Investigation of the characteristics and properties of mixed function oxidases (mfo) in commercially significant fish from SA waters and assessment of their induction as a potential early warning and hence biomarker of organic pollutant linked stress

Ms Kathryn Bellette

FISHERIES
RESEARCH &
DEVELOPMENT
CORPORATION

Project No. 1994/043

NON-TECHNICAL SUMMARY

1994/043	**Investigation of the characteristics and properties of mixed function oxidases (mfo) in commercially significant fish from SA waters and assessment of their induction as a potential early warning and hence biomarker of organic pollutant linked stress**

PRINCIPAL INVESTIGATOR: Ms Kathryn Bellette
ADDRESS: University of Adelaide
North Terrace
ADELAIDE SA 5005
Ph: 08 8303 5052 Fax: 08 8303 4350

OBJECTIVES:

1. To adapt methods currently developed overseas and in Port Phillip Bay to South Australian commercial fish species to indicate pollutant linked stress in fish.
2. To encourage the use of these methods (if acceptable) by the relevant authorities as part of a monitoring program.

NON TECHNICAL SUMMARY:

The results provide information on the preliminary characterisation of P-450 levels and activity in yellow eye mullet and yellow fin whiting and baseline data at two sites over a period of two years.

Although the data in this investigation is limited, it would appear that the yellow eye mullet may warrant further assessment as a species to be utilised in further investigations into the characteristics and properties of mixed functions oxidases and assessment of their induction as a potential early warning and hence biomarker of organic pollutant linked stress.

The preliminary investigations of this study, which provided details regarding to optimum assay parameters for yellow eye mullet would be useful for further investigations.

By comparisons, the data suggest that yellow fin whiting may be an unsuitable species for use in cytochrome P-450 biomarker related investigations.

The substrate PPO may also be worthy of further consideration as an alternative to EROD in P-450 IA1 activity investigations.

More detailed research on cytochrome P-450 levels and activities in fish of Australian coastal and estuarine waters has been undertaken by the Centre for Nutritional and Environmental Toxicology, Royal Melbourne Institute of Technology. It is suggested that those interested in the field of study obtain papers written by this group.

KEYWORDS: Fish stress, mixed function oxidases, pollution

Introduction

A variety of methods at differing levels of biological organisation have been utilised to evaluate or predict the stress effects of xenobiotic compounds on various animal species, including fish. Investigations have been undertaken at the molecular and biochemical level, the physiological, histopathological, immunological, behavioural and performance levels, community, population and ecosystem levels. Bioenergetics modelling approaches, organismic indices and autopsy based assessment have also been undertaken (Adams,1990).

Various biochemical parameters are for instance used to determine the impact of environmental contamination events, such as oil spills. The presence of polycyclic aromatic hydrocarbons (PAH's) within the oil mixture enables the Cytochrome P-450 IA1 monooxygenase (P-450) biomarker to be utilised.

The advantage of using an indicator at this level of biological organisation lies in the early warning potential, i.e. indications of biochemical change can be detected prior to those at the individual level, or population level change. It can therefore be used as a management tool to prevent higher level changes from occurring. However, characterisation of biochemical properties and then linkages to threshold pollutant levels must be ascertained in order that this level of bioindicator is useful as a predictive management tool.

This study undertakes a preliminary investigation into the feasibility of utilising Cytochrome P-450 IA1 monooxygenase as a biomarker of PAH sourced pollutant stress in two species of commercially significant fish of South Australian coastal waters; the yellow fin whiting (*Sillago schomburgkii*) and yellow eye mullet (*Aldrichetta forsteri*).

An increase in P-450 activity of 10-100 fold in a variety of species from baseline levels has been reported in the presence of PAH's and Polychlorinated biphenyls (PCB's) (Addison and Edwards, 1988). Other studies have demonstrated increases as little as 3 fold in killifish [2.3.7.8-tetrachlorodibenzo-p-dioxin, (TCDD dioxin)], (Haasch et al. 1992) and 2 fold in Cod and Haddock (oil) (Davies et al. 1984).

The initial November 1992 Port Pirie samples were collected two months after a 300 tonne bunker oil spill at Port Bonython in northern Spencer Gulf. The oil was transferred across the gulf to the mangrove estuarine region of Port Pirie, a fish breeding and nursery location. Both surface water oil sheen and

particularly sediment contamination was still evident in the sampling area during November 1992. Although the fish vacated the oil affected area for two-three weeks immediately after the spill, they returned during the month of September (SA Department of Primary Industries, pers. comm.).

A coinciding storm/flood event, resulting in an unusually large rural land sourced freshwater runoff flow to the coast, may have transported a residual agricultural chemical load to the mangrove estuarine region under investigation.

Aim

The aim of the current study was to determine baseline levels in wild populations of two fish species : yellow fin whiting (whiting) and yellow eye mullet (mullet), with a view to assessing the potential for cytochrome P450 mixed function oxidase (P-450) to be utilised as a biomarker of hydrocarbon pollutant related stress in commercial fish of South Australian coastal waters. Specifically, samples from these two species were collected from:

(1) Port Pirie, a site affected by

(i) an oil spill in northern Spencer Gulf (August 30 1992) which affected the mangrove estuarine area under investigation in early September 1992,

(ii) elevated levels of heavy metals in the coastal sediments within a radius of 10 km^2 from Port Pirie (lead, cadmium and zinc residues derived from the lead smelter at Port Pirie (Ward et al. 1982),

(iii) the possible presence of residual agricultural chemicals transported via freshwater runoff to a series of creeklines associated with the mangrove lined coast, resulting from flooding also during early September 1992.

(2) For the second period - May/June 1993, Port Broughton was used as a reference site, upon the advice of the then SA Department of Fisheries. However on arrival at Port Broughton, it was observed that this location has a large area of seagrass dieback, the cause of which is unknown.

Therefore this site was abandoned as a reference site for sampling during future time periods. Tickera, a clean water region approximately 60 km. south of Port Pirie, was utilised as a reference site for the remaining periods of sampling; November 1993 and May/June 1994.

The investigation of levels of blood serum sorbitol dehydrogenase (SDH) in both species post September 1992 at Port Pirie in addition to Port Pirie 1993 and 1994 in comparison to Port Broughton/Tickera 1993 and 1994 was subject to a separate investigation by the author. SDH, which is normally found in liver, is an indicator of liver damage when found in significant quantities in blood serum.

The analyses of fish tissue for PAH metabolites and metals have not been undertaken, nor have water and sediment samples for PAH's and metals for this study, due to time limitations. Similarly, data is available for the calculation of liver somatic index, however this has not yet been undertaken.

MATERIALS AND METHODS

Liver samples were collected from both species at the following times:

	1992		1993		1994	
	May/Jun	Nov/Dec	May	Nov/Dec	May/Jun	Nov/Dec
Port Pirie		•	•	•	•	
Port Broughton /Tickera			•	•	•	

This gave two sets of spring/summer and autumn/winter sampling between the years 1992 and 1994.

The method used for fish sampling was as follows:

(a) the fish were collected in the intertidal zone of the fourth, fifth and sixth creeks at Port Pirie, which was the oil spill affected area. Fish from Port Broughton/Tickera were collected from the shallow intertidal zones of these locations.

(i) Fish were caught by seine or purse net using commercial fishing methods. Length and sex was recorded for each fish.

(ii) Blood was taken from fish by closed cardiac puncture into a heparinised 5ml syringe fitted with a 21 gauge needle and immediately placed on ice.

(iii) Fish were killed by pithing, livers were immediately collected and placed into liquid nitrogen. Livers were collected to determine cytochrome P450 mixed function oxidase activity, and liver somatic index.

(b) Laboratory analysis

- The Laboratory preparation and assays were undertaken on a part time basis one day/week. Twelve livers/day were thawed from -80 C and retained on ice, weighed and prepared by homogenising and centrifugation to microsomal fraction. At a later date, 24 microsomal fractions/day were assayed to determine P-450 IA1 enzyme activity.

- Protein concentrations were undertaken (Lowry et al., 1951) to determine enzyme activity per protein (nmol/min/g protein) and Cytochrome P450 content (CO difference spectra) determined spectrophotometrically to determine nmol/mg protein. Enzyme activity was measured via product fluorescence using a spectrofluorimeter.

- Three Cytochrome P-450 activity assays were trialed using the substrates 7 - Ethoxyresorufin-o-deethylase (EROD), Ethoxycoumarin-o-deethylase (ECOD), and 2,5 Diphenyloxazole (PPO).

EROD

ethoxyresofufin *o*- deethylase

7-ethoxyresofufin----------> 7-hydroxyresorufin

ECOD

ethoxycoumarin *o*-deethylase

7-ethoxycoumarin ----------> 7-hydroxycoumarin

PPO

diphenyloxazole hydroxylase

2,5 Diphenyloxazole ----------> hydroxydiphenloxazole

- Both EROD and ECOD have been used extensively in overseas studies, EROD in particular. As EROD is very expensive to purchase, the less costly (PPO) was trialled as an alternative P-450IA1 substrate. The results from PPO and EROD assays were correlated with each other on a per protein and per cytochrome P450 basis. Cytochrome P-450 levels and EROD assays were undertaken on each sample. PPO and ECOD (yellow-eye mullet only) assays were undertaken on a randomly selected basis, to compare substrate activity.

- Preliminary results provided optimum assay parameters for the three assays - incubation time, pH, temperature and protein concentration, for the two fish species.

- After preliminary results, it was determined that the EROD assay was providing more consistent results than the ECOD assay, therefore only the EROD assay, and the PPO were used on a continuing basis. For the purpose of this report, the ECOD samples are not discussed as the results are too sparse to be the source of any meaningful discussion.

- Enzyme activity assay results were measured in nmol/min/g protein and nmol/min/cytochrome P450

RESULTS

CYTOCHROME P-450 LEVELS

Graphs and statistics are provided in Appendix 1

Sampling Sites

The results of analysis 1 demonstrate a significant difference in P-450 levels between Tickera mullet and Port Pirie mullet over the sampling period, the Pirie values being higher.

This suggests that the mullet may have been affected by a parameter of the Port Pirie waters which is not present at Tickera. Whether this parameter is related to the oil spill cannot be ascertained, as fish tissue oil content and water /sediment quality analyses have not been undertaken.

No significant difference was demonstrated between Port Pirie whiting and Tickera whiting for the same time period.

Sampling period

The results of analysis 2 demonstrate a significant difference in P-450 levels of Port Pirie mullet over time for each sampling site, where P-450 levels were high over summer and low over winter. Although only two seasons data are presented, Tickera mullet, P-450 levels are also higher in summer than winter.

No significant difference was demonstrated between Port Pirie whiting over time and Tickera whiting over time.

Species differences

The results of analysis 3 indicate a significant difference in cytochrome P-450 levels between the two species for each sampling site over the summer periods, but no significant differences over the winter periods.

In analyses 1 and 2, the whiting do not indicate any significant differences over the sampling period for spacial or temporal variables, the mullet however do show significant differences for both.

CYTOCHROME P-450 ACTIVITY

The parameters used for the assays are provided in appendix 2

Graphs and statistics for P-450 activities are provided in appendix 3

DISCUSSION

CYTOCHROME P-450 LEVELS

The levels of total microsomal P-450 in untreated fish liver ranges widely from <0.1 nmol/mg protein to <2.0 nmol/mg protein (microsomal protein). The high and low values do not necessarily characterise different species. At least 10 fold differences can occur within a single species, depending on strain (Pedersen et al. as in Stegeman 1989), or sex (Stegeman and Woodin, 1984).

Levels from this study range from between .028 nmol/mg protein at Tickera during winter, to .074 nmol/mg/protein at Port Pirie during summer for mullet and .015 nmol/min/g protein at Tickera during summer, to .031 nmol/min/g protein during winter for whiting.

Any discussion of P-450 content and activity in fish should be preceded by the acknowledgement of the influences of sexual hormones on the levels and activity of P-450, where induction of cytochrome P-450 content has been shown to be significantly higher in juvenile female fish than pre-spawning fish [Forlin et al. (1984), and George et al. (1990)]. These differences appear to be linked to plasma levels of the female hormone oestradiol (Elkus et al. 1992) which suppresses cytochrome P-450 related activity in female fish when elevated (Stegeman and Woodin 1984; Forlin et al., 1984).

A discussion of sexual hormonal influences in fish P-450 levels and activities can be gained from the literature review by the author (Bellette, unpublished).

The spawning times of the two species under investigation in this study are diametrically opposed, whereby the whiting spawn during spring/summer, and the mullet autumn/winter. Although analyses of P-450 content and activity has not been undertaken on pre-spawning fish in comparison to non-pre spawning fish (males and non-sexually mature females), seasonal differences were found in the mullet data for both sites.

An item of note from the raw data is that in some time periods, most of the mullet and whiting caught during their respective spawning seasons were female and pre-spawning, in other time periods however, this phenomena was not apparent.

Mullet also showed significant differences between sites, whereby P-450 levels at Port Pirie were greater than those of Tickera for the same time period, suggesting a response to an (unidentified) parameter difference between the two sites. Again, no significant differences were found between the two sites for whiting.

A comparison of mullet and whiting results (analysis 3) indicate a significant difference in cytochrome P-450 levels between the two species for each sampling site over the summer periods, where mullet P-450 levels are at their highest (and if whiting were showing any hormonal effect, they would be at their lowest) No such reciprocal difference was found in winter between the species.

In general, apart from the November 1992 sample, the P-450 levels are higher in the mullet than the whiting. As whiting may have suppressed P-450 levels during spring/summer, one would expect this difference to be exaggerated, but this has not occurred.

Five explanations are offered for these results;

1) a greater number of seasons are required to be monitored to be able to investigate species differences.

2) the species differences are attributable to non-hormonal effects such as physiological, biochemical, or a combination of hormonal and non-hormonal effects.

3) Mullet have the potential for further investigation as good indicator species as they provide consistent results, albeit with seasonal differences.

4) Whiting are not good indicator species for cytochrome P-450 investigations and there is therefore no purpose in comparing the results of the two species.

5) Whiting have the potential for further investigation as good indicator species as they do not demonstrate a seasonal effect. It would then follow that no significant differences, spacially or temporally would be found from this study.

In consideration of the above possible explanations, due to the fact that Analyses 1 and 2 demonstrated significant differences for mullet for both P-450 levels by site and sampling period, but not whiting, it is suggested that whiting may not be a good species to utilise in such investigations.

Analysis three, comparing the species indicates species differences which are not explainable by current knowledge in P-450 behaviour. This further suggests the unsuitability of this species of whiting as a species warranting further investigation for P-450 studies. Explanation 1) and 3) therefore appear reasonable conclusions.

As for all studies which incorporate the variable of seasonal change and fauna with relatively long life cycles spanning many seasons, several years of data collection is required to verify results.

An item of note is the physical differences between the mullet and whiting livers. The whiting livers were pink, contained more fat and were more brittle in comparison to the mullet livers, which were red and physically flexible. It is also possible that it may be the physiological characteristics of the livers which contribute to the differences between whiting and mullet.

CYTOCHROME P-450 ACTIVITY

Analysis 1 showed no significant difference between November 1993 samples and May/June 1994 samples, using either substrate. This suggests that data collected were baseline during these periods.

Analysis 2 showed significant differences in values between PPO and EROD for each sampling period.

Analysis 3, a linear regression between PPO and EROD showed a significant relationship, but a poor fit for November 1993, a significant relationship and good fit for May/June 1993. This suggests that it would be worthwhile to undertake research into the relationship between PPO and EROD.

Although the results indicate a relationship between PPO and EROD, and therefore justify further investigation into the possibility of using PPO as a low cost alternative substrate to EROD, the actual sample size of the cytochrome P-450 IA1 investigation is too small for any detailed discussion to be made.

Only those results indicating only minimal degradation of P-450 to P-420 were used for the P-450 activity assays. This resulted in only 2 sampling periods containing large enough data sets to perform statistical analysess - November 1993 and May/June 1994. The process of freezing the livers (liquid nitrogen), transfer to -80 C freezer for up to 6 months, defrosting to determine the P-450 content, re-freezing at -80 C for a further period of up to 6 months, then defrosting and undertaking P-450 activity levels quite possibly affected the results, via a gradual breakdown of the microsomes.

CONCLUSIONS

The results provide information on the preliminary characterisation of P-450 levels and activity in yellow eye mullet and yellow fin whiting and baseline data at two sites over a period of two years.

Although the data in this investigation is limited, it would appear that yellow eye mullet may warrant further assessment as a species to be utilised in further investigations into the characteristics and properties of mixed function oxidases and assessment of their induction as a potential early warning and hence biomarker of organic pollutant linked stress.

The preliminary investigations of this study, which provided details regarding to optimum assay parameters for yellow eye mullet would be useful for further investigations.

By comparison, the data suggest that yellow fin whiting may be an unsuitable species for use in cytochrome P-450 biomarker related investigations.

The substrate PPO may also be worthy of further consideration as an alternative to EROD in P-450 IA1 activity investigations.

More detailed research on Cytochrome P-450 levels and activities in fish of Australian coastal and estuarine waters has been undertaken by the Centre for Nutritional and Environmental Toxicology, Royal Melbourne Institute of Technology. It is suggested that those interested in this field of study obtain papers written by this group.

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APPENDIX 1
Cytochrome P-450 Levels

Statistical analysis of cytochrome P450 assay data

1. Introduction

Data of assayed cytochrome P450 levels of within two species of fish at two different sites during four different time periods were analysed using a simple one factor analysis of variance.

The two species were the Yellow Eye Mullet and the Yellow Fin Whiting. The two sampling sites were at Tickera/Port Broughton and Port Pirie. The four sampling times were November 1993, May/June 1994, May 1993 and November/December 1992. This is represented in Table 1.

Table 1: Layout of cytochrome P450 assay data

	Sampling Site			
Sampling Period	*Tickera/ Port Broughton*		*Port Pirie*	
November/December 1992	No data	No data	Yellow Eye Mullet	Yellow Fin Whiting
May 1993	No data	No data	Yellow Eye Mullet	Yellow Fin Whiting
November 1993	Yellow Eye Mullet	Yellow Fin Whiting	Yellow Eye Mullet	Yellow Fin Whiting
May/June 1994	Yellow Eye Mullet	Yellow Fin Whiting	Yellow Eye Mullet	Yellow Fin Whiting

As can be seen fom Table 1, there was not a full data coverage for each of the sampling periods, sampling sites and sampled species. This obviously has an impact upon the number of comparisons or analyses that can be undertaken, as well as the the accuracy of said analyses,

Three specific analyses were performed on the data.

1. to examine the null hypothesis (h_o) that there is no significant difference in the assayed cytochrome P450 levels between the two sampling sites for each sampling period and fish species
2. to examine the null hypothesis (h_o) that there is no significant difference in the assayed cytochrome P450 levels between the sampling periods for each sampling site and fish species.
3. to examine the null hypothesis (h_o) that there is no significant difference in the assayed cytochrome P450 levels between the two fish species for each sampling site and sampling period.

2. Methods

The data were manipulated and the analysis performed using the Microsoft Excel spreadsheet package, and the "add in" Data Analysis Tool Pak.

The data, analyses and charts of the analysis results can be found in Appendix A.

3. Analyses

3.1 Analysis 1: Sampling site

Examinantion of the null hypothesis (h_o) that there is no significant difference in the assayed cytochrome P450 levels between the two sampling sites for each sampling period and fish species.

The results of the ANOVA to examine h_o are summarised in Table 2, below. The actual data, analyses and charts can be found in the Appendix.

Table 2: Summary of P values from analysis of variance for assayed cytochrome P450 levels between the two sampling sites for each sampling period and fish species

	Fish Species	
Sampling period	*Yellow Eye Mullet*	*Yellow Fin Whiting*
May/June 1994	0.031709 *	0.132299
November 1993	0.031112 *	0.258992

Those analyses marked with an asterisk (*) have a value of $P<0.05$, which is the level of confidence sufficient for the null hypothesis h_o to be rejected, and the acceptance of the alternative hypothesis h_1 that there is a significant difference in the assayed cytochrome P450 levels between the two sampling sites for the sampling period and fish species.

3.2 Analysis 2: Sampling period

Examination of the null hypothesis (h_o) that there is no significant difference in the assayed cytochrome P450 levels between the sampling periods for each sampling site and fish species.

The results of the ANOVA to examine h_o are summarised in Table 3, below. The actual data, analyses and charts can be found in Appendix A.

Table 3: Summary of P values from analysis of variance for assayed cytochrome P450 levels between the sampling periods for each sampling site and fish species.

	Fish Species	
Sampled site	*Yellow Eye Mullet*	*Yellow Fin Whiting*
Port Pirie	3.16×10^{-6} *	0.12198
Tickera	0.003479 *	0.015332 *

Those analyses marked with an asterisk (*) have a value of $P<0.05$, which is the level of confidence sufficient for the null hypothesis h_o to be rejected, and the acceptance of the alternative hypothesis h_1 that there is a significant difference in the assayed cytochrome P450 levels between the sampling periods for each sampling site and fish species.

3.3 Analysis 3: Fish species

Examination of the null hypothesis (h_o) that there is no significant difference in the assayed cytochrome P450 levels between the two fish species for each sampling site and sampling period.

The results of the ANOVA to examine h_o are summarised in Table 4, below. The actual data, analyses and charts can be found in the Appendix.

Table 4: Summary of P values from analysis of variance for Analysis 3

	Sampling period			
Sampled site	*November 1992*	*May 1993*	*November 1993*	*May/June 1994*
Port Pirie	1.7×10^{-6} *	0.055155	8.32×10^{-11} *	0.057356
Tickera	No data	No data	1.235×10^{-6} *	0.354333

Those analyses marked with an asterisk (*) have a value of $P<0.05$, which is the level of confidence sufficient for the null hypothesis h_o to be rejected, and the acceptance of the alternative hypothesis h_1 that there is a significant difference in the assayed cytochrome P450 levels between the two fish species for each sampling site and sampling period.

4. Comments

The lack of total data coverage for each of the examined factors (ie: sampling site, sampling time, fish species) does not allow for as full an analysis as might be possible.

There were only 7 data points for Yellow Fin Whiting at Port Pirie, May 1993 for Analysis 3. If there were more representative data, the analysis result may have been different.

Analysis 1

May/June 94 - Yellow Fin Whiting - Cytochrome P450

Port Pirie	*Tickera*
0.659	1.648
3.846	1.758
5.604	1.319
1.978	1.648
4.396	1.099
0.879	2.747
2.527	1.978
3.187	3.626
1.868	4.725
1.538	3.077
2.198	1.978
3.407	1.209
4.835	2.747
0.549	2.308
5.604	4.396
5.275	1.099
3.846	2.198
1.868	0.33
4.396	2.637
	4.615

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Pirie	19	58.46	3.076842	2.784445
Tickera	20	47.142	2.3571	1.511747

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.047459	1	5.047459	2.368701	0.132299	4.105459
Within Groups	78.84321	37	2.130898			
Total	83.89067	38				

May/June 94 - Yellow Eye Mullet - Cytochrome P450

Port Pirie	*Tickera*
17.912	7.473
6.593	3.187
0.11	0.11
1.429	1.538
12.637	1.209
6.813	1.099
5.934	0.989
4.286	4.505
0.659	3.736
0.659	4.286
0.769	1.648
4.286	1.868
4.835	3.626
4.286	3.407
1.099	2.088
8.681	3.077
6.374	2.088
7.692	3.956
4.396	0.989
	5.385

Anova: Single Factor

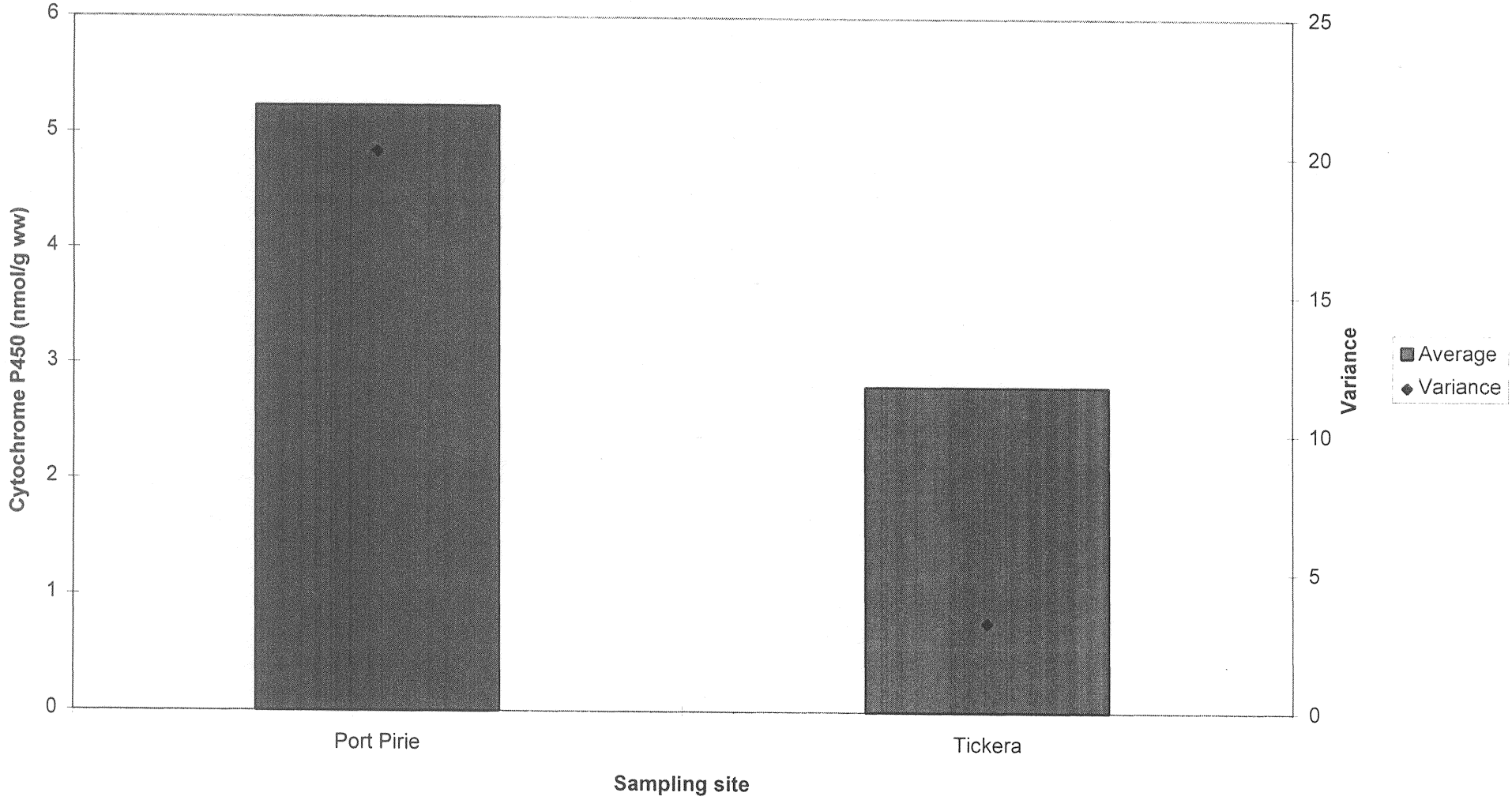
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Pirie	19	99.45	5.234211	20.15422
Tickera	20	56.264	2.8132	3.218054

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	57.11002	1	57.11002	4.984609	0.031709	4.105459
Within Groups	423.9191	37	11.45727			
Total	481.0291	38				

ANOVA results for Yellow Eye Mullet Cytochrome P450, May/June 1994



November 93 - Yellow Eye Mullet - Cytochrome P450

Port Pirie	*Tickera*
7.033	5.604
4.066	0.659
3.407	1.758
10	3.516
5.714	7.253
5.055	6.923
9.89	4.066
9.121	2.198
11.758	9.121
5.275	3.516
4.176	7.802
12.747	8.901
17.363	5.934
7.802	2.637
2.198	3.077
6.703	1.319
2.088	2.088
8.901	4.615
7.912	8.571
	4.725
	4.176
	8.791
	10.22

Anova: Single Factor

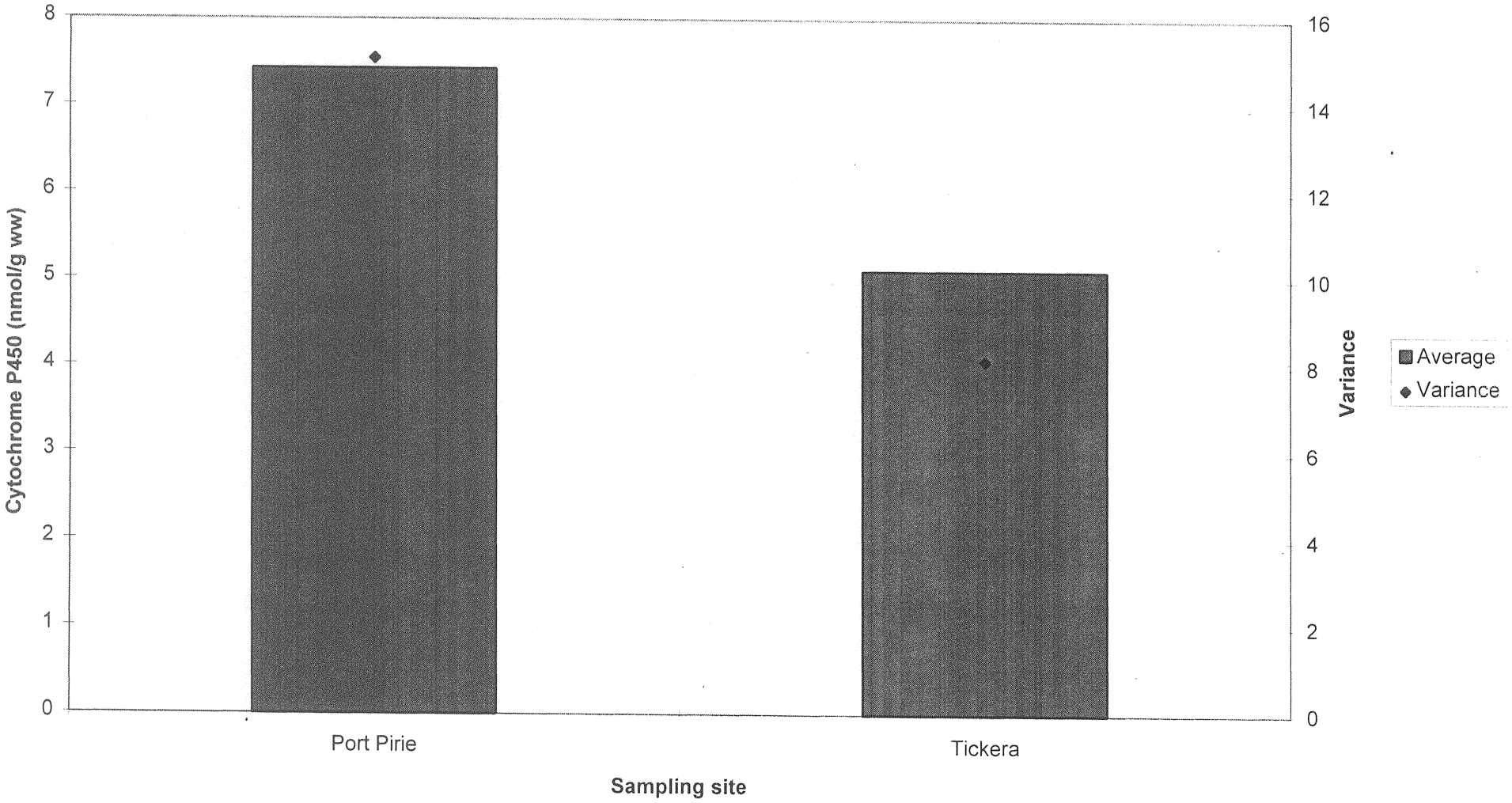
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Pirie	19	141.209	7.432053	15.09071
Tickera	23	117.47	5.107391	8.130299

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	56.22786	1	56.22786	4.992492	0.031112	4.08474
Within Groups	450.4994	40	11.26248			
Total	506.7272	41				

ANOVA results for Yellow Eye Mullet Cytochrome P450, November 93



November 93 - Yellow Fin Whiting - Cytochrome P450

Port Pirie	*Tickera*
0.879	0.44
1.538	0.549
0.549	0.44
4.286	0.989
3.516	0.879
1.319	1.978
1.429	0.879
0.549	0.22
0.44	1.099
0.879	2.198
1.538	2.527
1.648	2.308
1.648	1.758
2.857	2.527
3.187	0.989
1.758	1.648
0.33	1.538
2.637	1.099
1.099	1.648
1.758	1.538
0.549	3.956
0.769	2.308
2.088	
2.418	
8.022	
2.088	
2.088	
3.626	
2.418	

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Pirie	40	76.48	1.912	2.111664
Tickera	22	33.515	1.523409	0.793769

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.143267	1	2.143267	1.298634	0.258992	4.001194
Within Groups	99.02403	60	1.6504			
Total	101.1673	61				

1.319	
2.198	
0.769	
4.505	
3.077	
0.549	
0.549	
1.648	
1.538	
0.989	
1.429	

Analysis 2

Tickera - Yellow Fin Whiting - Cytochrome P450

November 93	*May/July 94*
0.44	1.648
0.549	1.758
0.44	1.319
0.989	1.648
0.879	1.099
1.978	2.747
0.879	1.978
0.22	3.626
1.099	4.725
2.198	3.077
2.527	1.978
2.308	1.209
1.758	2.747
2.527	2.308
0.989	4.396
1.648	1.099
1.538	2.198
1.099	0.33
1.648	2.637
1.538	4.615
3.956	
2.308	

Anova: Single Factor

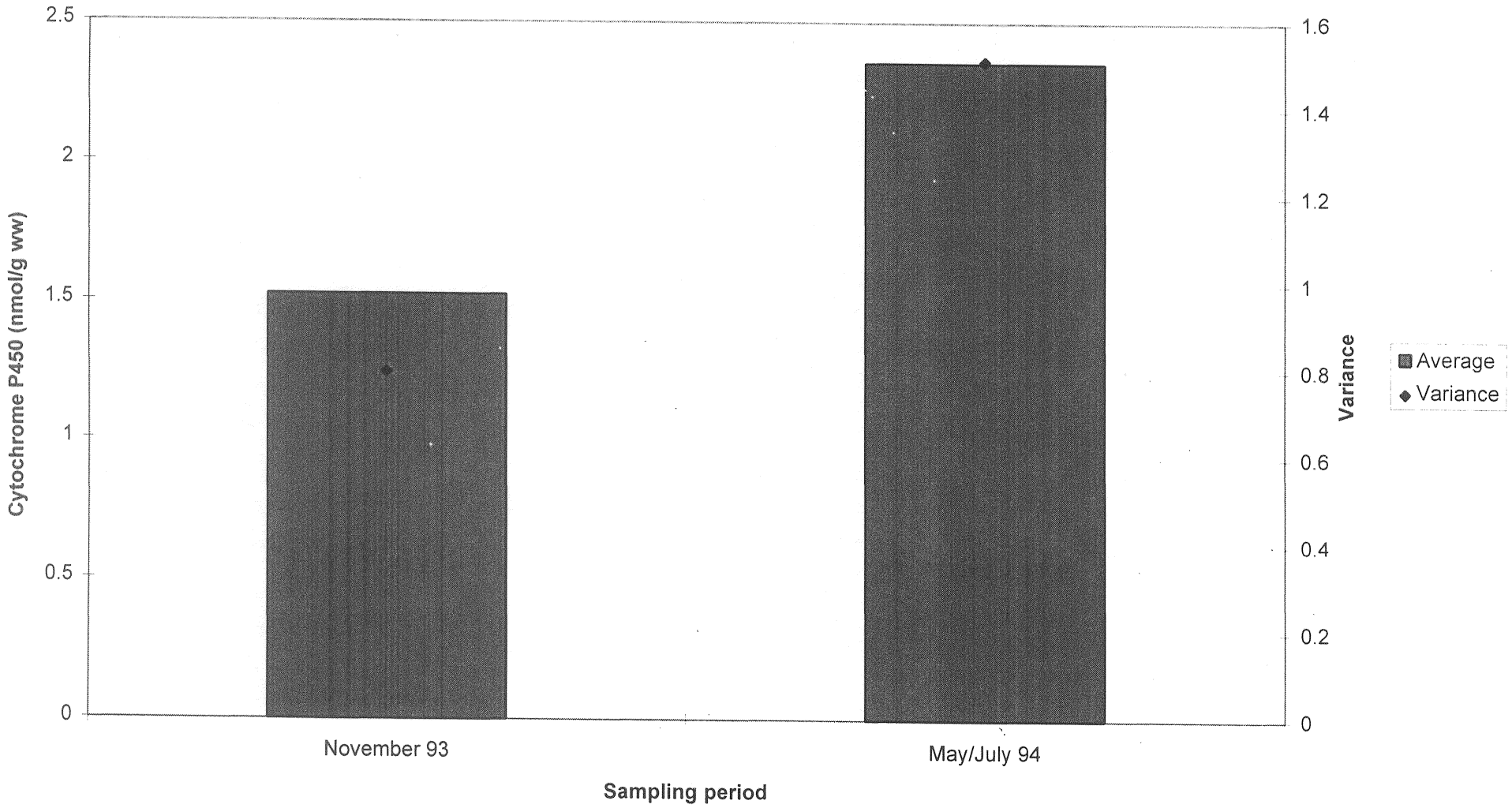
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
November 93	22	33.515	1.523409	0.793769
May/July 94	20	47.142	2.3571	1.511747

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.281377	1	7.281377	6.416392	0.015332	4.08474
Within Groups	45.39234	40	1.134809			
Total	52.67372	41				

ANOVA results for Yellow Fin Whiting Cytochrome P450, Tickera



Tickera - Yellow Eyed Mullet - Cytochrome P450 nmol/gww

Nov 93	*May/June 1994*
5.604	7.473
0.659	3.187
1.758	0.11
3.516	1.538
7.253	1.209
6.923	1.099
4.066	0.989
2.198	4.505
9.121	3.736
3.516	4.286
7.802	1.648
8.901	1.868
5.934	3.626
2.637	3.407
3.077	2.088
1.319	3.077
2.088	2.088
4.615	3.956
8.571	0.989
4.725	5.385
4.176	
8.791	
10.22	

Anova: Single Factor

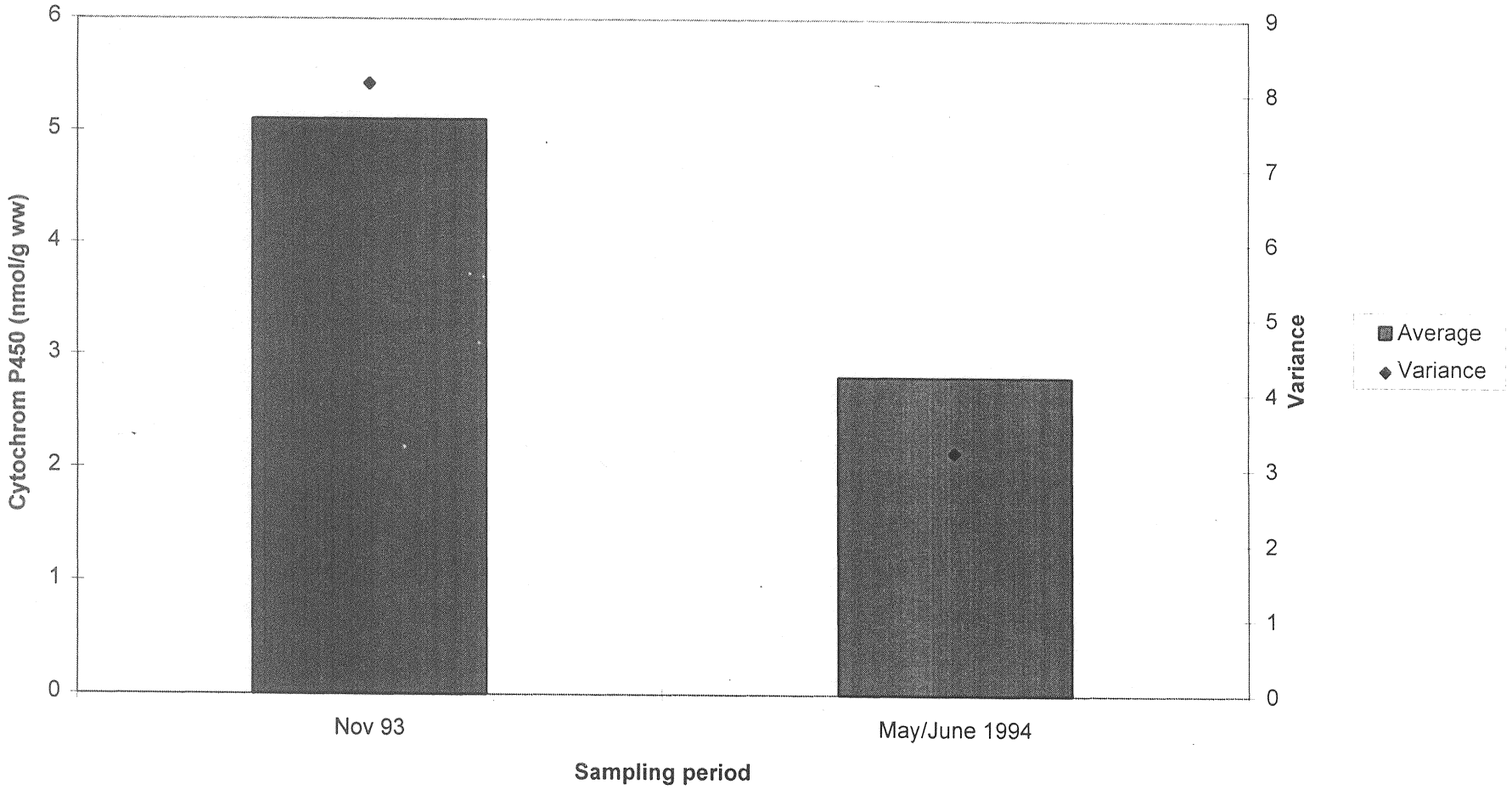
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Nov 93	23	117.47	5.107391	8.130299
May/June 1994	20	56.264	2.8132	3.218054

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	56.30522	1	56.30522	9.618423	0.003479	4.078544
Within Groups	240.0096	41	5.853893			
Total	296.3148	42				

ANOVA results for Yellow Eye Mullet Cytochrome P450, Tickera



Port Pirie - Yellow Eyed Mullet - Cytochrome P450 nmol/gww

May/June 1994	*May 1993*	*November 1993*	*November 1992*
17.912	7.253	7.033	7.363
6.593	5.495	4.066	6.593
0.11	4.945	3.407	9.484
1.429	5.824	10	12.088
12.637	3.956	5.714	15.055
6.813	0.33	5.055	10.44
5.934	2.967	9.89	10.44
4.286	4.286	9.121	8.901
0.659	2.857	11.758	8.352
0.659	2.308	5.275	6.264
0.769	4.176	4.176	7.582
4.286	4.396	12.747	8.791
4.835	1.648	17.363	7.802
4.286	3.956	7.802	5.385
1.099	5.165	2.198	2.088
8.681	2.308	6.703	13.736
6.374	9.341	2.088	6.813
7.692	4.176	8.901	11.319
4.396	0.44	7.912	10.659
	1.648		10
	4.615		6.813
	8.022		5.165
	2.967		8.791
	0.989		9.67
	0.549		8.901
			6.813
			6.923
			5.604
			10.33

Anova: Single Factor

SUMMARY

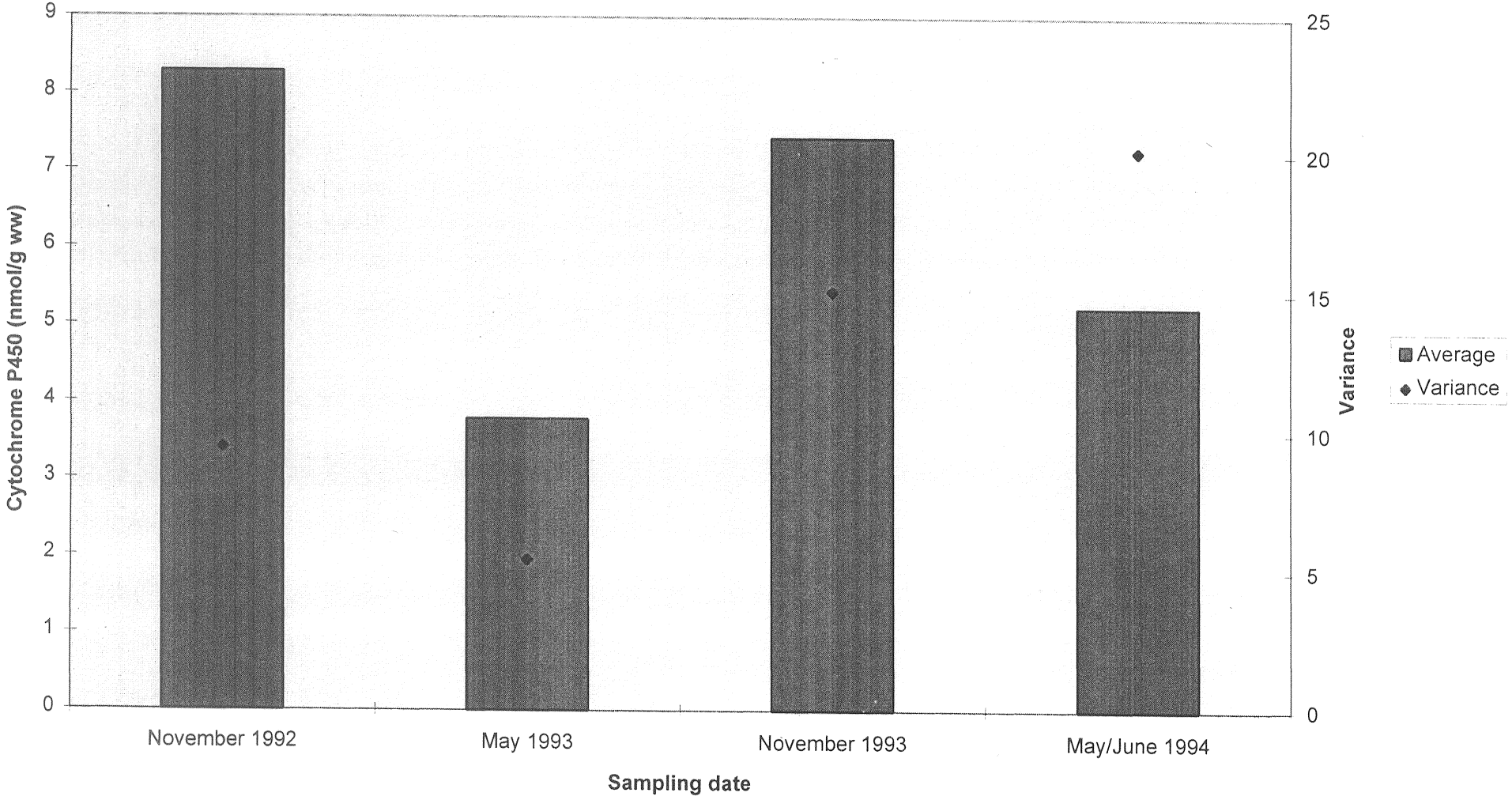
Groups	*Count*	*Sum*	*Average*	*Variance*
November 1992	43	356.516	8.291069767	9.448852543
May 1993	25	94.617	3.78468	5.41536681
November 1993	19	141.209	7.432052632	15.09071027
May/June 1994	19	99.45	5.234210526	20.15422473

ANOVA

Source of Variatio	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	369.14	3	123.0465045	10.80815128	3.16E-06	2.693724
Within Groups	1161.2	102	11.38460236			
Total	1530.4	105				

			16.044
			12.637
			6.374
			6.813
			10
			7.363
			2.747
			9.341
			8.791
			5.604
			3.736
			3.297
			10.659
			4.945

ANOVA results for Yellow Eyed Mullet Cytochrome P450, Port Pirie



Port Pirie - Yellow Fin Whiting - Cytochrome P450

November 1992	May 1993	November 1993	May/June 1994
0.659	6.484	0.879	0.659
0.549	0.879	1.538	3.846
1.099	0.879	0.549	5.604
1.319	1.758	4.286	1.978
0.769	0.33	3.516	4.396
0.33	1.319	1.319	0.879
19.67	1.209	1.429	2.527
1.648		0.549	3.187
0.769		0.44	1.868
0.438		0.879	1.538
0.22		1.538	2.198
1.209		1.648	3.407
1.648		1.648	4.835
1.209		2.857	0.549
4.066		3.187	5.604
4.615		1.758	5.275
4.945		0.33	3.846
3.626		2.637	1.868
6.374		1.099	4.396
9.341		1.758	
5.165		0.549	
4.396		0.769	
		2.088	
		2.418	
		8.022	
		2.088	
		2.088	
		3.626	
		2.418	
		1.319	
		2.198	
		0.769	

Anova: Single Factor

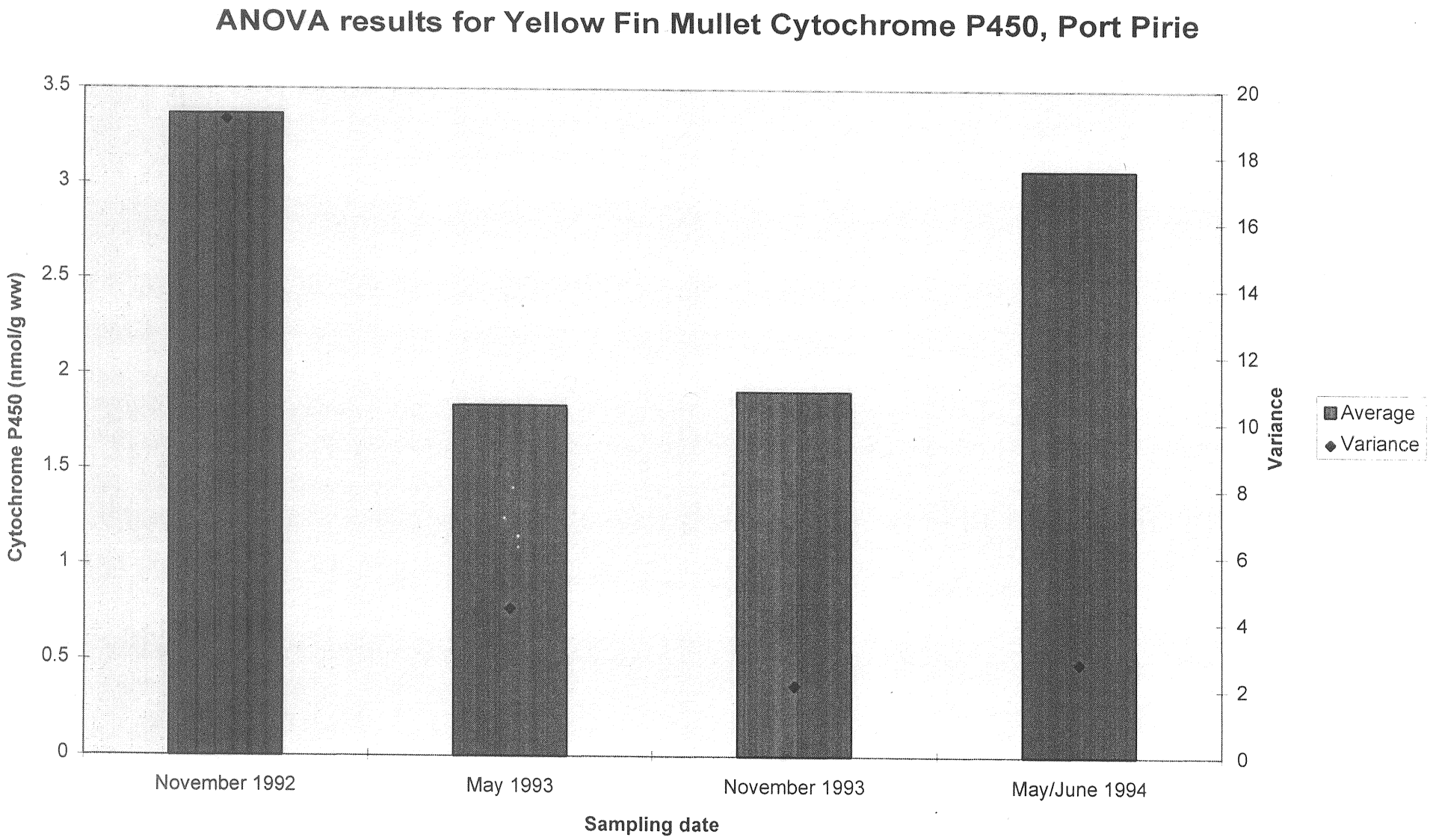
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
November 1992	22	74.064	3.366545	19.06929
May 1993	7	12.858	1.836857	4.395022
November 1993	40	76.48	1.912	2.111664
May/June 1994	19	58.46	3.076842	2.784445

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	39.71068	3	13.23689	1.988019	0.12198	2.713229
Within Groups	559.3001	84	6.658334			
Total	599.0107	87				

		4.505	
		3.077	
		0.549	
		0.549	
		1.648	
		1.538	
		0.989	
		1.429	


ANOVA results for Yellow Fin Mullet Cytochrome P450, Port Pirie
Cytochrome P450 (nmol/g ww)
3.5
3
2.5
2
1.5
1
0.5
0
20
18
16
14
12
10
8
6
4
2
0
Variance
Average
Variance
November 1992
May 1993
November 1993
May/June 1994
Sampling date

Analysis 3

Tickera -May/June 1994 - Cytochrome P450

Yellow Eye Mullet	*Yellow Fin Whiting*
7.473	1.648
3.187	1.758
0.11	1.319
1.538	1.648
1.209	1.099
1.099	2.747
0.989	1.978
4.505	3.626
3.736	4.725
4.286	3.077
1.648	1.978
1.868	1.209
3.626	2.747
3.407	2.308
2.088	4.396
3.077	1.099
2.088	2.198
3.956	0.33
0.989	2.637
5.385	4.615

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Yellow Eye Mullet	20	56.264	2.8132	3.218054
Yellow Fin Whiting	20	47.142	2.3571	1.511747

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.080272	1	2.080272	0.879645	0.354222	4.098169
Within Groups	89.86623	38	2.364901			
Total	91.9465	39				

Tickera - November 1993 - Cytochrome P450

Yellow Eye Mullet	*Yellow Fin Whiting*
5.604	0.44
0.659	0.549
1.758	0.44
3.516	0.989
7.253	0.879
6.923	1.978
4.066	0.879
2.198	0.22
9.121	1.099
3.516	2.198
7.802	2.527
8.901	2.308
5.934	1.758
2.637	2.527
3.077	0.989
1.319	1.648
2.088	1.538
4.615	1.099
8.571	1.648
4.725	1.538
4.176	3.956
8.791	2.308
10.22	

Anova: Single Factor

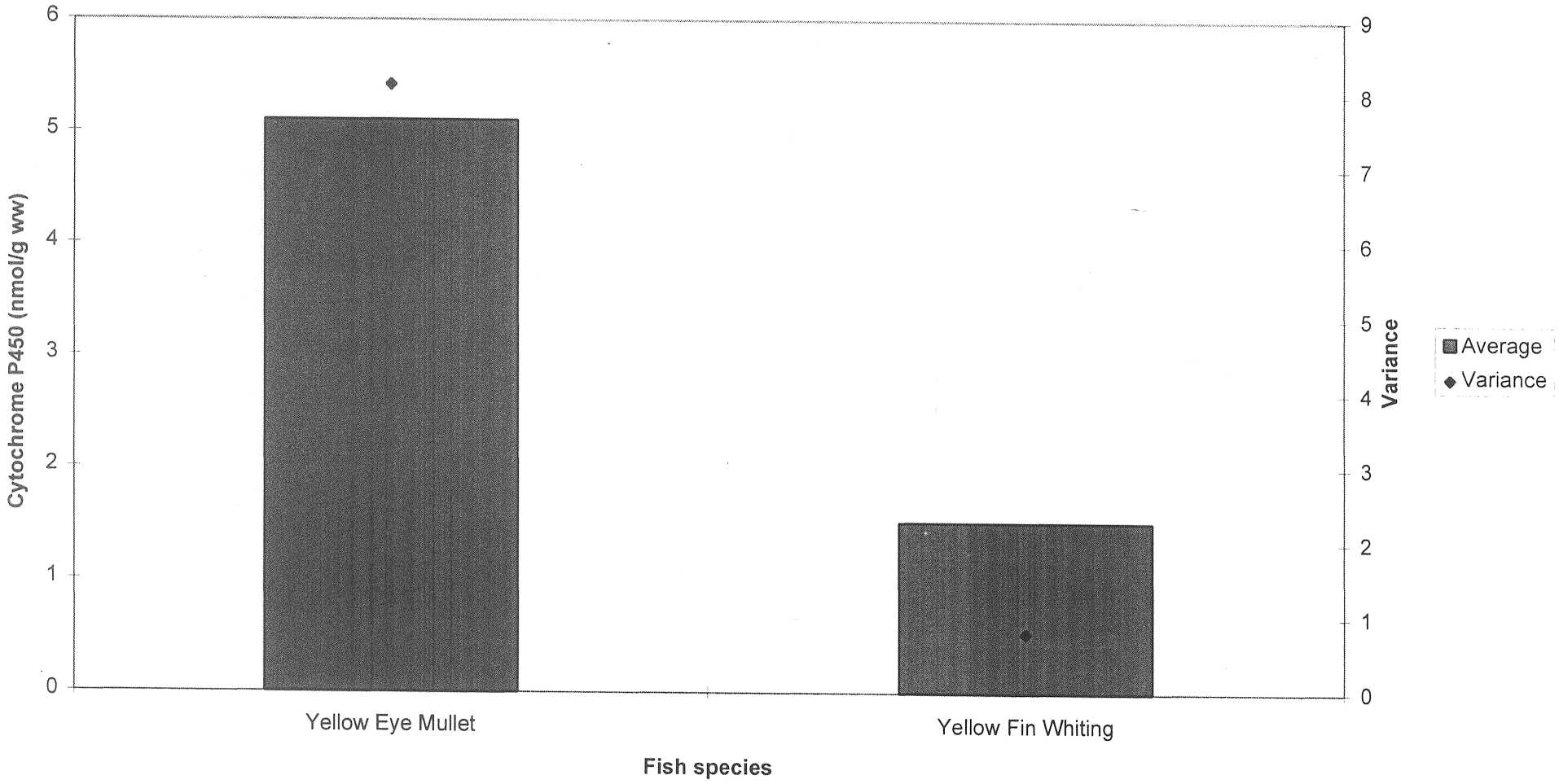
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Yellow Eye Mullet	23	117.47	5.107391	8.130299
Yellow Fin Whiting	22	33.515	1.523409	0.793769

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	144.4341	1	144.4341	31.76231	1.235E-06	4.067047
Within Groups	195.5357	43	4.547342			
Total	339.9698	44				

ANOVA results for cytochrome P450 assay levels at Tickera, November 1993



Port Pirie - November 1993 - Cytochrome P450

Yellow Eye Mullet	*Yellow Fin Whiting*
7.033	0.879
4.066	1.538
3.407	0.549
10	4.286
5.714	3.516
5.055	1.319
9.89	1.429
9.121	0.549
11.758	0.44
5.275	0.879
4.176	1.538
12.747	1.648
17.363	1.648
7.802	2.857
2.198	3.187
6.703	1.758
2.088	0.33
8.901	2.637
7.912	1.099
	1.758
	0.549
	0.769
	2.088
	2.418
	8.022
	2.088
	2.088
	3.626
	2.418
	1.319

Anova: Single Factor

SUMMARY

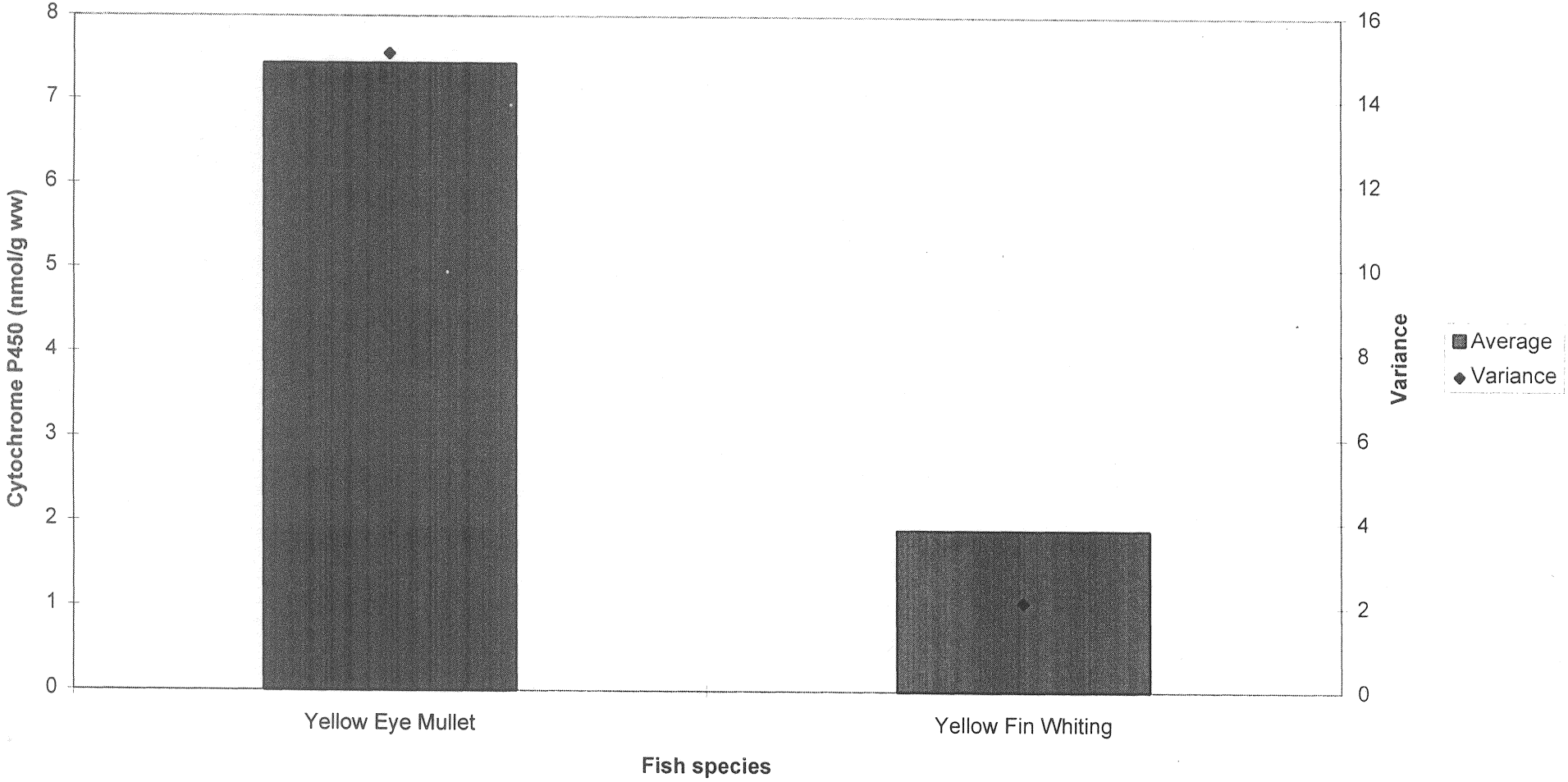
Groups	*Count*	*Sum*	*Average*	*Variance*
Yellow Eye Mullet	19	141.209	7.432053	15.09071
Yellow Fin Whiting	40	76.48	1.912	2.111664

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	392.5076	1	392.5076	63.20257	8.32E-11	4.009877
Within Groups	353.9877	57	6.21031			
Total	746.4952	58				

	2.198
	0.769
	4.505
	3.077
	0.549
	0.549
	1.648
	1.538
	0.989
	1.429

ANOVA results for cytochrome P450 assay levels at Prt Pirie, November 1993,



Port Pirie - May/June 1994 - Cytochrome P450

Yellow Eye Mullet	*Yellow Fin Whiting*
17.912	0.659
6.593	3.846
0.11	5.604
1.429	1.978
12.637	4.396
6.813	0.879
5.934	2.527
4.286	3.187
0.659	1.868
0.659	1.538
0.769	2.198
4.286	3.407
4.835	4.835
4.286	0.549
1.099	5.604
8.681	5.275
6.374	3.846
7.692	1.868
4.396	4.396

Anova: Single Factor

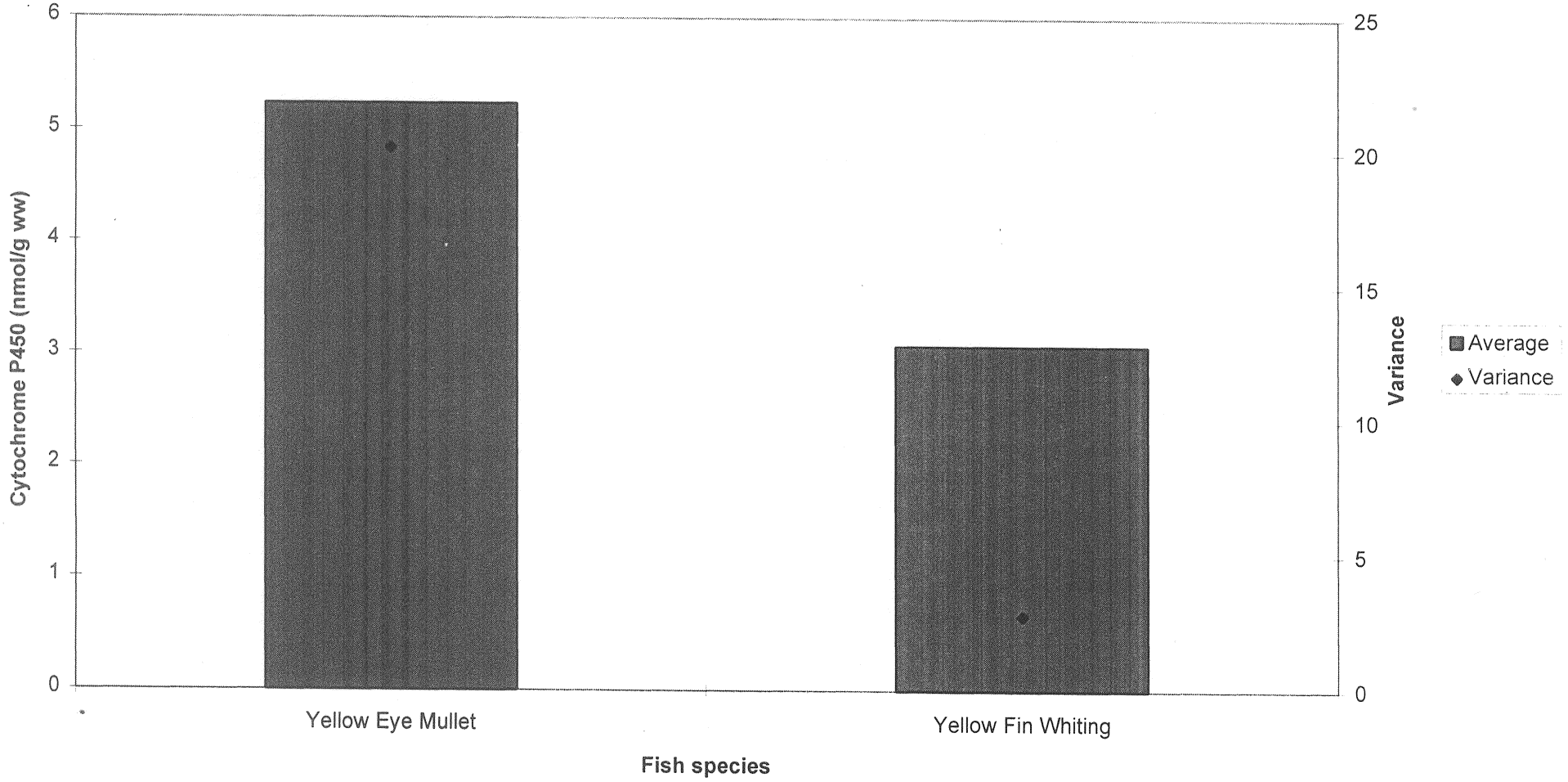
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Yellow Eye Mullet	19	99.45	5.234211	20.15422
Yellow Fin Whiting	19	58.46	3.076842	2.784445

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	44.21527	1	44.21527	3.855085	0.057356	4.113161
Within Groups	412.8961	36	11.46933			
Total	457.1113	37				

ANOVA results for cytochrome P450 assay levels at Prt Pirie, May/June 1994



Port Pirie - November 1992 - Cytochrome P450

Yellow Fin Whiting	*Yellow Eye Mullet*
0.659	7.363
0.549	6.593
1.099	9.484
1.319	12.088
0.769	15.055
0.33	10.44
19.67	10.44
1.648	8.901
0.769	8.352
0.438	6.264
0.22	7.582
1.209	8.791
1.648	7.802
1.209	5.385
4.066	2.088
4.615	13.736
4.945	6.813
3.626	11.319
6.374	10.659
9.341	10
5.165	6.813
4.396	5.165
	8.791
	9.67
	8.901
	6.813
	6.923
	5.604

Anova: Single Factor

SUMMARY

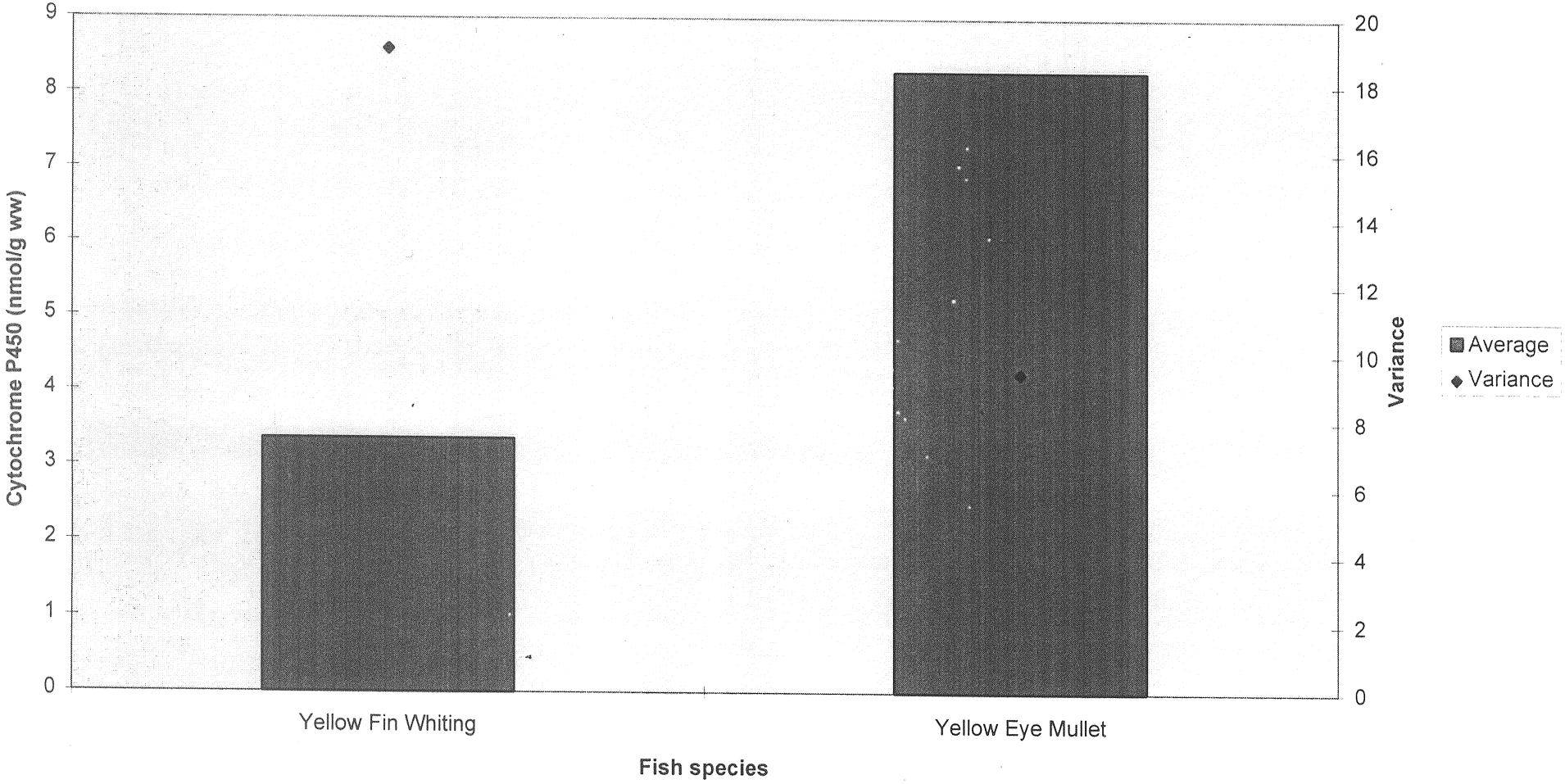
Groups	*Count*	*Sum*	*Average*	*Variance*
Yellow Fin Whiting	22	74.064	3.366545	19.06929
Yellow Eye Mullet	43	356.516	8.29107	9.448853

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	352.9444	1	352.9444	27.88826	1.7E-06	3.993364
Within Groups	797.3068	63	12.65566			
Total	1150.251	64				

	10.33
	16.044
	12.637
	6.374
	6.813
	10
	7.363
	2.747
	9.341
	8.791
	5.604
	3.736
	3.297
	10.659
	4.945

ANOVA results for cytochrome P450 assay levels at Port Pirie, November 1992



Port Pirie - May 1993 - Cytochrome P450

Yellow Eye Mullet	*Yellow Fin Whiting*
7.253	6.484
5.495	0.879
4.945	0.879
5.824	1.758
3.956	0.33
0.33	1.319
2.967	1.209
4.286	
2.857	
2.308	
4.176	
4.396	
1.648	
3.956	
5.165	
2.308	
9.341	
4.176	
0.44	
1.648	
4.615	
8.022	
2.967	
0.989	
0.549	

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Yellow Eye Mullet	25	94.617	3.78468	5.415367
Yellow Fin Whiting	7	12.858	1.836857	4.395022

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20.74851	1	20.74851	3.981448	0.055155	4.170886
Within Groups	156.3389	30	5.211298			
Total	177.0875	31				

APPENDIX 2
Cytochrome P-450 IA1 assay parameters

EROD

Both Yellow eye mullet and yellow fin whiting

pH	7.8 Tris buffer
Temp	25 C
Time	30 mins
Protein conc.	100%
Substrate conc.	As per B G Lake assay for EROD

ECOD

Yellow eye mullet

pH	7.6 Tris buffer
Temp	25 C
Time	30 mins
Protein conc.	100%
Substrate conc.	As per B G Lake assay for ECOD

PPO

Both Yellow eye mullet and yellow fin whiting

pH	7.4 Potassium phosphate buffer
Temp	25 C
Time	30 mins
Protein conc.	100%
Substrate conc.	0.44 mg/ml acetone

APPENDIX 3
Cytochrome P-450 Activities

1. Analysis 1: Comparison of data from each of the two collection periods for both analysis methods.

This analysis examines of the null hypothesis (h_o) that there is no significant difference in the values of assayed cytochrome P450 levels for each of the sampling periods, November 1993 and May/June 1994, acheived by using the two analysis methods, PPO and EROD.

The results of the ANOVA to examine h_o are summarised in Table 1, below. The actual data, analyses and charts can be found in the Appendix.

Table 1: Results of ANOVA for November 1993 vs May/June 1994 for PPO and EROD

	P-value
PPO	0.166402
EROD	0.172358

None of the analyses have a value of $P<0.05$, which is the level of confidence sufficient for the null hypothesis h_o to be rejected. Thus the null hypothesis h_o is retained. ie: there is not a significant difference in the values of assayed cytochrome P450 levels for each of the sampling periods, November 1993 and May/June 1994, acheived by using the two analysis methods, PPO and EROD.

2. Analysis 2: Comparison of data from the PPO and EROD methods for the two collection periods.

This analysis examines of the null hypothesis (h_o) that there is no significant difference in the values of assayed cytochrome P450 levels acheived by using the two analysis methods, PPO and EROD, for each of the sampling periods, November 1993 and May/June 1994.

The results of the ANOVA to examine h_o are summarised in Table 2, below. The actual data, analyses and charts can be found in the Appendix.

Table 2: Results of ANOVA for PPO vs EROD for May/June 1994 and November 1993

	P-value
May/June 1994	7.35×10^{-5} *
November 1993	3.67×10^{-5} *

Those analyses marked with an asterisk (*) have a value of $P<0.05$, which is the level of confidence sufficient for the null hypothesis h_o to be rejected, and the acceptance of the alternative hypothesis h_1 that there is a significant difference in the values of assayed cytochrome P450 levels acheived by using the two analysis methods, PPO and EROD, for each sampling period.

3. Analysis 3: Simple linear regression analysis of data from the PPO and EROD methods for the two sampling periods.

For those Analyses of Variance returning a significant result, it followed to perform a simple linear regresison to determine the nature of the relationship.

3.1 November 1993

In this analysis, the values for PPO and EROD were plotted against each other and a simple linear regression performed.

Table 3: Summary output from simple linear regression

Multiple R	0.51471
R Square	0.264927
Adjusted R Square	0.232967
Standard Error	532.4023
Observations	25

The full analysis and plots can be found in the appendix.

The regression has an ANOVA significance F=0.000828. This indicates that the linear regression has a significant relation to the data. However the R Square value of 0.264927 shows that the regression line is a very poor fit.. The residual plot shows that there is an uneven distribution of the residuals.

3.2 May/June 1994

In this analysis, the values for PPO and EROD were plotted against each other and a simple linear regression performed.

Table 4: Summary output from simple linear regression

Multiple R	0.83026
R Square	0.689331
Adjusted R Square	0.658265
Standard Error	204.6096
Observations	12

The regression has an ANOVA significance F=0.008472. This indicates that the linear regression has a significant relation to the data. The R Square value of 0.689331 shows the regression to be a good fit.The residual plot however shows that there is an uneven distribution of the residuals.

4. The take home message

- There is not a significant difference in the EROD and PPO levels for the two sampling periods. ie: EROD November 1993 vs May/June 1994 - no significant difference, PPO November 1993 vs May/June 1994- no significant difference, etc.
- There is a significant difference between the levels of EROD and PPO for each measuring period. ie: PPO November 1993 vs EROD November 1993 - significant difference, etc
- The simple linear regressions show that there is a statistically significant relationship between the levels of PPO and EROD for the two sampling periods, however the R Squared value for November 1993 is so low as to dismiss the regression as poor. This relationship can be expressed as an equation. The data shows a skewed distribution, however, and should be augmented with more data and further analyses.

Port Pirie - PPO and EROD data

PPO Nov-93	*PPO May/June 1994*
559.4	79.60
1070.4	506.20
1160.8	1092.60
574.4	583.30
387.1	620.90
1122.8	196.50
373.8	728.70
466.7	1223.10
427.3	322.40
908.3	492.90
789.6	186.20
476.2	378.40
1001.6	
644.2	
198.4	
1506.5	
910.6	
339.9	
1245.6	
785.4	
1582.9	
169.9	
341.7	
2950.8	
65.5	

EROD Nov-93	*EROD May/June 1994*
13.23	11.94
426.44	59.66
334.1	116.67
165.17	33.33
24.48	33.59
1385.74	16.84
10.81	60.47
266.67	60.15
87.88	26.14
404.5	37.91
9.96	3.99
14.99	32.43
46.24	
22.08	
3.39	
21.24	
7.82	
7.75	
22.95	
15.4	
20.57	
86.04	
6.01	
913.55	
34.4	

PPO Nov-93	*EROD Nov-93*
559.4	13.23
1070.4	426.44
1160.8	334.1
574.4	165.17
387.1	24.48
1122.8	1385.74
373.8	10.81
466.7	266.67
427.3	87.88
908.3	404.5
789.6	9.96
476.2	14.99
1001.6	46.24
644.2	22.08
198.4	3.39
1506.5	21.24
910.6	7.82
339.9	7.75
1245.6	22.95
785.4	15.4
1582.9	20.57
169.9	86.04
341.7	6.01
2950.8	913.55
65.5	34.4

PPO May/June 1994	*ROD May/June 1994*
79.60	11.94
506.20	59.66
1092.60	116.67
583.30	33.33
620.90	33.59
196.50	16.84
728.70	60.47
1223.10	60.15
322.40	26.14
492.90	37.91
186.20	3.99
378.40	32.43

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
EROD No	25	4351.41	174.0564	107287.6
EROD Ma	12	493.12	41.09333	912.302

ANOVA

rce of Vari	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between	143344.7	1	143344.7	1.940883	0.172358	4.121347
Within Gro	2584938	35	73855.39			
Total	2728283	36				

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PPO Nov-93	25	20059.8	802.392	369543.7
PPO May/June 1994	12	6410.8	534.2333	122507.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	583046.5	1	583046.5	1.997393	0.166402	4.121347
Within Groups	10216631	35	291903.7			
Total	10799677	36				

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PPO Nov-	25	20059.8	802.392	369543.7
EROD No	25	4351.41	174.0564	107287.6

ANOVA

rce of Vari	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between	4935070	1	4935070	20.69944	3.67E-05	4.042647
Within Gro	11443953	48	238415.7			
Total	16379023	49				

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PPO May/June 1994	12	6410.8	534.2333	122507.3
EROD May/June 19	12	493.12	41.09333	912.302

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1459122	1	1459122	23.6449	7.35E-05	4.300944
Within Groups	1357616	22	61709.82			
Total	2816738	23				

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.83026
R Square	0.689331
Adjusted R Square	0.658265
Standard Error	204.6096
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regression	1	928929.8	928929.8	22.18865	0.000828
Residual	10	418650.9	41865.09		
Total	11	1347581			

	Coefficient	*andard Err*	*t Stat*	*P-value*	*ower 95%*	*pper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	138.8693	102.6327	1.353071	0.205829	-89.8107	367.5493	-89.8107	367.5493
EROD May/June 19	9.621124	2.042492	4.710483	0.000828	5.070167	14.17208	5.070167	14.17208

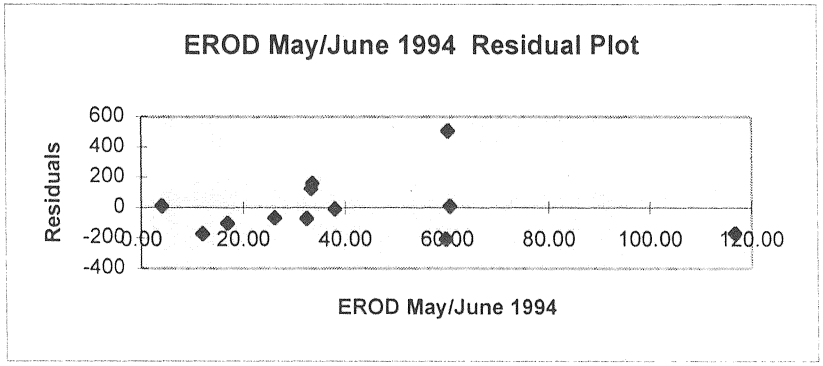
RESIDUAL OUTPUT

Observation	*PPO May/*	*Residuals*	*dard Residuals*
1	253.7455	-174.146	-0.85111
2	712.8655	-206.666	-1.01005
3	1261.366	-168.766	-0.82482
4	459.5413	123.7587	0.604853
5	462.0428	158.8572	0.776392
6	300.889	-104.389	-0.51019
7	720.6586	8.041362	0.039301
8	717.5799	505.5201	2.470657
9	390.3655	-67.9655	-0.33217
10	503.6061	-10.7061	-0.05232

PROBABILITY OUTPUT

Percentile	*May/June 1994*
4.166667	79.6
12.5	186.2
20.83333	196.5
29.16667	322.4
37.5	378.4
45.83333	492.9
54.16667	506.2
62.5	583.3
70.83333	620.9
79.16667	728.7

11	177.2576	8.942422	0.043705	87.5	1092.6
12	450.8823	-72.4823	-0.35425	95.83333	1223.1


EROD May/June 1994 Residual Plot
Residuals
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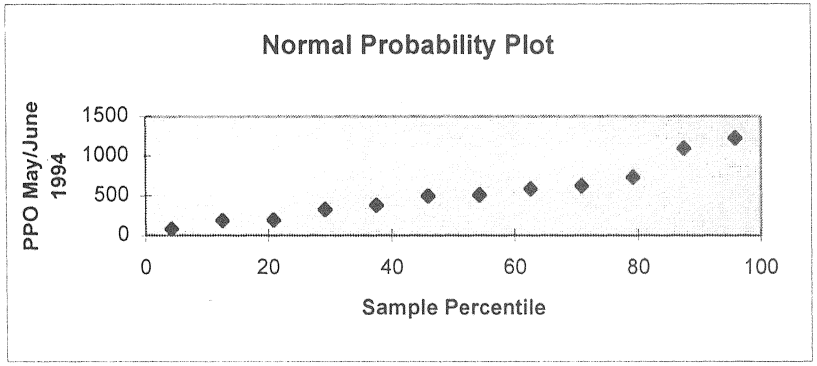
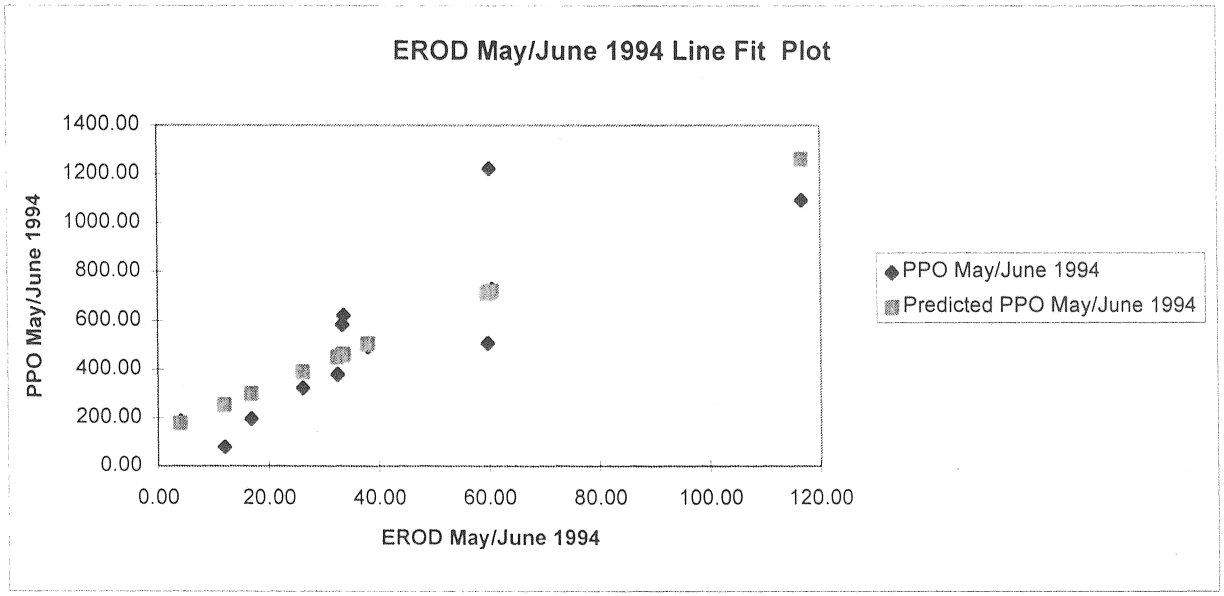
400
200
0
-200
-400
0.00
20.00
40.00
60.00
80.00
100.00
120.00
EROD May/June 1994


Normal Probability Plot
PPO May/June 1994
1500
1000
500
0
0
20
40
60
80
100
Sample Percentile


EROD May/June 1994 Line Fit Plot
PPO May/June 1994
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1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
0.00
20.00
40.00
60.00
80.00
100.00
120.00
EROD May/June 1994
PPO May/June 1994
Predicted PPO May/June 1994

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.51471
R Square	0.264927
Adjusted R Square	0.232967
Standard Error	532.4023
Observations	25

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regression	1	2349648	2349648	8.289397	0.008472
Residual	23	6519402	283452.3		
Total	24	8869050			

	Coefficient	*andard Err*	*t Stat*	*P-value*	*ower 95%*	*pper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	636.1231	121.1326	5.25146	2.51E-05	385.5415	886.7047	385.5415	886.7047
EROD Nov-93	0.955259	0.331787	2.879131	0.008472	0.268906	1.641612	0.268906	1.641612

RESIDUAL OUTPUT

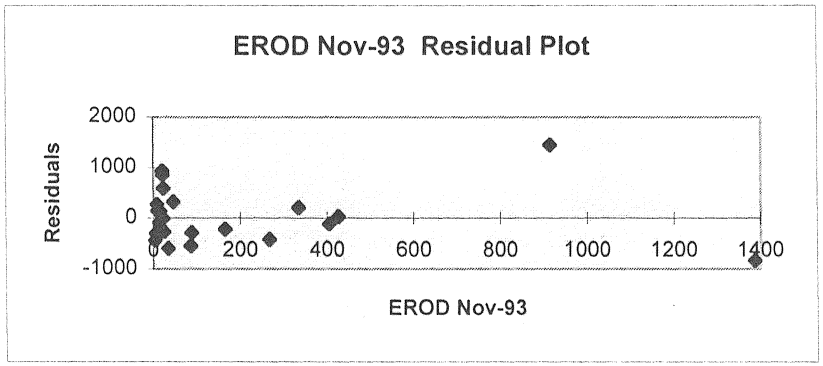
Observation	*ted PPO*	*Residuals*	*dard Residuals*
1	648.7612	-89.3612	-0.16785
2	1043.484	26.91639	0.050556
3	955.275	205.525	0.386033
4	793.9032	-219.503	-0.41229
5	659.5079	-272.408	-0.51166
6	1959.863	-837.063	-1.57224
7	646.4495	-272.649	-0.51211
8	890.8619	-424.162	-0.79669
9	720.0713	-292.771	-0.54991
10	1022.525	-114.225	-0.21455

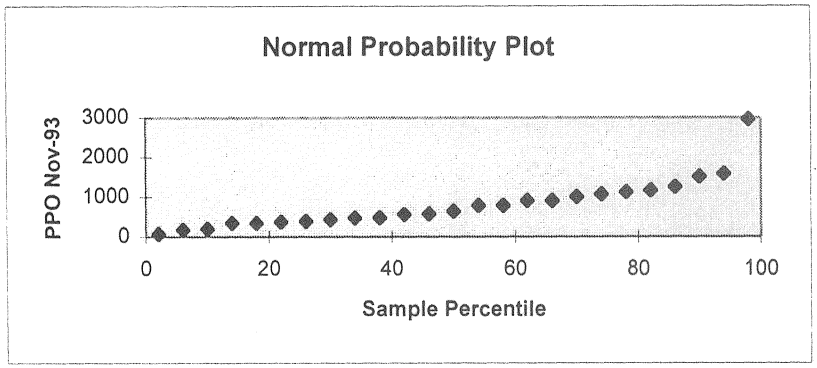
PROBABILITY OUTPUT

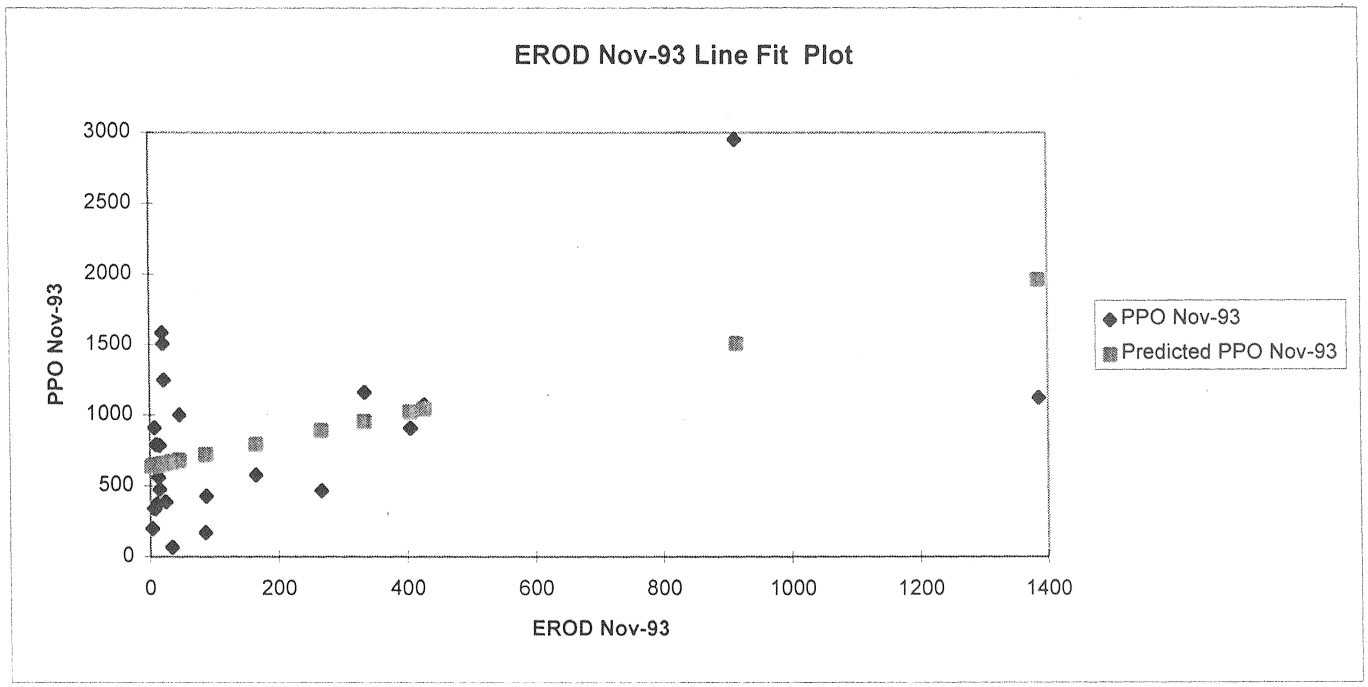
Percentile	*PO Nov-93*
2	65.5
6	169.9
10	198.4
14	339.9
18	341.7
22	373.8
26	387.1
30	427.3
34	466.7
38	476.2

11	645.6375	143.9625	0.270402
12	650.4425	-174.242	-0.32728
13	680.2943	321.3057	0.603502
14	657.2152	-13.0152	-0.02445
15	639.3615	-440.961	-0.82825
16	656.4128	850.0872	1.596701
17	643.5932	267.0068	0.501513
18	643.5264	-303.626	-0.57029
19	658.0463	587.5537	1.10359
20	650.8341	134.5659	0.252752
21	655.7728	927.1272	1.741403
22	718.3136	-548.414	-1.03007
23	641.8642	-300.164	-0.56379
24	1508.8	1442	2.708479
25	668.984	-603.484	-1.13351

42	559.4
46	574.4
50	644.2
54	785.4
58	789.6
62	908.3
66	910.6
70	1001.6
74	1070.4
78	1122.8
82	1160.8
86	1245.6
90	1506.5
94	1582.9
98	2950.8


EROD Nov-93 Residual Plot
Residuals
2000
1000
0
-1000
0
200
400
600
800
1000
1200
1400
EROD Nov-93


Normal Probability Plot
PPO Nov-93
3000
2000
1000
0
0
20
40
60
80
100
Sample Percentile


EROD Nov-93 Line Fit Plot
PPO Nov-93
3000
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
1200
1400
EROD Nov-93
PPO Nov-93
Predicted PPO Nov-93