A review of zooplankton communities in the Massachusetts Bay/Cape Cod Bay system

Massachusetts Water Resources Authority

Environmental Quality Department Report ENQUAD 2003-06



Citation:

Kropp, RK, JT Turner, D Borkman, S Emsbo-Mattingly, CD Hunt, and KE Keay. 2003. A review of zooplankton communities in the Massachusetts Bay/Cape Cod Bay system. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2003-06. 39 pp plus appendices.

A REVIEW OF ZOOPLANKTON COMMUNITIES IN THE MASSACHUSETTS BAY/CAPE COD BAY SYSTEM

submitted to

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June 2003

EXECUTIVE SUMMARY

One concern about the Massachusetts Water Resources Authority's (MWRA) Massachusetts Bay effluent discharge was whether it might adversely change the zooplankton community in Massachusetts Bay. Therefore, MWRA developed a zooplankton threshold for its outfall monitoring Contingency Plan. That threshold assumed the estuarine copepod genus Acartia was food limited, and therefore an increase in food offshore in response to the outfall relocation could result in increased Acartia abundance near the outfall. The threshold was a shift towards an inshore community in the offshore waters, measured as an increase in abundance near the outfall of either or both of A. hudsonica and A. tonsa, the two Acartia species found in Boston Harbor. Subsequently, it was determined that Acartia species are generally restricted to harbors and estuaries not because they have a selective advantage at higher levels of nutrients, but because their development requires the lower salinities found in estuaries. Therefore, Acartia abundance is not a good indicator of nutrient effects on zooplankton, and the Acartia threshold should be deleted from the Contingency Plan. Therefore, new analyses of the MWRA zooplankton data set were conducted to investigate whether or not properties of the zooplankton communities in the Bays suggested alternative thresholds that might be useful in monitoring the potential effects of the outfall discharge on the communities. Also, the hypothesis that zooplankton in the Bays resulted form the transport of individuals from the north to the south was tested.

The results of the analyses demonstrated that the zooplankton communities that occur in Massachusetts and Cape Cod Bays are not unique to the Bays, but are small components of the larger community characteristic of the Gulf of Maine. These zooplankton communities are numerically dominated by several species of copepods, all of which have widespread distributions in the Gulf of Maine or in the waters of the northwest Atlantic. Furthermore, the communities are numerically dominated by smaller zooplankters such as unidentifiable copepod developmental stages (nauplii and copepodites), and adults of the small, ubiquitous copepod *Oithona similis*. Larger taxa are also important in the Bays and include the estuarine species *Acartia tonsa, Acartia hudsonica*, and *Eurytemora herdmani*, as well as oceanic species *Calanus finmarchicus, Paracalanus parvus*, and species in the genera *Centropages* and *Pseudocalanus*. Meroplankton are seasonally important constituents of the zooplankton communities. The results supported here indicated that there is an overall high degree of similarity within most of the regions sampled in the Bays. This observation generally supports earlier studies that showed zooplankton communities were very similar over scales of about 10 km.

The zooplankton community structure in the Bays showed strong seasonal patterns that appear to be related primarily to intra-annual fluctuations in temperature. Colder-water taxa, such as barnacle nauplii and *Calanus finmarchicus*, are more abundant relatively early in the year than later, whereas taxa abundant at warmer temperatures, such as *Acartia tonsa*, *Centropages hamatus*, and *Paracalanus parvus*, reach peaks in abundance during summer. Patterns for some taxa, such as *Oithona similis* and copepod nauplii, are less clear. Some components of the communities, e.g., *Calanus finmarchicus* abundance, may respond to large-scale factors such as the North Atlantic Oscillation. Also important in influencing zooplankton communities in the Bays is the periodic occurrence of large numbers of zooplankton predators such as the ctenophore *Mnemiopsis leidyi*, which can dramatically reduce the abundance of many zooplankton taxa.

No evidence was found to support a conveyor belt hypothesis that would explain variations in zooplankton abundance as resulting from periodic infusions of individuals into northern Massachusetts Bay with subsequent transport to the south. Peak abundances of several taxa are often coincident at the boundary stations, the nearfield region, and in Cape Cod Bay. They also may occur earlier in the year at

the southern than at the northern stations. Similarly, there is no consistent north-south sequence in peak abundances of some taxa.

During the two years of monitoring since the initiation of effluent discharge through the relocated MWRA outfall, there have not been any apparent effects of the discharge on zooplankton abundance or community structure. Both were within the previously-established broad envelope-of-variability recorded during the baseline period.

Based on the analyses presented here, the development of a threshold that can effectively capture shifts in zooplankton species or abundance at local or regional scales that may be related to the relocation of the outfall is highly unlikely. The zooplankton in the system do not show a systematic pattern of occurrence from north to south in the Bays, generation times are long relative to the average transport time in the Bays, and zooplankton community or abundance responses to primary production as measured by chlorophyll biomass are not apparent in the data. The zooplankton communities tend to respond simultaneously to large-scale environmental factors at regional scales rather than local ones. Thus, defining a meaningful threshold for the zooplankton community is not possible. However, MWRA will continue to evaluate the zooplankton community at scales appropriate to the response features observed in this data set to ensure that potential outfall-related effects are detected.

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1.0 INTRODUCTION

One concern about the Massachusetts Water Resources Authority's (MWRA) Massachusetts Bay effluent discharge was whether it might adversely change the zooplankton community in Massachusetts Bay. Therefore, MWRA developed a zooplankton threshold for its outfall monitoring Contingency Plan (MWRA 1997). That threshold assumed the estuarine copepod genus *Acartia* was food limited, and therefore an increase in food offshore in response to the outfall relocation could result in increased *Acartia* abundance near the outfall. The threshold was a "shift towards an inshore community" in the offshore waters, measured as an increase in abundance near the outfall of either or both of *A. hudsonica* and *A. tonsa*, the two *Acartia* species found in Boston Harbor. Subsequent review (Libby *et al.* 1999) found that *Acartia* species are generally restricted to harbors and estuaries not because they have a selective advantage at higher levels of nutrients, but because their development requires the lower salinities found in estuaries (Tester & Turner 1991). Therefore, *Acartia* abundance is not a good indicator of nutrient effects on zooplankton, and the Outfall Monitoring Science Advisory Panel (OMSAP), the Environmental Protection Agency (EPA) and the Massachusetts Department of Environmental Protection (DEP) recommended that the *Acartia* threshold be deleted from the Contingency Plan. In place of an *Acartia*-based Contingency Plan threshold, OMSAP recommended:

"Since the Massachusetts and Cape Cod Bays system flows like a 'conveyor belt' from north to south, MWRA should develop a method for analyzing the current data spatially and temporally to contrast differences between the northern boundary stations and Cape Cod Bay." (OMSAP 2000).

This recommended evaluation was incorporated into the Contingency Plan (MWRA 2001). The "conveyor belt" hypothesis referred to by OMSAP suggested that MWRA's zooplankton data might reflect the overall counterclockwise circulation in the Bays, such that a population of zooplankton would be advected in at the northern boundary, transported through the nearfield (potentially receiving an inoculum of effluent nutrients), and be transported southward, ultimately into Cape Cod Bay. OMSAP suggested that the timing of peaks in important zooplankton species could be sequential, with taxa peaking first at the northern boundary, later in the nearfield and southern Massachusetts Bay, ultimately reaching Cape Cod Bay.

In further discussion with OMSAP (December 2000), suggestions were made for the framework of the evaluation, including:

- 1. A specific question/hypothesis be developed to guide the analysis (e.g., growth of adult and changes in stages across the study area relative to the transport scales of the Bays). The major question is whether Cape Cod Bay zooplankton can be predicted from northern boundary station data.
- 2. Complete a literature review and summarize succinctly the results prior to proceeding with the statistical analysis (e.g., summarize the findings of previous MWRA zooplankton synthesis) and place this information into the context of the conveyor belt hypothesis.
- 3. Use as much age structure data as defensibly feasible for the species evaluated.
- 4. Incorporate other diagnostic variables into the statistical analysis (i.e., salinity, temperature chlorophyll biomass).
- 5. Incorporate a succinct summary of the circulation patterns and scales into and through the Cape Cod Massachusetts/Cape Cod Bay system.
- 6. Define better the concept of "conveyor belt" transport in this system relative to the biological questions and the physical aspects.
- 7. Conduct the multivariate statistical analysis with and without the Harbor Stations in the data set. Use these data to demonstrate the separation of the Harbor and offshore zooplankton composition.

This report constitutes MWRA's evaluation. Chapter 1 includes a summary of previous work on the Massachusetts Bay zooplankton community, a description of the biological characteristics and life cycle information of relevant zooplankton taxa, and a summary of published molecular based studies on species similarity in the Gulf of Maine/Massachusetts Bay area and discussion of implications to the communities found in the Bay and to the conveyor belt theory. The detailed findings and results are presented in Appendix B. The statistical analysis and results are presented in Chapters 2 (Methods) and 3 (Results). Chapter 4 (Discussion) provides an update of the circulation of Massachusetts Bay and discusses the findings of the literature review and statistical analysis. Chapter 5 considers the implications of the results relative to a zooplankton threshold and recommendations regarding the zooplankton threshold and zooplankton monitoring in general.

1.1 Previous Zooplankton Reviews

MWRA sponsored two previous reviews of the zooplankton communities in Massachusetts Bay and the Gulf of Maine region. The first (Cibik *et al.* 1998) reviewed baseline (1992–1995) data for phytoplankton and zooplankton communities in Massachusetts Bay, Cape Cod Bay, and Boston Harbor to characterize conditions prior to relocation of effluent discharge from Boston Harbor to approximately 15 km offshore in Massachusetts Bay. During this review, Cibik *et al.* raised several potential issues regarding data comparability in the multi-year dataset that were subsequently discussed in detail in the 1998 Annual Water Column Report (Libby *et al.* 1999) and have been resolved. Cibik *et al.* characterized the Massachusetts Bay zooplankton community as showing strong regional, annual, and seasonal variation in abundance and in species composition and concluded that boreal species were most abundant early in the year, while warm water species predominated later. They reported that the coastal areas were characterized by *Acartia* spp. and the offshore areas by *Calanus* and *Pseudocalanus*. Cibik *et al.* advanced the *Acartia* hypothesis as a potential threshold parameter that could indicate that the nearfield zooplankton community had shifted towards a coastal community in response to eutrophication caused by the outfall discharge. Cibik *et al.* also recommended that MWRA sponsor a regional review of historical zooplankton data to better understand the community in Massachusetts Bay.

Lemieux *et al.* (1998) conducted the historical review recommended by Cibik *et al.*, and sponsored by MWRA, to examine broad-scale seasonal, annual, and regional zooplankton community patterns in the Gulf of Maine system, including Massachusetts and Cape Cod Bays. The study reviewed and analyzed data from many sources dating back to Bigelow's pioneering work in the 1920s. Lemieux *et al.* drew four primary conclusions from the review.

- Zooplankton patterns in Massachusetts and Cape Cod Bays were similar to those recorded elsewhere the Gulf of Maine and Georges Bank, except for coastal-offshore differences in Massachusetts Bay and somewhat different seasonal succession patterns between northern versus southern Massachusetts Bay.
- Comparisons of the Marine Resources Monitoring, Assessment, and Prediction Program
 (MARMAP) and MWRA data sets revealed that zooplankton taxonomic composition in
 Massachusetts Bay was similar to that in Cape Cod Bay, but that composition in Cape Cod
 Bay was more similar to that in coastal than in offshore Massachusetts Bay.
- Similarities between the MARMAP and MWRA data sets support use of MARMAP data to augment that of the MWRA.
- The Cape Cod Bay zooplankton community is not unique and the dense patches of zooplankton where whales have been observed to feed are likely based primarily on interaction of physical and behavioral factors that typically concentrate zooplankton.

1.2 Biology of Key Zooplankton Taxa

Plankton, by definition, drift through the oceans at the mercy of ocean currents. Thus, the plankton community in any given open-water location may consist primarily of transient individuals that are simply being carried through the area. Massachusetts Bay and the rest of the Gulf of Maine system is highly advective, with the Maine Coastal Current flowing from the north and east along the coasts of Maine, New Hampshire, and Massachusetts, exiting the system on both sides of Stellwagen Bank, and flowing to the east along the northern flank of Georges Bank (Bigelow 1927). Evidence for the transient nature of the zooplankton community in the MWRA sampling area is provided not only by physical oceanographic observations, but also from recent studies of copepod population genetics. Therefore, any large-scale impact on the zooplankton of Massachusetts Bay, whether of anthropogenic origin such as outfall discharge, or a natural event such as a catastrophic trophic cascade caused by ctenophore predation (Zaitsev 1992; Kideys 1994), would likely be short-lived. Before any long-term effects could be realized, the zooplankton populations involved would likely be carried out of Massachusetts Bay toward Georges Bank, and the area would be restocked by zooplankton transported from the north by the Maine Coastal Current.

Boston Harbor is partially isolated from the Maine Coastal Current; therefore, its zooplankton assemblages differ from those in Massachusetts Bay and are more influenced by "embayment" parameters such as lower salinities, warmer summer temperatures, shallower depths (which may influence copepod resting egg cycles and meroplankton retention), and higher turbidity. Thus, the Harbor is dominated by *Acartia* spp., *Eurytemora herdmani*, and meroplankton, whereas the Bay is dominated by plankton assemblages that are similar from Nova Scotia to Georges Bank because they are all part of the same physical oceanographic conveyor belt.

This section discusses general features of the biology of the zooplankton species that comprise the Massachusetts Bay zooplankton community, some of which may be useful in estimating the likelihood that certain taxa could be affected by outfall discharges. These are summarized in Table 1 for the major zooplankton species found in Massachusetts Bay. These species are characterized according to general distribution of the species in the Northwest Atlantic Ocean, abundance and seasonality in the Gulf of Maine, abundance and seasonality in Massachusetts Bay and Cape Cod Bays, breeding and generation time, and feeding behavior. Notes relevant to the evaluations undertaken in this report are also included. A more detailed discussion and references is included in Appendix B. Additionally, this section includes, for taxa where information is available (*Calanus finmarchicus*, *Pseudocalanus* spp., and *Acartia tonsa*), a summary of evidence from studies of population genetics that provide information relevant to the understanding of the Massachusetts Bay and Cape Cod Bay zooplankton communities.

Table 1. Summary of selected biological features of the Massachusetts Bay zooplankton community

Code Copepods	General Distribution	Abundance in the Gulf of Maine	Abundance in Massachusetts Bay and Cape Cod Bay	Breeding/ Generation Time	Feeding	Notes
Acartia hudsonica	Chesapeake Bay to Labrador; mainly embayments	Year-round, peak summer/fall;	Sporadic, usually low #s; most peaks in summer, late summer	Narragansett Bay forms diapause eggs at >16 °C, hatch only after chilled to 4–6 °C	Omnivorous	Can adjust feeding to fit food environment
Acartia tonsa	West Atlantic; Canada– Caribbean; estuarine	Peak summer/fall; absent in winter	Summer, late summer, fall only	3–4 weeks in Long Island Sound; summer; north of Cape Cod forms diapause eggs at 14–15 °C, hatch when spring temp >10 °C	Omnivorous	Can adjust feeding to fit food environment
Calanus finmarchicus	Amphi- Atlantic, cooler waters	Oceanic; year-round, peak in Feb- June	Very low numbers Oct–Mar; peaks can occur Apr– June	Gulf of Maine, ~2½ months nauplius—final molt; lives ~4 months	Omnivorous; wide variety of food	NW Atlantic individuals comprise a single population; abundance cycles may be tied to North Atlantic Oscillation
Centropages hamatus	Amphi-Atlantic temperate; neritic in GoM	Georges Bank: abundant late summer fall	Before 2000, absent most spring, peak late summer–fall. After 1999, low #s in spring, may peak summer–fall	21–25 d @ 12– 13 °C; produce resting eggs to overwinter	Omnivorous; major predator on copepods	
Centropages typicus	Amphi- Atlantic; North America, Cape Sable to Chesapeake Bay; neritic in GoM	Low abundance spring–early summer; peak late summer-fall	Low abundance spring-early summer; peak late summer-fall	Breeding only above 8–10 °C; 22 d @ 18–19 °C	Omnivorous; major predator on copepods; feeding sensitive to food fluctuations	Bigelow suggested 30 % may be lower limit
Copepod Nauplii	_	_	Year-round; peak late spring— summer	_	Omnivorous	MWRA #s may be mostly O. similis
Eurytemora herdmani	North America; coastal/harbors	Summer-fall; harbors	Sporadic; summer-late summer	?	Selective herbivore	Estuarine species
Metridia lucens	North Atlantic, temperate and boreal, not Arctic	Year-round; SW Gulf Feb-Apr	Low #s; summer- late summer; occasionally spring	?	Omnivorous; but voracious on copepod eggs/nauplii; Calanus fecal pellets	Luminescent; co- occurs with C. finmarchicus
Microsetella norvegica	widespread	Year-round; low #s	Year-round; low; late summer	32 d @ 20 °C	Phytoplankton?	Small
Oithona similis	worldwide	Year-round; abundant	Year-round; abundant; peak summer to late summer	20–21 d @ 15–16 °C	Broadly omnivorous	Small; euryhaline, eurythermal
Oithona atlantica	Circumglobal; oceanic	Sporadic; low #s	Sporadic; low #s, peak any season	Unknown	Unknown	Cold water species
Paracalanus parvus	Cosmopolitan, except polar	Spring-fall	Sporadic since 1995; fall "peaks"	< 30 d? (no specifics)	?	Tiny, smallest calanoid; euryhaline, eurythermal
Pseudocalanus spp.	Circumpolar	Year-round	Strong peak summer–late summer	Breeds Dec–July; 2 months @ 5– 10 °C	?	Includes <i>P. newmani</i> and <i>P. moultoni</i> ; 15–20 °C critical upper temp for <i>P. newmani</i>

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Code	General Distribution	Abundance in the Gulf of Maine	Abundance in Massachusetts Bay and Cape Cod Bay	Breeding/ Generation Time	Feeding	Notes
Paracalanus / Pseudocalanus copepodites	_	_	Low numbers most of year; peak in summer, occasionally late summer	_	?	(Lee et al. 2003)
Temora longicornis	Amphi- Atlantic; coastal	Year-round; peak Spring- fall	Year-round; peak summer-late summer	Late spring—early summer; Generation time?	Omnivorous	Lethal temp = 20 °C
Tortanus discaudatus	Brackish water?	?	Sporadic; low #s; summer—late summer	?	Carnivorous	
Non-copepods						
Cirripedia nauplii	Sporadically dominate inshore assemblages	Present April–May south-central Maine	Peak early-late spring; cool temperatures	_	Herbivorous	Peak occurs later in year as progress northward; release may be timed with peak phytoplankton bloom
Evadne spp. / Podon spp.	_	Neritic; peak in summer	Peak summer, late summer; occasionally fall; almost absent rest of year	Parthenogenic; populations may increase dramatically	Omnivorous on phytoplankton, zooplankton, detritus	
Penilia avirostris	Circumglobal in warm waters	Late summer peak in abundance	Sporadic; only Fall 1998 in nearfield samples	?	Omnivorous on bacterioplankton, small phytoplankton, also heterotrophic microflagellates	Excluded from analyses
Polychaete larvae	_	Probably year-round; peak spring- summer	Year-round; peaks usually early-late spring; low numbers late summer and fall (except 1998)	_	Herbivorous	
Oikopleura dioica	Amphi-Atlantic (?) common in Europe	Occurs most of year	Usually late spring; two peaks (late spring–fall- late summer) 1997–1999; virtually absent 2000, 2002	Life cycle marked by four events: hatching, shift of tail, gonad maturation, release of gametes, with three intervening periods of development (Touratier et al. 2003)	Filter feeder on small particles (to 0.1 μm)	
Salps	World-wide	Mostly summer	Usually late summer; only Fall 1998 in nearfield samples	Capable of very rapid population increases	Predators; feed on bacterioplankton and picoplankton	Excluded from analyses

Calanus finmarchicus

Biological notes. Calanus finmarchicus is perhaps the best known of all copepods, because of the extensive studies in British waters (reviewed by Marshall & Orr 1955). This species occurs in the North Atlantic from the Arctic to, in winter, Chesapeake Bay. It is broadly omnivorous, ingesting a wide size array of phytoplankton, protozooplankton and metazoan zooplankton prey (Turner 1984a), and adults as well as nauplii in some cases feed primarily on heterotrophic protists rather than phytoplankton (Levinsen *et al.* 2000, Turner *et al.* 2001). Individual females of this species exhibit considerable variation in feeding patterns (Turner *et al.* 1993), which are unrelated to food concentrations.

Calanus finmarchicus is an important species in the Gulf of Maine and Georges Bank regions because it is an significant component of the forage base of developing fish (Bigelow 1926, Wiebe *et al.* 2002) and for right whales. *C. finmarchicus* does not overwinter on Georges Bank, but may overwinter as juveniles in deep basins of the Gulf of Maine (Meise-Munns *et al.* 1990, Batchelder & Miller 1991), thus the source of animals to repopulate Georges Bank in spring is important to understand. Circulation patterns indicate that potential sources of *C. finmarchicus* for the Gulf of Maine include adjacent slope water (Miller *et al.* 1991), the Scotian Shelf (Sameoto & Herman 1990), and the Gulf of St. Lawrence (Herman *et al.* 1991, Plourde & Runge 1993).

Population genetics. *C. finmarchicus* mitochondrial DNA (mtDNA) haplotype frequencies, using 16S rRNA gene sequences, revealed moderate diversity levels in the Gulf of Maine (Bucklin 1995, Bucklin *et al.* 1996b, Bucklin & Kocher 1996). However, sample-to-sample variation (genetic "patchiness") did not distinguish among the Gulf of St. Lawrence, the Gulf of Maine, and Georges Bank, with the exception of a single sample from the northern Gulf of St. Lawrence, which might have resulted from advection from an adjacent oceanographic region such as from the Labrador Current via the Strait of Belle Isle (Bucklin *et al.* 1996b). Bucklin *et al.* (1996) also found low genetic diversity among individuals from the Norwegian Sea, but that there was significant diversity between Norwegian Sea and Gulf of Maine/Gulf of St. Lawrence samples. An additional study also revealed no significant differences in haplotype frequencies throughout the Gulf of Maine/Gulf of St. Lawrence region (Bucklin & Kocher 1996). Similar results were obtained by Kann & Wishner (1996) for studies of 16S rRNA genes of *C. finmarchicus* from the northern Gulf of Maine, Georges Bank and Great South Channel to the west of Georges Bank. Thus, *C. finmarchicus* in the Gulf of St. Lawrence, the Gulf of Maine, and Georges Bank most likely represents a single interbreeding population, with extensive gene flow throughout the region.

The low genetic diversity of *C. finmarchicus* in the Gulf of Maine and Norwegian Sea regions may reflect the impact of glaciation during the last 20,000 years (Bucklin & Wiebe 1998). The boreal/subarctic species *C. finmarchicus* may have experienced a 75% range reduction and latitudinal displacement to the south during the last glacial maximum 18,000 years ago, giving rise to a genetic bottleneck.

Pseudocalanus spp.

Biological notes. Many copepod genera contain sibling species that exhibit very subtle morphological differences and are virtually indistinguishable, even as adults, when examined by using routine light microscopy. That most individuals of a given copepod species in a zooplankton sample are nauplii and copepodites, both of which are particularly difficult to distinguish with microscopy, exacerbates the problem. Adults and juveniles of sibling species often co-occur in many areas further complicating the issue. Therefore, the life histories, ecological roles, distributions, and dynamics for such sibling species in a given ecosystem may be completely unknown.

The genus *Pseudocalanus* includes several sibling species that cannot easily be distinguished by using the secondary sexual morphological characteristics that distinguish most species of copepods (Frost 1989). Two such species, *P. moultoni* and *P. newmani*, have sympatric distributions in the northwestern Atlantic, including the Gulf of Maine and Georges Bank. *P. moultoni* is slightly larger than *P. newmani*, but size alone is not useful for separating adults of the two species (Frost 1989), much less copepodites. Prior to Frost's (1989) taxonomic revision of the genus, records for *Pseudocalanus* in the North Atlantic usually grouped all individuals into a single taxon assumed to be similar to the European species, *P. minutus*. Because of this taxonomic confusion, fundamental aspects of the geographic distributions, population structure, life history, and seasonal occurrence of *P. newmani* and *P. moultoni* are unknown (Bucklin *et al.* 1998a, 1999, 2001).

The distributions of these two species overlap, but differ somewhat. *P. newmani* is circumboreal and is abundant in coastal and oceanic waters of the North Atlantic and North Pacific, including off both coasts of North America and east Asia. *P. moultoni* is present off both coasts of North America, but is absent from Asia (Frost 1989). *P. moultoni* populations in the North Pacific and North Atlantic appear disjunct

(Frost 1989) and may be geographically isolated (Sevigny *et al.* 1989). *P. moultoni* distributions are primarily coastal, and on the Atlantic coast of North America, are restricted to waters between the New York Bight and Nova Scotia (Frost 1989). McLaren *et al.* (1989a, 1989b) concluded that while both species are present throughout the year over Browns Bank on the Scotian Shelf, they partition themselves somewhat seasonally. *P. moultoni* is more abundant in winter and spring, whereas *P. newmani* is more abundant in spring and summer. Both species occur in the MWRA samples, but have not been consistently distinguished throughout the program and are, therefore, considered a single taxon, *Pseudocalanus* spp.

Population genetics. Several genetic studies of *Pseudocalanus moultoni* and *P. newmani* distributions on Georges Bank (Bucklin *et al.* 1998a, 1999, 2001) have shown that the spring increase in *P. moultoni* over the crest of Georges Bank may result from the persistence of reproducing individuals and/or from advective transport from adjacent regions, whereas *P. newmani* were likely transported to Georges Bank from upstream regions on the Scotian Shelf and Browns Bank. The molecular evidence from Georges Bank is consistent with the biogeographic distributions described by Frost (1989) that *P. moultoni* is primarily a coastal species, and *P. newmani* is a cosmopolitan, oceanic species that also occurs in coastal waters (Bucklin *et al.* 2001).

McGillicuddy & Bucklin (2002) used a coupled physical-biological model, which uses an advection-diffusion-reaction equation for copepod concentrations, to examine the 1997 data for *Pseudocalanus* distributions on Georges Bank (reported in Bucklin *et al.* 2001) and concluded that the springtime distributions of *Pseudocalanus* spp. in the Gulf of Maine/Georges Bank region was driven by a complex mixture of hydrodynamic transport and species-specific population dynamics, including both growth and mortality.

Bucklin *et al.* (2003), using specimens from all over the world, examined DNA sequences for a 639 base pair region of mitochondrial cytochrome oxidase I (mtCOI) for 34 calanoid copepod species belonging to 10 genera in 2 families. Levels of mtCOI variation within species were small (1–4%), and were significantly less than those between species. However, molecular variation among the six described *Pseudocalanus* species was high (10–18%), despite the minimal morphological differences between these species. The small variation between conspecifics from different ocean basins suggests that gene flow between *Pseudocalanus* species populations is substantial, despite large dispersal distances, isolation of some populations over geological time scales, and present-day barriers to dispersal.

Acartia hudsonica / Acartia tonsa

Biological notes. The two species of *Acartia* that occur primarily in estuarine waters of eastern North America have received considerable research attention because of their accessibility to scientists. *A. hudsonica* is the more boreal of the pair, occurring from north of the mouth of Chesapeake Bay to Labrador (Turner 1981). *Acartia tonsa* occurs, at least seasonally, in estuarine waters of eastern North America from New Brunswick to the Caribbean (Turner 1981). Its global distribution in the western Atlantic extends as far south as the Falkland Islands (records summarized by Turner 1984).

The metazoan zooplankton of northeastern estuaries, for example Narragansett Bay (Jeffries 1962, 1967a), are usually dominated by the seasonally-alternating *Acartia* congeners. *Acartia hudsonica* is abundant from late fall to early summer, and *Acartia tonsa* is dominant in summer and fall (Frolander 1955, Martin 1965, 1970, Hulsizer 1976, Durbin & Durbin 1981, 1989). A similar situation occurs in the nearby estuarine waters of eastern Long Island (Turner 1982) and Cape Cod (Wheeler 1901, Fish 1925).

Many studies, using a variety of techniques, have shown *A. tonsa* and *A. hudsonica* to be broadly omnivorous, ingesting a variety of phytoplankton, microzooplankton, protozooplankton, and detrital food sources (see Turner 1984a, 2000, 2003, Turner & Roff 1993, and references therein). Also, grazing on natural phytoplankton is mostly non-selective, with different food items being ingested approximately in proportion to their abundance (Turner 1984b, Turner & Anderson 1983, Turner & Tester 1989a, 1989b).

Population genetics Although molecular evidence reveals that there is extensive gene flow for coastal or oceanic species such as Calanus finmarchicus and Pseudocalanus spp., the same is not the case for the estuarine copepod Acartia tonsa (Caudill 1995 as summarized in Bucklin et al. 1998b). Sequencing for mtDNA (16S rRNA gene) in individuals from estuaries on the east or Gulf coasts of the United States (Great Bay, New Hampshire; New Bedford Harbor, Massachusetts; Narragansett Bay, Rhode Island; Savannah River, Georgia; Port Aransas, Texas) over two years revealed that levels of molecular diversity were high and varied significantly among populations from different estuaries. For all the estuaries except Port Aransas, haplotypes could be mapped onto three statistically distinct lineages. These lineages were not restricted to a particular estuary, however, mtDNA variation revealed highly significant population genetic structuring of A. tonsa over small geographic distances. In particular, the genetics of A. tonsa populations differed significantly between New Bedford Harbor and nearby Narragansett Bay. In contrast to the distinct geographic differentiation, temporal variation in a given estuary was relatively stable among years. A. tonsa produces overwintering fertilized diapausing eggs, at least in the more temperate portion of its range, therefore, the high genetic diversity among estuaries suggests that A. tonsa retains high genetic diversity, as old and divergent mitochondrial lineages, likely as a result of population maintenance and protection from selection through benthic diapausing eggs (Marcus et al. 1994). The apparent restriction of gene flow between individuals in different estuaries, even adjacent ones in close proximity, supports distributional data (Turner 1981) indicating that that A. tonsa is an "embayment" animal, with limited dispersal through open coastal waters between adjacent estuaries.

Centropages hamatus

Kane (1996) used MARMAP data from 1977 to 1987 to summarize the interannual variation of *Centropages hamatus* for the northeastern United States continental shelf. After its annual low in winter abundance, this copepod increased slowly along the coast, expanding offshore with the northward progression of spring conditions. Maximum abundances were on Georges Bank in July. *Centropages hamatus* was generally prevalent in waters shallower than 61 m (Kane 1993), at surface temperatures of 12–17 °C, when chlorophyll levels were high and salinity was relatively low (27–36 PSU). During the decade of 1977–1987, *C. hamatus* increased in abundance in the Gulf of Maine and on Georges Bank, relative to other species, such as *Calanus finmarchicus*, *Pseudocalanus* spp., *Centropages typicus*, and *Metridia lucens* (Sherman *et al.* 1998), that were dominant contributors to zooplankton biomass (Kane 1993).

Centropages hamatus from waters near Woods Hole are omnivorous (Anraku & Omori 1963), and a simulation by Davis (1984a) suggested that this copepod was an important predator of smaller zooplankton. However, Conley & Turner (1984) found that in the Westport River Estuary on the southern coast of Massachusetts, *C. hamatus*, while omnivorous, ingested a higher proportion of its body carbon as natural phytoplankton (primarily small microflagellates) than as copepod nauplii (primarily *Acartia hudsonica* or *A. tonsa*).

Centropages typicus

Davis (1987) summarized the life history of *Centropages typicus* on Georges Bank. This species is typical of continental shelf waters, and in the Georges Bank/Gulf of Maine region is most abundant in fall. It is typically found in warmer surface waters above the thermocline (Clarke 1933). During rearing studies for taxonomic purposes (Lawson & Grice 1970), *C. typicus* had a generation time of 22 days at 18–19 °C. Davis (1987) suggested that in the Gulf of Maine, *C. typicus* had several generations during the fall on Georges Bank, and it was reported to have 5–6 generations per year off Delaware Bay (Deevey 1960) and 3–4 generations per year in Block Island Sound (Deevey 1952b).

Kane (1999), using the 1977–1987 MARMAP data, summarized interannual variations in abundance of *Centropages typicus*. Abundances were high in the fall, with declining abundances in winter. Abundances were highest near the mouths of major estuaries, usually where bottom depths were 20–

39 m. Interannual variability was substantial, but no clear long-term trends were detected. Counts on Georges Bank in autumn 1985 were nearly double all other years (Kane 1993), with extremely low values in 1986 and 1987. Additional data from Georges Bank from 1988 to 1996 indicated recovering abundances throughout most of the 1990's. Temperature and phytoplankton food appear to be key factors that determine *C. typicus* distributions and annual cycles. Abundances were high when temperatures exceeded 9 °C, and chlorophyll levels were above 1 mg m⁻³. Salinity variation appeared unimportant throughout the distributional range of *C. typicus* on the northeastern United States continental shelf.

Eurytemora herdmani

Jeffries (1967a) noted that *E. herdmani* is a "true estuarine" species, meaning that it propagates only in brackish waters, and is found in the open ocean only incidentally. Turner (1994) observed that within the MWRA sampling area, this species was recorded only for Boston Harbor, and not Massachusetts Bay or Cape Cod Bay. Since that report, *E. herdmani* has occurred sporadically in Massachusetts Bay, with a peak nearfield abundance of about 6,600 individuals/m³ occurring in July 2000. Turner (unpublished) also found that in the Newtown Creek sampling in New York Harbor, this copepod was most numerous at the station furthest up the Hudson River, particularly in spring. Jeffries (1967b) concluded that *Eurytemora* greatly outnumbers all other holoplanktonic forms during the winter-spring period when estuarine salinities are about 5–15 PSU, which is optimal for local *Eurytemora* species.

Oithona similis

The ubiquitous abundance of *Oithona similis* in epipelagic waters throughout the world (Gallienne & Robins 2001), which occurs over a wider range of temperatures and salinities than any other copepod species from coastal to oceanic and tropical to temperate to polar waters (Nishida, 1985), may be unique. In some locations, such as Massachusetts Bay, this species is the most abundant copepod year-round, over a temperature range sometimes exceeding 25 °C (Turner, 1994). *Oithona similis* is broadly omnivorous (reviewed by Turner 1984a). It feeds upon a variety of phytoplankton, heterotrophic protist, and copepod nauplii food sources, but primarily consumes ciliates and heterotrophic dinoflagellates (Nakamura & Turner 1997, and references therein). Nielsen & Sabatini (1996) found that specific egg production rates by *O. similis* in the North Sea in summer were correlated to abundance of heterotrophic protozooplankton, rather than to chlorophyll as was found for co-occurring *Paracalanus* species. Thus, the ability of *O. similis* to exploit the lower portion of the food size spectrum, which is more coupled to the microbial food loop than to phytoplankton blooms, may contribute to its maintaining a stable population almost continuously.

2.0 STATISTICAL METHODS

The analyses performed for this Task Order, and presented in this report, were designed to examine the zooplankton community in the Massachusetts Bay region to explore the conveyor belt hypothesis and to determine whether or not a likely indicator of possible effects of the outfall discharge on the community could be developed. The zooplankton data collected by MWRA from 1992 through 2002 were analyzed to identify the primary factors that structure the community. In particular, the regional, seasonal, and interannual variation in the community patterns and composition were identified. These patterns were used to evaluate whether or not the zooplankton community in the vicinity of the outfall was likely to suffer long-term effects from the discharge.

2.1 Zooplankton Stations Sampled and Surveys Conducted

The MWRA zooplankton database includes samples collected from 1992 through 2002. During that span, samples have been collected at various times from 22 stations (Figure 1). However, only five stations have been sampled every year of the monitoring (Table 2). One station, N04, was sampled every year except 1995. Generally two types of surveys have been conducted. Six farfield surveys have been conducted each year and have included all stations incorporated into the year-specific design. Nearfield surveys, which began in 1995, are now conducted 17 times per year and have included only those stations designated as "nearfield" ("N" stations). The stations included in that various types of analyses are listed in Table 2.

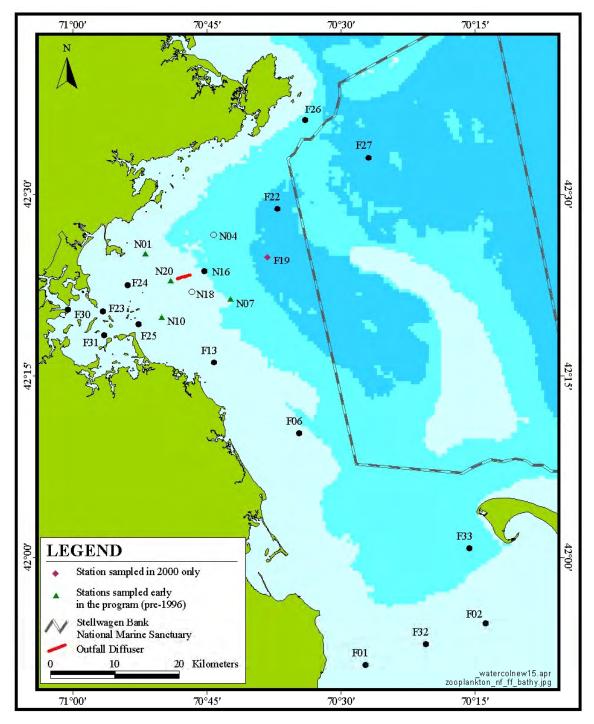


Figure 1. Stations in Massachusetts Bay sampled under the MWRA Outfall since 1992. Stations are characterized by when they were sampled, and by length of sampling.

Table 2. Stations included in the MWRA	zooplankton database, the years they were sampled, and
their use in the analyses.	

Station	Region	Years Sampled	Analytical Use
F01	Cape Cod Bay	1992-2002	PCA; FF Cluster
F02	Cape Cod Bay	1992-2002	PCA; FF Cluster
F06	Coastal	1995-2002	PCA
F13	Coastal	1992–2002 ¹	PCA; FF Cluster; NF Cluster
F19	Mid North Boundary	2000	PCA
F22	Mid North Boundary	2000-2002	PCA
F23	Boston Harbor	1992–2002 ²	FF Cluster
F24	Coastal Boston Harbor	1995-2002	PCA; NF, Season Cluster
F25	Coastal Boston Harbor	1995-2002	PCA; NF, Season Cluster
F26	Boundary	1995-2002	PCA
F27	Boundary	1995-2002	PCA
F30	Boston Harbor	1995-2002	None
F31	Boston Harbor	1995-2002	None
F32	Cape Cod Bay	1998-2002	PCA
F33	Cape Cod Bay	1998-2002	PCA
N01	Nearfield	1992-1994	PCA; NF, Season Cluster
N04	Nearfield	1992–1994; 1996–2002	PCA; FF, NF, Season Cluster
N07	Nearfield	1992-1994	PCA; NF, Season Cluster
N10	Nearfield	1992–1996	PCA; NF, Season Cluster
N16	Nearfield	1992–2002 ²	PCA; FF, NF, Season Cluster
N18	Nearfield	1997–2002	PCA; NF, Season Cluster
N20	Nearfield	1992–1994	PCA; NF, Season Cluster

2.2 Zooplankton Records

The MWRA zooplankton database contains about 70 taxa, some of which also contain lifestage and gender information. This list was reduced to exclude those taxa that would likely confound the analyses (Appendix B and subsequent discussions with MWRA). Most meroplankton were excluded because their abundance is driven by reproductive cycles of the benthic adults. Also excluded were taxa whose identifications were at a coarse level and likely include several species (e.g., *Pteropoda* spp., *Mysidacea* spp., *Amphipoda* spp.) and others whose occurrences were very sporadic and abundances were low (e.g., the ctenophore *Pleurobrachia pileus*). The final dataset included 16 copepod species or life stages and 7 noncopepod species (Table 3; Appendix B). After preliminary data analyses, two additional taxa (salps and *Penilia avirostris*) were excluded from the analyses because their occurrences in the dataset were extremely sporadic. Each taxon was also assigned a general association for harbor, oceanic, persistent species, or ephemeral.

2.3 Abiotic Factors

Five abiotic factors were included in the Principal Components Analysis because they were considered to have possible effects on the zooplankton community in the Massachusetts Bay system (Table 4). Data for all of the abiotic factors were obtained from the hydrographic profiles taken at the time the zooplankton sample was collected, thus are paired with the zooplankton data. To determine a single abiotic number to match the oblique zooplankton tow, the average value over the depth of the zooplankton tow (usually about 30 m except at very shallow stations) was calculated. Thus the abiotic values represent an average over the water column in which the zooplankton were collected. The averaging probably decreased the temperature separation that could occur in the summer as the net tows cross the thermocline and about half the tow was in warmer surface water and about half in cooler deeper water.

Table 3. Taxa included in the modified MWRA zooplankton dataset.

Taxon	Code	Data Included	General Association
Copepods	Code	Data Hiciadea	Association
Acartia hudsonica	Ahuds	Sum of all individuals	Harbor
Acartia tonsa	Atons	Sum of all individuals	Harbor
Calanus finmarchicus	Cfinm	Sum of all individuals	Oceanic
Centropages hamatus	Cham	Sum of all individuals	Harbor
Centropages typicus	Ctyp	Sum of all individuals	Oceanic
Copepod Nauplii	CopeN	Sum of all copepod nauplii, regardless of species	Persistent
Eurytemora herdmani	Eherd	Sum of all individuals	Harbor
Metridia lucens	Mluce	Sum of all individuals	Oceanic
Microsetella norvegica	Mnorv	Sum of all individuals	Oceanic
Oithona similis	Osim	Sum of all individuals	Persistent
Oithona atlantica	Oatl	Sum of all individuals	Oceanic
Paracalanus parvus	Pparv	Sum of all individuals	Oceanic
Pseudocalanus spp.	Pnewm	Sum of P. newmani, P. moultoni, and	Persistent
		Pseudocalanus spp.	
Paracalanus /	Pcop	Sum of copepodite stages of <i>Pseudocalanus</i> spp.	Persistent
Pseudocalanus		and Paracalanus spp.	
copepodites			
Temora longicornis	Tlong	Sum of all individuals	Persistent
Tortanus discaudatus	Tdisc	Sum of all individuals	Harbor
Non Copepods			
Cirripedia Nauplii	Cirr	Sum of barnacle nauplii	Ephemeral
Evadne spp.	Evad	Sum of Evadne nordmani, Evadne spp.	Harbor
Penilia avirostris	Pavir	Sum of all individuals	Excluded from
Dodon ann	Podon	Sum of Padan nahunhamaidas and Padan ann	analyses Harbor
Podon spp.		Sum of Podon polyphemoides and Podon spp.	
Polychaete spp.	Poly Odioi	Sum of polychaete larvae Sum of all individuals	Ephemeral
Oikopleura dioica	+		Ephemeral
Salps	Salps	Sum of Thaliacea and Salp spp.	Excluded from
			analyses

Table 4. Abiotic parameters included in the zooplankton principal components analyses.

Parameter	Code	Units	Association	Possible Biological Effects
Temperature	TEMP	° C	Seasonal effect	Metabolic and reproductive rates
Salinity	SAL	PSU	Coastal freshwater effect	Habitat or life cycle effects
Dissolved Oxygen	DO	mg/L	Seasonal effect	Primary productivity
Fluorescence	FLUOR	μg/L	Phytoplankton pigment level	Surrogate for zooplankton food
Transmissivity	TRANS	m ⁻¹	Particulates; Harbor influence	Surrogate for zooplankton food

2.4 Analytical Approach

2.4.1 Graphical Analyses

To examine the potential seasonal variation in the occurrence of zooplankton taxa and the possible "conveyor belt" effect on the zooplankton community in the Massachusetts Bay system, the abundances of various taxa were plotted by season for the nearfield data set (as described above) or a Cape Cod-Boundary data set that included stations F01, F06, and F27. Average abundance data per season were plotted in Microsoft ExcelTM spreadsheet software.

2.4.2 Principal Components Analyses

Principal components analysis (PCA) was employed to evaluate biotic (taxa) and abiotic (physical or chemical) variables that may reflect significant environmental effects attributed to the outfall pipe. PCA was used to succinctly visualize the intersample and intervariable relationships among the zooplankton data. PCA yields a distribution of samples in *n*-dimensional space, where *n* is the number of biotic and abiotic variables. The Euclidean distances between sample points on the PCA factor score plots are representative of the variance captured in each principal component (PC). Samples that cluster together are more similar and outliers are more distinct. A factor loading is calculated for each variable contributing to each PC. A crossplot of the factor loadings for the first two PCs reveals the individual variables responsible for the primary variance in each PC. PCA was performed by using Pirouette (Version 3.02; Infometrix, Inc., Seattle, Washington).

The zooplankton monitoring program included 28 primary biotic and abiotic factors (Tables 3, 4). The sample identifications were coded using "YYMMSSS"; where "YY" represents the year, "MM" represents the month, and "SSS" represents the sampling station identification. The data were normalized (raw counts of organisms were divided by the total number of organisms in each sample) to improve inter-sample comparability. In addition, the transformed data were z-score normalized to improve inter-analyte comparability. The abiotic variables were occasionally not determined. These data gaps required the omission of the associated samples from the PCA (Appendix C).

2.4.3 Cluster Analyses

Two types of cluster analyses were conducted; Q-mode to compare similarities among samples and R-mode to compare similarities among species (Boesch 1977). Abundance data were square-root transformed prior to the cluster analyses. The Bray-Curtis algorithm was used to determine similarities among samples of taxa, which then were clustered by using the unweighted pair group method. The software package BioDiversity Professional, Version 2 (© 1997 The Natural History Museum / Scottish Association for Marine Science) was used to perform the cluster analyses. The basic MWRA zooplankton dataset was modified for the various cluster analyses.

- The first analysis (called FF cluster in Table 2) was conducted to examine potential broad scale spatial or yearly zooplankton community patterns in the Massachusetts Bay system. This analysis included only those five stations (including Boston Harbor Station F23) sampled during farfield surveys for each year of monitoring and station N04, which was sampled every year except 1995. Station F13 sampled in 1995 was not included in the analyses because it was only visited during four surveys that year. The total abundances of each taxon for each station were summed within a year to reduce the effects of seasonal variability in abundance. This dataset was also used for selected individual station cluster analyses.
- The second analysis (NF cluster) was conducted to examine potential spatial or yearly zooplankton community patterns in the general nearfield area. This analysis included only the nearfield stations and farfield stations F13, F24, and F25, which are close to the nearfield. Only data from farfield surveys were used. The abundance of each taxon for each station was summed within a year. This data set was also used for the species (R-mode) cluster analysis.
- The third analysis (season cluster) was conducted to examine potential seasonal patterns in the zooplankton community in the general nearfield area. This analysis included the same set of stations included in the NF cluster analysis, but the average abundance for each species within a season was used because different numbers of stations were sampled within various seasons or years. Season labels, with their abbreviations and month sampled, were Early Spring (E_Spr, February), Spring (Spr, March), Late Spring (L_Spr, April), Early Summer (E_Sum, June), Late Summer (L_Sum, August), and Fall (Fall, October).

3.0 RESULTS

3.1 Graphical Analyses

The strong seasonal patterns evident in the zooplankton community structure are shown strikingly in the plots of average "seasonal" (i.e., month) abundance within the nearfield and coastal area (Figure 2). Despite the strong overall similarity between the plots for the oceanic taxa (e.g., *Calanus finmarchicus* and *Centropages typicus*) and the persistent taxa (e.g., *Oithona similis* and copepod nauplii), there are some noticeable differences. The seasonal pattern evident in the oceanic taxa is characterized by extremely low abundances, or total absence, during part of the year followed by a very dramatic increase in abundance (typically within a month's time) to the peak value for the year, followed by an equally swift decline (Figure 2a). The seasonal pattern typical for the persistent taxa is somewhat similar, but differs in that the taxa are present in at least moderate abundances throughout the year. The increase to peak abundance is rapid, but tends to occur more step-wise and usually declines less rapidly (Figure 2b). The only time the abundances of *Oithona similis* and copepod nauplii decreased to very low numbers was in the Fall 2002 as a result of intense ctenophore predation (Figure 2b). The two characteristic Harbor taxa, *Acartia hudsonica* and *A. tonsa*, only show sporadic occurrence in the nearfield/coastal region, occurring there primarily from summer to fall (Figure 2c).

Two sets of regional plots for key species were prepared. One set shows the seasonal occurrence of these taxa at the boundary stations to the north of Massachusetts Bay (F27), a station in the nearfield (N16), a station approximately in the midst of the north-south distribution of stations (F06, off Scituate), and one Cape Cod Bay station (F01). The plots for *Calanus finmarchicus* and *Oithona similis* examine the possibility that the species enter the Massachusetts Bay system from the north and are transported to the south. *C. finmarchicus* shows the sudden increases to peak abundances at these stations (Figure 3a) that were seen for the nearfield/coastal area (Figure 2a). The increases in *C. finmarchicus* abundance April to June at station F27 can be as dramatic as 17- to 35-fold. Such sharp increases in abundance also occurred at the other two stations, but did so at times that do not appear to be related to the abundance peak at station F27. Thus, it seems likely that the abundance of *C. finmarchicus* in Cape Cod Bay is not directly related to the transport of considerable numbers from the north. As noticed in the nearfield/coastal graphs, the abundance of *O. similis* increases and decreases somewhat more gradually than that of *C. finmarchicus* (Figure 3b). There also does not appear to be a direct link between the abundance peak at station F27 and those at either F06 or F01 that would support the notion of large-scale transport of the species from the north to the south.

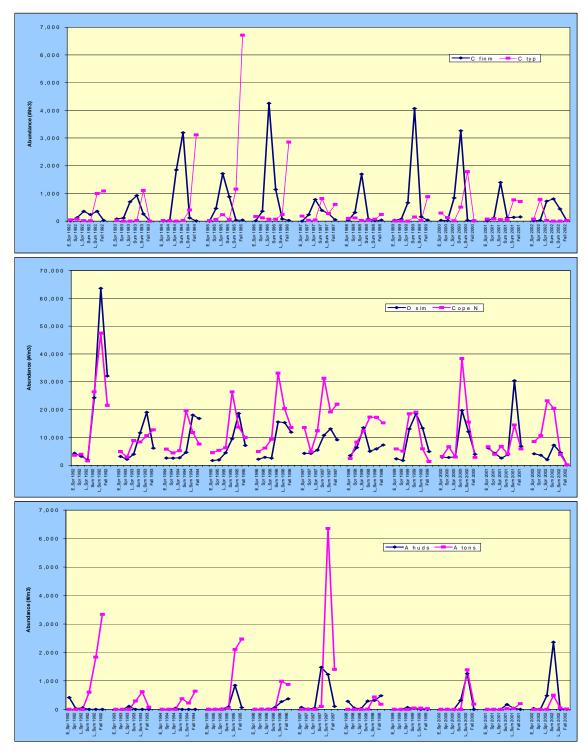


Figure 2. Plot of zooplankton abundance by season for oceanic taxa (a), persistent taxa (b), and Harbor taxa (c) in nearfield stations sampled on the six annual farfield surveys from 1992 through 2002. Abbreviations are as listed in Table 3.

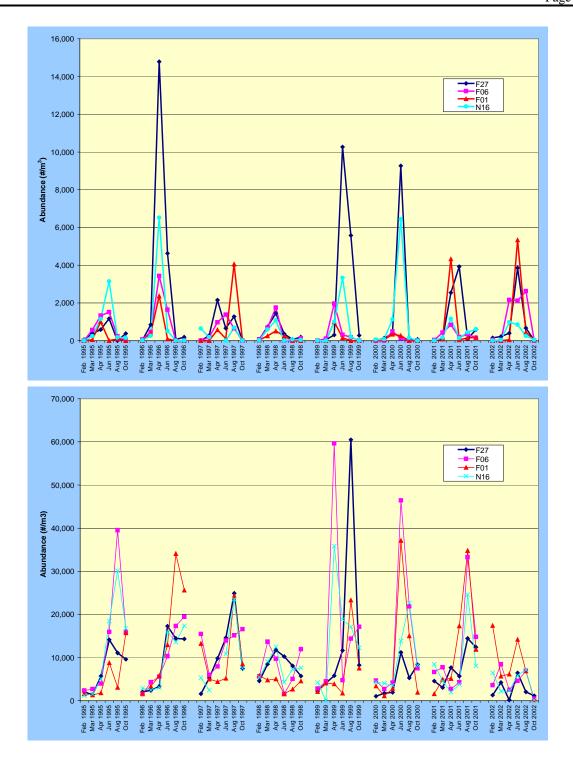


Figure 3. Plot of zooplankton abundance by month for *Calanus finmarchicus* (a) and *Oithona similis* (b) at selected nearfield and farfield stations sampled on the six annual farfield surveys from 1995 through 2002.

3.2 Principal Components Analyses

The general complexity and overall similarity of the zooplankton community in the Massachusetts Bay system is reflected in the results of the PCA that showed that the first two factors captured about 22% of the variability in the dataset. Factor 1 (X-axis Figure 4) accounted for about 13% of the variation and probably was most indicative of differences in temperature among samples, and to a lesser extent, the abundance of taxa such as *Oithona similis*, *Temora longicornis*, and copepod nauplii. Factor 2 (Y-axis in Figure 4) accounted for about 9% of the variation and primarily reflected differences in the abundance of *Oithona similis* and *Centropages typicus* (both negative) and *Acartia hudsoni* and *Eurytemora herdmani* (both positive). The former two species indicate an oceanic constituency, whereas the latter two reflect a Boston Harbor influence.

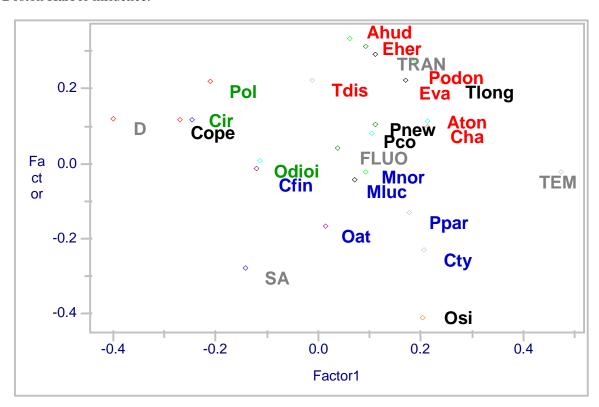


Figure 4. Loadings plot resulting from PCA analysis of MWRA zooplankton collected from 1992 to 2002.

Abiotic Factors.—Temperature, which ranged from -0.8 °C to 20.9 °C during the monitoring, is probably the most important abiotic factor affecting the zooplankton community characteristics of the Massachusetts Bay system. PCA plots show that temperature generally, but not absolutely, grades from samples having cooler temperatures towards the negative side of Factor 1 to those having warmer temperatures towards the positive side of the axis (Figure 5). Samples having temperatures in the two highest categories (Quartiles 3 and 4) completely overlapped in PC distribution and it is important to note that the samples with the highest temperatures do not occur at the extreme end of Factor 1.

Dissolved oxygen (DO) was the only other abiotic factor to show a pattern in the distribution of the quartile values (Appendix). Samples having high DO concentrations occurred towards the negative region of Factor 1, and those having lower concentrations occurred towards the positive side of the axis.

Thus, the general pattern was roughly inverse to that of temperature, as expected. Most DO values were greater than 5 mg/L (one was 1.6 mg/L), thus DO concentration is not expected to provide much towards discrimination among the zooplankton samples.

None of the other three abiotic factors, salinity, fluorescence, or transmissivity, showed distinctive distributions among samples shown in the PCA plots (Appendix) and provide no discriminatory power. Salinity ranged from 26.2 PSU to 33.3 PSU, but more than 95% of the samples had salinity values ≥ 31.5 PSU. Some lower salinity samples were located in the region of the PCA plot associated with Harbor taxa, but the samples were few, near the Harbor mouth, and collected in June. Therefore, it is most likely these low salinity samples were associated with periods of late spring/early summer runoff. Fluorescence values ranged up to 17.7 μ g/L, but 79% of the samples were in Quartile 1 with values ≤ 4.4 μ g/L. These were widely distributed the over the area of the PC plot. Transmissivity measurements ranged up to 6.4 m⁻¹, but 87% of the samples were in Quartile 1 with values ≤ 1.85 m⁻¹. These were widely distributed over the area of the PC plot.

PCA by Year.—The strong influence of temperature on the zooplankton community was evident for several of the monitoring years. These years, which showed somewhat bimodal distribution of samples along Factor 1 (appendix), included 1993, 1994, 1995 (weak), 1996 (weak), 1999, and 2001 (weak). The remaining years (1992, 1997, 1998, 2000, and 2002) did not show a bimodal temperature distribution (Appendix). The annual range of temperature for these years was still relatively large. Also indicated in these "year" plots (and others described later) is the general importance of *Oithona similis* as demonstrated by the strong orientation of the samples towards the negative region of Factor 2.

PCA by Station.—As shown for the analysis by year, most of the separation of the samples from each station is along Factor 1. Several of the stations show somewhat bimodal temperature distributions similar to those seen for years (Figure 6). These stations, which included F06, F13, F24, F25, F27 (weak) and perhaps N10 (weak), are primarily coastal stations near Boston Harbor and the south coast. There was no such bimodal temperature separation for the Cape Cod Bay or nearfield stations (F01, F02, N04, N16, N18), although there can still be a wide range along Factor 1 (Figure 6). The remaining stations did not have sufficient sample points to permit this type of characterization. Also shown by the station plots is the lack of any outfall effect on the community as most post-discharge samples are fully contained within the range of pre-discharge samples (Appendix C plots).

PCA Region Plots.—Examination of these plots allowed further examination of the potential effects of the outfall discharge at a regional level. Most, if not all, post-discharge points for the various regions (as defined in Table 1) were within the range of values determined for pre-discharge samples (Figure 6). Bimodal temperature distributions were evident for the boundary, coastal, and the coastal harbor regions, but not for the nearfield or Cape Cod Bay samples. The mid-north boundary regions had too few samples to permit an evaluation.

5a. Quartile 1 (n = 272)

Temperature Range: -0.771 °C to 4.35 °C The tight grouping of samples collected during the winter indicated the strong influence of temperature on the zooplankton composition.

5b. Quartile 2 (n = 162)

Temperature Range: 4.35 °C to 9.47 °C The group shifts to the right and begins to spread out as the temperature warms. These samples were mostly collected in March through June.

5c. Quartile 3 (n = 308)

Temperature Range: 9.47 °C to 14.6 °C The group shifts further to the right and spreads out as the temperature warms. These samples were mostly collected in June through November. Regional patterns arise – F 24 and F25 plot towards the upper right indicating a harbor influence.

5d. Quartile 4 (n = 62)

Temperature Range: 14.6 °C to 19.7 °C The group shifts further right and contains samples collected from August through November. The samples located furthest to the right are not necessarily the warmest.

5e. Loading Plot

Temperature is the dominant variable on the x-axis. Harbor influences (taxa in red plus transmissivity) draw some samples towards the upper right while oceanic influences (taxa in blue plus salinity) draw samples to the bottom of the plot. PC1 and 2 represent 13% and 9% of the variability, respectively.

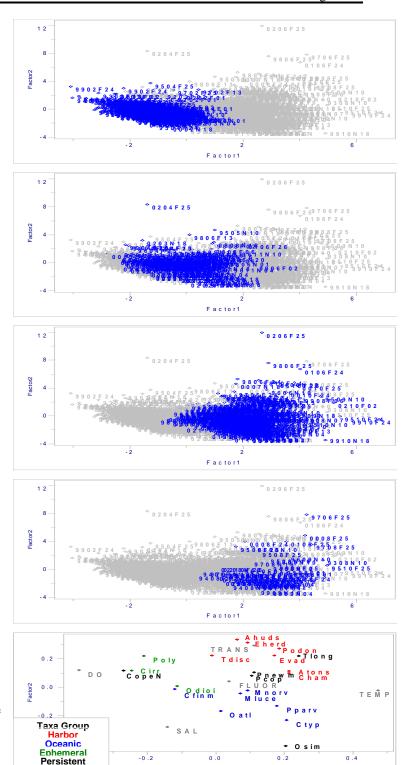


Figure 5. Temperature effects on zooplankton populations.

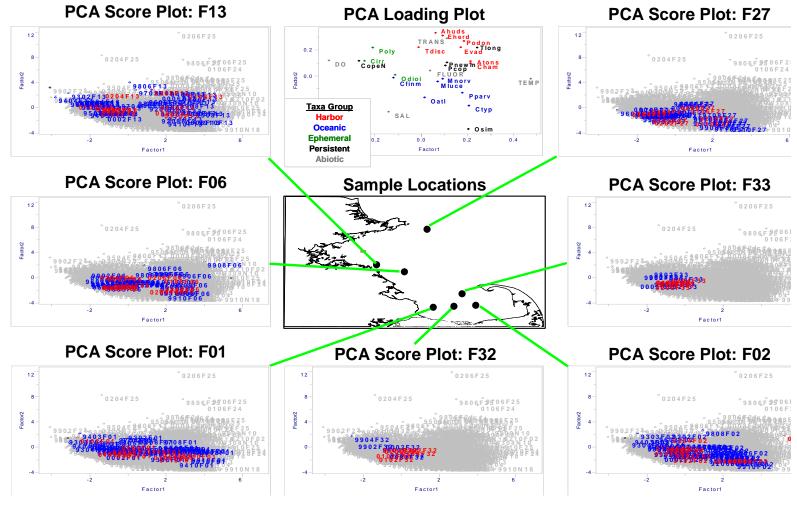


Figure 6a. Comparison of regional zooplankton sampling stations from the pre- (1992 to 2000) and post- (2001 to 2002) discharge periods using PCA. Score plots are replicated to demonstrate the relative spatial and temporal composition of selected regional sampling locations with a reasonable depth of pre- and post-discharge data colored blue and red, respectively.

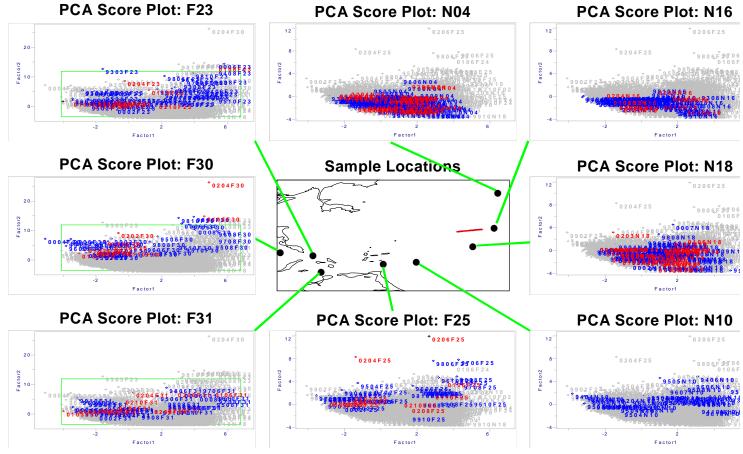


Figure 6b. Comparison of nearfield and harbor zooplankton sampling stations from the pre- (1992 to 2000) and post- (2001 to 2002) discharge periods using PCA. Score plots are replicated to demonstrate the relative spatial and temporal composition of selected regional sampling locations with a reasonable depth of pre- and post-discharge data colored blue and red, respectively.

PCA Species Plots.—Among the 21 taxa included in the PCA, the predominant abundance category for most was the lowest, Quartile 1. It is important to remember that Quartile 1 includes "zero" abundance values. For each taxon, this quartile was generally widely distributed across the samples shown on the PC plots. The only taxa for which Quartile 1 did not represent more than 90% of the samples were *Temora* longicornis (89%), Paracalanus/Pseudocalanus copepodites (84%), Oithona similis (36%), and Copepod nauplii (21%). Most taxa showed a distinct association between peak abundances and temperature; for others the relationship was less clear. Taxa showing peak abundances primarily associated with warmer temperatures included Acartia tonsa, Centropages hamatus, C. typicus, Paracalanus parvus, Evadne spp., Podon spp., and probably Temora longicornis (Figure 7). Taxa showing peak abundances associated with cooler temperatures included Calanus finmarchicus, Cirripedia nauplii, Oikopleura dioica, and Polychaete spp. (larvae) (Figure 8). Patterns for the predominant taxa, Copepod nauplii and Oithona similis, are less clear. The highest abundances (represented by Quartile 4) of Copepod nauplii were associated with cooler temperatures (except for the August 2000 sample) (Figure 9a). However, there were only 15 samples having temperatures in this quartile. Most (77%) of the samples containing Copepod nauplii had temperatures falling into Quartiles 2 and 3, both of which were broadly distributed across the range of temperatures indicated by Factor 1. Thus, while the highest abundances occurred when waters were cool, Copepod nauplii could also be very abundant at times during the year when water temperatures were warm. The highest abundances (Quartile 4) of the ubiquitous Oithona similis tended to be associated with warmer reaches along Factor 1 (Figure 9b). However, as for Copepod nauplii, most samples (60%) had abundances falling within Quartiles 2 and 3, both of which were broadly distributed across the range of temperatures indicated by Factor 1. Thus, while peak the highest abundances occurred when waters were warm, O. similis could also be very abundant at times during the year when water temperatures were cool.

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7a. Centropages typicus	7a.	Centropages	typicus
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Measurement	Ctyp	Frequency
< Q1 Max	0.0754	754
< Q2 Max	0.151	38
< Q3 Max	0.226	7
< Q4 Max	0.302	5
Median	0.00326	
Average	0.0152	

7b. Acartia tonsa

Measurement	Atons	Frequency
< Q1 Max	0.0986	789
< Q2 Max	0.197	10
< Q3 Max	0.296	3
< Q4 Max	0.394	2
Median	0	
Average	0.0137	

7c. Paracalanus parvus

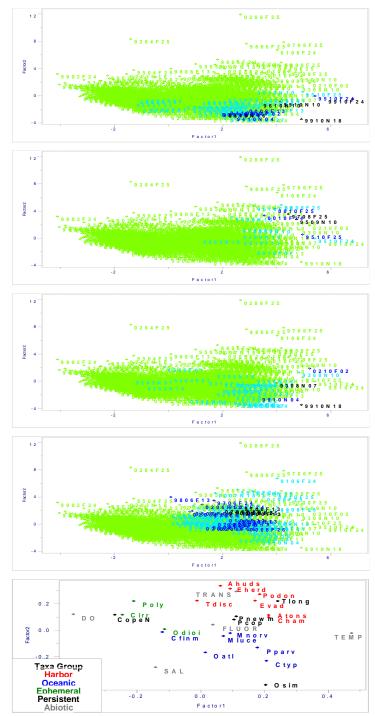
Measurement	Pparv	Frequency
< Q1 Max	0.0379	772
< Q2 Max	0.0759	28
< Q3 Max	0.114	2
< Q4 Max	0.152	2
Median	0	
Average	0.00501	

7d. Temora longicornis

Measurement	Tlong	Frequency
< Q1 Max	0.101	712
< Q2 Max	0.202	69
< Q3 Max	0.303	18
< Q4 Max	0.404	5
Median	0.0117	
Average	0.0339	

7e. Loading Plot

Temperature is the dominant variable on the x-axis.



Factor1

Figure 7. Selected zooplankton taxa that show peak abundance values associated primarily with warmer water temperatures. Quartile values are based on normalized abundances.

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8a. Cirripedia spp.

Measurement	Cirr	Frequency
< Q1 Max	0.223	775
< Q2 Max	0.446	19
< Q3 Max	0.67	10
< Q4 Max	0.893	0
Median	0	
Average	0.0431	

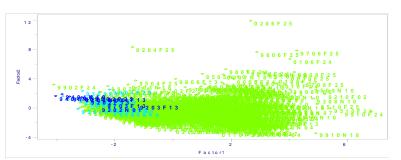
8b. Calanus finmarchicus

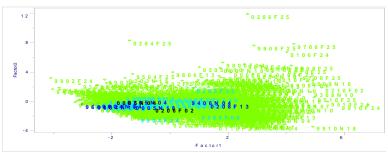
Measurement	Cfinm	Frequency
< Q1 Max	0.123	768
< Q2 Max	0.246	27
< Q3 Max	0.369	7
< Q4 Max	0.492	2
Median	0.00349	
Average	0.0222	

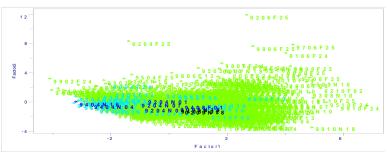
8c. Oikopleura dioica

Measurement	Odioi	Frequency
< Q1 Max	0.144	770
< Q2 Max	0.287	25
< Q3 Max	0.431	7
< Q4 Max	0.574	2
Median	0.00162	
Average	0.0257	

8d. Loading Plot Temperature is the dominant variable on the x-axis.







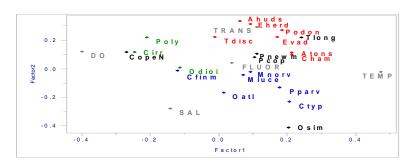


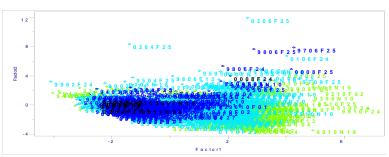
Figure 8. Selected zooplankton taxa that show peak abundance values associated primarily with cooler water temperatures. Quartile values are based on normalized abundances.

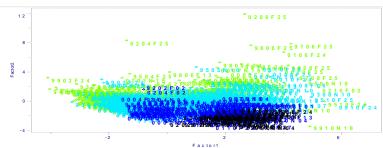
9a. Copepod Nauplii

Measurement	CopeN	Frequency
< Q1 Max	0.25	169
< Q2 Max	0.5	435
< Q3 Max	0.75	190
< Q4 Max	1	10
Median	0.397	
Average	0.389	

9b. Oithona similis

Measurement	Osim	Frequency
< Q1 Max	0.203	215
< Q2 Max	0.406	412
< Q3 Max	0.609	135
< Q4 Max	0.811	42
Median	0.258	
Average	0.278	





9e. Loading Plot Temperature is the dominant variable on the x-axis.

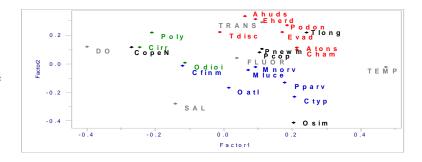


Figure 9. PCA plots showing abundances of the two predominant zooplankton taxa in Massachusetts Bay. Quartile values are based on normalized abundances.

3.3 Cluster Analyses

Stations Sampled All Years.—The first cluster analysis was conducted on the dataset that included those stations sampled in all years of the MWRA monitoring and also included station N04, which was sampled all years except 1995. The main feature of the dendrogram (Figure 10) is that all samples from the station at the mouth of Boston Harbor (F23, located off the tip of Deer Island) were much more similar to each other than they were to the Massachusetts Bay or Cape Cod Bay samples. A second important observation is the high overall similarity among the Massachusetts Bay and Cape Cod Bay samples, which were linked at a Bray-Curtis similarity of 74% or greater. Among the Bay samples, those from N04 and N16 from the same year clustered as part of a 2- or 3-sample cluster for 7 of the 11 years of monitoring. Samples from stations F01 and F02 (Cape Cod Bay) collected in the same year clustered as part of a 2- or 3-sample cluster 9 of the 11 years of monitoring. Station F13 samples clustered variably with those from the other four stations. Despite the general indication that there are differences between the Massachusetts Bay nearfield and Cape Cod Bay samples, the separation is not complete as the two primary non-Harbor clusters contain samples from both regions. Thus, this analysis showed that the Harbor zooplankton fauna differs from that in the Massachusetts Bay system and that the nearfield and

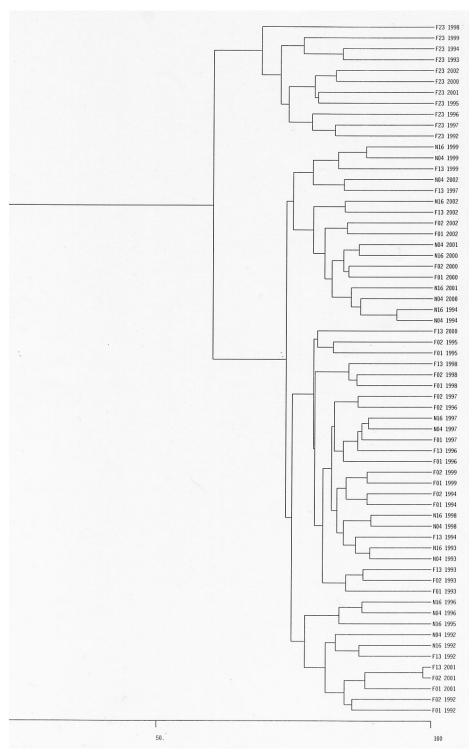


Figure 10. Dendrogram of Bray-Curtis similarities (X-axis) among samples collected during the nearfield zooplankton surveys conducted from 1992 to 2002. Station F13 sampled in 1995 is excluded.

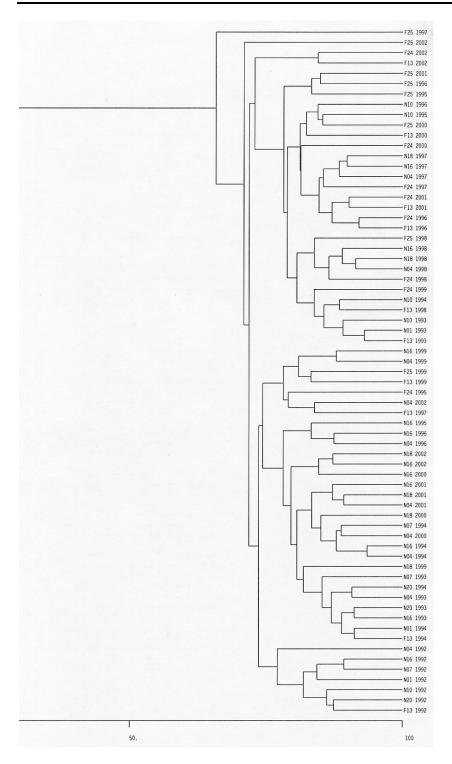


Figure 11. Dendrogram of Bray-Curtis similarities (X-axis) among nearfield/coastal samples collected during the nearfield zooplankton surveys conducted from 1992 to 2002.

Cape Cod Bay areas are generally similar, although each retains distinct characteristics relative to the zooplankton. The Harbor samples were not included in subsequent analyses.

Nearfield Area Stations.—The second cluster analysis, which included only the nearfield and nearby coastal stations, showed that station F25, located northeast of Hull, occasionally (1997 and 2002) clustered well apart from the two main sample clusters (Figure 11). Among the two main clusters of samples, the smaller group comprised of 29 samples includes most of the coastal station samples (F13, F24, F25) and a mix of nearfield samples. The larger group, comprised of 36 samples, primarily consisted of samples from the nearfield, but occasionally included coastal samples. Most noticeably, all 1992 samples clustered together. There was no indication of any potential effect of discharge from the outfall on the nearfield or coastal samples as those from the post-discharge period (2001–2002) clustered among samples from the pre-discharge period (1993–1999). (The year 2000 includes pre- and post-discharge samples and is not included in this descriptive comparison).

Seasonal Patterns.—When the zooplankton samples were analyzed by sampling "season" (i.e., month), several features appeared (Figure 12). First, the Fall 2002 samples were very different from the remaining samples. Zooplankton abundances then were much lower than at any other time and most likely reflected decimation of the community resulting from ctenophore predation. The remaining samples were organized into three main clusters, one primarily comprised of late spring or summer samples and two that generally separated by spring and summer/fall samples. The first cluster of seven samples was, in part, comprised of late spring samples from 1998, 1999, and 2002. These three samples were distinctive in having the highest total zooplankton abundances of any late spring samples. Also unusually abundant for the time period were Paracalanus/Pseudocalanus copepodites. Three summer samples (1997, 1999, 2002) also grouped together within this cluster. Again, Paracalanus/Pseudocalanus copepodites were more abundant for these three samples than for any other summer samples. The final sample in this cluster, late spring 1992, was also characterized by unusually high Paracalanus/Pseudocalanus copepodite abundance. Of the two remaining primary clusters, one was comprised of only summer, late summer, and fall samples. The other cluster was primarily comprised of early spring, spring, and late spring samples, but included two fall samples (1999, 2000). Thus, it appears that there is generally a bimodal separation of the zooplankton community based roughly on "season." The strength of the seasonal signal is further typified by the observation that consecutive seasons within a year cluster together only three times (E Spr, Spr 1992 and 2002; Sum, L Sum, Fall 1997). The greater tendency is for like seasons to group together independent of the year. For example the summer 1992, 1994, 1995, 1996, and 2000 samples are more similar to each other than they are to any other samples.

Single Stations Clusters.—Four stations (N04, N16, F01, F23) were analyzed individually to look for potential differences among samples that might be related to the outfall discharge (Figure 13). None of the stations showed any probable outfall effects as post-discharge samples clustered among pre-discharge samples.

Species Clusters.—Three groups of taxa were separated by the cluster analysis, with overall abundance apparently the primary discriminating factor. The two most abundant taxa, *Oithona similis* and Copepod nauplii, showed the highest similarity (89%) among any of the other pairs of taxa (Figure 14). Additionally, these two taxa clustered apart from the other 19 taxa included in the analysis. The remaining 19 taxa separated into two groups at an overall similarity of 43%. These two groups separate by total overall abundance. The larger group of 13 taxa includes those that ranked 3rd to 15th in abundance in the dataset. The smaller group of six taxa consists of the six least abundant taxa included in the analysis. Other factors such as general faunal association (see Table 3) or temperature have little influence on the clustering.

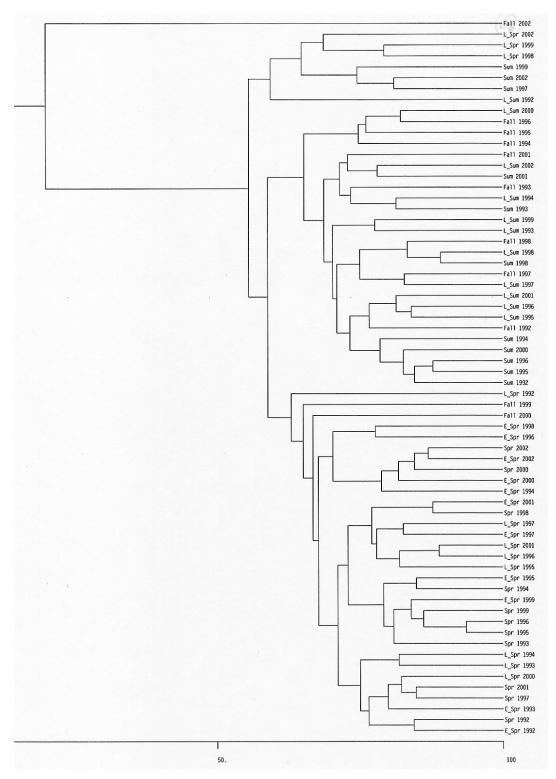


Figure 12. Dendrogram of Bray-Curtis similarities (X-axis) among seasons sampled during the nearfield zooplankton surveys conducted from 1992 to 2002.

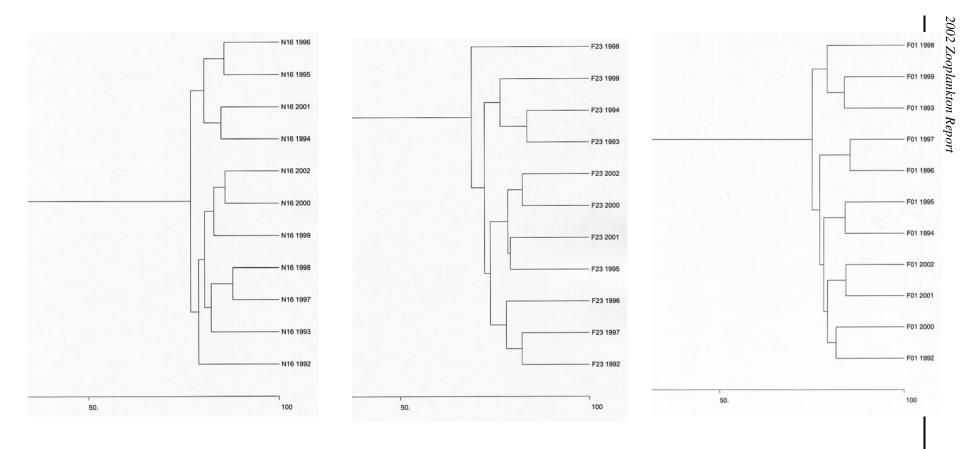


Figure 13. Dendrogram of Bray-Curtis similarities (X-axis) among years for a Boston Harbor station (F23), a nearfield station (N16), and a Cape Cod Bay station (F01).

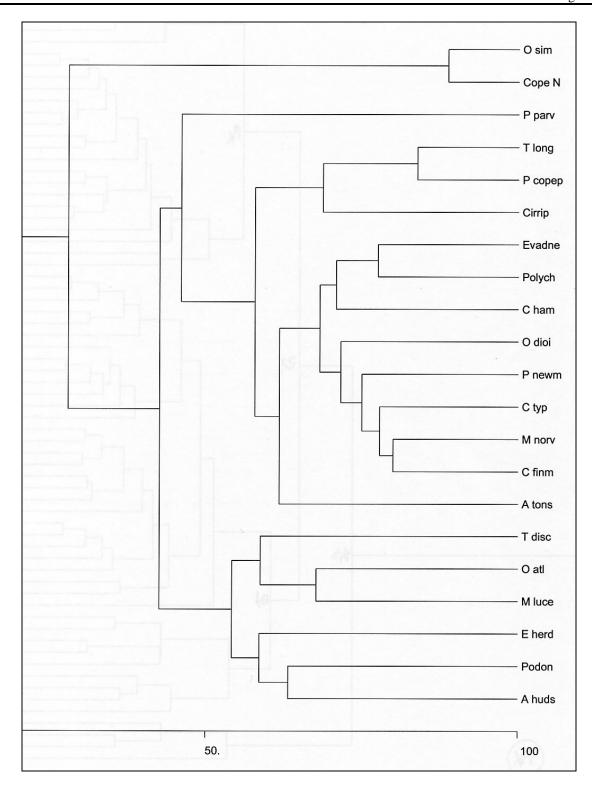


Figure 14. Dendrogram of Bray-Curtis similarities (X-axis) among species collected during the nearfield zooplankton surveys conducted from 1992 to 2002.

4.0 DISCUSSION

4.1 Zooplankton Community Characteristics

The zooplankton communities that occur in Massachusetts and Cape Cod Bays are not unique to the Bays, but are small components of the larger community characteristic of the Gulf of Maine. As such, the these zooplankton communities are numerically dominated by several species of copepods, all of which have widespread distributions in the Gulf of Maine or in the waters of the northwest Atlantic. MWRA's zooplankton monitoring is unusual in its use of finer mesh nets (0.1 mm) than are routinely used in other studies (0.3 mm and larger). Because of this, MWRA's data are numerically dominated by smaller zooplankters such as unidentifiable copepod developmental stages (nauplii and copepodites), and adults of the small, ubiquitous copepod *Oithona similis*, which are not captured at all or are under-reported in other studies. Larger taxa found the Bays include the estuarine species *Acartia tonsa, Acartia hudsonica*, and *Eurytemora herdmani*, as well as oceanic species *Calanus finmarchicus*, *Paracalanus parvus*, and species in the genera *Centropages* and *Pseudocalanus*. Polychaete and barnacle larvae are also seasonally important constituents of the zooplankton communities in Massachusetts Bay. The results supported here indicated that there is an overall high degree of similarity within most of the regions sampled in the Bays. This observation generally supports earlier studies that showed zooplankton communities were very similar over scales of about 10 km (Davis & Gallagher 2000).

Two of the major factors that affect zooplankton community structure in the Bays are abiotic features. Temperature, which is reflected in the strong seasonal patterns evident in the analyses presented in this report, is the primary factor affecting the zooplankton communities. Colder-water taxa, such as barnacle nauplii and *Calanus finmarchicus*, are more abundant relatively early in the year than later, whereas taxa abundant at warmer temperatures, such as *Acartia tonsa*, *Centropages hamatus*, and *Paracalanus parvus*, reach peaks in abundance during summer. Patterns for some taxa, such as *Oithona similis* and copepod nauplii, are less clear. Other abiotic factors measured during the MWRA monitoring (salinity, DO, fluorescence, and transmissivity) have little direct influence on the zooplankton communities in the Bays.

The second primary abiotic factor is somewhat related to geography. There appears to be a relatively strong estuarine-offshore gradient in community structure in the Bays that is most obvious in the separation of the Boston Harbor community from the rest of the system. Less obvious, but still present, is the relative similarity of samples from within western Massachusetts Bay and from within Cape Cod Bay.

Some taxa comprising the zooplankton communities within the Bays may respond to hemisphere-scale phenomena such as the North Atlantic Oscillation (NAO). Conversi *et al.* (2001) showed that the abundance of *Calanus finmarchicus* was positively correlated with the NAO index although there was a four-year lag time in the response to the index. Conversely, variation in the abundance of another calanoid copepod, *Metridia lucens*, could not be linked to fluctuations in the NAO index (Kane 2003).

Biological factors may at times exert considerable influence the zooplankton communities of the Bays. The major events since the discharge started that substantially altered the nature of the communities were two blooms of the ctenophore *Mnemiopsis leidyi* in the Bays in the fall of 2000 and 2002. Predation by *M. leidyi*, which was also unusually abundant during this period in Buzzards Bay, caused drastic declines in zooplankton abundance in Massachusetts Bay and Cape Cod Bay, and particularly in Boston Harbor. The effects of the ctenophore predation in 2002 were very evident in the cluster and graphical analyses, but were not indicated by PCA.

4.2 Conveyor Belt

The conveyor belt hypothesis suggested that MWRA's zooplankton data might reflect the broad counterclockwise circulation in the Bays, such that a population of zooplankton would be enter the Bays

at the northern boundary, would then be transported through the nearfield and southward, ultimately into Cape Cod Bay. The analyses presented in this report showed that the hypothesis that the general circulation patterns in the Bay consistently transport "pulses" of zooplankton from north to south probably is not valid. Peak *C. finmarchicus* abundances are often coincident at the boundary stations, the nearfield region, and in Cape Cod Bay. They also may occur earlier in the year at the southern than at the northern stations. Similarly, there is no consistent north-south sequence in peak abundances for *O. similis*, nor for other important copepod species including adults of *Paracalanus parvus*, *Pseudocalanus* spp., *Paracalanus/Pseudocalanus* copepodites, and *Centropages typicus*.

4.3 Outfall Effects

The relocated MWRA outfall began discharge into the nearfield, 15 km offshore from Boston Harbor, on September 6, 2000. It is now possible to evaluate issues and predictions about post-discharge effects from the report of Cibik et al. (1998), in light of more than two years of discharge from the relocated outfall. There have not been any apparent effects of the outfall discharge on zooplankton abundance or community structure in that both were within the previously-established broad envelope-of-variability recorded for the baseline for this period of the year. One of the more noticeable events following the discharge was the occurrence of unusually high chlorophyll levels throughout the MWRA sampling area during the fall of 2000. This bloom was later shown by Sea WiFS satellite data for chlorophyll to be part of a regional event that occurred over the entire northeastern United States. Following this high chlorophyll bloom, there was no eutrophication-induced shift toward zooplankton assemblages dominated by Acartia. That there has not been a detectable outfall discharge effect is also indicated by the analyses performed here. The principal components analyses also showed the post discharge samples were well within the overall boundaries of the baseline period. Cluster analyses showed that post discharge samples clustered among those from before the discharge. This observation held for analyses done at the nearfield scale and those done at the single-station scale. Overall, zooplankton abundance and community structure during the two years of post-relocation discharge, though variable, are similar to seasonal patterns revealed within the pre-discharge-relocation baseline.

5.0 CONCLUSIONS AND RECOMMENDATIONS

The results of the analyses presented in this report have shown that the zooplankton communities in Massachusetts Bay and Cape Cod Bay are not unique, but are part of the larger Gulf of Maine system. Within the Bays system, the communities exhibit a high degree of similarity across regions and are structured principally by large-scale environmental factors such as seasonal variation in water temperature. The community is numerically dominated by small zooplankton species, in particular, the ubiquitous calanoid, *Oithona similis*. Data presented here do not support the notion that the zooplankton communities in the Bays are derived from the wholesale transport of individuals from the north to the south by prevailing circulation patterns.

Threshold recommendation

Based on the analyses presented here, the development of a threshold that can effectively capture shifts in zooplankton species or abundance at local or regional scales that may be related to the relocation of the outfall is highly unlikely. The zooplankton in the system do not show a systematic pattern of occurrence from north to south in the Bays, generation times are long relative to the average transport time in the Bays, and zooplankton community or abundance responses to primary production as measured by chlorophyll biomass are not apparent in the data. The zooplankton communities tend to respond simultaneously to large-scale environmental factors at a regional scale rather than a local one. Thus, defining a meaningful threshold for the zooplankton community is not possible. However, MWRA will continue to evaluate the zooplankton community at scales appropriate to the response features observed in this data set to ensure that potential outfall-related effects are detected.

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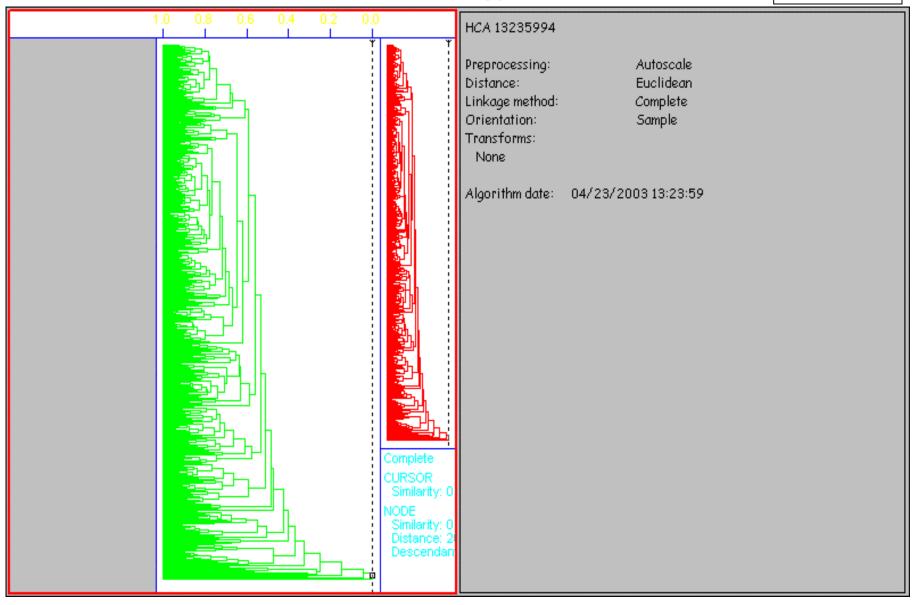
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APPENDIX A

PCA Plots

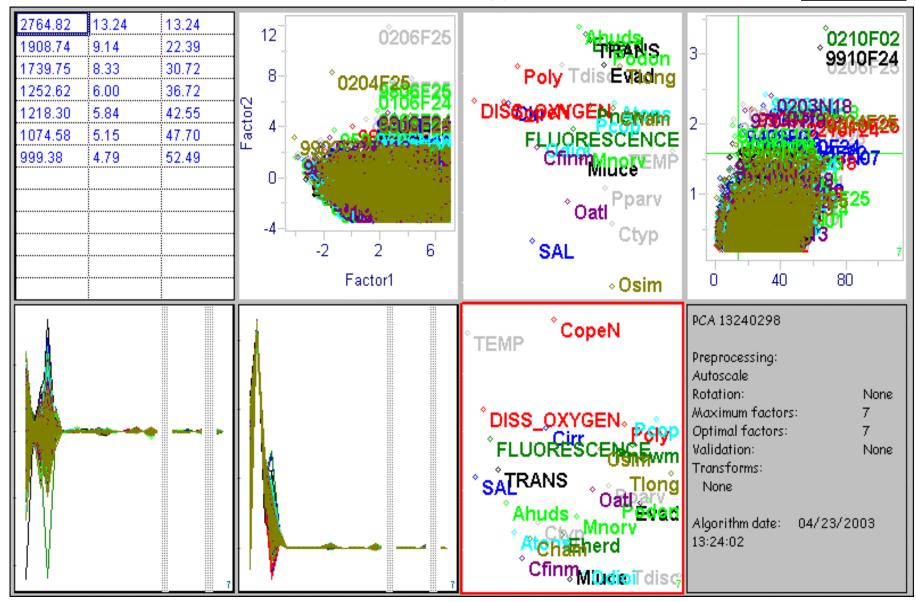
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Dendrogram



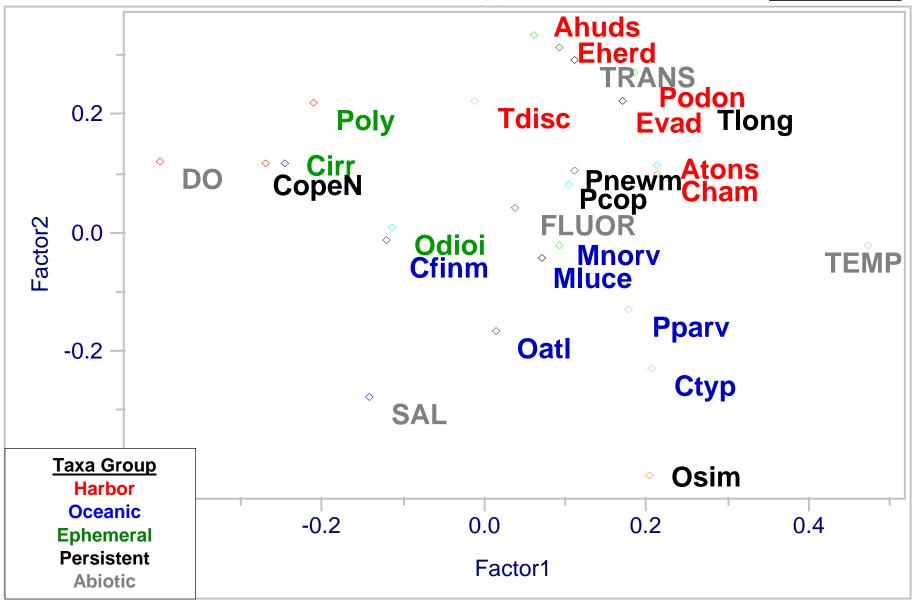
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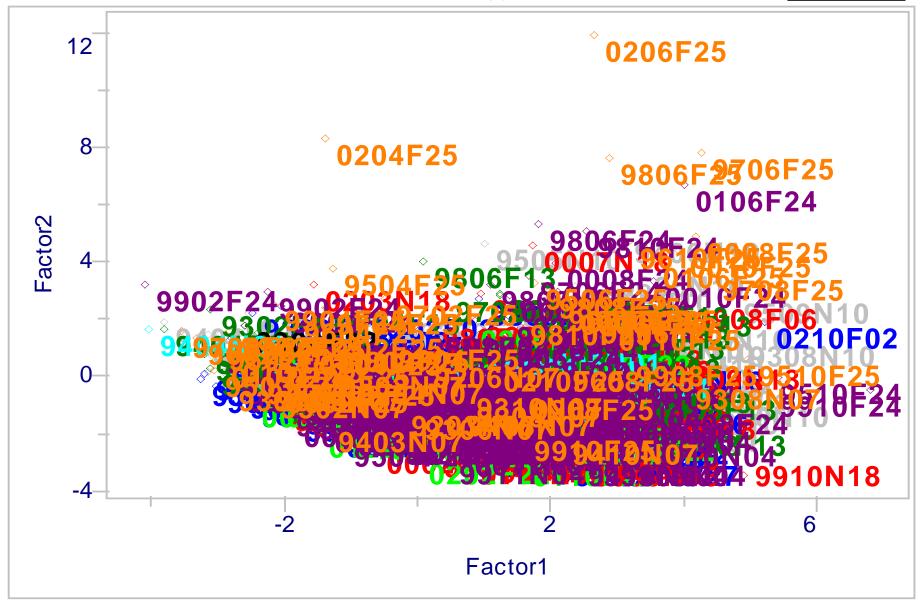
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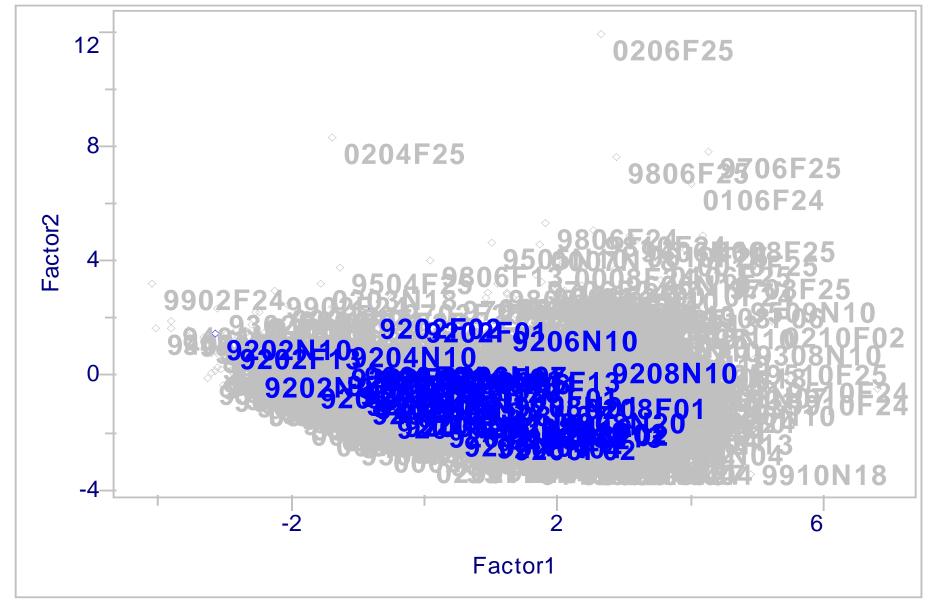


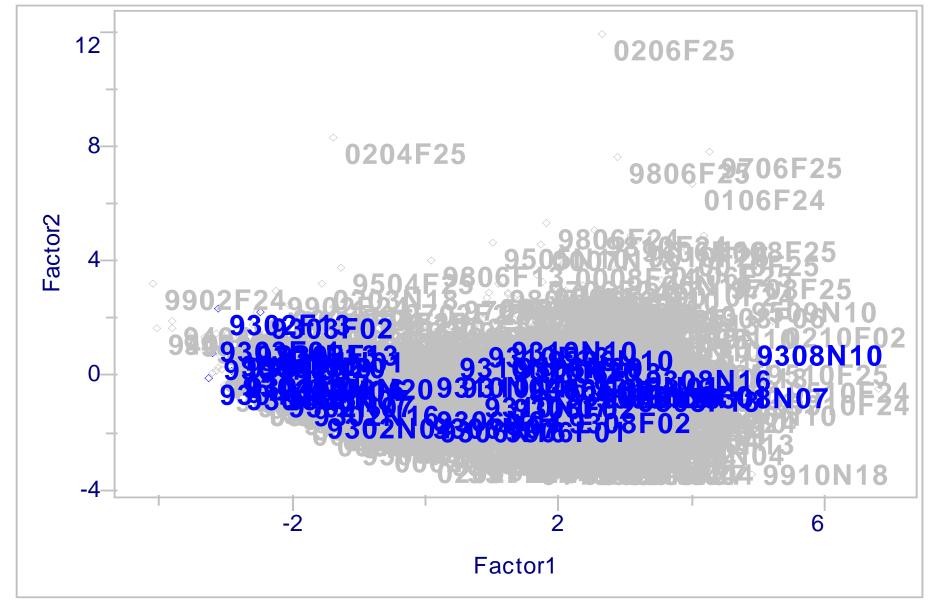
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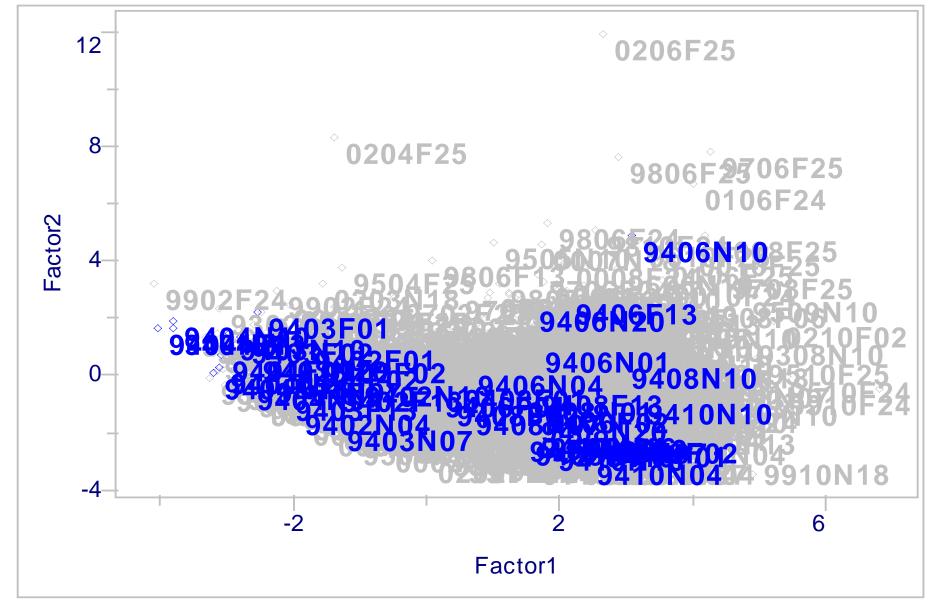
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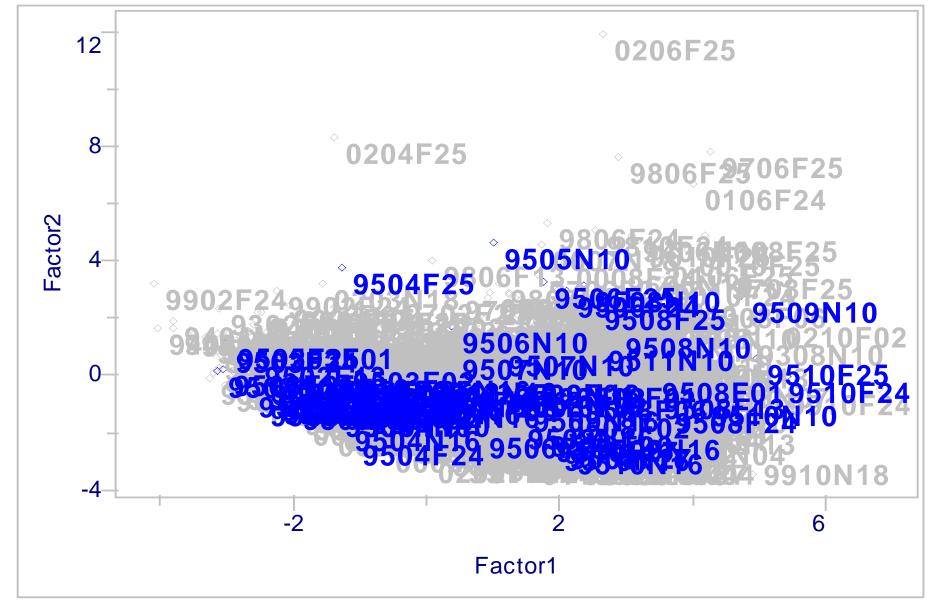


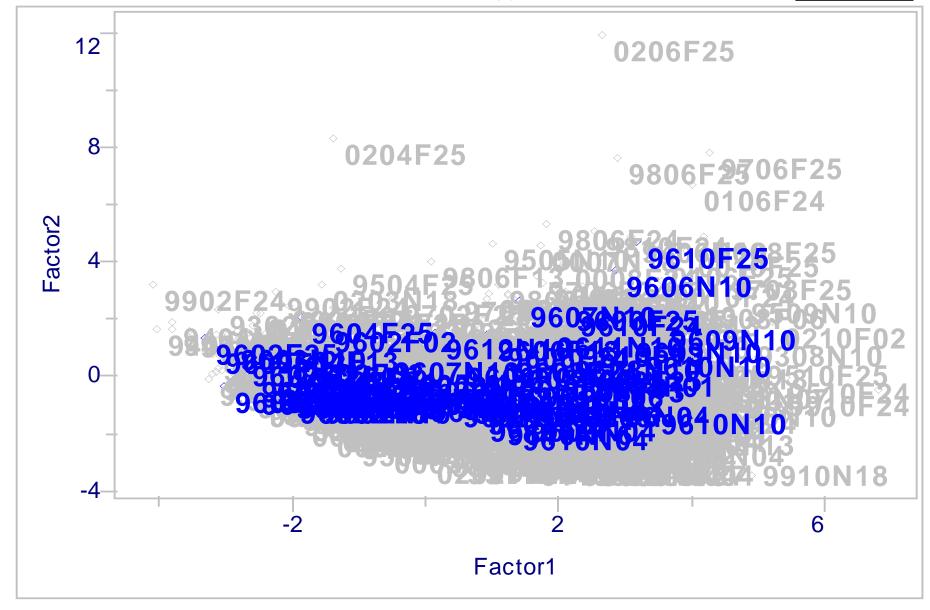


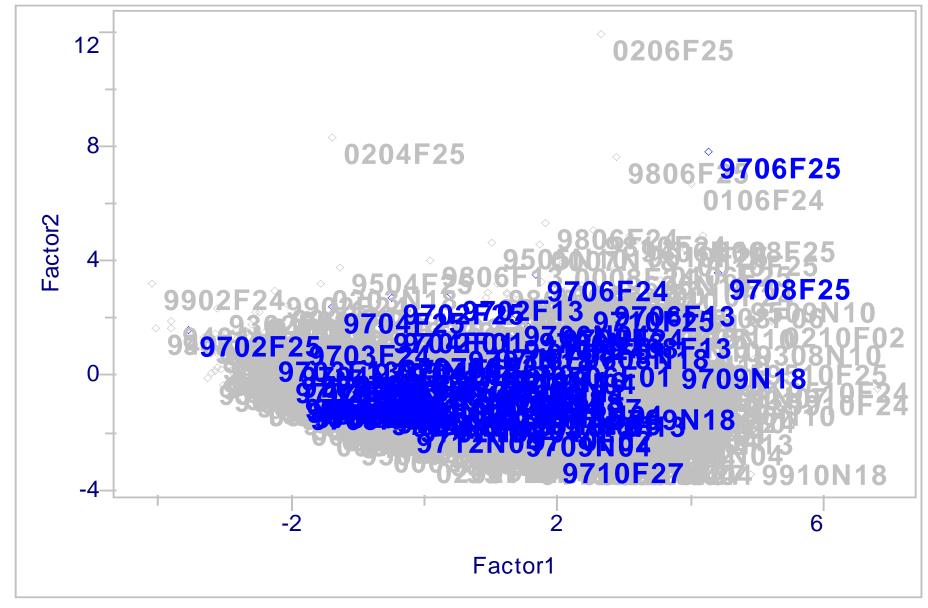


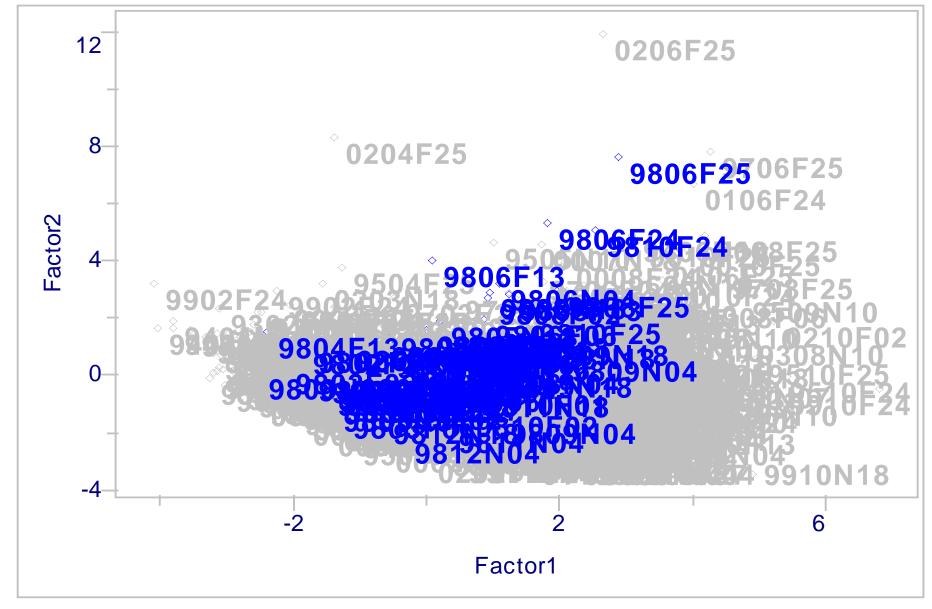


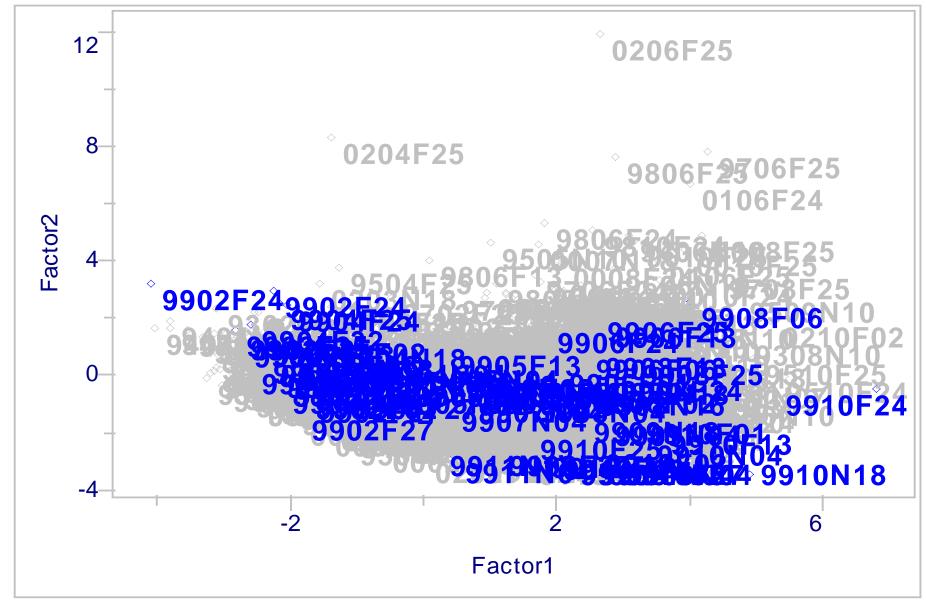


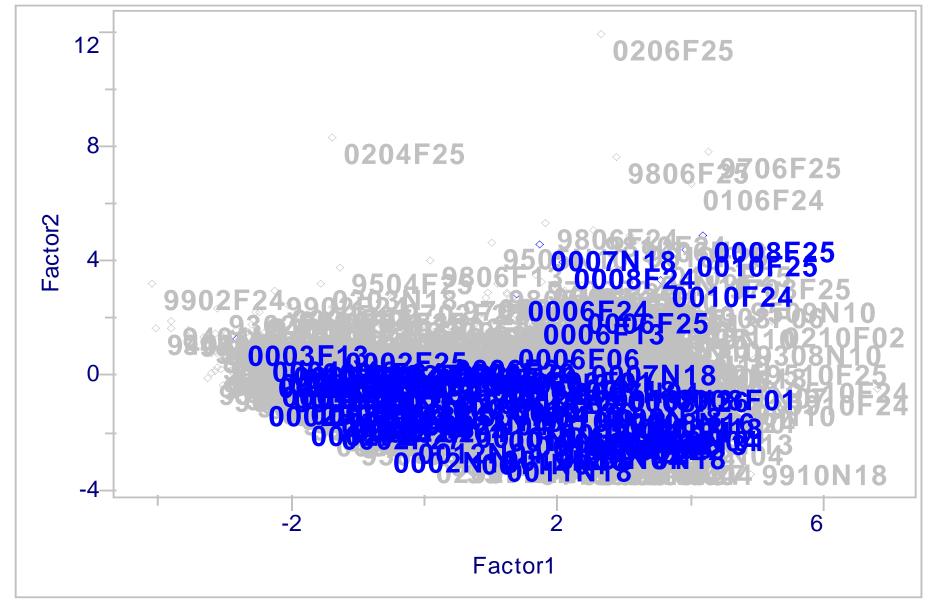


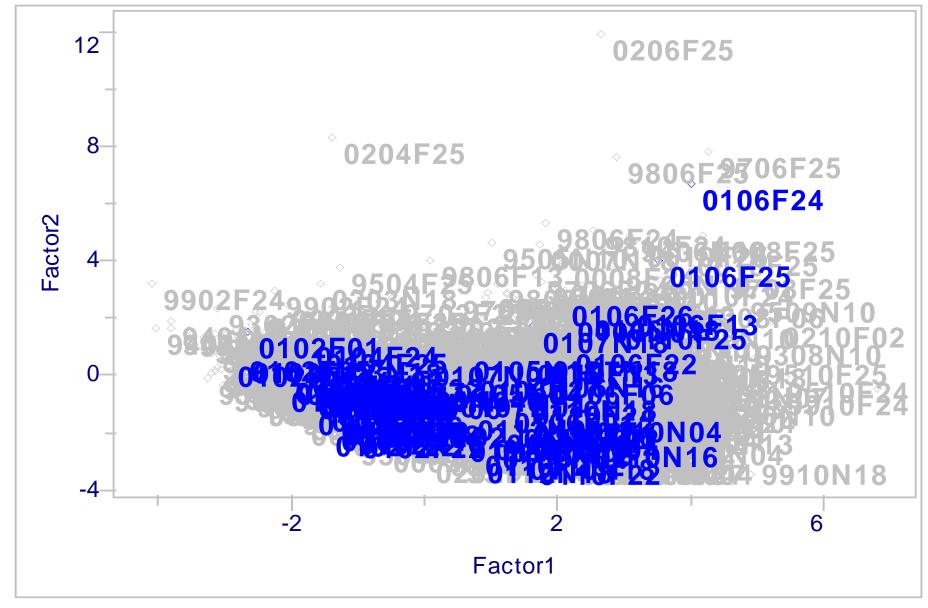


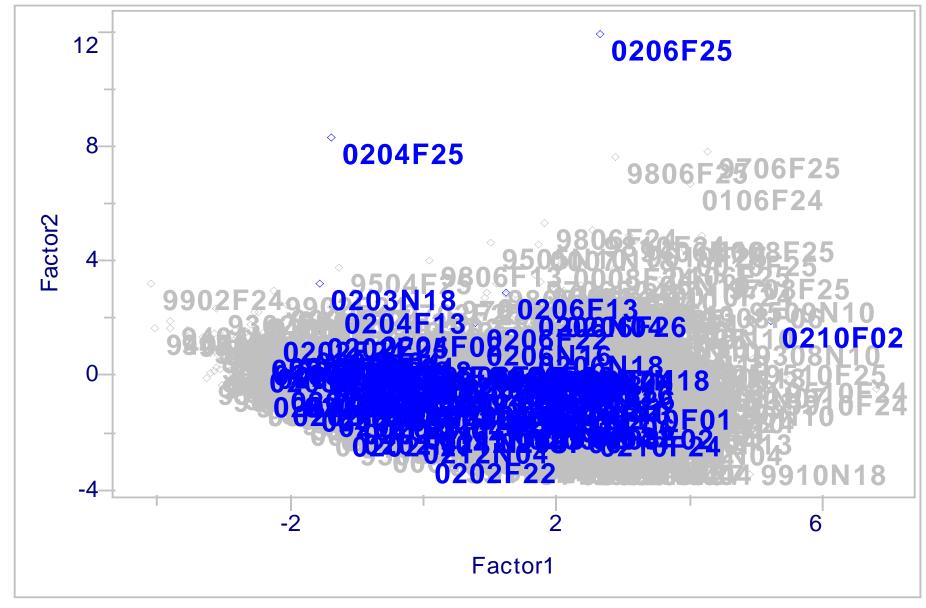


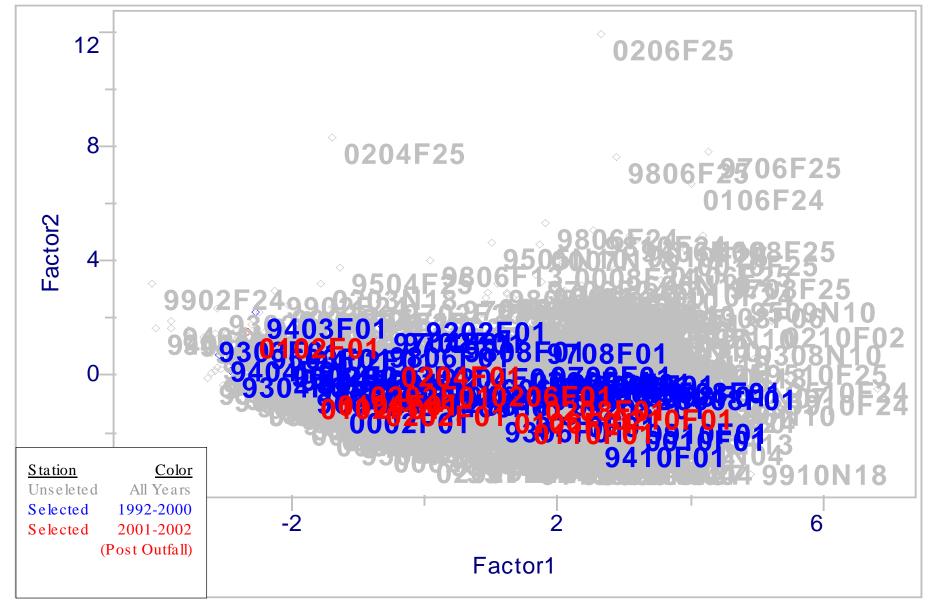


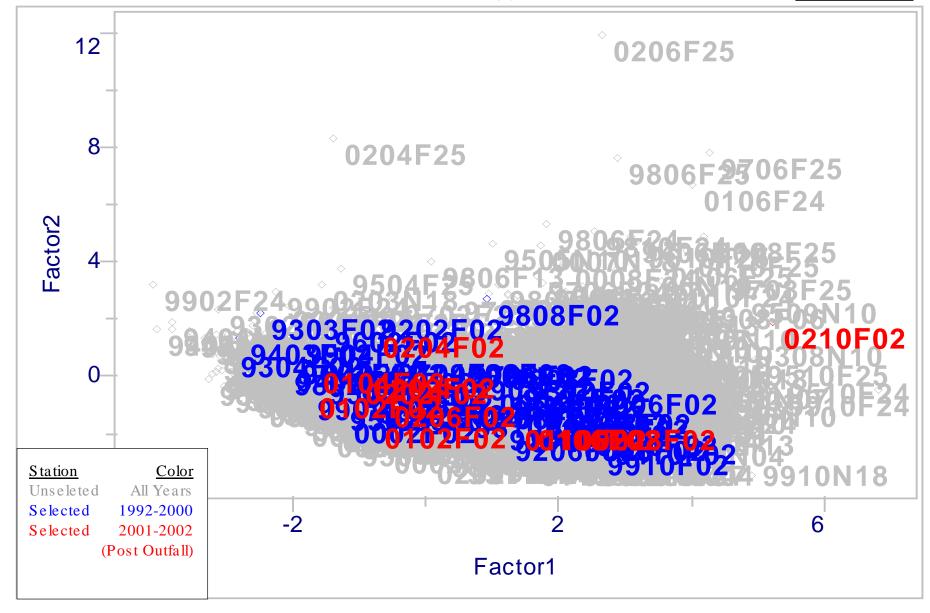


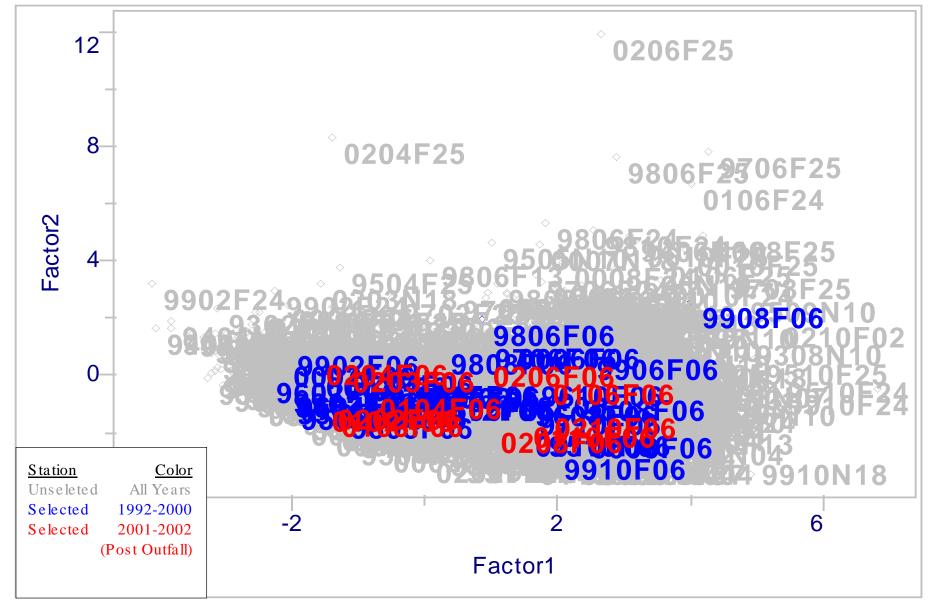


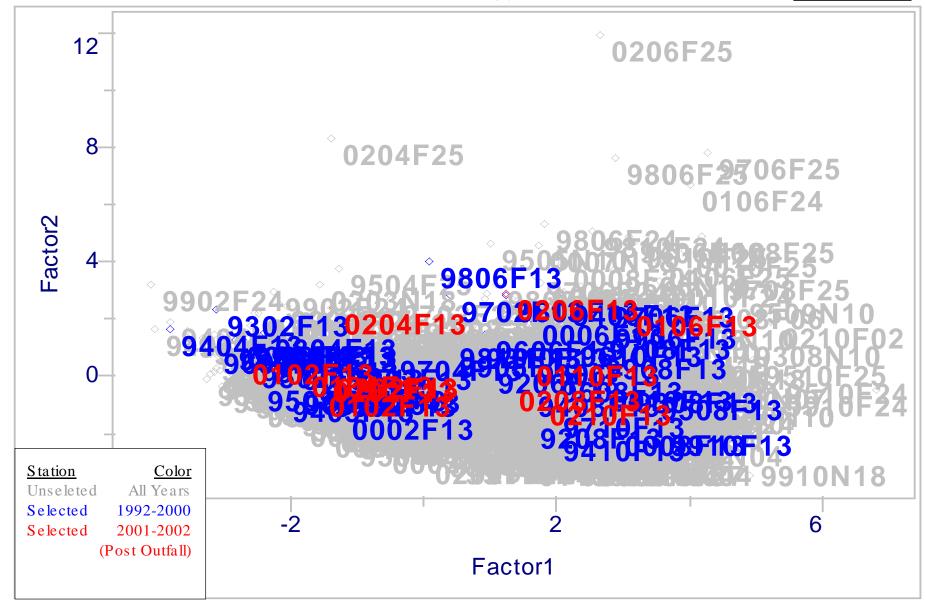


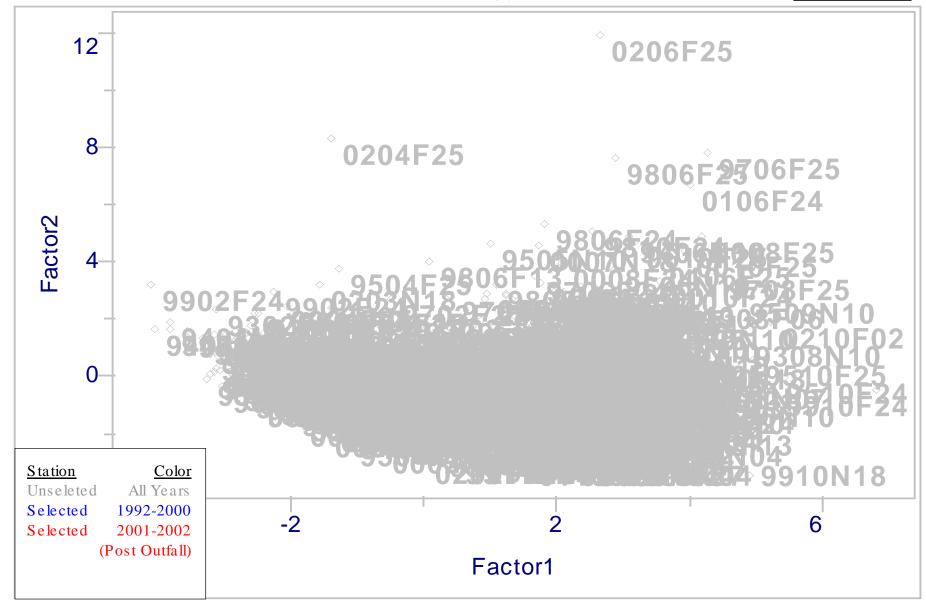


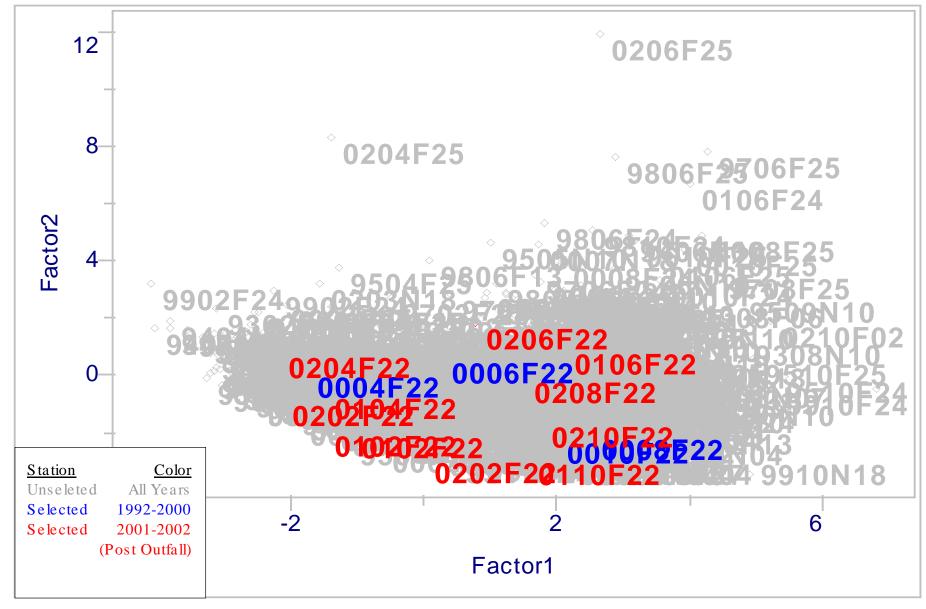


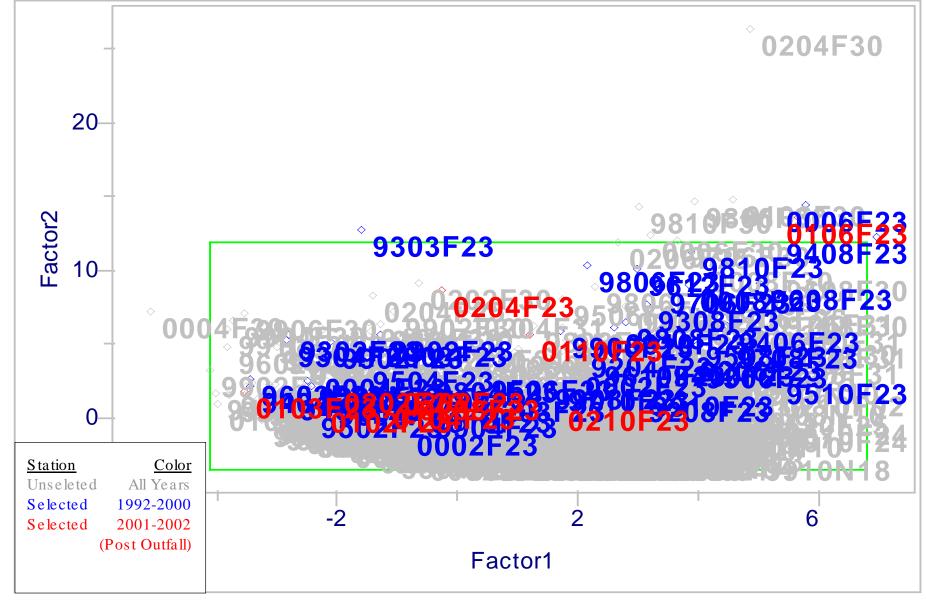


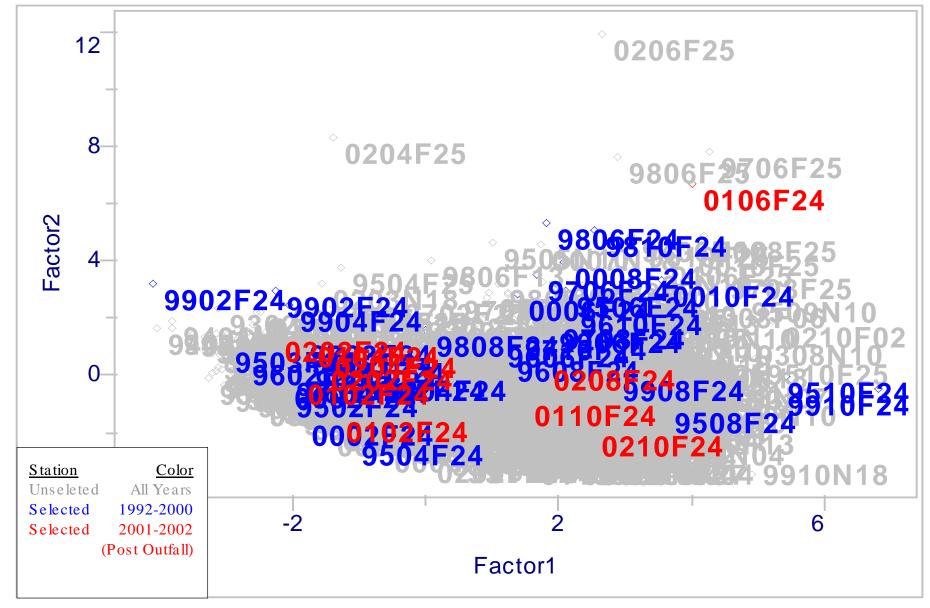


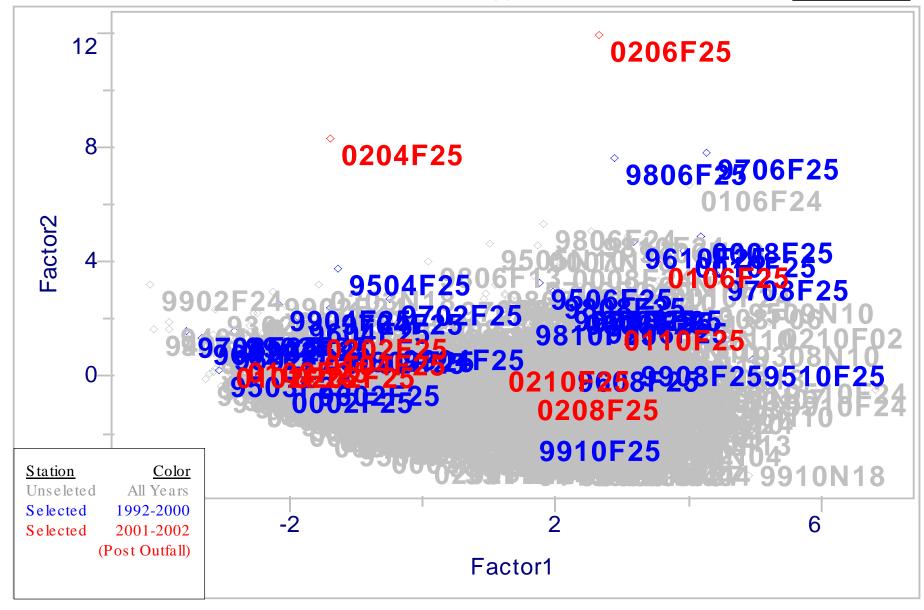


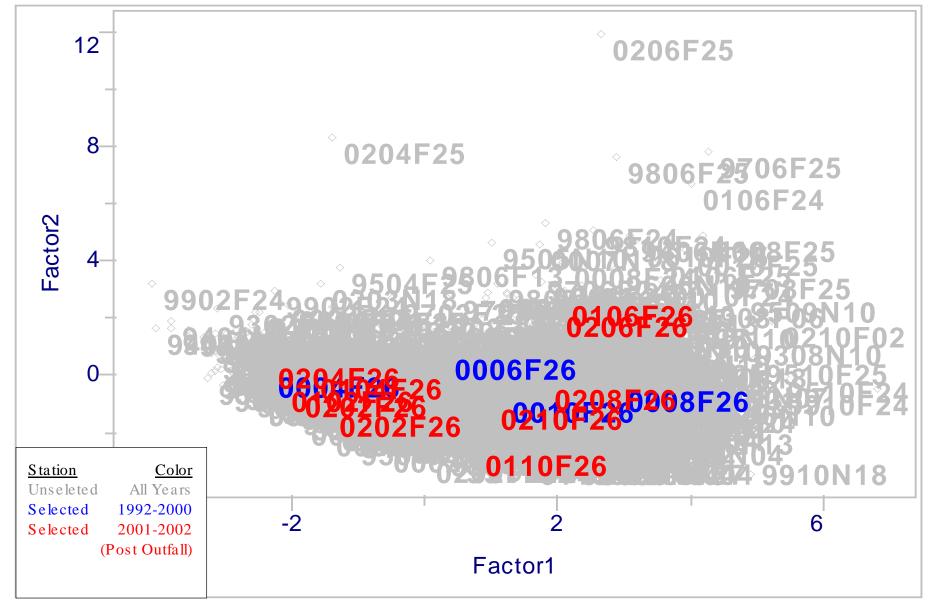




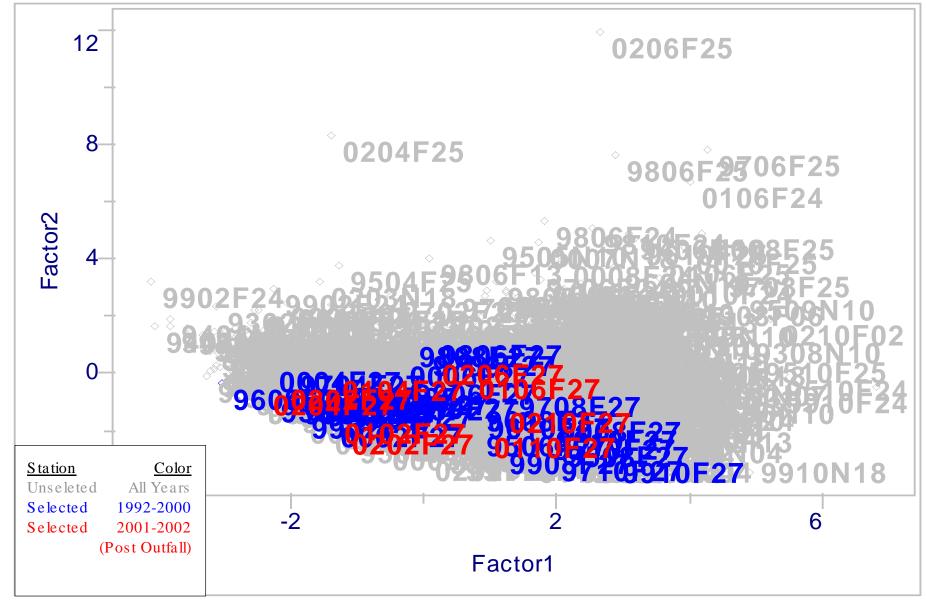


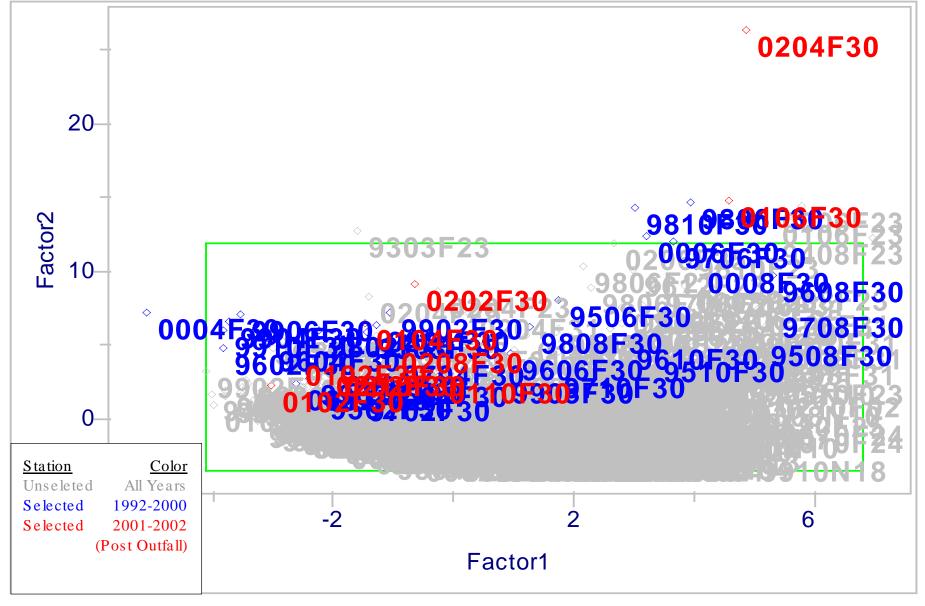


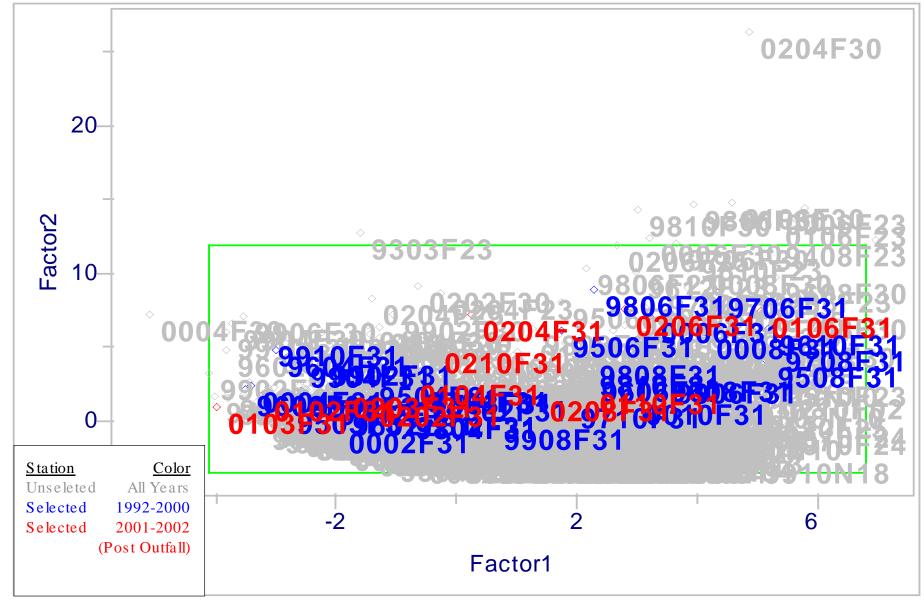


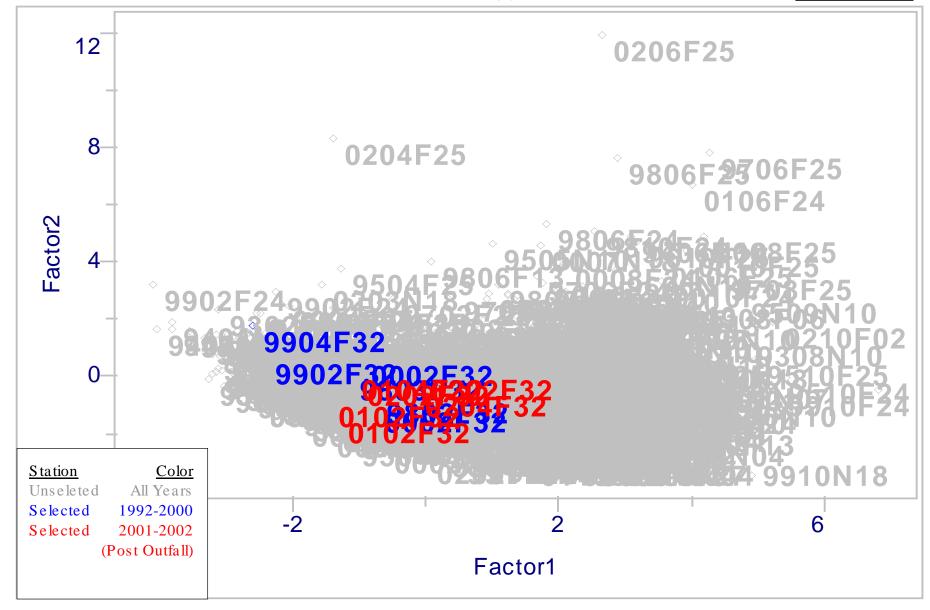


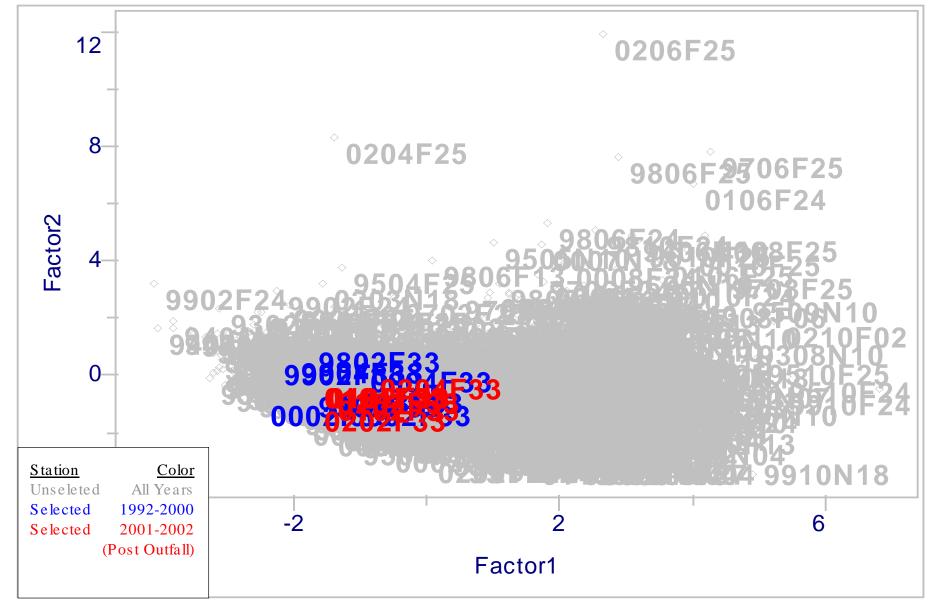
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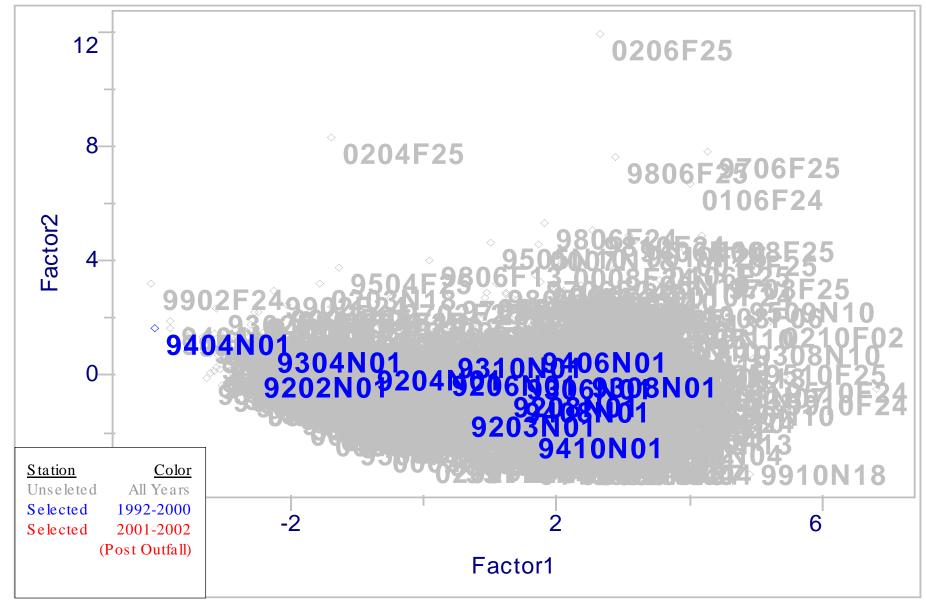


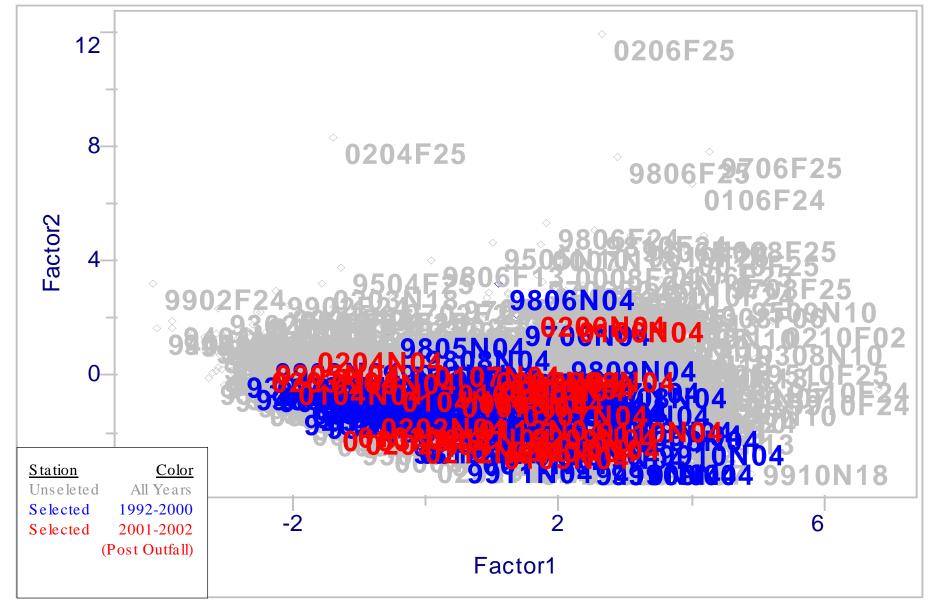


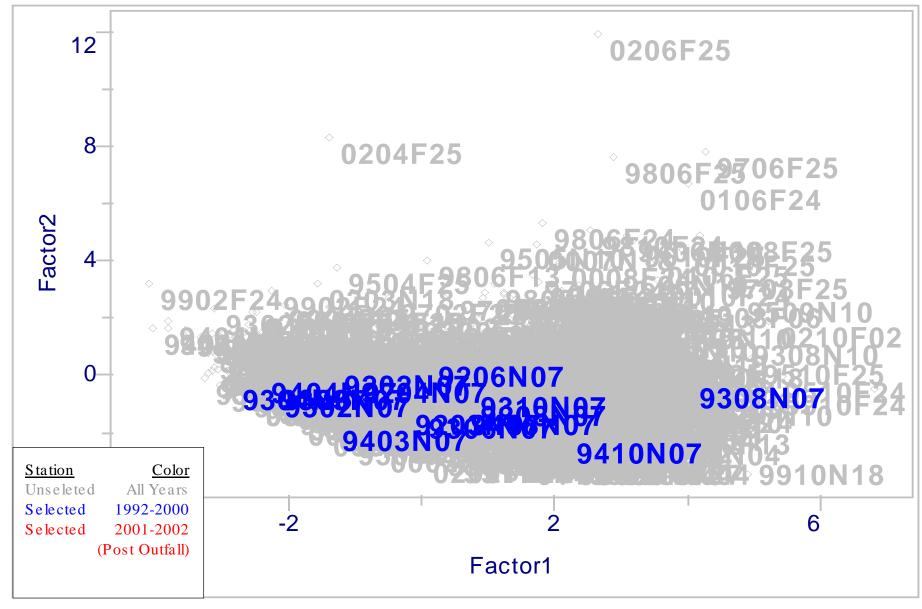


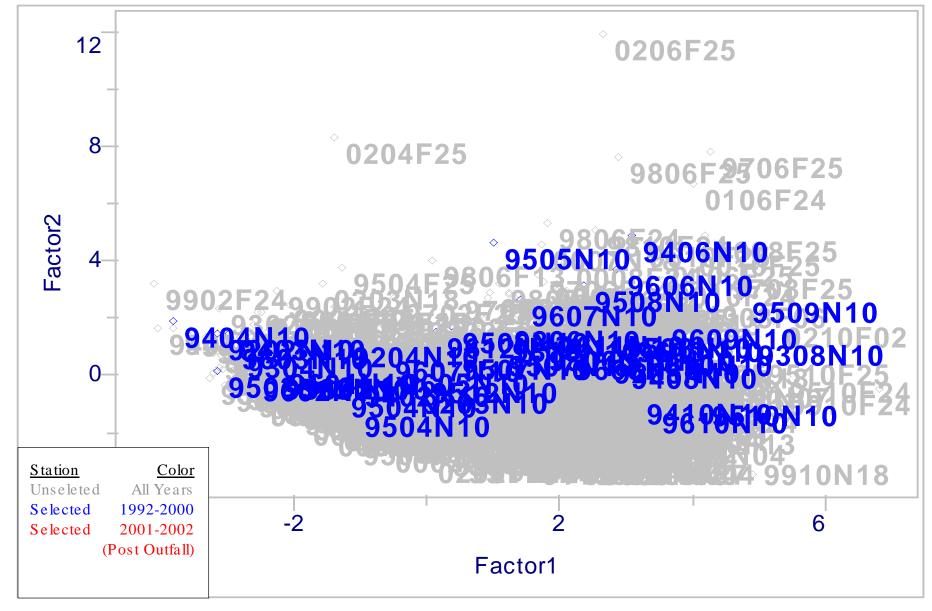


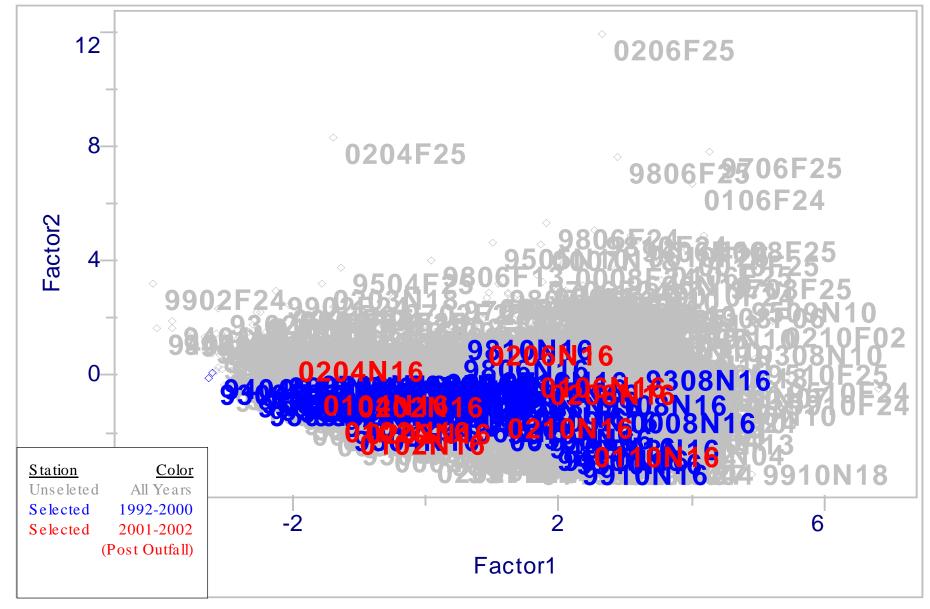


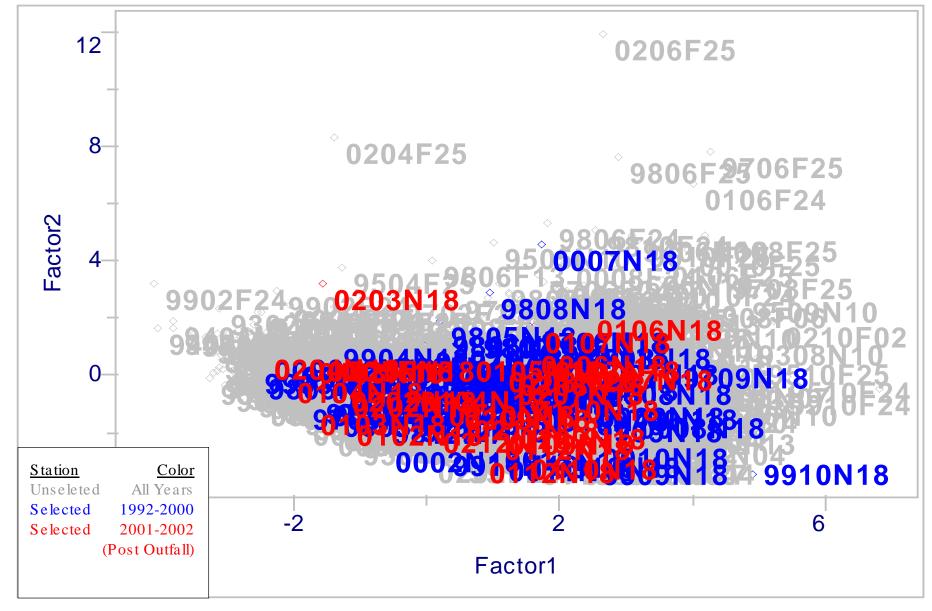


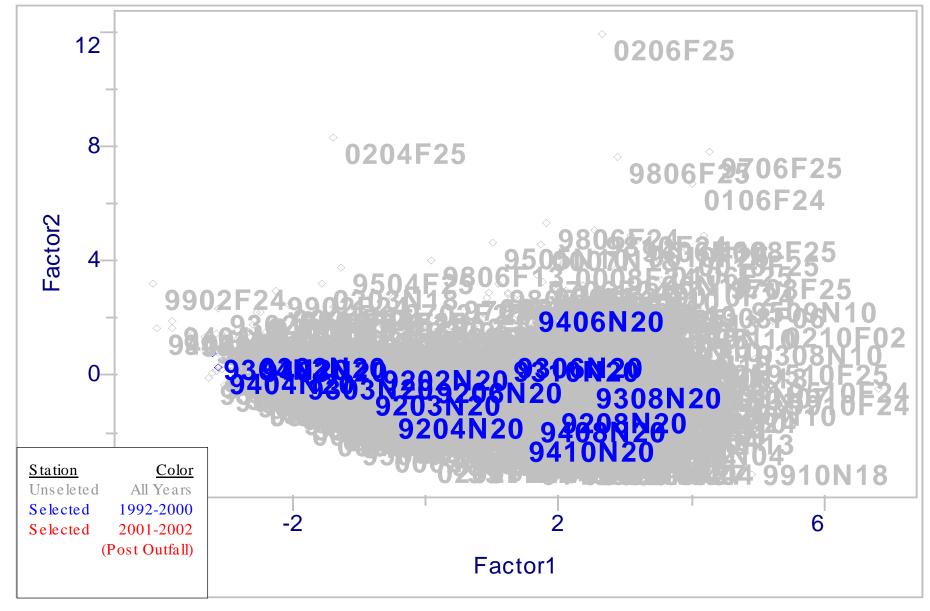


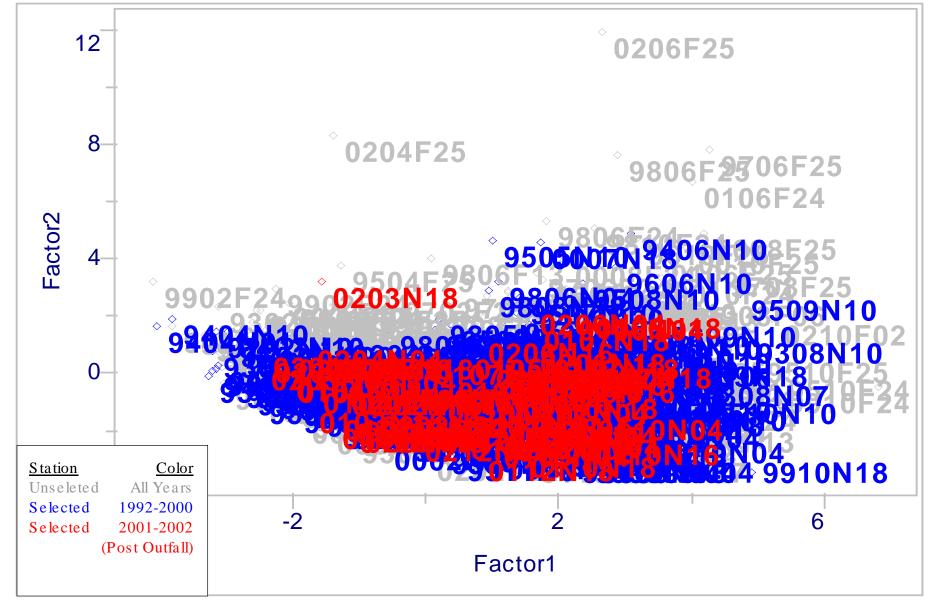


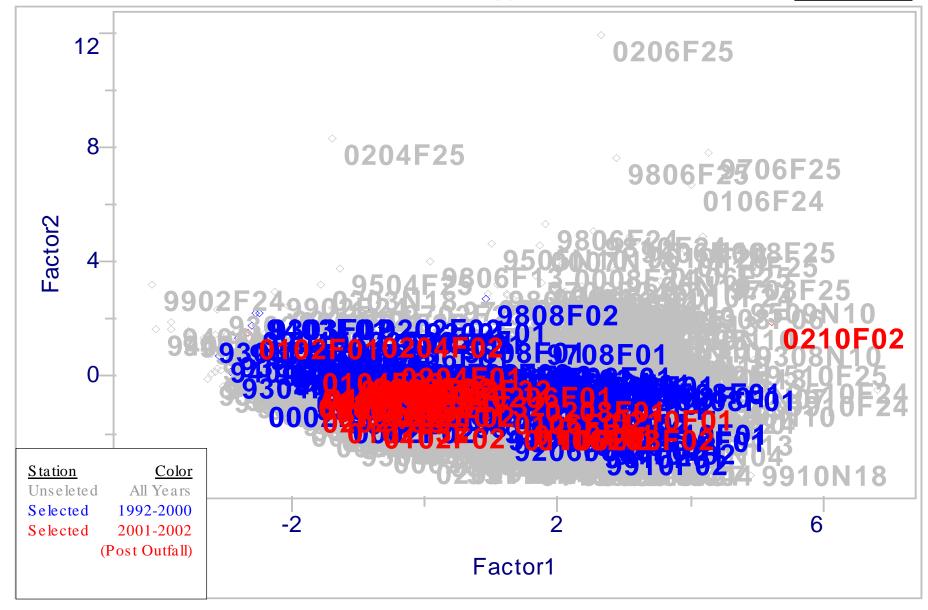






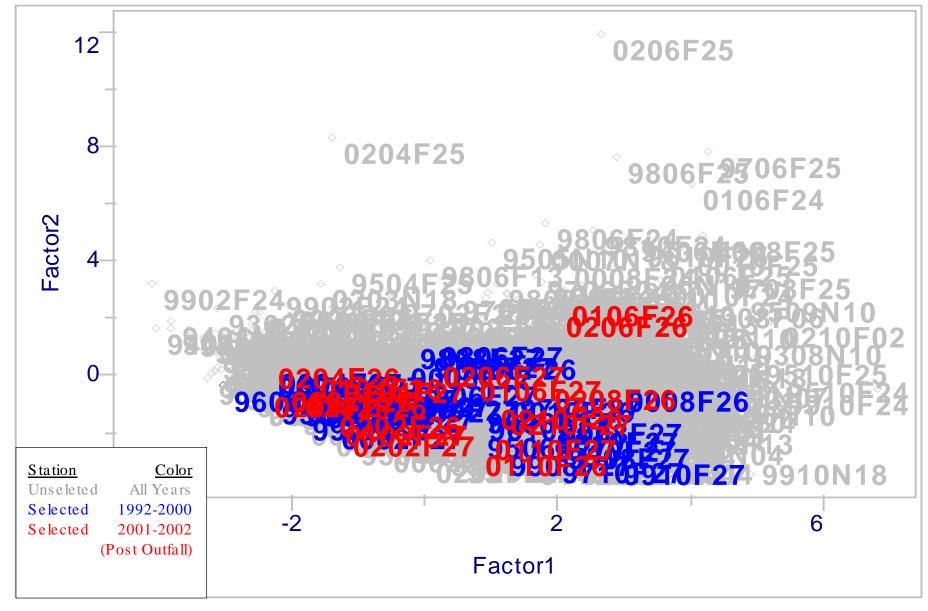


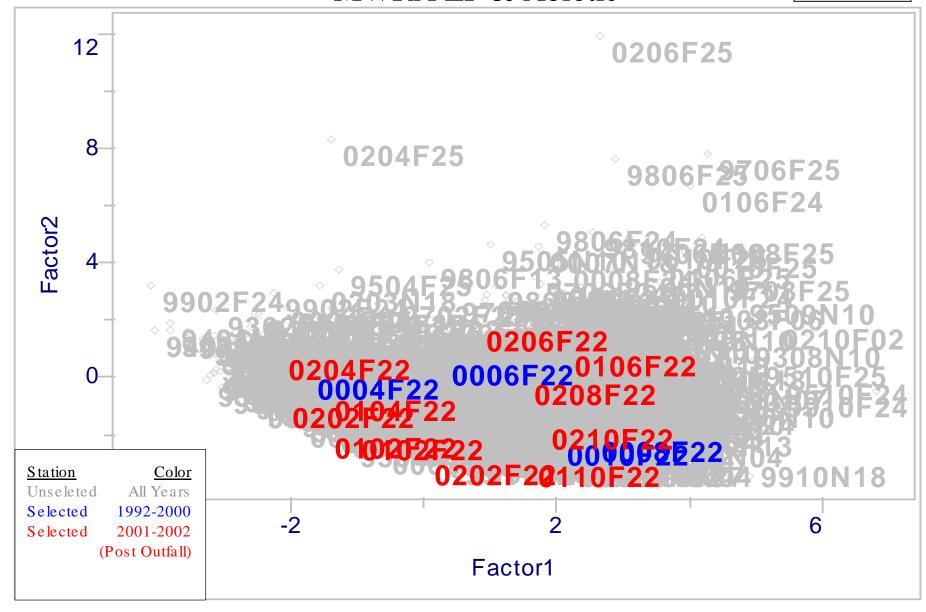


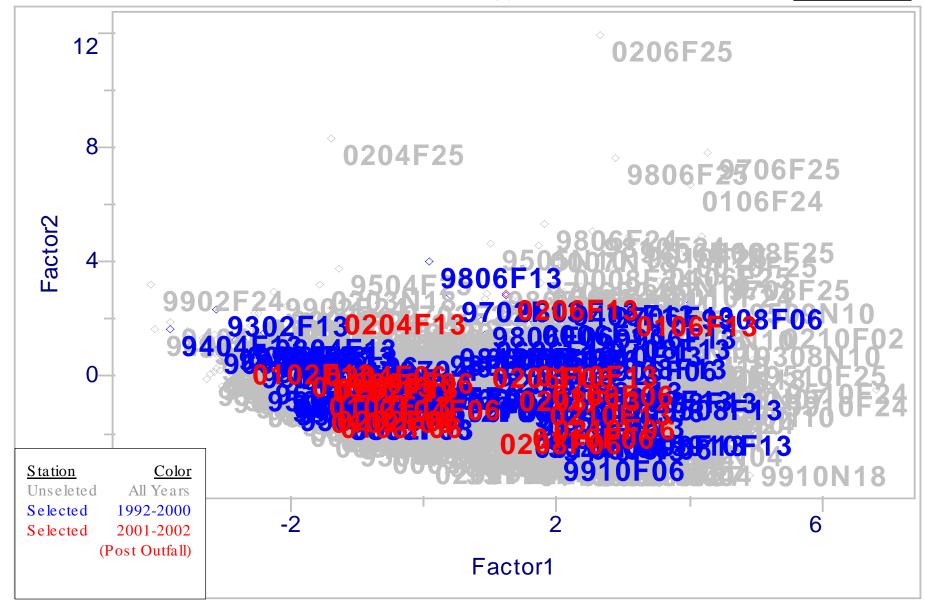


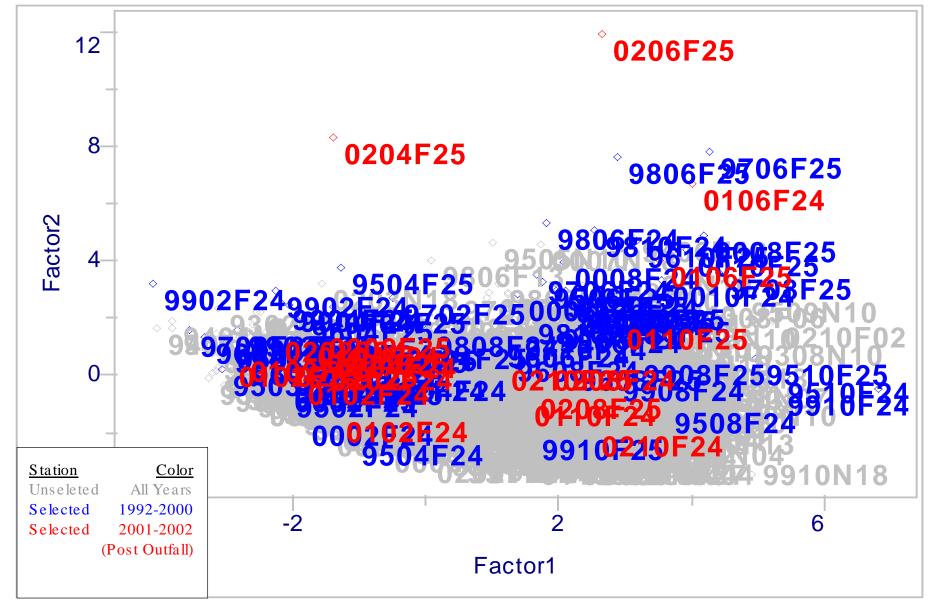


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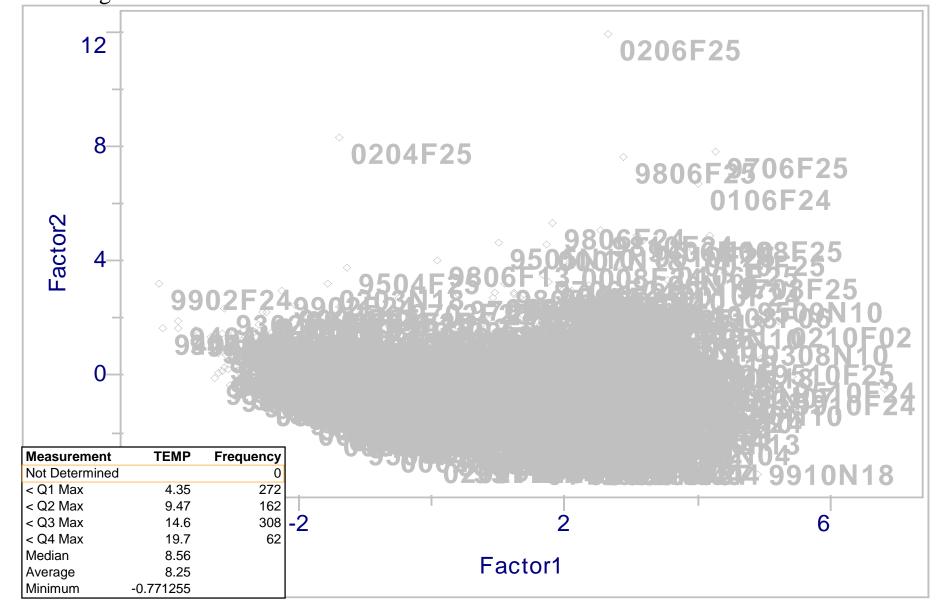




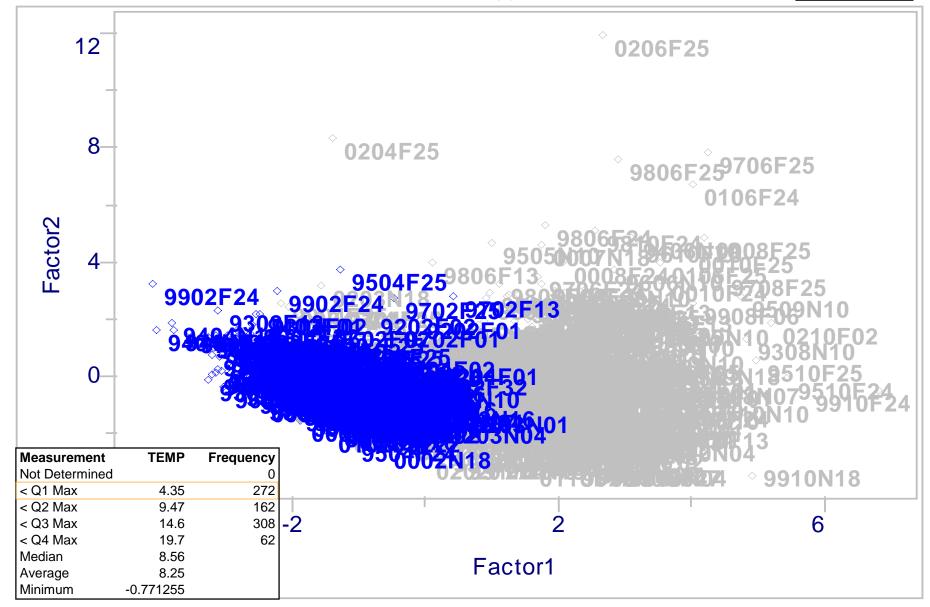


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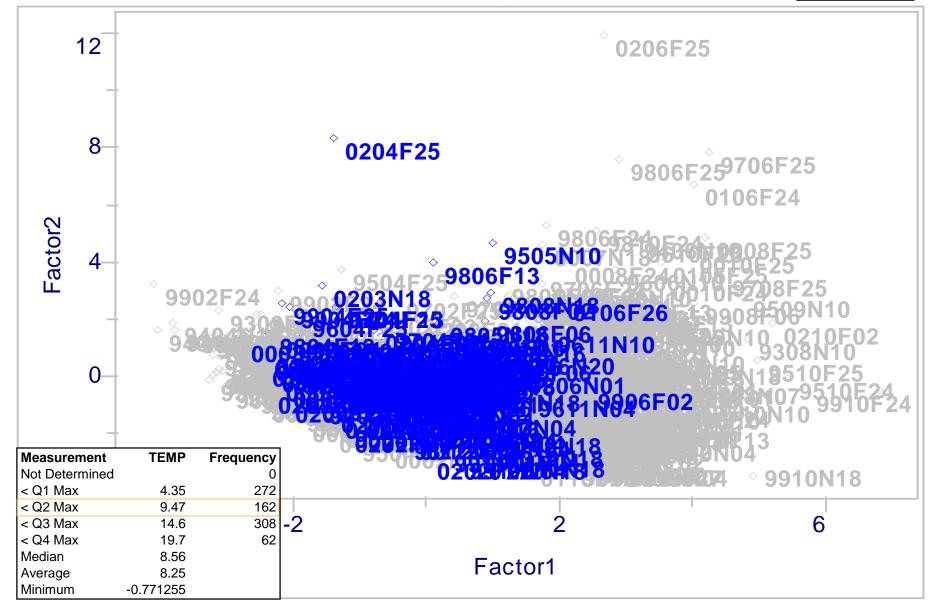
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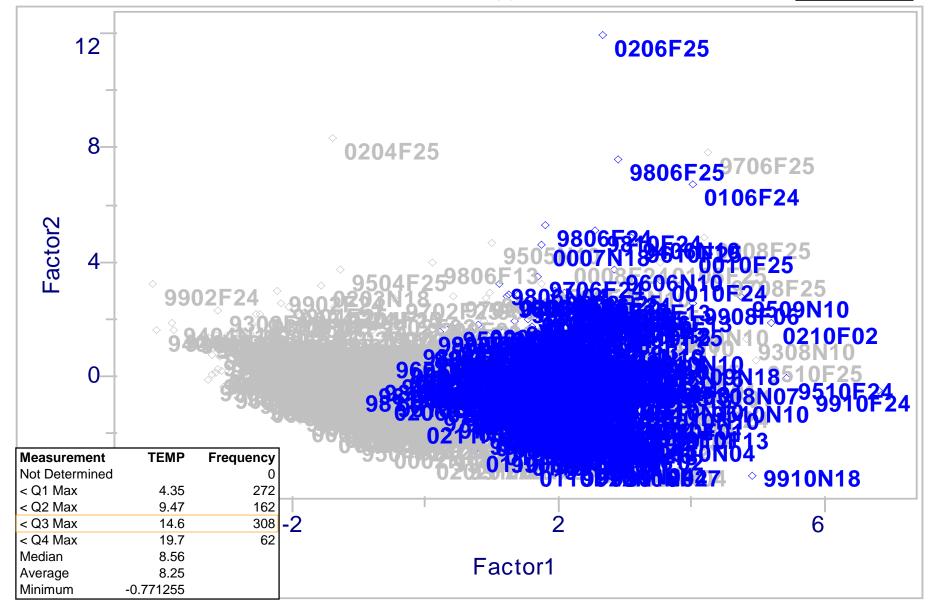
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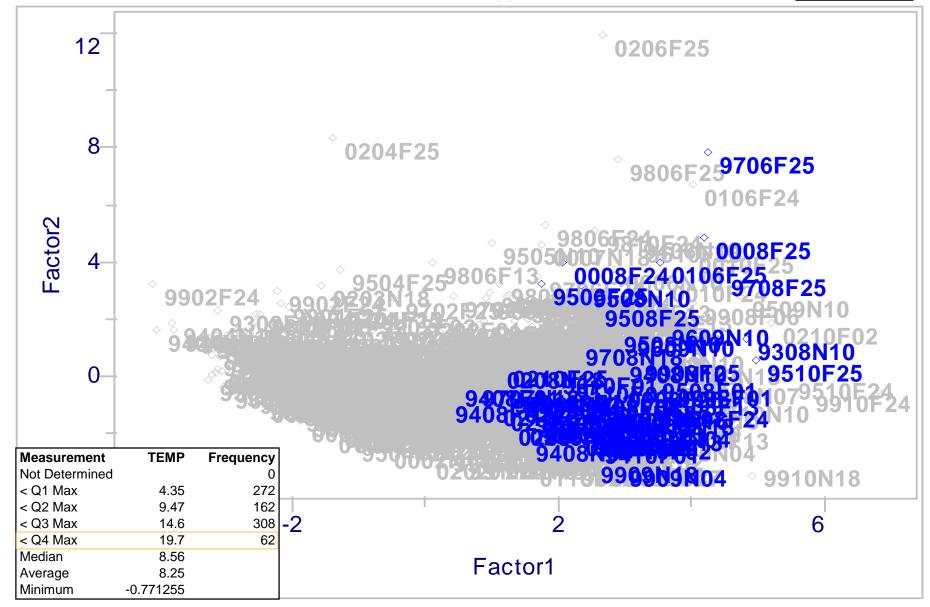
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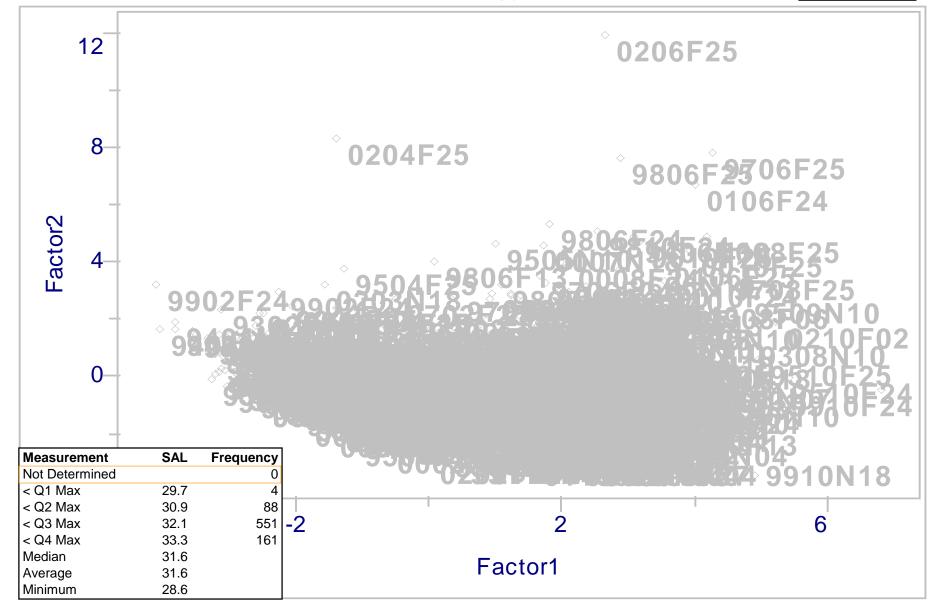
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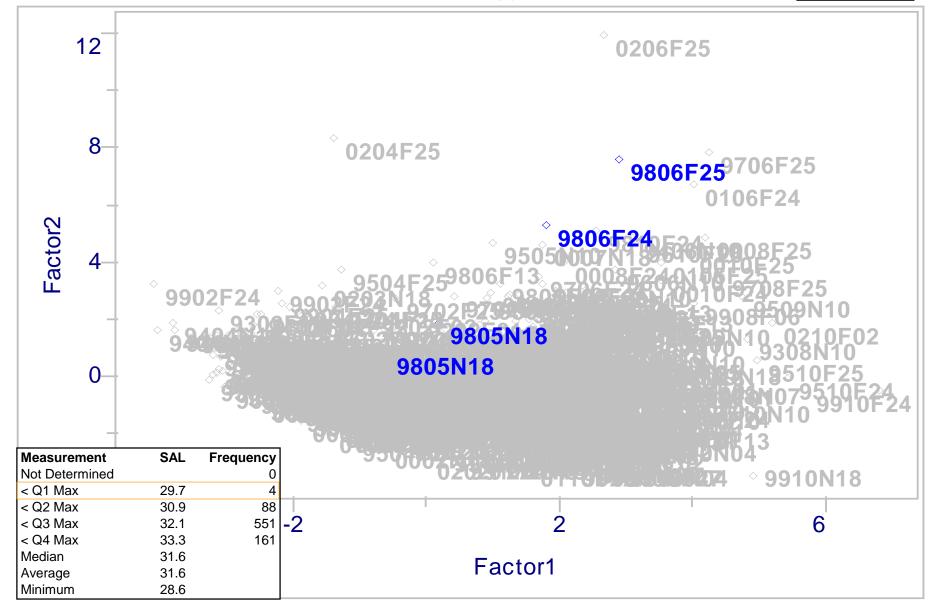


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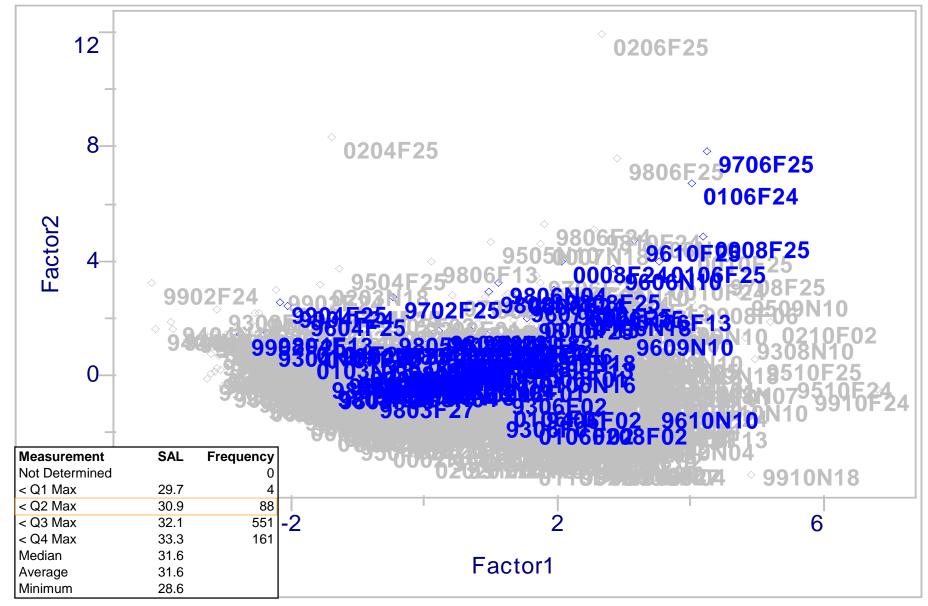
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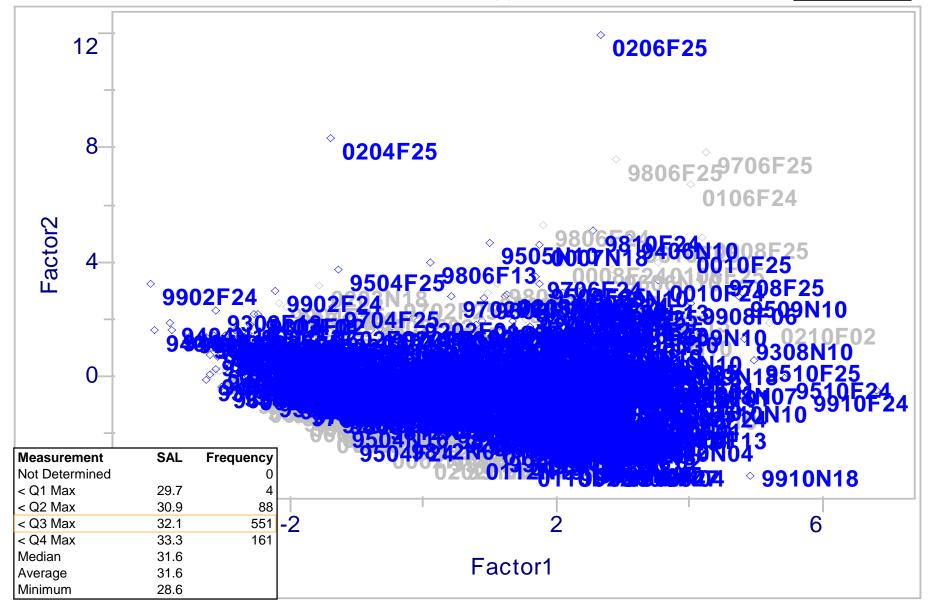


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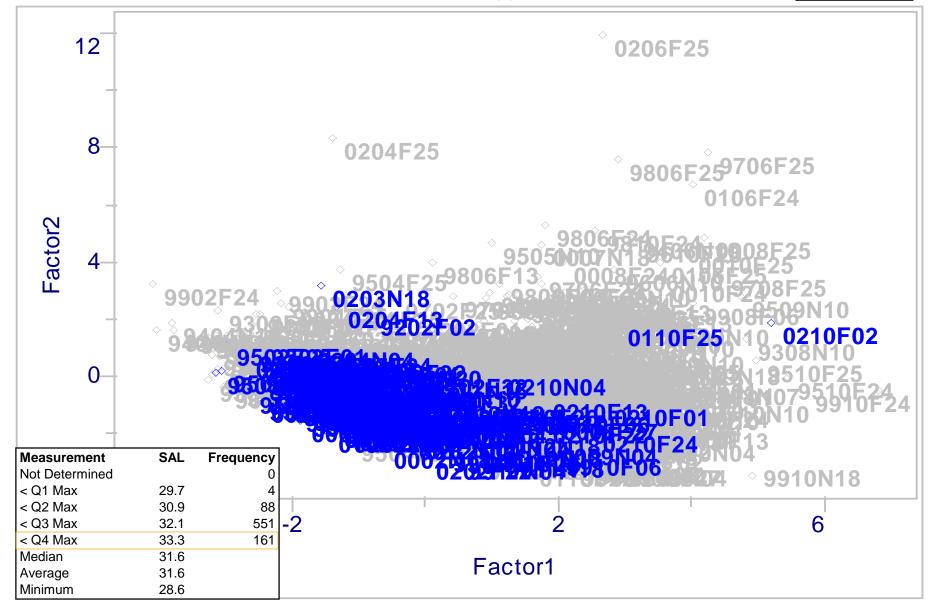
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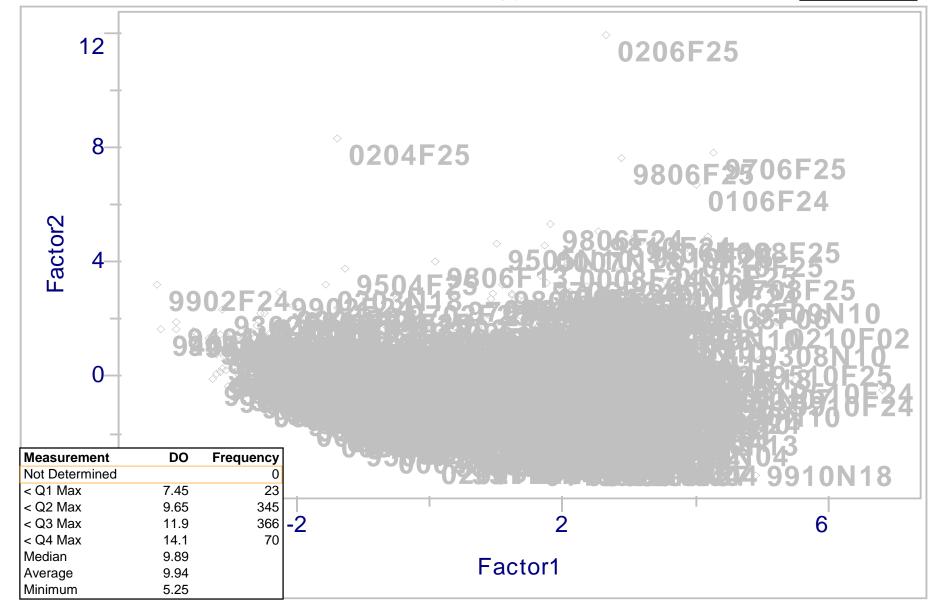
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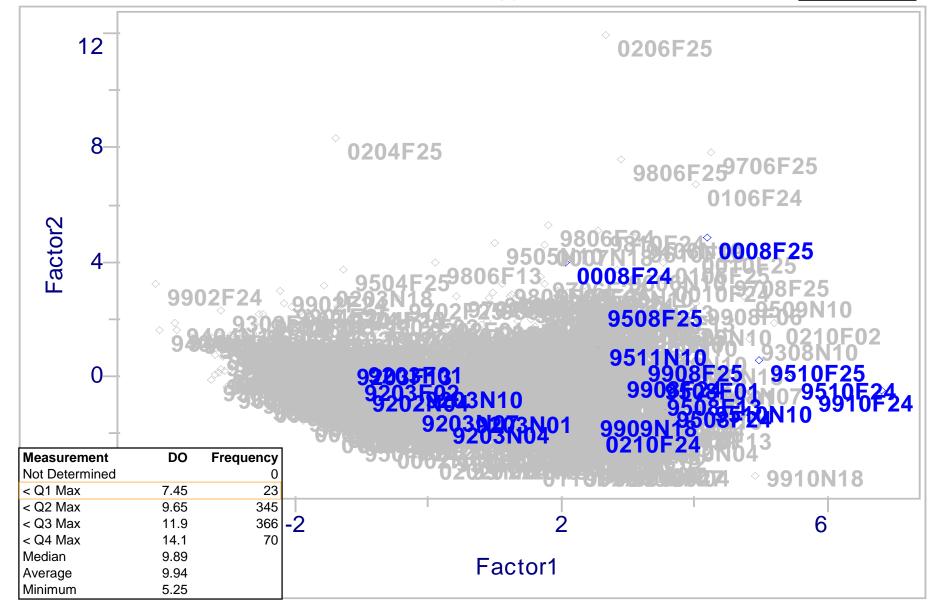
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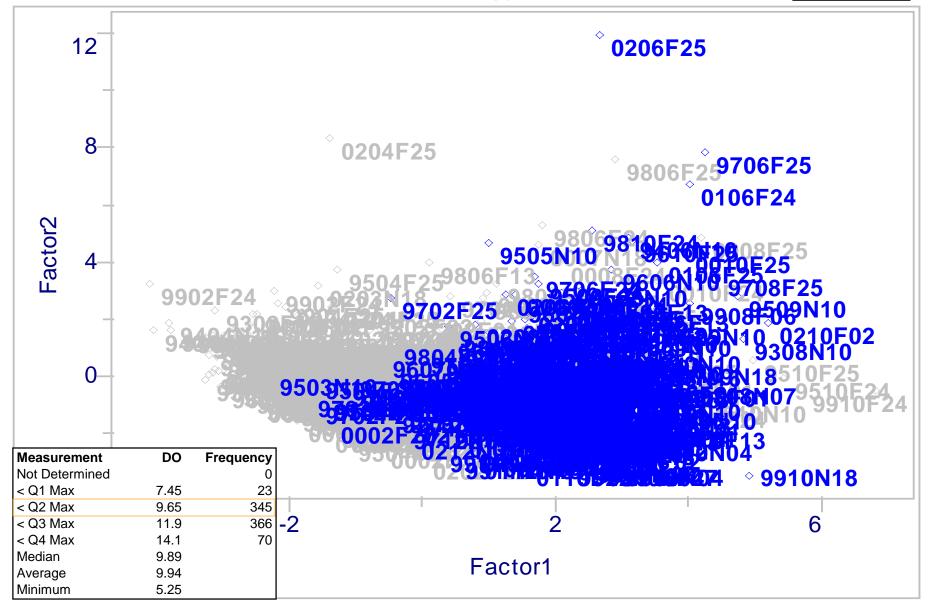
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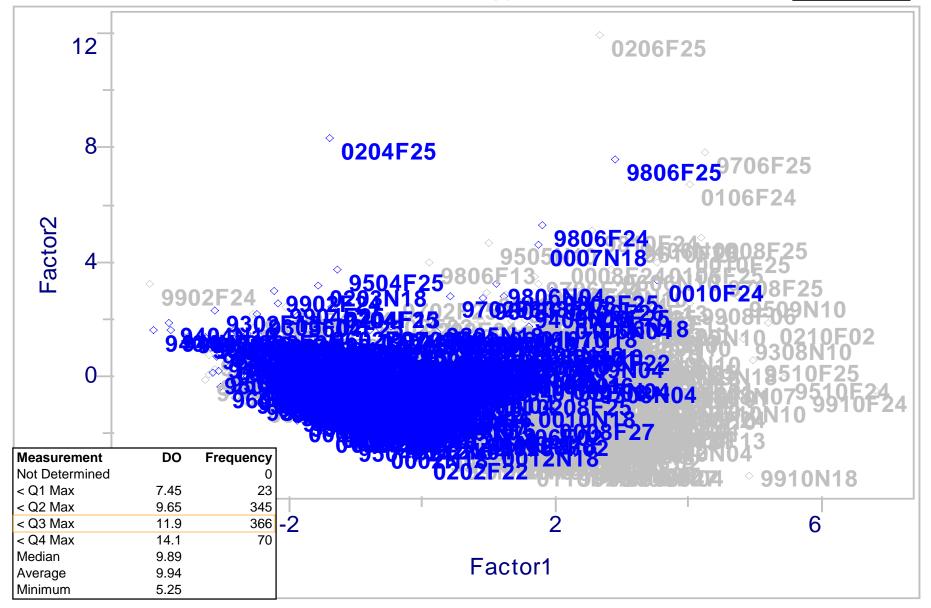
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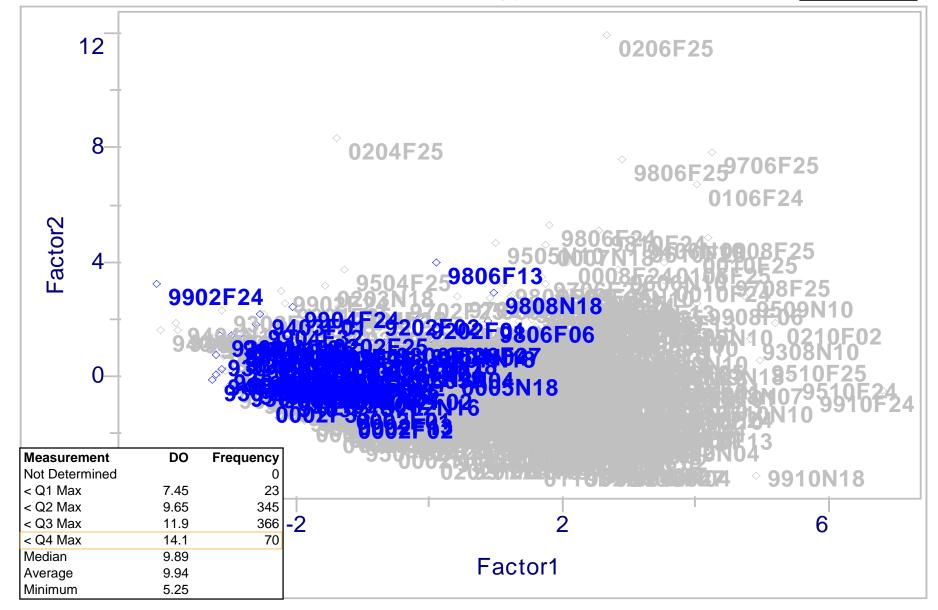
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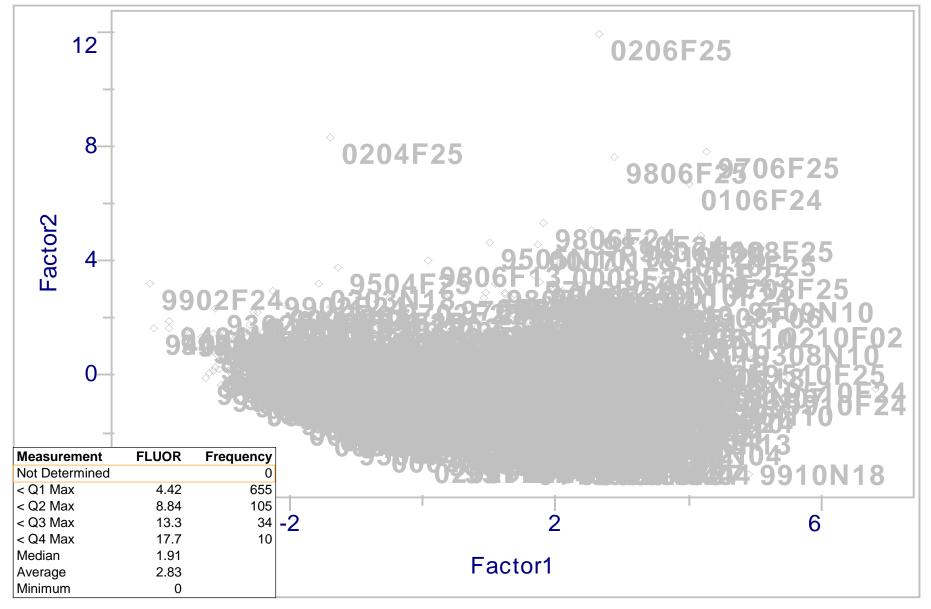


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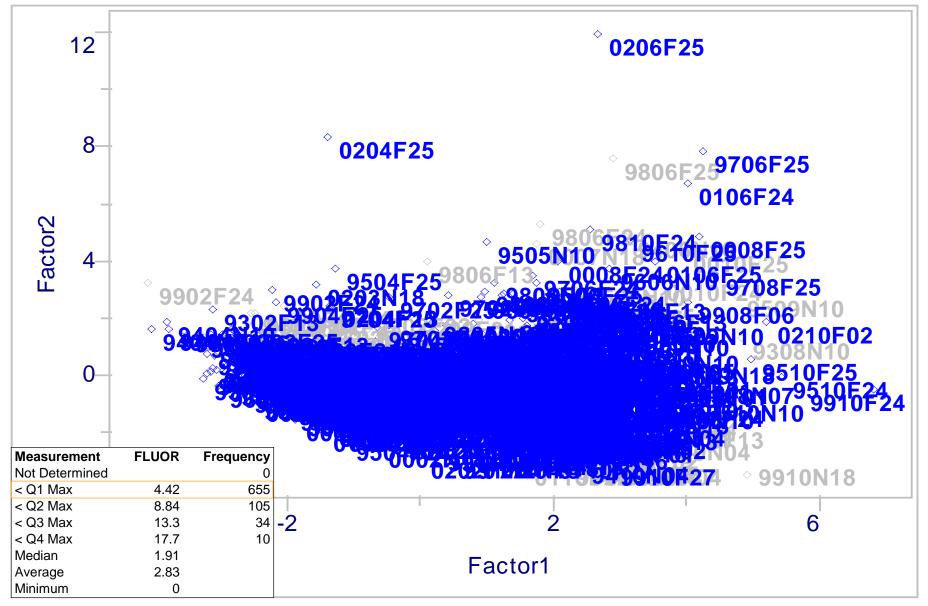
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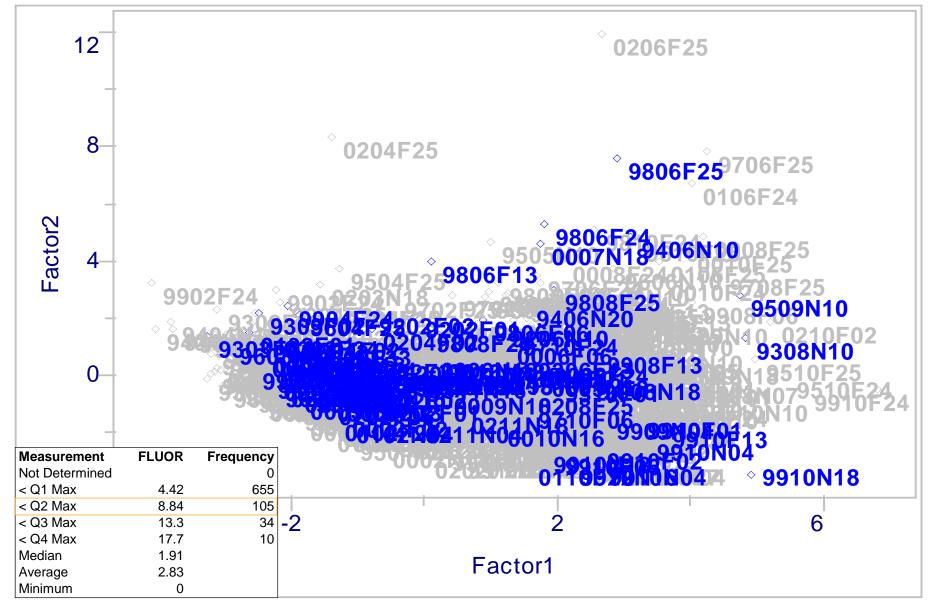
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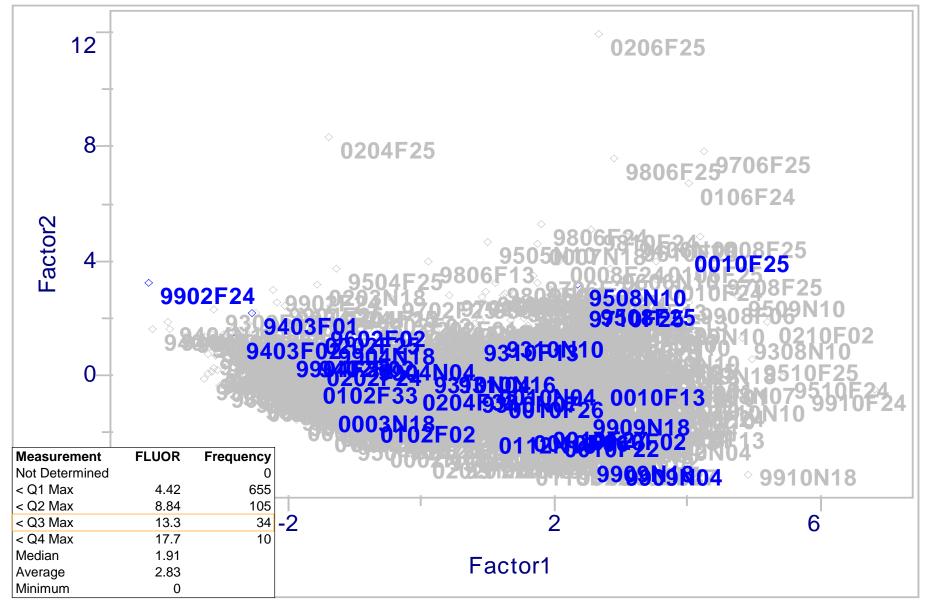
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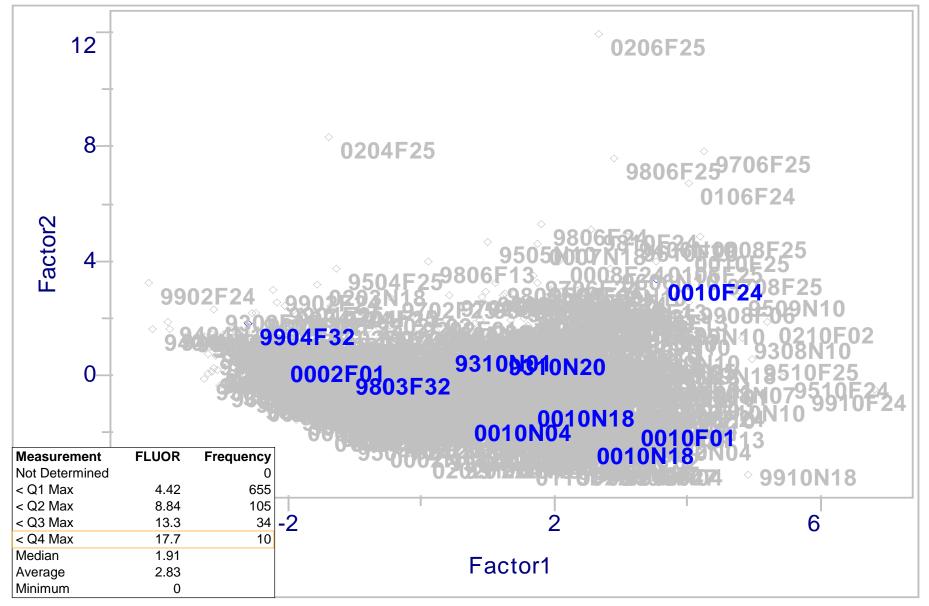
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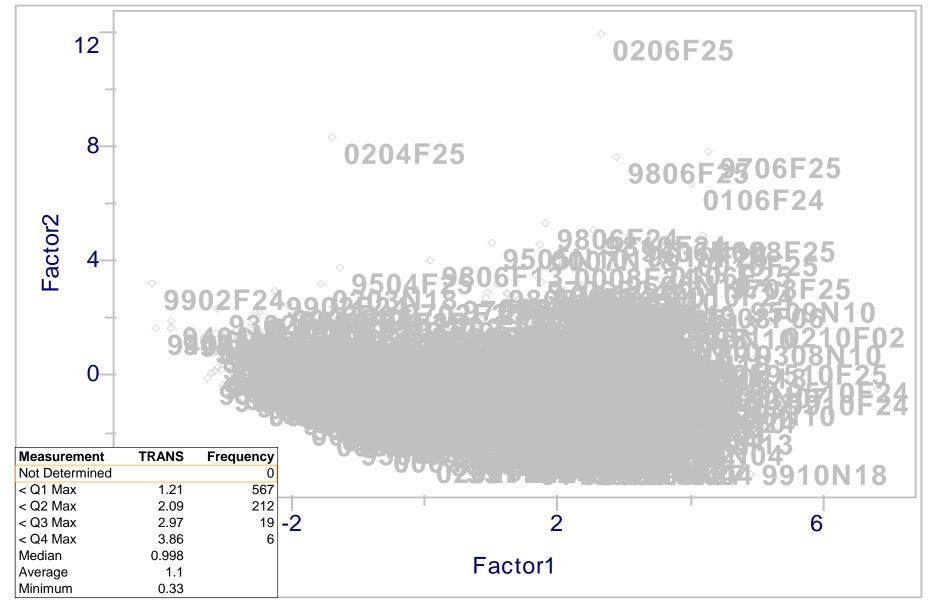


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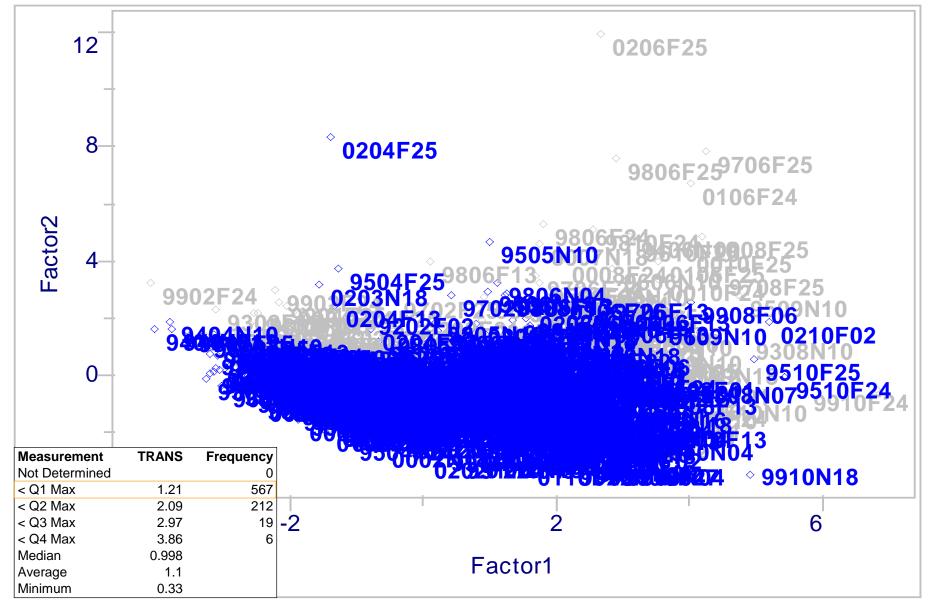
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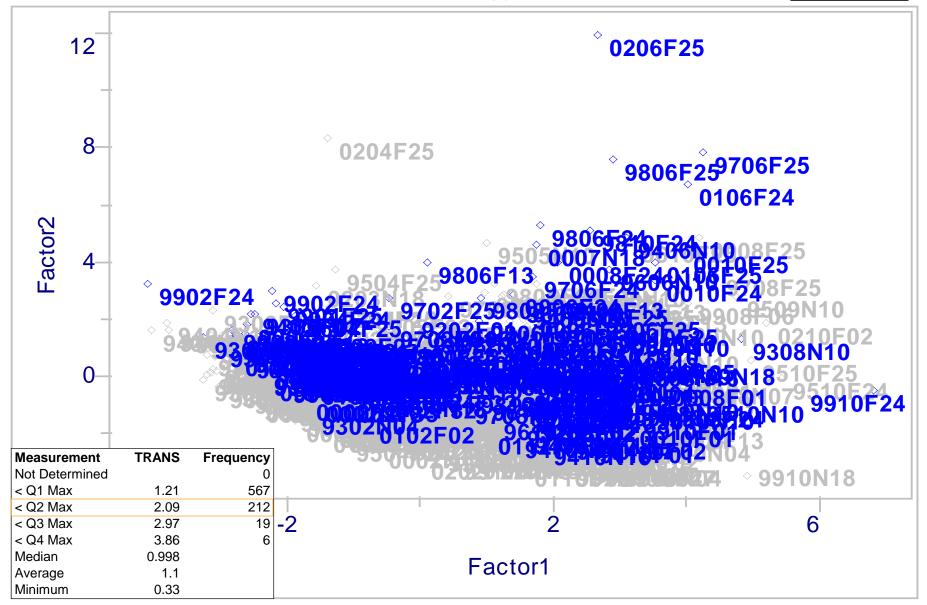


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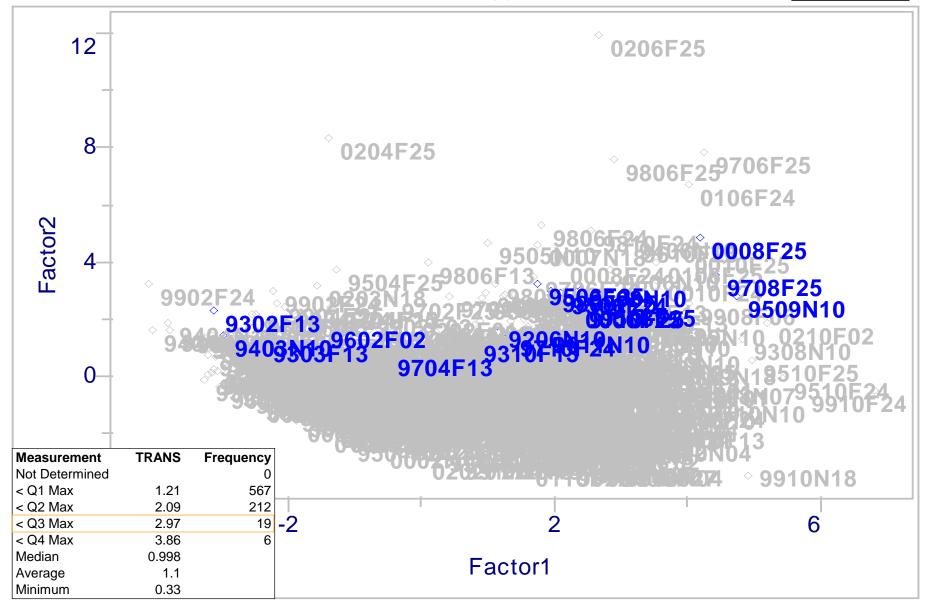


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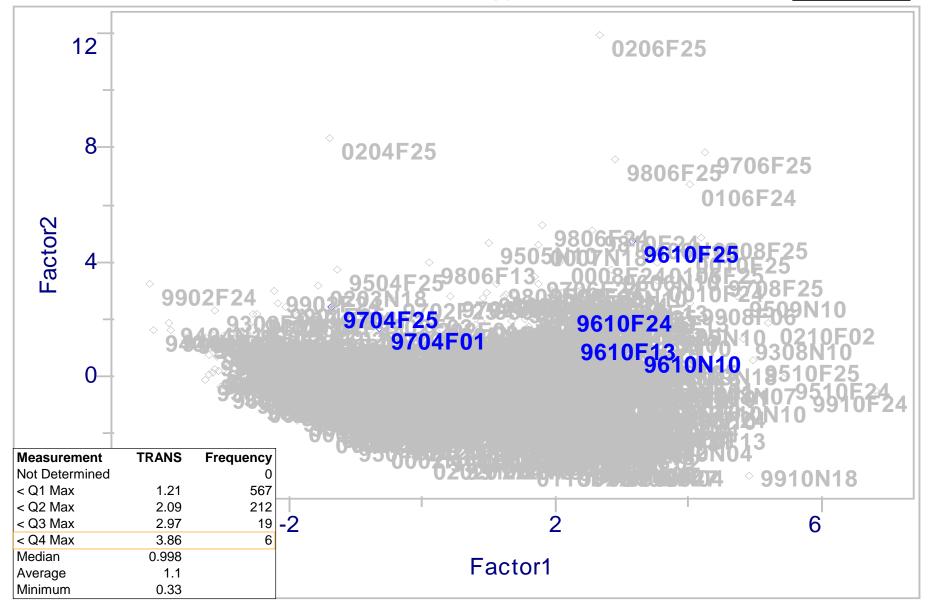


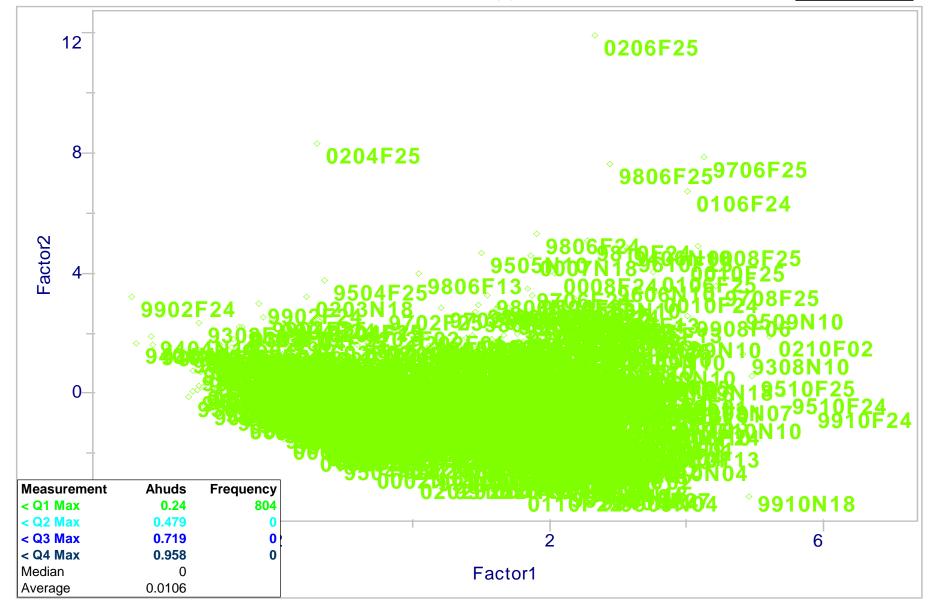


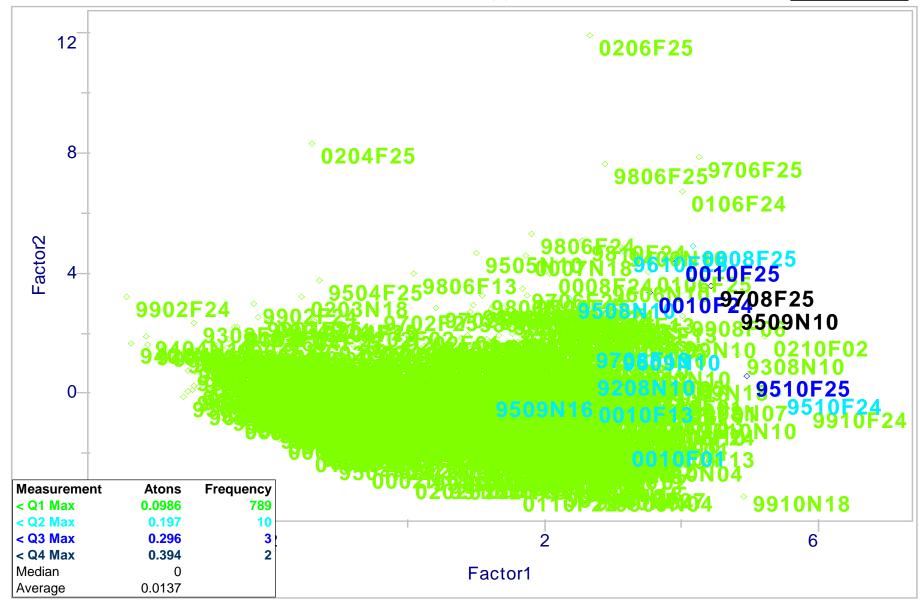
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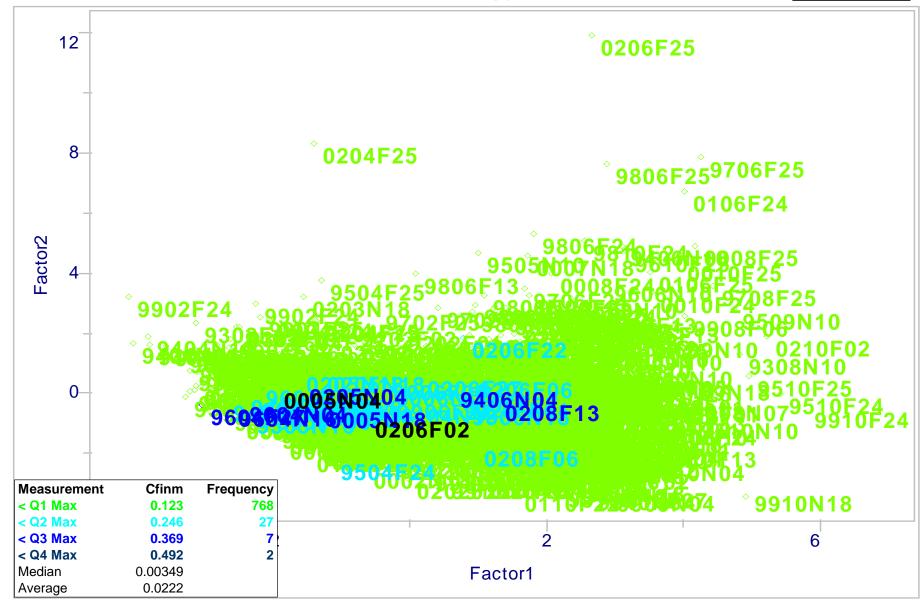


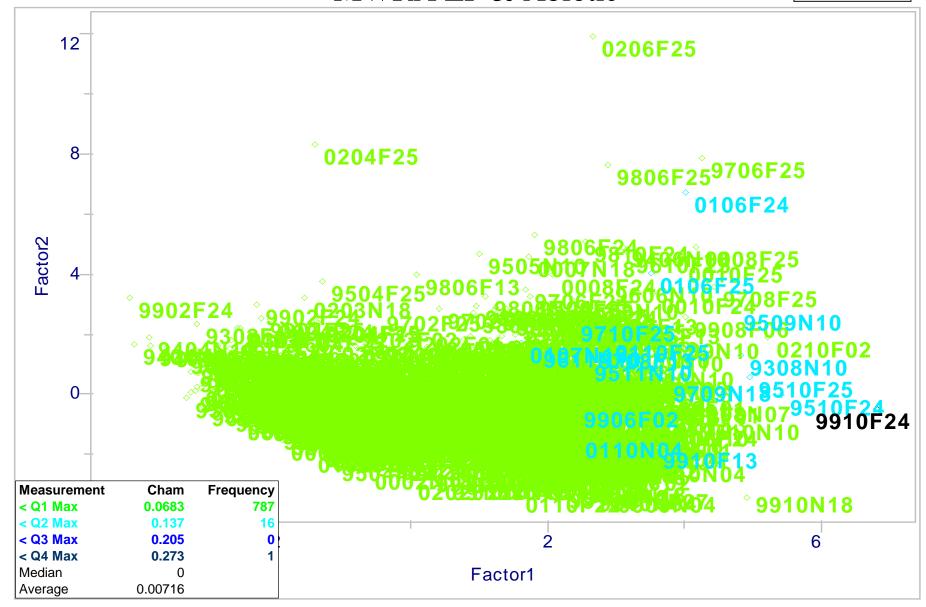


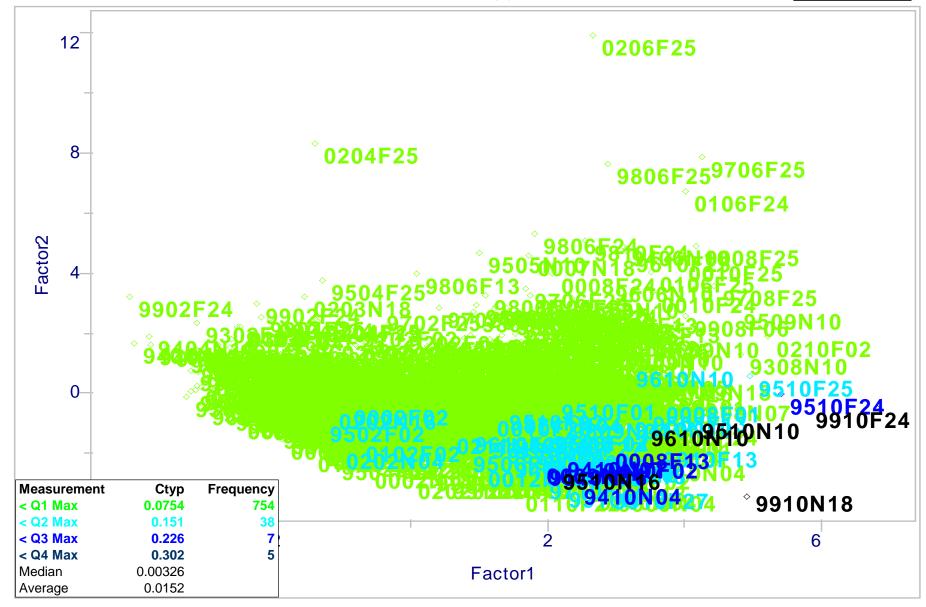


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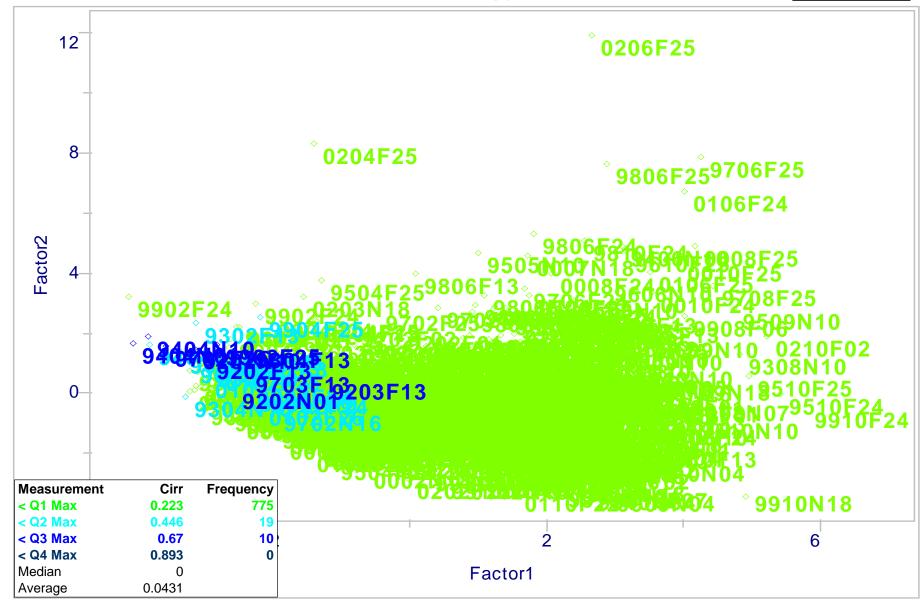


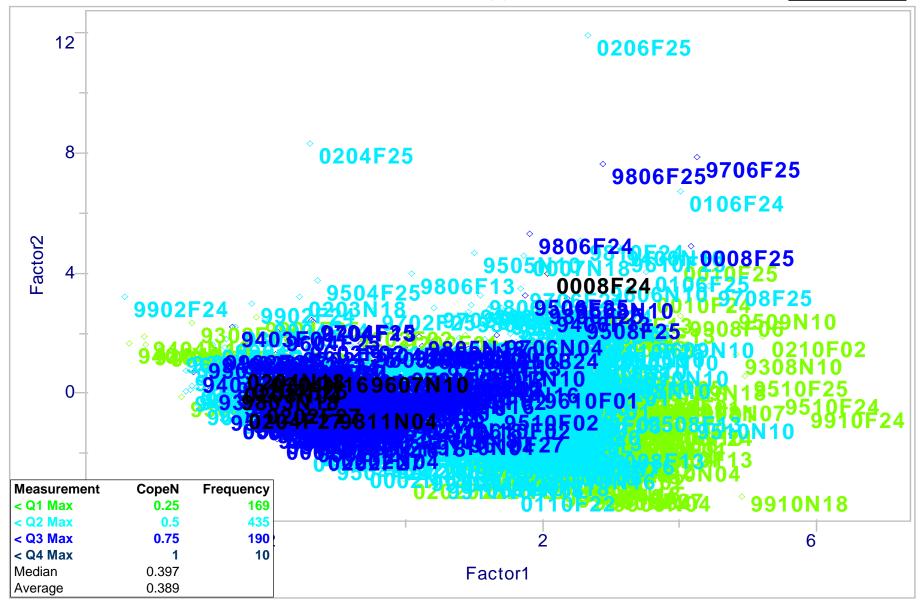


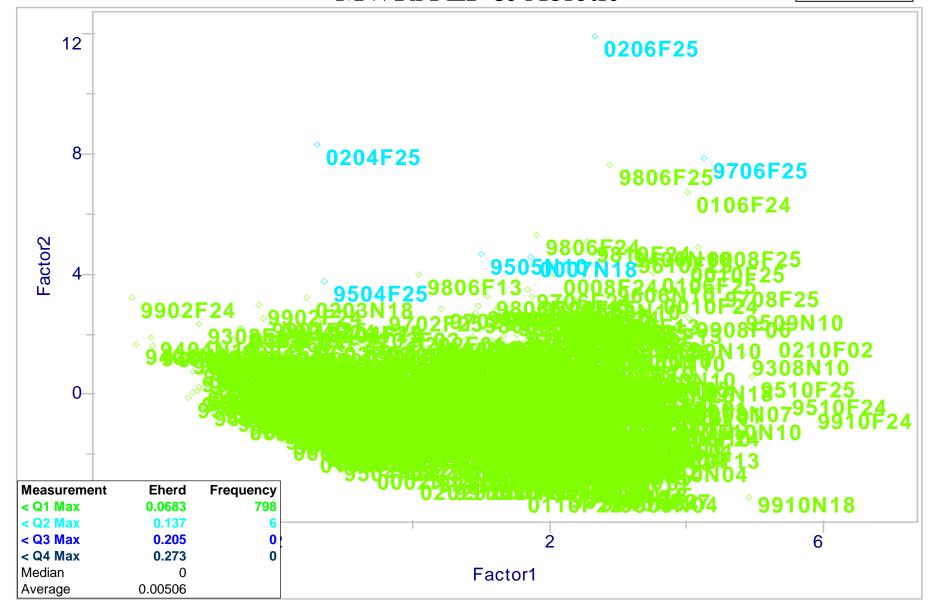


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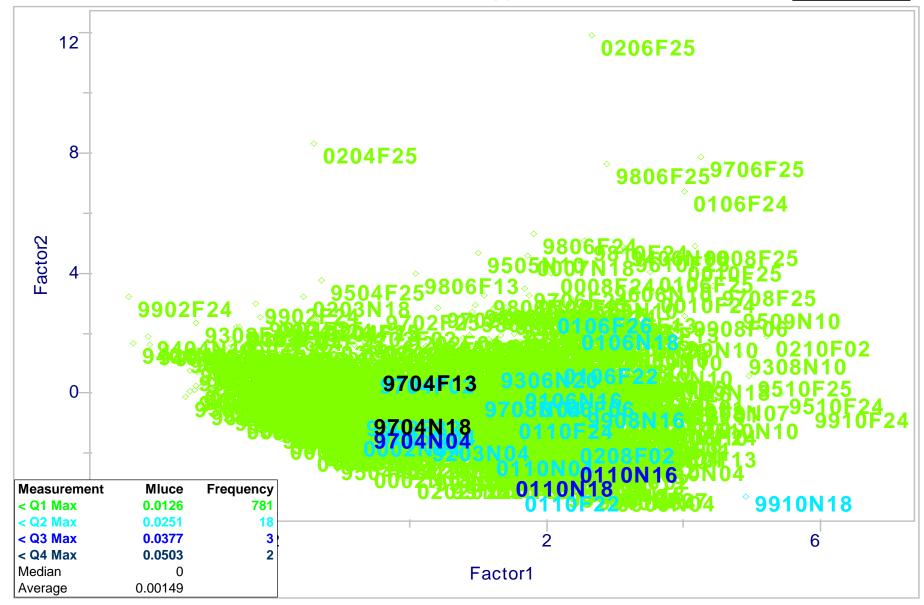






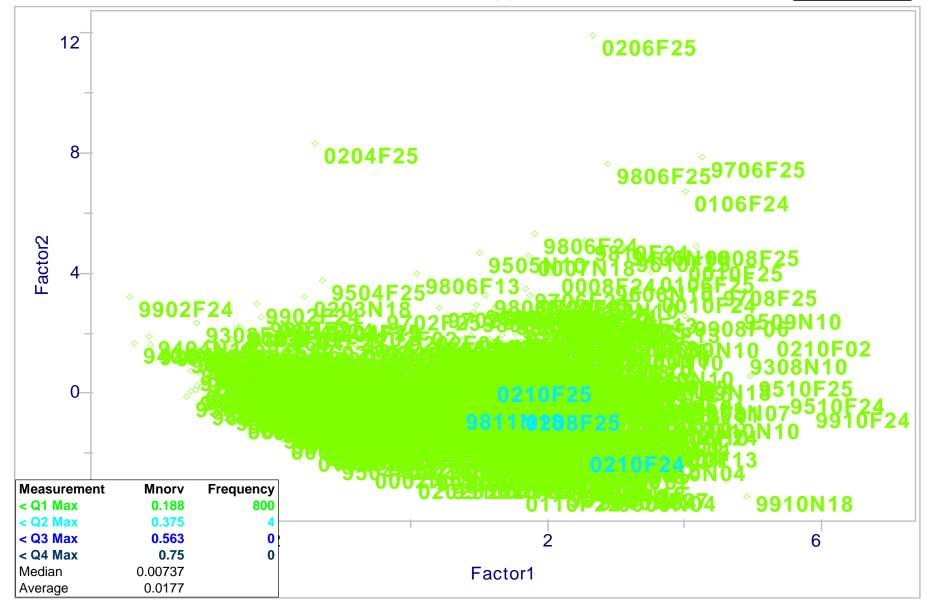
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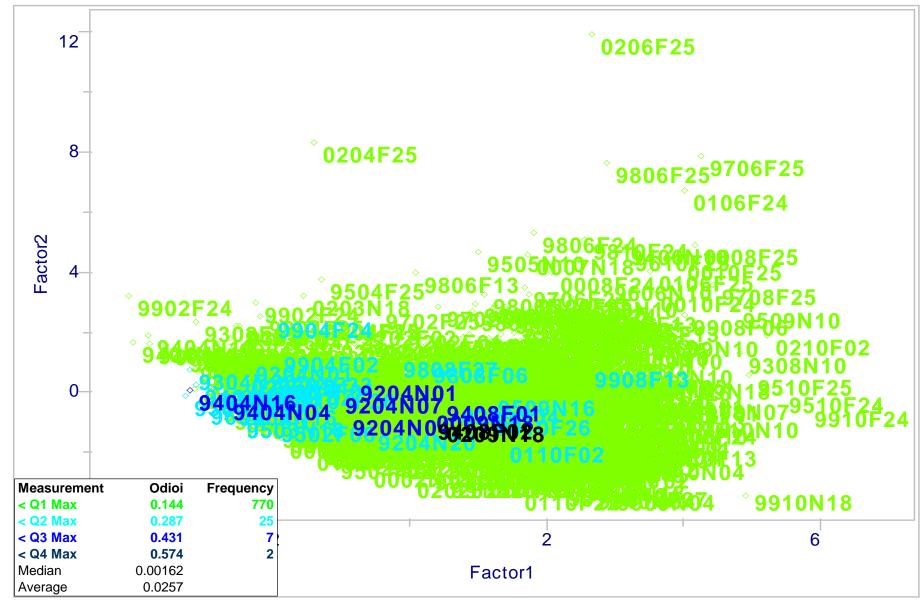
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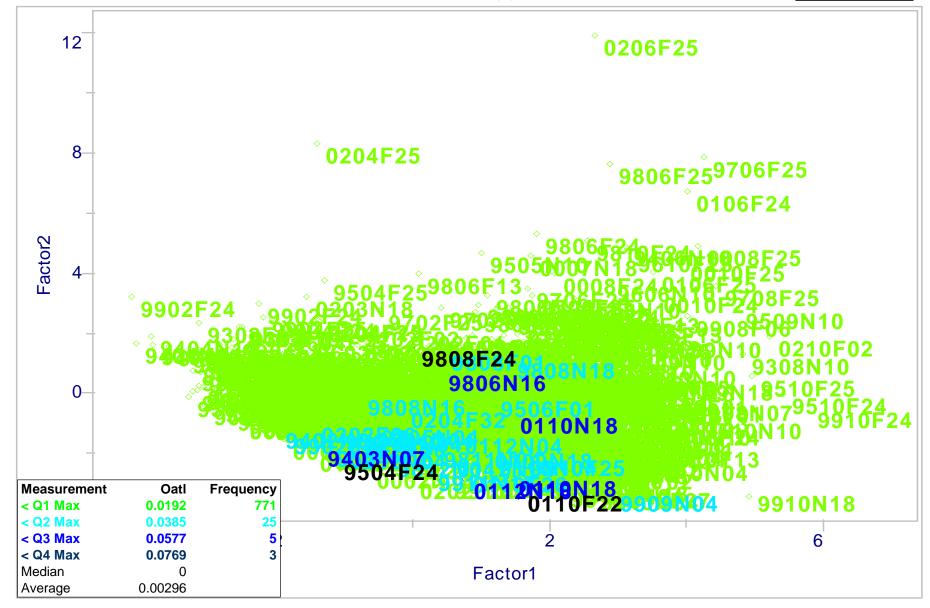


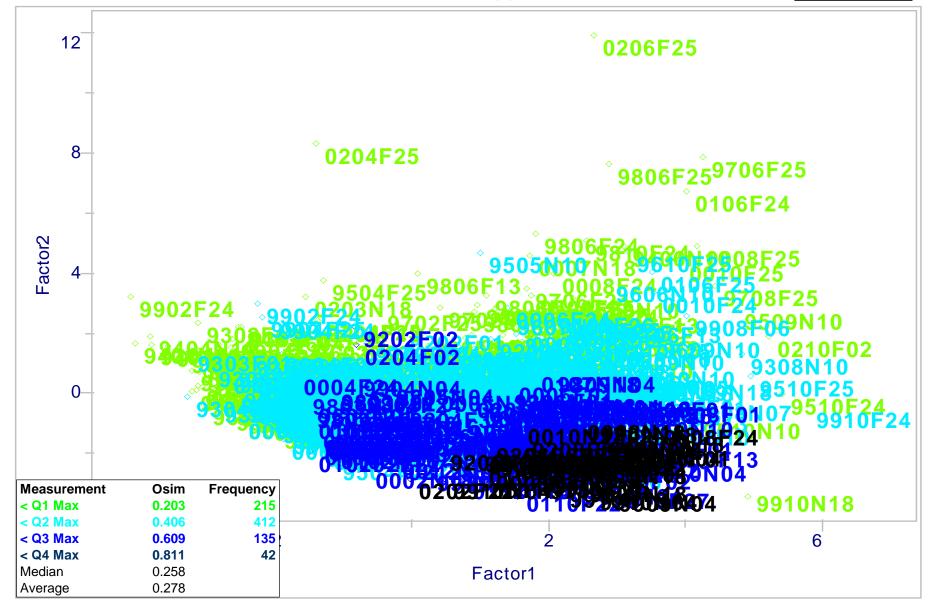
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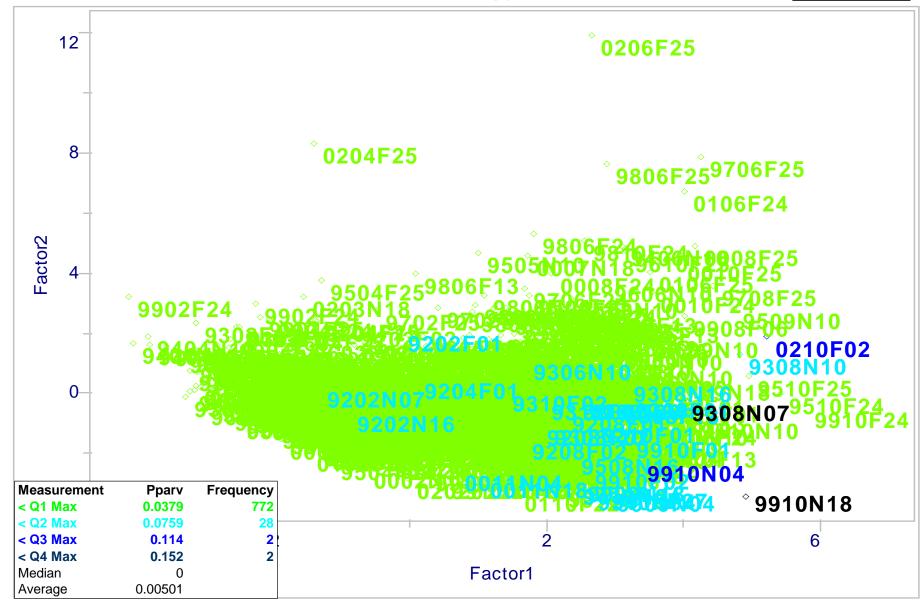
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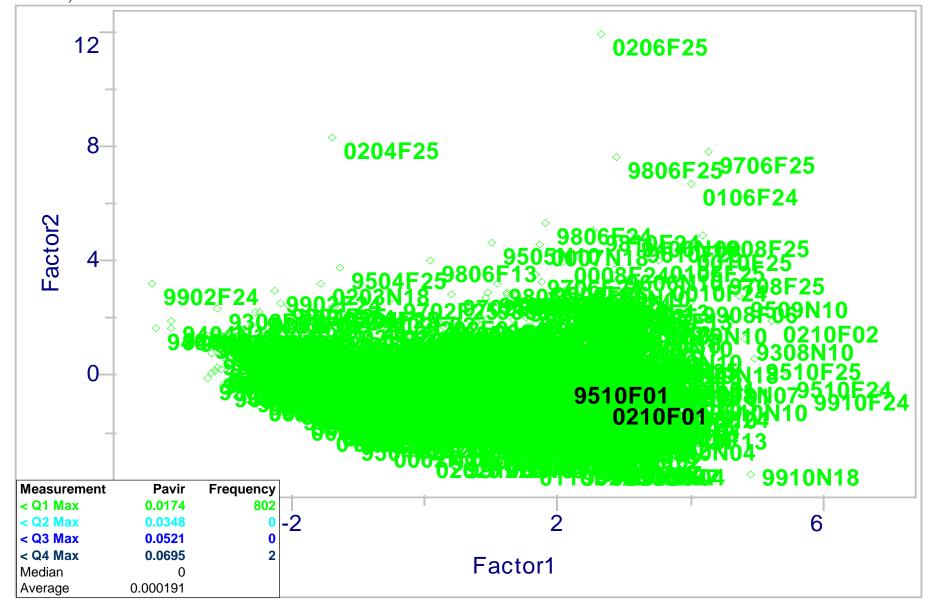


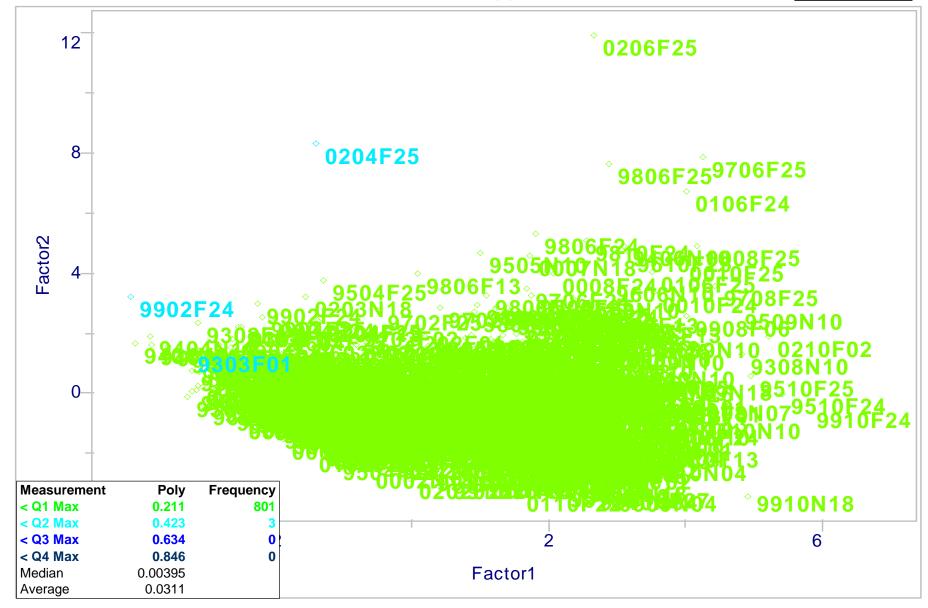


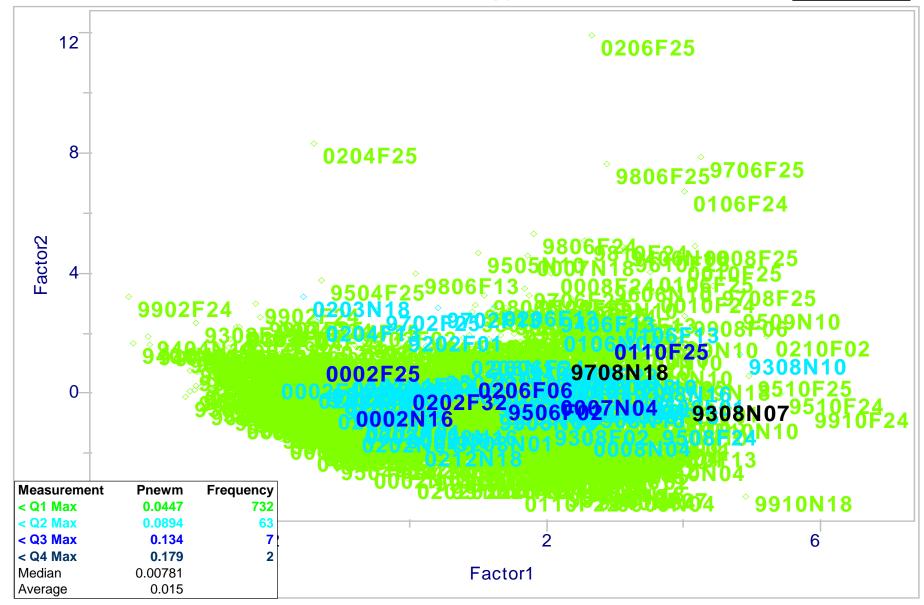


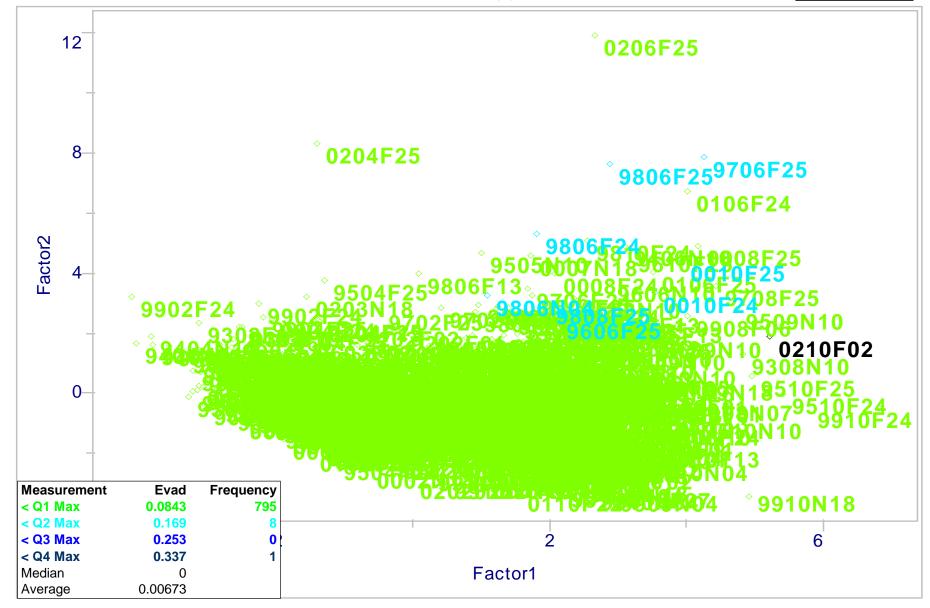
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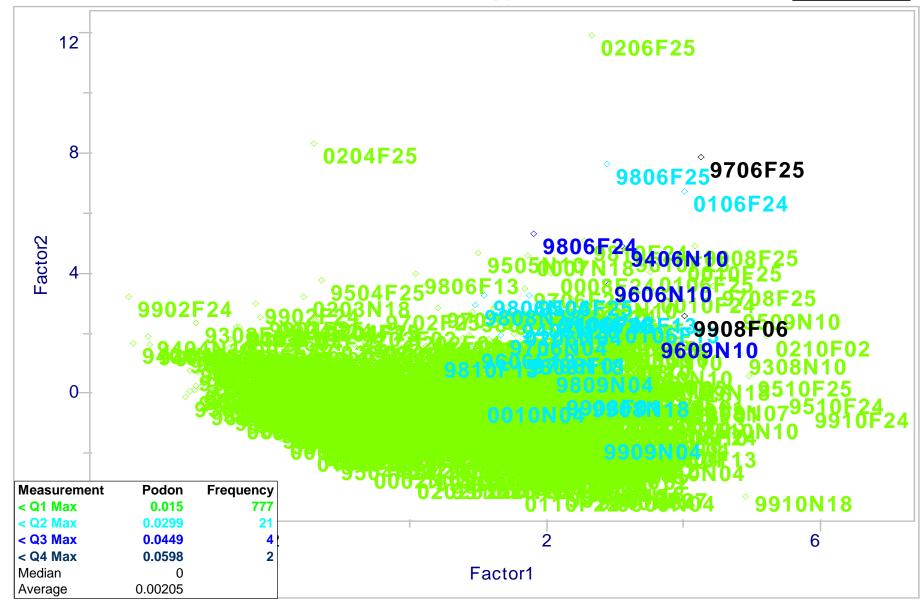
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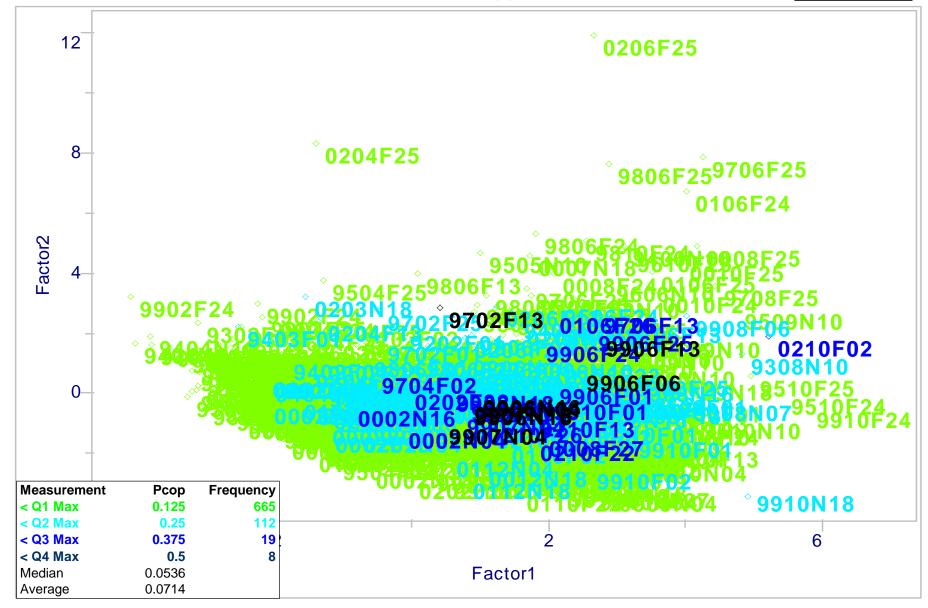


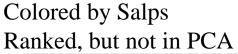


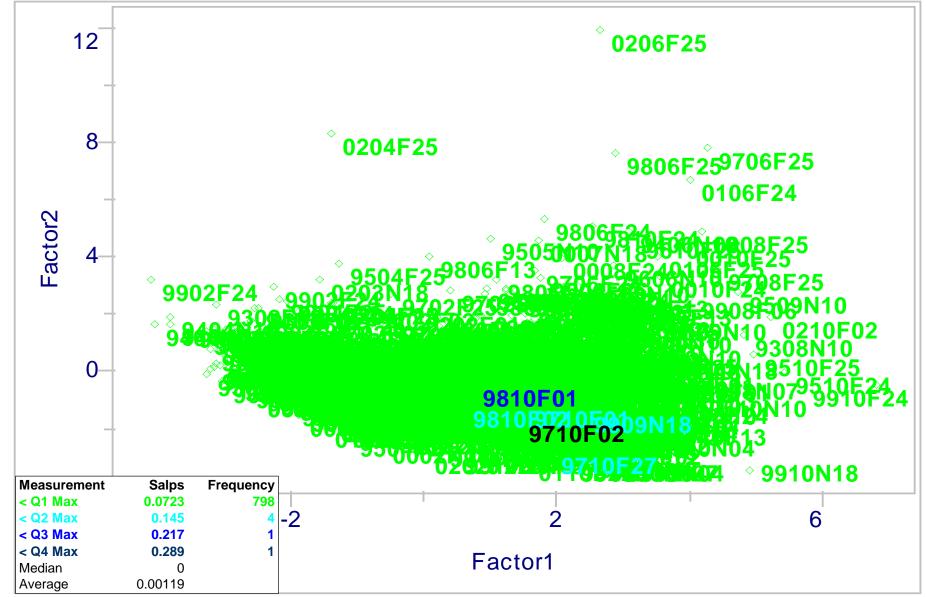


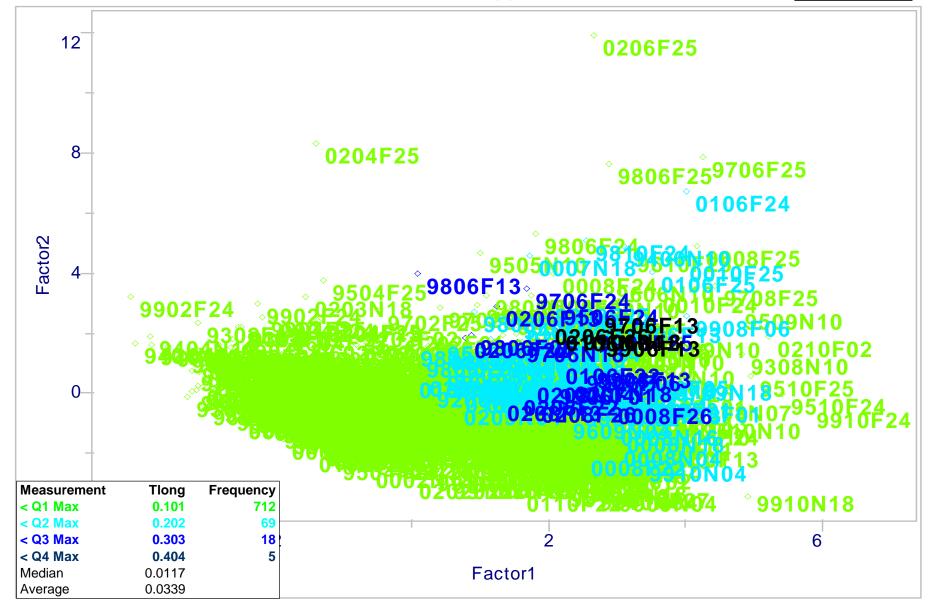


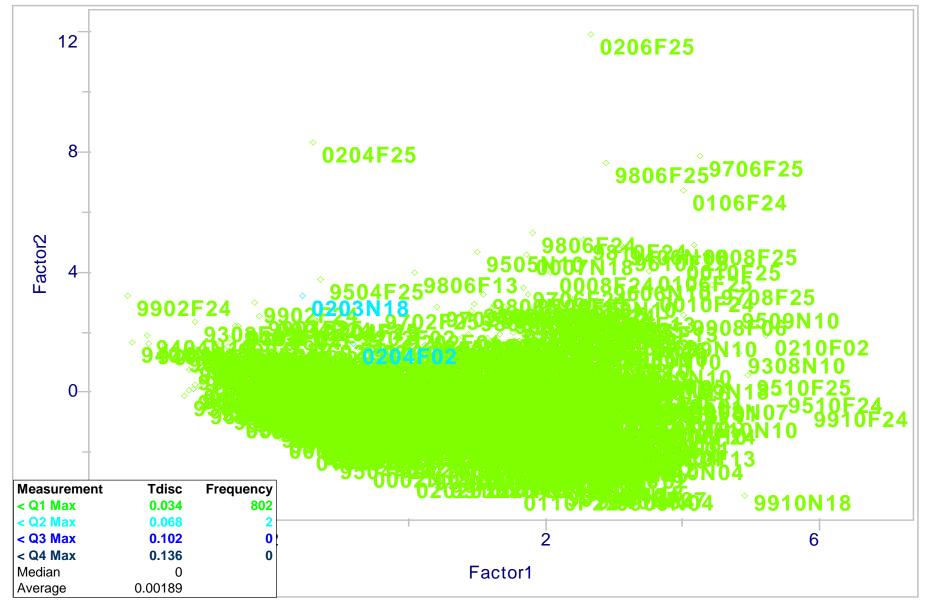














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