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Mobile autonomous process sampling within coastal ocean observing systems

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Abstract

Predicting when and where key oceanic processes will be encountered is problematic in dynamic coastal waters where diverse physical, chemical and biological factors interact in varied and rapidly changing combinations. Defining key processes often requires efficient sampling of water masses and prompt sample return for subsequent analyses. This compound challenge motivated our efforts to develop mobile autonomous process sampling (MAPS) for use with autonomous underwater vehicles (AUVs). With this system, features are recognized by artificial intelligence software that integrates AUV sensor data to estimate probabilistic states for adaptive control of survey navigation and triggering of targeted water samplers. To demonstrate the utility of the MAPS/AUV system, we focused on intermediate nepheloid layers (INLs), episodic features that are believed to play a role in the transport of zooplankton in the dynamic coastal waters of Monterey Bay, California. The MAPS/AUV system recognized, mapped and sampled INLs during multiple surveys. Invertebrate larvae contained in the water samples were subsequently characterized with molecular probes developed for high-throughput screening. Preliminary results obtained during these surveys support the hypothesis that INLs function as vehicles for episodic larval transport. Applying the MAPS/AUV system within a greater coastal ocean observing system permitted identification of a larval source region, oceanographic dynamics that forced transport, and spatio-temporal scales of transport.

Introduction

Understanding the hydrodynamic and biological processes affecting the connectivity of natural populations is one of the great challenges facing scientists and policy-makers involved in coastal ocean management, defining marine protected areas (MPAs) and regulating sustainable fisheries (Gawarkiewicz et al. 2007, Hare and Walsh 2007, Marinone et al. 2008). The connectivity of most benthic organisms is strongly influenced by interactions between pelagic larval stages and hydrodynamics that vary spatially, from local to basin-scale, and temporally, from episodic to climatic. Identifying larval species, determining their longevities in the water column, and assessing hydrodynamic processes that transport them are very difficult, however (Brown and Roughgarden 1985, Garland and Zimmer 2002, Shanks and Brink 2005). Approaches needed to advance this challenging area of research include in situ molecular detection of marine larvae (Goffredi et al. 2006, Jones et al. 2008), adaptive sampling methods capable of responding in real-time to event-driven transport processes (Pineda et al. 2007), and long-term studies that examine interactions between hydrodynamic and biological processes (Cowen et al. 2007).

Intermediate nepheloid layers (INLs) have the potential to influence geographical connectivity of benthic species having pelagic larval stages. Forced by diverse physical processes, INLs develop from transport of the turbid bottom boundary layer (BBL) in many ocean margin environments (Pak et al. 1980, Hickey et al. 1986, Washburn et al. 1993, Houghton and Visbeck 1998, McPhee-Shaw et al. 2004, Ryan et al. 2005). To effectively study INLs and evaluate their potential role in connectivity requires the abilities to search for, detect, map and sample these transient phenomena. Here we report on novel capabilities to robotically map and obtain water samples from INLs with autonomous underwater vehicles (AUVs). Engineering aspects of the water sampling system (AUV-Gulper) were previously documented (Bird et al. 2007). The artificial intelligence (AI) software capable of recognizing oceanic features with AUV sensor data and adaptively controlling the AUV has also been documented (Fox et al. 2007, McGann et al. 2008). The present study is the first demonstrative application of these novel advancements to a methods-limited area of marine ecology. We have coupled these engineering advancements for AUVs with (1) high-throughput molecular methods for the detection of larval invertebrates (Jones et al. 2008), and (2) data from a coastal ocean observing system, to illustrate how mobile autonomous process sampling (MAPS) can synergistically link these emergent technologies.

Materials and Procedures

We employed the MBARI AUV *Dorado*, an operational AUV with an extensive suite of physical, optical and chemical sensors for interdisciplinary research. Specifics of AUV sensors and methods of data processing have been published (Ryan et al. 2008). A custom AUV mid-section was developed for *Dorado* to support operation of ten *Gulper* water samplers (Fig. 1). For a complete engineering description of the *Gulper* sampler design, see Bird et al. (2007). Here we describe the science-motived functional requirements of the sampling system design. Each container intake opens directly to the outside of the AUV mid-body, so that samples cannot be contaminated by dead volume of a shared sample intake duct. All materials of the containers are non-toxic. The relatively large individual sample size of 2L was motivated by the need to sample biological constituents that may be at low concentrations. Rapid (1-2 s) sample intake, which motivated the name *Gulper*, serves two requirements: (1) it ensures that highly localized samples can be taken from vertical profiles of a rapidly moving AUV, and (2) it minimizes the opportunity for swimming plankton to escape capture. Sample acquisition start and end times are recorded by the AUV main computer to allow precise co-location of water samples with AUV sensor data.

Figure 1. The *Dorado* AUV water sampling system mid-section. The intake valves of individual *Gulper* water samplers penetrate the lower half of the AUV fairing (empty sample container slot at top left). When deployed for surveys, this subsystem is enclosed by the top half of the cylindrical mid-section fairing.

The AI advancements of this research employ a newly developed Teleo-Reactive Executive (TREX) control system that integrates probabilistic state estimation, planning and execution. The engineering of this system is described by McGann et al. (2008). State estimation is accomplished by clustering algorithms that are trained with field data and applied to real-time AUV data streams to detect target features with a quantified level of certainty (Fox et al. 2007). Training of the INL clustering algorithm utilized a 5-year (2003-2007) time-series of approximately monthly AUV vertical sections over Monterey Canyon (Fig. 2). This data set was also used to identify the location of most frequent and intense INL signals, which defined the domain for autonomous detection, mapping and sampling of INLs in this study (box in Fig. 2). INLs can be detected optically by elevated attenuation and backscattering of light caused by their relatively high concentrations of suspended particulate matter (SPM). Although phytoplankton cells also cause elevated attenuation and backscattering, they are distinguishable from INLs by the fluorescence of their chlorophyll. Therefore, INL clusters are characterized by a combination of high optical backscattering and low chlorophyll fluorescence. We used optical backscattering and chlorophyll fluorescence measurements from a HOBI Labs HS2 sensor for training and real-time application of the INL cluster analysis. In each survey, TREX was applied to (1) detect INLs, (2) adapt survey navigation for higher (lower) horizontal resolution of vertical sections in the presence (absence) of INLs, (3) trigger *Gulper* water sampling, and (4) control spatial allocation of samples within the survey volume, using a minimum separation distance of 1 km.

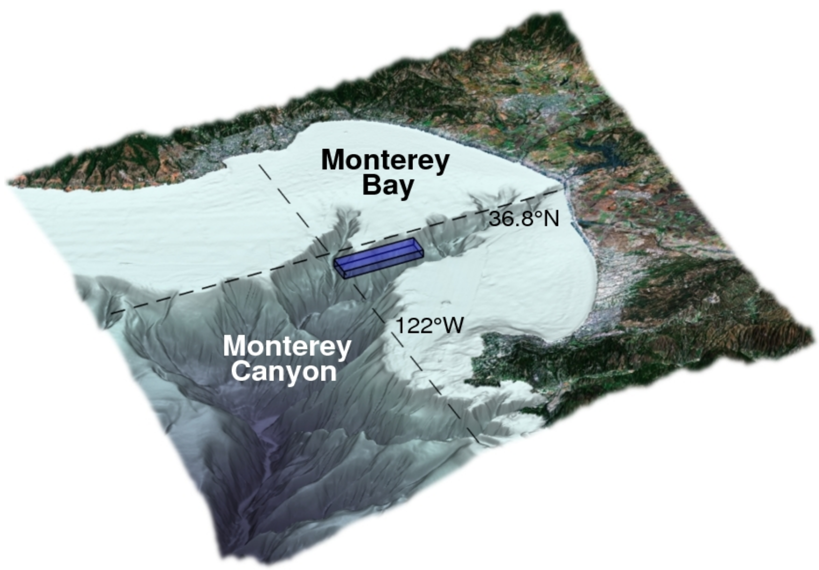

Figure 2. Environmental setting for testing mobile autonomous process sampling (MAPS) in Monterey Bay, California. The blue box shows the AUV survey domain with dimensions 7.8 km E-W, 1.2 km N-S, and 0-170 m in the vertical. Shading differentiates the continental shelf and the Monterey Submarine Canyon, which reaches depths of ~1000 m below the survey domain.

MAPS missions were conducted on 10 January and 10 and 13 November 2008. Following each ~7-hour survey, water samples were immediately returned to shore and transferred into clean carboys. Samples were then concentrated to 50 ml using a 100 µm mesh filter and sterile seawater for rinsing. All water samples were processed and analyzed using previously described molecular probes and sandwich-hybridization assays (Scholin et al. 1999, Goffredi et al. 2006, Jones et al. 2008). The molecular analyses applied in this study allowed detection of crustaceans, polychaetes, and mollusks.

Remote sensing data from satellite sensors and shore-based HF radar were used to examine event-driven transport phenomena related to the observed INL and larval variability. Methods for processing these remote sensing data have been published (Paduan et al. 2006, Ryan et al. 2008).

Assessment

During the first experiment, INL waters were detected in the pycnocline in all AUV profiles, and five INL samples were acquired (Fig. 3). Because the optical properties for INL detection in a vertical profile were first encountered within the gradients of INL upper and lower boundaries, samples were preferentially acquired as the AUV traversed these boundaries (Fig. 3b). The crustacean probe results were significant in all samples, while polychaete larvae were detected only in the two samples nearest the upper and lower INL boundaries (Table 1; Fig. 3b).

The second experiment permitted advancements in sampling and hypothesis testing. INL samples were acquired on multiple days to examine temporal variability, and they were acquired both within and immediately outside of INL patches. Modification of sample triggering control also allowed sample acquisition closer to the center of INLs (compare Fig. 3b and 4b).

Specifically, triggering control was separated into two steps: (1) activation of a ready state when an INL was detected, and (2) triggering of the sample acquisition only when the INL signal strength began to decrease, as would occur when passing the layer peak.

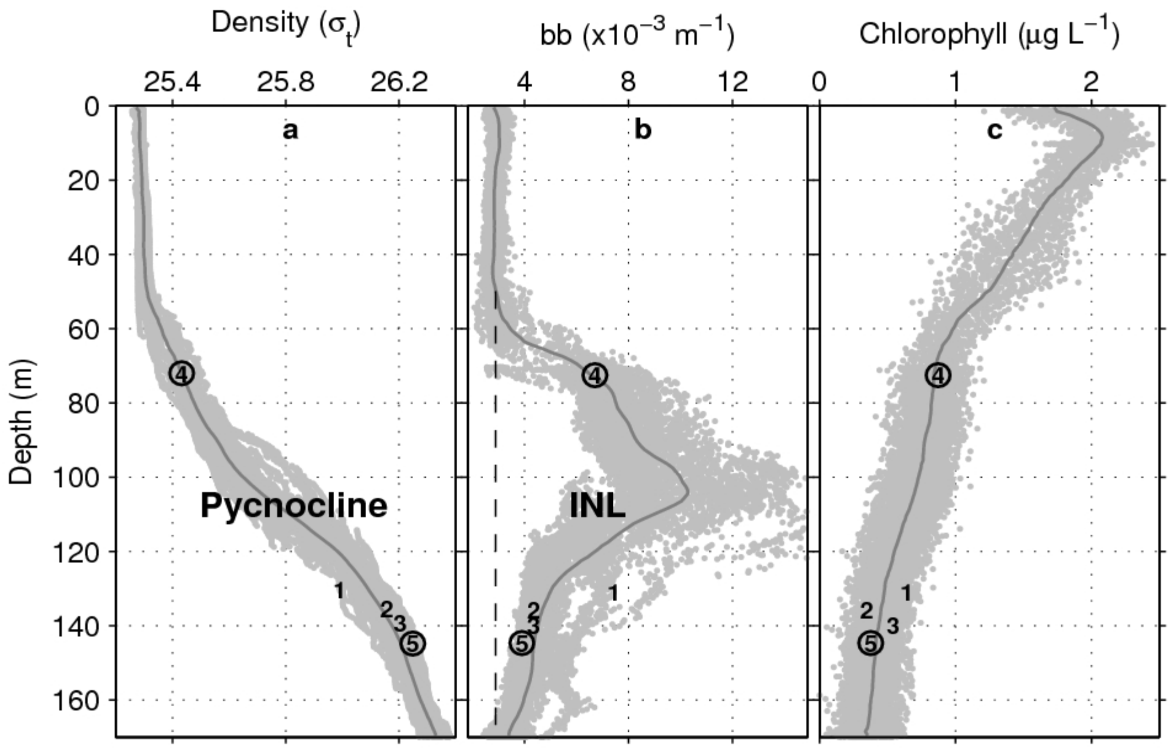

Figure 3. An INL observed in the pycnocline throughout the survey domain (Fig. 1) on 10 January 2008. Mean profiles (dark gray lines) are shown relative to all profile data for a) density, b) optical backscattering at 470 nm, and c) chlorophyll concentration. The dashed line in (b) defines the average optical backscattering level of the mixed layer; the INL is identified as the region of elevated optical backscattering below the homogeneous shallow mixed layer (below ~50 m). Numbers identify the depths and properties of water samples on which molecular analyses were conducted; circled numbers indicate detection of polychaete larvae (Table 1).

Major changes were observed in oceanographic conditions between 10 and 13 November. Decreased temperature and increased salinity resulted in a significant density increase throughout the upper 100 m (Fig. 4a). Observations from a mooring 3 km west of the AUV survey domain showed intensification of upwelling-favorable (poleward) winds during 10-13 November and hydrographic changes like those observed in the AUV surveys (mooring data not presented). These changes indicated movement of recently upwelled waters into the study site.

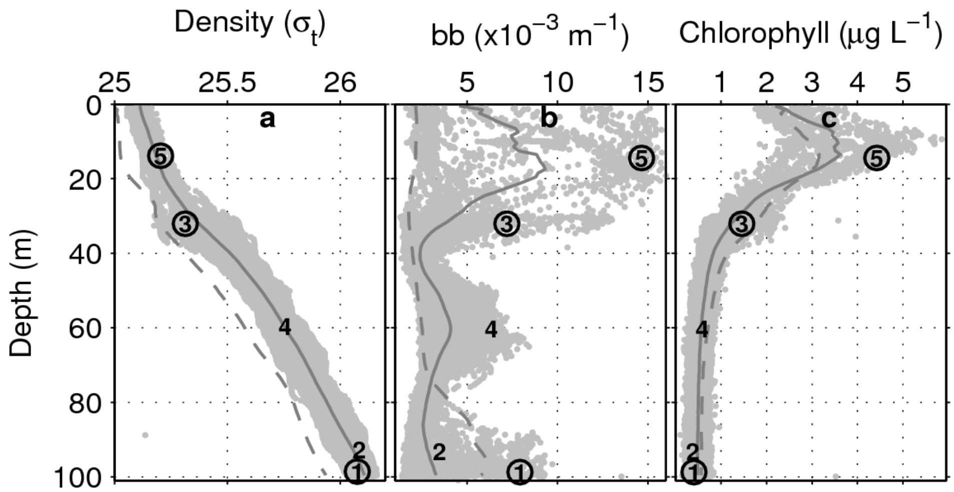

Figure 4. Significant changes in water mass distributions and INL intensity, linked to larval detection in INLs, 10-13 November 2008. Data presentation is as in Fig. 3. Gray data points show the full data set for 13 November only; dashed lines are mean profiles for 10 November. Numbers identify the depths and properties of water samples on which molecular analyses were conducted; circled numbers indicate detection of polychaete larvae (Table 1).

Accompanying the 10-13 November hydrographic shift were changes in the intensity and vertical distribution of INLs. INL patches were detected over a greater depth range on 13 November, when they were found between ~15 and 100 m, than on 10 November, when they were only below 80 m (Fig. 4b). By 13 November, average optical backscattering (bb) increased significantly above 80 m depth, with peaks centered near 15 and 60 m depth, and bb decreased below 80 m. Because SPM optical signal in the surface mixed layer is influenced by phytoplankton and other biogenic constituents, INLs are more readily discernible below the mixed layer. When INLs are transported into the mixed layer, information on temporal variation in the ratio of optical backscattering to chlorophyll fluorescence can aid discrimination. While the average chlorophyll profile remained very similar between 10 and 13 November (Fig. 4c), average bb increased significantly in parts of the water column, by a factor of up to ~ 5 x between 15 and 20 m (Fig. 4b). These patterns indicate a major increase in SPM of the surface mixed layer that was not caused by increased phytoplankton abundance, and it is consistent with the introduction of an INL into the surface mixed layer.

The in situ data showing changes in regional water mass distributions and INL activity were supported by remote sensing data. Satellite imagery showed a plume of whitish waters emanating from the Point Año Nuevo upwelling center (Fig. 5). The brightest waters were the coldest (label c), linking them to upwelling. A filament of this sediment-laden water extended into Monterey Bay and the AUV survey domain (box in Fig. 5), and southward past the bay. The indication of sediment loading was supported by MODIS derived products (not presented), including elevated particulate backscatter and calcite concentrations throughout the contiguous upwelling plume and filament. Average HF radar surface velocity during 10-13 November showed the southward transport of sediment-laden waters and convergent flow in the filament transport zone (Fig. 5).

As in the January experiment, the general crustacean probe results were significant for all samples from both November surveys (Table 1). The most interesting larval signal was the absence of polychaetes in INL samples from 10 November, before the upwelling pulse, and their presence in INL samples from 13 November, coincident with the environmental and INL changes linked to the upwelling pulse (Figs. 4, 5; Table 1). Detection of barnacle and mussel larvae also increased between 10 and 13 November (Table 1). Polychaete larvae were detected in the samples having the highest particle backscattering, near 15, 35 and 100 m depth (circled in Fig. 4b). The samples without significant signal for polychaete larvae (Fig. 4b) were sampled immediately outside of an INL patch (#2), and within a distinct INL patch centered at 60 m depth (#4). Of all INL samples, #4 had the lowest ratio of red:blue backscattering, suggesting a somewhat different color, and possibly sediment composition, in this spatially isolated patch.

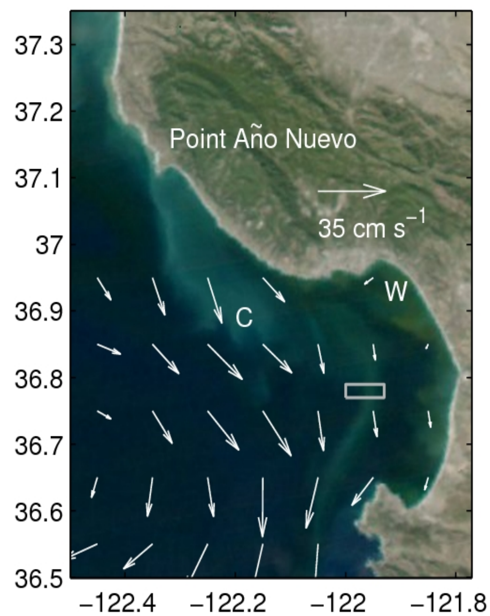

Figure 5. Coastal ocean dynamics underlying the observed oceanographic, INL and larval variability. The true-color satellite image is from the Moderate Resolution Imaging Spectroradiometer (MODIS) on 14 November 2008, and surface velocity vectors are averaged from hourly CODAR measurements of 11-13 November 2008. The gray rectangle shows the AUV survey domain, as in Fig. 2. Bright waters emanating from the upwelling center at Point Año Nuevo, and extending in a filament across the AUV survey domain, indicate sediment transport forced by wind-driven upwelling (see text). The label c and w indicate the locations of the coldest and warmest sea surface temperature, which differed by 3°C.

Of the organisms targeted with molecular probes, polychaete larvae exhibited the clearest patterns. Polychaetes comprise the largest component of soft-sediment infauna, yet the complete life history is known for only about 5% of the > 8000 described polychaete species (Ramey, 2008). Presence of polychaete larvae only in INL samples, and not in water sampled immediately outside of an INL patch, suggest that INLs function as transport vehicles for their larval stages. Absence of polychaete larvae in INL patches sampled near the shelfbreak immediately before an upwelling pulse, and their presence in a more patchy INL environment during an upwelling pulse define a linkage between benthic habitat of an upwelling center and event-driven larval transport into boundary current and bay environments.

The data showed significant lateral and vertical dispersion of larvae transported with INLs. The filament of sediment-laden waters containing polychaetes flowed at least 100 km from its origin at Point Año Nuevo, extending both into Monterey Bay and southward past the bay (Fig. 5). Detection of polychaetes in INL patches sampled between ~15 and 100 m also suggests significant vertical dispersion, consistent with an episodic transport event in which vertical circulation was enhanced. In addition to the enhanced vertical flows of the upwelling source region at Point Año Nuevo, strong vertical circulations would have occurred in the convergent flow evident in the zone of filament transport. These results offer a glimpse of a larger paradigm for dispersal – that small and mesoscale dynamics having enhanced lateral and vertical transports (fronts, jets, eddies) create a prism through which radiation of transport pathways can occur. These different pathways, in turn, expose larvae to different planktonic habitat with regard to physical-biological interactions, trophic dynamics, temperature (metabolic rates), and recruitment potential.

Discussion

The aspect of marine ecology that we approached with the newly developed MAPS system, population connectivity, is extremely challenging. By their very nature as episodic transport events that couple near/benthic habitat to the greater pelagic environment, INLs have a potentially significant role in the population connectivity of benthic species having pelagic larval stages. MAPS detected, mapped, and sampled INLs in each of the field studies. Recognizing the boundary sampling inclination of the initial INL model in the first survey, we improved sample acquisition control to more consistently acquire samples from INL centers. Because of the effective INL sampling by MAPS, we could apply molecular methods to precisely targeted water samples and identify larval constituents. Because these samples were acquired within the environmental context provided by AUV sensors and a greater coastal ocean observing system, we could link larval patterns to oceanographic processes. The preliminary data set produced by this integration of autonomy, oceanography and molecular ecology yielded insights into the constituents, dynamics and scales of INL transport, and thereby a compelling glimpse of the role of INL transport in population connectivity. The MAPS system is being applied for more intensive INL surveying to more thoroughly study this multidisciplinary, multifaceted process.

The methodological requirements of the MAPS application demonstrated here are representative of the requirements for advancing studies of many other important coastal ocean processes, for example episodic harmful algal blooms (HABs) or development of riverine and estuarine plumes that follow intense flushing of a land drainage basin. The stochastic nature of complex marine ecosystems and the inability to adequately sample these dynamic environments necessitate advanced methods that intelligently focus limited observing and sampling resources when and where collection of data and samples are most needed. The MAPS system represents a widely applicable approach to meeting this fundamental need.

Comments and Recommendations

The critical requirements for applying MAPS to diverse problems in coastal ocean science include (1) a robust AUV with a sensor suite sufficient to detect processes of interest, (2) a water sampling system with appropriate sample attributes (sample volume, number, avoidance of contamination), (3) a data set sufficient to develop an accurate model for target detection, and (4) development of adaptive AUV control strategies appropriate to the process of interest, e.g. increasing spatial resolution of mapping when a target feature is detected. Expanding the application of MAPS will benefit from other developments. Two-way satellite communication with the AUV, a capability recently demonstrated with the MAPS system, allows shore-side scientists to optimize or alter AUV survey characteristics based on real-time information coming from the AUV or from other ocean observing system assets. AUV onboard sample processing will support real-time alert capabilities for processes such as HABs that may have immediate human health or fisheries impacts. State estimation models used to detect features may be improved by continuously refining feature identification as new data becomes available, and by incorporating greater plasticity for adapting to variable signal intensities and tracking multiple target states simultaneously. Ongoing development of molecular probes and methods will target a broader spectrum of marine life and access more specific genes to identify organisms at lower taxonomic levels and to assess metabolic activities.

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Table 1. Sandwich hybridization assay (SHA) absorbance levels for capture probes (A_{450}) for larval detection in AUV-acquired water samples from intermediate nepheloid layers (INLs).

Date	Sample Number	Sample Volume (L)	Crustacean, general	Polychaete, general	Barnacle, general	Mytilus spp.
1/10/2008	1	0.9	1.20	-		-
1/10/2008	2	0.9	0.61	-		-
1/10/2008	3	0.9	0.56	-		-
1/10/2008	4	0.9	0.86	0.11		-
1/10/2008	5	0.9	0.96	.14		-
11/10/2008	1	4.0	1.05	-	.12	-
11/10/2008	2	2.0	0.42	-	-	-
11/10/2008	3	4.0	3.56	-	.19	.13
11/10/2008	4	4.0	2.21	-	-	-
11/10/2008	5	2.0	3.11	-	.12	.13
11/13/2008	1	3.0	1.46	.12	.15	.17
11/13/2008	2	2.0	1.15	-	.13	.10
11/13/2008	3	4.0	2.44	.10	.12	.13
11/13/2008	4	4.0	0.45	-	.12	.11
11/13/2008	5	4.0	3.01	.12	-	.11