HEAVY METALS IN *PENAEUS ESCULENTUS* IN TORRES STRAIT PILOT STUDY

Prawns for the pilot study were collected from both the north and south site (see figure 1) in May/June 1992. Samples were collected aboard the commercial prawn trawlers FV 'Maggie Jo' (in the north), and FV 'Smithy' (in the south). Each site was sampled over 2 or 3 nights, with 3 or 4 separate shots being trawled each night. The objective of the pilot program was to assess variability in metal levels in prawns at various scales in order to choose the optimum sampling unit for the main study. In addition, the comparability and relative merits of various sample handling procedures was compared, in order to assess the poolability of samples collected under various regimes.

Initially, the intention was to remove the head from other tissues immediately after capture and prior to freezing, in a portable clean air 'glove-box' on board the trawler. In practice, this procedure was soon abandoned due to the space and time limitations on board the trawlers. Various 'next best' procedures were tried, until the optimum method, as adopted in the main study, was evolved. Treatment variations were further confounded by freezer and transport problems, which allowed some samples to defrost and re-freeze at least once. The resulting suite of different sample handling treatments raised the important question of whether or not samples collected under such different regimes should be treated separately.

Treatments can be categorised as follows:

1. Freeze immediately, dissect at later date while par frozen.
2. Keep samples chilled until 12–36 hours (with intention of dissecting ASAP), freeze and dissect at later date while par frozen.
3. Dissect immediately, within 4 hours (the original goal).
4. Dissect within 12–36 hours.

After considering previously published records of metal levels in prawns, high variability was anticipated. It was suggested that since the hepatopancreas is a major storage site for many metals, smaller variance may be achieved by analysis of this tissue alone. This idea was also trialed in this pilot study.

This pilot study was based on cadmium data, since this had been identified as the metal of most concern.

Pilot analysis of prawn data:

1 Tiger prawn samples were selected across nights and shots, in the following way. Only large females were selected, to eliminate variability due to sex and size. It was not possible to standardise gonad and moult stage.
Northern Site: 3 shots selected from all 3 nights, 3 reps/shot
Southern Site: 4 shots selected from both nights, 3 reps/shot

2 Dry weight concentrations are used because they are less variable.

3 In considering whether levels of a factor can be pooled, Winer's (1971) criteria (as described in Underwood 1981) of setting $a>0.25$ in an F- or t-test was used.

4. As a whole, variances in ANOVA's were heteroscedastic. However, this was not a concern because the aim of the analyses was to investigate the probability that the null hypothesis could explain the data. (see Underwood 1981 for a discussion on this.) Thus, transformations were not performed.

Can dissection codes be pooled?

5. Comparison 1:
The two main handling methods 1 and 3, were reproduced within nights in the south, for tiger prawns.
F tests did not support pooling these two codes at a>0.25 and on the whole levels were higher from dissection code 1 (see table 3).

Table 3 Cadmium levels in tissues for dissection codes 1 and 3. Samples were tiger prawns, from the southern site. In each cell, n=6, representing pooled samples from 2 shots. Values are on a dry weight basis in mg/kg (ppm).

Dissection Method		Head total night 1	night 2	Tail Muscle night 1	night 2
3	x	27.50	19.03	0.48	0.25
	SE	3.15	3.60	0.08	0.03
1	x	38.68	39.78	0.62	0.48
	SE	16.31	9.56	0.06	0.08

6. Comparison 2:
The tail flesh of 5 endeavour prawns each from handling methods 3 and 4 (reproduced in one shot) was analysed for the southern site only.
A t-test supported pooling these two methods at a>0.25.

7. Overall however, there was insufficient evidence to support pooling different handling methods. For this reason, samples collected during the pilot program were excluded from the main study.

Is it an advantage to analyse hepatopancreas instead of total head?

8. At dissection code 1 only (since it is the one that is standard for subsequent analyses), the precision was calculated for these two tissue types, for use as a comparable measure of variability.
n=6 per night (shots were dropped). Results were:

Night	Precision estimate Hepatopancreas	Head total
3	.26	.21
4	.21	.42
5	.20	.24

(Dissection code 1 was not used on nights 1 and 2)

Except on night 4, analysis of hepatopancreas did not improve the precision of estimates of levels in the head. Thus, the option of using it as an alternative to total head was not pursued.

Can shots be pooled?

9. In 6 ANOVAs performed using shots as a factor, 5 supported pooling shots at a>0.25. Also, in 5 out of 6 cases, the variance attributable to shots was 0. Thus, the factor shots will be dropped from future analyses.

10. After pooling shots however, there was no consistent evidence to support pooling nights.

11. Thus, the random factors that remain for the design of future analyses are nights and replicates.

How many nights and how many replicates?

12. To allow enough analyses to cover all prioritised factors (see proposal), the maximum total number of replicates per random unit (=nights x reps) should not exceed 20.

13. The effect of varying number of nights and replicates on overall variance was calculated according to formulae in Andrew and Mapstone (1987). In summary, increasing the number of nights had a much greater benefit to overall variance than increasing the number of replicates. The optimal combination was the maximum possible number of nights, i.e. three and seven replicates.

14. In the north, only two nights are available because samples from one night were included in the freezer problem already described. In these cases, two nights with 10 replicates will be used.