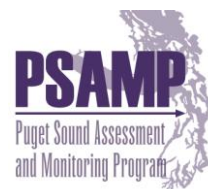




Eelgrass Stressor-Response Report 2007-2008

Zostera marina L. (eelgrass) transplant growth and survival
along a spatial and tidal gradient in Westcott Bay

February 2010



WASHINGTON STATE DEPARTMENT OF
Natural Resources
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By Anja Schanz
Hannah Julich
Lisa Ferrier
Helen Berry

Nearshore Habitat Program
Aquatic Resources Division



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Enter search term ‘nearshore habitat’ on DNR home page: <http://www.dnr.wa.gov>

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EXECUTIVE SUMMARY

The Washington State Department of Natural Resources (DNR) is the steward of 2.6 million acres of state-owned aquatic land. DNR manages these aquatic lands for the benefit of current and future citizens of Washington State. As part of its stewardship responsibilities, DNR investigates the causes of eelgrass (*Zostera marina* L.) losses in greater Puget Sound through the Eelgrass Stressor-Response Project (ES-RP).

Z. marina is a flowering plant that grows in shallow coastal waters. It provides important habitat for many economically and ecologically important fishes and invertebrates, and is linked to multiple functions and services in coastal ecosystems. *Z. marina* beds are recognized as an indicator of the health and stability of coastal ecosystems, since they are very sensitive to changes in their physical environment.

Identifying stressors related to *Z. marina* declines is an important first step toward formulating management responses to environmental degradation. Guidance regarding stressors of greatest concern is needed by multiple efforts to restore and protect Puget Sound, most notably the regional Puget Sound Partnership's Action Agenda.

Recently, observed *Z. marina* losses in shallow embayments in the San Juan Island Archipelago have generated widespread concerns about the condition of *Z. marina* in these areas. In order to explore driving factors related to patterns of *Z. marina* losses, DNR conducted a case study in Westcott Bay, an embayment with extensive documented loss. The study combined *Z. marina* transplant experiments and continuous environmental monitoring to assess habitat suitability. Hypothesizing that unfavorable physical conditions prevent *Z. marina* growth at sites in the inner and head of Westcott Bay, we transplanted *Z. marina* in currently and formerly vegetated areas along a spatial gradient of decreasing eelgrass abundance from the mouth to the bay head at three different tidal elevations, and related transplant performance to environmental parameters. Study objectives included:

- Assess current *habitat suitability* of various sites and different tidal elevations to support *Z. marina* growth and survival
- Assess the role of eelgrass *air exposure* on transplant performance
- Relate transplant performance to hypothesized *elevated water temperatures* at the head of the bay
- Update the conceptual model for *Z. marina* stressors in Westcott Bay
- Identify future research priorities

Key Findings:

1. *Z. marina* performance in transplant experiments indicated two gradients in decreasing habitat suitability throughout Westcott Bay. The strongest gradient is a spatial gradient from the mouth toward the head of Westcott Bay. In addition, in the inner bay, habitat suitability decreased on a vertical gradient from the subtidal toward the intertidal area. These results are supported by four main findings:
 - a. *Z. marina* transplants did not survive at the head of the bay at any tidal elevation.
 - b. *Z. marina* transplants did not survive at the inner bay site in the lower intertidal area, but did survive in the transition and subtidal areas.
 - c. All transplants were lost at the head of the bay and in the lower intertidal zone at the inner bay site in two consecutive years (2007 and 2008).
 - c. *Z. marina* currently persists at sites near the entrance of Westcott Bay, as demonstrated by the survival of transplants and naturally growing *Z. marina* plants in this area.
2. A strong negative correlation between transplant performance and air exposure suggests that prolonged air exposure during extreme low tides in spring and summer contributed to rapid *Z. marina* loss in the lower intertidal area at sites of the inner and the head of the bay in 2007, and perhaps to the loss of intertidal transplants at the inner bay site in 2008. Prolonged air exposure most likely affected *Z. marina* plants either exclusively by desiccation stress due to water loss in leaves, or by a combined effect of desiccation and heat stress resulting from warm air and sediment temperatures, depending on site specific environmental characteristics. In contrast, comparably short air exposure of transplants at the entrance and the head of the bay in 2008 corresponded with initially stable transplant performance. The eventual loss of transplants at the bay head in 2008 suggests a stressor other than air exposure.
3. A moderate negative correlation between transplant performance and water temperatures suggests that elevated water temperature in summer is a contributing stressor at the head of Westcott Bay. In the head of the bay, consistently higher water temperatures (sometimes reaching critical values) were recorded at all tidal elevations relative to other sites at the bay in two consecutive years (2007 and 2008). Distinct decreases in *Z. marina* transplant performance coincided with distinct increases in water temperature.
4. Based on our study findings, we updated the conceptual model of *Z. marina* stressors in Westcott Bay. Results suggest a suite of unfavorable conditions (rather than a single stressor) affecting *Z. marina* performance. Increasing or consistently warm water temperatures in conjunction with low oxygen conditions or anoxic events may preclude growth and survival of *Z. marina* in the presence of high sediment sulfide concentrations. These effects may be even more pronounced in the lower intertidal area, where prolonged air exposure, resulting in desiccation and/or heat stress, affect *Z. marina* growth and survival during extreme low tides in spring and summer. This hypothesis is supported by global studies on sudden seagrass die-offs during summer months in other regions.

The suggested suite of stressors may also explain *Z. marina* declines in other quiescent shallow embayments in the San Juan Island Archipelago, and other areas in Puget Sound with comparable environmental characteristics.

Recommendations

The study demonstrates that current environmental conditions do not support *Z. marina* survival at three tested tidal elevations at the head of Westcott Bay and in the intertidal at the site of the inner bay. This suggests that the current observed *Z. marina* distribution in Westcott Bay most likely represents the extent of suitable habitat. Therefore, planting of *Z. marina* in order to restore the historical *Z. marina* sites in Westcott Bay is currently *not* recommended.

Mechanisms behind the factors which affect *Z. marina* must be addressed in future studies to quantitatively and qualitatively understand the relative importance of stressors in Puget Sound. An important step for future work includes investigation of cascading effects caused by increasing temperature and/ or eutrophication, such as events of anoxia and high sediment sulfides that probably decrease habitat suitability for *Z. marina*.

An inherent challenge in identifying stressors is to tease apart complex site-specific differences from plant responses to stressors. Further work could benefit from the enhanced understanding of site characteristics and physical processes in Westcott Bay gained in this and previous studies.

In quiescent shallow embayments such as the head of Westcott Bay, extreme climatic events such as exceptionally low tides and elevated water temperatures may amplify the effect of the identified suite of stressors and related processes. These changes can be triggered by climate change, as well as climatic events such as the 18.6 year tidal epoch in the Northeast Pacific, El Niño Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO). Therefore, we recommend that future studies on *Z. marina* stressors and restoration in Puget Sound consider potential effects of climate change and climate variability.

ES-RP Priorities

The overall goal of the ES-RP is to identify and understand *Z. marina* stressors by investigating sites with observed losses in greater Puget Sound. Future work priorities to support the project goal include:

- 1 Complete analysis of *Z. marina* monitoring data recorded in Westcott Bay and other shallow embayments in the San Juan Archipelago in 2008 and 2009 to assess changes in *Z. marina* distribution in other related areas of concern.
- 2 Assess the carbohydrate reserves in root and rhizome tissue of *Z. marina* transplants from Westcott Bay in order to identify the potential early depletion of the carbohydrate reserve and to better understand causes of *Z. marina* losses.
- 3 Analyze existing water column nutrient data in Westcott Bay in order to characterize nutrient variability along a spatial scale from the entrance to the head of the bay.
- 4 Assess the combined effect of elevated water temperature and sediment sulfides on *Z. marina* survival in Westcott Bay.

-
- 5 Analyze water column oxygen in Westcott Bay in order to identify hypoxic or anoxic events.
 - 6 Analyze 2009 PAR data (recorded at different tidal elevations) to evaluate light availability in late summer and fall. Reduced light levels in late summer and fall may prove to be critical to plant survival, e.g., during high plant respiration due to stress.



1 INTRODUCTION

1.1 IMPORTANCE OF *ZOSTERA MARINA*

Zostera marina L. (eelgrass) is an aquatic flowering plant that inhabits the intertidal and subtidal areas in greater Puget Sound. Seagrass beds, such as *Z. marina* beds, are of a high ecological and economic value, and are linked to various functions in shallow coastal ecosystems; they modify the food web and material exchange, enhance productivity and biodiversity, and serve as ecosystem engineers in coastal areas (den Hartog 1970, Fonseca *et al.* 1990, Heck *et al.* 1995, Matilla *et al.* 1999, Thomas *et al.* 2000, Bos *et al.* 2007). *Z. marina* beds improve the water quality by reducing particle loads and acting as a sink for nutrients (e.g., Short & Short 1984, Gacia *et al.* 1999, Asmus & Asmus 2000). The plant root and rhizome system binds and stabilizes the sediment, thus counteracting erosion processes (Harlin *et al.* 1982, Fonseca 1996), and the complex canopy structure provides shelter and serves as a feeding, spawning, and nursery habitat for many fish and crustaceans (e.g., Heck *et al.* 1995, Matilla *et al.* 1999, Valentine & Heck 1999, Polte *et al.* 2005), thus benefiting the commercial coastal fishery. On a global scale, the total economic value of all ecosystem services provided by seagrass/algae beds has been estimated at US \$3.8 trillion yr⁻¹ (cf. the total value of forests: US \$4.7 trillion yr⁻¹) (Constanza *et al.* 1997). In the Pacific Northwest, *Z. marina* habitat sustains important migratory and residential animal species, such as the Dungeness crab (*Cancer magister*), black brant (*Branta bernicla*) (Wilson & Atkinson 1995) and juvenile salmon (*Oncorhynchus spp.*) (Simenstad 1994). In addition it is a spawning ground for the Pacific herring (*Clupea harengus pallasii*) (Phillips 1984).

Z. marina beds are biotic communities that indicate the health and stability of coastal ecosystems since they require a high quality of environmental conditions and consequently respond sensitively to changes in their environment. The decline of *Z. marina* has often been attributed to human-induced disturbances as well as climatic changes, which often lead to elevated nutrients and reduced light availability as well as changes in temperature and salinity (Short & Wyllie-Echeverria 1996, Short & Neckles 1999).

1.2 THE EELGRASS STRESSOR-RESPONSE PROJECT

Cases of *Z. marina* decline throughout Puget Sound have been documented by the Nearshore Habitat Program (NHP) of the Washington Department of Natural Resources (DNR) through its long-term Submerged Vegetation Monitoring Project (SVMP) (Gaeckle *et al.* 2008). The Eelgrass Stressor-Response Project (ES-RP) was initiated by DNR in 2005 to investigate and understand the nature of stressors that lead to declines of *Z. marina* in Puget Sound, observed in SVMP monitoring data and other data sets (Dowty *et al.* 2007). A key emphasis of the ES-RP is to deliver information to resource managers and decision makers that will guide management actions to protect and restore these valuable habitats. Guidance regarding stressors of greatest concern is needed by multiple groups working to restore and protect Puget Sound, especially in the context of the regional Action Agenda of Puget Sound Partnership (PSP).

In 2007 and 2008, ES-RP focused its research in the San Juan Island Archipelago where *Z. marina* declines have been observed, and have led to concern of further loss (Dowty *et al.* 2007, Gaeckle *et al.* 2007, 2008) (Fig. 1-1).

1.3 INVESTIGATING EELGRASS STRESSORS IN WESTCOTT BAY- A CASE STUDY FOR *Z. MARINA* LOSSES IN THE SAN JUAN ISLAND ARCHIPELAGO

1.3.1 GENERAL BACKGROUND

In the San Juan Island Archipelago, *Z. marina* losses have been most prominent in the heads of shallow embayments, such as at Westcott Bay (San Juan Island), Blind Bay and Picnic Cove (Shaw Island), and Watmough Bay (Lopez Island) as documented by the SVMP and other studies (e.g., Wyllie-Echeverria *et al.* 2003, Gaeckle *et al.* 2007, 2008) (Fig. 1-1). The major effort of the ES-RP work in 2007 and 2008 was associated with investigations of *Z. marina* stressors in the San Juan Island Archipelago, with special focus on Westcott Bay, a site of substantial loss of *Z. marina* (Fig. 1-2).

The total loss of *Z. marina* at the head of Westcott Bay between 2001 and 2003 is the largest and most recognized *Z. marina* decline known in the San Juan Island Archipelago. The presence of *Z. marina* throughout Westcott Bay was documented by DNR's SVMP in 2000 and 2001 (Berry *et al.* 2003). In February 2003, an extensive loss of approximately 20 hectares of *Z. marina* was discovered during an annual Pacific herring spawn survey conducted by the Washington State Department of Fish and Wildlife (WDFW) (Wyllie-Echeverria *et al.* 2003) (Fig. 1-2).

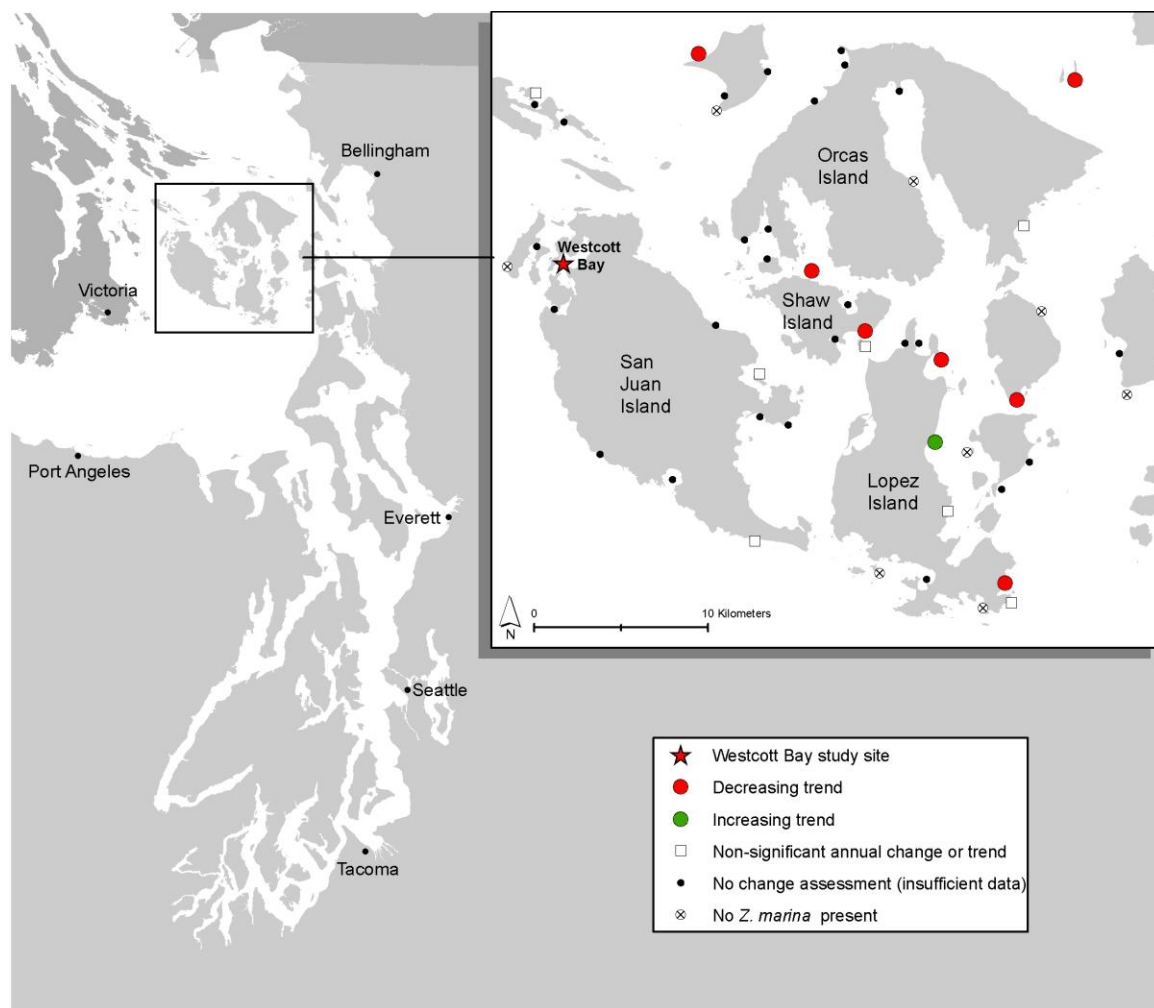


Fig. 1-1 ES-RP research focused on the San Juan Island Archipelago in 2007 and 2008. *Zostera marina* losses have been most prominent in the heads of shallow embayments, first observed and most widely recognized in Westcott Bay at the northwest corner of San Juan Island. Inset map shows increasing and decreasing *Z. marina* area trends at 80% confidence intervals (SVMP unpubl. data) and Westcott Bay, where a dramatic loss of *Z. marina* was observed in 2003.

Moreover, there seems to be evidence that the last remaining *Z. marina* population at the inner Westcott Bay site (Bell Point) that had been documented as being healthy by WDFW in 2003 (Fig. 1-2), may have declined between 2003 and 2007 (Wyllie-Echeverria & Britton Simmons pers. communication, Schanz *et al.* pers. observation). This is supported by a photo showing extensive *Z. marina* vegetation throughout the lower intertidal zone up to the exposed shore line at Bell Point in 2003 (Fig. 1-3), while *Z. marina* was absent in these areas in 2007. Absence of *Z. marina* along the shoreline of Westcott Bay was further documented in a *Z. marina* survey conducted in July 2007 (Dethier & Berry 2008). The authors found that the overall distribution of *Z. marina* changed from a virtually continuous ring along the shoreline of Westcott Bay in 1998 to very small and scattered *Z. marina* stands in 2007 that only persisted in close proximity to the entrance of the bay.



Fig. 1-2 Map of Westcott Bay with *Zostera marina* observations in 2000, 2001 and 2003. Presence of *Z. marina* in the inner Westcott Bay documented by SVMP in 2000 (orange) and in 2001 (green) along monitored transect lines (white). Areas shaded in orange present *Z. marina* loss observed in a 2003 survey completed by WDFW.

Alarmed by the drastic *Z. marina* losses in Westcott Bay, several scientific groups including scientists from the University of Washington (UW), Friday Harbor Laboratories (FHL), U.S. Geological Survey (USGS), Pacific Science Center (PSC), Friends of San Juan (FOSJ), and DNR conducted initial multidisciplinary investigations in Westcott Bay and other sites in the San Juan Island Archipelago in order to identify the causes of the observed *Z. marina* declines (e.g. Wyllie-Echeverria *et al.* 2003, Dowty *et al.* 2007).

Field observations of high turbidity at the head of Westcott Bay (particularly turbidity plumes from tidal resuspension) led to the initial investigation of *Z. marina* stressors that focused on low light availability due to high turbidity in Westcott Bay (Dowty *et al.* 2007, Dowty & Ferrier 2009). This and other key biotic and abiotic observations resulting from initial surveys between 2003 and 2007 underlie the initial conceptual thinking about Westcott Bay stressors (Dowty *et al.* 2007).



Fig. 1-3 Photo from the northeast side of Bell Point showing extensive *Z. marina* vegetation throughout the lower intertidal zone up to the exposed shore line in May 2003. *Z. marina* was absent in these areas in 2007.

Observations of other bays in the San Juan Islands Archipelago show similar patterns of *Z. marina* distribution with evidence of decline at the heads of the bays, suggesting that key stressors responsible for *Z. marina* loss at the head of Westcott Bay may also operate at a regional scale rather than just locally at Westcott Bay.

Table 1-1 summarizes hypothesized stressors and other priority research questions identified by the ES-RP during initial strategic planning and field work. Results of field research conducted in 2007 and 2008 are being reported in a series of different reports.

Table 1-1 Initial research questions related to *Z. marina* stressors in Westcott Bay

Hypothesis / Parameter of Interest	Related observations	Source of observations	Status of assessment (DNR)	References
1 <i>Z. marina</i> will not survive under the current conditions at the head of the bay in the formerly vegetated area.	No vegetation observed at the head of WB since 2003 . <i>Z. marina</i> is decreasing on a spatial and tidal gradient towards the head of WB.	FOSJ underwater monitoring 2005, Wyllie-Echeverria & Britton Simmons, pers. comm. DNR staff, pers. observations March 2007, Dethier & Berry 2008.	Focus of this study	This report
2 Limited light availability at the head of the bay due to high turbidity precludes growth of <i>Z. marina</i> .	Frequent/persistent high turbidity observations in 2005 and 2006.	Takesue, USGS, Wyllie-Echeverria & Britton-Simmons, FHL, pers. communication.	Underwater photosynthetically active radiation (PAR) is not a key factor controlling <i>Z. marina</i> abundance in WB. PAR was strongly reduced at the head of WB by roughly 20-25 %, but was nearly threefold greater (8.4 mol m ⁻² day ⁻¹) than the minimum requirements for <i>Z. marina</i> survival in Pacific Northwest estuaries (3 mol m ⁻² day ⁻¹ ; Thom et. al. 2008). Mean level of turbidity and chlorophyll are elevated at bay head. Topic for future research.	Dowty & Ferrier (2009)
3 Mean turbidity and intensity and duration of discrete turbidity events increases from the mouth to the head of WB.				
4 Mean turbidity and the intensity and duration of discrete turbidity events increases from the subtidal to the upper intertidal for sites at the inner WB.				
5 Limited nutrient supply at the head of the bay prevents the re-establishment and growth of <i>Z. marina</i> .	Low summer water column nutrients (N, P, and Si) in Aug'04, June'05, July'06 .	Takesue, USGS, unpublished data.	Preliminary water column nutrient data collected. Topic for future research.	-
6 High water temperature events in 2002 led to direct stress on <i>Z. marina</i> in the head of the bay (and promoted an out break of the wasting disease <i>Labyrinthula zosterae</i>).	No previously documented observations.	-	Annual water temperatures (Source: NOAA, Friday Harbor data) presented in discussion chapter.	This report
7 High sediment sulfide levels resulting from high sediment organic matter and anoxic conditions led to <i>Z. marina</i> decline.	High sediment organic matter content at the head of WB.	Shoemaker & Wyllie-Echeverria, FHL, unpublished data, this report.	Initial field observations suggest high sediment sulfides at bay head, moderate sediment organic matter at the inner and head of WB. Topic for future research	This report
	Low sediment redox values at the head of WB in July 2006.	Takesue, USGS, unpublished data.		
8 There is a negative correlation between air exposure and <i>Z. marina</i> performance during extreme low tides in the lower intertidal area.	Symptoms of desiccation stress on intertidal eelgrass plants ("crispy" dry brown eelgrass leaves) during periods of extreme low tides in spring and summer 2007 .	DNR research April to July 2007.	Focus of this study	This report
9 There is a negative correlation between increasing water temperature and <i>Z. marina</i> performance toward the head of WB.	High water temperature at the head of WB in spring/summer 2007.	DNR research May to July 2007.	Focus of this study	This report
10 Carbon & nitrogen contents in leaf tissue potentially indicate nutrient limitation at the head of WB.	No previously documented observations.	-	Topic of current research	Report in prep.
11 Carbohydrate reserves remain high, eliminating slow-acting stressors that affect long-term plant carbon balance, and highlighting quick-acting stressors.				
12 Survey trends in <i>Z. marina</i> distribution in WB and other selected shallow embayments in the San Juan Island (SJI) Archipelago.	<i>Z. marina</i> distribution in WB decreased between 1998 and 2007. Other bays in the SJI Archipelago show similar patterns of <i>Z. marina</i> decline at the heads of the bays .	DNR research (2007, 2008, 2009) Wyllie-Echeverria 2005a,b. DNR research 2004 and 2009.	<i>Z. marina</i> distribution in WB dramatically decreased from 2001 to 2007. <i>Z. marina</i> trend assessment in WB and other selected shallow embayments in the SJI in progress. General <i>Z. marina</i> trend assessment in focus region SJI (SVMP monitoring 2004 & 2009).	Dethier & Berry (2008) Ferrier et al. in prep. Dowty et al. (2004), Gaeckle et al. in prep.

1.3.2 PURPOSE OF THE PRESENT STUDY

The purpose of this study was to assess the current habitat suitability for *Z. marina* growth and survival in order to identify current *Z. marina* stressors in Westcott Bay. Hypothesizing that unfavorable environmental conditions prevent *Z. marina* growth in the inner and head of the bay, we conducted transplantations in currently and formerly vegetated areas along a spatial gradient of decreasing *Z. marina* abundance from the entrance to the head of Westcott Bay at three different tidal elevations, and related transplant performance to environmental parameters.

Research was designed to take advantage of an observed spatial gradient of *Z. marina* abundance as well as a hypothesized gradient in multiple interrelated environmental conditions (turbidity, nutrients, temperature, currents, etc.) within the bay. Environmental data were collected at multiple sites in order to:

- a) Identify relationships between the time series of environmental parameters and the performance of *Z. marina*.
- b) Identify thresholds in environmental parameters beyond which *Z. marina* growth and survival is not supported (supported by values documented in the literature).

In addition to human impacts (e.g. eutrophication), *Z. marina* beds in shallow embayments are exposed to a variety of environmental extremes, such as wide temperature fluctuations and, if growing in the intertidal area, exposure to air and resulting desiccation (all of which are influenced by the prevailing tidal conditions). Though many seagrass species have evolved anatomical and physiological mechanisms to deal with such environmental conditions (Pérez-Lloréns & Niell 1993, Abal *et al.* 1994), extreme events can produce conditions beyond the range of physiological tolerance. The effect of temperature extremes (Reusch *et al.* 2005, Ehlers *et al.* 2008) and desiccation (Leuschner *et al.* 1998, Seddon & Cheshire 2001) have been recognized in marine macrophytes. However, the effect of these factors and underlying plant physiological processes in seagrasses are not yet well understood.

Based on initial field research and data analysis in 2007, elevated water temperature at the head of Westcott Bay, as well as desiccation of *Z. marina* in the lower intertidal during extreme low tides in spring and summer was identified as potential stressors controlling *Z. marina* growth and survival in Westcott Bay. In order to examine the potential role of elevated water temperatures and air exposure on *Z. marina* transplant development, this study assesses water temperatures and events of *Z. marina* air exposure at various sites and different tidal elevations in Westcott Bay in spring and summer 2007 and 2008, and correlates these environmental parameters with the performance of *Z. marina* transplants.

Note to Table 1-1

1-7 Identified in the ES-RP 2005-2007 report (Dowty *et al.* 2007). Hypotheses # 1-5 were identified as priorities for investigation, while hypotheses # 6 and # 7 describe potential historical scenarios that may not be directly tested.

8-12 Hypotheses and parameter of interest identified in 2007.

SPECIFIC OBJECTIVES OF THE PRESENT STUDY ARE

- 1) assess the current habitat suitability of various sites and different tidal elevations to support *Z. marina* development and survival in intertidal and shallow subtidal areas throughout Westcott Bay;
- 2) assess the role of air exposure on *Z. marina* transplant development and survival in the lower intertidal area relative to the transition area and subtidal area across Westcott Bay;
- 3) assess the relationship between water temperatures and *Z. marina* performance along a gradient of increasing water temperature toward the inner Westcott Bay at different tidal elevations;
- 4) update the conceptual model of eelgrass stressors in Westcott Bay and priorities for future research based on plant performance and temperature results.

IT IS HYPOTHESIZED THAT

- a. *Z. marina* transplant performance decreases at sites towards the inner bay (in areas of observed eelgrass loss) over the main growing season at different tidal elevations.
- b. *Z. marina* transplant performance decreases with increasing time of plant exposure to air.
- c. *Z. marina* transplant performance decreases with increasing water temperatures in a gradient from the mouth to the head of Westcott Bay.



2 MATERIALS AND METHODS

2.1 STUDY AREA WESTCOTT BAY

Research was conducted in Westcott Bay, which is located in the San Juan Island Archipelago along the northwest coast of San Juan Island (Puget Sound, Washington) (Fig. 1-2, 2-1). It is a small shallow embayment of about 3 km in length and averages 800 m in width with a maximum depth of approximately 8.5 m. A majority of the bay (83%) varies between depths of 2 to 3 m. Westcott Bay is oriented in WSW to ENE directions and because of its narrow entrance, receives little swell from wind waves originating from summertime northwest and periodic wintertime southwest fetch. Tides are mixed semi-diurnal with a tidal range of about 3.5 - 4 m. Current velocities of up to 1.0 m s^{-1} were measured at the entrance of Westcott Bay and decreased with distance into the head of the bay (Grossman *et al.* 2007). The substrate is characterized by coarse, sandy sediments, including gravel and cobbles at the entrance of Westcott Bay, with fine sediments in the center, whereas the head of the bay is dominated by silt with a significant portion of clay (Grossman *et al.* 2007) (Table 2-1). *Z. marina* grows in dense, perennial populations adjacent to the channel and the entrance of the bay at Mosquito Pass (MP) and White Point (WP) and decreases in abundance within Westcott Bay (Fig. 2-1, Table 2-1). One sparse, residual *Z. marina* population persists in the inner Westcott Bay at Bell Point (BP) in the low intertidal and subtidal area, whereas *Z. marina* has been absent from the head of Westcott Bay (WBS & WBN) since 2002/2003. Table 2-1 characterizes the conditions of the different *Z. marina* research sites during the experimental period in 2007 and 2008.

2.2 Z. MARINA EXPERIMENTS

2.2.1 EXPERIMENTAL DESIGN

In order to investigate the present habitat suitability for *Z. marina* growth and survival in lower intertidal and shallow subtidal areas throughout Westcott Bay, two different seagrass transplantation experiments were carried out at various sites and different tidal elevations in currently and formerly vegetated sites along a gradient of decreasing abundance of *Z. marina* from the entrance toward the inner bay in May 2007 and 2008. In 2007, core transplantations were carried out at five different sites at -0.7 m MLLW in order to assess *Z. marina* performance across the sites in the intertidal area. In 2008, transplantations of *Z. marina* shoots in transplant units (TPUs) were carried out at three sites at -0.7 m (lower intertidal area), -0.9 m (transition zone) and -1.5 m MLLW (subtidal area) in order to



Fig. 2-1 Color infrared aerial photography of the area of Westcott Bay showing the locations of *Z. marina* study sites (2007: blue lines; 2008: red, violet and green lines) and adjacent water quality monitoring stations (red circles) in 2007 (WP, BP, WBS, WBN) and 2008 (WP, BP, WBN). MP represents the site of the visually healthy, dense donor population at the bay entrance (see section 2.3 and 2.4 for more details).

accommodate the assessment of *Z. marina* transplant performance at different tidal elevations in 2008 (Fig. 2-1, 2-3). The transplant experiments were designed to allow for the assessment of the role of air exposure and elevated water temperature in *Z. marina* transplant development and survival at individual sites across Westcott Bay (Fig. 2-2, 2-3, 2-6). *Z. marina* transplants located in the lower intertidal area were expected to be regularly exposed to air during periods of extreme low tides in spring and summer, whereas transplants in the transition zone were assumed to be exposed to air only infrequently. Subtidal transplants remained submerged perpetually. At all transplant sites temperature loggers were placed at the center of each transplant transect (Fig. 2-3).

Table 2-1 Physical and biological characteristics of *Z. marina* transplant sites in spring and summer 2007 and 2008. **MP** Mosquito Pass, **WP** White Point, **BP** Bell Point, **WBS** Westcott Bay South, **WBN** Westcott Bay North.

Site	MP (Donor site)	WP	BP	WBS	WBN
Location in bay	Entrance of Bay		Inner Bay	Head of Bay	
Sediments					
Type of substrate ^a	medium sand, partly with pebbles	fine to very fine sand	very fine sand	coarse silt	coarse to medium silt
Mean grain size (mm)	0.25 - 0.5	0.125 - 0.25	0.062 - 0.125	0.0331 - 0.062	0.016 - 0.063
Organic matter (%) ^b	0.81 (±0.06)	1.541 (±0.10)	2.91 (±0.43)	2.35 (±0.20)	0.279 (±0.09)
Water motion ^c	high	high/moderate	moderate	low	low
Light availability ^d					
Daily PAR (mol m ⁻² day ⁻¹) ^e	-	5.46 - 15.28	5.69 - 16.01		2.80 - 12.92 ^g
Attenuation ^f	-	0.26 - 0.76	0.33 - 0.65		0.52 - 0.82 ^g
Seagrass presence ^h					
Intertidal area	dense	sparse	no	no	no
Subtidal area	dense	dense	sparse	no	no
Other observations					
Algae presence ⁱ	Moderate to dense green algae cover in lower intertidal zone	Moderate to dense green algae cover in lower intertidal zone	Dense green algae mats with high coverage in lower intertidal zone in July/August	no	no

Notes to Table 2-1

- ^a Grossman *et al.* (2007). Mean grain size in mm was classified by predominant grain size classes.
- ^b Mean sediment organic matter (%) (±SEM) at the lower intertidal zone. Sediment core samples (n=6) were collected at -0.7 m MLLW in August 2007 (Schanz, unpubl. data).
- ^c Moderate currents at MP of approximately 0.2 - 0.4 m s⁻¹ decreased steadily with distance into the head of the bay (Grossman *et al.* 2007); Observations of Kitaeff *et al.* (pers. observations during subtidal field work in 2008) and Schanz *et al.* (pers. observations during intertidal and subtidal fieldwork in 2007 and 2008) support the classification.
- ^d Dowty and Ferrier (2008). Photosynthetically active radiation (PAR) and attenuation at -1.5 m MLLW (see e and f).
- ^e Minimum and maximum monthly averages of daily photosynthetically active radiation (PAR) 30 cm above the sediment surface from April to September 2007 and 2008.
- ^f Minimum and maximum of the mean monthly attenuation from April to September 2007 and 2008. Attenuation was calculated from simultaneous PAR measurements 30 cm and 70 cm above the sediment surface.
- ^g Data from WBS (2007) and WBN (2008) sites were combined to represent the conditions at the head of the bay.
- ^h Schanz *et al.* (pers. observations) and data presented in this report.
- ⁱ Kitaeff *et al.* 2008 (pers. communication), Schanz *et al.* (pers. observations) this report.
- Water quality monitoring stations not established.

	Objectives	Parameters	Methods		
Objective I	Capacity of various sites and different tidal elevations to support <i>Z. marina</i> development and survival	<i>Z. marina</i> shoot density Above- and belowground biomass Transplant survival	Assessment of transplant performance	Environmental monitoring	<i>Z. marina</i> Transplantation experiments
Objective II	Role of air exposure on <i>Z. marina</i> transplant performance	<i>Z. marina</i> shoot density Above- and belowground biomass Transplant survival Air exposure Air temperature	Correlation of transplant performance with air exposure		
Objective III	Role of elevated temperatures on <i>Z. marina</i> performance	<i>Z. marina</i> shoot density Above- and belowground biomass Transplant survival Water temperature	Correlation of transplant performance with water temperature		

Fig. 2-2 Summary of parameters and methods used during this study to achieve the specific objectives.

2.2.2 SITE SELECTION

In order to determine the current approximate distribution of *Z. marina* for transplantation experiments, sites of existing *Z. marina* populations across Westcott Bay were identified using color-infrared 1:12,000 aerial photography collected in summer 2006 (Berry 2007) and confirmed by ground-truthing conducted by foot and boat during low tide periods in March 2007 (Fig. 2-1).

Z. marina transplant sites were selected to characterize a gradient in *Z. marina* condition as well as related physical and biological habitat properties from the entrance to the head of Westcott Bay (Table 2-1).

2.2.3 Z. MARINA TRANSPLANTATION

Seagrass cores (2007)

Core transplantations of *Z. marina* were conducted in the intertidal area at Mosquito Pass (MP), White Point (WP), Bell Point (BP), Westcott Bay South (WBS) and Westcott Bay North (WBN) in 2007 (Fig. 2-4, 2-6). *Z. marina* cores, including intact sediment, were taken from a donor population (MP) at the entrance of the bay by carefully pushing a plastic tube (core area: 182 cm², high: 20 cm) into the sediment without disturbing the leaves of the plants (Fig. 2-4). Seagrass cores were transported and kept separately in their tubes covered by a moist towel to prevent desiccation. Twelve seagrass cores from the

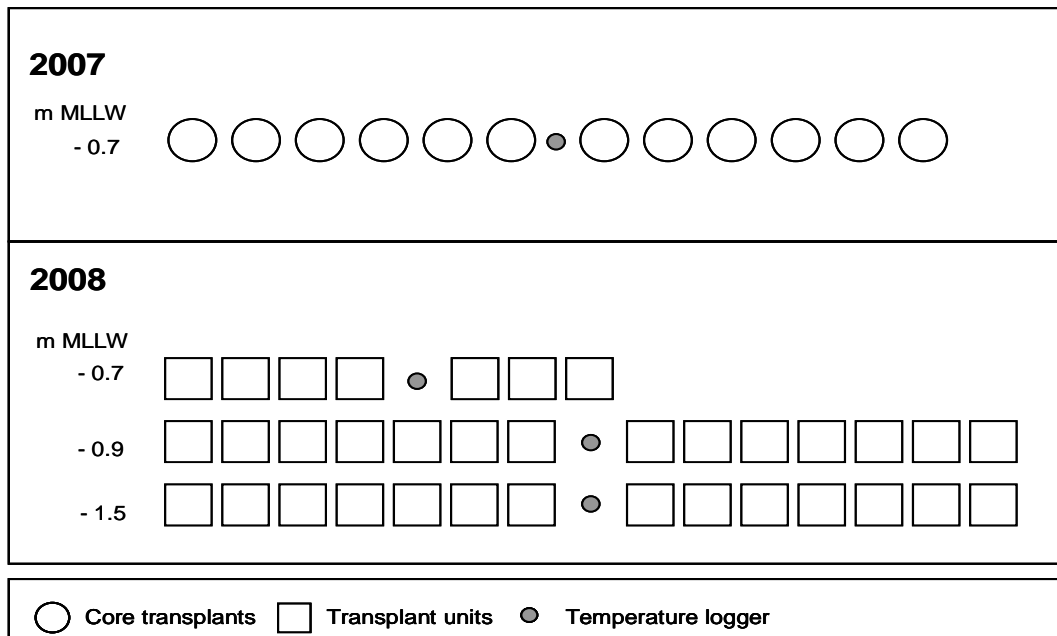


Fig. 2-3 Schematic of the experimental set-up. Core transplantations were conducted at -0.7 m MLLW at five sites in 2007, and transplantations in transplant units were conducted at -0.7, -0.9, and -1.5 m MLLW at three sites in 2008.

donor population were transplanted into each site (WP, BP, WBS and WBN) along a 60 m transect line at 5 m intervals, and 12 cores were transplanted back into the donor population (MP) to check for transplantation effects. All extracted *Z. marina* cores were transplanted into a new site during the same low tide.

Transplant units (TPU) (2008)

Transplant units (TPU) transplantations of *Z. marina* were carried out in the intertidal area, the transition zone and the subtidal area at MP, BP and WBN (Fig. 2-1, 2-5). The TPU method is a modification of the Transplanting Eelgrass Remotely with Frame Systems (TERFS) method that uses weighted wire mesh frames and biodegradable ties (twisted crepe paper) to attach the seagrass shoots (Short *et al.* 2002).

The TPUs in this study consisted of a metal frame (900 cm²) divided in sub-quadrats with biodegradable jute cord. Opposing pairs of *Z. marina* shoots including rhizomes (4-5 internodes) were attached with jute ties to the grid (Fig. 2-5). Nine pairs of seagrass shoots were tied to each frame approximately 10 cm apart, resulting in a total of 18 *Z. marina* shoots per transplant unit.

Deployment of the TPU required that the metal frame be slightly pressed into the sediment, thereby pushing the rhizomes carefully into the surface layer of the sediment. The flexible jute grid of the TPU allowed close contact of the rhizomes with the sediment surface



Fig. 2-4 Transplantation of *Zostera marina* in cores. **A)** Extraction of a *Z. marina* transplant core. **B)** Mosquito Pass: *Z. marina* transplantation along a transect line within the donor population

on uneven substrate. After successful rooting, the frame can be removed by cutting the jute cord along the outer metal frame. However, TPU frames remained in place during the experimental period in 2008 to facilitate the assessment of transplanted seagrass units in the dense donor population or to easily identify transplant areas if total loss of *Z. marina* occurred.

Z. marina shoots were collected from the donor population at MP and transported in a container with seawater. Shoots were tied to the TPU frames and stored in seawater overnight. Seven TPUs were spaced at 10 m intervals along a 70 m transect line at each site in the intertidal area (-0.7 m MLLW), and fourteen TPUs were spaced at 5 m intervals at each site in the transition (-0.9 m MLLW) and the subtidal area (-1.5 m MLLW). To facilitate recovery of transplants, the TPUs were marked with flagging tape. Red flagging tape characterized TPUs that were to remain totally undisturbed after the transplantation, whereas green flagging tape marked TPUs from which *Z. marina* plants were collected for further plant analysis. All seagrass plants were transplanted within 24 h of removal from the donor site. A total of 105 TPU (1890 individual *Z. marina* shoots) were transplanted in 2008.

Both transplantation methods conducted in Westcott Bay are well established and have been documented to successfully establish seagrass in a variety of restoration projects (e.g., Fonseca *et al.* 1998, Short *et al.* 2002).

TRANSPLANT LOCATIONS

Z. marina transplant locations were recorded using a portable GPS data logging system (Trimble Navigation GPS Pathfinder). We recorded the coordinates of individual transplant units in the intertidal- and transition area, and the end points of the transect lines



Fig. 2-5 Transplantation of *Zostera marina* in transplant units (TPU). **A.** Preparation of a seagrass TPU. **B.** Completed TPU. **C.** Freshly deployed transplant unit in the subtidal.

in the subtidal zone (Fig. 2-6). Since the *Z. marina* transplants were placed based on the use of predicted tides for Hanbury Point in Mosquito Pass Nobeltec Tides & Currents, some discrepancy between the transplantation and target elevations was anticipated. Individual core elevation data were collected once in May 2007 at WP, BP, WBS, and WBN. Data were not collected at MP. The water column depth was recorded at each core along a transect with a meter stick at an appropriate tidal height (i.e. 5-80 cm). Hanbury Point predicted tides, interpolated to the time of water column measurements from 15 minute predictions, were later corrected for observed tides at the Friday Harbor tide station (interpolated from 6 minute data). This estimated actual tide was used with the water column observations to estimate core elevations.

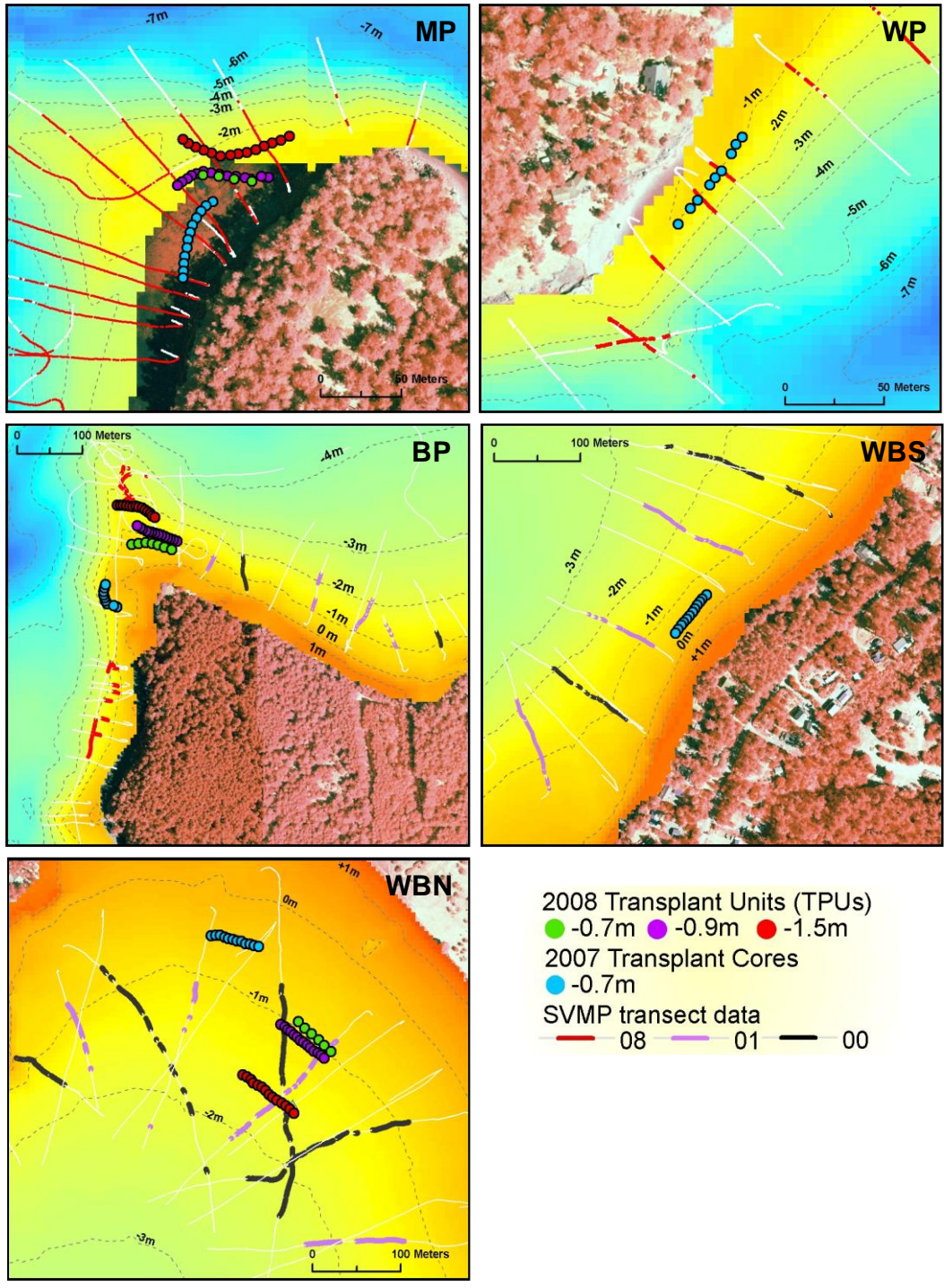


Fig. 2-6 Close-up views of *Z. marina* research sites across Westcott Bay. Estimated locations of core transplants at all sites in the intertidal zone in 2007 (blue circles). Locations of transplant units at MP, BP and WBN in the intertidal (green circles), the transition (violet circles) and the subtidal zone (red circles) in 2008. *Z. marina* distribution documented in 2000 (black lines) and 2001 (purple lines) by the SVMP, and in 2008 (red lines) along monitored transect lines (white) documented by joint SVMP/ES-RP study (Ferrier *et al.* in prep.) Single red circle indicates water quality monitoring stations. **MP**=Mosquito Pass, **WP**=White Point, **BP**=Bell Point, **WBS**=Westcott Bay South and **WBN**=Westcott Bay North. Bathymetry provided by Grossman *et al.* (2008).

2.3 TRANSPLANT PERFORMANCE

To assess the transplant performance the shoot density and above- and belowground biomass of *Z. marina* transplants was estimated in 2007 and 2008.

SHOOT DENSITY

The shoot density was monitored by counting *Z. marina* shoots within transplanted cores and TPUs. In addition, the shoot density at donor population was estimated by counting all shoots within a frame (50 x 50 cm) (n=6-10), randomly thrown within the proximity of the transplants at the donor site.

BIOMASS

In 2007, above- and belowground biomass of transplanted shoots was calculated at each site by extracting *Z. marina* core samples of 182 cm² (n=6). Seagrass cores were rinsed to remove sediment, adherent fauna and algae, and leaves were separated at the meristem (aboveground biomass) from the rhizomes and roots (belowground biomass). Seagrass leaves were carefully cleaned of epiphytes using a razor blade and all plant material was dried at 55 °C until constant weight (2-5 days). Core transplant biomass was calculated as biomass (g DW m⁻²).

In 2008, above- and belowground biomass of transplants was measured for individual *Z. marina* shoots (separated in above- and belowground biomass) (n= 8-20), as the TPU transplantation method did not allow core sampling for biomass estimates in a given area. Individual *Z. marina* shoots were collected from TPUs and the donor population, gently washed, and leaves separated from rhizomes and roots at the meristem. *Z. marina* tissue was then cleaned of epiphytes and dried as described for biomass samples in 2007. The TPUs transplant biomass is presented as biomass (g DW shoot⁻¹).

TRANSPLANT MONITORING

In 2007, core transplant shoot density in the intertidal area was monitored every two weeks between May 15 and July 12 for a total of 5 sampling efforts. In addition, the initial and final biomass of core transplants was estimated once in May and once in July. In April 2008, three selected 2007 core transplants sites (MP, BP, WBN) were revisited and transplant shoot densities estimated.

In 2008, TPU shoot density in the intertidal area was monitored monthly between May 4 and July 4. The initial and final biomass of intertidal transplants was estimated once in May and July, respectively.

In 2008, shoot density of transplants in the transition zone and the subtidal area were monitored every two weeks between May 4 and August 6, and once in November. In the transition zone, transplants were monitored from shore during low tides (> -0.9 m MLLW) at monthly intervals, and by scuba divers in the middle of each month, thereby enabling a biweekly monitoring of transplants at this elevation. Subtidal transplants were consistently

and exclusively monitored by scuba divers. Biomass of transplants was estimated at both tidal elevations monthly between May and August 2008.

In May 2009, 2008 transplants at all three tidal elevations were revisited, and shoot density counted.

During each sampling event, qualitative ancillary data was recorded, such as the presence of macro algae and bubble snail spawn, turbidity events, and the presence of sulfide odor.

2.4 ENVIRONMENTAL MONITORING

WATER TEMPERATURE

To characterize the water temperature (°C) in the direct proximity of *Z. marina* plants, small temperature data loggers (HOBO TidBit v2) were deployed at the sediment surface at the midpoint of each transplant transect at all sites and tidal elevations across Westcott Bay (Fig. 2-3). Temperature in the intertidal area was recorded at 15-minute intervals from May to July in 2007 and 2008 concurrently to the transplantation experiment. In the transition zone and subtidal area, temperatures were recorded from May to August 2008. Temperature data were filtered and values recorded during air exposure during events of extreme low tides were extracted (Appendix A). Extracted air temperature values were further used to provide additional information on transplant air exposure.

AIR EXPOSURE

The total duration of *Z. marina* transplant air exposure time was estimated by filtering temperature data and extracting values recorded during instances when the data logger (and *Z. marina* transplants) was exposed to air. Air exposure of transplants was estimated in the intertidal- and transition area during low tides in spring and summer from May to July in 2007 and 2008. Estimated tide levels for the area of Westcott Bay (see below) of -0.7 m and -0.9 m MLLW were used as thresholds to identify all observations where *Z. marina* plants were exposed to air (Appendix A). The total air exposure time of transplants was calculated as the sum of individual air exposure events observed over the transplantation experiment from May to July.

ESTIMATED TIDES FOR THE AREA OF WESTCOTT BAY

To estimate the tides in Westcott Bay, the mean hourly predicted tide for Roche Harbor (RH) and Hanbury Point (HP) (Nobeltec Tides & Currents) was averaged to produce a predicted tide for Westcott Bay. This predicted tide was then adjusted by the departure of the observed tide at Friday Harbor (FH) (<http://tidesandcurrents.noaa.gov>; station 9449880) from the predicted tide at Friday Harbor (also predicted by Nobeltec Tides & Currents). Estimated tides for Westcott Bay (WB est.) were calculated by (Dowty & Ferrier 2009) using the following equation:

$$((RH + HP)/2) + (FH \text{ obs.} - FH \text{ pred.}) = WB \text{ est.}$$

2.5 DATA ANALYSIS

Differences in shoot density over time were tested for significance in the intertidal area in 2007 between the bi-weekly monitoring intervals from May 15 to July 12, and in 2008 between monthly monitoring intervals from May 4 to July 4. Differences in the above- and belowground biomass over time were tested between the initial biomass (May) and the final biomass (July) in the intertidal area in both years. In the transition zone and the subtidal area, differences in shoot density over time were tested between bi-weekly monitoring intervals from May 4 to August 6 as well as November 22. Changes in above- and belowground biomass were tested monthly between May and August 2008.

One-way ANOVAs were conducted to test for differences in transplant performance (shoot density, above- and belowground biomass) between the sites across Westcott Bay at individual tidal elevations in July 2007 and 2008.

For the purpose of relating the transplant performance to the total time of air exposure, the relative transplant shoot density (%) and aboveground biomass across all sites and tidal elevations in July were correlated with the cumulative transplant air exposure time through June (including all air exposure events that the transplants experienced at different sites and tidal elevation in both years).

Two-way ANOVAs were conducted to test for differences in mean water temperature between months and sites separately for water temperatures in individual years and tidal elevation).

For the purpose of relating transplant performance to water temperature, the relative transplant shoot density (%) of each month (intertidal transplants: June & July, and transition and subtidal transplants: June, July and August) was correlated with the mean and maximum monthly water temperatures of the previous month (May, June, July).

The percentage of the relative shoot density was calculated from the ratio between the initial number of shoots transplanted and the remaining shoot density in individual replicate transplant units at each sampling date.

All results are presented either as arithmetic or geometric means (\pm SE). If variables were log-normally distributed, data were log-transformed prior to analysis, and back transformed means were used as a central measure. Corresponding standard errors were calculated according to Mood *et al.* (1974). Differences between sites and experimental effects were analyzed by means of analysis of variance (ANOVA), followed by a Tukey's honest-significant-differences (HSD) multiple comparison test (Sokal & Rohlf's 1995). Data were tested for homoscedasticity of variance by using Cochran's test to fulfill the assumption of ANOVA. Differences were considered to be statistically significant, if p -values were < 0.05 . All statistical tests were conducted by using STATISTICA (StatSoft Inc.; Tulsa, Oklahoma).

3 RESULTS

3.1 *Z. MARINA* TRANSPLANTATION EXPERIMENTS

3.1.1 *Z. MARINA* TRANSPLANT ESTABLISHMENT

All *Z. marina* transplants were established successfully across the different sites and tidal elevations in Westcott Bay in 2007 (-0.7 m MLLW) and 2008 (-0.7 m, -0.9 m, -1.5 m MLLW). In June 2007, the mean shoot density of transplants remained at initial values at all sites 4 weeks after the transplantation, except at BP, where the shoot density decreased (Fig. 3-2, BP: Tukey tests of means $p < 0.001$; all other sites showed no differences in shoot density between May and June 2007, as confirmed by Tukey tests following a one-way ANOVA testing for differences over time at individual sites). Similarly, in June 2008, the mean shoot density met or exceeded initial values at all sites and tidal elevations 4 weeks after the transplantation (Fig. 3-4, differences in shoot density over time were determined as described above).

The development of transplants at MP generally followed the trends in shoot density and biomass of the surrounding donor population over the experimental period in 2007 and 2008, suggesting a low transplantation stress in both years, although there was a varying trend in the aboveground and belowground biomass between transplants and the donor population at MP at -1.5 m MLLW (Fig. 3-4, 3-5). The observed differences between transplants and the donor population at this particular elevation might have been caused by the dislocation of the transplant transect line (Fig. 2-6) (see further explanation about estimated locations of transplants below). This reduced the number of transplant replicates ($n=4$) and relocated them across a range of deeper tidal elevations (>-1.5 m MLLW).

3.1.2 ESTIMATED LOCATIONS OF TRANSPLANTS

Plotted coordinates of transplants at the intertidal zone in 2007 in relation to Westcott Bay bathymetry shows the core transplants at WP were placed approximately 0.2 m deeper (mean depth -0.89 m) than the expected -0.7 m MLLW (Fig. 3-1). Estimated mean depth of core transplants at BP and WBS were at the same elevation at -0.74 m. Mean transplant depth at WBN was measured at -0.66 m, slightly shallower than the former intertidal *Z. marina* population.

In 2008, the subtidal transect line at -1.5 m MLLW at MP was partly displaced (presumably by boaters) without notice prior to transplantation. This led to deeper placement of some transplants at MP at the subtidal zone (Fig. 2-6), and hindered the recovery of individual TPUs during the transplant monitoring, resulting in a reduced number of replicates at times. The estimated locations of *Z. marina* transplants at WBN

suggest that the transplants located at the intertidal and transition zone might have been placed slightly deeper than the intended -0.7 m and -0.9 m MLLW (Fig. 2-6). However, all *Z. marina* shoots transplanted at the different sites and elevations in Westcott Bay in 2008 were placed in areas formerly or currently vegetated by *Z. marina* (Fig. 2-6).

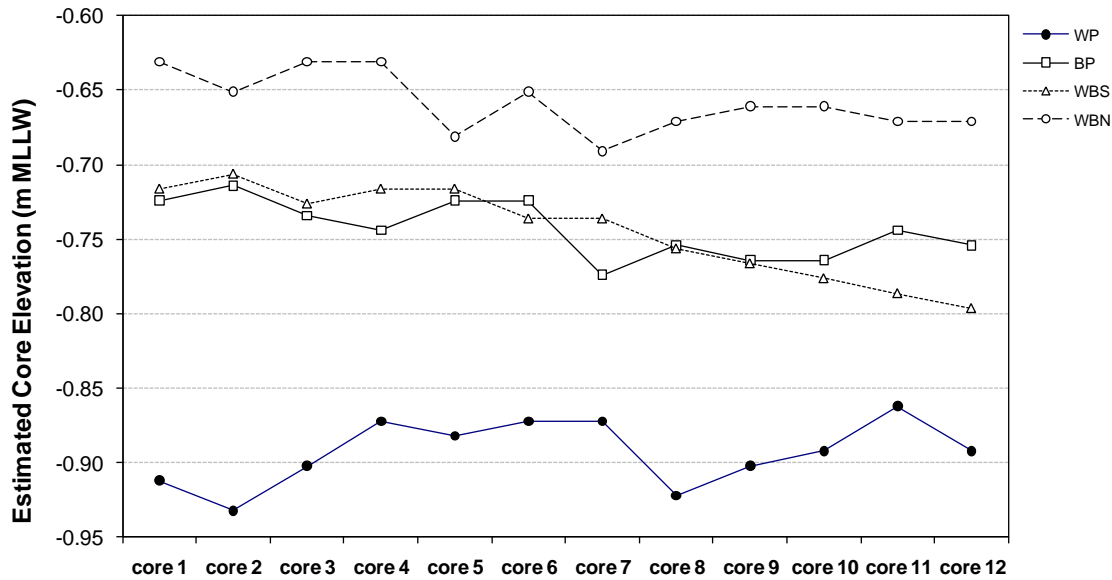


Fig. 3-1 Estimated elevations of individual core transplants along transects in the intertidal zone at WP=White Point, BP=Bell Point, WBS=Westcott Bay South and WBN=Westcott Bay North in 2007. Mosquito Pass (MP) core elevations were not verified using these methods.

3.2 TRANSPLANT PERFORMANCE & SURVIVAL

3.2.1 *Z. MARINA* AT THE INTERTIDAL ZONE (-0.7 M MLLW)

2007

After the transplantation of *Z. marina* at the intertidal zone in May 2007, shoot density and aboveground biomass varied over time at all sites across Westcott Bay, except at White Point, where shoot densities remained stable from May to July (Fig. 3-2, 3-3A, Table 3-1).

Both shoot density and aboveground biomass of transplants decreased from the entrance (MP, WP) towards the inner bay (BP) and the head (WBS, WBN) of the bay in summer 2007 (Fig. 3-2, 3-3A, Table 3-1). The mean shoot density followed a distinct inter-monthly zig-zag trend in *Z. marina* transplants at MP and the surrounding donor population over the season, while the same trend was apparent (but not significant) at White Point (Fig. 3-2, Table 3-1). At BP, the initial shoot density was reduced by 60% at the end of May, and thereafter remained stable through July. In contrast, the shoot density at WBS and WBN approached zero in July (Fig. 3-2, 3-3A).

Concurrently, the mean aboveground biomass of transplants was lowest at the sites at the head of bay, where the initial aboveground biomass was reduced by 95% at WBN and WBN in July 2007 (Fig. 3-3A, Table 3-1). Interestingly, the belowground biomass remained stable at all sites over the entire experimental period and showed no differences across the sites in July 2007 (Fig. 3-3A, Table 3-1).

Follow-up monitoring of the 2007 transplants at selected sites (MP, BP, and WBN) in April 2008 revealed that *Z. marina* did not survive at BP or WBN in the intertidal zone. In contrast, the transplants at MP intermingled with the surrounding donor population, and could not be differentiated.

2008

After the transplantation of *Z. marina* at the intertidal zone at -0.7 m MLLW in 2008, there was no apparent variation in the mean shoot density of transplants at MP or WBN, or the donor population at MP from May to July. In contrast, the mean shoot density at BP strongly varied over the season, and was significantly reduced in July 2008 (Fig. 3-4A, Table 3-1).

While shoot densities at MP and WBN were stable, the mean initial aboveground and belowground biomass of individual *Z. marina* shoots decreased at all sites over the season (Table 3-1). Aboveground biomass was reduced by 72% at MP, by 80% at BP and by 66% at WBN in July 2008 (Fig. 3-3B). Lowest values in the mean belowground biomass were reached at BP in July 2008 (Fig. 3-3B).

2007 Shoot density at -0.7 m MLLW

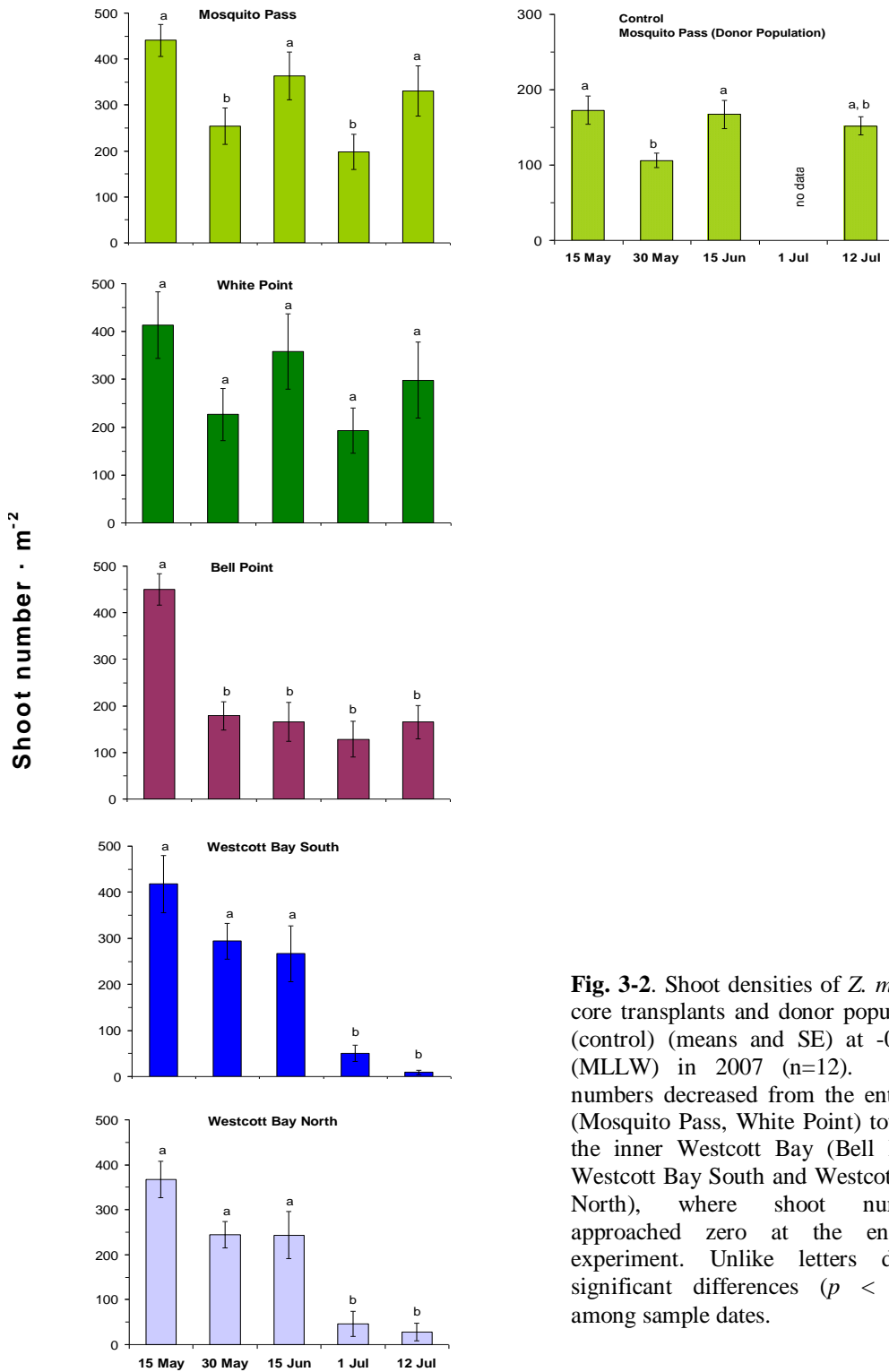


Fig. 3-2. Shoot densities of *Z. marina* core transplants and donor population (control) (means and SE) at -0.7 m (MLLW) in 2007 (n=12). Shoot numbers decreased from the entrance (Mosquito Pass, White Point) towards the inner Westcott Bay (Bell Point, Westcott Bay South and Westcott Bay North), where shoot numbers approached zero at the end of experiment. Unlike letters denote significant differences ($p < 0.05$) among sample dates.

3.2.2 *Z. MARINA* AT THE TRANSITION ZONE (-0.9 M MLLW)

At the transition zone at -0.9 m MLLW, the mean shoot density varied over the study period, with a general trend of decreasing shoot density towards July and August at all sites (Fig. 3-4B, 3-6, Table 3-2). The initial mean shoot density at BP was distinctly reduced in July (BP: Tukey's tests, $p < 0.05$), followed by a strong reduction in initial shoot density at WBN and the donor population in August 2008 (WBN and donor population: Tukey's tests, $p < 0.001$) (Fig. 3-4B, Table 3-2). By November 2008, the shoot density at BP and the donor site had recovered to initial values (BP: Tukey's test, $p = 0.33$, donor site: Tukey's test, $p = 0.66$, Fig. 3-4B). In contrast, the shoot density at WBN approached zero in November 2008 (WBN: Tukey's test, $p < 0.0001$) (Fig. 3-4B, Table 3-2).

The aboveground and belowground biomass followed similar trends. The initial mean aboveground biomass decreased at all sites from May to July 2008, except at WBN where the aboveground biomass remained stable through July (WBN Tukey's tests, $p = 0.073$) (Fig. 3-5A, Table 3-2). In August, the initial aboveground biomass was reduced by 72% at BP and by 59% at WBN, whereas the aboveground biomass recovered to initial values at MP transplants and the donor population (Fig. 3-5). Contrary to the development in the aboveground biomass, the mean belowground biomass exhibited no differences over the season at MP or WBN transplants or the donor population between May and August, whereas the initial belowground biomass showed a decrease of 52% at BP by August 2008 (Fig. 3-5A).

3.2.3 *Z. MARINA* AT THE SUBTIDAL ZONE (-1.5 M MLLW)

Z. marina transplants at the subtidal zone (-1.5 M MLLW) showed less inter-seasonal variability in shoot density and biomass over the study period than transplants at the transition zone (Fig. 3-4C, Table 3-2). However, there was a strong effect of time from May to November on the mean shoot density at all sites, with a strong decrease in the initial shoot density at WBN by 70% in August that approached zero in November 2008 (Fig. 3-4C, Table 3-2). In contrast, the mean shoot density was distinctly increased at BP in November 2008 (Fig. 3-4).

There was no effect of time on the aboveground or belowground biomass of *Z. marina* shoots at any site except MP, where the aboveground biomass was reduced and the belowground biomass increased in July 2008, possibly attributable to the location of transplants at different tidal elevation (Fig. 3-5B, Table 3-2).

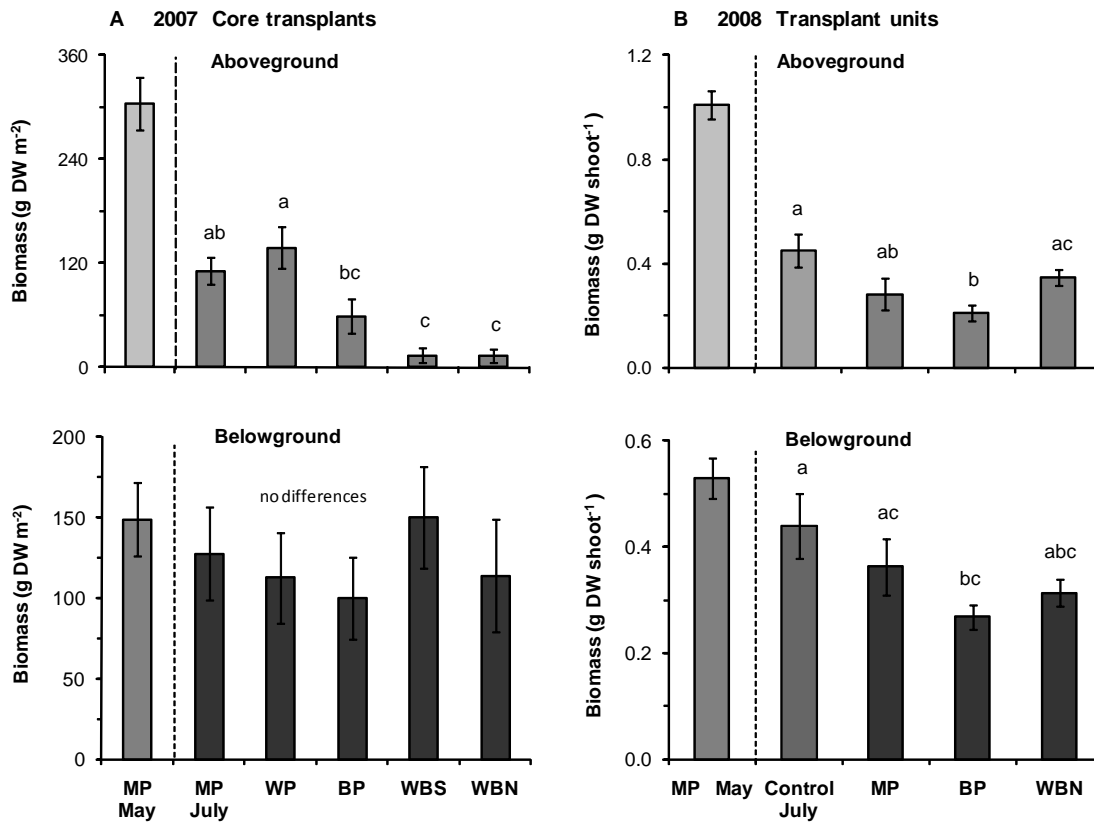


Fig. 3-3 A, B Mean (\pm SE) initial (May) and final (July) aboveground and belowground biomass of *Z. marina* transplants at -0.7 m MLLW across Westcott Bay **A** in 2007 (n=6-7) and **B** 2008 (n=6-19). *Note:* A one-way ANOVA was conducted comparing the shoot densities between the sites in July 2007 and 2008. Unlike letters denote significant differences ($p < 0.05$) among sites.

3.2.4 TRANSPLANT PERFORMANCE THROUGH 2009

Results of the continued monitoring of the 2008 *Z. marina* transplant performance across the sites in Westcott Bay in May 2009 revealed the same shoot densities at MP in the intertidal and the transition area in May 2009 as observed in these areas in May 2008 (Fig. 3-4, 3-6). The subtidal transplants at MP could not be recovered. However, *Z. marina* shoot density within the surrounding donor population revealed a decrease in initial shoot density at the subtidal area at MP of approximately 30% from May 2008 through May 2009. In contrast, at BP the *Z. marina* transplants did not survive in the intertidal area. In the transition area at BP the transplant shoot densities were decreased by approximately 65%, and in the subtidal area by approximately 68%. However, no *Z. marina* transplants could be recovered at WBN. Thus, transplants did not survive at the head of the bay at any tidal elevation through May 2009.

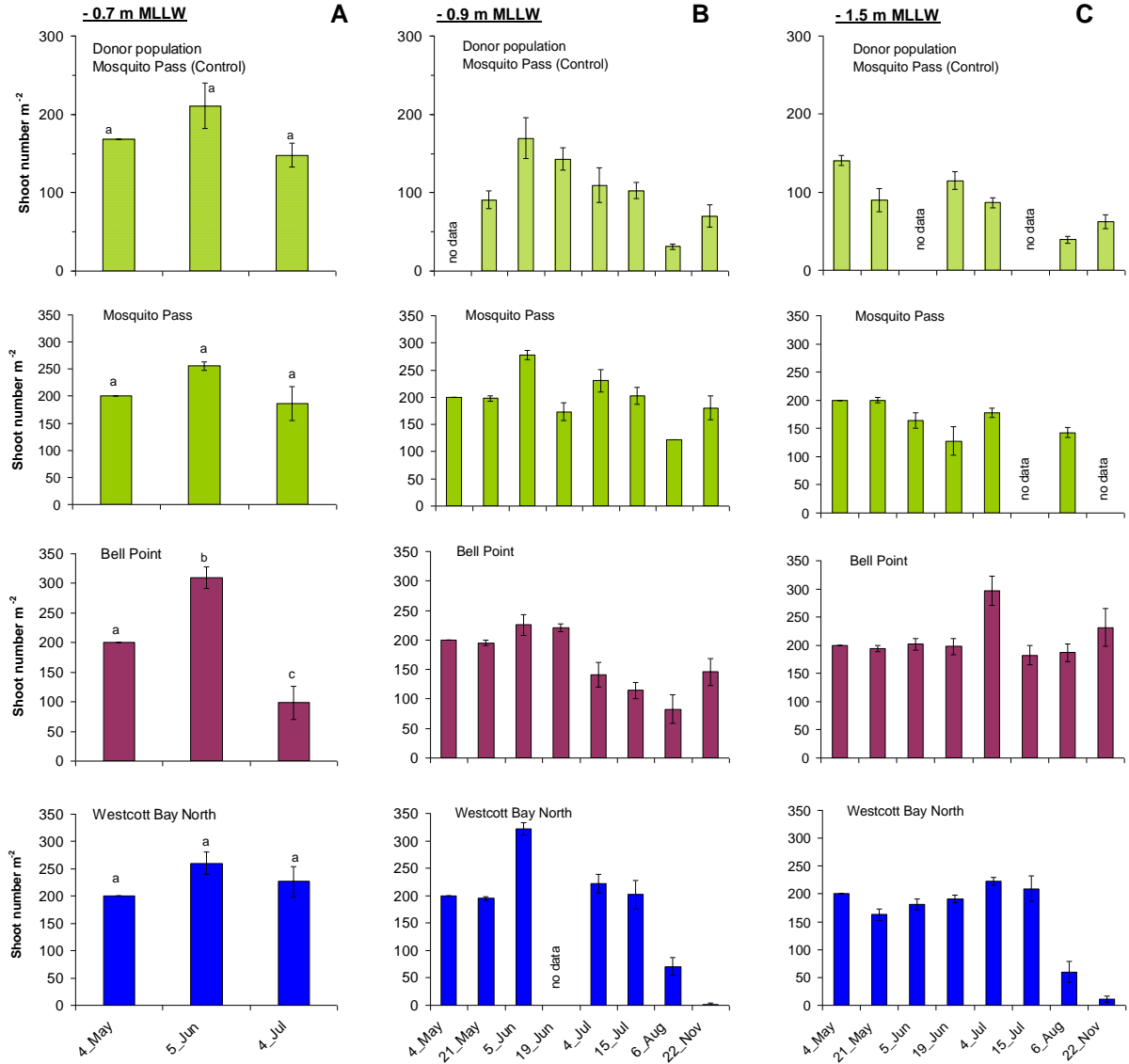


Fig. 3-4 A, B, C Shoot densities of *Z. marina* transplant units (TPUs) and donor population at **A** the intertidal area (-0.7 m); Unlike letters denote significant differences ($p < 0.05$) among sample dates., **B** the transition zone (-0.9 m) and **C** the subtidal area (-1.5 m) (MLLW) in 2008 (means \pm SE). Note: No SE is presented in May 2008 since the transplant experiment started with equal shoot density at all sites and tidal elevations.

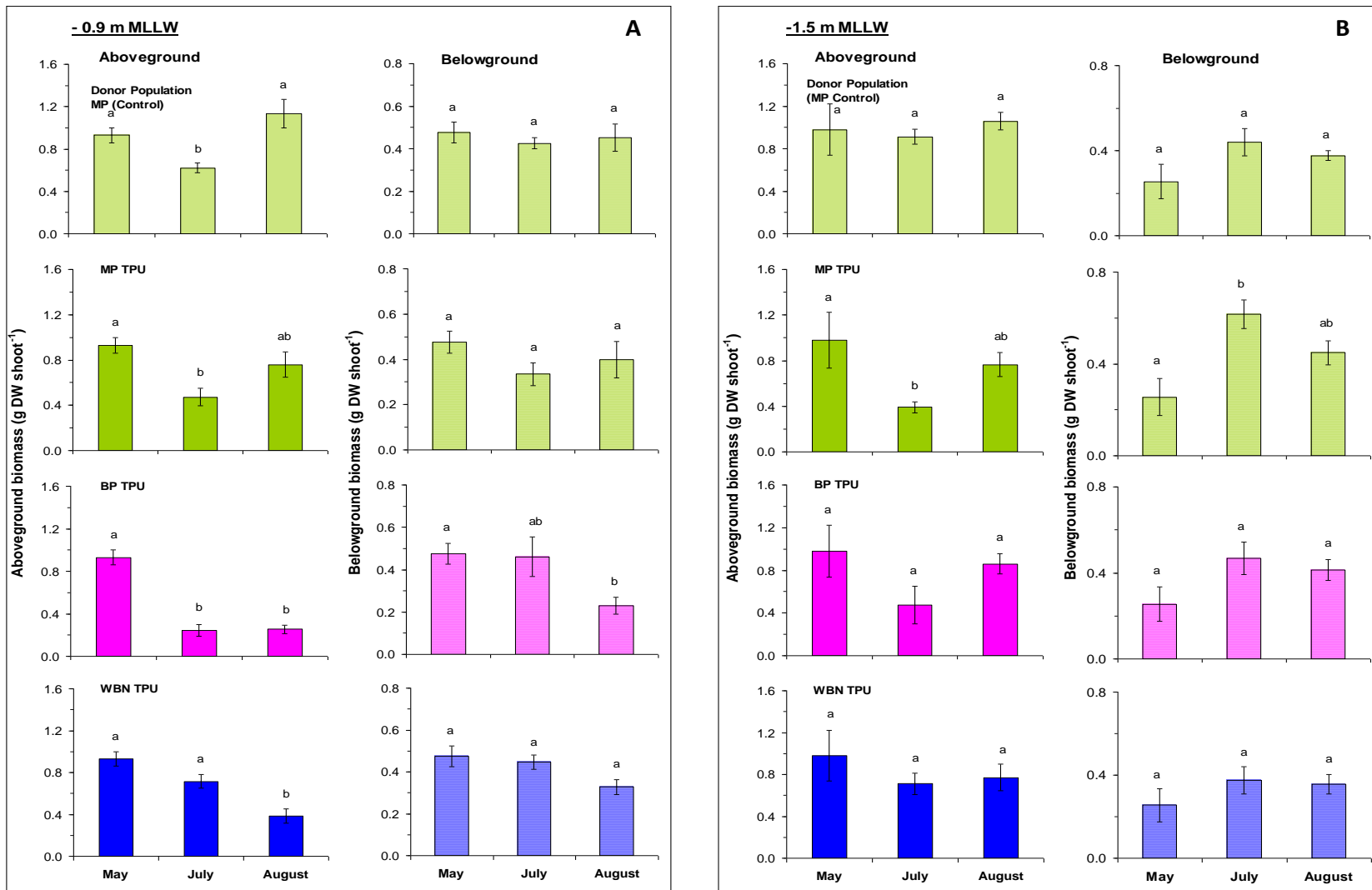


Fig. 3-5 A, B Mean aboveground and belowground biomass of individual *Z. marina* shoots at transplant units (TPUs) and the donor population (control) at **A** the transition zone (-0.9 m) and **B** the subtidal area (-1.5 m) (MLLW) in 2008 (means \pm SE). Unlike letters denote significant differences ($p < 0.05$) among sample dates.

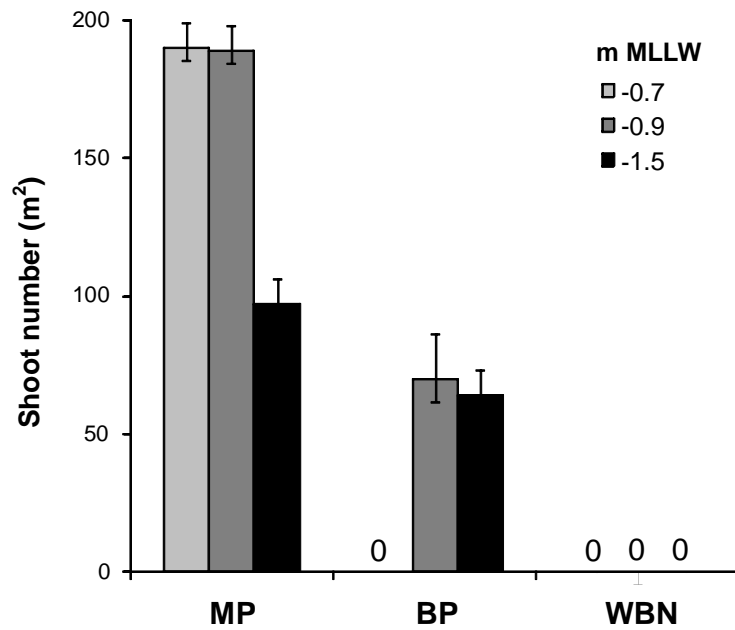


Fig. 3-6 Status of *Z. marina* shoot density (mean \pm SE) of the 2008 transplants at the entrance (MP), the inner (BP) and the head (WBN) of Westcott Bay at three tidal elevation (-0.7 m, -0.9 m, -1.5 m MLLW) in May 2009. *Z. marina* did not survive in the intertidal area of the site in the inner bay, and at all tidal elevations at the head of Westcott Bay.

Table 3-1 One-way ANOVA table testing for the effect of time on *Z. marina* density and biomass (aboveground and belowground biomass) at individual sites in the intertidal zone (-0.7 m MLLW) over the study period from May to July in 2007 and 2008.

Treatment	2007				2008			
	-0.7 m MLLW				-0.7 m MLLW			
	df	MS	F	P	df	MS	F	P
<u>Donor population</u>								
Shoot density	3	9182.27	3.906	< 0.05	2	6147.56	2.848	ns
Aboveground biomass	-	-	-	-	1	1.43	27.972	< 0.0001
Belowground biomass	-	-	-	-	1	0.04	1.332	ns
<u>Transplantations</u>								
Mosquito Pass								
Shoot density	4	90453.84	4.016	< 0.01	2	5400.15	3.987	ns
Aboveground biomass	1	111038.68	31.779	< 0.001	1	2.97	58.668	< 0.0001
Belowground biomass	1	1378.57	0.344	ns	1	0.06	5.296	< 0.05 ^a
White Point								
Shoot density	4	108697.71	2.295	ns	-	-	-	-
Aboveground biomass	1	88496.98	19.020	< 0.01	-	-	-	-
Belowground biomass	1	5879.85	1.326	ns	-	-	-	-
Bell Point								
Shoot density	4	90453.84	4.016	< 0.01	2	78014.96	28.896	< 0.0001
Aboveground biomass	1	193317.62	48.121	< 0.0001	1	5.38	137.245	< 0.0001
Belowground biomass	1	3696.85	0.961	ns	1	0.57	29.088	< 0.0001
Westcott Bay South								
Shoot density	4	69.42	31.912	< 0.0001 ^a	-	-	-	-
Aboveground biomass	1	251728.34	83.721	< 0.0001	-	-	-	-
Belowground biomass	1	2.99	0.001	ns	-	-	-	-
Westcott Bay North								
Shoot density	4	65.05	23.802	< 0.0001 ^a	2	6389.08	2.356	ns
Aboveground biomass	1	252765.44	84.204	< 0.0001	1	4.17	107.968	< 0.0001
Belowground biomass	1	3679.19	0.712	ns	1	0.44	22.000	< 0.0001

^a Logarithmic transformation

- not assessed

Table 3-2 One-way ANOVA table testing for the effect of time on *Z. marina* density and biomass (aboveground and belowground biomass) at individual sites in the transition zone (-0.9m MLLW) and subtidal zone (-1.5 m MLLW) over the study period from May to August in 2008.

Treatment	2008							
	-0.9 m MLLW				-1.5 m MLLW			
	df	MS	F	P	df	MS	F	P
<u>Donor population (control)</u>								
Shoot density	6	90583.65	92.28	< 0.0001 ^a	5	11862.4	17.4848	< 0.0001
Aboveground biomass	2	0.91	11.12	< 0.001	2	0.07	0.66	ns
Belowground biomass	2	0.01	0.36	ns	2	0.06	2.00	ns
<u>Transplantations</u>								
Mosquito Pass								
Shoot density	7	10442.86	16.09	< 0.0001	5	5391.93	11.64	< 0.0001
Aboveground biomass	2	0.55	7.39	< 0.01	2	0.17	4.81	< 0.05 ^a
Belowground biomass	2	0.05	1.51	ns	2	0.19	7.57	< 0.01
Bell Point								
Shoot density	7	23398.51	11.78	< 0.0001	7	8577.54	4.63	< 0.001
Aboveground biomass	2	1.39	27.27	< 0.0001	2	0.31	2.05	ns
Belowground biomass	2	0.13	4.48	< 0.05	2	0.06	2.06	ns
Westcott Bay North								
Shoot density	6	90583.65	92.28	< 0.0001	7	37030.33	32.68	< 0.0001
Aboveground biomass	2	0.85	12.56	< 0.0001	2	0.12	0.70	ns
Belowground biomass	2	0.07	2.67	ns	2	0.02	0.80	ns

^a Logarithmic transformation

3.3 ENVIRONMENTAL STRESSORS INVESTIGATED

3.3.1 AIR EXPOSURE

2007

In spring/summer 2007, the duration and the number of air exposure events of *Z. marina* transplants at the intertidal zone (-0.7 m MLLW) varied among sites, and corresponded to observed differences in the transplant elevation at individual sites (Fig. 3-1, Fig. 3-7A, Table 3-3). The highest values of the duration and the number of exposure events were observed at BP, WBS and WBN, with a maximum cumulative value of transplant air exposure of 34 hours (h) at WBN, accumulated during exposure events from May 21 to July 13 (Fig. 3-7A, 3-8A). Interestingly, the air temperature at the sediment surface indicates constantly higher values at extensively air exposed sites, such as WBS and WBN, throughout the study period in 2007 (Fig. 3-7A, Table 3-3).

After the transplantation of *Z. marina* in the intertidal zone in 2007, the transplants at BP and MP, and *Z. marina* plants at the donor site, were observed to change their leaf color from green to brown and leaves frequently appeared to be dry and crispy during the air exposure events at extreme low tides in May and June. At the end of the experiment in July 2007, the mean transplant shoot density and the aboveground biomass was distinctly lower at WBS and WBN as compared to WP and MP, whereas there was no difference in the belowground biomass of transplants across the sites in Westcott Bay (Fig. 3-3A, 3-8A, Table 3-4, 3-5).

2008

In 2008, there were no distinct patterns in the duration and number of transplant air exposure events at the intertidal zone and in the transition zone respectively, and the overall time and number of exposure events during low tides was reduced by more than 50% at MP, BP, and WBN in 2008 as compared to air exposure events at these particular sites in 2007 (Fig. 3-7B, 3-9A, Table 3-3). The maximum air/sediment temperature during air exposure events was consistently higher at all transplants sites in 2007 than in 2008, where the maximum air temperature at WBN in 2007 (25.0°C) exceeded the temperature of WBN in 2008 (18.0°C) by 7°C (Table 3-3).

After the transplantation of *Z. marina* at the intertidal zone in 2008, the plants looked visually green and healthy at MP and WBN over the experimental period from May to July. In contrast, the transplanted shoots at BP were found to be very dry and crispy during the air exposure events at extreme low tides in June 2008, where intertidal transplants were exposed about 4.5 hours longer at BP (8.5 h) than at MP, and 3 hours longer than at WBN (Table 3-3). Moreover, the highest air temperature at the sediment surface was about 2.0 °C higher in June at BP (21.6 °C) than at the other sites (Table 3-3). These observations correspond to the results of the transplant performance and survival at the intertidal in 2008, indicating no distinct difference in the mean shoot density between transplants at MP and WBN, whereas the mean shoot density of transplants was slightly lower at BP than at WBN (Fig. 3-9B, Table 3-4, 3-5). Similarly, the mean aboveground biomass was slightly reduced at BP as compared to WBN and the donor population. The mean belowground biomass differed between BP and the donor population (Fig. 3-3B, Table 3-3, 3-4).

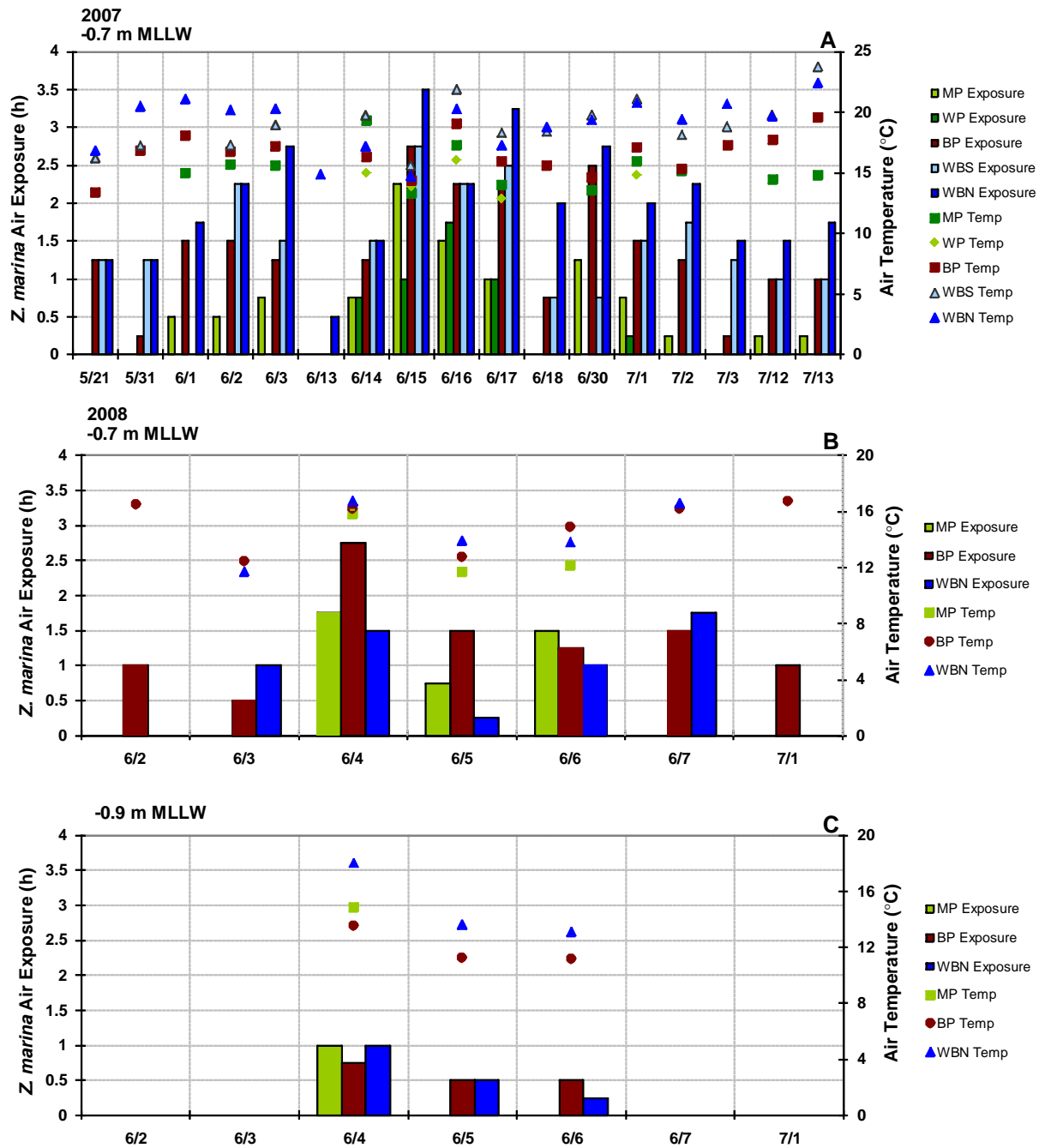


Fig. 3-7 A, B, C Duration of *Z. marina* transplant air exposure hours (h) and related mean air/sediment temperatures (°C) during the exposure events at **A** the intertidal zone in 2007, **B** at the intertidal zone in 2008, and **C** at the transition zone in 2008. Both transplant experiments in the intertidal zone were conducted from May to July in both years, and lasted for a total of 8 weeks.

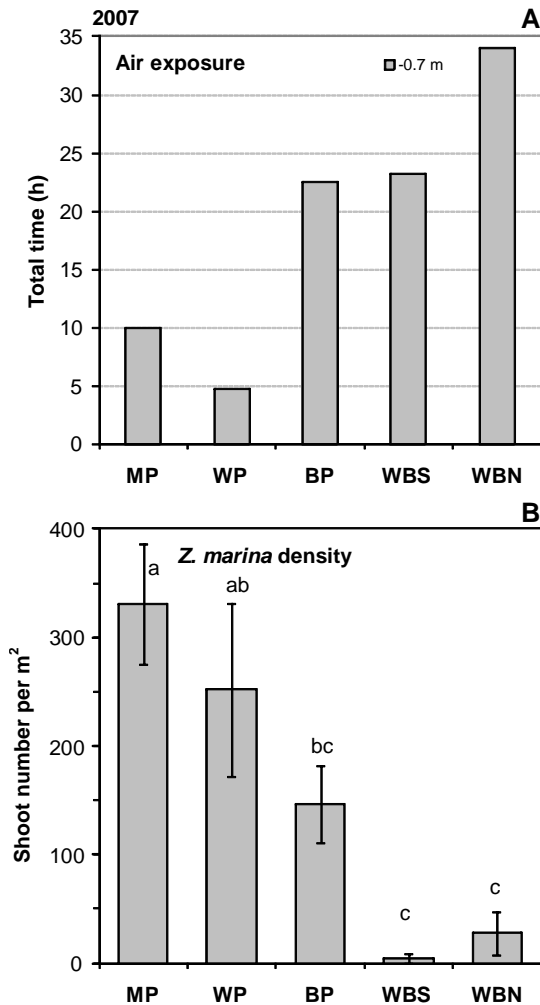


Fig. 3-8 A, B *Z. marina* transplant experiment at the intertidal (-0.7 m MLLW) zone in 2007. **A** Total duration of transplant air exposure (h) was highest at WBN during the experimental period from May to July 2007. **B** Transplant shoot number (mean \pm SE) was distinctly lower at WBS and WBN relative to other sites at the end of the experiment in July 2007. Unlike letters denote significant differences ($p < 0.05$) among sites.

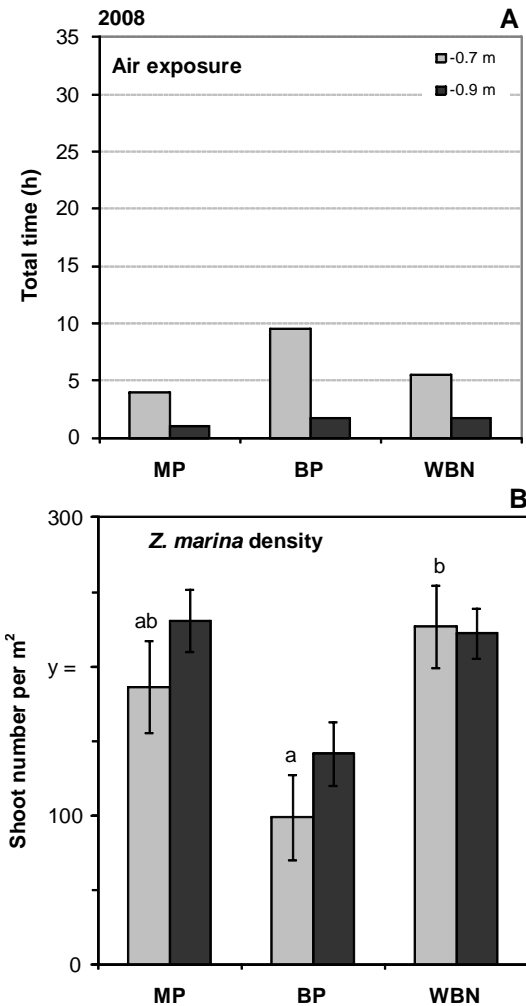


Fig. 3-9 A, B *Z. marina* transplant experiment in the intertidal (-0.7 m) and the transition (-0.9 m MLLW) zone in 2008. **A** Total duration of transplant air exposure (h) in the intertidal zone was highest at BP during the experimental period from May to July in 2008. **B** Intertidal transplant shoot number (mean \pm SE) was significantly lower at BP than at WBN at the end of the experiment in July 2008. Unlike letters denote significant differences ($p < 0.05$) between sites in the intertidal area (grey bars). There are no differences among sites in the transition zone (black bars).

At the transition zone, which was 0.2 m deeper than the intertidal zone, the estimated exposure times were much shorter (MP 25%, BP 21%, WBN 33%), and the number of exposure events were fewer (MP 33%, BP 50%, WBN 60%), as compared to the intertidal

Table 3-3 Characteristics of air exposure events (total air exposure time (h), number of exposure events (d), and related maximum air/sediment temperature (°C)) of *Z. marina* transplants in the intertidal and the transition zone during the experimental period in June and July in 2007 and 2008.

	Month	2007					2008					
		- 0.7 m					- 0.7 m			- 0.9 m		
		MP	WP	BP	WBS	WBN	MP	BP	WBN	MP	BP	WBN
Total exposure (h)	June	8.5	4.5	16.0	14.3	22.5	4	8.5	5.5	1.0	1.8	1.8
	July	1.5	0.3	5.0	6.5	9.0	-	1	-	-	-	-
Number of exposure (d)	June	8	4	9	8	10	3	6	5	1	3	3
	July	4	1	5	5	5	-	1	-	-	-	-
Max air/sediment temperature (°C)	June	23.2	13.2	23.4	24.1	25.0	19.7	21.6	19.8	15.4	14.2	18.0
	July	13.7	14.8	20.9	23.1	24.1	-	16.7	-	-	-	-

- no desiccation event

zone in summer 2008 (Fig. 3-7C, Table 3-3). The transplant exposure times and number of events were slightly higher at BP and WBN as compared to MP, and the air temperature at the sediment surface was about 4°C higher at WBN (18.0°C) than at BP. Both sites experienced the same total exposure time and number of exposure events (Fig. 3-9A, Table 3-3).

Correspondingly, lowest values in the mean shoot density at the transition zone were observed at the donor population and BP in July 2008, although differences between the sites were rather weak (donor population and BP: $p < 0.05$ Tukey tests) (Fig. 3-4B, 3-9B, Table 3-4, 3-5). Similarly, the mean aboveground biomass was lower at BP ($p < 0.05$ Tukey's test) than at WBN, whereas there were no differences between the sites in the belowground biomass in July 2008 (Fig. 3-5A, Table 3-4).

The correlation of the transplant performance with the total air exposure time revealed a strong negative correlation of *Z. marina* shoot density in July with the total air exposure time across all sites and tidal elevations in Westcott Bay, calculated as cumulative exposure in June 2007 and 2008 (Pearson correlation, $r = -0.86$) (Fig. 3-10A). Similarly, the aboveground biomass of transplants was negatively correlated with the total air exposure time (Pearson correlation, $r = -0.64$) (Fig. 3-10B).

Table 3-4 *Z. marina* transplant shoot density, above- and belowground biomass at different tidal elevations (- 0.7 m, -0.9 m, -1.5 m MLLW) across the sites in Westcott Bay in July and August 2008 (means ± SE).

	2008					
	July			August		
	- 0.7	- 0.9	-1.5	- 0.9	-1.5	m MLLW
Donor population (control)						
Shoot density	148 (5.5)	109 (22.2)	86.5 (6.6)	31.0 (3.8)	39.0 (4.6)	
Aboveground biomass	0.45 (0.06)	0.62 (0.04)	0.91 (0.07)	1.13 (0.14)	1.06 (0.08)	
Belowground biomass	0.44 (0.06)	0.43 (0.03)	0.44 (0.06)	0.45 (0.07)	0.38 (0.02)	
Mosquito Pass (MP)						
Shoot density	186 (30.89)	230.5 (21.0)	177.8 (7.9)	122.2 ¹	142.6 (9.3)	
Aboveground biomass	1.01 (0.06)	0.47 (0.08)	0.39 (0.05)	0.76 (0.11)	0.76 (0.11)	
Belowground biomass	0.36 (0.05)	0.33 (0.05)	0.62 (0.06)	0.40 (0.08)	0.45 (0.05)	
Bell Point (BP)						
Shoot density	98.40 (28.4)	141.3 (21.1)	296.3 (25.8)	82.5 (24.3)	187.0 (15.8)	
Aboveground biomass	0.21 (0.03)	0.25 (0.06)	0.48 (0.18)	0.25 (0.04)	0.86 (0.09)	
Belowground biomass	0.27 (0.02)	0.46 (0.09)	0.47 (0.08)	0.23 (0.04)	0.41 (0.05)	
Westcott Bay North (WBN)						
Shoot density	227 (27.6)	222.2 (17.0)	222.2 (7.0)	70.4 (16.1)	59.7 (18.8)	
Aboveground biomass	0.35 (0.03)	0.72 (0.07)	0.71 (0.10)	0.38 (0.07)	0.77 (0.13)	
Belowground biomass	0.31 (0.02)	0.45 (0.03)	0.37 (0.07)	0.33 (0.04)	0.36 (0.05)	

¹ n=1

Table 3-5 F-values and significance level (P) of ANOVA for differences in transplant performance (shoot density and above- and belowground biomass) between the sites in Westcott Bay at the intertidal (-0.7 m MLLW), the transition (-0.9 m MLLW), and subtidal zone (-1.5 m MLLW) describing the conditions in July 2007 and 2008.

	Depth	Parameter	df	MS	F	P
2007	-0.7 m	Shoot density	4	214973.10	12.83	< 0.0001 ^a
		Aboveground biomass	4	20400.60	10.84	< 0.0001
		Belowground biomass	4	1829.40	0.33	ns
2008	-0.7 m	Shoot density	3	20443.29	4.83	< 0.05
		Aboveground biomass	3	0.10	5.17	< 0.01
		Belowground biomass	3	0.04	3.53	< 0.05
	-0.9 m	Shoot density	3	16437.26	6.49	< 0.01
		Aboveground biomass	3	0.29	5.70	< 0.01
		Belowground biomass	3	0.03	1.41	ns
	-1.5 m	Shoot density	3	1.95	60.17	< 0.0001 ^a
		Aboveground biomass	3	0.47	5.94	< 0.01
		Belowground biomass	3	0.09	2.26	ns

^a Logarithmic transformation

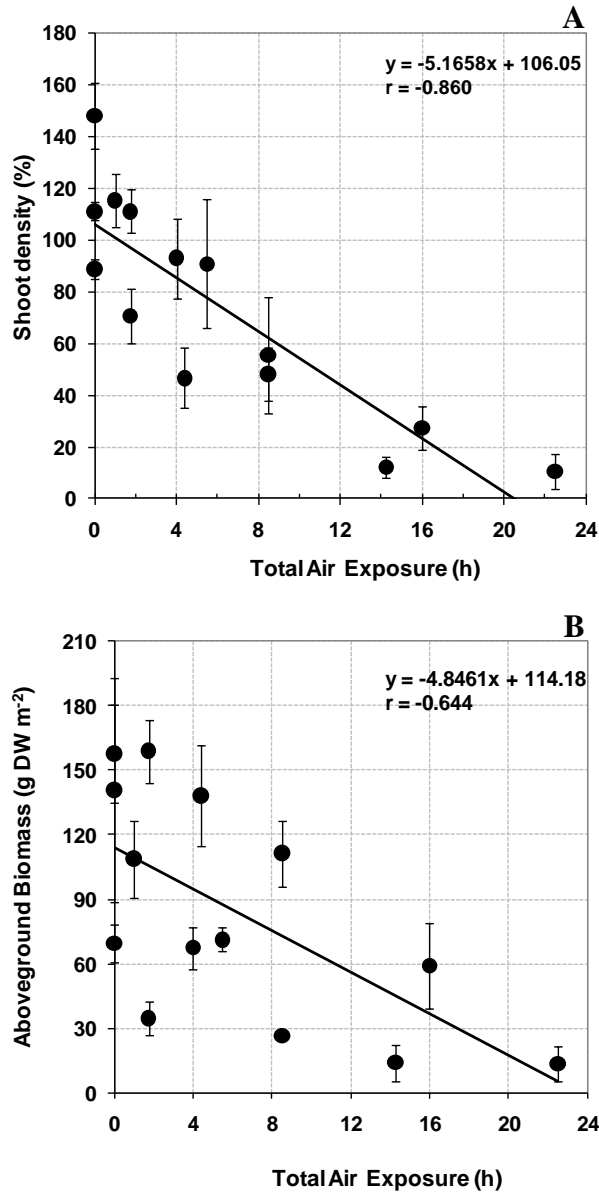


Fig. 3-10 Correlation of *Z. marina* transplant performance with total hours of air exposure (h). **A** Relative shoot density (%), **B** Aboveground biomass (g DW m⁻²) (mean ± SE).

3.3.2 WATER TEMPERATURE

The mean daily water temperature and the monthly mean of daily water temperatures were consistently higher at sites at the head of the bay compared to sites in the rest of the bay at all tidal elevations over the entire season in 2007 (WBS and WBN at -0.7 m MLLW) and in 2008 (WBN at -0.7 m, -0.9 m, -1.5 m MLLW) (Fig. 3-11 to 3-14), Table 3-6, 3-7). Similarly, the maximum daily temperatures over the season tend to be higher at the sites at the head of the bay relative to other sites in the bay at all tidal elevations in both years (Fig. 3-11 to 3-14, Table 3-6).

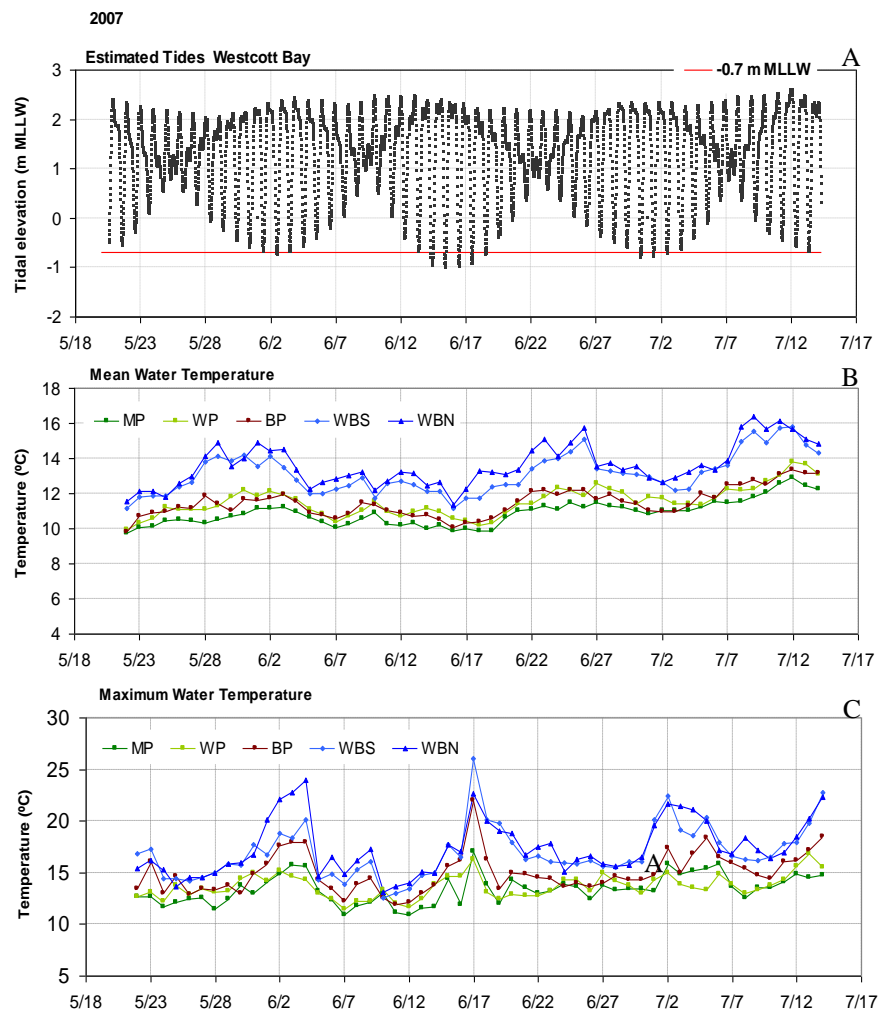


Fig. 3-11 A, B, C 2007 Intertidal zone (-0.7 m MLLW). A Estimated Tides (m MLLW) in Westcott Bay in 2007, B mean daily water temperature (°C), C maximum daily water temperature (°C) at the entrance (MP, WP), the inner bay (BP) and the bay head (WBS and WBN).

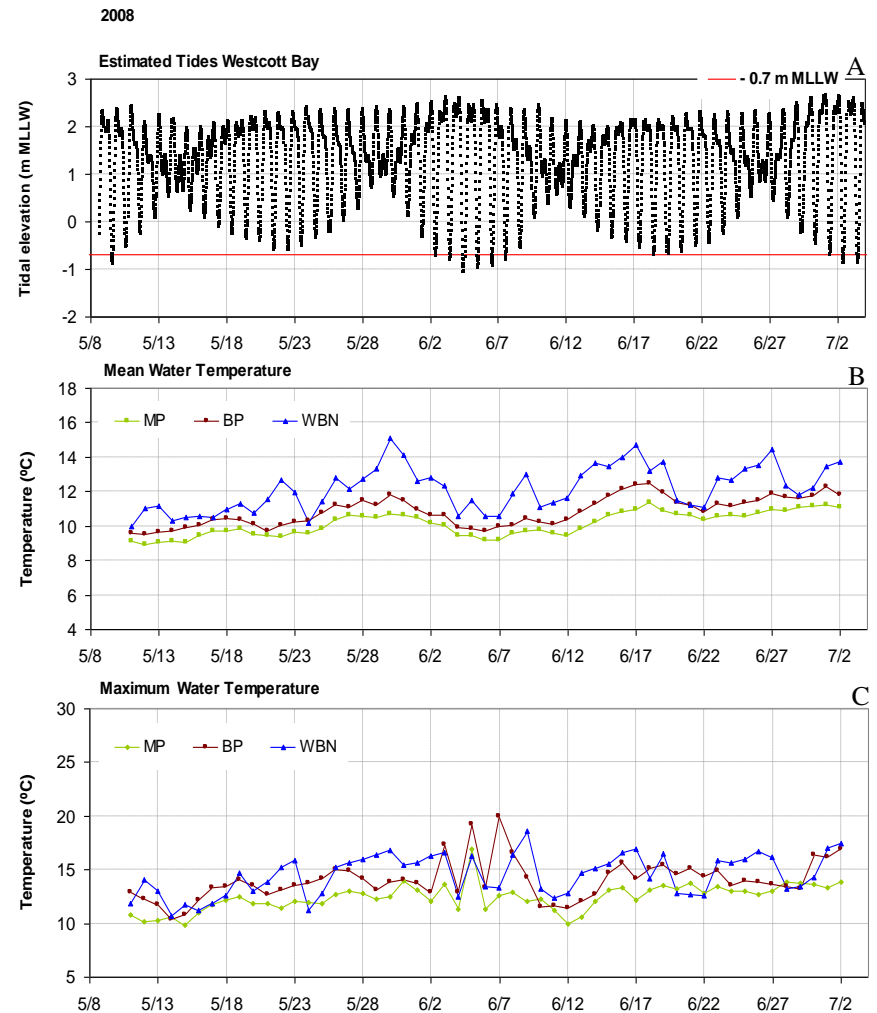


Fig. 3-12 A, B, C 2008 Intertidal zone (-0.7 m MLLW). A Estimated Tides (m MLLW) in Westcott Bay in 2008, B mean daily water temperature (°C), C maximum daily water temperature (°C) at the entrance (MP), the inner bay (BP) and the bay head (WBN).

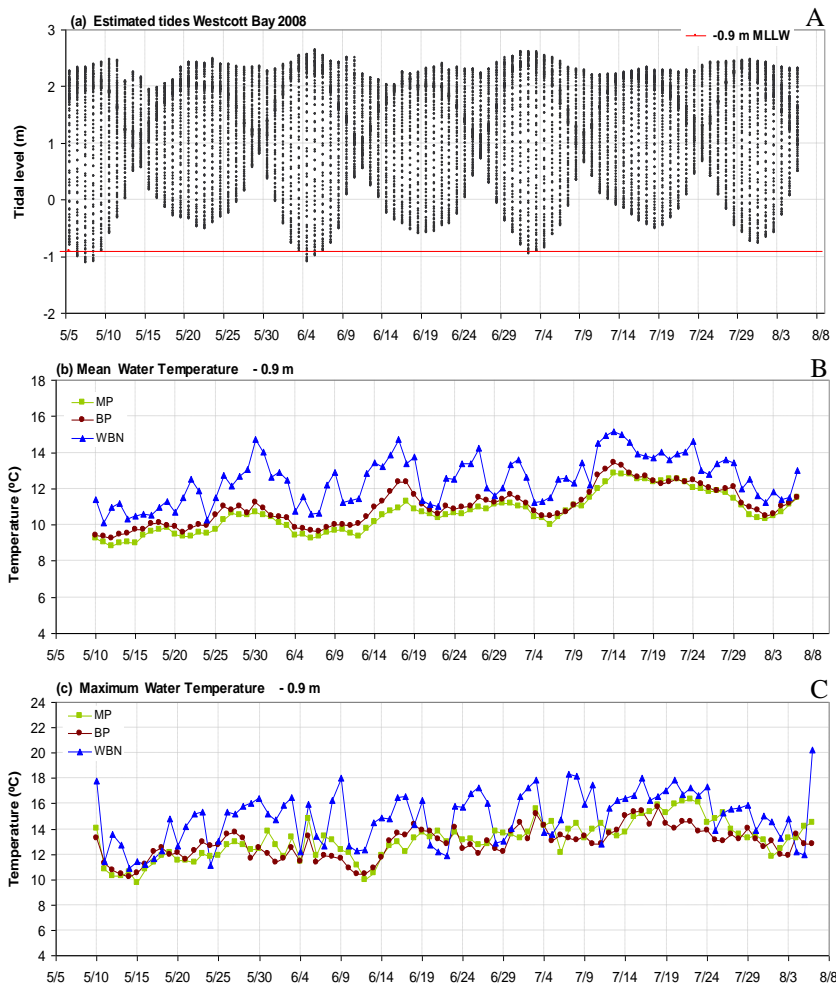


Fig. 3-13 A, B, C 2008 Transition zone (-0.9 m MLLW). A Estimated Tides (m MLLW) in Westcott Bay in 2008, B mean daily water temperature (°C), C maximum daily water temperature (°C) at the entrance (MP), the inner bay (BP) and the bay head (WBN).

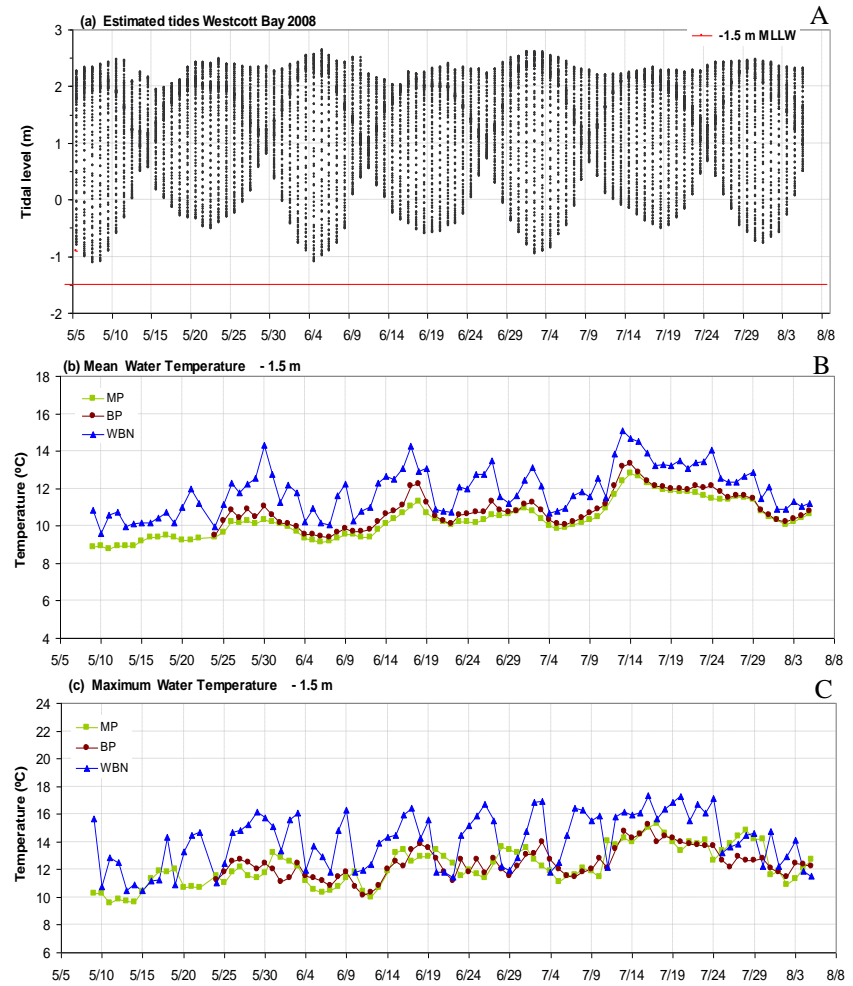


Fig. 3-14 A, B, C 2008 Subtidal zone (-1.5 m MLLW). A Estimated Tides (m MLLW) in Westcott Bay in 2008, B mean daily water temperature (°C), C maximum daily water temperature (°C) at the entrance (MP), the inner bay (BP) and the bay head (WBN).

3.3.2.1 INTERTIDAL WATER TEMPERATURE

DAILY TEMPERATURE OVER SEASON

In 2007, the mean daily water temperatures at the intertidal area ranged from a daily minimum of 9.8°C at MP in May to the highest mean daily temperature of up to 16.4°C at WBN in July (Fig. 3-11B). Highest maximum daily temperatures, exceeding 24.0°C, were recorded at WBN and WBS (26.0°C) in June 2007 (Fig. 3-11C).

In 2008, the lowest value of the mean daily temperature was observed at MP (8.9°C) in May, whereas the highest mean daily temperature of 15.2°C was recorded at WBN at the end of May 2008 (Fig. 3-12B). The highest maximum daily temperature in 2008 was recorded at BP in June with 20.0°C (Fig. 3-12C).

Interestingly, highest values of maximum daily water temperatures coincided with the events of extreme low tides in both years, whereas the values of the mean daily temperatures tend to increase in periods between the extreme low tide events at rather moderate tidal amplitudes (Fig. 3-11, 3-12).

Table 3-6 Mean (\pm SE) and maximum water temperature at different sites and tidal elevations in Westcott Bay describing the conditions during the experimental period in spring and summer 2007 and 2008.

	Depth (m MLLW)	Month	Water Temperature								
			Mosquito Pass			Bell Point			Westcott Bay North		
			Mean	SEM	Max	Mean	SEM	Max	Mean	SEM	Max
2007	-0.7	May	10.45	(0.12)	14.05	11.13	(0.17)	16.03	13.15	(0.37)	20.13
		Jun	10.68	(0.10)	17.11	11.24	(0.11)	21.99	13.36	(0.17)	24.00
2008	-0.7	May	9.80	(0.13)	13.93	10.46	(0.15)	15.01	11.71	(0.29)	16.77
		Jun	10.34	(0.12)	16.87	11.09	(0.15)	19.98	12.45	(0.21)	18.60
	-0.9	May	9.72	(0.13)	14.07	10.11	(0.12)	13.76	11.67	(0.26)	17.77
		Jun	10.31	(0.12)	14.77	10.81	(0.14)	14.53	12.40	(0.21)	17.25
		Jul	11.63	(0.16)	16.34	11.88	(0.16)	15.72	13.28	(0.20)	18.37
		Aug	11.33	(0.11)	14.94	11.53	(0.13)	15.08	12.69	(0.22)	20.22
		Sep	11.35	(0.14)	13.98	11.47	(0.15)	14.77	12.26	(0.18)	14.70
	-1.5	May	9.50	(0.11)	13.21	-	-	-	11.14	(0.24)	16.18
		Jun	10.10	(0.11)	13.62	10.45	(0.14)	13.86	11.82	(0.20)	16.73
		Jul	11.23	(0.15)	15.34	11.49	(0.16)	15.25	12.68	(0.21)	17.37

- available values not comparable, due to low number of days (n=9)

COMPARISONS BETWEEN MONTHS

In 2007, there was no change in the means of daily water temperatures in the intertidal zone between the months. In contrast, in 2008, the monthly mean daily water temperature increased from May to June at BP (Tukey test of means $p < 0.0001$) (Fig. 3-15A, B, Table 3-6, 3-7). The comparison of the monthly mean daily temperatures between the sites in 2007 revealed the highest temperature at WBN in June (13.36°C), where temperature exceeds the values at BP (11.24°C) by approximately 2.1°C (Fig. 3-15A, Table 3-6, 3-7). Similarly, in 2008, the highest mean daily temperature was observed in June at WBN (12.45°C). The monthly mean daily temperatures differed significantly across all sites (Tukey's test of means $p < 0.0001$ for all sites) (Fig. 3-15B, Table 3-6, 3-7).

COMPARISON BETWEEN YEARS

The comparison of the monthly means of daily temperatures revealed distinctly higher temperatures at all sites in Westcott Bay in May 2007 than in May 2008 (Tukey's test of means $p < 0.01$ for all sites, following a one-way ANOVA testing for differences between sites). In contrast, the mean daily temperatures in June were distinctly higher at MP and WBN in 2007 as compared to 2008. In contrast, there were no differences between the temperatures in June 2007 and 2008 at BP (BP: ANOVA, $df=1$, $F=0.625$, $p=0.432$; Fig. 3-15 A, B, Table 3-6, 3-7).

Table 3-7 Two-way ANOVA testing for differences in mean water temperature between month and sites at -0.7 m MLLW in 2007, and -0.7 m, -0.9 m and -1.5 m MLLW in 2008.

	Depth	Source of Variation	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
2007	-0.7	Site	4	43.55	72.02	< 0.0001
		Month	1	0.82	1.36	ns
		Month x Site	4	0.09	0.14	ns
2008	-0.7	Site	2	58.33	69.81	< 0.0001
		Month	1	27.90	33.39	< 0.0001
		Month x Site	2	1.94	2.32	ns
	-0.9	Site	2	83.25	101.42	< 0.0001
		Month	2	65.02	79.20	< 0.0001
		Month x Site	4	0.39	0.48	ns
	-1.5	Site	2	43.29	51.65	< 0.0001
		Month	1	46.64	55.64	< 0.0001
		Month x Site	2	0.29	0.34	ns

3.3.2.2 TRANSITION & SUBTIDAL ZONE WATER TEMPERATURE

-0.9 m MLLW

In the transition zone, there was no difference in the monthly means of daily temperatures between May and June at any site, whereas the mean daily temperatures strongly increased at all sites from June to July 2008 (Tukey's test of means $p < 0.0001$ at all sites) (Fig. 3-15C, Table 3-6, 3-7).

In accordance with temperature patterns at the intertidal zone, the monthly mean daily temperature at the transition zone was significantly higher at WBN than at MP and BP in May, June and July 2008 (Tukey's test: WBN x MP; WBN x BP; $p=0.0001$ for each month) (Fig. 3-15C, Table 3-6, 3-7). In this period, no significant difference in water temperature was found between MP and BP. Highest values of monthly maximum daily water temperatures were recorded at WBN at each month from May to August (Table 3-6). In September, maximum temperatures at WBN were similar to those at BP (Table 3-6).

-1.5 m MLLW

Similarly, the monthly mean daily temperature at the subtidal zone did not differ at any site between May and June, but increased at all sites between June and July by roughly 1°C (Fig. 3-15D, Table 3-6, 3-7). Likewise, the monthly mean daily temperatures were significantly higher in the inner bay at WBN than at BP and MP during summer in June and July (Tukey's HSD: WBN x MP; WBN x BP; $p=0.0002$ for each month). No significant difference in temperature was observed between MP and BP during June and July 2008 (Fig. 3-15D, Table 3-6, 3-7). Maximum daily water temperatures in the subtidal zone were consistently highest at WBN throughout the sampling period from May to July (Table 3-6).

3.3.2.3 WATER TEMPERATURES & *Z. MARINA* PERFORMANCE

Following a period of approximately 2 weeks of elevated mean daily temperatures at the transition and the subtidal zone at WBN in the middle of July, with maximum values of up to 18.4°C at -0.9 m MLLW, and 17.4°C at -1.5 m MLLW, the mean shoot density of transplants substantially decreased in August 2008 (Fig. 3-4B, C, 3-5B, 3-15C, D, Table 3-2, 3-6). Moreover, the continued recording of water temperatures at the transition zone revealed maximum daily temperatures above 20°C in August 2008 (Table 3-6), that were followed by the loss of all *Z. marina* transplants in the transition and subtidal zones by November 2008.

A correlation of transplant shoot density across all sites and tidal elevations over the experimental period from May to August confirmed a moderate negative correlation of the *Z. marina* transplant shoot density with increasing maximum temperatures (Pearson correlation, $r = -0.48$) (Fig. 3-16).

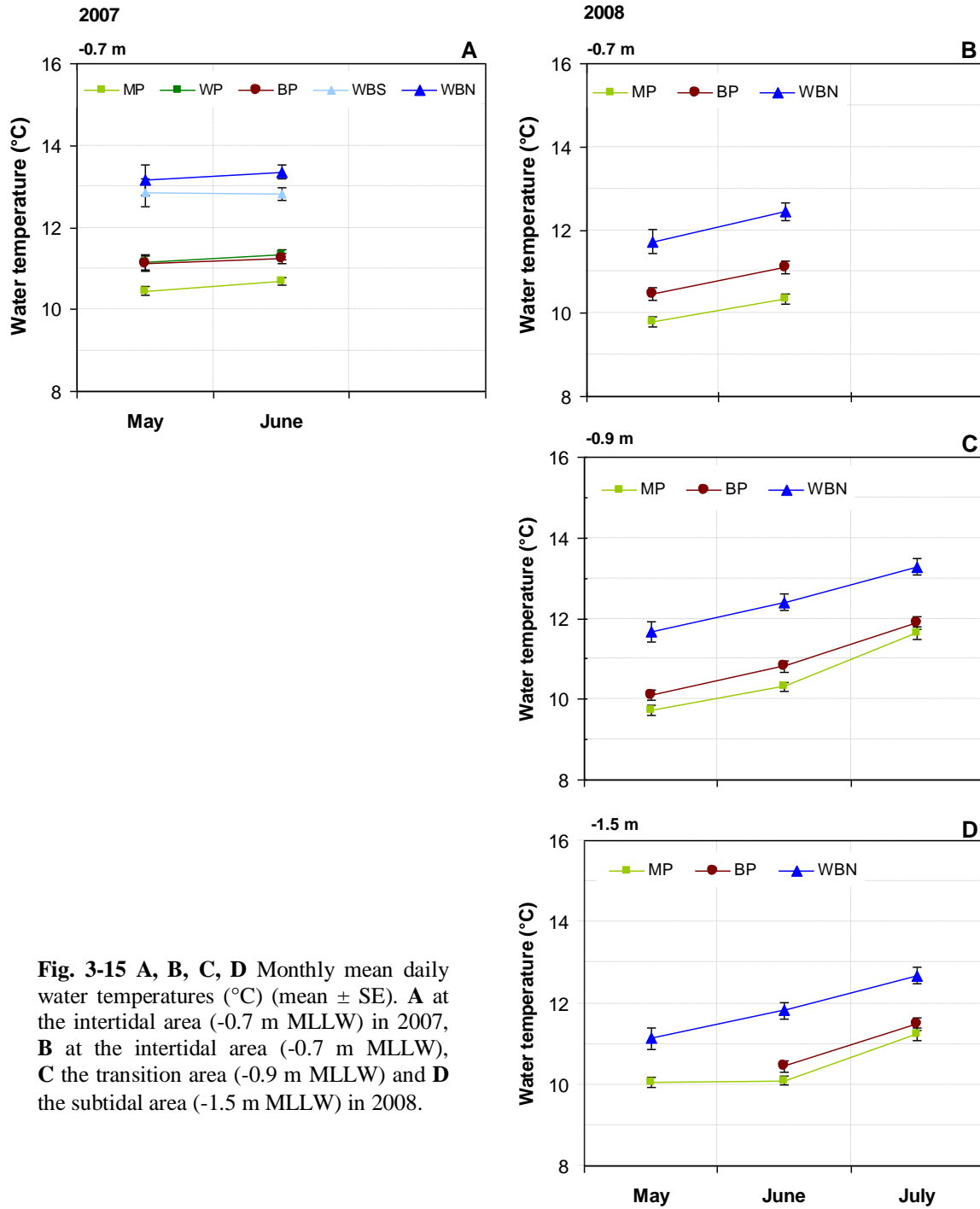


Fig. 3-15 A, B, C, D Monthly mean daily water temperatures (°C) (mean \pm SE). **A** at the intertidal area (-0.7 m MLLW) in 2007, **B** at the intertidal area (-0.7 m MLLW), **C** the transition area (-0.9 m MLLW) and **D** the subtidal area (-1.5 m MLLW) in 2008.

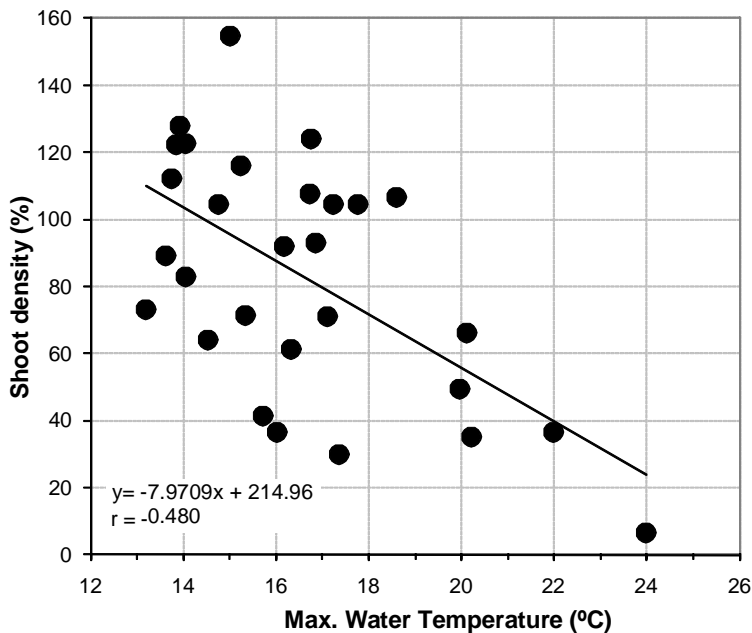


Fig. 3-16 Correlation of relative *Z. marina* transplant shoot density (%) of with monthly maximum daily water temperature (°C).

3.3.6 ADDITIONAL POTENTIAL STRESSORS OBSERVED

SPAWN OF THE BUBBLE SNAIL (*HAMINOEA VESICULA*)

In 2007 and 2008, bubble snail (*Haminoea vesicula*) egg cases attached to *Z. marina* shoots were observed at all sites in June and July, with the highest coverage at the head of Westcott Bay (Fig. 3-17C). In the beginning of July 2008, increasing amounts of bubble snail egg masses were observed covering *Z. marina* transplants at WBN, smothering and depressing the *Z. marina* transplants at all three transplantation depths (-0.7 m, -0.9 m, -1.5 m MLLW) (Fig. 3-17). Visual inspection by divers during the bi-weekly transplant monitoring indicated that some *Z. marina* transplants were completely covered with egg masses in July, while the amount of egg cases further increased at all tidal elevations until August 2008. In August, the plants appeared to be dead and were covered in silt (Kitaeff *et al.* pers. communication). The analysis of mean egg mass loads attached to individual *Z. marina* transplant shoots (wet weight of egg mass in mg cm⁻² leaf) revealed the highest mean value of 45.0 mg cm⁻² leaf⁻¹ (± 12.55 SE) at the transition zone at WBN in July, with maximum values of up to 220.64 mg (Fig. 3-17C). In addition, bubble snail egg cases, which are sticky, were observed trapping and accumulating resuspended particles from the water column, thereby further contributing to the smothering of transplants (Fig. 3-17A).

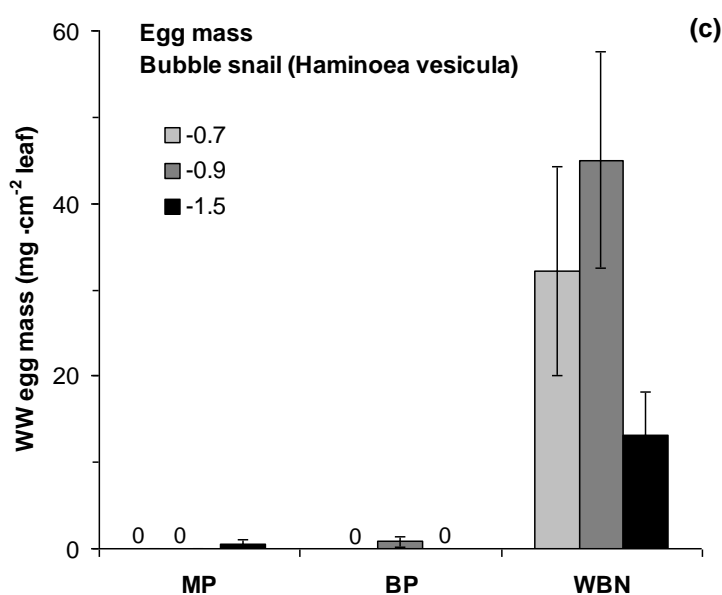
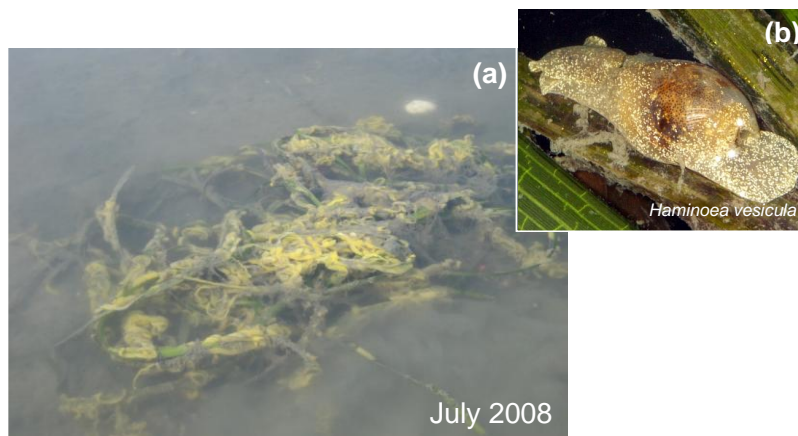


Fig. 3-17 A, B, C Bubble snail (*Haminoea vesicula*) egg masses smothering *Z. marina* shoots. **A** *Z. marina* transplant covered with bubble snail eggs at WBN at -0.9 m MLLW in July 2008. **B** Bubble snail *Haminoea vesicula*. **C** Wet weight of bubble snail eggs ($\text{mg}\cdot\text{cm}^{-2}\text{ leaf}^{-1}$) attached to individual shoots of *Z. marina* transplants at different tidal depths across the sites in Westcott Bay in summer 2008 (means \pm SE; pooled data of July and August).

SEDIMENT SULFIDES

An obvious, strong increase in sulfur odor was recognized in both air and sediment at the head of Westcott Bay during the transplant monitoring in July and August in both years (Schanz *et al.* pers. observation and Kitaeff *et al.* pers. com.). Moreover, in July 2008, *Z. marina* transplants at the head of Westcott Bay were observed to be pressed to the sediment by heavy bubble snail spawn (3-17A); when these plants were lifted, a strong sulfur odor was present. Rhizomes were often observed to be partially rotten and “mushy” under these conditions. Additionally, the leaves and meristem of some *Z. marina* plants were observed to be rotten and/or covered with a white film of sulfur bacteria (*Beggiatoa* spp.) on the leaf surface likely indicating the presence of sulfide (e.g. Nelson *et al.* 1986,

Holmer *et al.* 2005). In November 2008, the rhizomes of the few remaining *Z. marina* transplants looked black, and were mushy and partly decomposed. In May 2009, there was barely any rhizome material from the 2008 transplants left.

GREEN ALGAL COVER

Large amounts of green algae, in particular *Ulva spp.*, were observed in the intertidal zone at BP, partly smothering the *Z. marina* transplants in early summer 2007. Furthermore, a high coverage of algae was observed in the intertidal and transition zone at MP and BP in June and July 2008, with highest algae coverage of transplants observed in the transition zone at BP (Fig. 3-18). *Z. marina* shoots in this area were observed to be partly brown, broken, or mostly dead. In some cases, only the plant's sheath remained. Moreover, many plants had buried leaf tips, whereas roots and rhizomes were exposed from erosion of sediment.

TURBIDITY

High water column turbidity was observed at the head of Westcott Bay relative to other sites of the bay in 2007 and 2008. Furthermore, turbidity tended to be higher in June and July than in May, August, and November, and visibility was described as consistently 'very bad or poor' at the transition zone and the subtidal zone as reported during transplant monitoring events in these months (Kitaeff pers. com.).

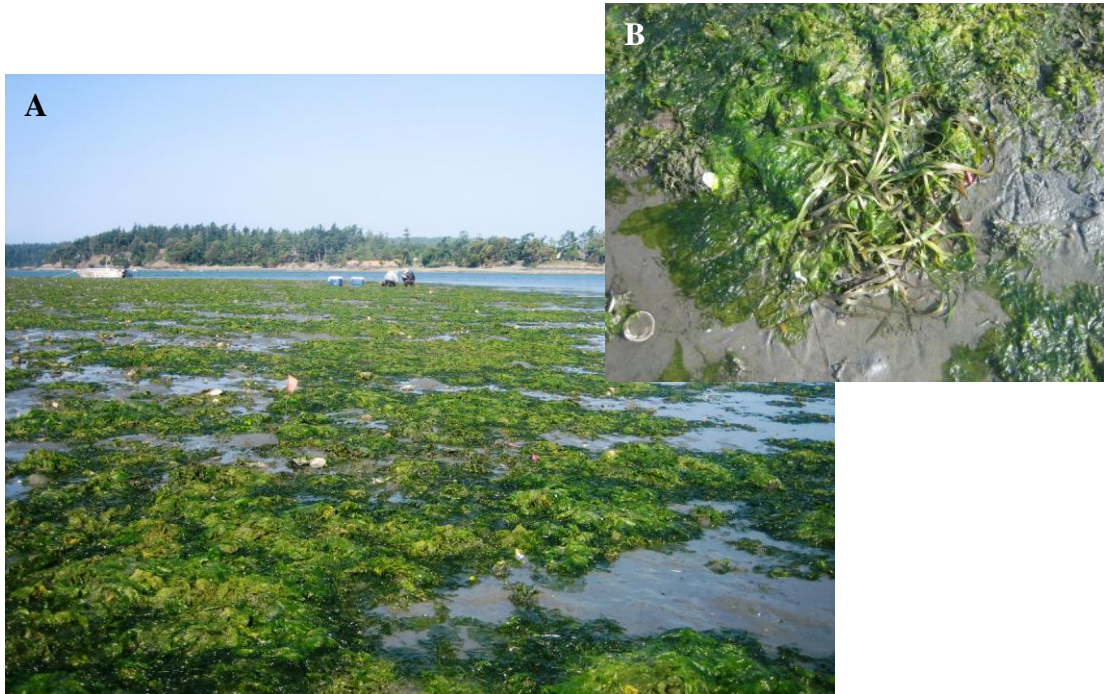


Fig. 3-18 **A** Green algae mats (*Ulva spp.*) in the lower intertidal- and transition area (-0.7 to -0.9 m MLLW) at Bell Point in July 2008. **B** Green algae smothering *Z. marina* transplant at -0.9 m MLLW at Bell Point.



4 DISCUSSION

4.1 EXPERIMENTAL DESIGN AND TRANSPLANT ARTIFACTS

It is unlikely that the early loss of *Z. marina* transplants in 2007 (compared to the transplant performance in 2008) was due to differences in the transplantation methods used in Westcott Bay. Both techniques are well established and have been documented to successfully transplant *Z. marina* and other seagrass species in research and restoration projects worldwide (Fonseca *et al.* 1998, Short *et al.* 2002). Furthermore, performance of plants transplanted with the same method was highly site specific. Performance of *Z. marina* that was re-transplanted into donor beds was not compromised, thereby negating the effect of the transplantation technique.

Failure in seagrass transplantation and/or restoration projects is generally related to unsuitable environmental conditions at the targeted transplant area (Short *et al.* 2002, van Katwijk *et al.* 2009). In 2007, the *Z. marina* transplant location in the intertidal area at the head of Westcott Bay (WBN) was slightly higher than the former *Z. marina* distribution (Fig. 3-1). This might partly explain an early loss of these transplants, since the margin of seagrass beds is controlled by tides and water dynamics (Leuschner *et al.* 1998, van Katwijk & Hermus 2000, Koch 2001, Boese *et al.* 2005), which are related to elevation. However, transplants in the intertidal area did not survive at any site in the inner bay and the head of Westcott Bay in 2007, and thus a difference in elevation of ≤ 0.1 m at WBN in 2007 might have been only an ancillary factor causing the observed transplant loss at this site. In contrast, the stable transplant shoot densities in the intertidal area at WP (Fig. 3-1) might have been a result of the comparably deeper location of the transplants at this site relative to the other transplants in the lower intertidal area in 2007 (further information in section 4.3). Therefore, the horizontal gradient in the performance of transplants of the intertidal area in 2007 is at least partly confounded with the elevation at WP. However, survival and persistence of *Z. marina* at MP in 2007 and 2008, and transplant performance at the sites of the inner and the head of the bay at different tidal elevations in 2008 confirmed an existing spatial gradient of decreasing *Z. marina* performance from the entrance to the head of Westcott Bay.

4.2 DOES *Z. MARINA* CURRENTLY GROW AND SURVIVE IN WESTCOTT BAY?

Zostera marina transplantation experiments in Westcott Bay indicate that:

- *Z. marina* does currently not survive at the head of Westcott Bay at any of the three tidal elevations tested (intertidal-, transition- and subtidal area)
- *Z. marina* does not survive in the intertidal area at Bell Point. Both conclusions are supported by the fact that *Z. marina* transplants did not survive in these areas in two consecutive years (2007 and 2008)
- *Z. marina* growth and survival is currently supported at sites at the entrance of Westcott Bay, as demonstrated by the persistence of *Z. marina* transplants as well as the documented natural *Z. marina* distribution at Mosquito Pass and White Point by the SVMP/ES-RP in 2008 and 2009 (Gaeckle *et al.* 2009, Ferrier *et al.* in prep.) (Fig. 2-6, Table 2-1)
- The current capacity of sites to support *Z. marina* growth and survival decreases along a spatial gradient from the mouth to the head of Westcott Bay, as shown by comparably stable transplant shoot densities and aboveground biomasses at the entrance of the bay that decreased toward the site of the inner bay and approached zero at the head of the bay in both years of transplantations
- The current capacity of sites to support *Z. marina* growth and survival decreases vertically from the subtidal toward the intertidal area. This is shown by comparably stable transplant shoot density and biomass in the subtidal area across all sites through July 2008, whereas the shoot density and aboveground biomass decreased at in the transition zone (BP: July 2008; WBN: August 2008), and in the intertidal area (BP: July 2007, 2008; WBS & WBN: July 2007 and WBN: transplants were lost after July 2008) at sites in the inner and head of bay

Distinct differences in the performance and survival of *Z. marina* transplants across the sites in Westcott Bay indicate that there are differences among sites that are limiting *Z. marina* re-colonization and growth. Since neither *Z. marina* transplants nor infrequently observed single seedlings in spring survived at the head of Westcott Bay or in the intertidal area at Bell Point in areas that have been historically vegetated (DNR staff pers. observations in 2007 and 2008), the lack of *Z. marina* re-growth in these areas has not been simply due to a lack of propagule recruitment. Thus, it is suggested that the current distribution of *Z. marina* across the sites in Westcott Bay, with no vegetation at the bay head and at the intertidal area at Bell Point, at this time likely represents the extent of suitable environmental conditions in this area.

Temporal differences in transplant response between 2007 and 2008 suggest that stressors that lead to the decline of *Z. marina* transplants may have differed between years, tidal elevations and sites. In 2007, the transplant shoot density and aboveground biomass decreased rapidly in the intertidal area at Bell Point and at the head of the bay, where values approached zero within 8 weeks after the transplantation. In contrast, in 2008, a decrease in transplant shoot density and biomass was initially observed in the transition area and the subtidal area at the head of Westcott Bay after 12 weeks, although there was a

decline at Bell Point after only 8 weeks. Transplant shoot density at the bay head approached zero in November 2008 at the transition and the subtidal area.

Although bioturbation often causes transplant losses along the eastern and southern shores of the U.S. (Fonseca *et al.* 1994, 1998, Davis *et al.* 1998), we found little evidence of disruption of the *Z. marina* transplants at Westcott Bay by burrowing activities of crustaceans or fish during the transplant experiments. Observations indicate that Dungeness Crabs (*Cancer magister*) and Rock Crabs (*Cancer productus* and *Cancer gracilis*) were abundant within *Z. marina* transplants shortly after the transplantation, particularly at the head of the bay. A decrease in shoot density, however, was measured 8 weeks after transplantation. This long time span does not support a likelihood of effects by crab bioturbation. Additionally, in both years no differences in the belowground biomass were observed between sites (with the exception of the slightly lower belowground biomass of transplants in the intertidal- and transition area at Bell Point in 2008). Rhizomes could usually be found in the sediments at the locations of the individual transplant units. This confirmed that the plants died *in situ*, and were not simply up-rooted by burrowing activities.

The following sections discuss the role of air exposure and elevated water temperatures as well as other environmental factors in the performance of *Z. marina* transplants in order to explain *Z. marina* transplant loss, and to identify current *Z. marina* stressors in Westcott Bay.

4.3 ROLE OF AIR EXPOSURE IN INTERTIDAL TRANSPLANT PERFORMANCE

In coastal zones influenced by large tidal amplitudes, exposure to air during low tides is an important environmental factor affecting biota inhabiting the littoral zone. With respect to seagrass condition, air exposure can cause plant desiccation stress by decreasing the water content in seagrass leaves (Leuschner *et al.* 1998, Boese *et al.* 2003, 2005), and is further suggested to limit the upper distribution of seagrasses in the intertidal zone (Leuschner *et al.* 1998, Koch 2001, Boese *et al.* 2005).

During this study, prolonged air exposure of intertidal transplants at sites of the inner and the head of Westcott Bay (21-34 h) in 2007 was associated with low transplant shoot density and aboveground biomass relative to transplants at the entrance of the bay with comparably short air exposure times (5-10 h) (Fig. 3-8A). Similarly in 2008, longest air exposure time of transplants in the intertidal area at site of the inner bay (9.5 h) corresponded with the lowest transplant shoot density and aboveground biomass relative to transplants at the entrance and the head of Westcott Bay (4-5.5 h). The observed transplant performance suggests that prolonged total air exposure time, additive over the period of extreme low tides in May and June, may have contributed to *Z. marina* transplant loss in the lower intertidal area at the sites of the inner and the head of Westcott Bay in 2007. In addition, comparably longer air exposure and resulting substantial plant desiccation presumably also contributed to transplant loss in the intertidal at the inner bay site in 2008. Results of the transplant performance correlated with the total time of air exposure

confirmed a strong negative correlation of *Z. marina* shoot density and aboveground biomass with increasing total time of air exposure in Westcott Bay. These findings are supported by several studies identifying air exposure and resulting desiccation as the probable cause of changes in seagrass density and morphology for *Z. marina* growing in the temperate zone across a tidal gradient (Bayer 1979, Jacobs 1979, Kentula and Mc Intire 1986, Keddy 1987, Koch and Beer 1996, Boese *et al.* 2003). Under extreme conditions (e.g., long air exposure in combination with high sun radiation, warm air temperatures, and/or windy conditions) the upper margins of *Zostera noltii* beds have been observed to be visibly ‘burned’ because seagrass leaves turned brown (Van Lent *et al.* 1991, Schanz pers. observations in *Z. noltii* beds in the German Wadden Sea). Comparable patterns of changes in leaf color were observed frequently for *Z. marina* in the lower intertidal area at Mosquito Pass (2007) and Bell Point (2007 and 2008) during air exposure at low tides in spring and summer. However, while at Mosquito Pass only the top layer of *Z. marina* leaves appeared brown, at Bell Point all transplant leaves turned brown, dry and ‘crispy’. In Yaquina Bay, Oregon (USA) large non-pigmented necrotic patches have been observed on *Z. marina* leaves following spring and summer low tide exposures, presumably as a result of desiccation stress (Boese *et al.* 2003). Desiccation damage further contributes to shorter leaf length since damaged leaf sections tend to break more easily (Boese *et al.* 2003). In tropical seagrasses, it has been shown that air exposure during daytime low tides in spring resulted in seasonal losses of aboveground biomass (Vermaat *et al.* 1993, Erfteimeijer and Herman 1994, Stapel *et al.* 1997). The findings of Boese *et al.* (2003) and the reported total losses of the aboveground biomass in tropical seagrass beds may explain the distinctly lower transplant shoot densities and aboveground biomasses at sites with long air exposure in Westcott Bay, while the belowground biomass remained comparably stable across the sites (with the exception of Bell Point in 2008). The fact that the belowground eelgrass biomass did not show site-specific or seasonal variations after the extreme air exposure events might also point to stressors that acted above the sediment surface in 2007, such as desiccation stress.

While transplants that were exposed to prolonged air exposure time showed a decrease in shoot density and aboveground biomass, shoot densities of transplants that were only briefly exposed to air remained stable. Short air exposure duration in the intertidal area at WP (as a result of the approximately 0.2 m deeper elevation relative to other intertidal transplants) might also explain the stable transplant shoot densities at this site in 2007. In 2008, total air exposure time of transplants in the intertidal area that was less than half as long as transplant air exposure times at the inner and the head of the bay in 2007. This might explain why transplant shoot density and biomass remained stable at the intertidal transplants through July 2008 (except at the inner bay (Bell Point) which showed a decrease in shoot density by July). A study in the German Wadden Sea by Leuschner *et al.* (1998) reported that after 5 h of exposure to air during low tide, leaves of *Z. noltii* lost up to 50% of their water content. The degree of leaf water loss rapidly increased with the distance from the low water line and, hence, was a function of the duration of leaf air exposure. Intertidal seagrass species such as *Z. noltii* and *Z. marina* are able to tolerate desiccation stress for a certain period (Leuschner & Rees 1993, Leuschner *et al.* 1998, Shafer *et al.* 2007). As long as leaf water contents are favorable, elevated photosynthetic rates are supported in *Zostera* species by both high irradiance and a readily available carbon source due to the rapid diffusion of CO₂ in air (Leuschner *et al.* 1998). Experiments

with *Zostera* leaves in air under both laboratory and field conditions showed that moderate losses in water content in *Zostera* leaves resulted in a reversible reduction in light-saturated net photosynthesis rate (H_{\max}) (Leuschner *et al.* 1998, Shafer *et al.* 2007), whereas a depletion of the leaf water content by more than 50% resulted in a reduction in light-saturated net photosynthesis by 50 to 90% in *Z. noltii* and *Z. marina* (Leuschner *et al.* 1998). The above named study clearly demonstrated that the photosynthetic potential of *Zostera spp.* is limited by desiccation during low tide.

Although eelgrass transplant performance in Westcott Bay in 2007 was most likely affected by desiccation as a result of air exposure during extreme spring and summer low tides, the plant response, especially survival and recovery of transplants, differed distinctly between sites (Table 4.1). As described by Boese *et al.* (2005), stress by desiccation events might be mitigated by several local factors, among them the morphology and density of the affected eelgrass stands. A layer of residual water trapped between the leaves of a dense canopy at low tide (Polte *et al.* 2005) probably also lowers desiccation effects in intertidal seagrass beds. At the entrance of the bay such a residual water layer was retained during the extreme exposure events by the dense *Z. marina* vegetation at Mosquito Pass. This kept the lower layer of *Z. marina* leaves moist, and thus presumably mitigated the effect of air exposure (while the top layer of leaves desiccated). Sparse patches of *Z. marina* are unlikely to retain a layer of water, and thus might be more susceptible to desiccation stress. Consequently, transplants at the inner bay at Bell Point that grow on sandy substrate substantially desiccated during air exposure events in the absence of other vegetation.

In Table 4.1 we offer interpretations for the site specific differences of the transplant response in relation to air exposure and resulting desiccation in Westcott Bay. Furthermore, by means of a conceptual diagram (Fig. 4-2) we explain how different physical factors control air exposure and the resulting desiccation stress on *Z. marina* plant response (Section 4.2.6).

Continuously recorded environmental parameters in Westcott Bay show that high air temperature and the corresponding high temperature at the sediment surface during prolonged periods of extreme air exposure coincided with elevated water temperatures at the head of the bay and that, in turn, led to a constant warming of the plants without distinct cooling during flood tides. Cooling phases during periods of flood tides with strong water movements, such as events of large tidal fluctuations, presumably lessen the cumulative daily stress from desiccation, thus allowing the seagrass population to survive over the long-term (Thom *et al.* in prep.). This could have contributed to the observed zig-zag trend in the intertidal area at the entrance of the bay in 2007, where transplant shoot densities showed alternating decreases after strong desiccation events and increases two weeks later, probably related to cooler water temperatures.

In conclusion, it may be assumed that prolonged air exposure and resulting desiccation as well as related elevated air/sediment and water temperatures in May and June 2007 affected the transplant performance in the lower intertidal area at the sites of the inner and the head of Westcott Bay, and presumably contributed to the irreversible decline of transplant shoot density by July 2007. In contrast, poor transplant performance in the intertidal area of the inner bay, and at all tidal elevations at head of the bay in 2008, in the absence of prolonged air exposure suggest that stressors other than desiccation led to the

eventual loss of *Z. marina* in 2008. However, desiccation stress might have been a contributing factor at the inner bay in 2008.

Table 4-1 Interpretation of *Z. marina* transplant response (shoot density) related to air exposure time and site specific characteristics in the lower intertidal area in Westcott Bay in 2007 (*SD* = shoot density).

Site	Air exposure	Sediment composition	Canopy residual water layer	Plant response	Interpretation
Entrance (MP)	moderate	coarse sand	yes	Zig-zag trend in SD, fast recovery	Desiccation-induced shoot loss limited to the air exposed <i>Z. marina</i> canopy top layer; recovery by remaining underlying shoots that were kept moist by a residual water layer.
Inner bay (BP)	long	sand	no	Distinct decrease in SD within 2 weeks, delayed die off (after July), no recovery	Low sediment moisture due to high drainage and evaporation promotes substantial desiccation resulting in rapid irreversible shoot loss.
Head of bay (WBN)	long	silt	no	Drastic decrease in SD after 6 weeks, die off by July, no recovery	High sediment moisture due to low water drainage and evaporation diminishes immediate desiccation effects; irreversible shoot loss might not be only induced by desiccation but by consistently elevated ambient (air/sediment & water) temperatures during the entire tidal cycle.

4.4 ROLE OF ELEVATED WATER TEMPERATURE ON *Z. MARINA* PERFORMANCE

Within its biogeographic distribution and seasonal growth periods, *Zostera marina* tolerates a wide range (0-30°C) of temperatures (Phillips 1984, Bulthuis 1987, Lee *et al.* 2007). However, extreme water temperatures can affect all aspects of the plant’s life cycle (Marsh *et al.* 1986, Olesen & Sand-Jensen 1993). While the geographic distribution of *Z. marina* is well documented, little is known about the plant’s tolerance to extreme temperature fluctuations at a given location. Temperate seagrasses might be generally adapted to lower water temperatures and might have limited tolerances to high temperatures. Accordingly, for temperate *Z. marina* in Denmark, Nejrup & Pedersen (2008) documented that low water temperatures (5°C) did not affect plant mortality whereas sustained high temperatures (25-30°C) increased mortality by 12 fold within 6 weeks. In their study, photosynthesis rates were highest between 10°C and 15°C, while shoot mortality “increased sharply” at 20°C. Setchell (1929) documented that *Z. marina* shoots died and detached from the substrate at temperatures above 20-25°C. More recently,

extensive summer die-offs of *Z. marina* have been observed in coastal waters of the Danish and German Baltic Sea, related to extreme heat waves in Europe (Reusch *et al.* 2005, Ehlers *et al.* 2008, Nejrup & Pedersen 2008). High shoot mortality in periods where local water temperatures exceeded 25°C for longer periods have caused extensive disappearance of *Z. marina*. It has been suggested that elevated temperatures in conjunction with anoxic conditions and high sulfide concentrations in the sediment were the major reason for the plants' decline (e.g. Greve *et al.* 2003, Pedersen *et al.* 2004, Borum *et al.* 2005, Holmer *et al.* 2005). In contrast, the study of Nejrup & Pedersen (2008) suggested that the effect of high water temperature alone may explain part of the summer die-offs, because their aquaria were well aerated, which prevents sulfide intrusion into roots and rhizomes (Greve *et al.* 2003, Pedersen *et al.* 2004). However, results on temperature optima and thresholds for plants growing in the Northeast Atlantic and Baltic region might not be adopted globally without regard to potentially different ecological and genetic adaptation (Ehlers *et al.* 2008, Reusch *et al.* 2008) of *Z. marina* according to climatic regions and seasonal ranges in water temperatures.

While plants in the Northeast Atlantic and Baltic Sea are regularly exposed to immense seasonal temperature fluctuations from the freezing point in winter to 22-25°C in summer (e.g., Sand-Jensen 1975), *Z. marina* in the Northeast Pacific region grows under a remarkably constant temperature regime with less fluctuation (8-14°C) (Phillips 1984). Due to the more stable climatic environment, the latter might react even more sensitively to temperature extremes since genetic and strategic (metabolic) adaptations might not be as developed. For *Z. marina* in Puget Sound, Phillips (1984) described vegetative growth and reproductive activity in a temperature range from 6-12.5°C.

The results of the water temperature measurements in our study show that mean and maximum daily water temperatures in Westcott Bay were consistently higher at the head of the bay at all tidal elevations relative to other sites in the bay during May through July in 2007 and 2008. *Z. marina* transplants did not survive at the head of the bay at any tidal elevation in either year. The correlation of *Z. marina* transplant performance with water temperatures recorded in Westcott Bay indicates a moderate negative impact of elevated maximum temperatures on shoot density.

A detailed view of transplant performance related to water temperatures in the intertidal zone in 2007 reveals that temperature peaks in June were most pronounced in the inner bay (BP: max. 22°C) and the head of the bay (WBN: max. 24°C), while temperatures at the entrance of the bay remained somewhat cooler (MP: max. 17°C). Corresponding transplant performance decreased rapidly and distinctly at sites in the inner bay and the head of the bay. However, maximum water temperatures above 20°C in mid-June do not explain the earlier decrease in shoot density at Bell Point at the end of May 2007 which is probably related to desiccation (see previous section). During summer 2008, temperature peaks were not as high or as frequent as in the previous year. However, following a distinct temperature peak in June (max. 20°C) the corresponding transplant performance in the intertidal zone of Bell Point decreased in July, and these transplants eventually died. During the same period, the temperature peak at the head of the bay was 1.4°C lower (WBN: max. 18.6°C), and the transplants survived for a longer period but did not survive through the end of summer.

In the Puget Sound area Thom *et al.* (2003) found a (weak) significant, negative correlation of *Z. marina* mean shoot densities and temperature. Although they observed a great deal of variation in plant response over a temperature range of about 15-18°C, their data showed that sites with lowest temperatures in summer contained the densest *Z. marina*. In general, temperature is known to control seagrass productivity (Kentula & McIntire 1986, Bulthuis 1987, Zimmerman *et al.* 1989, Cabello-Pasini *et al.* 2003). Although high temperatures can increase productivity, at some point increasing respiration rates will outweigh the potential benefit (Zimmerman *et al.* 1989). In general, fluctuations of water temperatures are not limited to geographic regions and seasons but also occur vertically on different tidal elevations. Plants growing mostly (transition zone) or constantly (subtidal zone) submerged may display more narrow tolerance ranges than intertidal plants that are regularly exposed to temperature fluctuations and a variety of environmental extremes (including high solar radiation) related to exposure to air during the tidal cycle. However, Thom *et al.* (in prep.) showed that *in situ* *Z. marina* summer growth rates measured at three tidal elevations at Sequim Bay, WA decreased with increasing temperature.

Throughout Westcott Bay, deeper *Z. marina* transplants were not so much exposed to extreme temperature peaks in early summer but to consistently elevated water temperatures with an average of about 15°C over a period for more than a month. However, in mid-summer water temperatures at the head of the bay reached maximum values of 20°C in the transition zone and even 17.4°C in the subtidal area. Correspondingly, none of the transplants survived in the head of the bay. Transplants in the transition zone of Bell Point approached critical values although max temperatures were 2.65°C (July) to 5.14°C (August) lower than at the head of the bay. It can only be assumed that prolonged periods of consistently elevated temperatures exceeding optimal values (above 15°C max) lead to an impairment of transplant condition. This is supported by the fact that transplants in the transition zone and subtidal area of Mosquito Pass and in the subtidal zone of Bell Point, where maximum temperatures barely approached 15°C, showed no losses in transplant density and biomass.

According to Thom *et al.* (in prep.), *Z. marina* in Puget Sound is healthiest within a narrow temperature range of 5-8°C, although optimal productivity within Northeastern Pacific estuaries is reported to occur at 11-15°C. At temperatures above 15°C the productivity to respiration ratio is very low, indicating heat stress to the plants (Thom *et al.* 2003). Laboratory experiments showed that net productivity was greatest at about 10-17°C, but the productivity to respiration ratio showed a very narrow and pronounced peak between 4-7°C. Maximum net productivity measured in winter conditions (winter plants) (7-10°C) was up to six times higher than rates in summer plants under summer conditions (12-15°C) (Thom *et al.* in prep.). These findings correspond to our results in Westcott Bay, where the decrease in shoot density at the head of the bay coincided with highest mean daily temperatures approaching 15°C in the transition zone and in the subtidal area, while maximum daily temperatures frequently exceeded 15°C (with highest temperatures of approximately 18°C recorded at both tidal elevations). Transplants in the transition zone at the entrance of the bay and at Bell Point exposed to maximum water temperatures of 15°C also showed a decrease in shoot density, whereas shoot densities of transplants in the subtidal area that experienced only a single short temperature peak of 15°C remained comparably stable. Therefore, we conclude that *Z. marina* transplants in Westcott Bay that constantly experience daily water temperatures of 15°C and higher suffer from heat stress.

The results of this study not only demonstrate a correlation between temperature extremes and plant response at different tidal elevations, but might also hint at certain temperature thresholds for short term temperature pulses that potentially cause irreversible damage and lead to *Z. marina* die off. This possibility must be thoroughly investigated if a local threshold value is to be established.

The mechanisms behind temperature stress and its consequences for seagrass are not well understood. However, most publications on the subject deal with temperature effects on the plant's respiration and photosynthetic activity. Exposure to high water temperatures increases respiration relative to photosynthesis, and consequently, leads to reduced photosynthesis to respiration (P:R) ratios (Marsh *et al.* 1986). Therefore, growth can be limited by high water temperatures during summer months (Marsh *et al.* 1986, Lee *et al.* 2005). Photosynthesis and respiration rates usually increase with increasing water temperatures (Bulthuis 1983, Dennison 1987, Marsh *et al.* 1986, Pérez and Romeo 1992, Masisni & Manning 1997, Moore *et al.* 1997). At progressively higher temperatures respiration increases beyond photosynthesis rates leading to a reduction in net energy budgets (Bulthuis 1983, Dennison 1987, Marsh *et al.* 1986, Pérez and Romeo 1992, Masisni & Manning 1997, Moore *et al.* 1997).

Interestingly, optimal temperatures for seagrass growth are usually lower than those for photosynthesis (Lee *et al.* 2007). The optimum temperature for photosynthesis can vary with underwater irradiance (Bulthuis 1987). For example, the optimum temperature decreases as irradiance decreases for *Heterozostera tasmanica* (Bulthuis 1987). This implies that seagrasses growing in low light conditions have lower optimum temperatures for photosynthesis than plants in high light conditions. Plants at higher temperatures likely need more light to maintain a positive carbon balance than those at lower temperatures. Thus, photosynthetic production in seagrasses is more susceptible to higher water temperatures in reduced light conditions. Consequently, reductions in underwater light may be more harmful to seagrasses during summer rather than in winter.

When taken together, the results of this study in conjunction with findings of Thom *et al.* (2001a, 2001b, 2003) suggest that intertidal and shallow subtidal *Z. marina* in Puget Sound is affected by even relatively short periods of exposure to extreme temperatures in the range of 20(+)^oC, or prolonged periods of exposure to high daily mean water temperatures of 15(+)^oC during late summer. In the context of sea surface warming as a consequence of climate change, research on effects of increased temperature on aquatic vegetation will probably increase in the near future. This is necessary and will presumably contribute to the understanding of the role of heat stress in temperate seagrass species.

4.5 OTHER ENVIRONMENTAL CHARACTERISTICS RELATED TO TRANSPLANT PERFORMANCE

4.5.1 LIGHT LIMITATION

Data on the light climate in Westcott Bay are documented by Dowty & Ferrier (2009). Their analysis of continuously recorded photosynthetic active radiation (PAR) over the experimental period in Westcott Bay revealed distinctly lower daily PAR values at the

head of the bay relative to other sites in the bay in 2007 and 2008. Although PAR values at the bay head were reduced by roughly 20%, their results show that the available PAR throughout Westcott Bay exceeded the critical threshold value of $3 \text{ mol m}^{-2} \text{ day}^{-1}$ which was identified as a minimum requirement for *Z. marina* survival in Pacific Northwest estuaries (Thom *et al.* 2008). Therefore, light limitation is excluded as a significant stressor in this study.

However, high water turbidity was observed by DNR staff and divers primarily during June and July, particularly at the head of Westcott Bay at all tidal elevations and at Bell Point in the intertidal and transition area. Turbidity is often increased by boat wakes resulting from high boat traffic during vacations or holidays, as observed by DNR staff during transplant monitoring events. Even if the observed turbidity did not bring PAR underneath literature thresholds for eelgrass survival, it seems likely that decreased light penetration might contribute to decreasing performance of plants that are already suffering from heat (air/sediment temperature during air exposure and warm water temperature during high tides) and desiccation stress.

4.5.2 BUBBLE SNAIL SPAWN ACCUMULATION AT THE HEAD OF THE BAY

Egg cases of the bubble snail *Haminoea vesicula* were found attached to *Z. marina* shoots at all sites across Westcott Bay in summer 2007 and 2008, with highest coverage of egg spawn on *Z. marina* shoots at the head of Westcott Bay, smothering and depressing the *Z. marina* transplants at all tidal elevations (Fig. 3-17). Furthermore, *Z. marina* transplants at the head of the bay were observed to be totally covered by egg masses in July, while the amount of egg cases further increased at all tidal elevations until August 2008. The highest amounts of bubble snail spawn attached to *Z. marina* shoots at the head of the bay in summer corresponded with a sudden decrease in transplant shoot densities in late summer, which then approached zero at all tidal elevations in fall.

The high leaf coverage by egg cases alone probably decreased the light available for photosynthesis substantially. However, the egg cases also trapped and accumulated re-suspended particles from the water column, further contributing to the smothering of transplants (Fig. 3-17A). Accumulation of snail spawn on transplants that are isolated from naturally occurring eelgrass stands might be due to an “oasis” effect caused by the absence of adequate spawning substrate and leading to a concentration of spawn on single eelgrass patches or shoots. Similar heavy use of small patches of eelgrass by juvenile cod (*Gadus spp.*) has been demonstrated in the absence of sufficient habitat (Laurel *et al.* 2003). The potential for an oasis effect is supported by the observation that bubble snail spawn found at Mosquito Pass was sparse and rather well distributed without large accumulations. If it was indeed an oasis effect, bubble snail spawn accumulation at the head of the bay might appear to be an experimental artifact. However, it might represent a serious stressor for sparse eelgrass stands already weakened by other factors. Furthermore, an overload of snail spawn might be a relevant danger for single pioneer shoots in the process of re-colonization, and might further become an issue in respect of eelgrass restoration activities.

4.5.3 MACROALGAE

Large accumulations of macroalgae have been shown to affect *Z. marina* by direct smothering (den Hartog & Polderman 1974, den Hartog 1994, Hauxwell *et al.* 1998, 2003, Cummis *et al.* 2004). Furthermore, it is reported that decaying algae mats can increase the sediment concentrations of potentially toxic sulfides (Carlson *et al.* 1994, Holmer & Nielsen 2007). In this study, the occurrence of macroalgae, in particular green algae (*Ulva spp.*), was observed in the intertidal- and transition area at the entrance of Westcott Bay and at Bell Point in spring and summer 2007 and 2008 (Fig. 3-18), whereas no algae were observed at the head of the bay during this study. Large accumulations of green algae, partly smothering *Z. marina* transplants in the transition zone at Bell Point in 2008, coincided with visibly 'unhealthy' shoots that were brown, had broken leaves, or had only sheaths and belowground biomass, while other plants were found to be dead. Moreover, the occurrence of macroalgae corresponded with decreases in transplant shoot density in the intertidal area and the transition zone at Bell Point in summer and fall, with shoot densities partly recovering in the transition zone during late fall/winter when macroalgae were no longer present. In accordance with our findings, Boese and Robbins (2008) observed that the timing of large fall accumulations of green macroalgae (*Ulva spp.*) corresponded to reduced *Z. marina* shoot densities in intertidal areas in the Yaquina Estuary, OR (USA).

Smothering of *Z. marina* plants by macroalgae reduces the light availability by shading the lower parts of the plants (Hauxwell *et al.* 2001). In addition, high respiration rates in the mats of macroalgae during the night or during degradation of the macroalgae in late summer and fall further limit the oxygen availability near the sediment surface, which may result in sediment hypoxia under the alga cover, and this in turn can directly inhibit seagrass and also leads to the build up of potentially toxic sediment sulfides (Carlson *et al.* 1994). Boese & Robbins (2008) speculated that increased sediment sulfides might persist into late fall as decaying algal material is incorporated into sediments. *Z. marina* is tolerant of hypoxic sediment conditions as it transports oxygen from leaves to roots (Kraemer & Alberte 1993, Holmer & Bondgaard 2001). However, in late summer and fall, decreasing light availability, due to shorter day length, coupled with a large amount of macroalgae partially shading *Z. marina* leaves may reduce the ability of plants to cope with sediment hypoxia at the time where sediment sulfides are at their highest concentration, possibly reducing the belowground biomass of *Z. marina* (van Lent & Verschuure 1994). In accordance with findings of Boese & Robbins (2008) and van Lent & Verschuure (1994), results of the transplant performance show that transplant shoot densities at Bell Point started to decrease in the intertidal and transition zone in July, when algae masses seemed to be highest, and continued to decline through fall. Moreover, only the transplants at the intertidal area and the transition zone at Bell Point showed a distinct decrease in the belowground biomass, which remained stable at the reduced level in the transition zone through August 2008. No further observations of the belowground biomass of intertidal plants exist after July 2008.

Although we did not observe that algae accumulation at Bell Point covered *Z. marina* completely, since parts of the seagrass plants still floated in the water column during inundation, partial smothering of *Z. marina* plants has been shown to reduce shoot densities of *Z. marina* beds (Nelson & Lee 2001). Considering the accumulation of algae

and the corresponding results of the transplant performance at Bell Point as well as related findings of other studies as stated above, we assume that the green algae coverage at Bell Point is a contributing factor affecting *Z. marina* transplant performance in the intertidal- and transition area at Bell Point in 2007 and 2008.

4.5.4 SEDIMENT SULFIDES

Worldwide, sediment sulfides have been documented to have a negative impact on seagrass survival (e.g., Goodman *et al.* 1995, Borum *et al.* 2005, Holmer *et al.* 2005). Sulfides act as a phytotoxin by inhibiting photosynthesis (Goodman *et al.* 1995) and nutrient uptake (Koch *et al.* 1990), and often result decreased productivity (Lee & Dunton 2000), and overall meadow decline observed in late summer and fall (Borum *et al.* 2005).

Field observation in this study included a strong sulfur odor in both air and sediment at the head of Westcott Bay in July and August in both years. When lifted, *Z. marina* shoots in transplants at the head of the bay emitted a strong sulfur odor, and rhizomes were found to be partially rotten and mushy under these conditions. In addition, all rhizomes seemed to be decomposed by November, while remaining parts of rhizomes were observed to be black and mushy. Rotten seagrass leaves and plant meristems as well as rhizome material can be indicative for an intrusion of sulfide into the plants (Holmer *et al.* 2005).

An investigation in 2007 highlighted a potential elevation of sulfide levels in sediment porewater at the head of Westcott Bay (Takesue unpubl. data). The findings of this study imply that sediment sulfides should be incorporated in future research as a potential factor contributing to a suite of stressors affecting *Z. marina* growth in Westcott Bay. This is supported by studies documenting that *Z. marina* (and other seagrasses, such as *Thalassia testudinum*) growth and survival are particularly hampered by the interaction of multiple stressors, such as elevated temperature, anoxia, and sulfide (Holmer & Bondgaard 2001, Koch *et al.* 2007). The combined effect of sulfide and plant anoxia has been suggested as key factor behind seagrass die-off events (Carlson *et al.* 1994, Greve *et al.* 2003). Sulfides enter the plant through the roots if the oxygen pool in the plant is depleted (Pedersen *et al.* 2004). Reduced photosynthetic activity and increased mortality have been documented for *Z. marina* experiencing exposure to anoxia and sulfides (Goodman *et al.* 1995, Holmer & Bondgaard 2001).

4.6 CURRENT CONCEPTUAL THINKING FOR WESCOTT BAY

The Eelgrass Stressor-Response Project 2005-2007 report formulated a series of initial research questions and hypotheses to be evaluated (Table 1-1). Furthermore, the report states that once the hypotheses are tested, the conceptual model explaining the *Z. marina* loss in Westcott Bay will be revised (Dowty *et al.* 2007).

The present study investigated a key hypothesis identified in the 2005-2007 report (table 1.1, hypothesis #1). The results show that current habitat conditions do not support *Z. marina* growth and survival at the head of Westcott Bay at the intertidal, transition- and subtidal area, as well as at the inner bay in the intertidal area. By correlating *Z. marina*

transplant performance along a spatial and tidal gradient with concurrently monitored environmental conditions, the results of this study further identified a suite of different stressors that are suggested to synergistically affect *Z. marina* survival at different tidal elevations Westcott Bay.

In the following sections we present two conceptual diagrams addressing *Z. marina* stressors as identified in this study in order to provide an understanding of how these stressors and related processes can control *Z. marina* growth and survival in Westcott Bay. In addition, we present a conceptual model that summarizes potential *Z. marina* stressors identified in Westcott Bay, and includes the current understanding of these stressors based on study findings.

4.6.1 SYNERGISTIC EFFECTS OF ELEVATED WATER TEMPERATURE AND OTHER STRESSORS

Results of the present study highlight the potential role of elevated water temperature as a contributing stressor controlling *Z. marina* growth and survival in Westcott Bay. Elevated water temperatures can affect *Z. marina* performance either alone, through different plant physiological processes (e.g., decrease in the photosynthesis-respiration rate or acute heat shock), or in conjunction with other physical (e.g., reduced underwater light, enhanced nutrients) and/or biological (e.g., algae mats) stressors as well as associated processes. In Fig. 4-1 we present a conceptual diagram describing how elevated water temperature in conjunction with other potential stressors and related plant physiological processes controls *Z. marina* growth and survival.

Generally, water temperature is controlled by climatic conditions, such as solar radiation and air temperature. Moreover, it is influenced by physical factors, such as hydrodynamics including e.g., water mixing or water volume. Mixing of water is not only influenced by tides and wind (wind waves), but also depends on physical features, such as the geomorphology (e.g., a narrow bay or lagoon, or orientation (e.g., embayment that is protecting from prevailing wind)). Reduced mixing indirectly promotes an increase in water temperatures, and lasting conditions. Increasing water temperature leads to both an increase in photosynthesis and respiration of marine plants.

In *Z. marina*, the rate of leaf respiration increases more rapidly with rising temperatures than does that of photosynthesis (Marsh *et al.* 1986, Masini & Manning 1997). In contrast to photosynthesis, which exhibits optimum rates at moderate temperatures, respiration continues to increase up to high temperatures, leading to a steady decrease in the photosynthesis-to-respiration (P:R) ratio with increasing temperature. If increasing temperatures exceed an optimum temperature, the oxygen content in shoots declines dramatically and plant tissue may turn anoxic even in the light (Greve *et al.* 2003).

At less extreme temperatures, the strength of the respiratory oxygen sink may become so high that the transport of oxygen to belowground tissue is insufficient to maintain aerobic radial oxygen loss to the sediment (Caffrey & Kemp 1991). This situation represents a threat to plant survival, because toxic anaerobic metabolites may accumulate within roots and rhizomes (Pregnall *et al.* 1984, Smith *et al.* 1984).

The plant oxygen balance is seen to primarily depend on changes in surface irradiance in the light, and controlled by changes in water column oxygen in the dark. The oxygen content at high light is determined by the balance between light saturated oxygen evolution in leaves and the oxygen loss due to plant respiration and the oxygen efflux to the water column and sediment. In darkness, the relationship between plant and water column oxygen is determined by the balance between oxygen supply from the water column and oxygen losses due to plant respiration and the oxygen efflux to the sediment.

Tissue anoxia in seagrass impairs growth of roots, nutrient uptake and translocation of nutrients and carbohydrates (Smith *et al.* 1984, Zimmerman & Alberte 1996). Moreover, the disappearance of the oxic microshield around roots and rhizomes normally provided by the radial oxygen loss allows the invasion of reduced phytotoxins from sediment to the plant tissues. Periodic invasion of sulfide from the sediment into roots of *Z. marina*, and invasions of gaseous sulfide into seagrass lacunae, has been measured both under laboratory and field conditions for *Z. marina* and *Thalassia testudinum* (Pedersen *et al.* 2004, Borum *et al.* 2005).

The events of sudden seagrass die-off (e.g., tropical turtle grass in Florida Bay) which have been reported for temperate and tropical seagrass beds are suggested to be influenced by a series of unfavorable environmental conditions and related plant physiological processes, including increased water temperatures, anoxia in plant tissue, sediments, and the water column, as well as high sediment sulfide concentrations and sulfide intrusion and/ or toxicity of the plants (Roblee *et al.* 1991, Ziemann *et al.* 1999, Greve *et al.* 2003, Borum *et al.* 2005, Koch *et al.* 2007). However, the complex synergetic effects of the suite of stressors and underlying mechanisms are not well understood.

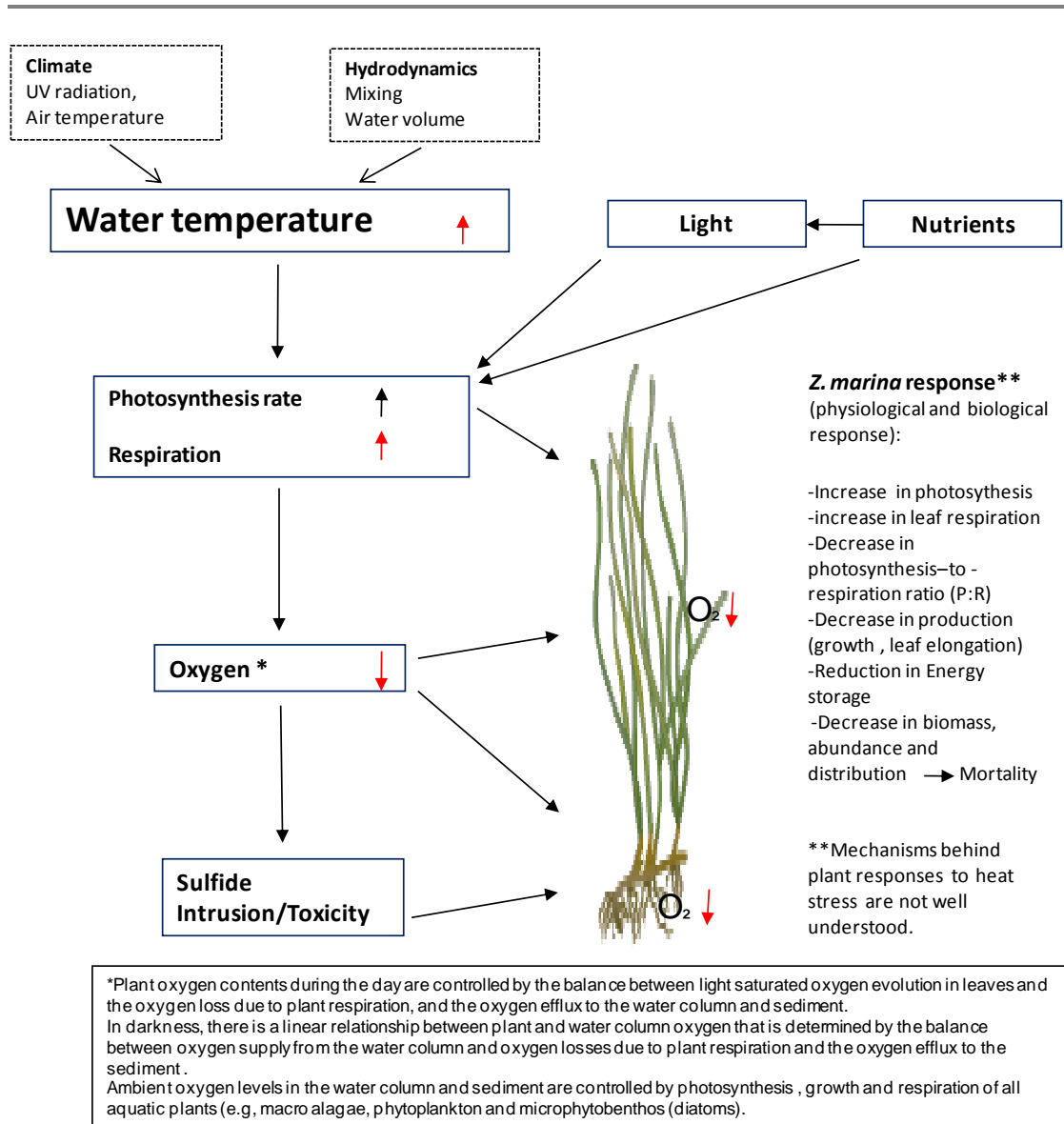


Fig. 4-1 Conceptual diagram showing the synergistic effect of increasing water temperature and other stressors as well as underlying plant biological and physiological response on *Z. marina* performance

4.6.2 INTERTIDAL *Z. MARINA* AIR EXPOSURE MODEL

In the context of this study desiccation as a result of prolonged air exposure during extreme low tides in spring and summer was identified as a contributing stressor affecting the shoot density and biomass of *Z. marina* plants growing in the lower intertidal area. In the Northeast Pacific low tides in spring and summer (March to September) occur during daytime hours while low tides in fall and winter (October to February) occur during nighttime hours. Consequently, during periods of prolonged air exposure at extreme low tides (spring tides) in spring and summer *Z. marina* has to cope with the predominant climatic conditions (Fig. 4-2).

Air exposure of *Z. marina* is controlled by tides (tidal cycle, amplitudes, and annually by the 18.6-year nodal tidal cycle in the North Pacific) as well as the depth distribution (tidal elevation) of the plants. Furthermore, air exposure can be minimized by topographical features (Jacobs 1979, Kentula & McIntire 1986) such as the presence of water-retaining vegetation (Boese *et al.* 2005) or sediment micro-depressions retaining water (Silva *et al.* 2005) that keep the leaves moist.

Desiccation of *Z. marina* as a consequence of prolonged air exposure is influenced by several environmental factors. Climatic variables, such as UV radiation, air temperature, precipitation and wind control the degree of desiccation stress. Furthermore, sediment composition, grain size and sediment porosity influence desiccation stress by affecting water drainage and evaporation rates.

Generally, seagrasses are well adapted to their surrounding environment. Therefore, it can be expected that *Z. marina* plants that are regularly exposed to air during low tides are better adapted to frequently occurring desiccation stress than plants that are only infrequently or rarely exposed to air during extreme events. One mechanism of *Z. marina* to cope with desiccation is differences in plant morphology or differing morphotypes of *Z. marina* along a tidal gradient (as documented for *Z. marina* in the Yaquina Estuary, Newport, Oregon, USA, Boese *et al.* 2005, and in the Wadden Sea, North Sea, van Katwijk *et al.* 2000) and may explain higher susceptibility to desiccation stress of plants at lower intertidal elevations. At the upper extent of its range, *Z. marina* sheaths tend to be flexible and lay flat on the moist sediment, which provides protection from desiccation. At lower elevations, plants have stiff sheaths, which are vulnerable to desiccation during extreme low tides, but more resistant to stronger water dynamics. Other plant morphology factors also differ among morphotypes, such as shoot and leaf length, and tissue robustness. In the Wadden Sea, van Katwijk *et al.* (2000) documented that between the two seagrass zones, a bare zone existed, where the habitat is too dynamic for the high, flexible morphotype, and the periods of emergence last too long for the more robust morphotype. In this study, *Z. marina* sheaths in the lower intertidal and transition area were observed to be stiff and upright when exposed to air, which suggests they belonged to the morphotype that is more sensitive to air exposure. Presumably, this resulted in the eventual desiccation of the essential basal meristem during prolonged air exposure.

Response of *Z. marina* to desiccation varies with the duration and degree of plant desiccation including the water loss in leaves and/ or plant sheaths, reduction in CO₂ assimilation and photosynthesis (decrease in effective quantum yields), change in morphology (e.g., shoot length, leaf number), reduction in shoot density, biomass and mortality. However, mechanisms of the effect of air exposure, climate, and resulting desiccation stress are not yet well understood. In Figure 4-2 we present a conceptual diagram explaining how prolonged air exposure in conjunction with other physical parameters controls the effect of desiccation on the biological and physiological response of *Z. marina*.

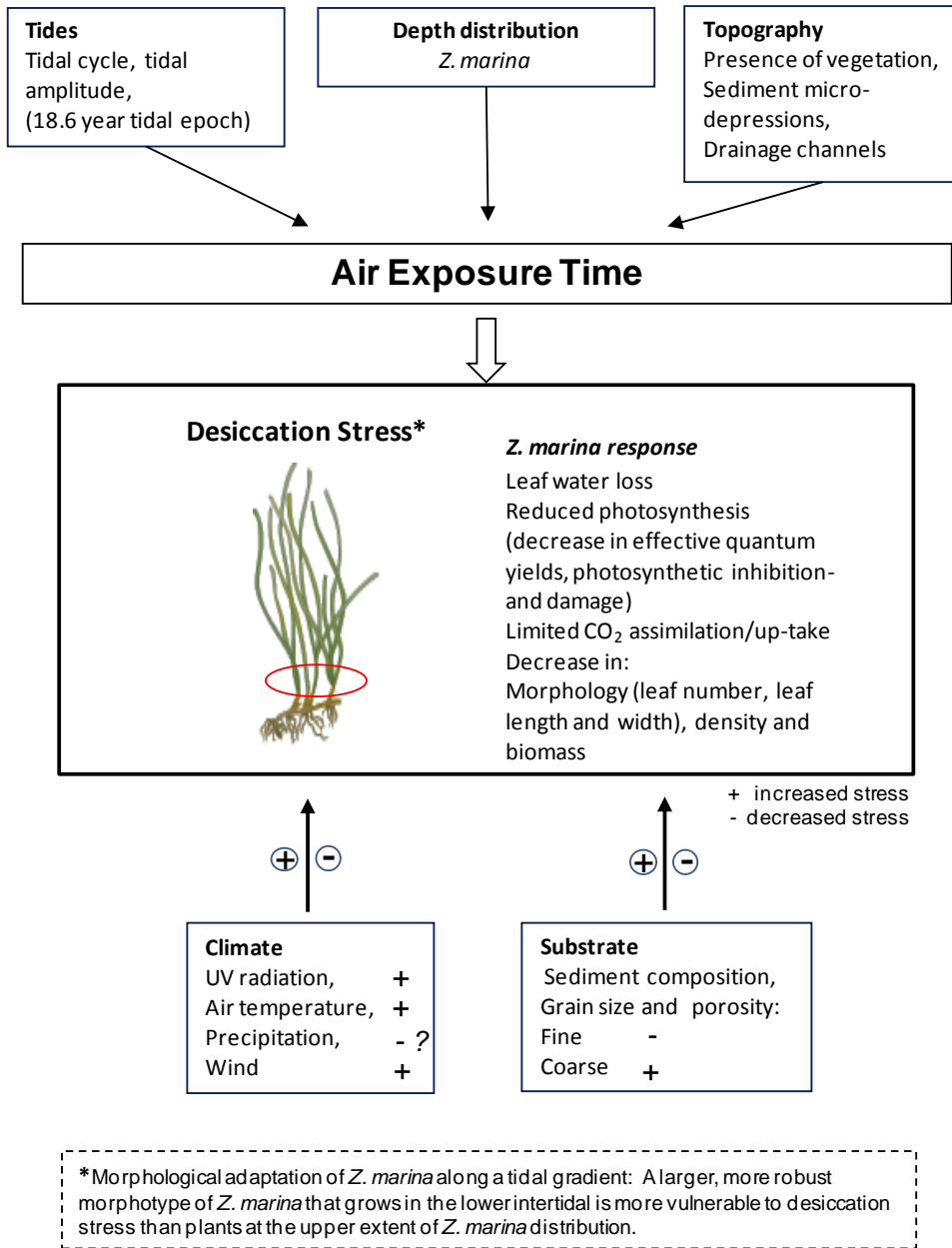


Fig. 4-2 Conceptual diagram showing how different physical factors control air exposure and the resulting desiccation stress on *Z. marina* plant response.

4.6.3 POTENTIAL SCENARIOS EXPLAINING *Z. MARINA* LOSS IN WESTCOTT BAY

Regional experts hypothesize that there was a gradual decline of *Z marina* in Westcott and Garrison Bays, which began no later than 1998 and accelerated in the early 2000s, leading to a crash in or around 2003 (Wyllie-Echeverria *et al.* 2003). Mapping conducted by Berry *et al.* (2003) corroborates this view. A report by Friends of the San Juan (FoSJ) (2004) also notes the near-complete loss of eelgrass in Westcott Bay between 2001 and 2003. Below, we present a potential scenario based on available records of tides, sea surface temperatures, and air temperatures.

Subtidal populations thinned throughout the period of overall decline (Wyllie-Echeverria *et al.* 2003), suggesting that a chronic stressor was present at the time. According to data collected from the NOAA station in Friday Harbor, sea surface temperatures were unusually high during 1997 and 1998 (Fig. 4-3). At the same time, tidal amplitudes were near their minimum; that is, the difference between the high and low tides was near its natural minimum during this period. In a shallow, quiescent bay such as Westcott Bay, this results in very poor flushing. The lack of cold water influx and mixing in the bay at a time of high temperatures could conceivably result in chronic heat stress in subtidal populations better adapted to frequent cold pulses. Although little is known of the nutrient and light regime during the late 1990s, warm and stagnant water conditions seem favorable for a plankton bloom and the resulting anoxia and turbidity.

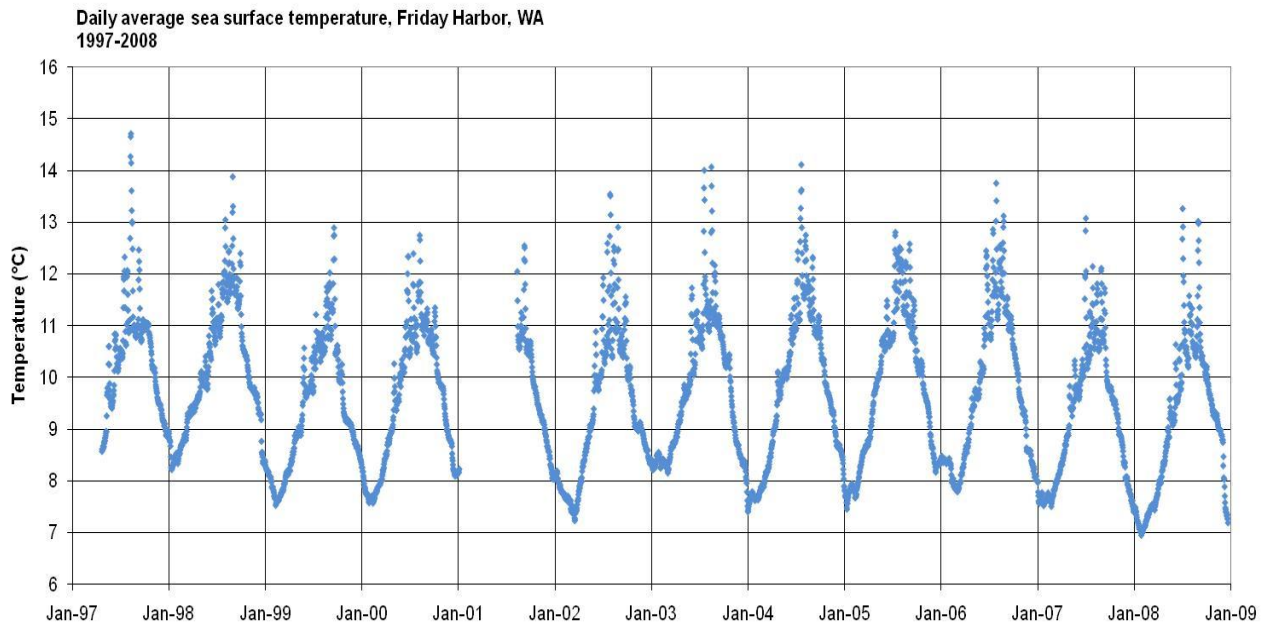


Fig. 4-3 Daily average sea surface temperatures in Friday Harbor, WA (<http://tidesandcurrents.noaa.gov>; station 9449880). Data between 1/8/2001 and 8/14/2001 are missing.

While subtidal populations thinned, intertidal *Z. marina* populations retracted (Wyllie-Echeverria *et al.* 2003). Presumably, the conditions experienced in the subtidal also affected intertidal plants, in that even less cold water could have reached them from the Strait of Juan de Fuca in the absence of large tidal fluctuations. In addition, the stable shallow water column under these conditions would experience more extreme temperature fluctuations due to solar radiation. The slow decline during the late 1990s and into the early 2000s was followed by the increase in tidal amplitudes associated with the shift in the 18.6-year nodal regime. It was also associated with yet another peak in sea surface and air temperatures (Fig. 4-3, 4-4). Plants which had already been in decline may have been challenged by increased exposure to both warm water and warm air temperatures (Fig. 4-3, 4-4, 4-5). The combination of chronic heat and more frequent and prolonged desiccation is a plausible scenario for the loss of intertidal *Z. marina* populations.

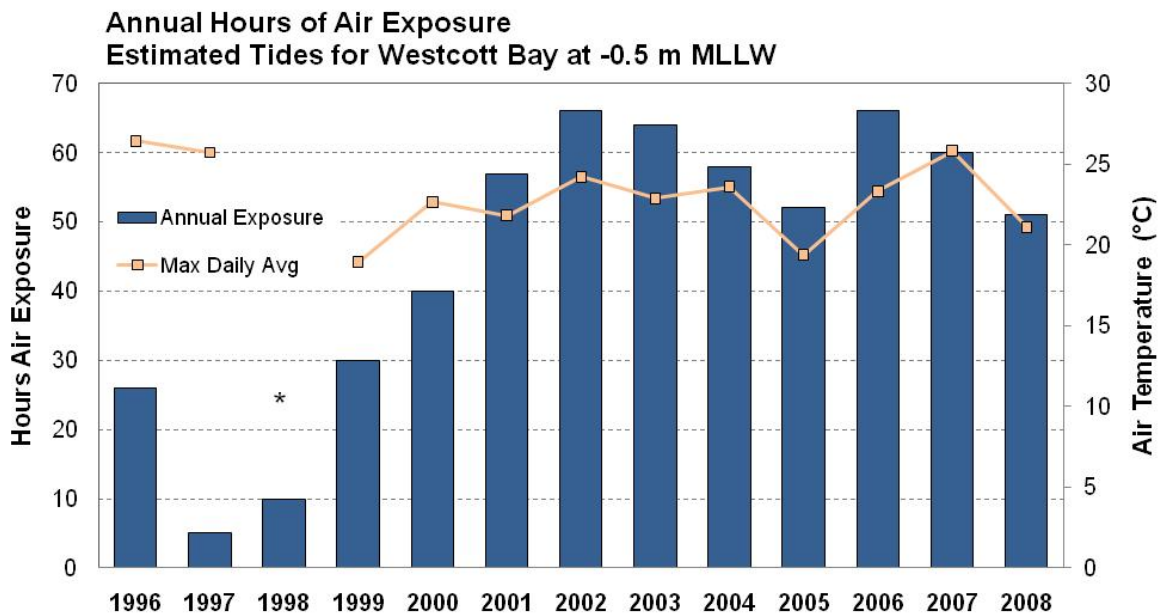


Fig. 4-4 Annual hours of exposure based on Westcott Bay estimated tides between 1996 and 2008 vs. maximum daily air temperatures. Temperature data are missing for 1998. Exposure follows the pattern of tidal extremes caused by the 18.6-year nodal tidal cycle.

Extreme tidal amplitudes and resulting air exposure time of *Z. marina* continued to increase through the period leading up to the crash of 2003 (Fig. 4-3, 4-4). This naturally brings with it an increase in turbidity when fine sediments are left unprotected by the loss of *Z. marina*. It is not unlikely that this resulted in further decline of subtidal populations, already stressed by heat, which were now subjected to poor water quality and the resultant loss of adequate insolation. The remnant population left at Bell Point after the 2003 crash has since retracted to a very narrow band present only in the subtidal area with the greatest influence of cold water from the Straits. Indeed, the only naturally-occurring populations

still left in Westcott Bay are in areas with the greatest influence of cold, clear water from the Strait of Juan de Fuca.

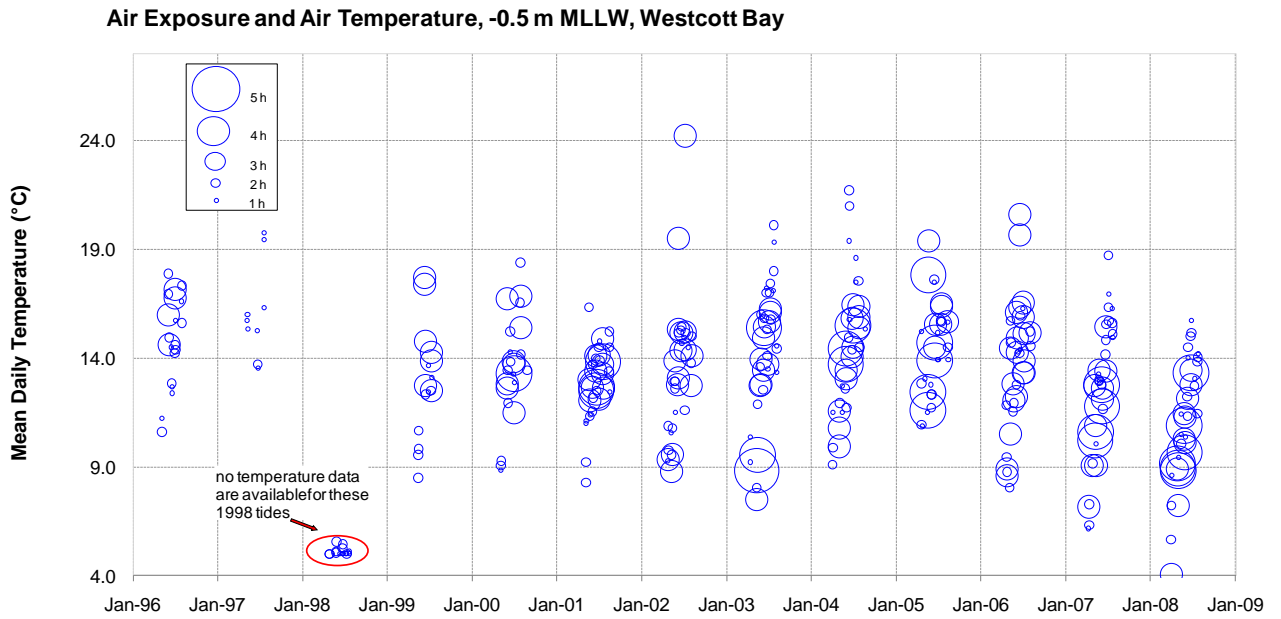
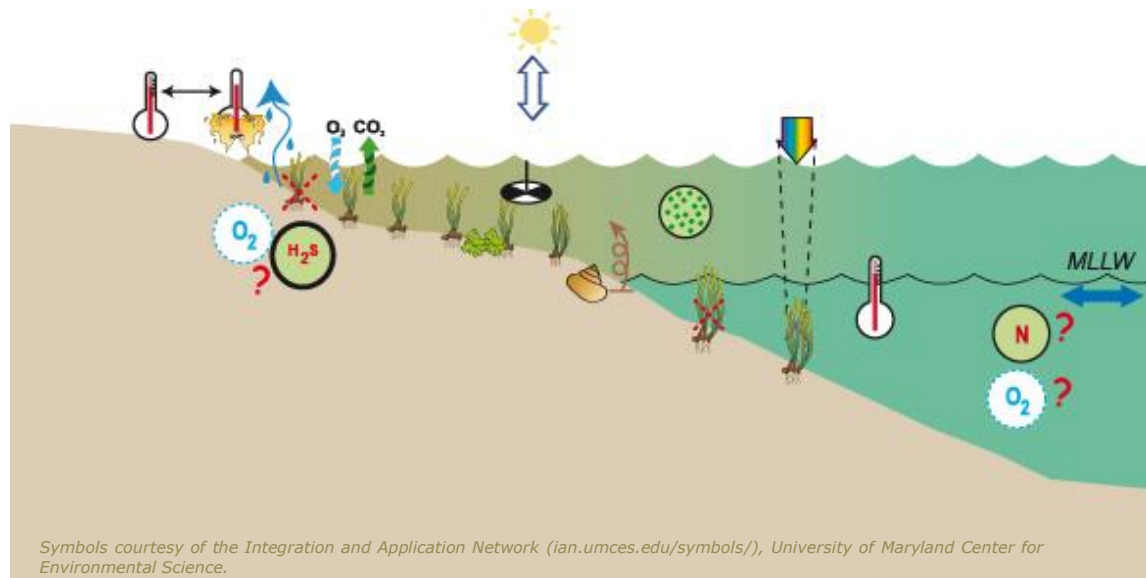


Fig. 4-5 Mean daily air temperature related to length of daytime air exposure (9am – 3pm PST) at -0.5 m MLLW in Westcott Bay. Source air temperatures: NOAA National Climatic Data Center. Global Summary of Day, Friday Harbor station. (<http://www7.ncdc.noaa.gov/CDO/cdo>).

4.6.4 Current Conceptual Model for Westcott Bay

The following conceptual model for Westcott Bay incorporates the findings of this study into the existing framework of initial hypotheses for *Z. marina* stressors in Westcott Bay (Table 1-1). It provides a summary of all potential *Z. marina* stressors identified, and states the current understanding and/or status of these stressors (where this information is available) based on DNR study findings in Westcott Bay between 2007 and 2008.



Synergy of potential *Z. marina* stressors in Westcott Bay

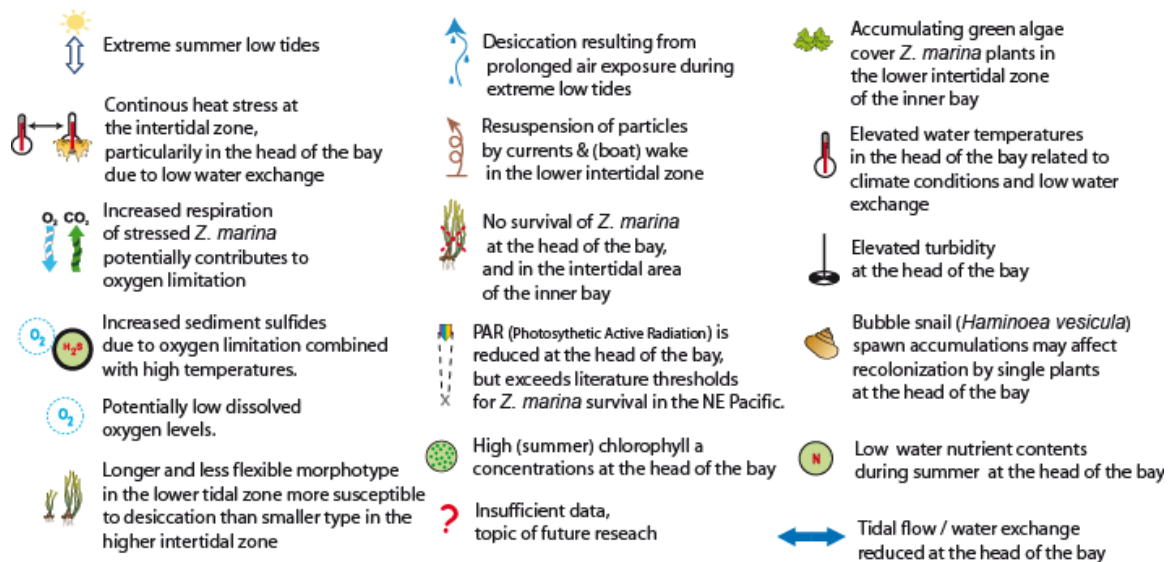


Fig. 4-6 Conceptual model showing synergistic effects of potential *Z. marina* stressors as identified for Westcott Bay. The Westcott Bay conceptual model updates the initial research questions and hypotheses reported in table 1-1 based on findings in this report. Human induced indirect stressors linked to these explicitly above are not explicitly considered.



5 CONCLUSIONS AND RECOMMENDATIONS

Based on our results it can be concluded that i) Current environmental conditions do not support *Z. marina* growth or survival at any tidal elevation at the head of Westcott Bay or at the intertidal area at Bell Point. The distribution of *Z. marina* across the sites in Westcott Bay as demonstrated in this study most likely represents the current extent of suitable habitat conditions in this area. ii) The potential for growth and survival of *Z. marina* transplants decreases along a spatial gradient from the entrance towards the inner Westcott Bay. iii) *Z. marina* survival and performance decreases on a vertical gradient from the shallow subtidal towards the lower intertidal zone. The fluctuations of growth and survival on both gradients are most probably due to a combination of unfavorable environmental factors (Table 5-1). Site-specific differences on both gradients in Westcott Bay are likely a consequence of the different magnitudes of impacts of individual factors (Table 5-2), which are dependent on site-specific characteristics in landscape structure (topography, bathymetry, hydrology, etc.). These environmental stressors are largely related, and impacts are probably increased by cascading effects during summer.

In respect of the performance of *Z. marina* transplants in Westcott Bay, a combination of different factors, including high water temperatures (daily maxima as well as mean values), in combination with green algae coverage and accumulation of snail spawn were identified to be stressful for *Z. marina*. Moreover, *Z. marina* transplants, particularly at the head and inner bay, might have suffered from high sediment sulfide concentrations during hypoxic or anoxic events in late summer and fall. Although light limitation was generally eliminated as a significant stressor (Dowty & Ferrier 2009), pulsed increases in turbidity and high sulfide contents, as have been hypothesized for the head of Westcott Bay, could have contributed to a decrease of the already-stressed *Z. marina* transplants.

In the intertidal zone, a significant stressor controlling *Z. marina* growth and survival in 2007 might have been desiccation in combination with heat stress as a result of air exposure during extreme spring and summer low tides.

Table 5-1 summarizes the conclusions of this study in order to address the initial hypotheses.

Table 5-1 Summary of study findings related to initially formulated hypotheses and related explanation

Objectives	Findings	Explanations
<p>A) To assess current habitat suitability for <i>Z. marina</i> development and survival in Westcott Bay (WB). <i>Z. marina</i> transplant experiment: H_A: Transplant performance decreases towards the inner bay at different tidal elevations during the main vegetation period in summer. H_0 [Null]: There is no effect of a spatial and tidal gradient on <i>Z. marina</i> transplant performance.</p>	<p>H_A supported by <i>Z. marina</i> performance</p> <p>Null hypothesis rejected</p>	<p><i>Z. marina</i> transplant performance indicated two gradients of habitat condition in WB. 1) A spatial gradient with decreasing performance from the mouth toward the inner and head of WB. 2) A vertical gradient with decreasing habitat condition in the inner and bay from the subtidal toward the intertidal area.</p>
<p>B) To evaluate the role of air exposure in <i>Z. marina</i> performance. <u>Correlation:</u> Transplant performance with air exposure. H_B: <i>Z. marina</i> transplant performance decreases with increasing time of air exposure. H_0 [Null]: There is no relation between <i>Z. marina</i> performance and air exposure time.</p>	<p>Strong negative correlation</p> <p>Null hypothesis rejected</p>	<p>1) Prolonged air exposure during extreme spring and summer low tides contributed to a decrease in <i>Z. marina</i> density in the lower intertidal area at the inner and the head of WB in summer 2007, and at the inner bay in summer 2008. In contrast, plants that were only briefly exposed to air in summer 2008 remained stable at first, but were lost by November. 2) Prolonged air exposure most likely affected <i>Z. marina</i> plants either by desiccation stress or by a combined effect of desiccation and heat stress resulting from warm air/sediment temperatures during exposure events. 3) It is assumed that a different morphotype of <i>Z. marina</i> in the lower intertidal (stiff in plant sheath) is more susceptible to prolonged desiccation stress than the more flexible morphotype in the higher intertidal area. 4) The relationship of <i>Z. marina</i> performance (and higher vulnerability of a different morphotype in the lower intertidal to desiccation stress) is demonstrated in the literature (see air exposure text).</p>
<p>C) To evaluate the role of water temperature in transplant performance along a gradient of increasing temperatures toward the head of the bay . <u>Correlation:</u> Transplant performance with water temperature. H_C: Decreasing performance of <i>Z. marina</i> is related to increasing water temperatures toward the head of WB. H_0 [Null]: There is no relation between increasing water temperature and <i>Z. marina</i> performance.</p>	<p>Moderate negative correlation</p> <p>Null hypothesis rejected</p>	<p>1) Decreases in <i>Z. marina</i> performance coincided with consistently higher water temperatures recorded at the head of WB at all tidal elevations during two consecutive years (2007 and 2008). 2) Monthly performance of <i>Z. marina</i> transplants showed a moderate negative correlation of shoot density with increasing water temperature. 3) Water temperatures measured at the head of the bay exceeded optimal values and reached critical values for <i>Z. marina</i> performance as reported in the literature.</p>

Table 5-2 Summary of a suite of stressors resulting in *Z. marina* loss in Westcott Bay, San Juan Island Archipelago, USA.

Tidal level	Lower intertidal area (-0.7m)			Transition area (-0.9 m MLLW)			Subtidal area (-1.5 m MLLW)		
Stressor/ Site	Bay entrance	Inner bay	Bay head	Bay entrance	Inner bay	Bay head	Bay entrance	Inner bay	Bay head
Air exposure ^a									
Daily water temperature ^b									
T _{mean}									
T _{max}									
Algal cover ^c									
Bubble snail spawn accumulation ^c									
<i>Z. marina</i> performance ^d May through Jul./Nov.									
Final status									
Interpretation	Prolonged air exposure periods along with high T _{max} and potential light limiting factors, such as algal cover and snail spawn accumulation result in <i>Z. marina</i> loss.			High T _{mean} along with long periods of moderate T _{max} and potential light limiting factors, such as snail spawn accumulation result in <i>Z. marina</i> loss.			High T _{mean} along with prolonged periods of moderate T _{max} and potential light limiting factors, such as snail spawn accumulation result in <i>Z. marina</i> loss.		
Additional potential stressors related to those above	none	Sulfide toxicity as a result of anoxia related to high temperatures.		none	Sulfide toxicity as a result of anoxia related to decomposing algac.	Sulfide toxicity as a result of anoxia related to high temperatures and light limiting bubble snail spawn.	none	none	Sulfide toxicity as a result of anoxia related to high temperatures and light limiting bubble snail spawn.

Note Table 5-2

^a Air exposure classifications in the lower intertidal area are representing air exposure observations in 2007; classifications in the transition and subtidal area are based on 2008 observations.

^b Water temperature characteristics are rough estimates based on Westcott Bay observations in 2007 and 2008.

Daily mean temperatures: high: ≥ 15 °C, moderate: >12 °C <15 °C, low: ≤ 12 °C

Daily max. temperatures: high: ≥ 20 °C, moderate: ≥ 15 °C <20 °C, low: <15 °C

* 'Moderate' daily T_{\max} water temperatures were distinctly higher relative to other sites, and exceeded daily T_{\max} temperatures above 15 °C (range from 15 °C to 20.2 °C) in the transition zone for more than 45 days, and in the subtidal area for about 34 days.

^c Classifications in algal cover and bubble snail spawn in the intertidal area are based on 2007 and 2008 observations, and in the transition and subtidal area on 2008 observations.

^d Performance of *Z. marina* in the intertidal area is based on shoot densities from May through July 2007 and 2008; in the transition and subtidal area performance is based on shoot densities from May through November 2008. The final status of performance at all tidal elevations is recorded in May 2009.

In summary, transplant performance in relation to environmental characteristics recorded in Westcott Bay suggests that a suite of stressors (rather than a single stressor), depending on site-specific environmental differences, affect the growth and survival of *Z. marina*. Site-specific stressor expressions in the context of transplant performance are summarized in Table 4-3.

Furthermore, it appears likely that the discussed combination of stressors also contribute to explain *Z. marina* losses in other shallow embayments in the San Juan Island Archipelago and greater Puget Sound with comparable environmental characteristics.

Recommendations and Applications

The ES-RP investigates observed *Z. marina* losses in Puget Sound in order to identify and understand the nature of stressors that lead to declines of *Z. marina* beds. A key emphasis of the project is to deliver information to resource managers and decision makers that will guide management actions to protect and restore this ecologically and economically valuable habitat. Current research of the ES-RP focuses on the San Juan Island Archipelago, where the most prevalent *Z. marina* losses in shallow embayments are assessed in the frame of a comprehensive case study in Westcott Bay.

Our findings indicate that *Z. marina* distribution in Westcott Bay most likely represents the current extent of suitable habitat in the area. The study clearly demonstrates that environmental conditions at the head of Westcott Bay and in the lower intertidal area at Bell Point are currently not suitable for growth of *Z. marina*. Thus, from a management perspective, efforts to restore *Z. marina* in Westcott Bay, and perhaps other shallow embayments with comparable environmental conditions where *Z. marina* losses have been observed, is currently not recommended. As demonstrated in this study, “test” transplantations in areas with observed seagrass losses can help to determine current habitat suitability prior to restoration efforts, thereby minimizing the costs due to potential restoration failure.

Besides the combination of factors discussed within this study, we identified important knowledge gaps in *Z. marina* ecology that should be the focus of further research. The

mechanisms behind how different factors affect *Z. marina* must be addressed in future studies in order to qualitatively and quantitatively understand the importance of individual stressors and their interactions in Puget Sound. Another important step for future work includes investigation of cascading effects caused by increasing temperature and/or eutrophication, such as events of anoxia and high sediment sulfides that probably decrease habitat condition for *Z. marina*. We recommend continuing efforts to further identify and understand the contributions of individual stressors in relation, but not limited to, elevated water temperatures in Westcott Bay. Future work will benefit from the enhanced understanding of complex site-specific differences and physical processes in Westcott Bay, as well as long term data and stressor interactions identified by the Westcott Bay case study since 2007.

Hood Canal, another area of concern where distinct losses of *Z. marina* have been observed (Gaeckle *et al.* 2008, Gaeckle *et al.* 2009), is known to experience periodic warm water temperatures and anoxic events. There is a promising potential to apply our study findings and assess *Z. marina* losses in Hood Canal in relation to the identified suite of environmental conditions including warm water temperatures, anoxic events, and sediment sulfides. However, the case study in Westcott Bay would be valuable to maintain, considering the extent of data collected in the bay, and further investigation on environmental stressors can profit from long term data and knowledge of seasonal and inter-annual variation.

Findings of this study identified the potential importance of elevated sediment sulfide concentrations in Westcott Bay. We hypothesize that elevated water temperatures (and the resulting increase in *Z. marina* respiration) in conjunction with low oxygen concentrations may preclude successful growth and survival of *Z. marina* in Westcott Bay in the presence of high sediment sulfide concentrations. Thus, it would be a straightforward process to investigate the driving mechanisms based on our previous observations in the field.

Other site specific environmental factors, such as elevated water column nutrient concentrations (that result in deteriorating light conditions due to phytoplankton blooms as well as accumulation of macroalgae) may amplify the effect of the above described processes by increasing organic matter that further increases the system respiration and thus contributes to lower oxygen levels in the water column and sediment. However, it is hypothesized that limited nutrient supply at the head of Westcott Bay prevents the re-establishment and growth of *Z. marina*. Thus, we recommend further characterization of water column nutrient concentrations in Westcott Bay in order to better understand nutrient levels and related plant response across the sites.

Moreover, based on the findings of this study it is recommended that the carbohydrate energy reserves in the root and rhizome systems be investigated further in order to better understand the mechanisms and causes of observed *Z. marina* mortality. Under stressful environmental conditions, such as elevated temperatures and high sulfide concentrations, respiration increases and this in turn increases the demand for light, perhaps exceeding reported literature thresholds (Thom *et al.* 2008). As a result, the production to respiration ratio of the plant decreases, and the plant must mobilize its energy reserves, leading to an early depletion of reserves and eventual loss of the plant.

In quiescent shallow embayments such as at the head of Westcott Bay, the effect of the identified suite of stressors and related processes may be amplified by extreme climatic events, such as extreme low tides and warm water temperatures resulting from extreme tidal amplitudes within the 18.6 year tidal node epoch in the Northeast Pacific, climatic variability due to the El Niño Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO) as well as predicted climate change. The greatest impact of climate change on seagrass systems is expected to result from elevated water temperatures, initially during extreme events, but eventually in a chronic manner (Waycott *et al.* 2007). Climate change has been recently identified and classified as a very high threat in marine waters in Puget Sound (WA) by the Puget Sound Partnership (Neuman *et al.* 2009). We recommend that future research on *Z. marina* stressors and restoration in Puget Sound include potential impacts of climate change (e.g, rise in water temperature, extreme tidal amplitudes, increased turbidity due to higher storm frequency, etc.) and resulting biological and physical processes.

Research Priorities Include:

- Assess observed *Z. marina* losses in areas of concern in Puget Sound to fulfill the mandate and the goals identified by WDNR and the Puget Sound Partnership
- Analyze the SVMP/ES-RP monitoring data recorded in Westcott Bay and other shallow embayments in the San Juan Island Archipelago in 2008 and 2009 and report potential changes and trends between years
- Assess the carbohydrate contents in root and rhizome tissues of *Z. marina* plants from Westcott Bay in order to identify any depletion of the plant energy reserves and to better understand causes of *Z. marina* losses (e.g. carbohydrates remain high, eliminating slow acting (chronic) stressors and highlighting quick acting (acute) stressors)
- Analyze water column nutrient data in Westcott Bay in order to characterize nutrient variability along a spatial scale from the entrance to the head of the bay
- Assess the effect of sediment sulfides and increasing water temperature on *Z. marina* survival in Westcott Bay in field and tank experiments
- Analyze oxygen data recorded in Westcott Bay (2007 - 2009) in order to identify hypoxic or anoxic events
- Analyze 2009 PAR data (YSI and Odyssey light logger) with focus on light availability in late summer and fall

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7 APPENDIX

APPENDIX A: FILTERING TEMPERATURE DATA

In order to estimate water temperatures and air exposure times temperature data were filtered, and water temperatures were separated from those temperatures that were recorded during air exposure of the data logger (by Hannah Julich):

Tide predictions are based on the WB correction by Pete Dowty:
 $(RH \text{ Pred} + HP \text{ Pred})/2 + (FH \text{ Obs} - FH \text{ Pred}) = WB \text{ Estimation}$

Temperatures were recorded using HOBO Tidbits logging at 15-minute intervals. HOBO Tidbits have a precision of $\pm 0.2^\circ\text{C}$; any change smaller than this is assumed to be undetectable. This level of precision is suggested by the manufacturer and has been confirmed in laboratory and field experiments by Julich and Becker (unpublished data).

Exposure was initially estimated using the WB tide predictions based on the above formula. However, questions about bathymetry at individual sites and personal field observations led to site-specific estimations based on tide predictions, temperature records, and field observations. Raw data was first filtered by the tide column to show only tides estimated to be equal to or less than -70cm. These were highlighted in gray.

Next, a "Difference" column was added next to each site's HOBO temperature data. The difference between each point and the one before was calculated to give an estimation of any change in temperature over the previous 15-minute period. Each "Difference" column was filtered individually to show only points that were greater than or equal to 0.4°C to show increasing temperatures. These were highlighted in yellow. The same was done for points that were less than or equal to -0.4°C ; these were highlighted in blue to show decreasing temperatures.

Next, the data were examined for patterns in changing temperatures that might indicate air exposure. Sharp increases in temperature over the previous 15-minute period (generally greater than $0.5 - 0.8^\circ\text{C}$) in coincidence with a sufficiently low tide cycle were examined closer. Data were tagged as air temperatures (in gold) if the temperature increases agreed generally with tide predictions and demonstrated a subjectively strong pattern of increase, followed by a similarly strong pattern of decreasing temperatures. In cases where there was some dispute, field observations were employed to confirm or negate entire exposure cycles or individual points. Estimations of exposure are conservative; if no agreement could be reached about whether to include a point, or if the pattern of increase or decrease was not clear, then the point was excluded. This decision was based on the need to avoid including temperatures that may have been indicative of a thin layer of water still present over the plants, as desiccation stress was the main focus of the exercise.

Potential limitations of this method include both instrumental and human error, as well as difficulties with sampling interval and logger placement. It is known that some Tidbits did not rest directly on the sediment, but were suspended a few centimeters off of the bottom; this could cause small, but potentially significant, errors in temperature records. Furthermore, using subjective measures to delineate tidal shifts inevitably introduces an element of human bias, although this was remedied as much as possible throughout processing. Finally, the 15-minute sampling interval used for temperature measurements and tidal estimations may introduce an element of error. For example, it is known that toward the end of May 2008, plants were briefly exposed at the head of the bay. However, the 15-minute sampling interval is not sensitive enough to record this exposure. Whether the loss of sensitivity when switching from a 6-minute interval to a 15-minute interval is significant is unknown; it has been suggested that this loss is statistically negligible.