Effect of field exposure to 38-year old residual petroleum hydrocarbons on growth, condition index, and filtration rate of the ribbed mussel, *Geukensia demissa*

Jennifer B. Culbertson*[1,2], Ivan Valiela[1,4], Ylva S. Olsen[1], and Christopher M. Reddy[2]

[1] Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA, USA

[2] Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

[4] Ecosystems Center, Marine Biological Laboratory, Woods Hole MA 02543

* Corresponding author: J.B. Culbertson, MBL, 7 MBL Street, Woods Hole, MA 02543, jbculber@bu.edu

Abstract

In September 1969, the *Florida* barge spilled 700,000 L of No. 2 fuel oil into the salt marsh sediments of Wild Harbor, MA. Today a substantial amount, approximately 100 kg, of moderately degraded petroleum remains within the sediment and along eroding creek banks. The ribbed mussels, *Geukensia demissa*, which inhabit the salt

marsh creek bank, are exposed to the spilled oil. Examination of short-term exposure was done with transplantation of *G. demissa* from a control site, Great Sippewissett marsh, into Wild Harbor. We examined the effects of long-term exposure with transplantation of mussels from Wild Harbor into Great Sippewissett. Both the short- and long-term exposure transplants exhibited slower growth rates, shorter mean shell lengths, lower condition indices, and decreased filtration rates. Our results add new knowledge about long-term consequences of spilled oil, a dimension that should be included when assessing oil-impacted areas and developing management plans designed to restore, rehabilitate, or replace impacted areas.

Capsule

Ribbed mussels exposed to residual petroleum in salt marsh sediments, in short-term and long-term transplant experiments, exhibited significant effects that should be considered when assessing oil-impacted areas and developing management plans.

Keywords: Florida; oil pollution; petroleum hydrocarbons; *Geukensia demissa*; salt marsh

1. Introduction

The lingering effect on coastal ecosystems following long-term exposure to petroleum hydrocarbons remains one of the largest unknowns (Burns and Teal, 1971; Sanders et al., 1972; Sanders et al., 1980; Teal et al., 1992, NRC 2003, Peterson et al., 2003). An opportunity to examine the long-term effect of residual spilled petroleum

presents itself in Wild Harbor, MA, where oil spilled in 1969 still remains buried in marsh sediment (Reddy et al., 2002; Peacock et al., 2005).

In September 1969, the barge *Florida* ran aground in Buzzards Bay, Massachusetts (Fig. 1) and spilled 700,000 L of No. 2 fuel oil. Spilled oil entered Wild Harbor and impacted the salt marsh ecosystem (Blumer and Sass 1972a, b; Blumer et al., 1970; Burns and Teal, 1971; Sanders et al., 1972; Sanders et al., 1980; Teal et al., 1992). Studies over the past 38 years have shown the continued presence and effects of residual oil in Wild Harbor marsh sediments (Frysinger et al., 2003; Peacock et al., 2005; Reddy et al., 2002; Sanders et al., 1972; Slater et al., 2005; Teal et al., 1978; Teal et al., 1992; White et al., 2005a, b; Culbertson et al., 2007a, b). Recent work found that a substantial residue of the No. 2 fuel oil was still present 8 to 20 cm below the salt marsh surface across Wild Harbor (Frysinger et al., 2003; Reddy et al., 2002; Slater et al., 2005; White et al., 2005a, b; Peacock et al., 2005) and that the surface in heavily oiled locations is eroding (Culbertson et al., 2007b).

The concentrations of residual total petroleum hydrocarbons (TPHs) within the 8 to 20 cm layer are in roughly the same range as those measured in 1973 in surface sediments (Teal et al., 1978; Teal et al., 1992; Reddy et al., 2002; Peacock et al., 2005). The petroleum hydrocarbons present at depth are moderately degraded: volatile and water-soluble compounds and *n*-alkanes have been removed, concentrations of acyclic isoprenoids have been reduced, but alkyl cyclohexanes, alkylbenzenes, and polycyclic aromatic hydrocarbons (PAHs) as well as many other unidentified hydrocarbons still persist (Reddy et al., 2002; Frysinger et al., 2003; White et al., 2005b). Total PAHs

(mostly alkylated naphthalenes and phenanthrenes/anthracenes) in a core collected in 2000 were 134 μg g^{-1} in the 14-16 cm horizon (White et al., 2005b). This current PAH content is 40x greater than thresholds thought to be associated with the incidence of biological effects (Long et al., 1995), assuming that the biological response for parent and alkylated PAHs is the same. Furthermore, the PAHs found in these sediments are characteristic of PAHs shown to chronically persist within bivalve tissues (Boehm and Quinn, 1977; Stegeman 1981; Farrington et al., 1982; Booth et al., 2007).

Among the many species present in Wild Harbor we have selected marsh grasses, fiddler crabs, and ribbed mussels as biota that, owing to their biology, are exposed to the buried oil residue. We reported on grasses and crabs in earlier papers (Culbertson et al., 2007a, b); here we deal *G. demissa*, the ribbed mussel. Ribbed mussels, occur in dense assemblages within salt marshes over the span of the intertidal zone along the Atlantic coast of North America and into the Gulf of Mexico (Brousseau, 1984; Franz, 1996, 1997). *G. demissa* is a keystone species in salt marsh ecosystems due to high rates of production and large biomass and their active processing of particulate carbon and nitrogen (Kuenzler, 1961 a, b; Jordan and Valiela, 1982; Bertness 1984; Evgenidou and Valiela, 2002; Chintala et al., 2006). The abundance of ribbed mussels in these coastal ecosystems, their sessile nature, tolerance to environmental contaminants, suspension feeding, and ease to transplant make them useful organisms for pollution monitoring (Widdows and Donkin, 1992; Sericano et al., 1995).

Many studies have used mussels as potential bioindicators for PAHs (Widdows and Donkin 1992; Capuzzo 1996; Babcock et al., 1996, 1998; Axelman et al., 1999;

Baumard et al., 1999; Carls et al., 2004; Page et al., 2005). Through filtering large quantities of water, mussels are exposed to lipophilic PAHs, which are known to bioconcentrate by 2-5 orders of magnitude within mussel tissue (Neff 2002). This exposure to hydrocarbons can decrease population (Peteiro et al., 2006) and individual growth rates (Stromgren et al., 1986; LeFloch et al., 2003), lower condition indices (Bayne and Worrall, 1980), and lower feeding rates (clearance rates) (Widdows et al., 1981; Widdows et al., 1987; Widdows et al., 1996)..

Unambiguous definition of the effects of a contaminant in the field is possible by means of transplant experiments (Thiesen 1982; Widdows et al., 1984; Chapman 1986; Myrand and Gaudreault 1995; Hoonkoop et al., 2003). *Mytilus edulis* transferred from a clean to an oiled area and then back to a clean area were able to depurate 90% of aromatics from their tissues (DiSalvo et al., 1975). When *M. edulis* originating from the oiled site were placed into the clean site only 50-60% of the aromatics were depurated. Therefore, chronic exposure of mussels to petroleum may irreversibly impair the ability for depuration of petroleum hydrocarbons.

A reciprocal transplant of mussels between polluted and non-polluted sites allows answering two additional questions: is there any effect of long-term chronic exposure versus short–term exposure and is the recovery of the mussels significant in the non-polluted environment. The use of mussel transplant experiments has been critical in the examination of effects from petroleum contamination in coastal environments (Widdows et al., 1981; Cheung et al., 2001; Honkoop et al., 2003; Peteiro et al., 2006).

A reciprocal transplantation experiment, coupled to measurement of responses, can be useful to determine whether there are long- or short-term effects on growth, health and filtration rates of *G. demissa* following exposure to residual petroleum. Here we report whether residual oil in Wild Harbor sediments, after being in situ for almost four decades, still affects population size classes and growth, individual growth rates, condition indices, and filtration rates of *Geukensia demissa* found in Wild Harbor.

2. Methods

2.1 Study and control sites

Geukensia demissa from Wild Harbor (WH) and Great Sippewissett (GS) salt marsh were collected for both the field and laboratory experiments. The latter is a neighboring salt marsh that has similar flora, fauna, sediments, and geological history (Krebs and Burns 1977). In addition, using routine methods (Reddy et al., 2002), we found no detectable oil residues in sediment cores collected at this site in 2006. In this study and as in the past, we assume that most of the hydrocarbons in our operationally defined TPHs are from the *Florida* spill and consist mainly of an unresolved complex mixture (UCM) in the boiling range from *n*-C_{10} to *n*-C_{26} alkanes. Our estimated method detection limit is 0.015 mg of TPH g^{-1} of dry sediment (Reddy et al., 2002). Mussel tissues from Wild Harbor and GSM were analyzed for parent and alkylated PAHs by Alpha Woods Hole Laboratories using standards tissue extraction and gas chromatography mass spectrometry analysis.

2.2 Field measurements

To assess sizes and growth of *G. demissa*, we studied mussels along creek banks of Wild Harbor, where oil is still present, and Great Sippewissett salt marsh, our reference site, in the summer of 2003 (Fig. 1). We measured shell lengths of all *G. demissa* found in 0.25 m^2 quadrats within four representative transects along the creek bank (> 45 cm below Mean High Tide) in Wild Harbor (*n*=284) and Great Sippewissett (*n*=163) marshes. The total number of quadrats sampled differed due to slight elevational differences between the two marshes, therefore results are reported as percent of total observations.

To measure growth of mussels in the quadrats, we took advantage of the presence of annual growth layers in the shells of ribbed mussels, that can be used to determine the age of the mussel (Lutz and Castagna, 1980; Evgenidou and Valiela, 2002). At each site, 50-75 mussels of different size classes were collected and brought to the laboratory. Soft tissue was removed and the shells cleaned. Annual growth lines in the cross section of the shells near the umbo were counted under a dissecting microscope.

2.3 Transplant experiments

To assess the effect of exposure to petroleum residues, we conducted a transplant experiment. The manipulation involved moving mussels of three size classes (40-49 mm, 50-59 mm, and 60-69 mm) reciprocally between Wild Harbor and Great Sippewissett marshes (*n*=180) (Table 1). Fifteen mussels (5 from each size class) were randomly placed into metal cages covered with 0.5-cm pore size plastic mesh. Each location (Wild

Harbor and Great Sippewissett) received three replicate cages with either mussels from within the same location or with mussels from the other location. These reciprocal transplants were placed in the field in July 2005 and were brought into the lab in late October 2006. The mussels from the transplants were then used to obtain data on growth, condition index, and filtration rate, as a way to assess possible impacts of chronic and short-term exposure to residual petroleum.

2.3.1. Growth measurements

To estimate the increase in shell length, and hence estimate growth during the experiment, we painted a number on the edge of the shell of each mussel with red enamel, before placing the mussel into a cage as outlined previously. The painted number allowed us to identify the mussel at the end of the experiment, and then mussel growth over the period of transplantation was determined as the difference between the initial and final shell length. The shell length was measured as the longest distance from the umbo to the shell edge.

2.3.2. Condition index measurements

To examine the condition index of mussels in each treatment of the transplant experiment we dissected and weighed every surviving mussel used in the transplant experiment. The mussels were weighed for total weight wet and then tissue was separated from the shell and shell weight was recorded. All tissues were dried at 60° C for 3-5 days. Condition index (CI) of each mussel was calculated as the tissue dry weight/shell weight x 100 (Shriver et al., 2002).

2.3.3. Filtration rate measurements

To measure the filtration rate of mussels in each treatment of the transplant experiment, we placed a mussel within a 2-L glass beaker with 1-L of seawater containing an abundant amount of phytoplankton. The water in each jar was aerated to ensure oxygen saturation throughout the experiment. Salinity and temperature were measured in each experiment to ensure no differences between jars. As soon as each mussel started to filter, a 120-mL water sample was removed (t=0). Water samples were then taken at t = 5, 10, 20 and 30 minutes, and placed on ice in the dark until filtered on GF/F Whatman 47 mm 0.7 μM glass fiber filters. Chlorophyll *a* concentration was determined according to Lorenzen (1967) by acetone extraction and fluorometry. Filtration rates were determined according to Kraak et al. (1997) using initial and final chlorophyll *a* concentrations. This procedure was repeated for two mussels from each size class within each cage (18 mussels per treatment).

2.4 Data analysis

Chi-square tests were used to test the hypotheses related to differences in population shell lengths (Fig. 2), analysis of covariance (ANCOVA) was used to test the hypotheses related to differences in population growth rates (Fig. 3), and analysis of variance (ANOVA) along with the post-hoc Tukey's test for the differences between means of each group (Table 2, Figs. 4, 5, and 6) (SPSS, v. 11.0.4). All data were examined to meet the underlying assumptions. F_s indicates the F-statistic from the ANCOVA analysis. In all figures, a single asterisk indicates a probability of < 0.05, a

double asterisk indicates a probability of < 0.01, and a triple asterisk indicates a probability of < 0.001.

3. Results

3.1 Study and control sites

We have found no other potential pollutant that might confound the interpretation we offer here. The most evident contrast is the presence of the oil residue in one marsh but not the other. White et al. (2005) found that the C2-phenanthrenes were the abundant PAHs in sediment cores collected in Wild Harbor in 2000. Wild Harbor mussel tissue contained significantly higher content of C2-phenanthrenes than found in Great Sippewissett mussel tissue (t-test, t-stat=2.89, $P<0.05$). While these concentrations of all PAHs were only 2 to 10 times above the method detection limit, the Wild Harbor sample did contain slightly more PAHs. The PAH concentrations found in mussels at Wild Harbor are similar to those found in other studies examining the uptake of PAH compounds from industrial sites (Francioni et al., 2007).

3.2. Field measurements

The frequency distribution of ribbed mussel lengths for Great Sippewissett and Wild Harbor marshes differed significantly (Fig. 2), with larger sizes more frequent in Great Sippewissett than in Wild Harbor. However, mussel density was not significantly different in Wild Harbor (mean ± s.e. = 200.44 ± 1.36 m^{-2}) than in Great Sippewissett

(mean ± s.e. = 196.0 ± 4.00 m^{-2}). Therefore, the difference in size frequency may not be attributed to the number of mussels per m^{-2}.

To discern whether the different sizes were a result of different growth rates, we determined the age of Great Sippewissett and Wild Harbor mussels, and then estimated growth rates per year (Fig. 3). Growth rates during initial years were similar, but growth of mussel populations in Great Sippewissett (mean ± s.e. = 6.7 ± 0.26 mm yr^{-1}) and Wild Harbor (mean ± s.e. = 7.6 ± 0.41 mm yr^{-1}) became significantly different in mussels >7 years of age (*t*-stat = -2.38, $p < 0.01$, Fig. 3). This implies that differences in frequency distribution seen in Fig. 2 were a result of differential growth rather than a result of different recruitment and survival between the two marshes.

The growth of ribbed mussels was fastest during earlier years and tapered off through the later years (Fig. 3). This is typical of this species (Evgenidou and Valiela, 2002). In fact, the rates of growth of Great Sippewissett ribbed mussels were similar to those from an earlier study in Waquoit Bay, Massachusetts (Evgenidou and Valiela, 2002) and in Jamaica Bay, New York (Franz 1997) with growth rates in the smaller size classes of 7-9 mm $year^{-1}$, in the middle size classes of 4-6 mm $year^{-1}$, and in the largest size classes of 1-2 mm $year^{-1}$.

3.3. Transplant experiments

3.3.1. Growth measurements

Direct growth rates measured over the course of the transplantion period differed significantly only in the smaller ribbed mussels (< 50 mm length) (Table 2, Fig. 4). The

lower growth rates in larger mussels may have led to the apparant lack of significant differences in the two larger size classes. Growth of Wild Harbor mussels transplanted to Great Sippewissett reached rates similar to those of Great Sippewisett mussels that remained within Great Sippewissett. This suggests that decadal-level effects on growth were not significant and that short-term oil exposure led to the treatment effect seen in growth of younger mussels.

3.3.2. Condition index measurements

CI differed significantly in mussels reciprocally transplanted into the oiled and non-oiled sites (Table 2, Fig. 5). Mussels transplanted into the oiled Wild Harbor site from both Wild Harbor and Great Sippewissett had lower CI than mussels transplanted into Great Sippewissett marsh (Tukey's, $P < 0.001$). Further, mussels transplanted from Wild Harbor into the non-oiled Great Sippewissett marsh showed a significant increase in CI over mussels from Wild Harbor that remained within Wild Harbor (Tukey's, $P < 0.05$). There were no significant differences in CI among different size classes of mussels (Table 2), so we pooled all size classes in Fig. 5. These results suggest an effect of the oil in Wild Harbor on the condition index of ribbed mussels transplanted there from Great Sippewissett (short-term) and from Wild Harbor (long-term).

The CI of mussels transplanted from Wild Harbor into Great Sippewissett marsh was significantly larger but their CI was significantly lower than those collected from Great Sippewissett and placed in Great Sippewissett (Tukey's, $P < 0.01$). Even after one year of potential depuration, the CI of mussels from Wild Harbor still appear lower,

affected by the residual oil (Fig. 5). This suggests that chronic decadal-scale effects on CI were significant.

3.3.3. Filtration rate measurements

The filtration rates of ribbed mussels differed significantly in mussels reciprocally transplanted into the oiled and non-oiled sites (Fig. 6, Table 2). Filtration rates in mussels transplanted into the oiled site from Wild Harbor and from Great Sippewissett were lower than those of mussels transplanted into Great Sippewissett marsh (Tukey's, $P < 0.05$). Further, mussels transplanted from Wild Harbor into the non-oiled Great Sippewissett marsh show a significant increase in filtration rates over mussels from Wild Harbor that remained within Wild Harbor (Tukey's, $P < 0.05$). There were no differences in filtration rate among size classes (Table 2), so we pooled size classes in Fig. 6. These results suggest an effect of the oil in Wild Harbor on the filtration rate of ribbed mussels transplanted there from Great Sippewissett (short-term) and from Wild Harbor (long-term).

Filtration rates of mussels transplanted from Wild Harbor into Great Sippewissett marsh had lower filtration rates than mussels from Great Sippewissett placed back into Great Sippewissett. Even after one year of possible depuration, the mussels from Wild Harbor still appeared somewhat affected by the residual oil (Fig. 6).

4. Discussion

Petroleum residue still present almost forty years after the *Florida* spill continues to have biological effects. Long-term exposure to petroleum may irreversibly impair the

ability for depuration of petroleum hydrocarbons in bivalves (DiSalvo et al., 1975; Boehm and Quinn, 1977; Farrington et al., 1982; Black et al., 1983; Carls et al., 2004). In our study, oil exposure made for shorter mean shell lengths, slower growth rates, lower condition indices, and decreased filtration rates for all mussels regardless of origin. These effects were similar to those reported in previous studies of mussels exposed to petroleum that found a depression in feeding rates (Gilfillan 1975; Widdows et al., 1982; Widdows et al., 1985), growth rates (Keck et al., 1978; Stekoll et al., 1980; LeFloch et al., 2003), and condition indices (Peteiro et al., 2006). LeFloch et al. (2003) found an approximate 50% decrease in shell length growth in *Mytilus edulis* exposed to crude oil over a 10-month study period, comparable to results seen here with small size class *G. demissa*.

The use of reciprocal transplants in our study helped define decadal-scale effects (Wild Harbor mussels transplanted to Great Sippewissett and within Wild Harbor) and shorter-term (annual) effects (Great Sippewissett mussels transplanted to Wild Harbor). Mussels transplanted into Great Sippewissett showed some recovery with increased growth, in the small size class, and filtration rates. The response of Wild Harbor mussels was, however, often less marked than those of Great Sippewissett mussels, indicating persistent decadal effects on mussel health. Mussels from Wild Harbor transplanted into Wild Harbor, exhibited lower growth rates, lower CI, and decreased filtration rates, indicating long-term exposure does not confer resistance to petroleum. On a short-term scale, mussels from Great Sippewissett placed into Wild Harbor exhibited decreased growth rates, condition index, and filtration rates indicating that short-term exposure (~1 year) to the oil significantly effects mussel health. The results of lingering effects of the

residual oil following long-term (decadal) and short-term (annual) exposure are similar to those reported in a previous transplant study (DiSalvo et al., 1975).

The burial of the spilled oil does not necessarily reduce exposure. The impacts of the petroleum on salt marsh vegetation have led to increased erosion of the oiled sediments, which has led to exposure of the mussels to the residual petroleum. The results presented here show that the buried petroleum is still biologically active, and does have harmful long-term effects on populations that are exposed to the contaminated layer, such as *G. demissa*. Further, these results demonstrate that population growth rates, size structure, condition index and filtration rates of *G. demissa* may be used as successful bioindicators of environmental contaminants.

Recovery from oil spills depends on the species, on the post-spill history, and on characteristics of the specific environment (Baker 1999). In anoxic accreting salt marshes that have been exposed to spilled oil the residual undegraded biologically active oil may last for many decades with the potential for chronic lingering effects. Information on such effects is essential when determining whether to restore, rehabilitate, or replace oil-impacted areas, as required under legislation enacted in many countries, such as the United States Oil Pollution Act of 1990 (33 U.S.C. 2701-2761). It is evident that we need more than mere visual inspection of environments or chemical analysis of coastal environments to evaluate recovery of oil-impacted areas.

5. Conclusions

Examination *G. demissa* in field studies found long-term effects from petroleum exposure resulting in shorter mean shell lengths and slower growth rates. Reciprocal transplants of *G. demissa* from oiled and non-oiled locations examined effects following short- and long-term exposure. The transplants from Great Sippewissett to Wild Harbor exhibited slower growth rates, lower condition indices, and lower filtration rates following short-term residual petroleum exposure. The transplants from Wild Harbor to Great Sippewissett exhibited some recovery with increased growth and filtration rates but not in condition indices following long-term petroleum exposure. The oil residues are therefore biologically active and affect *G. demissa* populations.

Acknowledgments

This work is the result of research sponsored by NOAA National Sea Grant College Program Office, Department of Commerce, under Grant No. NA16RG2273, Woods Hole Oceanographic Institution Sea Grant Project No. R/P-73. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. Additional support was provided by funding from the NSF-funded Research Experience for Undergraduates program, award 0453292, an Office of Naval Research Young Investigator Award (N00014-04-01-0029) to C. Reddy. The authors thank R. Carmichael, H. White, B. Nelson, L. Xu, S. Carroll, E. Halchak, M. Rodela, N. Lysiak and D. B. Madison for assistance with data collection and analysis, Anne Giblin for a review of this manuscript,

and the WHOI Graphics Department for their assistance. We also thank the Bayshores Homeowners for site access and the Ford family for their hospitality and support.

References

Axelman, J., Naes, K., Naf, C., Broman, D., 1999. Accumulation of polycyclic aromatic hydrocarbons in semipermeable membrane devices and caged mussels (*Mytilus edulis*) in relation to water column phase distribution. Environmental Toxicology and Chemistry 18, 2454-2461.

Babcock, M.M., Irvine, G., Harris, P.M., Cusick, J., Rice, S.D., 1996. Persistence of oiling in mussels beds three and four years after the *Exxon Valdez* oil spill, in: Rice, S.D., Spies, R.B., Wolfe, D.A., Wright, B.A. (Eds.), Proceedings of the *Exxon Valdez* Oil Spill Symposium, American Fisheries Society, Bethesda, MD, pp. 286-297.

Babcock, M.M., Harris, P.M., Carls, M.G., Brodersen, C.C., Rice, S.D., 1998. Mussel bed restoration and monitoring. *Exxon Valdez* oil spill restoration project final report (Restoration Project 95090). National Oceanic & Atmospheric Administration, National Marine Fisheries Service, Juneau, AK.

Baker, J.M., 1999. Ecological effectiveness of oil spill countermeasures: How clean is clean? Pure Applied Chemistry 71, 135-151.

Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J.F., Burgeot, T., Michel, X., Bellocq, J., 1999. Polycyclic aromatic hydrocarbons (PAH) burden of mussels

(*Mytilus* sp.) in different marine environmental in relation with sediment PAH contamination, and bioavailabilty. Marine Environmental Research 47, 415-439.

Bayne, B.L., Worrall, C.M., 1980. Growth and production of mussels *Mytilus edulis* from two populations. Marine Ecology Progress Series 3, 317-328.

Bertness, M.D., 1984. Ribbed Mussels and *Spartina alterniflora* production in a New England salt marsh. Ecology 65, 1794-1807.

Black, J.A., Birge, W.J., Westerman, A.G., Francis, P.C., 1983. Comparative aquatic toxicology of aromatic hydrocarbons. Fundamentals of Applied Toxicology 3, 353-358.

Blumer, M., Sass, J., Souza, G., Sanders, H.L., Grassle, J.F., Hampson, G.R., 1970. The West Falmouth oil spill – The persistence of the pollution eight months after the accident. Woods Hole Oceanographic Institution Technical Report 70-44.

Blumer, M., Sass, J., 1972a. Oil pollution: Persistence and degradation of spilled fuel oil. Science 176, 1120-1122.

Blumer, M., Sass, J., 1972b. Indigenous and petroleum-derived hydrocarbons in a polluted sediment. Marine Pollution Bulletin 3, 92-94.

Boehm, P.D., Quinn, J.G., 1977. The persistence of chronically accumulated hydrocarbons in the hard shell clam *Mercenaria mercenaria*. Marine Biology 44, 227-233.

Booth, A.M., Sutton, P.A., Lewis, C.A., Lewis, A.C., Scarlett, A., Chau, W., Widdows, J., Rowland, S.J., 2007. Unresolved complex mixtures of aromatic hydrocarbons:

thousands of overlooked persistant, bioaccumulative, and toxic contaminants in mussels. Environmental Science and Technology 41, 457-464.

Brousseau, D., 1984. Age and growth rate determination for the Atlantic ribbed mussel *Geukensia demissa* Dillwyn (Bivalvia: Mytilidae). Estuaries 7, 233-241.

Burns, K.A., Teal, J.M., 1971. Hydrocarbon incorporation into the salt marsh ecosystem after the West Falmouth oil spill. Woods Hole Oceanographic Institution Technical Report 71-69.

Capuzzo, J.M., 1996. The bioaccumulation and biological effects of lipophilic organic contaminants, in: Kennedy, V.S., Newell, R.I.E., Eble, A.F. (Eds.), The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant College, MD, pp. 539-557.

Carls, M.G., Harris, P.M., Rice, S.D., 2004. Restoration of oiled mussel beds in Prince William Sound, Alaska. Marine Environmental Research 57, 359-376.

Chapman, M.G., 1986. Assessment of some controls in experimental transplants of intertidal gastropods. Journal of Experimental Marine Biology and Ecology 103, 181-201.

Cheung, C.C.C., Zheng, G.J., Li, A.M.Y., Richardson, B.J., Lam, P.K.S., 2001. Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis*. Aquatic Toxicology 52, 189-203.

Chintala, M.M., Wigand, C., Thursby, G., 2006. Comparison of *Geukensia demissa* populations in Rhode Island fringe salt marshes with varying nitrogen loads. Marine Ecology Progress Series 320, 101-108.

Culbertson, J.B., Valiela, I., Peacock, E.E., Reddy, C.M., Carter, A., VanderKruik, R., 2007a. Long-term biological effects of petroleum residues on fiddler crabs in salt marshes. Marine Pollution Bulletin doi:10.1016/j.marpolbul.2007.02.015.

Culbertson, J.B., Valiela, I., Pickart, M., Peacock, E.E., Reddy, C.M., 2007b. Response of salt marsh grass to residual petroleum spilled 38 years ago in Wild Harbor, MA. Journal of Applied Ecology submitted.

DiSalvo, L.H., Guard, H.E., Hunter, L., 1975. Tissue hydrocarbon burden of mussels as a potential monitor of environmental hydrocarbon insult. Environmental Science and Technology 9, 247-251.

Evgenidou, A., Valiela, I., 2002. Response of growth and density of a population of *Geukensia demissa* to land-derived nitrogen loading in Waquoit Bay, Massachusetts. Estuarine, Coastal and Shelf Science 55, 125-138.

Farrington, J.W., Davis, A.C., Frew, N.M., Rabin, K.S., 1982. No. 2 fuel oil compounds in *Mytilus edulis*, retention and release after an oil spill. Marine Biology 66, 15-26.

Franz, D.R., 1996. Size and age at first reproduction of the ribbed mussel *Geukensia demissa* (Dillwyn) in relation to shore level in a New York salt marsh. Journal of Experimental Marine Biology and Ecology 205, 1-13.

Franz, D.R., 1997. Resource allocation in the intertidal salt-marsh mussel *Geukensia demissa* in relation to shore level. Estuaries 20, 134-148.

Frysinger, G.S., Gaines, R.B., Xu, L., Reddy, C.M., 2003. Resolving the unresolved complex mixture in petroleum-contaminated sediments. Environmental Science and Technology 37, 4754-4760.

Gilfillan, E.S., Mayo, D.W., Page, D.S., Donovan, D., and Hanson, S., 1977. Effects of varying concentrations of petroleum hydrocarbons in sediments on carbon flux in *Mya arenaria*, in: Vernberg, F.J., Calabrese, A., Thurberg, F.P., Vernberg, W.B (Eds.), Physiological Responses of Marine Biota to Pollutants. Academic Press, NY, pp. 299-314.

Honkoop, P.J.C., Bayne, B.L., Underwood, A.J., Svensson, S., 2003. Appropriate experimental design for transplanting mussels (*Mytilus* sp.) in analyses of environmental stress: an example in Sydney Harbor (Australia). Journal of Experimental Marine Biology and Ecology 297, 252-268.

Jordan, T.E., Valiela, I., 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a New England salt marsh. Limnology and Oceanography 27, 75-90.

Keck, R.T., Heess, R.C., Wehmiller, J., Maurer, D., 1978. Sublethal effects of the water soluble fraction of Nirgerian crude oil on the juvenile hard clams, *Mercenaria mercenaria* (Linn). Environmental Pollution 15, 109-119.

Kraak, M.H.S., Ainscough, C., Fernandez, A., van Vlaardingen, P.L.A., de Voogt, P., Admirall, W.A., 1997. Short-term and chronic exposure of the zebra mussel (*Dreissena polymorpha*) to acridine: Effects and metabolism. Aquatic Toxicology 37, 9-20.

Krebs, C.T., Burns, K.A., 1977. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. Science 197, 484-487.

Kuenzler, E.J., 1961a. Structure and energy flow of a mussel population in a Georgia salt marsh. Limnology and Oceanography 6, 191-204.

Kuenzler, E.J., 1961b. Phosphorus budget of a mussel population. Limnology and Oceanography 6, 400-415.

Le Floch, S., Guyomarch, J., Merlin, F., Borseth, J.F., Le Corre, P., Lee, K., 2003. Effects of oil and bioremediation on mussel (*Mytilus edulis* L.). Environmental Technology 24, 1212-1219.

Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19, 81-97.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. Limnology and Oceanography 30, 343-346.

Lutz, R.A., Castagna, M., 1980. Age composition and growth rate of a mussel (*Geukensia demissa*) population in a Virginia salt marsh. Journal of Molluscan Studies 46, 106-115.

Myrand, B., Gaudreault, J., 1995. Summer mortality of the blue mussel (*Mytilus edulis* Linneaus, 1785) in the Magdalen Islands (southern Gulf of St Lawrence, Canada). Journal of Shellfish Research 14, 395-404.

Neff, J.M., 2002. Bioaccumulation in Marine Organisms. Effects of Contaminants from Oil Well Produced Water. Elsevier Science Publishers, Amsterdam.

National Research Council of the National Academies, 2003. Oil in the Sea III, The National Academies Press, Washington, D.C.

Page, D.S., Boehm, P.D., Brown, J.S., Neff, J.M., Burns, W.A., Bence, A.E., 2005. Mussels document loss of bioavailable polycyclic aromatic hydrocarbon and the return to baseline conditions for oiled shorelines in Prince William Sound, Alaska. Marine Environmental Research 60, 422-436.

Peacock, E.E., Nelson, R.K., Solow, A.R., Warren, J.D., Baker, J.L., Reddy, C.M., 2005. The West Falmouth oil spill: ~ 100 kg oil found to persist decades later. Environmental Forensics 6, 273-281.

Peterson, C.H., Rice, S.D., Short, J.W., Ester, D., Bodkin, J.L., Ballachey, B.E., Irons, D.B., 2003. Long-term ecosystem response to the Exxon Valdez oil spill. Science 302, 2082-2086.

Peteiro, L.G., Babarro, J.M.F., Labarta, U., Fernandez-Reiriz, M.J., 2006. Growth of *Mytilus galloprovincialis* after the *Prestige* oil spill. Journal of Marine Science 63, 1005-1013.

Reddy, C.M., Eglinton, T.I., Hounshell, A., White, H.K., Xu, L., Gaines, R.B., Frysinger, G.S., 2002. The West Falmouth oil spill: The persistence of petroleum hydrocarbons in marsh sediments. Environmental Science and Technology 36, 4754-4760.

Sanders, H.L., Grassle, J.F., Hampson, G.R., 1972. The West Falmouth oil spill. I. Biology. Woods Hole Oceanographic Institution Technical Report 72-20.

Sanders, H.L., Grassle, J.F., Hampson, G.R., Morse, L.S., Garner-Price, S., Jones, C.C., 1980. Anatomy of an oil spill: long-term effects from the grounding of the barge *Florida* off West Falmouth, Massachusetts. Journal of Marine Research 38, 265-380.

Sericano, J.L., Wade, T.L., Jackson, T.J., Brooks, J.M., Tripp, J.W., Farrington, J.W., Mee, L.D., Readman, J.W., Villeneuve, J.P., Goldberg, E.D., 1995. Trace organic contamination in the Americas: an overview of the US national status and trends and the international 'Mussel Watch' Programmes. Marine Pollution Bulletin 31, 214-225.

Shriver, A.C., Carmichael, R.H., Valiela, I., 2002. Growth, condition, reproductive potential, and mortality of bay scallops, *Argopecten irradians*, in response to eutrophic-driven changes in food resources. Journal of Experimental Marine Biology and Ecology 279, 21-40.

Slater, G. F., White, H. K., Eglinton, T. I., Reddy, C. M., 2005. Determination of microbial carbon sources in petroleum contaminated sediments using molecular ^{14}C analysis. Environmental Science and Technology 39, 2552-2558.

Stegeman, J.J., 1981. Polynuclear aromatic hydrocarbons and their metabolism in the marine environment, in: Gelboin, H.V., Ts'o, P.O.P. (Eds.), Polycyclic Aromatic Hydrocarbons and Cancer, Academic Press, New York, Vol. 3 pp. 1-60.

Stekoll, M.S., Clement, L.E., Shaw, D.G., 1980. Sublethal effects of chronic oil exposure on the intertidal clam, *Macoma balthica*. Marine Biology 57, 51-60.

Stromgren, T., Nielsen, M.V., Ueland, K., 1986. Short-term effect of microencapsulated hydrocarbons on shell growth of *Mytilus edulis*. Marine Biology 91, 33-39.

Teal, J.M., Burns, K., Farrington, J., 1978. Analyses of aromatic hydrocarbons in intertidal sediments resulting from two oil spills of No. 2 fuel oil in Buzzards Bay, Massachusetts. Journal of the Fisheries Research Board of Canada 35, 510-520.

Teal, J.M., Farrington, J.W., Burns, K.A., Stegeman, J.J., Tripp, B.W., Woodin, B., Phinney, C., 1992. The West Falmouth oil spill after 20 years: Fate of fuel oil compounds and effects on animals. Marine Pollution Bulletin 24, 607-614.

Theisen, B.F., 1982. Variation in size of gills, labial palps, and adductor mussel in *Mytilus edulis* L. (Bivalvia) from Danish waters. Ophelia 21, 49-63.

White, H.K., Reddy, C.M., Eglinton, T.I., 2005a. Isotopic constraints on the fate of petroleum residues sequestered in salt marsh sediments. Environmental Science and Technology 39, 2545-2551.

White, H.K., Xu, L., Lima, A.L.C., Eglinton, T.I., Reddy, C.M., 2005b. Abundance, composition, and vertical transport of PAHs in marsh sediments. Environmental Science and Technology 39, 8273-8280.

Widdows, J., Phelps, D.K., Galloway, W., 1981. Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. Marine Environmental Research 4, 181-194.

Widdows, J., Bakke, T., Bayne, B.L., Donkin, P., Livingstone, D.R., Lowe, D.M., Moore, M.N., Evans, S.V., Moore, S.L., 1982. Responses of *Mytilus edulis* L. on

exposure to the water accommodated fraction of North Sea oil. Marine Biology 67, 15-31.

Widdows, J., Donkin, P., Salkeld, P.N., Cleary, J.J., Lowe, D.M., Evans, S.V., Thomson, P.E., 1984. Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). Marine Ecological Progress Series 17, 33-47.

Widdows, J., Donkin, P., Evans, S.V., 1985. Recovery of *Mytilus edulis* L. from chronic oil exposure. Marine Environmental Research 17, 250-253.

Widdows, J., Donkin, P., Evans, S.V., 1987. Physiological response of *Mytilus edulis* during chronic oil exposure and recovery. Marine Environmental Research 23, 15-32.

Widdows, J., Donkin, P., 1992. Mussels and environmental contaminants: Bioaccumulation and physiological aspects, in: Gosling, E. (Eds.), The Mussel *Mytilus*: Ecology, Physiology, Genetics, and Culture. Elsevier, Amsterdam, pp. 383-424.

Widdows, J., Nasci, C., Fossato, V.U., 1996. Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. Marine Environmental Research 43, 69-79.

Table 1. Experiment set-up for mussel transplants. Each x= one cage with 15 mussels [5 from each size class (40-49 mm, 50-59 mm, 60-69 mm)]. There were three cages per treatment. Total n = 180 mussels.

	Mussels from Wild Harbor	Mussels from Great Sippewissett Marsh
Cages in Wild Harbor	xxx	xxx
Cages in Great Sippewissett Marsh	xxx	xxx

Table 2. Analysis of variance of mussel population growth rate, individual growth, condition index, and filtration rates under the effects of treatment and shell length. The transplant data are shown in Figs. 4, 5, and 6. The four treatments in those analyses are the reciprocal transplants: Great Sippewissett to Great Sippewissett, Great Sippewissett to Wild Harbor, Wild Harbor to Wild Harbor, and Wild Harbor to Great Sippewissett. The interaction terms for each ANOVA were not significant.

	df	ms	*F*	*P* value
Population growth rate (Fig. 3)				
Treatment (Site)	Total = 114	572	8.12^{**}	$P < 0.01$
Growth (Fig. 4)				
Treatment	3	34.9	2.76^{*}	$P < 0.05$
Length	2	402	31.7^{***}	$P < 0.001$
40-49 mm	3	60.9	3.25^{*}	$P < 0.05$
50-59 mm	3	12.6	0.73 ns	$P > 0.05$
60-69 mm	3	3.6	1.13 ns	$P > 0.05$
Condition index (Fig. 5)				
Treatment	3	128	15.6^{***}	$P < 0.001$
Length	2	2.7	0.33 ns	$P > 0.05$
Filtration rate (Fig. 6)				
Treatment	3	6400	10.2^{***}	$P < 0.001$
Length	2	1030	1.64 ns	$P > 0.05$

Figure legends

Fig. 1. Map of Wild Harbor and Great Sippewissett Marsh on Cape Cod, MA. Star symbol indicates the grounding site of the *Florida* barge, on the way to the Cape Cod canal, within Buzzards Bay in September 1969.

Fig. 2. Frequency histogram of shell length (mm) of *Geukensia demissa*, as percent of total observations, found along the creekbank elevations in Wild Harbor and Great Sippewissett marsh.

Fig. 3. *Geukensia demissa* length (mm) in Wild Harbor and Great Sippewissett marsh relative to age determined from growth rings (ANCOVA, $F_s = 8.12$ **).

Fig. 4. Ribbed mussel growth rates (mm year^{-1}) ± standard error (s.e) for each treatment (GS = Great Sippewissett; WH = Wild Harbor), for mussels in three size classes (a) 40 – 49 mm (Table 2), (b) 50 – 59 mm (Table 2) and (c) 60 – 69 mm (Table 2). There were significant differences between treatments (Table 2) in the 40 - 49 mm size class with (a) significantly different than (b) at $P < 0.05$ (Tukey's).

Fig. 5. Ribbed mussel mean condition index ± s.e. for each treatment (GS = Great Sippewissett; WH = Wild Harbor). There were no significant differences between size classes (Table 2). There

were significant differences between treatments (Table 2) with (a) significantly different than (b) and (c) at $P < 0.001$ and (b) significantly different than (c) at $P < 0.05$ (Tukey's).

Fig. 6. Ribbed mussel mean filtration rates (mL water mussel^{-1} hour^{-1}) ± s.e. for each treatment (GS = Great Sippewissett; WH = Wild Harbor). There were no significant differences between size classes (Table 2). There were significant differences between treatments (Table 2) with each comparison significantly different at $P < 0.001$ except WH to GS compared WH to WH which are significantly different at $P < 0.05$ (Tukey's). There were no significant differences in initial chlorophyll *a* concentrations so these data were not included here.

Fig. 1

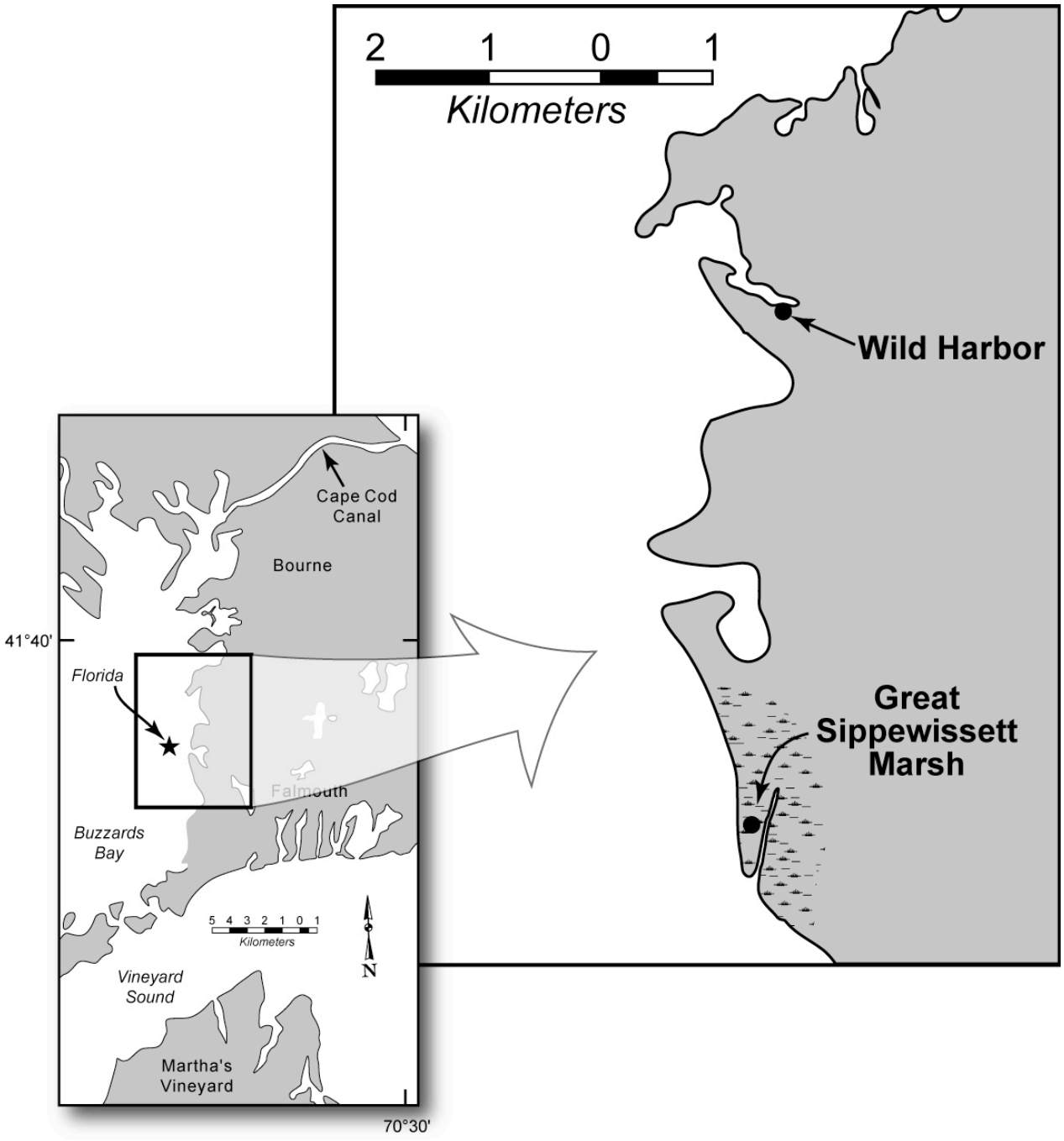

2 1 0 1
Kilometers
Wild Harbor
Great Sippewissett Marsh
Cape Cod Canal
Bourne
41°40'
Florida
Falmouth
Buzzards Bay
5 4 3 2 1 0 1
Kilometers
N
Vineyard Sound
Martha's Vineyard
70°30'

Fig. 2

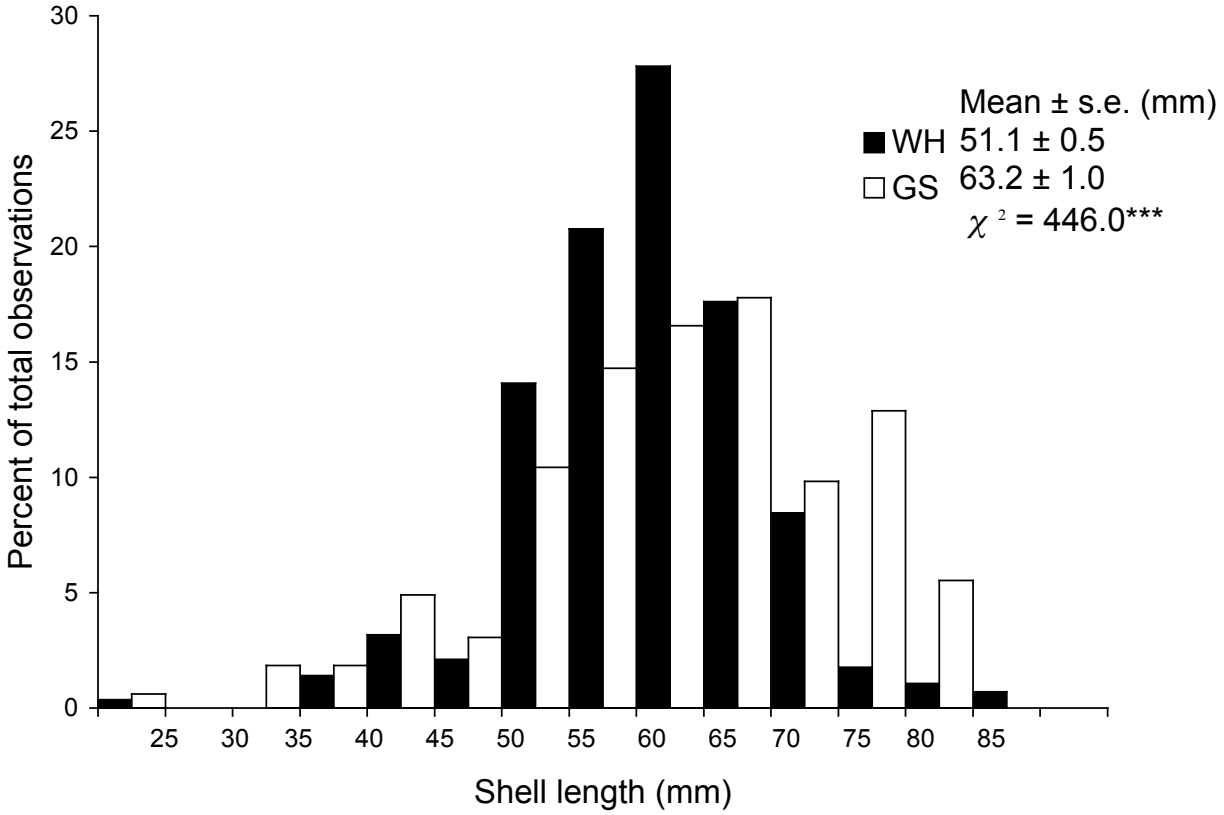

Percent of total observations
Shell length (mm)
Mean ± s.e. (mm)
WH 51.1 ± 0.5
GS 63.2 ± 1.0
χ² = 446.0***

Fig. 3

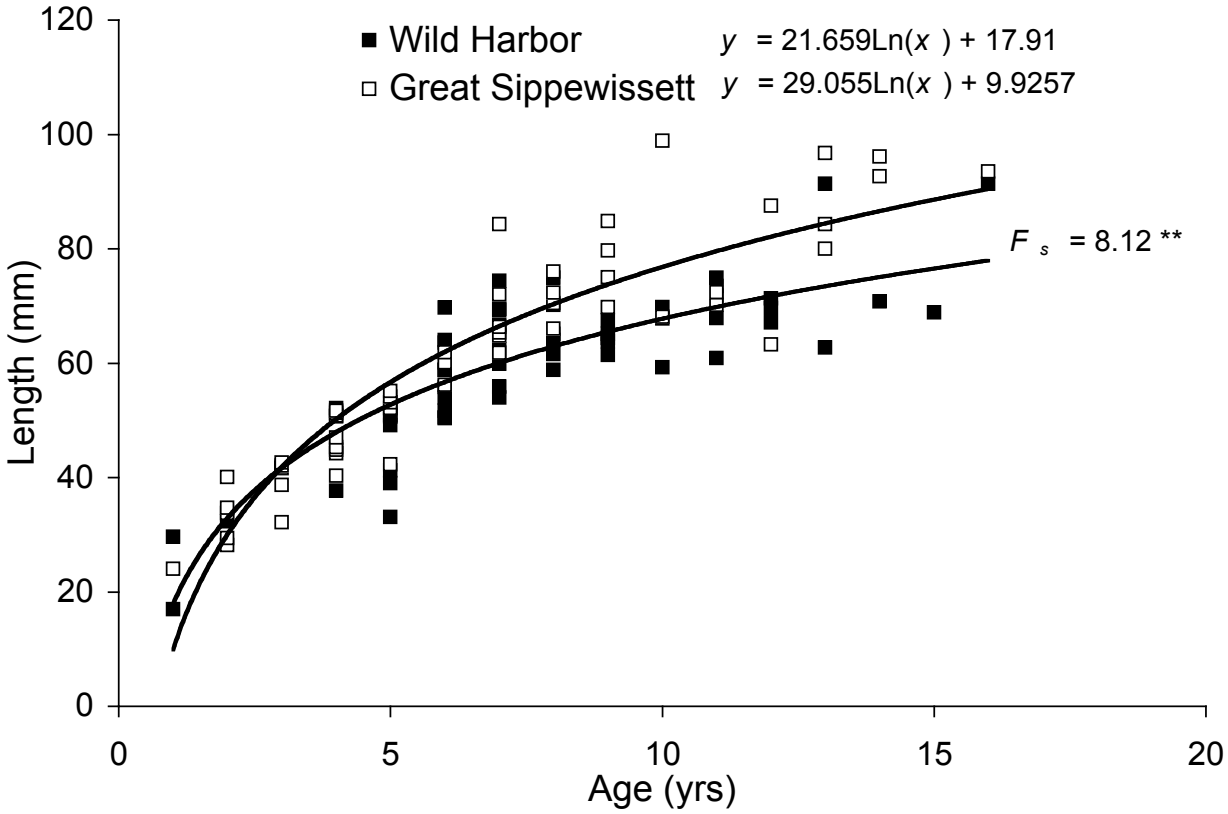

Wild Harbor
Great Sippewissett
y = 21.659Ln(x) + 17.91
y = 29.055Ln(x) + 9.9257
F s = 8.12 **
Length (mm)
Age (yrs)
0
20
40
60
80
100
120
0
5
10
15
20

Fig. 4

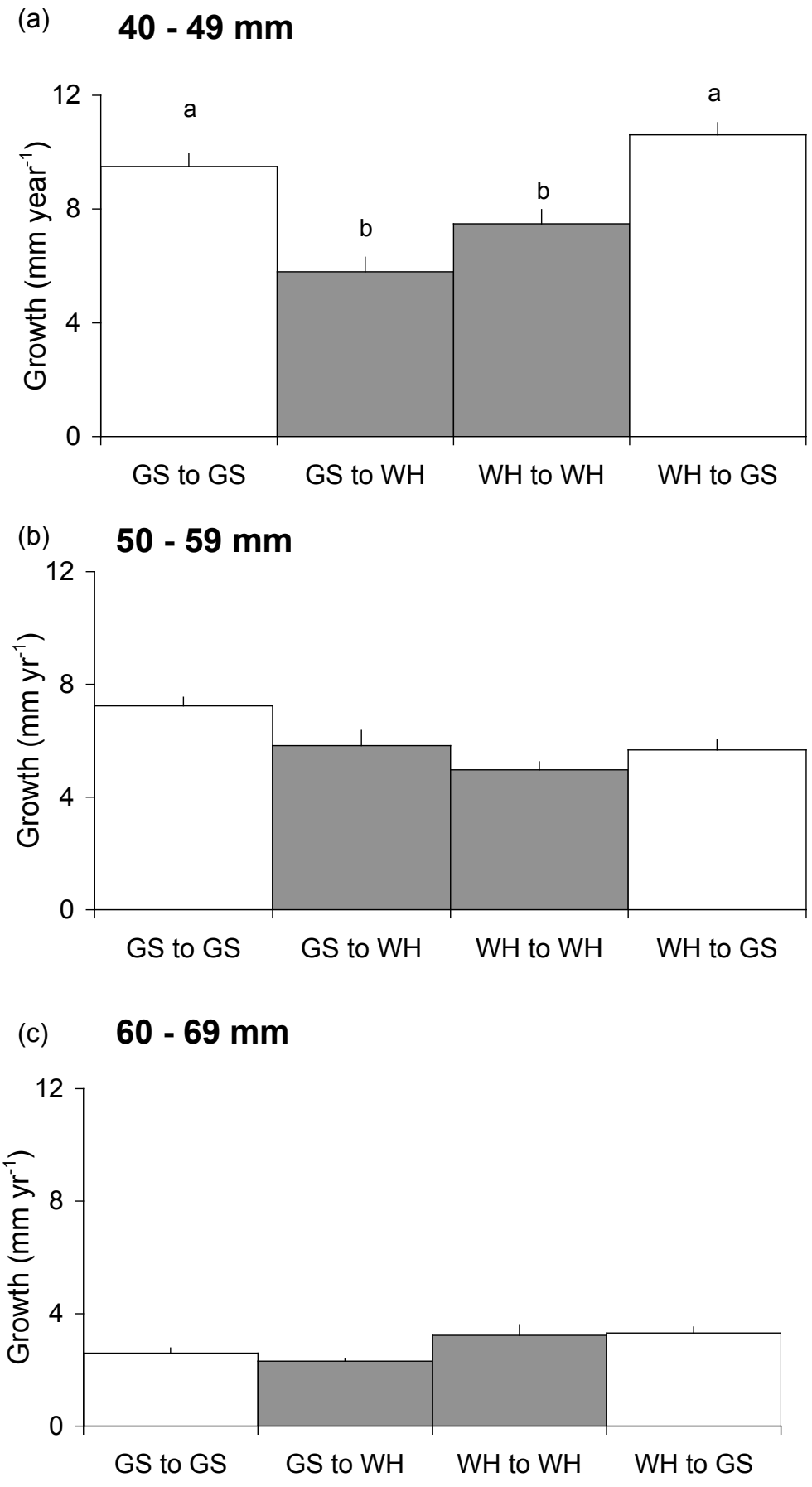

(a)
40 - 49 mm
Growth (mm year-1)
12
8
4
0
a
b
b
a
GS to GS
GS to WH
WH to WH
WH to GS
(b)
50 - 59 mm
Growth (mm yr-1)
12
8
4
0
GS to GS
GS to WH
WH to WH
WH to GS
(c)
60 - 69 mm
Growth (mm yr-1)
12
8
4
0
GS to GS
GS to WH
WH to WH
WH to GS

Fig. 5

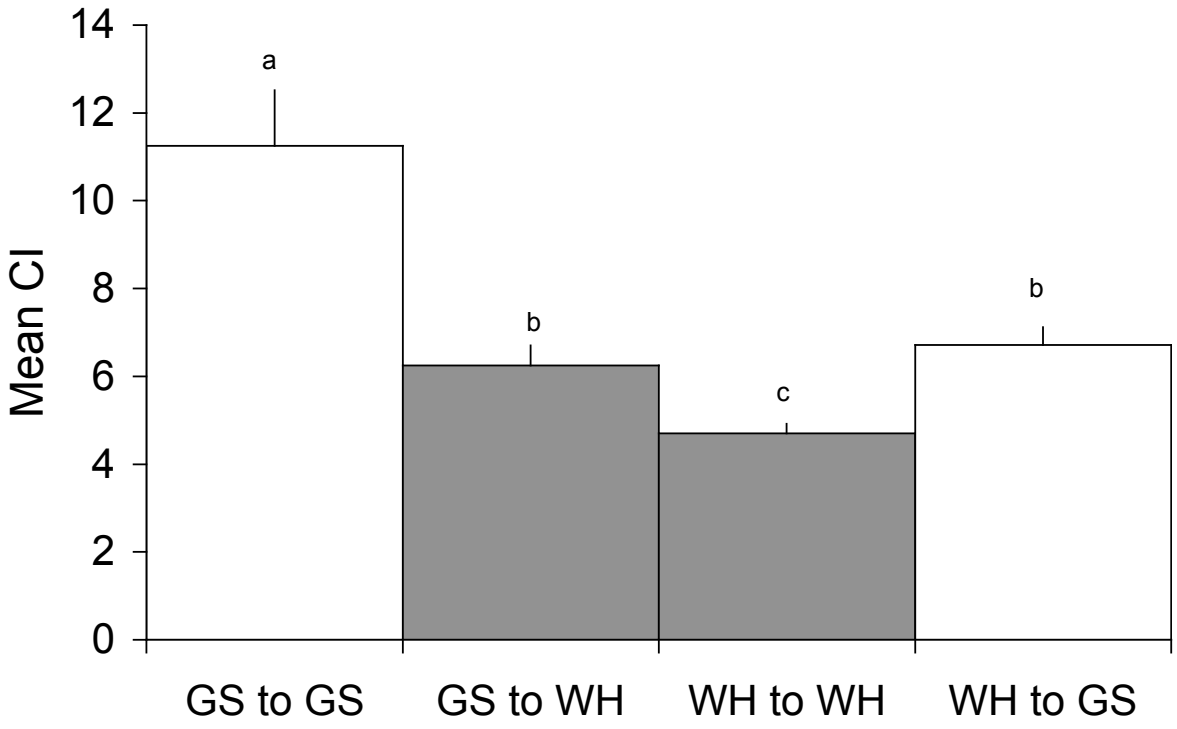
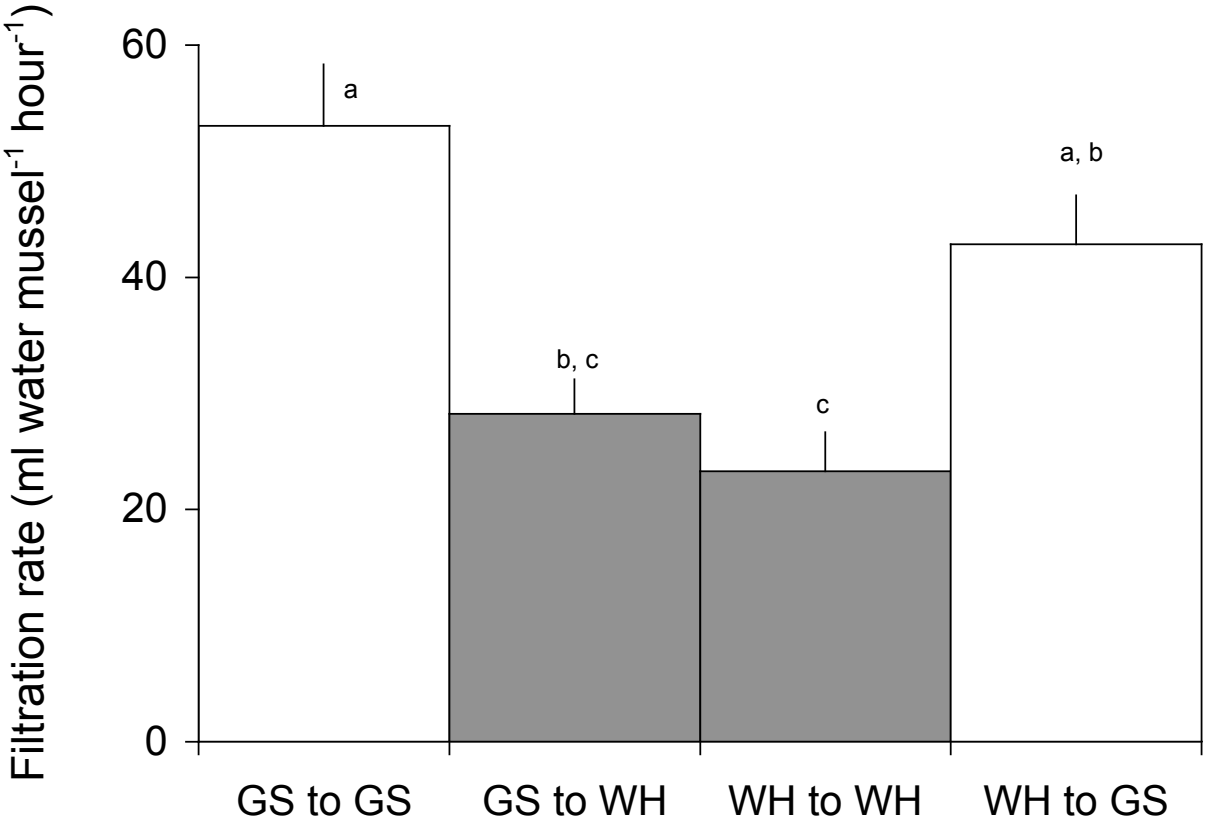


Fig. 6


Filtration rate (ml water mussel-1 hour-1)
60
40
20
0
a
b, c
c
a, b
GS to GS
GS to WH
WH to WH
WH to GS