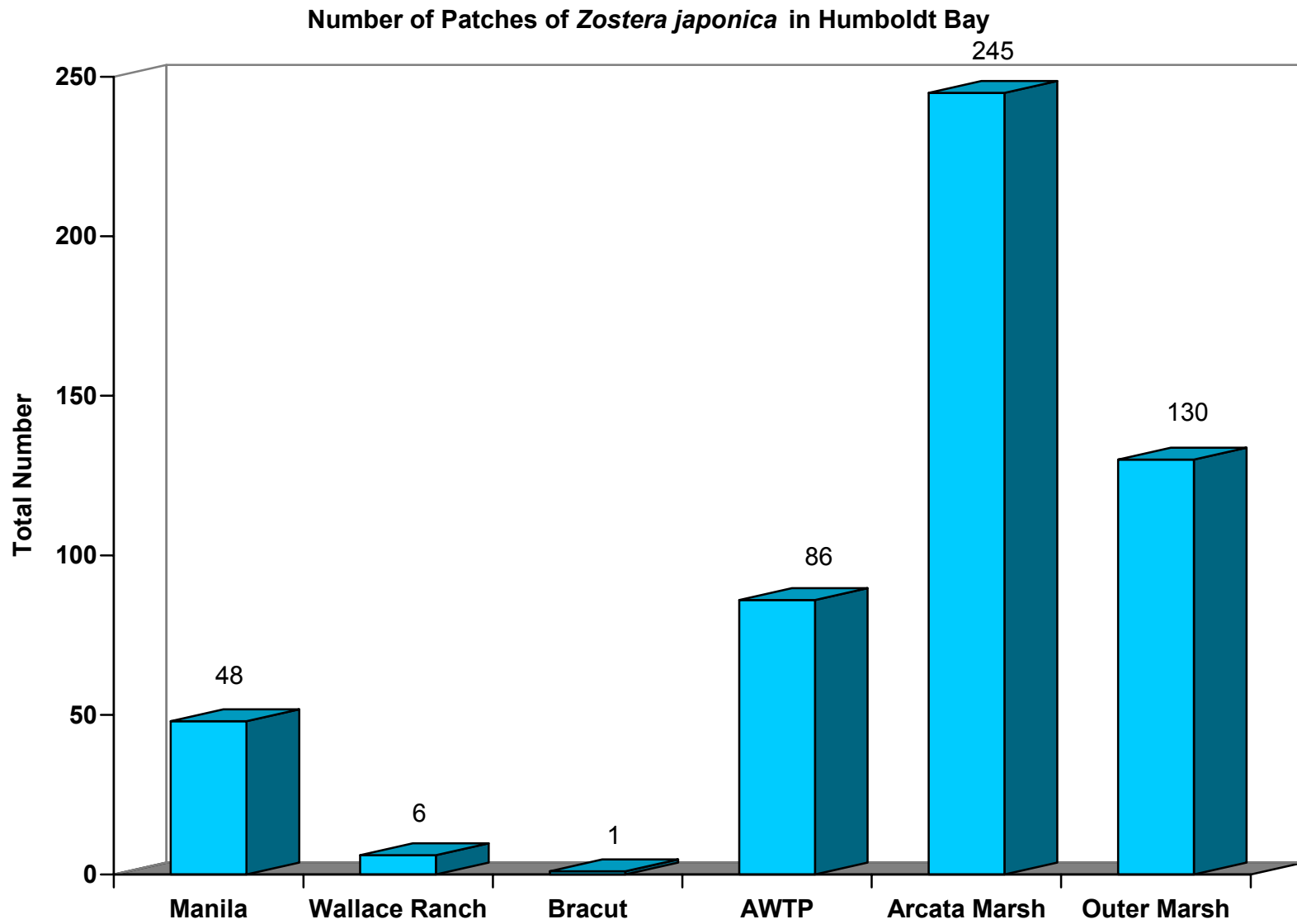


Figure 2. Number of *Zostera japonica* patches around Humboldt Bay, 2010.

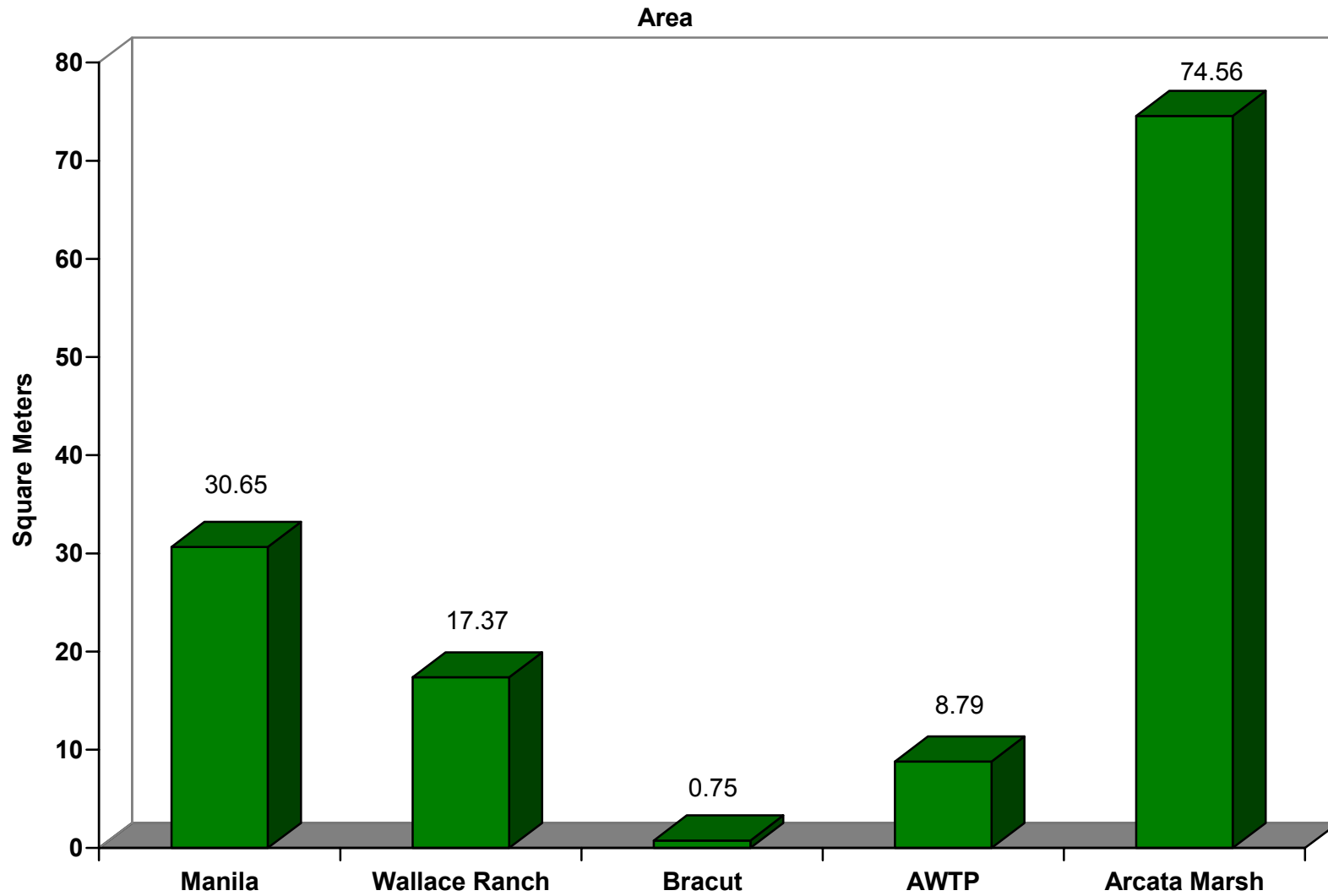


Figure 3. Total area (m²) of *Zostera japonica* present in Humboldt Bay, 2010.

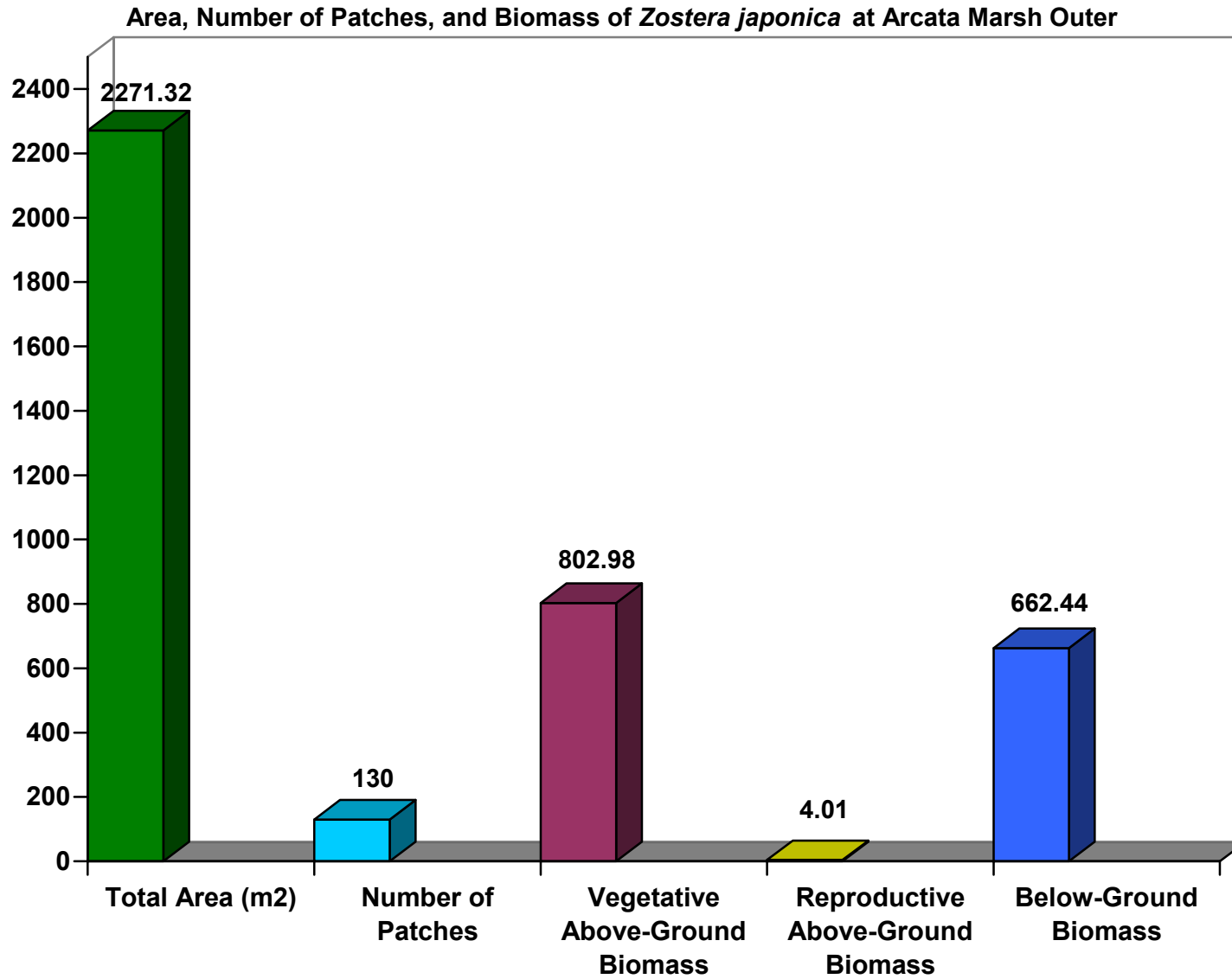


Figure 4. Amount of *Zostera japonica* (total area, number of patches, and biomass) removed from Arcata Marsh Outer, Humboldt Bay, 2010.

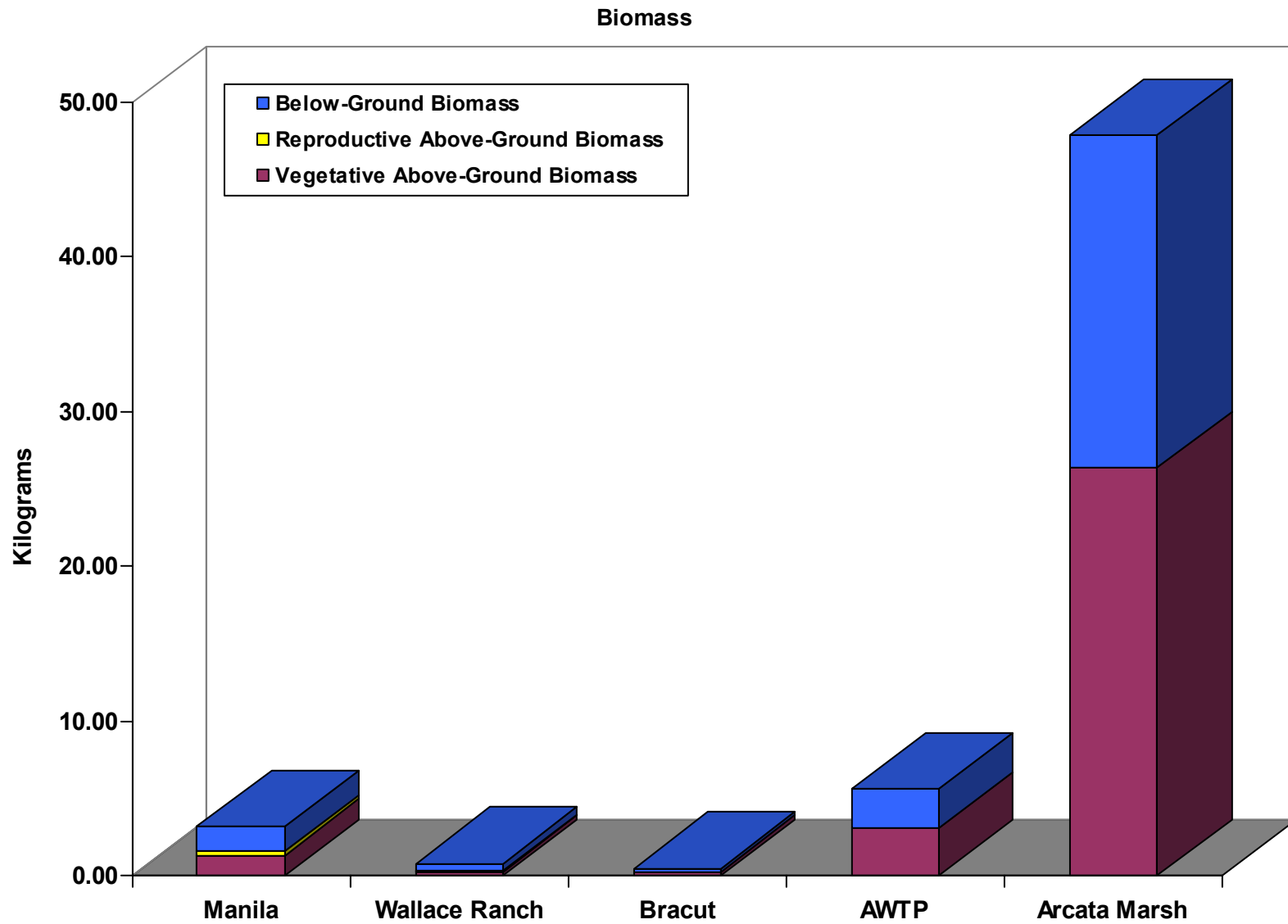


Figure 5. Amount of *Zostera japonica* biomass (kg) removed from Humboldt Bay, 2010.

Number of Patches of *Zostera japonica* at Indian Island

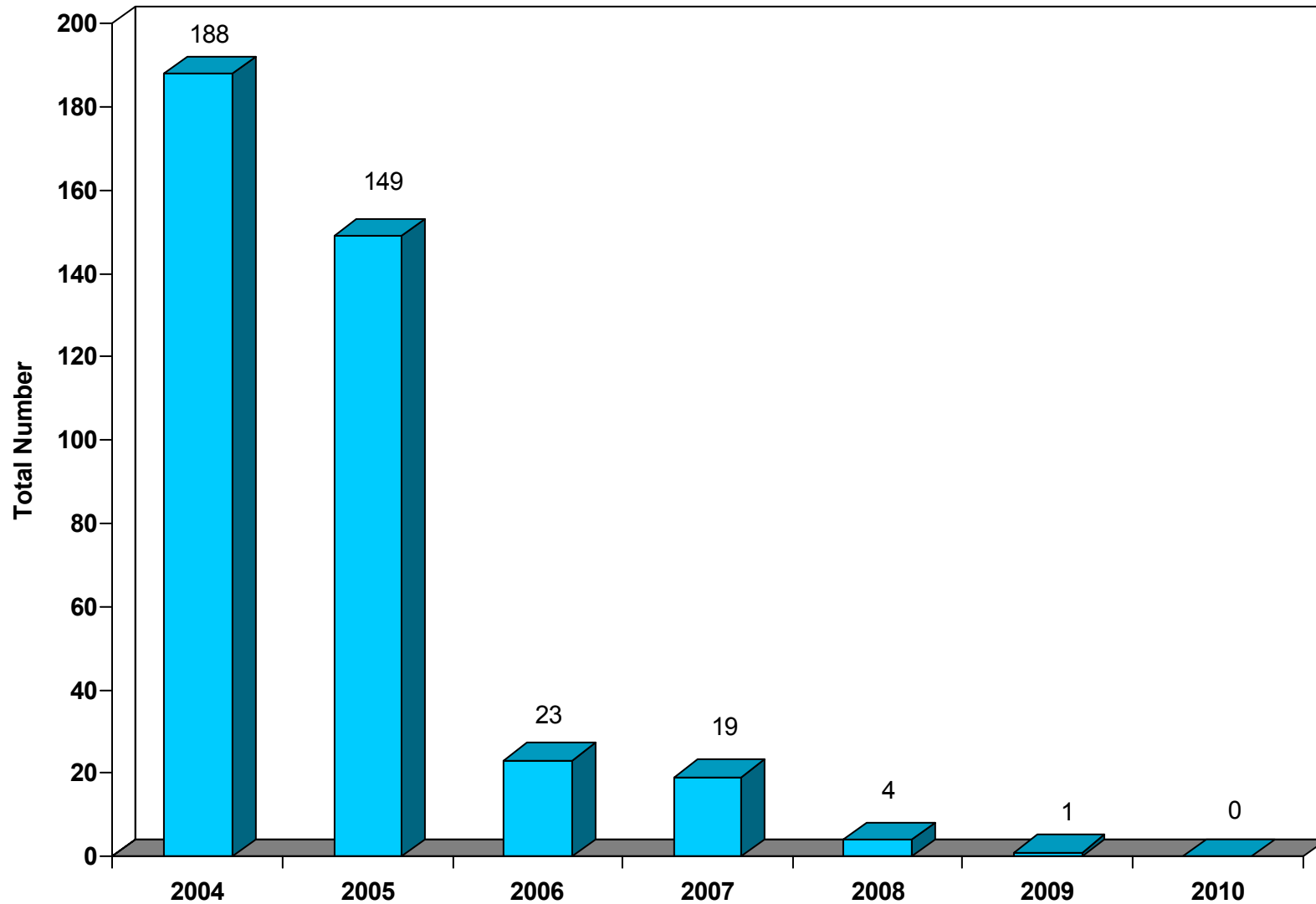


Figure 6. Number of *Zostera japonica* Patches at Indian Island, Humboldt Bay 2004-2010.

Table 2. Macro invertebrate study design

	Treatment Plots				Control Plots			
	Group A (8 plots)	Group B (8 plots)	Group C (8 plots)	Group D (8 plots)	Group A (8 plots)	Group B (8 plots)	Group C (8 plots)	Group D (8 plots)
Time 0	Cover	Cover	Cover	Cover	Collect core sample			
Time Period 1 – 6 wks post cover	Uncover Collect core sample					Collect core sample		
Time Period 2 – 12 wks post cover	Collect core sample	Uncover Collect core sample					Collect core sample	
Time Period 3 – 18 wks post cover	Collect core sample	Collect core sample	Uncover Collect core sample					Collect core sample
Time Period 4 – 24 wks post cover				Uncover Collect core sample				

References

Abe M, Yokota K, Kurashima A, Maegawa M (2009) Temperature characteristics in seed germination and growth of *Zostera japonica*, Ascherson & Graebner, from Ago Bay, Mie Prefecture, central Japan. Fisheries Science 75: 921-927