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October 15, 2013

Amy Trainer
Executive Director
Environmental Action Committee of West Marin
Via email: amy@eacmarin.org

RE: 2013 Survey of *Didemnum vexillum* in Drakes Estero

Dear Amy:

Based on my several years of experience working on invasive species removal projects throughout the San Francisco Bay Area, you requested that I perform a survey of Drakes Estero to document the abundance of *Didemnum vexillum* (Dvex), also known as "marine vomit." Attached you will find my report: **Survey of the presence, local abundance, and substrate use of *Didemnum vexillum* (Dvex) in Drakes Estero-2013.**

While conducting this survey in Drakes Estero, I observed much of the well-documented, highly abundant, and extremely invasive colonial tunicate, Dvex living on the infrastructure (oyster shells, plastic tubes, lines) used for non-native oyster cultivation by the Drakes Bay Oyster Company (DBOC). I also observed and documented significant amounts of live Dvex colonies thriving on the live and dead eelgrass plants on the Estero floor directly below and at varying distances from some of the DBOC oyster cultivation racks.

In August, 2012 I and other Dvex researchers observed live Dvex colonizing dead eelgrass (*Zostera marina*) on the Estero floor. This discovery was alarming because of the long-held belief that Dvex was not capable of living on eelgrass or colonizing the soft-bottom floor of Drakes Estero.

More alarming are the observations made during this current survey of Dvex colonizing both dead and live eelgrass on the floor of Drakes Estero. This finding indicates that Dvex is now using the substrate of the native eelgrass and persisting in relatively large colonies on this substrate on the Estero floor. Overall, the relative abundance and varying substrate use and growth characteristics of Dvex observed in this partial survey appear to represent a trend of increasing Dvex infestation and its increasing ability to utilize eelgrass as a substrate within Drakes Estero.

Dvex colonies are aggressive and rapidly-spreading invasive marine organisms. Once established in a new location, Dvex may persist and become the dominant member of the infected community. In some cases, these rapid colonial expansions are known to reduce the abundance of previously established species and cause significant changes in the natural community structure. ***Because eelgrass dominates the bottom habitat of Drakes Estero, the potential for the colonization of much of the Estero floor by Dvex is high and the ongoing and future harm to this natural resource is cause for serious concern***.

My understanding is that the management policies of a national wilderness area require the removal of impacts that would cause “impairment” or “unacceptable impacts” to any key park resource, such as eelgrass and the associated benthic community. My observations and documentation of the successful colonization by Dvex on eelgrass in the federally designated wilderness area of Drakes Estero should be considered very seriously and Dvex removal efforts should be initiated immediately to prevent further Dvex colonization of the eelgrass habitat.

It is very well documented that a rapid response to the detection of an infestation of Dvex is critical to the success of managing its removal and that waiting too long can make the removal much more difficult, expensive, and threatening to the affected natural ecosystem.

Based on my observations in the attached report, it is my best professional opinion that:

1) a complete survey of the entire Estero should be conducted as soon as possible to document the prevalence of this Dvex infestation,
2) the Dvex should be immediately removed from all sources in the Estero, including infested oysters, infested oyster cultivation infrastructure, infested live and dead eelgrass, and any other infested natural substrate, and
3) A regular Estero-wide Dvex inventory-monitoring and removal program should be implemented to manage the future control of this highly invasive species. <u>Such removal actions must be done in a manner that prevents the fragments from any removed Dvex from re-entering the Estero waters, which may require the complete removal of these infested sources from the Estero</u>.

Thank you for the opportunity to conduct this survey and for your consideration of my concerns regarding the survey observations. Please do not hesitate to contact me with questions or for additional information.

Sincerely,

Jude Stalker

Jude Stalker
Biologist, Invasive Species Specialist

Report to the Environmental Action Committee of West Marin

Survey of the presence, relative abundance, and substrate use of *Didemnum vexillum* (Dvex) in Drakes Estero 2013

Prepared by Jude Stalker[1]

Survey Summary

A partial, visual survey of Drakes Estero complex (Drakes Estero and its five bays including the Estero de Limantour) was conducted by kayak over a three-week period to document the presence, relative abundance and substrate use of *Didemnum vexillum (Dvex).*

Dvex is a non-native, invasive colonial tunicate, considered to be a significant threat to the health of marine ecosystems because of its documented ability to rapidly spread, reducing the abundance of previously established benthic species and causing changes in benthic community structure (Coutts and Forrest 2007: Lengyel et al 2009). No prior research has quantified the abundance of Dvex in Drakes Estero or identified the source of the Dvex larvae and fragments that provide for its continuous colonization within the Estero. Documentation of the use of eelgrass (*Zostera marina*) as substrate by Dvex in Drakes Estero is very limited (Grosholz 2011).

This Report documents the abundant presence of Dvex living on the oyster shells and oyster equipment used by the Drakes Bay Oyster Company (DBOC) for the cultivation of non-native oysters (*Crassostrea gigas*) and suggests that this infestation is the source of the continued presence of Dvex within Drakes Estero. It also documents the recently detailed phenomenon of the presence of Dvex living on dead and live eelgrass both on the distal portions of the blades of live eelgrass plants (growing upright in water) and on dead and live eelgrass plants on the Estero floor, a phenomenon indicating that Dvex is now colonizing the eelgrass habitat in Drakes Estero.

Estero de Limantour was found to have no Dvex present in a preliminary inventory and assessment conducted over a four-year period from 2000-2004 (Fisk et al. 2005), and was surveyed as a comparison site in this 2013 survey. This current survey also found no Dvex present within the area surveyed in the Estero de Limantour which also suggests that the oyster shells and other equipment used for oyster cultivation in Drakes Estero provide the substrate for the source of the continuing infestations of Dvex in Drakes Estero.

Historical Presence of Dvex in Drakes Estero and Background Information

The area of the Drakes Estero complex encompasses approximately 2,300 acres of relatively shallow and sheltered tidal lands and is comprised of a system of five branching bays. Four of these bays (Schooner Bay, Home Bay, Creamery Bay and Barries Bay) branch off of the central

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area of Drakes Estero (Figure 1). The bottom substrate in all of the Drakes Estero complex is predominately comprised of sand and soft sediment and surrounded by a cobble-strewn, partially rocky shoreline (Figures 5, 6 and 7). Eelgrass is the dominant form of submerged aquatic vegetation and covers a significant portion of the bottom habitat throughout the Drakes Estero complex (Figures 8 and 9). Estero de Limantour, the eastern-most bay of the Drakes Estero complex, is somewhat isolated from the rest of Drakes Estero by the Drakes Head peninsula but is subjected to the same tidal action. Although it is smaller in size, the Estero de Limantour has very similar physical characteristics to the rest of Drakes Estero (Figure 1).

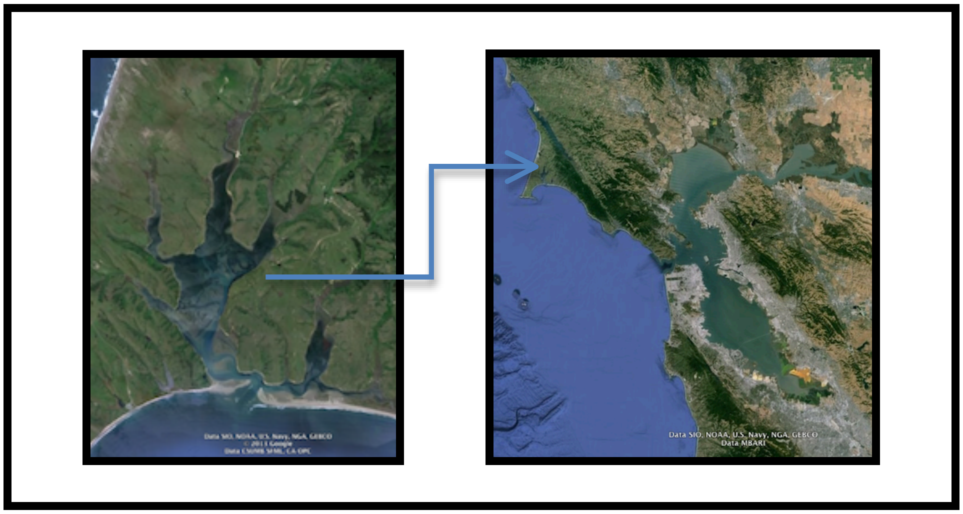

Figure 1. Regional location of the Drakes Estero Complex.

The presence of Dvex colonizing the hard substrate of oyster cultivation infrastructure (Shells, racks, lines, plastic tubes) has been well documented in the past in Drakes Estero and beyond (Fisk et al. 2005; Bullard et al. 2007a; NAS 2009; Grosholz 2011), but very little prior survey information relating to the abundance of Dvex in Drakes Estero exists. Both the Fisk and Grosholz studies were limited to the sampling of specific sites (oyster rack areas) within the Estero for the recruitment and presence of any fouling species. Although both of these studies did document the presence of Dvex (although Fisk et al. misnamed it *D. lahillei*), the information is limited to specific study areas and substrate and has very little documentation of the presence of Dvex on substrate other than that of the oyster racks and experimental settling plates.

There has been a general assumption that Dvex can only persist on hard substrate and is not capable of inhabiting the soft-bottom habitat within the Estero (Bullard et al. 2007a; NAS 2009), as it has done extensively on the cobble-bottom seafloor in New England (Valentine et al. 2007) and on the rip-rap seafloor in an area in New Zealand (Coutts and Forest 2007).

Until 2010 when Dr. Mary Carmen documented the presence of live Dvex on eelgrass in a tidal lagoon in Massachusetts (Carman and Grunden 2010), it was believed that Dvex could not grow on eelgrass. In 2011, Dr. Grosholz noted the observation of large colonies of Dvex growing on the distal portions of the leaf shoots of the native eelgrass in the area of an oyster rack in Drakes Estero (Grosholz 2011).

In 2012, while kayaking Drakes Estero with two San Francisco State University researchers and *Didemnum* specialists, we observed significant amounts of Dvex colonizing dead eelgrass near DBOC oyster cultivation racks on the Estero floor. The researchers made a positive visual identification of the Dvex at that time.

Survey Objectives

The objective of this survey was to conduct a partial assessment of the extent of the Dvex infestation living on the artificial substrate of the DBOC infrastructure and to assess the likelihood that this infestation is serving as the source of the continuous presence of Dvex in Drakes Estero. A second objective was also to locate, investigate, and document the abundance and growth characteristics of the Dvex reported to be living on the dead and live eelgrass plants (both upright in the water column and on the floor of Drakes Estero).

The Estero de Limantour was surveyed as a comparison site to the rest of the Drakes Estero complex because:

1) it is an isolated extension of Drakes Estero and has very similar natural physical features,
2) there is an absence of oyster operation infrastructure,
3) it is subjected to the same tidal action as the rest of Drakes Estero, and
4) it had no presence of Dvex observed on the experimental settling plates in the 2005 Fisk study.

Survey Methods and Observations

Drakes Estero

DBOC Oyster Cultivation Racks

Several of the DBOC oyster cultivation racks (including some of those surveyed by both Fisk et al. and Dr. Grosholz) were visually inspected by kayak for the presence, abundance and substrate use of Dvex. This information was recorded and documented by photographs. Location was recorded using a hand-held GPS unit.

Many of the oyster racks used for “hanging culture” that were inspected had a high density of Dvex living on the substrate of the oyster shells and ‘French tubes’ used for non-native oyster cultivation. In some cases, the majority of the lines of tubes and shells were completely enveloped by Dvex with long drooping tendrils hanging from the colony (Table 1, Figures 2, 10, 11 and 12).

Drakes Estero Floor (below racks)

The area of the Estero floor directly below the inspected oyster cultivation racks was visually surveyed for the presence, abundance, substrate use, and growth characteristics of Dvex. Observations were noted and documented with photographs. Location was recorded using a hand-held GPS unit.

There were some Dvex colonies observed growing on substrate of fallen shells and eelgrass directly below the oyster racks. The eelgrass distribution under the racks is relatively sparse and the Dvex present there was observed as using primarily fallen oyster shells as substrate. Observations of this substrate were limited due to low visibility and lack of access below the oyster racks (Table 1, Figure 13).

Drakes Estero Floor (eelgrass beds)

A partial area of the floor of Drakes Estero beyond the oyster cultivation racks was visually surveyed for the presence, abundance and substrate use of Dvex. This habitat consisted of areas of both live and dead eelgrass plants as well as of areas of bare (sand and silt) sediment.

Two areas (Figure 2, A and B) located away from the oyster cultivation racks were observed to be populated with Dvex colonies living attached to dead and live eelgrass plants on the floor of the Estero. These areas are at least 100 meters from oyster cultivation racks (although one is adjacent to a culture bed area), and were approximately (A) 2,500 Square meters (m^2) and (B) 1,500 m^2 in area and had average Dvex colony densities of approximately 5% cover. The Dvex colonies observed within these areas ranged in size from a few centimeters (cm) to up to approximately 50 cm in length and were multi-lobed.

Another area (Figure 2, C) was observed adjacent to oyster rack #2 (Fisk 2005 Sampling Station #1, Rack E and Grosholz 2011 Active Production Rack #1) that had a significant amount of Dvex living on live and dead eelgrass plants on the Estero bottom. This area had a Dvex cover of approximately 5% over a 2,100 m^2 area. The Dvex colonies on the Estero floor within this area also ranged in size from a few cm to approximately 50 cm in length and were multi-lobed. GPS locations were recorded (Table 1, Figures 2 and 17-22).

Also in area C, smaller colonies of Dvex were observed to be attached to and living on the distal portions of the live eelgrass blades. These colonies ranged in size from 1 cm to approximately 12 cm. The colonies were relatively flat, thin, uniform and lacking major lobes or tendrils, indicating that they were relatively young (SeaGrant 2005). One of the affected eelgrass plants was pulled up and photographed. The GPS location was recorded (Table 1, Figures 2 and 14-16).

Intact Dvex colonies living on live/dead eelgrass plants on the Estero bottom from each of these observed areas were pulled up from the Estero bottom and photographed. No samples were collected for further analysis. All surveying was conducted during tidal heights of between 2-4 feet (Drakes Bay). The location of these three observed areas (A, B, and C) is shown in Figure 2.

Figure 2. Drakes Estero with observation locations**.**

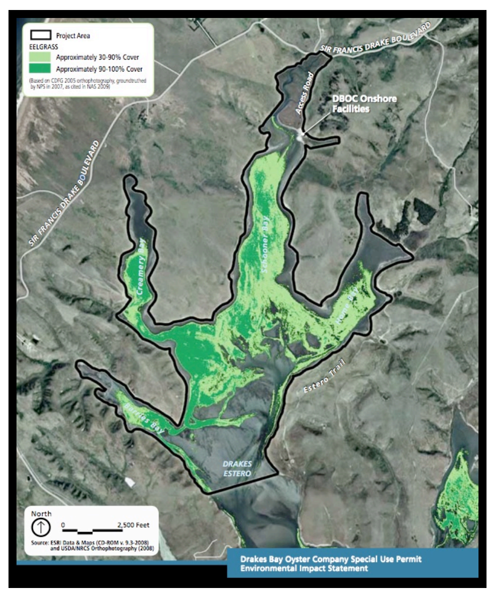

Figure 3. Eelgrass coverage in Drakes Estero.

Estero de Limantour

On September 27, 2013 I conducted an extensive visual inspection survey of the bottom, eelgrass, and shoreline habitats of Estero de Limantour, including the four grid sampling areas that were studied by Fisk et al (2005). The Estero area was traversed by kayak (Figure 4) and the shoreline was partially scanned from the kayak and partially walked on foot. The wind was mild (0-6 mph) making the visibility into the water and eelgrass and onto the bottom very good. No Dvex was observed in the Estero de Limantour.

Figure 4. Estero de Limantour survey area with kayak track (yellow) and 2005 grid locations (blue).

Discussion

Drakes Estero

Although earlier studies have documented the presence of Dvex in Drake's Estero, little documentation of the abundance and extent of its coverage, substrate use or growth characteristics has been made available. The scope of this survey did not allow for an inspection of the entire Estero. **The relative abundance and varying substrate use and growth characteristics of Dvex observed in this partial survey however, appear to represent a trend of increasing infestation and ability to utilize eelgrass as a substrate within Drakes Estero.**

It is widely known that existing colonies of Dvex can reproduce sexually, releasing larvae into the water where they can attach to hard substrate and form new colonies. Dvex larvae are short lived and likely settle on suitable substrate near the point or origin. Dvex can also reproduce asexually when fragments of lobes or tendrils break away from a colony, drift to a new site, settle and grow out over suitable substrate (Lengyel et al. 2009; Bullard et al. 2007a). Fragments from a colony can settle near the point of fragmentation or drift for longer distances before settling and colonizing new areas (Lengyel et al. 2009).

Factors that may cause Dvex fragmentation include boat induced wave action (Reinhardt et al. 2012), the scraping of Dvex colonies against adjacent hard substrate (such as oyster shells and other oyster farming infrastructure) (Bullard et al. 2007a), movements of fouled aquaculture stock and cleaning of Dvex infested substrate and gear in or near the water (Lambert 2009; Morris and Carman 2012).

The abundant population of Dvex observed on the DBOC infrastructure provides a significant source of Dvex in both larval and fragmental form in Drakes Estero. The release of larvae from the existing Dvex colonies and the DBOC harvesting practices of pulling hanging culture lines and tubes from the racks, transporting them on open barges, separating and cleaning the Dvex-ridden oyster shells on the dock and returning the fragmented Dvex colonies back into the water are all likely facilitating the spread and persistence of this highly invasive species (Figures 23-27) (Morris and Carman 2012, NAS 2009).

The observations and documentation of the Dvex colonization of both live and dead eelgrass (in relatively great abundance in some areas) is potentially of great significance. This recently observed phenomenon indicates that relatively young colonies (flat, thin and uniform without major lobes or tendrils) of Dvex are able to recruit to and grow on eelgrass plants in Drakes Estero. Further research is needed to determine if these younger colonies on live eelgrass are eventually weighted down, resulting in the observed Dvex colonies on the live and dead eelgrass on the floor of Drakes Estero.

Regardless of the process, the documentation of the Dvex colonization of live and dead eelgrass on the Estero floor make it evident that the eelgrass-laden floor of Drakes Estero is now providing suitable substrate for the Dvex to live and expand on. Eelgrass cover in Drakes Estero has been estimated to be between thirty and ninety percent over an area of approximately 737 acres (Figure 3)(FEIS 2012). With such an abundant source of Dvex known to be living on the oyster cultivation infrastructure, there is cause for concern considering the potential threat of Dvex colonization to the native eelgrass habitat and to the rich community of flora and fauna that this habitat supports.

Estero de Limantour

The absence of Dvex observed during the visual survey of Estero de Limantour and the complete absence of Dvex colonization on settling plates over a four-year period in the Fisk et al. study (2005) suggests that the greater distance and relative isolation of Estero de Limantour from the significant source of Dvex larvae and fragments in Drakes Estero have prevented the dispersal and settlement of Dvex there so far. It may also suggest that without the ideal hard substrate of the DBOC oyster shells and other infrastructure, any larvae or fragments that may have entered into the Estero de Limantour were unable to survive. However, if there had been Dvex larvae or fragments in the water of the incoming tides while the Fisk study was conducted, it is very likely that they would have observed a presence of Dvex on the settling plates in the Estero de Limantour as they did in Drakes Estero. That Fisk et al., after four years, did not observe Dvex on the hard substrate of the settling plates in the Estero de Limantour, suggests that the abundant presence of Dvex on the hard substrate of oyster shells and other equipment used for oyster cultivation is the primary source of the Dvex infestation in Drakes Estero.

Recommendations for Future Monitoring and Management of Dvex in Drakes Estero

Future professional inventory and assessment surveys of the entire Estero (including the shoreline) are recommended to document the full extent of the colonization of this aggressive and highly invasive species. Without further inventory surveys and research, it will remain unclear as to what the entire extent of the Dvex infestation in Drakes Estero is, if the Dvex colonies living on the standing eelgrass are an early form of what becomes the Dvex colonies enveloping the dead and live eelgrass on the Estero bottom, and if the Dvex on the bottom is capable of expanding throughout the eelgrass habitat of the Estero.

Dvex research elsewhere indicates that the environmental and economic damage from an uncontrolled Dvex infestation is great (Bullard et al. 2007a; Coutts and Forrest 2007). This survey indicates an increasing potential for that outcome in Drake Estero.

Research has also shown that a rapid response to a Dvex infestation is essential to successfully manage and ensure its removal from a natural area (Coutts and Forrest 2007; Cohen et al. 2011). In my professional opinion, the best response to this Dvex infestation is the immediate removal of Dvex from all sources in the Estero (including infested oysters, infested oyster cultivation infrastructure, infested live and dead eelgrass, and any other infested natural substrate) followed by regular, thorough, estero-wide monitoring for Dvex and the subsequent removal of any newly discovered Dvex colonies.

Such removal actions must be done in a manner that prevents fragments from any removed Dvex colonies from re-entering the Estero waters, which may require the complete removal of these infested sources from the Estero. Because Fisk did not observe a presence of Dvex in the Estero de Limantour, it is likely that the Dvex population in the rest of Drakes Estero has remained local and that once the source and present population of Dvex is removed from Drakes Estero, that infestation may be controlled.

Table 1. Dates, locations, substrate and approximate extent of Dvex observed in Drakes Estero.

Date	Latitude	Longitude	Map Ref.	Dvex substrate	Approx. area of infestation	Approx. Dvex cover on substrate	Photo #
9/10/2013	38° 04' 05.94"	122° 56' 04.98"	A	Dead & live eelgrass on Estero Floor	2,500 m²	5%	16 & 17
9/11/2013	38° 03' 47.52"	122° 56' 03.24"	Rack 1	Oyster shells, lines and French tubes	100 m²	70%	10
9/11/2013	38° 03' 58.10"	122° 56' 07.70"	B	Dead & live eelgrass on Estero Floor	1,500 m²	5%	18 & 19
9/11/2013	38° 03' 47.52"	122° 56' 03.24"	Rack 1	Estero Floor-below racks	100 m²	5%	12
9/24/2013	38° 04' 33.3"	122° 56' 18.30"	C	Dead & live eelgrass on Estero Floor	2,000 m²	5%	20 & 21
9/24/2013	38° 04' 31.45"	122° 56' 16.21"	Rack 2	French tubes and oyster shells	580 m²	40%	11
9/24/2013	38° 04' 33.3"	122° 56' 18.30"	C	Eelgrass blades and shoots	2,000 m²	<1%	13, 14 & 15

Photographs - Figures 5-27

Unless otherwise indicated, all photographs were taken by Jude Stalker.

Figure 5. Drakes Estero Shoreline.

Figure 6. Estero de Limantour Shoreline.

Figure 7. Sand/silt bottom habitat of Estero de Limantour.

Figure 8. Bottom habitat in Estero de Limantour.

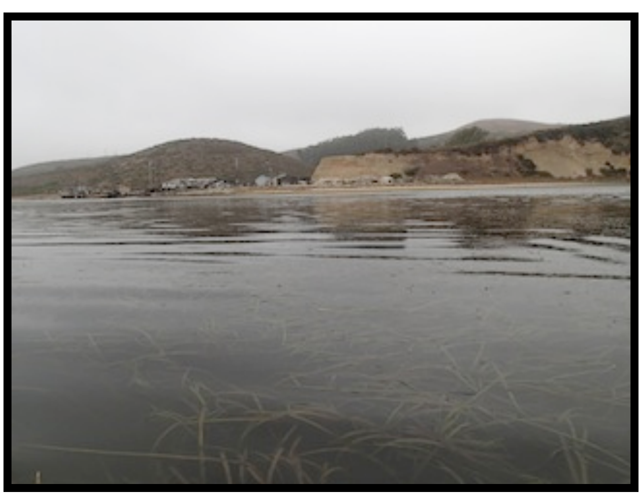

Figure 9. Eelgrass habitat-Drakes Estero.

Figure 10. DBOC oyster racks in Drakes Estero.

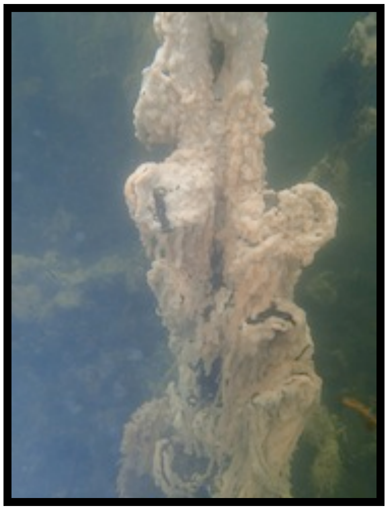

Figure 11. Rack 1: Dvex on hanging French tube and oyster shells.

Figure 12. Rack 2: Dvex on French tubes and oyster shells.

Figure 13. Rack 1: Dvex below rack.

Figure 14. Area C: Dvex on distal blades and shoots of live eelgrass.

Figure 15. Area C: Dvex on distal blades of live eelgrass.

Figure 16. Area C: Dvex on distal blades of live eelgrass.

Figure 17. Area A: Dvex colonies on estero floor.

Figure 18. Area A: Dvex on live and dead eelgrass from estero floor**.**

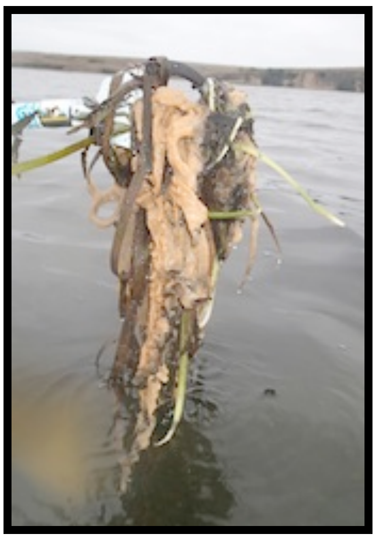

Figure 19. Area B: Dvex on live and dead eelgrass from Estero floor**.**

Figure 20. Area B: Dvex on live and dead eelgrass on Estero floor.

Figure 21. Area C: Dvex colony on live eelgrass on Estero floor.

Figure 22. Area C: Dvex on live eelgrass from Estero floor.

Figure 23. DBOC staff harvesting Dvex-infested oysters.
Photo by Christopher Chung Press Democrat 2012.

Figure 24. DBOC staff using pneumatic hammers at DBOC's cleaning station to chip Dvex off infested oysters.
Photo by Alvin Jornada-SF Chronicle 2011.

Figure 25. Water from oyster cleaning station can carry small Dvex fragments back into the Estero.

Figure 26. DBOC transporting Dvex-infested oysters on open barge on Drakes Estero.

Figure 27. DBOC's oyster cleaning and processing station at edge of Estero.

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