Final Data Report:
UC Davis Bodega Marine Laboratory - Tier 1 Study for Assessing Mussel Reproductive State and Collection of Tissues for Potential Future Histopathology

The scope of this study was to collect mussels for reproductive status and future histopathology from sites in the East Bay following the October 30, 2009 fuel oil spill. Mussels from the same sites were collected by the Trustees for other analyses as well. The scope of this work includes collections of mussels from four assessment sites and one reference site as follows: (1) from two assessment sites (Alameda Point and Ballena Bay Marina) on November 5, 2009 and (2) from one reference site (San Leandro Marina) and two assessment sites (Bay Farm Island/Harbor Bay and Elsie Roemer "groin" wall/Crown Beach wall) on November 30, 2009 or December 1, 2009. From each site, 30 individual mussels (*Mytilus spp.*) were collected, placed in mesh bags, and transported back to the Bodega Marine Laboratory (BML) at 4ºC dry. Once back at the BML, animals were maintained dry at 4ºC in a moist environment (not immersed) until dissection.

Gonadal condition was evaluated on mussels collected on November 5, 2009 to make an initial assessment of whether mussels were reproductively active. If possible, all animals had gonadal tissue excised and placed into fixative (4% paraformaldehyde in phosphate buffer) for subsequent histological analyses. Non-fixed gonadal tissue samples from the same individuals were placed on a microscope slide, squashed using coverglass, and viewed using interference contrast optics on an upright microscope with 10, 20, and 40X objective lenses. As appropriate, representative digital images were recorded for each animal. If gonadal tissue was detectable at the time of dissection, the gender and stage of oogenesis and spermatogenesis was determined. For immature gonads, samples would need to be processed for histological examination at some point in the future.

Mussels were collected from three sites on November 30 and/or December 1, 2009 and tissues were dissected and fixed for subsequent histopathological evaluation. Animals collected on November 30, 2009, and December 1, 2009 were opened and organ tissue excised and placed into fixative (4% paraformaldehyde in phosphate buffer) for subsequent histological analyses (not included in this study). Tissue from the gills, gonad, mantle, kidney and digestive gland were dissected, labeled and preserved. In addition, some whole animals were preserved from each collection area. Length measurements were recorded for each mussel collected (see Mussel Tissue Collection Data Sheets). As appropriate, representative digital images were recorded for each animal.

Based on preliminary observations of the gonadal status of the mussels collected from Alameda Point and Ballena Bay Marina (November 5, 2009) there are differences between the two locations (see Gonad Status Report). Mussels collected from the Alameda Point area were more mature and at later stages of development than those collected from Ballena Bay Marina. The ratio between males and females also differed slightly between sites with 15 males and 9 females from the Alameda Point and 9 males

and 12 females from the Ballena Bay Marina. However, these differences may not be due solely to location, but to the fact that the species may be different.

The taxonomy of the common species of smooth shelled mussels in the genus *Mytilus* was uncertain before the advent of allozyme genetics (Gosling, 1992), but there are now three recognized species of closely related mussels. Mussels of the *Mytilus* species complex, *M. edulis, M. trossulus amd M. galloprovincialis* are geographically widespread in temperate marine waters. Members of this group can not be reliably diagnosed by shell characters (Light & Smith Manual, 2007). *M. edulis* is native to the cold-temperate areas of the Atlantic, *M. trossulus* is native to the north Pacific and north Atlantic, the northern form of *M. galloprovincialis* is native to the Mediterranean Sea and Western Europe and the southern form of *M. galloprovincialis* is found on the coasts of Argentina, Chile, New Zealand, and Australia. The three species maintain distinct genetic identities over large parts of their ranges, but where their ranges overlap they often hybridize. The northern form of *M. galloprovincialis* was introduced into the Pacific coast, where it ranges from southern California to British Columbia. *M. galloprovincialis* occurs locally, mostly in protected waters such as San Francisco Bay, Tomales Bay, and Bodega Bay. *M. trossulus* and hybrids may also occur in these waters. The native north Atlantic M. edulis is subject to experimental mariculture in British Columbia and may spread and hybridize as well. The different *Mytilus* species and their hybrids are morphologically similar and to definitively identify them as one of the three species has typically required genetic testing. Historically, along the Pacific coast, the protected bay mussel was classified as *M. edulis*, thus older research and data references to *M. edulis* cannot be separated into which of the three recognized genetically different species the earlier data is related.

Since the original samples collected after the oil spill in San Francisco Bay (October 2009) were not classified by genetic testing, we have no idea if these samples represent one species or a combination of species and hybrids.

Tier 1 Gonadal Condition Results

Mytilus spp. Gonad Status Report

Date: November 12, 2009 **Sampling Area and Date**: Alameda Point on November 5, 2009

Lab Staff E.H. Smith, K. Menard **Species** *Mytilus spp.*

Sample #	Micro Photo	Dig photo	Length (mm)	Sex	Gonad Status	Comments	Gill Cilia
A1	a1c	1,2	91	♂	Mature	Slight gape/NR	yes
A2	a2c,a2c2	3,4	63	♀	Immature	Slight gape	yes
A3	a3c	5	83	♂	Mature		
A4	A4c	6	72	♂	Mature		
A5	A5c	7	60	♂	Mature	White gonad	
A6	A6c	8	79	♀	Mature	Slight gape /R	
A7	A7c	9	81	♀	Mature		
A8	A8c	10	73	♀	Mature		
A9	A9c	11	81	♂	Mature		
A10	A10c	12	78	♂	Mature		
A11	A11c	13	56	♂	Immature		
A12	A12c	14	82	♂	Mature		
A13	A13c	15	54	♂	Mature?		
A14	A14c	16	64	♂	Immature		
A15	A15c	17	66	♀	Immature		
A16	A16c	18	57	♂	Immature	Histo needed	
A17	A17c	19	82	♀	Mature	Slight gape/R	
A18	A18c	20	53	♂	Immature		
A19	A19c	21	59	♀	Mature		
A20	A20c	22*	63	♂	Immature	Histo needed	
A21	A21c	24	47	♂	Mature	Different species?	
A22	A22c	25	67	♂	Immature	Slight gape, histo	
A23	A23c	26	46	Ud	Immature	Histo needed	
A24	A24c	27	52	Ud	Immature	Histo needed	
A25	Np	28	43	Ud	Immature	Thin gonad, histo	
A26	A26c	29	77	♀	Mature	Slight gape/R	
A27	A27c	30	59	♀	Immature/spawn	Histo needed	
A28/28a	Np	31	41	Ud	Thin gonad	Histo-no tissue ph	
A29	Np	32,33	52	Ud	Thin gonad	Histo	

Legend

- Sample# (collection locations): A=Alameda Point
- Dig Photo=macro photo of entire open mussel, Micro Photo= digital photo through compound microscope, Length= shell length measured along the long axis.
- ?= could be developing gonad or reabsorbing gonad after spawning, histological analysis needed to make determination.
- *Digital photo 23 = whole mussels (A21, A22, A23)
- Ud = undetermined, Np = no micro photo, ND = no gonad tissue visible, NR = not responding to stimulus, R = responding to stimulus.

Mytilus spp. Gonad Status Report

Date: November 12, 2009 **Sampling Area and Date:** Ballena Bay Marina on November 5, 2009

Lab Staff E.H. Smith, K. Menard **Species** *Mytilus spp.*

Sample #	Micro Photo	Dig photo	Length mm	Sex	Gonad Status	Comments	Gill Cilia
B1	Np	34*,35	57	Ud	ND	*Trossulus?* Histo	
B2	B2c	36	57	♀	Immature	Histo needed	
B3	B3c	37,38	48	♂	Immature?	Histo needed	
B4	Np	39	50	Ud	Immature	Histo needed	
B5	B5c	40	42	♀	Mature?	Histo needed	
B6	B6c	41	66	♂	Immature		
B7	B7c	42,43	67	♀	Immature?	Histo needed	
B8	B8c	44*-47	73	♀	Mature		
B9	B9c	48	52	Ud	Thin gonad	Histo needed	
B10	Np	49	53	Ud		Histo needed	
B11	B11c	50	63	Ud	Thin gonad	Histo needed	
B12	B12c	51	65	Ud			
B13	B13c	52	56	Ud			
B14	B14c	53	59	♂	Mature	Motile sperm	
B15	B15c1, b15c2	54	47	♀	Immature	Histo needed	
B16	B16c	55*	65	♀	Immature?	Histo needed	
B17	B17c	56	66	♀	Immature?	Histo needed	
B18	B18c	57	54	♀	Immature?	Histo needed	
B19	B19c	58	55	♀	Immature?	Histo needed	
B20	B20c	59	46	♀	Immature?	Histo needed	
B21	B21c*	60	61	♀	Immature?	Histo needed	
B22	B22c	61	60	♂	Mature	Motile sperm	
B23	B23c	62, 63	60	♀	Immature?	Histo needed	
B24	B24c	64	53	♂	Mature	Motile sperm	
B25	B25c	65	55	♂	Mature	Motile sperm	
B26	B26c	66	54	♂	Mature	Motile sperm	
B27	B27c	67	48	♂	Immature		
B28	B28c	68	52	♂	Immature		
B29	B29c	69	49	Ud	ND	Histo needed	
B30	B30c	70	46	Ud	ND	Histo needed	

Legend:

- Sample# (collection locations): B=Ballena Island Marina
- Dig Photo=macro photo of entire open mussel, Micro Photo= digital photo through compound microscope, Length= shell length measured along the long axis.
- ?= could be developing gonad or reabsorbing gonad after spawning, histological analysis needed to make determination. *Digital photo 34 = whole mussels (B1, B2, B3, B4, B5) *Mytilus trossulus* like? *Digital photos 44= focus issue (44, 45, 46, 46 all same mussel)
- Ud = undetermined, Np = no micro photo, ND = no gonad tissue visible, NR = not responding to stimulus, R = responding to stimulus.

Mussel Tissue Collection Data Sheet

Date Dec. 1, 2009 **Sampling Area** Harbor Bay (Bay Farm Island) on November 30, 2009 (ALD12-R11-113009-4mu)
Lab Staff E.H. Smith, K. Menard **Species** *Mytilus spp.*

Specimen label	(A)	(B)	(C)	(D)	(E)	Size (mm)	Comments
HB1	√	√	√	√	√	52	
HB2	√	√	√	√	√	70	
HB3	√	√	√	√	√	52	
HB4	√	√	√	√	√	61	
HB5	√	√	√	√	√	61	
HB6	√	√	√	√	√	53	
HB7	√	√	√	√	√	47	
HB8	√	√	√	√	√	54	
HB9	√	√	√	√	√	46	
HB10	√	√	√	√	√	54	
HB11	√	√	√	√	√	61	
HB12	√	√	√	√	√	65	
HB13	√	√	√	√	√	71	
HB14	√	√	√	√	√	66	
HB15	√	√	√	√	√	64	Small tissue amounts
HB16	√	√	√	√	√	64	
HB17	√	√	√	√	√	54	
HB18	√	√	√	√	√	57	
HB19	√	√	√	NC	√	53	
HB20	√	√	√	NC	√	54	
HB21							Whole animal preserved
HB22							Whole animal preserved
HB23							Whole animal preserved
HB24							Whole animal preserved
HB25							Whole animal preserved
							Only 25 animals collected

Legend:

- **A**= Gill, **B**= Gonad, **C**= Mantle, **D**= Kidney, **E**= Digestive Gland,
- **NC=** not collected, √**=** tissue collected,
- **Size**= length of mussel shell measured along the long axis.

Mussel Tissue Collection Data Sheet

Date Dec. 1, 2009 **Sampling Area** San Leandro Marina on November 30, 2009 (ALE-04-R11-113009-2-mu)
Lab Staff E.H. Smith, K. Menard **Species** *Mytilus spp.*

Specimen label	(A)	(B)	(C)	(D)	(E)	Size (mm)	Comments
SLM1	√	√	√	√	√	70	
SLM2	√	√	√	√	√	63	
SLM3	√	√	√	√	√	56	
SLM4	√	√	√	√	√	71	
SLM5	√	√	√	√	√	53	
SLM6	√	√	√	√	√	52	
SLM7	√	√	√	√	√	71	
SLM8	√	√	√	√	√	68	
SLM9	√	√	√	√	√	46	
SLM10	√	√	√	√	√	61	
SLM11	√	√	√	NC	√	56	
SLM12	√	√	√	NC	√	52	
SLM13	√	√	√	NC	√	43	
SLM14	√	√	√	NC	√	44	
SLM15	√	√	√	NC	√	40	
SLM16	√	√	√	√	√	57	
SLM17	√	√	√	√	√	59	
SLM18	√	√	√	√	√	61	
SLM19	√	√	√	√	√	55	
SLM20	√	√	√	√	√	56	
SLM21						57	Whole animal preserved
SLM22						56	Whole animal preserved
SLM23						52	Whole animal preserved
SLM24						54	Whole animal preserved
SLM25						52	Whole animal preserved
SLM26						53	Whole animal preserved
SLM27						38	Whole animal preserved
SLM28						36	Whole animal preserved
SLM29						45	Whole animal preserved
SLM30						47	Whole animal preserved

Legend:

- **A**= Gill, **B**= Gonad, **C**= Mantle, **D**= Kidney, **E**= Digestive Gland,
- **NC=** not collected, **√=** tissue collected,
- **Size**= length of mussel shell measured along the long axis.

Mussel Tissue Collection Data Sheet

Date Dec. 1, 2009 **Sampling Area** Crown Beach Wall (Elsie Roemer groin wall) on December 1, 2009 (ALD03-120109-R11-mu)
Lab Staff E.H. Smith, K. Menard **Species** *Mytilus spp.*

Specimen label	(A)	(B)	(C)	(D)	(E)	Size (mm)	Comments
CBW1	√	√	√	√	√	71	
CBW2	√	√	√	√	√	73	
CBW3	√	√	√	√	√	61	
CBW4	√	√	√	√	√	71	
CBW5	√	√	√	√	√	80	
CBW6	√	√	√	NC	√	66	
CBW7	√	√	√	√	√	69	
CBW8	√	√	√	√	√	71	
CBW9	√	√	√	√	√	69	
CBW10	√	√	√	√	√	63	
CBW11	√	√	√	√	√	72	
CBW12	√	√	√	√	√	62	
CBW13	√	√	√	√	√	63	
CBW14	√	√	√	√	√	64	
CBW15	√	√	√	√	√	66	
CBW16	√	√	√	√	√	69	
CBW17	√	√	√	√	√	60	
CBW18	√	√	√	√	√	65	
CBW19	√	√	√	√	√	62	
CBW20	√	√	√	√	√	59	
CBW21						61	Whole animal preserved
CBW22						56	Whole animal preserved
CBW23						54	Whole animal preserved
CBW24						55	Whole animal preserved
CBW25						59	Whole animal preserved
CBW26						56	Whole animal preserved
CBW27						55	Whole animal preserved
CBW28						61	Whole animal preserved
CBW29						58	Whole animal preserved
CBW30						52	Whole animal preserved

Legend:

- **A**= Gill, **B**= Gonad, **C**= Mantle, **D**= Kidney, **E**= Digestive Gland,
- **NC=** not collected, √**=** tissue collected,
- **Size**= length of mussel shell measured along the long axis.