# Appendix B – Statistical report: Gladstone Harbour sampling trips 2012

**OVERVIEW**

This report describes the statistical analyses of the fisheries data collected on two sampling trips, from targeted sites around Gladstone Harbour as well as from two reference sites (Fitzroy River and Bundaberg). Section 1 outlines the statistical methods employed. Section 2 covers the results for the species that were not submitted to the laboratory, and illustrates that there were no apparent problems amongst these species. The comprehensive Section 3 outlines the results for the important and targeted fish species submitted to the laboratory (barramundi, bull sharks, mullet and queenfish), at both the trip / location level, and then combined into regional comparisons. Sections 4 to 6 then repeat this comprehensive presentation of results separately for three key species, namely barramundi, mullet and mudcrabs respectively. For bull sharks, there were insufficient numbers to conduct separate analyses. For queenfish, the observed percentages of diseased individuals were too low to warrant separate analyses.

**1. STATISTICAL METHODS**

All analyses were conducted using GenStat (2011). General linear models (McCullagh and Nelder 1989) were used for the continuous variables. Generalised linear models (McCullagh and Nelder 1989) were adopted for the discrete (categorical) data, using the Poisson distribution with a log link for counts, and the Binomial distribution with the logit link function for binary (percentage) data.

As there was incomplete coverage in the three-way design matrix (fish by location by trip – see Table 1.1), it was important to account for this in the analyses, and to correctly adjust the resultant means. Figure 1.1 demonstrates the large differences between fish species for the hepatosomatic index. This shows that any 'raw' averages (for each trip and location) will depend heavily on the balance of fish being caught, in particular, the number of bull sharks.

**Table 1.1.** Distribution of catches by species and locations.

| | Barra. | Bull shark | Grinner | Herring | Mullet | Prawn | Queenfish | Threadfin |
|---|---|---|---|---|---|---|---|---|
| Fitzroy R | 68 | 7 | 115 | 115 | 21 | 200 | 12 | 200 |
| Bundaberg | 20 | 1 | 18 | 110 | 26 | 200 | 36 | 125 |
| Hamilton Pt | 31 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Calliope R | 23 | 3 | 0 | 0 | 18 | 0 | 14 | 0 |
| Harbour | 7 | 0 | 46 | 120 | 0 | 174 | 0 | 220 |
| Spoil Grounds | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| Upp. Boyne R | 21 | 1 | 0 | 0 | 20 | 0 | 0 | 0 |
| Low Boyne R | 32 | 10 | 0 | 0 | 20 | 0 | 4 | 0 |
| Rodds Bay | 9 | 0 | 0 | 0 | 20 | 0 | 0 | 0 |
| Lake Awoonga | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

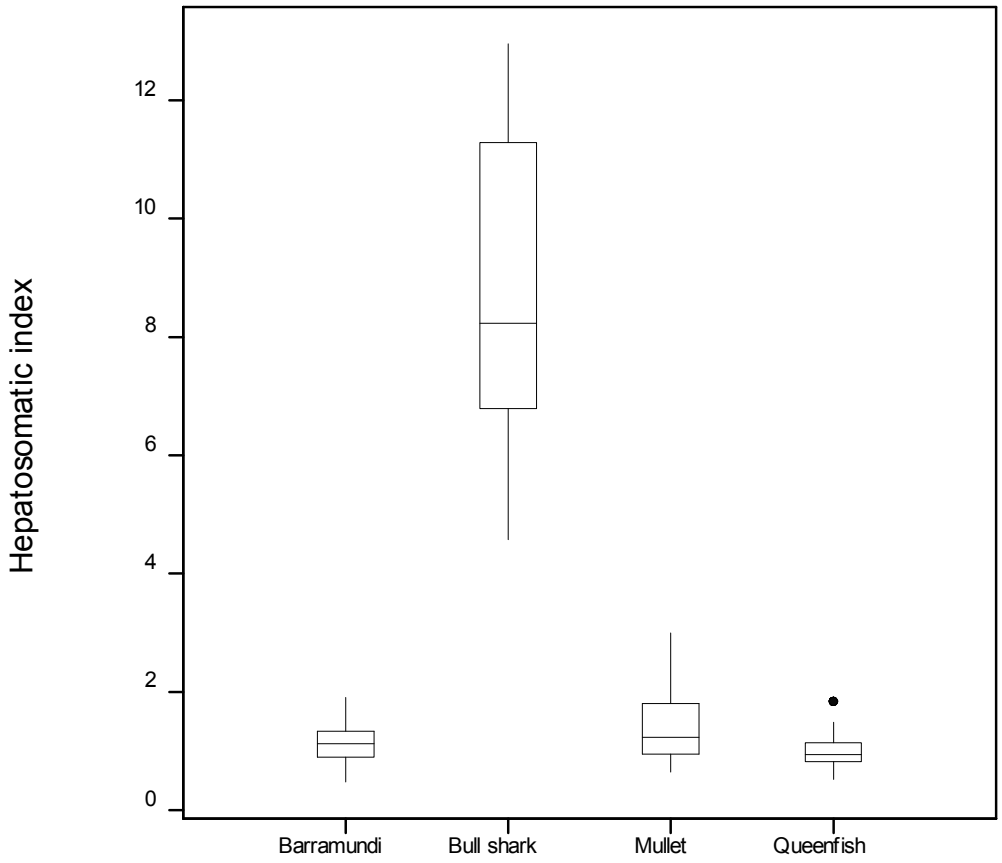


**Figure 1.1.** Boxplot (showing the means, quartiles and ranges) of hepatosomatic index for the laboratory-assessed fish species.

To avoid any apparent biases due to the varying combinations of fish caught at each location and trip, 'fish type' was included as a main effect in all models, and the 'location by trip' tables were extracted using the 'full' option. This effectively estimates missing (fish) values for those location/trip combinations when each fish type was not caught, and correctly adjusts each mean to the overall expectation.

The deliberate laboratory sampling of both diseased and unaffected fish (the target was for five of each, for each location/trip combination) introduced further complexity into the analyses. In the field, each fish was defined as 'not diseased' if its skin condition, lesions category and eye condition were all zero, and 'diseased' otherwise. For the variables measured in the laboratory, any unweighted analyses for locations and times would only reflect the proportions of diseased fish that were submitted to the laboratory, rather than the overall population (as best measured by all the fish obtained in the field). To correctly adjust for this stratification, weighting factors were calculated as:

Proportion (diseased or not diseased) in the whole sample
Proportion (diseased or not diseased) in the laboratory subsample

These weighting factors were determined at the location by trip by fish species level, and then used to correctly weight each of the respective observations (diseased or not) in the analyses.

Fish size (length or weight) was trialed as an overall covariate, but showed inconsistent trends. Problems were encountered due to the greatly varying size ranges of the species – there was evidence that this term was merely fitting to the overall differences in size between the fish types. Adjusting all results back to a common mean size also made little sense, given the large difference between the average lengths for bull sharks *vs.* the others. Hence size was omitted from all of the cross-species analyses, leaving size effects to be captured in with the 'fish type' factor. Length was included as a covariate in the separate barramundi

analyses, but this was not done for mullet, as (within this group of fish) lengths were confounded with the actual species of mullet.

'Time since death' was trialed as a covariate for those laboratory ratings for which there was a reasonable hypothesis that they would increase after death. This was tested at the individual species level, and for the instances where this relationship was not significant, time since death was then dropped for the final model.

In addition to conducting the primary weighted analyses of location by trip by fish species (along with their interactions), second analyses were conducted for each variable including 'disease status' in the model. This allowed the direct testing and reporting of the impact of disease status on each variable.

The analyses of each of the variates (in turn) are followed by a multivariate summarisation. This adopts a principal components representation of all the data, taking the first two orthogonal dimensions, to obtain an overview as to which locations and times are most similar.

## 2. RESULTS - TRAWL AND NET DATA (SPECIES NOT SUBMITTED TO THE LABORATORY)

### 2.1 Prawns

Of the 574 prawns observed, there were only five with shell erosions – three from the Harbour and two from the Fitzroy River, with none from the Bundaberg samples. There was no statistical difference in the percentages of prawns with erosions, with mean values (± s.e.) of 1.8% (± 1.0%) for Gladstone Harbour, 1.0% (± 0.7%) for the Fitzroy River, and 0.0% (n/a) for Bundaberg.

### 2.2 Fish

All of the grinner (179), herring (345) and threadfin (545) were field-classified as 'not diseased', having a rating of zero for skin condition, lesion grade and eye condition. The grinners and herring also had no parasites. For threadfin, there were two found with parasites, one from Bundaberg (a reference site) and one from Gladstone Harbour. Similarly, all 71 Queenfish had ratings of zero for the skin and eye ratings, with two fish having lesions – one each from Bundaberg and the Calliope River. There were 36 queenfish with externally-observed parasites, with 28 from the reference sites and 8 from the Gladstone region.

## 3. TRAWL AND NET DATA (SPECIES WHICH WERE SUBMITTED TO THE LABORATORY)

Table 3.1 shows the distributions of the fish numbers that were submitted to the laboratory. In many of the location / trip combinations, these were the whole sample.

**Table 3.1.** Distribution of numbers submitted to the laboratory.

| | Barra. | | Bull shark | | Mullet | | Queenfish | |
|---|---|---|---|---|---|---|---|---|
| | Trip 1 | Trip 2 | Trip 1 | Trip 2 | Trip 1 | Trip 2 | Trip 1 | Trip 2 |
| Fitzroy R | 10 | 10 | 7 | 0 | 10 | 11 | 1 | 10 |
| Bundaberg | 10 | 7 | 1 | 0 | 10 | 10 | 10 | 10 |
| Hamilton Pt | 10 | 10 | 1 | 0 | 0 | 0 | 0 | 0 |
| Calliope R | 10 | 10 | 3 | 0 | 7 | 10 | 0 | 0 |
| Spoil Grounds | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| Upp. Boyne R | 10 | 6 | 0 | 0 | 10 | 10 | 0 | 0 |
| Low Boyne R | 10 | 10 | 0 | 0 | 10 | 10 | 0 | 1 |
| Rodds Bay | 7 | 2 | 0 | 0 | 10 | 10 | 0 | 0 |
| Lake Awoonga | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Excluding net damage, there were only three fish with a recordable ***eye condition*** – one barramundi in the Calliope River, one barramundi at Bundaberg, and one sea mullet in the Fitzroy River. Notably, the latter two were from the reference sites. Due to this low incidence level, eye condition was not analysed.

Similarly, for ***general condition***, only one fish was classified as 'emaciated' – a barramundi from Hamilton Point. All others were rated as 'normal'.

For ***heart condition***, six fish were rated as 'abnormal', with four of these being barramundi. These six appeared randomly spread across the trips (four *vs.* two, for trips 1 and 2 respectively), locations (one from a reference site and the other five from four different locations), and 'disease status' (three each).

Similarly, only six fish had a ***hindgut rating*** other than 'zero' – three tiger mullet from the lower Boyne River on trip 1, and from trip 2 there were two barramundi from the Calliope River and one from the Fitzroy River (reference site). Five of these six fish were rated as 'not diseased'.

Nine fish had a ***kidney rating*** of 'other' (*vs.* 'normal'). These were all mullet from trip 2 - six coming from the upper Boyne River, two from the lower Boyne River, and one from Rodd's Bay. Whilst being excluded for the overall (cross-species) analyses, this variable was included in the separate analyses conducted for mullet.

Most of the ***spleen categories*** were rated as red or black granular, which are considered normal. There was only one enlarged spleen, a barramundi from the lower Boyne River. There were no apparent patterns in ratings of 'nodular' (four from the reference sites *vs*. three from the Gladstone sites), nor for the 'other' category (eight from the reference sites *vs*. four from the Gladstone sites).

***Parasites*** (as determined in the field) had generally low incidence, with 30 from Bundaberg and 6 from the Fitzroy River (the reference sites) *vs.* a total of 11 from the seven sites around Gladstone.

### 3.2 Comparing the laboratory and field assessments

The skin and lesion categories for each individual were re-assessed in the laboratory. For ***skin categories***, the respective counts are listed in Table 3.2. There was a significant ($P < 0.01$) degree of association, with perfect agreement in 63% of the cases (the light-grey cells). There was no apparent bias, as the field rating was higher than the laboratory rating in 18% of the cases, and lower in 19%. This shows that the ratings conducted in the field are unbiased, and as these were done on all 1504 fish (*vs*. the 294 that were also rated in the laboratory), forms the best overall sample of skin category ratings.

**Table 3.2.** Counts for skin categories for fish (F = field assessment; L = laboratory assessment).

| | F - 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| L - 0 | 163 | 6 | 26 | 1 | 0 |
| 1 | 28 | 2 | 7 | 4 | 2 |
| 2 | 8 | 1 | 11 | 5 | 2 |
| 3 | 6 | 0 | 0 | 7 | 0 |
| 4 | 13 | 0 | 0 | 0 | 2 |

As shown in Table 3.3, ***lesion categories*** tell a similar story. Again there was a significant ($P < 0.01$), but higher, degree of association, with perfect agreement in 89% of the cases. The lack of bias is again evident, with 4.5% having a field rating higher than the rating in the laboratory, and 6.5% for vice versa.

**Table 3.3.** Counts for lesion categories for fish (F = field assessment; L = laboratory assessment).

| | F - 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| L - 0 | 246 | 3 | 6 | 1 | 0 |
| 1 | 2 | 2 | 3 | 0 | 0 |
| 2 | 2 | 3 | 11 | 0 | 0 |
| 3 | 0 | 0 | 5 | 0 | 0 |
| 4 | 6 | 0 | 1 | 0 | 3 |

### 3.3 Overview of analyses

Of the 13 variables that had sufficient data for analyses, 'fish species' was significant ($P < 0.05$) for 11, indicating the necessity to correctly adjust the other means for this important effect. Interestingly, 'disease status' (as assessed in the field) only had a significant effect on four of the variables. Significant differences were found between locations in 11 analyses and between trips in four. Importantly, there

was a significant ($P < 0.05$) location by trip interaction in seven of the 13 analyses (54%). As this is notably higher than the random expectation of 5%, it was concluded that the changing patterns of responses over space and time are a real effect, so the data are reported on this two-way basis (locations by times).

**Time since death –** This ranged from 0 to 17 hours. It significantly ($P < 0.05$) affected the laboratory ratings for skin and fin condition (% of fish affected), and there was a significant interaction with fish species. Of the 12 bull sharks submitted to the laboratory, most (8) had a recordable skin condition, yet for all 12 the rated fin condition was zero. The analyses for laboratory-rated skin and fin condition thus included time since death (along with the species interaction), and the resultant means are standardised for this.

The observed patterns for the fish species are of interest. As binary (0 or 1) data cannot easily be visually interpreted, we condensed these data into five levels of time since death, separately for the species (which did display different coverage), and also using the numbers of observations of each species in each class as a weighting factor for these analyses. For the percentage of fish with a recordable skin condition, there was a relationship with time since death for both barramundi ($R^2 = 58\%$; $P = 0.010$) and mullet ($R^2 = 64\%$; $P = 0.056$; mullet had fewer observations over times). For both these species, there was no evidence of a difference in the relationship between the reference and the Gladstone sites ($P > 0.17$ for all four of the significance tests). Figures 3.1 and 3.2 show the mean values for the regions, along with the fitted lines.

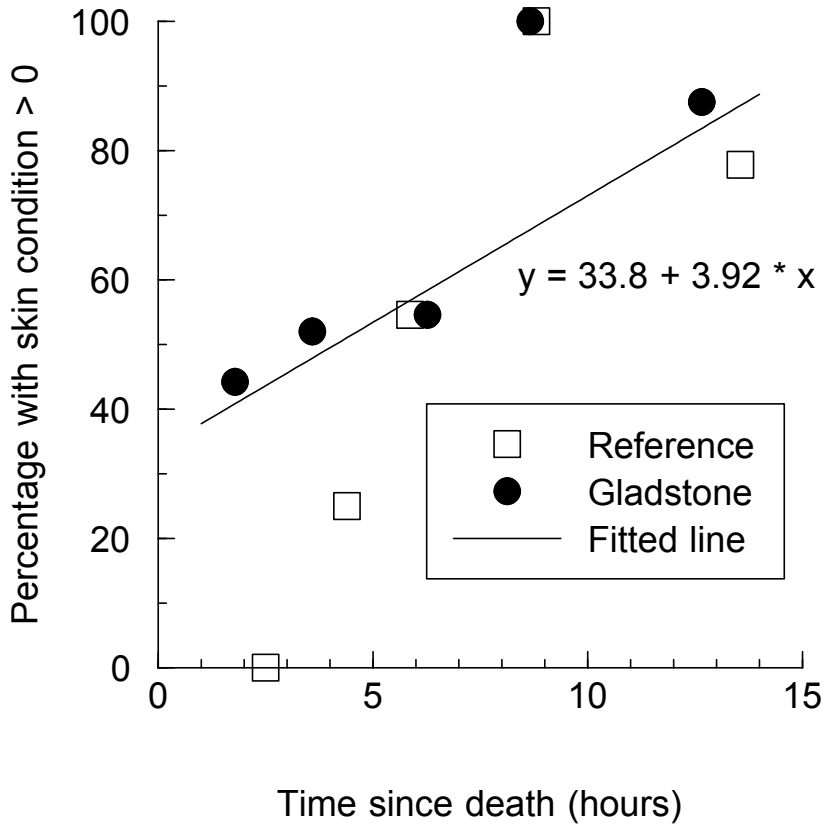


**Figure 3.1.** Barramundi skin condition ratings *vs*. time since death, by regions.

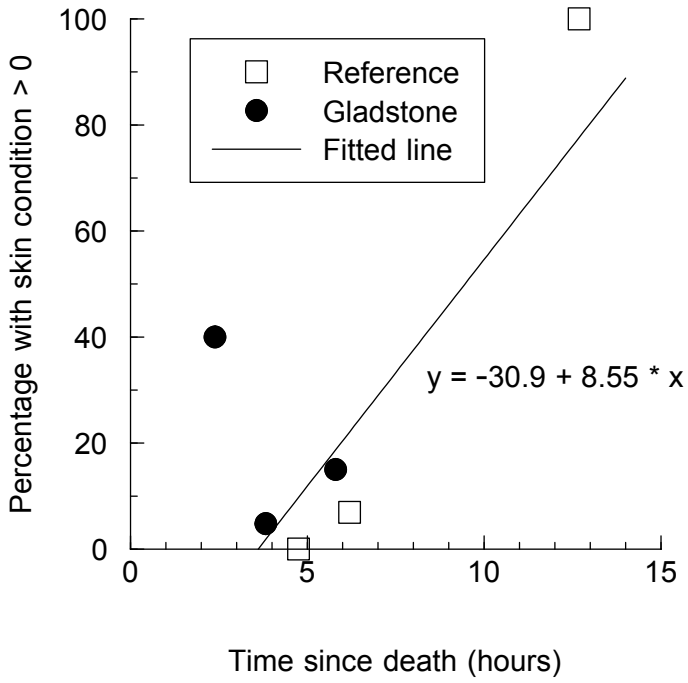


**Figure 3.2.** Mullet skin condition ratings *vs*. time since death, by regions.

For the percentage of fish with a recordable fin condition, for barramundi there was a significant (P = 0.006) difference between the regions in the intercepts. The data plus these lines are shown in Figure 3.3 (overall $R^2$ = 70%; *P* = 0.016). Whilst this relationship was not significant for mullet ($R^2$ = 11%; *P* = 0.52), it is shown in Figure 3.4 for comparison.

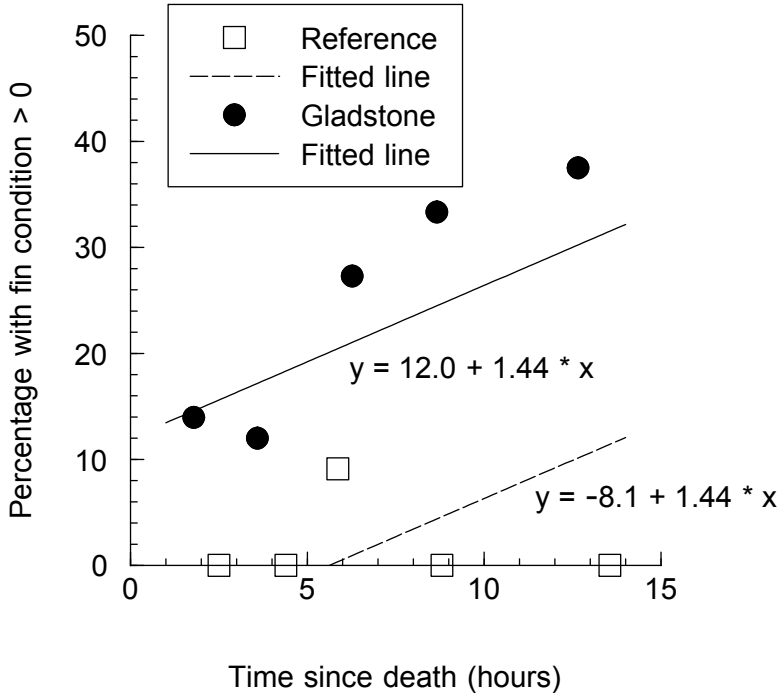


**Figure 3.3.** Barramundi fin condition ratings *vs*. time since death, by regions.

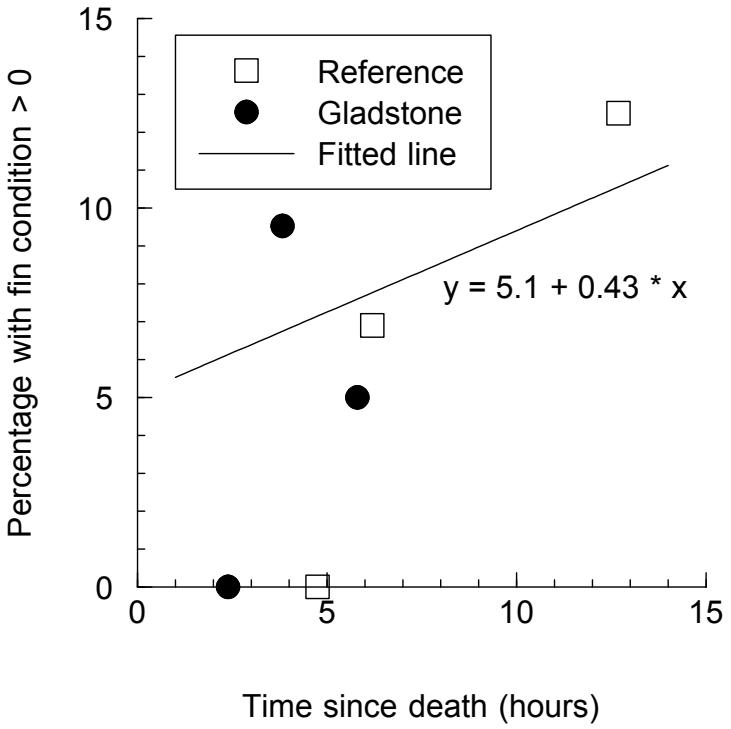


**Figure 3.4.** Mullet fin condition ratings *vs*. time since death, by regions.

**Fish species –** The adjusted means, the tests of significance between these, and their average standard errors are listed in Table 3.4. These results (particularly the number of significant values) confirm the need for the important 'location by time' means to be correctly adjusted for the numbers of fish taken in each sample.

**Table 3.4.** Effects of fish species on the response variables.

| | Sig. level | Barra. | Bull shark | Mullet | Queenfish | avg. s.e. |
|---|---|---|---|---|---|---|
| Hepatosomatic index | ** | 1.1 | 8.9 | 1.4 | 0.9 | 0.1 |
| Condition score | ** | 1.3 | 1.5 | 1.5 | 1.1 | 0.0 |
| HAI score | * | 25.8 | 32.3 | 18.1 | 31.0 | 3.9 |
| % diseased (field) | ** | 28.7 | 85.2 | 23.7 | 4.3 | 4.8 |
| Skin % > zero (field) | ** | 21.5 | 81.6 | 29.3 | 0.0 | 3.9 |
| Lesions % > zero (field) | ** | 12.7 | 44.3 | 0.7 | 4.1 | 4.0 |
| Fins % abnormal | | 13.4 | 0.0 | 7.1 | 22.0 | 3.7 |
| Gills % abnormal | | 13.1 | 0.0 | 16.2 | 19.7 | 3.3 |
| Abdomen % tucked up | ** | 21.9 | 0.0 | 1.3 | 48.0 | 2.8 |
| Liver form % abnormal | ** | 16.1 | 35.9 | 9.7 | 43.0 | 7.1 |
| Liver colour % discoloured | ** | 17.0 | 40.1 | 14.3 | 55.0 | 7.2 |
| Parasites % > zero | ** | 17.7 | 19.2 | 1.5 | 30.9 | 5.9 |
| Mesentary fat (%) | ** | 42.6 | 0.0 | 12.4 | 11.4 | 4.6 |

* $P < 0.05$; ** $P < 0.01$

**Disease status (as assessed in the field) –** The adjusted means, tests of significance and average standard errors for the continuous variables follow in Table 3.5.

**Table 3.5.** Effects of disease status on the response variables.

| | Sig. level | Not diseased# | Diseased# | avg. s.e. |
|---|---|---|---|---|
| Hepatosomatic index | | 1.27 | 1.48 | 0.06 |
| Condition score | | 1.35 | 1.32 | 0.02 |
| HAI score | ** | 18.3 | 31.0 | 2.1 |
| Fins % abnormal | ** | 7.4 | 17.7 | 2.9 |
| Gills % abnormal | | 15.0 | 12.4 | 3.1 |
| Abdomen % tucked up | | 15.9 | 20.2 | 2.7 |
| Liver form % abnormal | ** | 10.9 | 21.1 | 2.7 |
| Liver colour % discoloured | * | 15.4 | 20.8 | 3.1 |
| Parasites % > zero | | 11.6 | 10.7 | 2.5 |
| Mesentary fat (%) | | 22.5 | 24.5 | 2.9 |

#as assessed in the field; * $P < 0.05$; ** $P < 0.01$

The tabulations and histograms of the full distributions of counts against field-rated disease status have not been presented, but as indicated by the general lack of significant results in Table 3.5, these were not informative. In the analyses, the effect of disease on the resultant distributions of counts was significant ($P < 0.05$) only for spleen, bile and mesentary fat.

### 3.4 Location by trip means

These are the results that are of primary interest, and are listed in Tables 3.6a to 3.6c. For most of the categorical measures, these tables show the binary contrast ('normal' *vs.* 'other'), listed as percent not-normal. Mesentary fat (% cover) has been analysed as an approximately-continuous variable. Of the five categories here, three are direct numbers (namely 'none' = 0% cover, '0.5' = 50%, and 'completely covered' = 100%), and the centre-points were adopted for the remaining two categories: 25% for '<50%', and 75% for '>50%'.

It is notable that there were actually significant differences ($P < 0.05$) between the two reference sites (Fitzroy River and Bundaberg) for hepatosomatic index, condition factor, HAI, abdomen % tucked and liver colour (trip 1); and for condition factor, % diseased, % skin condition > 0, lesions % > 0, abdomen % tucked, liver form and liver colour (trip 2).

**Table 3.6a.** Effects of locations and trips on the response variables.

| Location | Trip | Hepat. index | Condition factor | HAI score | %# diseased | %# skin cond. > 0 |
|---|---|---|---|---|---|---|
| Fitzroy | 1 | 0.82 | 1.34 | 11.8 | 7.6 | 7.8 |
| Bundy | 1 | 1.74 | 1.46 | 32.8 | 7.9 | 6.6 |
| Hamilton | 1 | 1.17 | 1.22 | 9.3 | 7.4 | 8.5 |
| Calliope | 1 | 2.00 | 1.36 | 18.7 | 3.6 | 0.0 |
| Harbour | 1 | | | | 4.0 | 4.9 |
| Spoil | 1 | 1.57 | 1.28 | 3.6 | 0.0 | 4.8 |
| UpBoyne | 1 | 1.26 | 1.31 | 30.9 | 10.0 | 3.1 |
| LwBoyne | 1 | 1.31 | 1.29 | 32.7 | 13.6 | 3.8 |
| Rodds | 1 | 1.66 | 1.36 | 21.6 | 5.6 | 4.9 |
| Lake Awoonga | 1 | 1.66 | 1.41 | 3.7 | 0.0 | 0.0 |
| Fitzroy | 2 | 1.56 | 1.25 | 19.8 | 10.0 | 9.0 |
| Bundy | 2 | 1.49 | 1.42 | 24.6 | 3.5 | 3.6 |
| Hamilton | 2 | 1.49 | 1.23 | 19.9 | 12.4 | 13.6 |
| Calliope | 2 | 1.79 | 1.39 | 22.2 | 13.4 | 11.6 |
| Harbour | 2 | | | | 7.8 | 7.3 |
| UpBoyne | 2 | 1.80 | 1.24 | 38.8 | 7.6 | 5.2 |
| LwBoyne | 2 | 2.08 | 1.36 | 28.2 | 10.8 | 10.2 |
| Rodds | 2 | 1.57 | 1.35 | 14.9 | 5.7 | 4.4 |
| Sig. of - | Locations | ** | ** | ** | * | * |
| | Trips | ** | | | | ** |
| | Interaction | ** | | | * | ** |

#as assessed in the field; ** $P < 0.01$; * $P < 0.05$

**Table 3.6b.** Effects of locations and trips on the response variables.

| Location | Trip | Lesions %[#] > 0 | Fins % abnormal | Gills % abnormal | Abdomen % tucked |
|---|---|---|---|---|---|
| Fitzroy | 1 | 0.3 | 16.6 | 9.4 | 0.0 |
| Bundy | 1 | 2.3 | 1.8 | 16.4 | 9.8 |
| Hamilton | 1 | 1.5 | 45.1 | 11.4 | 34.3 |
| Calliope | 1 | 2.1 | 31.3 | 17.9 | 11.1 |
| Harbour | 1 | 0.0 | | | |
| Spoil | 1 | 0.0 | 7.0 | 0.0 | 0.0 |
| UpBoyne | 1 | 11.5 | 12.0 | 28.3 | 15.9 |
| LwBoyne | 1 | 11.6 | 16.5 | 5.0 | 28.6 |
| Rodds | 1 | 0.0 | 17.7 | 11.6 | 22.1 |
| Lake | | | | | |
| Awoonga | 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Fitzroy | 2 | 6.0 | 0.0 | 23.2 | 35.3 |
| Bundy | 2 | 0.0 | 2.0 | 19.7 | 10.9 |
| Hamilton | 2 | 3.7 | 0.0 | 0.0 | 33.4 |
| Calliope | 2 | 5.0 | 19.8 | 24.4 | 16.8 |
| Harbour | 2 | 3.0 | | | |
| UpBoyne | 2 | 12.0 | 12.8 | 12.2 | 39.0 |
| LwBoyne | 2 | 4.1 | 11.1 | 4.7 | 13.8 |
| Rodds | 2 | 14.1 | 61.9 | 0.0 | 0.0 |
| Sig. of - | Locations | ** | | | ** |
| | Trips | | | | |
| | Interaction | ** | ** | | ** |

[#]as assessed in the field; ** $P < 0.01$; * $P < 0.05$

**Table 3.6c.** Effects of locations and trips on the response variables.

| Location | Trip | Liver form % abnormal | Liver % discoloured | Parasites % > zero | Mesentary fat (%) |
|---|---|---|---|---|---|
| Fitzroy | 1 | 7.2 | 15.3 | 13.5 | 42.7 |
| Bundy | 1 | 12.9 | 37.0 | 2.3 | 42.3 |
| Hamilton | 1 | 0.0 | 0.0 | 7.8 | 2.3 |
| Calliope | 1 | 14.4 | 10.9 | 15.9 | 18.1 |
| Harbour | 1 | | | | |
| Spoil | 1 | 6.1 | 6.3 | 0.0 | 31.7 |
| UpBoyne | 1 | 25.2 | 23.6 | 0.0 | 29.0 |
| LwBoyne | 1 | 33.5 | 24.8 | 7.4 | 28.3 |
| Rodds | 1 | 11.4 | 37.1 | 20.0 | 14.1 |
| Lake | | | | | |
| Awoonga | 1 | 23.0 | 0.0 | 0.0 | 70.1 |
| Fitzroy | 2 | 4.6 | 9.3 | 17.2 | 20.0 |
| Bundy | 2 | 35.9 | 43.6 | 4.9 | 16.0 |
| Hamilton | 2 | 0.0 | 10.0 | 51.6 | 0.0 |
| Calliope | 2 | 0.0 | 0.0 | 27.4 | 1.5 |
| Harbour | 2 | | | | |
| UpBoyne | 2 | 47.7 | 18.7 | 12.3 | 30.3 |
| LwBoyne | 2 | 39.5 | 23.6 | 24.0 | 9.4 |
| Rodds | 2 | 0.0 | 0.0 | 25.3 | 38.7 |
| Sig. of - | Locations | ** | ** | ** | ** |
| | Trips | | | ** | ** |
| | Interaction | | | | ** |

** $P < 0.01$; * $P < 0.05$

### 3.5 Full categorical data

In addition to just the binary contrasts (of 'class zero' *vs.* 'the others', in Table 3.6), we also looked at the distributions of counts across all categories. However, these showed little of apparent interest, except that most categories were observed at most of the locations (including the reference sites). These overall counts are summarised in Table 3.7a to 3.7f.

**Table 3.7a.** Skin categories – counts by locations.

| | SK0 | SK1 | SK2 | SK3 | SK4 |
|---|---|---|---|---|---|
| Fitzroy R | 505 | 1 | 29 | 3 | 0 |
| Bundaberg | 326 | 0 | 8 | 2 | 0 |
| Hamilton Pt | 18 | 3 | 6 | 4 | 1 |
| Calliope R | 47 | 4 | 5 | 2 | 0 |
| Harbour | 392 | 1 | 0 | 0 | 0 |
| Spoil Grounds | 5 | 0 | 0 | 0 | 0 |
| Upper Boyne R | 36 | 0 | 0 | 6 | 0 |
| Lower Boyne R | 47 | 1 | 15 | 0 | 3 |
| Rodds Bay | 24 | 0 | 2 | 1 | 2 |
| Lake Awoonga | 5 | 0 | 0 | 0 | 0 |

**Table 3.7b.** Lesion categories – counts by locations.

| | L0 | L1 | L2 | L3 | L4 |
|---|---|---|---|---|---|
| Fitzroy R | 526 | 2 | 3 | 1 | 6 |
| Bundaberg | 334 | 1 | 1 | 0 | 0 |
| Hamilton Pt | 28 | 1 | 3 | 0 | 0 |
| Calliope R | 52 | 1 | 4 | 0 | 1 |
| Harbour | 393 | 0 | 0 | 0 | 0 |
| Spoil Grounds | 5 | 0 | 0 | 0 | 0 |
| Upper Boyne R | 28 | 3 | 9 | 0 | 2 |
| Lower Boyne R | 41 | 0 | 24 | 0 | 1 |
| Rodds Bay | 27 | 0 | 2 | 0 | 0 |
| Lake Awoonga | 5 | 0 | 0 | 0 | 0 |

**Table 3.7c.** Gonad categories – counts by locations.

| | F1 | F2 | M1 | M2 | Unclear |
|---|---|---|---|---|---|
| Fitzroy R | 13 | 3 | 5 | 2 | 36 |
| Bundaberg | 4 | 22 | 3 | 6 | 23 |
| Hamilton Pt | 4 | 13 | 0 | 1 | 3 |
| Calliope R | 5 | 23 | 0 | 1 | 11 |
| Harbour | 0 | 0 | 0 | 0 | 0 |
| Spoil Grounds | 1 | 1 | 0 | 1 | 2 |
| Upper Boyne R | 4 | 11 | 1 | 2 | 18 |
| Lower Boyne R | 2 | 14 | 0 | 1 | 24 |
| Rodds Bay | 8 | 4 | 3 | 0 | 14 |
| Lake Awoonga | 0 | 0 | 0 | 0 | 5 |

**Table 3.7d.** Spleen categories – counts by locations.

| | Black enlarged | Black granular | Black nodular | Other | Red granular | Red nodular |
|---|---|---|---|---|---|---|
| Fitzroy R | 0 | 40 | 0 | 7 | 11 | 1 |
| Bundaberg | 0 | 42 | 3 | 1 | 12 | 0 |
| Hamilton Pt | 0 | 20 | 0 | 1 | 0 | 0 |
| Calliope R | 0 | 27 | 0 | 3 | 9 | 1 |
| Harbour | 0 | 0 | 0 | 0 | 0 | 0 |
| Spoil Grounds | 0 | 5 | 0 | 0 | 0 | 0 |
| Upper Boyne R | 0 | 26 | 0 | 0 | 10 | 0 |
| Lower Boyne R | 1 | 25 | 0 | 0 | 14 | 1 |
| Rodds Bay | 0 | 8 | 1 | 0 | 20 | 0 |
| Lake Awoonga | 0 | 5 | 0 | 0 | 0 | 0 |

**Table 3.7e.** Mesentary fat categories – counts by locations.

| | None | <50% | 50% | >50% | Completely covered |
|---|---|---|---|---|---|
| Fitzroy R | 37 | 6 | 3 | 3 | 10 |
| Bundaberg | 24 | 20 | 9 | 0 | 5 |
| Hamilton Pt | 20 | 1 | 0 | 0 | 0 |
| Calliope R | 29 | 5 | 5 | 0 | 1 |
| Harbour | 0 | 0 | 0 | 0 | 0 |
| Spoil Grounds | 3 | 1 | 1 | 0 | 0 |
| Upper Boyne R | 22 | 1 | 2 | 0 | 11 |
| Lower Boyne R | 29 | 3 | 0 | 1 | 8 |
| Rodds Bay | 11 | 15 | 1 | 0 | 2 |
| Lake Awoonga | 0 | 0 | 0 | 2 | 3 |

**Table 3.7f.** Bile categories – counts by locations.

| | Dark green | Empty or not visible | Light green | Yellow |
|---|---|---|---|---|
| Fitzroy R | 12 | 3 | 26 | 18 |
| Bundaberg | 11 | 13 | 19 | 15 |
| Hamilton Pt | 1 | 0 | 7 | 13 |
| Calliope R | 1 | 4 | 20 | 15 |
| Harbour | 0 | 0 | 0 | 0 |
| Spoil Grounds | 4 | 0 | 1 | 0 |
| Upper Boyne R | 7 | 4 | 11 | 14 |
| Lower Boyne R | 4 | 1 | 13 | 23 |
| Rodds Bay | 0 | 0 | 3 | 26 |
| Lake Awoonga | 0 | 5 | 0 | 0 |

### 3.6 Overall (multivariate patterns)

Relationships amongst the continuous variables can be measured by the correlation coefficient, r. Table 3.8 lists these values for the size measures and the variables in Table 3.6. Correlations between the binary variables are indicative only, but do show the direction and relative strength of these relationships.

**Table 3.8.** Correlation matrix for the laboratory-measured variables. Correlations are significant ($P <$ 0.05) where the absolute value of r is greater than 0.13.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Length | 1.00 | | | | | | |
| Weight | 0.93 | 1.00 | | | | | |
| Liver wt | 0.43 | 0.50 | 1.00 | | | | |
| Hepat. indx | 0.00 | -0.01 | 0.71 | 1.00 | | | |
| Cond. fact. | -0.36 | -0.17 | 0.07 | 0.20 | 1.00 | | |
| HAI_score | 0.30 | 0.34 | 0.23 | 0.10 | 0.00 | 1.00 | |
| Fins % | 0.16 | 0.16 | 0.02 | -0.08 | -0.11 | 0.30 | 1.00 |
| Abdom. % | 0.17 | 0.08 | -0.05 | -0.15 | -0.38 | 0.21 | 0.02 |
| Gills % | 0.05 | 0.05 | -0.03 | -0.06 | -0.03 | 0.50 | 0.06 |
| Heart % | 0.13 | 0.17 | 0.06 | -0.02 | 0.01 | 0.18 | -0.05 |
| Kidney % | -0.11 | -0.12 | -0.06 | 0.02 | 0.05 | 0.19 | -0.06 |
| Hindgut % | 0.01 | 0.06 | 0.01 | -0.04 | 0.07 | 0.03 | -0.05 |
| Liver frm% | 0.23 | 0.26 | 0.23 | 0.15 | -0.02 | 0.54 | 0.15 |
| Liver col.% | 0.12 | 0.15 | 0.17 | 0.11 | 0.02 | 0.58 | 0.11 |
| Skin % | 0.45 | 0.45 | 0.34 | 0.16 | -0.01 | 0.51 | 0.24 |
| Lesions % | 0.29 | 0.33 | 0.12 | -0.07 | -0.03 | 0.39 | 0.14 |
| Parasites% | 0.20 | 0.15 | 0.03 | -0.01 | -0.24 | 0.15 | -0.06 |
| Mes. fat % | 0.17 | 0.17 | 0.04 | -0.10 | 0.14 | 0.19 | 0.05 |
| | Length | Weight | Liver wt | Hepat. | Cond. f. | HAI score | Fins % |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Abdom. % | 1.00 | | | | | | |
| Gills % | 0.04 | 1.00 | | | | | |
| Heart % | 0.12 | 0.08 | 1.00 | | | | |
| Kidney % | -0.03 | -0.01 | -0.02 | 1.00 | | | |
| Hindgut % | 0.01 | 0.02 | -0.02 | -0.02 | 1.00 | | |
| Liver frm% | 0.16 | 0.06 | 0.13 | 0.04 | -0.06 | 1.00 | |
| Liver col.% | 0.16 | 0.12 | 0.17 | -0.09 | -0.07 | 0.65 | 1.00 |
| Skin % | 0.10 | 0.08 | 0.10 | 0.01 | 0.02 | 0.10 | 0.09 |
| Lesions % | 0.08 | -0.04 | 0.09 | 0.00 | 0.03 | 0.20 | 0.11 |
| Parasites% | 0.27 | -0.03 | 0.02 | 0.00 | 0.03 | -0.09 | -0.04 |
| Mes. Fat% | -0.07 | -0.04 | 0.10 | -0.10 | -0.05 | 0.14 | 0.13 |
| | Abdm. % | Gills % | Heart % | Kidney% | Hindgut% | Liver f.% | Liv c.% |

| | | | | |
|---|---|---|---|---|
| Skin % | 1.00 | | | |
| Lesions % | 0.44 | 1.00 | | |
| Parasites% | 0.08 | -0.03 | 1.00 | |
| Mes. Fat% | 0.28 | 0.44 | -0.19 | 1.00 |
| | Skin % | Lesion% | Paras.% | Mes.fat% |

The principal components spatial representation for all of the variables (Table 3.6) is shown in Figure 3.5.

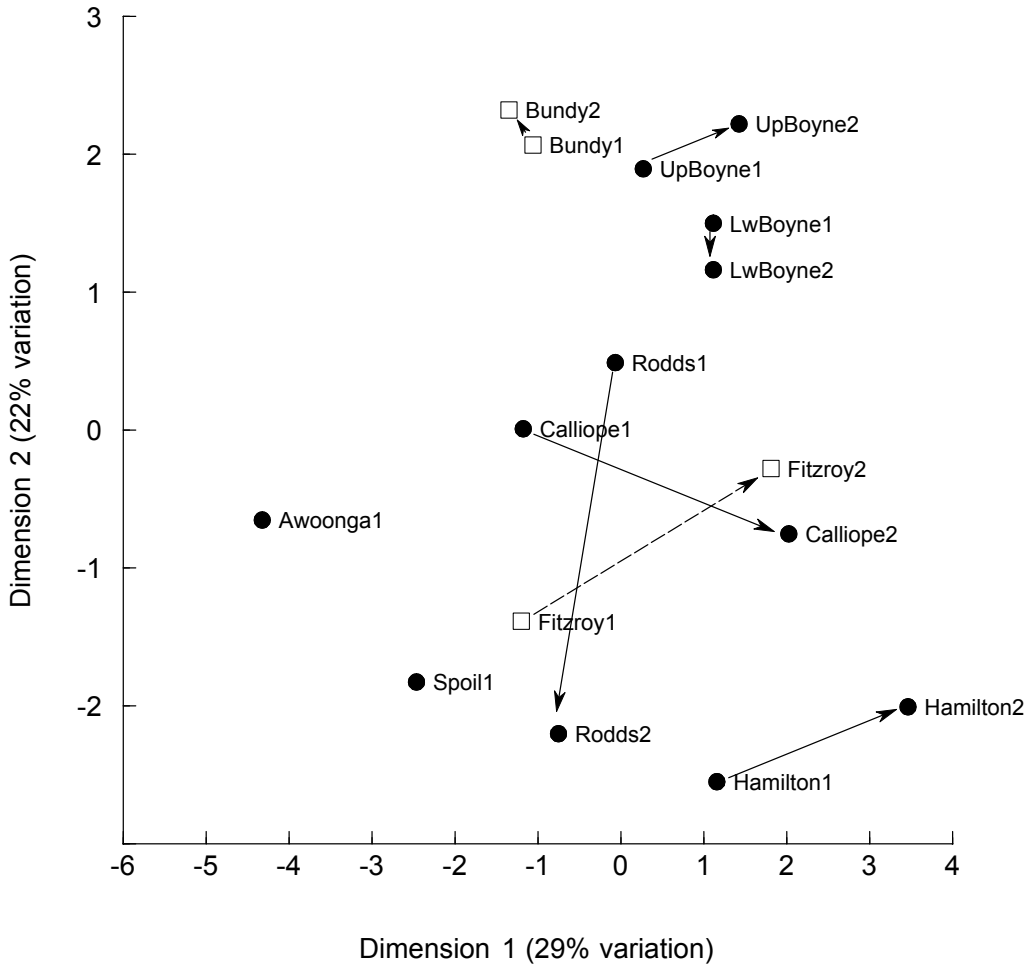


**Figure 3.5.** Movements of the overall location and trip principal component means in multidimensional space. The reference sites are indicated by the squares with the dotted lines.

Given that the four reference-site points represent 'normality', it is pleasing to see that these scatter around the mid-top area of this figure, and that most of the Gladstone sites are in this proximity. One corner-point here is the upper Boyne River at trip 2, which did have the highest HAI score, and the highest rates of abnormalities in the abdomen and liver-form.

Lake Awoonga does appear somewhat removed, and this is due to the 'very good' condition of the barramundi (the only species sampled there) – these were all 'undiseased' and had low HAI scores. Similarly, the Spoil Grounds had generally healthy fish. Hamilton Point is also somewhat remote, but recall that this sample consisted of 31 barramundi and one bull shark (hence see the separate 'barramundi' analyses and discussion for this site).

The 'Harbour' location is not plotted on this graph, as no samples were submitted to the laboratory from this site. Hence its status can only be judged by considering the percentages of diseased fish, and skin and lesion condition greater than zero. On the basis of these three variables, on trip 1 the Harbour samples were very similar to those from Rodds Bay. For trip 2, the Harbour samples were very similar to the samples from trip 1 at Bundaberg. Neither result appears a reason for concern.

### 3.7 Summarising by regions

The means for the individual locations can be combined into regional groupings, and also into the overall Gladstone area (comprising Hamilton Point, the Calliope and Boyne Rivers, and Rodds Bay), as listed in Tables 3.9a to 3.9c.

**Table 3.9a.** Regional group means and significance-difference testing.

| Trip 1 – | Hepat. index | Condition factor | HAI score | % diseased | % skin cond. > 0 |
|---|---|---|---|---|---|
| Fitzroy R / Bundaberg | 1.28[b] | 1.40[a] | 22.3[ab] | 7.7[b] | 7.2[a] |
| Hamilton Pt / Calliope R | 1.58[a] | 1.29[b] | 14.0[b] | 5.5[b] | 4.2[ab] |
| Upper & Lower Boyne R | 1.29[b] | 1.30[b] | 31.8[a] | 11.8[a] | 3.4[b] |
| Rodds Bay | 1.66[a] | 1.36[ab] | 21.6[ab] | 5.6[b] | 4.9[ab] |
| Gladstone area (overall) [#] | 1.48 | **1.31** | 22.7 | 8.0 | **4.1** |
| Trip 2 – | | | | | |
| Fitzroy R / Bundaberg | 1.53[b] | 1.33 | 22.2 [b] | 6.7[b] | 6.3[b] |
| Hamilton Pt / Calliope R | 1.64[ab] | 1.31 | 21.1[b] | 12.9[a] | 12.6[a] |
| Upper & Lower Boyne R | 1.94[a] | 1.30 | 33.5[a] | 9.2[ab] | 7.7[b] |
| Rodds Bay | 1.57[ab] | 1.35 | 14.9[b] | 5.7[b] | 4.4[b] |
| Gladstone area (overall) [#] | 1.75 | 1.32 | 24.8 | 10.0 | 9.0 |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).

[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

**Table 3.9b.** Regional group means and significance-difference testing.

| Trip 1 – | Lesions %[#] > 0 | Fins % abnormal | Gills % abnormal | Abdomen % tucked |
|---|---|---|---|---|
| Fitzroy R / Bundaberg | 1.3[b] | 9.2[b] | 12.9 | 4.9[b] |
| Hamilton Pt / Calliope R | 1.8[b] | 38.2[a] | 14.6 | 22.7[a] |
| Upper & Lower Boyne R | 11.6[a] | 14.2[ab] | 16.7 | 22.3[a] |
| Rodds Bay | 0.0[c] | 17.7[ab] | 11.6 | 22.1[ab] |
| Gladstone area (overall) | **5.3** | **24.5** | 14.8 | **22.4** |
| Trip 2 – | | | | |
| Fitzroy R / Bundaberg | 3.0[b] | 1.0[b] | 21.5[a] | 23.1[a] |
| Hamilton Pt / Calliope R | 4.3[ab] | 9.9[b] | 12.2[a] | 25.1[a] |
| Upper & Lower Boyne R | 8.0[a] | 12.0[b] | 8.5[ab] | 26.4[a] |
| Rodds Bay | 14.1[a] | 61.9[a] | 0.0[b] | 0.0[b] |
| Gladstone area (overall) | **7.8** | **21.1** | **8.3** | 20.6 |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).

[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

**Table 3.9c.** Regional group means and significance-difference testing.

| Trip 1 – | Liver form % abnormal | Liver % discoloured | Parasites % > zero | Mesentary fat (%) |
|---|---|---|---|---|
| Fitzroy R / Bundaberg | 10.0[b] | 26.2[a] | 7.9 | 42.5[a] |
| Hamilton Pt / Calliope R | 7.2[b] | 5.4[b] | 11.8 | 10.2[c] |
| Upper & Lower Boyne R | 29.4[a] | 24.2[a] | 3.7 | 28.7[b] |
| Rodds Bay | 11.4[ab] | 37.1[a] | 20.0 | 14.1[bc] |
| Gladstone area (overall) | 16.9 | 19.3 | 10.2 | **18.4** |
| Trip 2 – | | | | |
| Fitzroy R / Bundaberg | 20.2[b] | 26.4[a] | 11.1[b] | 18.0[b] |
| Hamilton Pt / Calliope R | 0.0[c] | 5.0[bc] | 39.5[a] | 0.8[c] |
| Upper & Lower Boyne R | 43.6[a] | 21.2[ab] | 18.1[b] | 19.8[b] |
| Rodds Bay | 0.0[c] | 0.0[c] | 25.3[ab] | 38.7[a] |
| Gladstone area (overall) | 17.4 | **10.5** | **28.1** | 16.0 |

[a,b,c] Within columns and trips, means for the individual regions that do not have a common superscript letter are significantly different ($P < 0.05$).

[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

## 4. SEPARATE ANALYSES FOR BARRAMUNDI DATA

### 4.1 Variables not analysed

For barramundi, there were too few 'non-normal' ratings for the analyses of eye condition, general condition, heart condition, hindgut rating, kidney condition and spleen rating.

### 4.2 Overview of analyses

Of the thirteen continuous variables that had sufficient data for analyses, fish length was significant ($P < 0.05$) for nine, so this was retained in all models. Time since death was only included for the analyses of HAI index, and the rated condition for skin, fins and gills. 'Disease status' (as assessed in the field) was only significant for four of the variables. Table 4.1 lists these adjusted means, tests of significance and average standard errors.

**Table 4.1.** Effects of disease status (field-rated) on the response variables.

| | Sig. level | Not diseased[#] | Diseased[#] | avg. s.e. |
|---|---|---|---|---|
| Hepatosomatic index | | 1.07 | 1.16 | 0.04 |
| Condition score | | 1.24 | 1.19 | 0.03 |
| HAI score | ** | 16.6 | 32.6 | 2.8 |
| Fins % abnormal | | 10.4 | 18.9 | 3.7 |
| Gills % abnormal | | 15.1 | 9.3 | 3.2 |
| Abdomen % tucked up | | 25.7 | 35.0 | 5.0 |
| Liver form % abnormal | * | 3.7 | 13.8 | 2.7 |
| Liver colour % discoloured | * | 6.7 | 16.7 | 3.1 |
| Parasites % > zero | | 20.0 | 14.9 | 3.7 |
| Mesentary fat (%) | * | 33.1 | 50.2 | 5.1 |

[#]as assessed in the field; ** $P < 0.01$; * $P < 0.05$

### 4.3 Location by trip means

The categorical measures have again been analysed and listed here as the binary contrast (per cent not-normal). Significant differences ($P < 0.05$) were found between locations in ten analyses, between trips in five, with a significant location by trip interaction in seven (54%). Hence, for most variables there are proven changing patterns over space and time, so again the data are reported on this two-way basis, in Tables 4.2a to 4.2c.

**Table 4.2a.** Effects of locations and trips on the response variables.

| Location | Trip | Hepat. index | Condition factor | HAI score | %[#] diseased | %[#] skin cond. > 0 |
|---|---|---|---|---|---|---|
| Fitzroy | 1 | 1.05 | 1.36 | 16.5 | 16.6 | 14.4 |
| Bundy | 1 | 1.24 | 1.36 | 22.1 | 21.5 | 22.8 |
| Hamilton | 1 | 0.84 | 1.11 | 10.6 | 21.7 | 24.2 |
| Calliope | 1 | 1.08 | 1.15 | 15.0 | 6.6 | 0.0 |
| Harbour | 1 | | | | 12.6 | 13.4 |
| UpBoyne | 1 | 1.17 | 1.27 | 47.7 | 56.0 | 18.1 |
| LwBoyne | 1 | 1.12 | 1.18 | 41.8 | 63.3 | 3.6 |
| Rodds | 1 | 1.25 | 1.16 | 12.8 | 42.2 | 42.6 |
| Lake Awoonga | 1 | 1.25 | 1.42 | 29.1 | 0.1 | 0.0 |
| Fitzroy | 2 | 1.19 | 1.32 | 27.2 | 42.4 | 29.4 |
| Bundy | 2 | 1.37 | 1.45 | 21.4 | 0.0 | 0.0 |
| Hamilton | 2 | 1.08 | 1.12 | 20.2 | 44.8 | 47.2 |
| Calliope | 2 | 0.98 | 1.30 | 13.1 | 32.9 | 38.8 |
| UpBoyne | 2 | 1.43 | 1.32 | 48.5 | 63.0 | 39.4 |
| LwBoyne | 2 | 1.18 | 1.17 | 30.0 | 42.1 | 41.6 |
| Rodds [@] | 2 | 1.06 | 0.93 | 19.5 | 99.9 | 100.0 |
| Sig. of - | Locations | * | ** | ** | ** | |
| | Trips | | | | * | ** |
| | Interaction | | | | * | * |

[#]as assessed in the field; [@] based on two (diseased) fish; ** $P < 0.01$; * $P < 0.05$

**Table 4.2b.** Effects of locations and trips on the response variables.

| Location | Trip | Lesions %[#] > 0 | Gills % abnormal | Fins % abnormal | Abdomen % tucked |
|---|---|---|---|---|---|
| Fitzroy | 1 | 0.0 | 0.0 | 19.0 | 0.0 |
| Bundy | 1 | 0.0 | 30.0 | 0.0 | 8.9 |
| Hamilton | 1 | 0.0 | 10.0 | 31.1 | 53.5 |
| Calliope | 1 | 0.0 | 20.0 | 12.1 | 11.6 |
| Harbour | 1 | 0.0 | | | |
| UpBoyne | 1 | 56.5 | 50.0 | 12.7 | 17.6 |
| LwBoyne | 1 | 59.1 | 0.0 | 11.5 | 47.4 |
| Rodds | 1 | 0.0 | 0.0 | 13.1 | 27.9 |
| Lake Awoonga | 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Fitzroy | 2 | 35.1 | 20.0 | 0.0 | 40.4 |
| Bundy | 2 | 0.0 | 14.3 | 0.0 | 0.0 |
| Hamilton | 2 | 12.6 | 0.0 | 0.0 | 51.4 |
| Calliope | 2 | 9.8 | 0.0 | 0.0 | 23.8 |
| UpBoyne | 2 | 61.6 | 16.7 | 28.3 | 41.6 |
| LwBoyne | 2 | 19.9 | 0.0 | 9.0 | 17.2 |
| Rodds [@] | 2 | 100.0 | 0.0 | 100.0 | 0.0 |
| Sig. of - | Locations | ** | | | * |
| | Trips | ** | | | |
| | Interaction | ** | ** | * | * |

[#]as assessed in the field; [@] based on two (diseased) fish; ** $P < 0.01$; * $P < 0.05$

**Table 4.2c.** Effects of locations and trips on the response variables.

| Location | Trip | Liver form % abnormal | Liver % discoloured | Parasites % > zero | Mesentary fat (%) |
|---|---|---|---|---|---|
| Fitzroy | 1 | 0.0 | 16.8 | 0.0 | 94.9 |
| Bundy | 1 | 1.9 | 19.5 | 11.1 | 56.4 |
| Hamilton | 1 | 0.0 | 0.0 | 10.3 | 5.4 |
| Calliope | 1 | 3.7 | 5.9 | 29.6 | 21.1 |
| Harbour | 1 | | | | |
| UpBoyne | 1 | 10.7 | 27.5 | 0.0 | 61.5 |
| LwBoyne | 1 | 17.1 | 24.8 | 8.9 | 68.1 |
| Rodds | 1 | 0.0 | 0.0 | 28.6 | 3.4 |
| Lake | | | | | |
| Awoonga | 1 | 88.3 | 0.0 | 0.0 | 80.4 |
| Fitzroy | 2 | 0.0 | 0.0 | 0.0 | 43.5 |
| Bundy | 2 | 5.7 | 48.8 | 14.1 | 35.0 |
| Hamilton | 2 | 0.0 | 5.1 | 71.2 | 2.5 |
| Calliope | 2 | 0.0 | 0.0 | 39.3 | 7.9 |
| UpBoyne | 2 | 93.0 | 48.0 | 0.0 | 73.5 |
| LwBoyne | 2 | 8.0 | 8.9 | 40.2 | 20.6 |
| Rodds @ | 2 | 0.0 | 0.0 | 49.6 | 3.4 |
| Sig. of - | Locations | ** | ** | ** | ** |
| | Trips | | | ** | ** |
| | Interaction | | | | * |

@ based on two (diseased) fish; ** $P < 0.01$; * $P < 0.05$

### 4.4 Overall (multivariate patterns)

The principal components spatial representation of the combined data is shown in Figure 4.1.

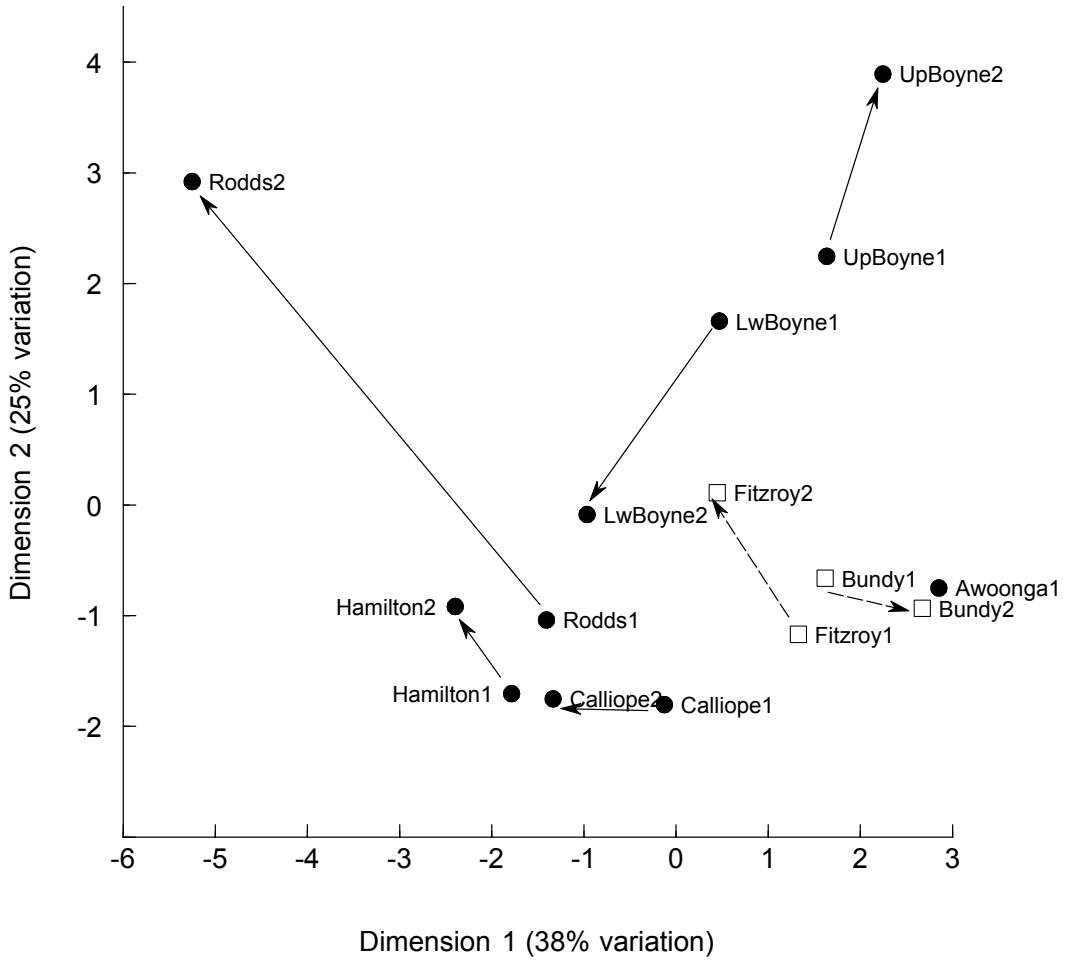


**Figure 4.1.** Movements of the overall location and trip principal component means for barramundi in multidimensional space. The reference sites are indicated by the squares with the dotted lines.

Most points are in the lower-right region, reasonably close to the reference sites. The 'slightly-removed' group at the bottom (Rodds Bay Trip 1, and Calliope and Hamilton Trip 2) generally represent 'good-quality' fish, with low scores for HAI and most abnormality rates.

The most notable outlying points are –

Rodd's Bay (Trip 2) – this total sample consisted of only two fish, both of which were diseased, so these gave 'poor quality' values across most of the variables.

Upper Boyne (both trips) – generally had higher values for most variables, except this site did have zero parasites.

Lower Boyne, Trip 1 – had high HAI and lesions; however by trip 2 this site had 'reverted' back into the general pack.

Samples from the Harbour (Trip 1 only) were not submitted to the laboratory, hence for this location there are only values for field-assessed disease, skin condition and lesion ratings. Based on these three variables, the barramundi from the Harbour were very similar to those sampled from the Fitzroy River on Trip 1.

### 4.5 Summarising by regions

The means for the individual locations have again been combined into regional groupings, and also into the overall Gladstone area. These are listed in Tables 4.3a to 4.3c.

**Table 4.3a.** Regional group means and significance-difference testing.

| Trip 1 – | Hepat. index | Condition factor | HAI score | % diseased | % skin cond. > 0 |
|---|---|---|---|---|---|
| Fitzroy River / Bundaberg | 1.15[a] | 1.36[a] | 19.3[b] | 19.1[b] | 18.6 |
| Hamilton Pt / Calliope River | 0.96[b] | 1.13[b] | 12.8[b] | 14.1[b] | 12.1 |
| Upper and Lower Boyne R | 1.14[a] | 1.22[b] | 44.7[a] | 59.6[a] | 10.8 |
| Rodds Bay | 1.25[a] | 1.16[b] | 12.7[b] | 42.2[a,b] | 42.6 |
| Gladstone area (overall) [#] | 1.09 | **1.17** | 25.6 | **37.9** | 17.7 |
| Trip 2 – | | | | | |
| Fitzroy River / Bundaberg | 1.28[a] | 1.38[a] | 24.3[b] | 21.2[c] | 14.7[c] |
| Hamilton Pt / Calliope River | 1.03[b] | 1.21[b] | 16.6[b] | 38.9[b,c] | 43.0[b] |
| Upper and Lower Boyne River | 1.31[a] | 1.24[b] | 39.3[a] | 52.5[b] | 40.5[b,c] |
| Rodds Bay | 1.06[a,b] | 0.93[c] | 19.5[a,b] | 99.9[a] | 99.9[a] |
| Gladstone area (overall) [#] | 1.15 | **1.17** | 26.3 | **56.5** | **53.4** |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).
[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy River / Bundaberg).

**Table 4.3b.** Regional group means and significance-difference testing.

| Trip 1 – | Lesions %[#] > 0 | Gills % abnormal | Fins % abnormal | Abdomen % tucked |
|---|---|---|---|---|
| Fitzroy River / Bundaberg | 0.0[b] | 15.0[a,b] | 9.5 | 4.5[b] |
| Hamilton Pt / Calliope River | 0.0[b] | 15.0[a,b] | 21.6 | 32.5[a] |
| Upper and Lower Boyne River | 57.8[a] | 25.0[a] | 12.1 | 32.5[a] |
| Rodds Bay | 0.0[b] | 0.0[b] | 13.1 | 27.9[a,b] |
| Gladstone area (overall) | **23.1** | 16.0 | 16.1 | **31.6** |
| Trip 2 – | | | | |
| Fitzroy River / Bundaberg | 17.6[b,c] | 17.1 | 0.0[b] | 20.2[a] |
| Hamilton Pt / Calliope River | 11.2[c] | 0.0 | 0.0[b] | 37.6[a] |
| Upper and Lower Boyne River | 40.7[b] | 8.3 | 18.6[b] | 29.4[a] |
| Rodds Bay | 99.9[a] | 0.0 | 99.9[a] | 0.0[b] |
| Gladstone area (overall) | **40.77** | 3.3 | **27.4** | 26.8 |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).
[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

**Table 4.3c.** Regional group means and significance-difference testing.

| Trip 1 – | Liver form % abnormal | Liver % discoloured | Parasites % > zero | Mesentary fat (%) |
|---|---|---|---|---|
| Fitzroy River / Bundaberg | 0.9 | 18.2[a,b] | 5.6 | 75.6[a] |
| Hamilton Pt / Calliope River | 1.9 | 3.0[b] | 19.9 | 13.2[b] |
| Upper and Lower Boyne River | 13.9 | 26.1[a] | 4.5 | 64.8[a] |
| Rodds Bay | 0.0 | 0.0[b] | 28.6 | 3.4[b] |
| Gladstone area (overall) | 6.3 | 11.6 | 15.5 | **31.9** |
| Trip 2 – | | | | |
| Fitzroy River / Bundaberg | 2.8[b] | 24.4[a,b] | 7.1[b] | 39.3[a] |
| Hamilton Pt / Calliope River | 0.0[b] | 2.5[a,b] | 55.2[a] | 5.2[b] |
| Upper & Lower Boyne River | 50.5[a] | 28.4[a] | 20.1[b] | 47.1[a] |
| Rodds Bay | 0.0[b] | 0.0[b] | 49.6[a,b] | 3.4[b] |
| Gladstone area (overall) | **20.2** | 12.4 | **40.1** | 21.6 |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).
[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy River / Bundaberg).

## 5. SEPARATE ANALYSES FOR MULLET DATA

### 5.1 Variables not analysed

For mullet, there were too few 'non-normal' ratings to allow analyses of eye condition, general condition, heart condition, hindgut rating, abdomen condition and spleen rating.

### 5.2 Overview of analyses

Fish length was confounded with species of mullet, so the analyses were not adjusted for this variable. Time since death was only included for the analyses of HAI index, and the laboratory-rated condition for skin. 'Disease status', as assessed in the field, showed no significant effects (Table 5.1).

**Table 5.1.** Effects of disease status (field-rated) on the response variables.

| | Sig. level | Not diseased[#] | Diseased[#] | avg. s.e. |
|---|---|---|---|---|
| Hepatosomatic index | 0.84 | 1.53 | 1.38 | 0.07 |
| Condition score | 0.62 | 1.52 | 1.45 | 0.04 |
| HAI score | 0.89 | 19.6 | 21.8 | 3.8 |
| Fins % abnormal | 0.76 | 6.1 | 5.4 | 2.7 |
| Gills % abnormal | 0.88 | 15.9 | 14.1 | 4.2 |
| Kidney % abnormal | 0.60 | 9.5 | 14.7 | 2.6 |
| Liver form % abnormal | 0.66 | 12.0 | 9.9 | 3.8 |
| Liver colour % discoloured | 0.44 | 15.8 | 11.1 | 4.4 |
| Parasites % > zero | 0.68 | 1.1 | 1.4 | 1.2 |
| Mesentary fat (%) | 0.15 | 13.1 | 7.0 | 2.9 |

[#]as assessed in the field

### 5.3 Location by trip means

The categorical measures have again been analysed and listed here as the binary contrasts (per cent not-normal). Significant differences were found between locations in seven of the 13 analyses, trips in five, with a significant ($P < 0.05$) location by trip interaction in seven (54%). Again, Tables 5.2a to 5.2c list these means on the 'location by trip' basis.

**Table 5.2a.** Effects of locations and trips on the response variables.

| Location | Trip | | Hepat. index | Condition factor | HAI score | %[#] diseased | %[#] skin cond. > 0 |
|---|---|---|---|---|---|---|---|
| Fitzroy | 1 | | 0.89 | 1.46 | 5.7 | 100.0 | 100.0 |
| Bundy | 1 | | 1.22 | 1.62 | 15.7 | 25.0 | 25.0 |
| Calliope | 1 | | 1.75 | 1.66 | 24.0 | 0.0 | 0.0 |
| UpBoyne | 1 | | 0.81 | 1.42 | 0.0 | 0.0 | 0.0 |
| LwBoyne | 1 | | 0.96 | 1.43 | 13.7 | 0.0 | 0.0 |
| Rodds | 1 | | 1.52 | 1.61 | 2.9 | 0.0 | 0.0 |
| Fitzroy | 2 | | 1.57 | 1.31 | 19.9 | 54.6 | 54.6 |
| Bundy | 2 | | 1.07 | 1.54 | 3.0 | 20.0 | 20.0 |
| Calliope | 2 | | 2.06 | 1.51 | 24.0 | 45.5 | 45.5 |
| UpBoyne | 2 | | 1.64 | 1.35 | 35.5 | 10.0 | 10.0 |
| LwBoyne | 2 | | 2.54 | 1.65 | 44.8 | 40.0 | 40.0 |
| Rodds | 2 | | 1.46 | 1.57 | 17.1 | 0.0 | 0.0 |
| Sig. of - | | Locations | ** | ** | * | ** | * |
| | | Trips | ** | | ** | | |
| | | Interaction | ** | | ** | ** | ** |

[#]as assessed in the field; ** $P < 0.01$; * $P < 0.05$

**Table 5.2b.** Effects of locations and trips on the response variables.

| Location | Trip | | Lesions %[#] > 0 | Gills % abnormal | Fins % abnormal | Kidney % abnormal |
|---|---|---|---|---|---|---|
| Fitzroy | 1 | | 0.0 | 10.0 | 20.0 | 0.0 |
| Bundy | 1 | | 0.0 | 25.0 | 12.5 | 0.0 |
| Calliope | 1 | | 0.0 | 14.3 | 14.3 | 0.0 |
| UpBoyne | 1 | | 0.0 | 0.0 | 0.0 | 0.0 |
| LwBoyne | 1 | | 0.0 | 10.0 | 0.0 | 0.0 |
| Rodds | 1 | | 0.0 | 20.0 | 0.0 | 0.0 |
| Fitzroy | 2 | | 9.1 | 27.3 | 0.0 | 0.0 |
| Bundy | 2 | | 0.0 | 10.0 | 0.0 | 0.0 |
| Calliope | 2 | | 0.0 | 49.1 | 20.0 | 0.0 |
| UpBoyne | 2 | | 10.0 | 10.0 | 0.0 | 60.0 |
| LwBoyne | 2 | | 0.0 | 10.0 | 0.0 | 20.0 |
| Rodds | 2 | | 0.0 | 0.0 | 20.0 | 10.0 |
| Sig. of - | | Locations | | | | ** |
| | | Trips | | | | ** |
| | | Interaction | | | | |

[#]as assessed in the field; ** $P < 0.01$; * $P < 0.05$

**Table 5.2c.** Effects of locations and trips on the response variables.

| Location | Trip | | Liver form % abnormal | Liver % discoloured | Parasites % > zero | Mesentary fat (%) |
|---|---|---|---|---|---|---|
| Fitzroy | 1 | | 0.0 | 10.0 | 10.0 | 0.0 |
| Bundy | 1 | | 0.0 | 31.3 | 0.0 | 39.1 |
| Calliope | 1 | | 14.3 | 14.3 | 0.0 | 28.6 |
| UpBoyne | 1 | | 0.0 | 0.0 | 0.0 | 5.0 |
| LwBoyne | 1 | | 0.0 | 0.0 | 0.0 | 0.0 |
| Rodds | 1 | | 10.0 | 50.0 | 0.0 | 22.5 |
| Fitzroy | 2 | | 18.2 | 27.3 | 0.0 | 2.3 |
| Bundy | 2 | | 0.0 | 0.0 | 0.0 | 0.0 |
| Calliope | 2 | | 0.0 | 0.0 | 0.0 | 7.7 |
| UpBoyne | 2 | | 30.0 | 0.0 | 10.0 | 0.0 |
| LwBoyne | 2 | | 50.0 | 30.0 | 0.0 | 0.0 |
| Rodds | 2 | | 0.0 | 0.0 | 0.0 | 37.5 |
| Sig. of - | | Locations | | | | ** |
| | | Trips | * | | | ** |
| | | Interaction | * | ** | | ** |

** $P < 0.01$; * $P < 0.05$

### 5.4 Overall (multivariate patterns)

The principal components spatial representation is shown in Figure 5.1.

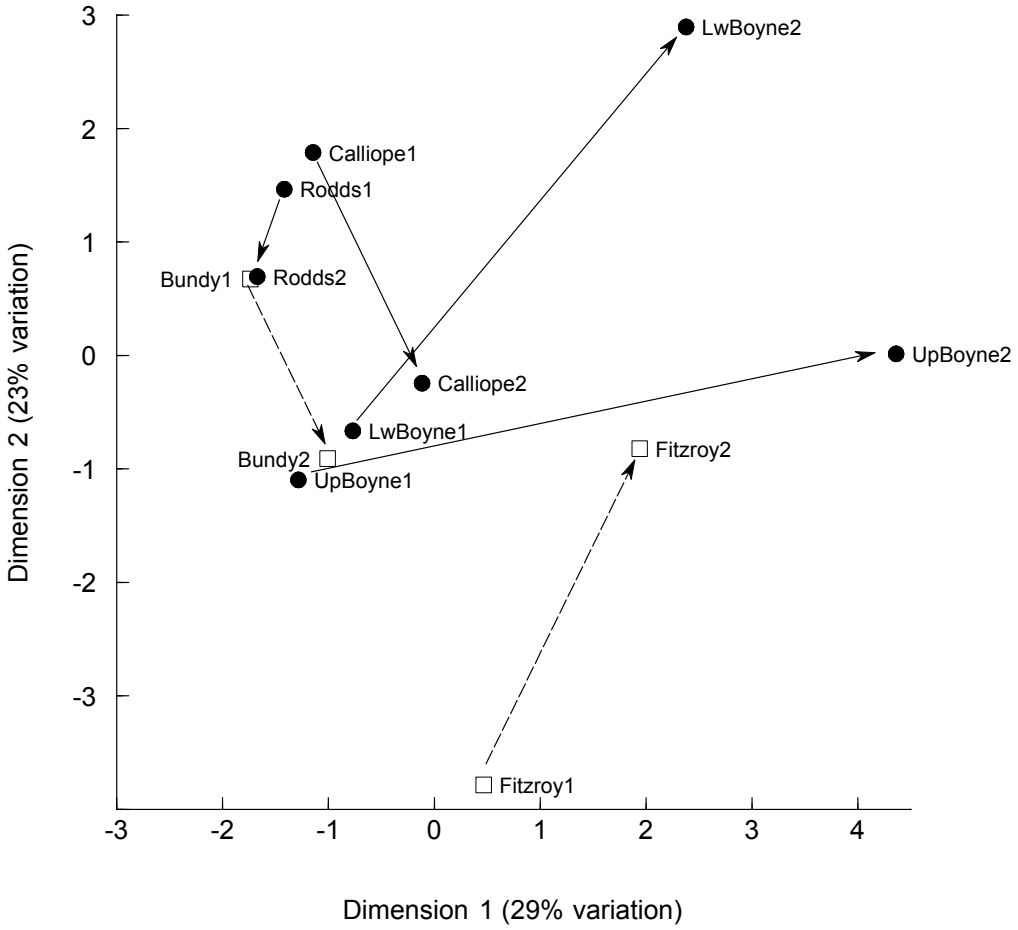


**Figure 5.1.** Movements of the overall location and trip principal component means for mullet in multidimensional space. The reference sites are indicated by the squares with the dotted lines.

Most points appear in the top-left cluster, which contains both times for the Bundaberg reference site. The Fitzroy River, particularly at trip 1, is somewhat removed from the other locations. This was primarily due to 100% of the sample being 'diseased' – all 10 mullet from trip 1 were rated as category 2 for skin condition. Notably, this was a reference site.

The main other outlying points are the upper Boyne River at trip 2 (mainly due to the high value for kidney abnormalities – 6 out of the 10 mullet were rated as 'other'), and the lower Boyne River also at trip 2 (which had high values for the hepatosomatic index, HAI score and liver form abnormalities).

## 5.5 Summarising by regions

The means for the individual locations have been combined into regional groupings, as listed in Tables 5.3a to 5.3c.

**Table 5.3a.** Regional group means and significance-difference testing.

| Trip 1 – | Hepat. index | Condition factor | HAI score | % diseased | % skin cond. > 0 |
|---|---|---|---|---|---|
| Fitzroy R / Bundaberg | 1.05[b] | 1.54[a,b] | 10.7 | 62.5[a] | 62.5[a] |
| Calliope R | 1.75[a] | 1.66[a] | 24.0 | 0.0[b] | 0.0[b] |
| Upper & Lower Boyne R | 0.89[b] | 1.43[b] | 6.9 | 0.0[b] | 0.0[b] |
| Rodds Bay | 1.52[a] | 1.61[a] | 2.9 | 0.0[b] | 0.0[b] |
| Gladstone area (overall) [#] | 1.26 | 1.53 | 10.1 | **0.0** | **0.0** |
| Trip 2 – | | | | | |
| Fitzroy R / Bundaberg | 1.32[b] | 1.42 | 11.5[b] | 37.3[a] | 37.3[a] |
| Calliope R | 2.06[a] | 1.51 | 24.0[b] | 45.5[a] | 45.5[a] |
| Upper & Lower Boyne R | 2.09[a] | 1.50 | 40.1[a] | 25.0[a] | 25.0[a] |
| Rodds Bay | 1.46[b] | 1.57 | 17.1[b] | 0.0[b] | 0.0[b] |
| Gladstone area (overall) [#] | **1.93** | 1.52 | **30.3** | 23.9 | 23.9 |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).
[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

**Table 5.3b.** Regional group means and significance-difference testing.

| Trip 1 – | Lesions %[#] > 0 | Gills % abnormal | Fins % abnormal | Kidney % abnormal |
|---|---|---|---|---|
| Fitzroy R / Bundaberg | 0.0 | 17.5 | 16.3[a] | 0.0 |
| Calliope R | 0.0 | 14.3 | 14.3[a,b] | 0.0 |
| Upper & Lower Boyne R | 0.0 | 5.0 | 0.0[b] | 0.0 |
| Rodds Bay | 0.0 | 20.0 | 0.0[b] | 0.0 |
| Gladstone area (overall) | 0.0 | 11.1 | 3.6 | 0.0 |
| Trip 2 – | | | | |
| Fitzroy R / Bundaberg | 4.5 | 18.6[a,b] | 0.0 | 0.0[b] |
| Calliope R | 0.0 | 49.1[a] | 20.0 | 0.0[b] |
| Upper & Lower Boyne R | 5.0 | 10.0[b,c] | 0.0 | 40.0[a] |
| Rodds Bay | 0.0 | 0.0[c] | 20.0 | 10.0[b] |
| Gladstone area (overall) | 2.5 | 17.3 | **10.0** | **22.5** |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).
[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

**Table 5.3c.** Regional group means and significance-difference testing.

| Trip 1 – | Liver form % abnormal | Liver % discoloured | Parasites % > zero | Mesentary fat (%) |
|---|---|---|---|---|
| Fitzroy R / Bundaberg | 0.0 | 20.6[a] | 5.0 | 19.5[a] |
| Calliope R | 14.3 | 14.3[a,b] | 0.0 | 28.6[a] |
| Upper & Lower Boyne R | 0.0 | 0.0[b] | 0.0 | 2.5[b] |
| Rodds Bay | 10.0 | 50.0[a] | 0.0 | 22.5[a] |
| Gladstone area (overall) | 6.1 | 16.1 | 0.0 | 14.0 |
| Trip 2 – | | | | |
| Fitzroy R / Bundaberg | 9.1[b] | 13.6[a] | 0.0 | 1.1[b] |
| Calliope R | 0.0[b] | 0.0[b] | 0.0 | 7.7[b] |
| Upper & Lower Boyne R | 40.0[a] | 15.0[a] | 5.0 | 0.0[b] |
| Rodds Bay | 0.0[b] | 0.0[b] | 0.0 | 37.5[a] |
| Gladstone area (overall) | 20.0 | 7.50 | 2.50 | **11.3** |

[a,b,c] Within columns and trips, means for the individual regions which do not have a common superscript letter are significantly different ($P < 0.05$).
[#] For the overall 'Gladstone area' means, those which are bolded are significantly different from the mean for the pooled reference sites (Fitzroy R / Bundaberg).

## 6. SEPARATE ANALYSES FOR MUDCRAB DATA

### 6.1 Overview

Shell condition (as measured at capture) had 1599 observations of which only 57 (3.6%) were classified as abnormal. These were then further classified according to their lesion grades. The sub-samples (140 individual crabs) were submitted to laboratory study for re-checking the lesion gradings as well as measuring overall size (carapace width and weight) and hepatopancreas weight. Again, analyses of the laboratory sample data were appropriately weighted to accurately reflect the observed damaged proportions in the overall sample.

Crab class (defined as small females, large females, undersized males, and retained males) was included in all analyses, as this effect was always significant ($P < 0.05$). Carapace width and total weight were trialled separately as a covariate for percentage damage, under the hypothesis of a positive relationship (increasing amounts of damage with increasing size). However, these relationship were nowhere near significant ($P > 0.20$), and actually fitted as slightly negative, possibly due to 'crab class' capturing the majority of any effect here. Hence 'crab class' was retained in the final model but size was omitted.

### 6.2 Comparing the laboratory and field assessments

The comparison between the lesion grade ratings showed a significant ($P < 0.01$) degree of association, with the counts listed in Table 6.1. There was agreement in 72% of these cases. Interestingly, the field assessment was higher than the laboratory assessment in 23% of these cases, whereas the reverse only occurred in 5% of the crabs.

**Table 6.1.** Highest lesion grade for each crab (Field = field assessment; Lab. = laboratory assessment).

| | Field - 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Lab. - 0 | 88 | 2 | 7 | 6 | 7 | 0 |
| 1 | 3 | 1 | 2 | 0 | 2 | 1 |
| 2 | 1 | 1 | 2 | 0 | 1 | 1 |
| 3 | 0 | 0 | 0 | 1 | 0 | 0 |
| 4 | 0 | 0 | 1 | 0 | 1 | 2 |
| 5 | 0 | 0 | 0 | 0 | 1 | 5 |

### 6.3 Results

There was no significant difference ($P$ = 0.68) between the hepatosomatic index of normal and abnormal crabs (0.057 ± 0.002 vs. 0.054 ± 0.002). Missing appendages (any *vs*. none) appeared to be random occurrences, with an overall mean rate of 16.9%, and not related to any of the design factors.

The key variables analysed were the percentage abnormal (field-assessed using all 1599 observations; a Binomial proportions model), the distribution of counts according to highest lesion class (a Poisson linear model), and hepatosomatic index (Normal model). In these analyses 'crab category' was always significant ($P$ < 0.05), justifying its inclusion as a stratifying term, with the means shown in Table 6.2.

**Table 6.2.** Mean values and standard errors (s.e.) for the crab categories.

| | Hepatosomatic index | s.e. | Percent abnormal | s.e. |
|---|---|---|---|---|
| Female large | 0.059 | 0.002 | 3.8 | 0.8 |
| Female small | 0.064 | 0.004 | 1.8 | 0.7 |
| Male retained | 0.043 | 0.003 | 7.1 | 1.7 |
| Male undersize | 0.056 | 0.002 | 2.5 | 0.7 |

Regarding locations and trips, the only significant ($P$ < 0.01) effect was for the hepatosomatic index, where trip 2 (overall, 0.061 ± 0.002) was higher than trip 1 (0.050 ± 0.002). Despite the interaction between location and trip being non-significant ($P$ > 0.66) for all variables, it is the relationships amongst these values that are of prime interest, and the two-way means are presented here for consistency with the results for the fish species. Table 6.3 lists these means, and the two-dimensional spatial representation is shown in Figure 6.1. No principal components analysis was needed to summarise these results, as there are only these two key continuous variates.

**Table 6.3.** Mean values and average standard errors for locations and trips.

| | Hepatosomatic index - Trip 1 | Trip 2 | Percent abnormal - Trip 1 | Trip 2 |
|---|---|---|---|---|
| Fitzroy River | 0.046 | 0.060 | 4.5 | 1.4 |
| Bundaberg | 0.050 | 0.061 | 3.7 | 3.1 |
| The Narrows | 0.055 | 0.059 | 3.2 | 5.2 |
| Port | 0.057 | 0.068 | 8.8 | 4.4 |
| Hamilton Point | 0.050 | 0.061 | 3.1 | 3.7 |
| Calliope River | 0.049 | 0.063 | 5.8 | 2.7 |
| Rodds Bay | 0.043 | 0.056 | 2.3 | 2.4 |
| | (average s.e.) | 0.004 | (average s.e.) | 2.0 |

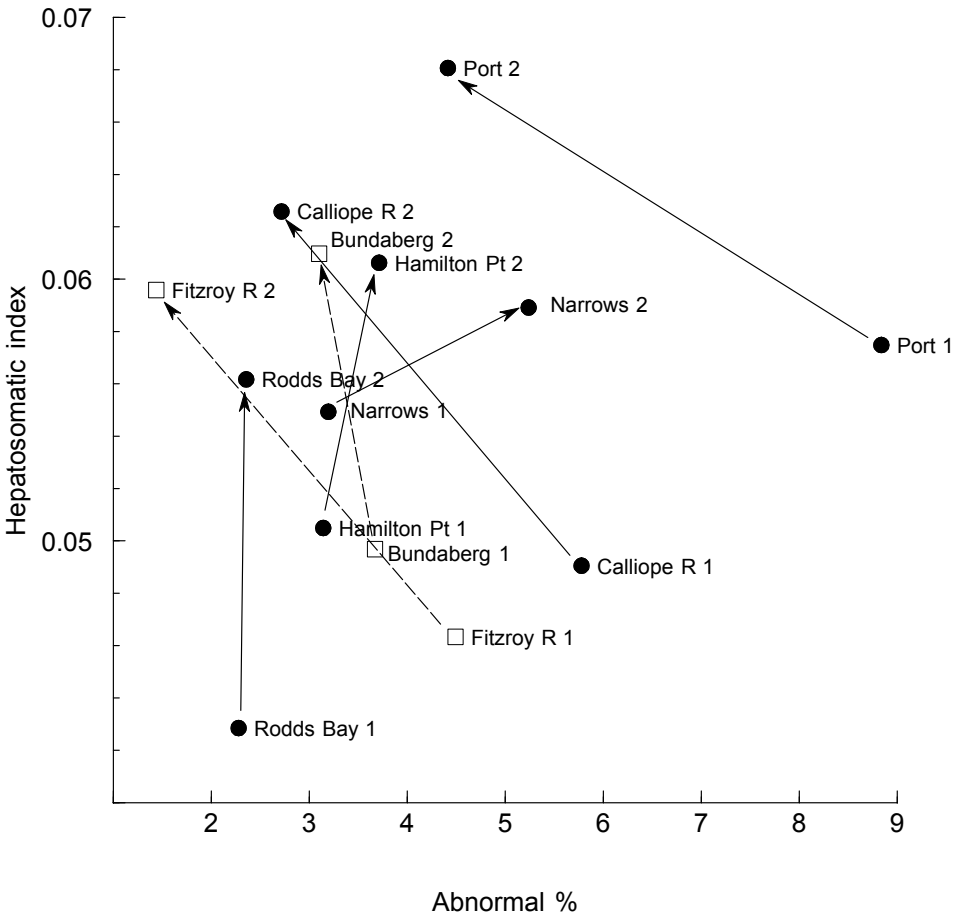


**Figure 6.1.** Movements of the mudcrab location means over trips. The reference sites are indicated by the squares with the dotted lines.

For all sites, the hepatosomatic index was higher for trip 2. Trip 2 also sees similar or lower percentages of abnormals than trip 1, for all sites except for the Narrows. Except for Port, all sites are in the same general spatial vicinity (and hence not notably different from) the two reference sites, at each trip.

Possibly due to the low number of abnormal crabs (57), the distributions of these counts across the highest observed lesion class also showed no significant effects for location or trip ($P$ = 0.14 and 0.13 respectively). Again, all categories were observed both at the reference sites and across the Gladstone region. These counts are listed in Table 6.4 for completeness.

**Table 6.4.** Lesion categories – counts by locations.

| | L1 | L2 | L3 | L4 | L5 |
|---|---|---|---|---|---|
| Fitzroy River | 2 | 6 | 1 | 1 | 4 |
| Bundaberg | 0 | 1 | 1 | 2 | 2 |
| The Narrows | 1 | 0 | 2 | 2 | 1 |
| Port | 2 | 5 | 1 | 1 | 1 |
| Hamilton Point | 0 | 2 | 1 | 2 | 2 |
| Calliope River | 1 | 1 | 2 | 1 | 0 |
| Rodds Bay | 1 | 3 | 0 | 4 | 1 |

Table 6.5 lists the mean values when combining the locations into their respective regions. The reference sites are Bundaberg and the Fitzroy River, and Gladstone includes the locations of the Narrows, Port, Hamilton Point, the Calliope River and Rodds Bay. There were no significant differences between the reference sites and Gladstone region, across both variables and both trips.

**Table 6.5.** Mean values and average standard errors for the pooled regions.

| | Hepatosomatic index - Trip 1 | Trip 2 | Percent abnormal - Trip 1 | Trip 2 |
|---|---|---|---|---|
| Reference sites | 0.048 | 0.060 | 4.1 | 2.3 |
| Gladstone region | 0.051 | 0.061 | 4.6 | 3.7 |
| | (average s.e.) | 0.002 | (average s.e.) | 1.0 |

**References**

GenStat (2011). GenStat for Windows, Release 14.1. VSN International Ltd., Oxford.

McCullagh, P. and Nelder, J. A. (1989). Generalized Linear Models (2nd ed.). Chapman and Hall, London.