The Impacts of Feedlot Effluent on Aquatic Freshwater Systems

By

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<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE OF CONTENTS</td>
<td>II</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>IX</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>XII</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>XVI</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>XVIII</td>
</tr>
<tr>
<td>OPSOMMING</td>
<td>XIX</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>XX</td>
</tr>
<tr>
<td>LIST OF NOTATIONS USED</td>
<td>XXII</td>
</tr>
<tr>
<td>CHAPTER 1</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1. STUDY MOTIVATION</td>
<td>1</td>
</tr>
<tr>
<td>1.2. RESEARCH HYPOTHESIS</td>
<td>3</td>
</tr>
<tr>
<td>1.3. AIMS</td>
<td>3</td>
</tr>
<tr>
<td>1.4. SPECIFIC RESEARCH OBJECTIVES</td>
<td>3</td>
</tr>
<tr>
<td>1.5. DISSERTATION OUTLAY</td>
<td>4</td>
</tr>
<tr>
<td>CHAPTER 2</td>
<td>5</td>
</tr>
<tr>
<td>LITERATURE REVIEW AND COMPONENTS ADDRESSED IN THIS STUDY</td>
<td>5</td>
</tr>
</tbody>
</table>
2.1. PHARMACEUTICALS IN THE ENVIRONMENT ............................................. 5

2.1.1. Growth hormones used in meat production ...................................................... 5

2.1.2. Bioaccumulation of the pharmaceuticals ............................................................. 6

2.1.3. Growth hormone residues in the environment ..................................................... 6

2.1.4. Effects at the individual level .............................................................................. 6

2.2. WATER AND SEDIMENT QUALITY CHANGES IN RELATION TO FEEDLOT ACTIVITY ................................................................................................................... 7

2.2.1. In situ variables .................................................................................................. 7

2.2.2. Nutrients ........................................................................................................... 9

2.2.3. Metals .............................................................................................................. 9

2.2.4. Sediment composition ....................................................................................... 12

   Percentage grain size ................................................................................................. 13

   Percentage organic matter ......................................................................................... 13

2.3. DETERMINING THE EFFECT OF FEEDLOT ACTIVITY ON THE PRESENT ECOLOGICAL STATE ........................................................... 13

2.3.1. Aquatic biota as Indicators ............................................................................... 14

2.3.2. Habitat assessments ......................................................................................... 14

2.3.3. Aquatic macroinvertebrate community structures .......................................... 15

2.4. USING BIOMARKER RESPONSES TO DETERMINE THE EFFECTS OF EXPOSURE TO GROWTH PROMOTING HORMONES ................................. 15

2.4.1. Cellular Energy Allocation .............................................................................. 16

2.4.2. Metabolomics .................................................................................................. 17

CHAPTER 3 ............................................................................................................. 19

STUDY AREA AND SITE DESCRIPTION ..................................................................... 19
3.1. INTRODUCTION ................................................................. 19

3.2. FEEDLOT A ................................................................. 20
  3.2.1. Introduction ................................................................. 20
  3.2.2. Upstream site ............................................................. 21
  3.2.3. Downstream site ........................................................ 22

3.3. INTRODUCTION ................................................................. 23
  3.3.1. Upstream site ............................................................. 24
  3.3.2. Downstream site ........................................................ 25

3.3. FEEDLOT C ................................................................. 26
  3.3.1. Introduction ................................................................. 26
  3.3.2. Upstream site ............................................................. 27
  3.3.3. Downstream site ........................................................ 28

CHAPTER 4 ......................................................................................... 30

DETERMINING THE PRESENT ECOLOGICAL STATE (PES) OF AQUATIC
ECOSYSTEMS THAT ARE INFLUENCED BY FEEDLOT ACTIVITY .......... 30

4.1. INTRODUCTION ................................................................. 30
  4.1.1. Water quality .............................................................. 30
  4.1.2. Sediment composition .................................................. 31
  4.1.3. Ecological Classification ............................................... 32
    Habitat assessment ............................................................. 32
    South African Scoring System version 5 ................................... 33
    Fish Assemblage Integrity Index ............................................. 33

4.2. MATERIALS AND METHODS ............................................... 34
4.2.1. Field survey ................................................................. 34

4.2.2. Water quality ............................................................. 34
   In situ variables and nutrients ........................................... 34
   Metals ............................................................................ 34

4.2.3. Sediment composition ................................................. 35

4.2.4. Ecological classification and PES determination ............... 35
   Habitat assessment .......................................................... 37
   Invertebrate assessment .................................................. 37
   Fish assemblage assessment ............................................ 38

4.2.4. Statistical analysis ........................................................ 39

4.3. RESULTS ........................................................................... 40

4.3.1. Water quality ............................................................. 40
   In situ variables comparison ............................................. 40
   Nutrient variable: Feedlot A ............................................ 41
   Nutrient variables: Feedlot B ............................................ 41
   Nutrient variables: Feedlot C ............................................ 45
   Metals ............................................................................ 48

4.3.2. Sediment composition .................................................. 52
   Grain size composition ..................................................... 52
   Organic contents ............................................................. 52

4.3. ECOLOGICAL CLASSIFICATION (EC) AND PRESENT ECOLOGICAL
STATE (PES) DETERMINATION .................................................. 55

4.3.1. Habitat assessments ..................................................... 55
   Index of Habitat Integrity ................................................ 55
   Invertebrate Habitat Assessment Score ............................ 57
   Invertebrate assessment .................................................. 60
   Fish assemblage assessment .......................................... 62

4.4. DISCUSSION .................................................................... 66

4.4.1. Water quality ............................................................. 66

4.4.2. Sediment composition .................................................. 67
4.4.3. Present Ecological State ......................................................................................................................... 69
Habitat integrity associated with feedlots .............................................................................................................. 69
South African Scoring Systems version 5 .............................................................................................................. 69
Fish Assemblage Integrity Index .......................................................................................................................... 71

4.5. CHAPTER SUMMARY AND CONCLUSION ................................................................................................. 73

CHAPTER 5 ......................................................................................................................................................... 75

ALTERATION TO AQUATIC MACROINVERTEBRATE COMMUNITY
STRUCTURES WITH REFERENCE TO FEEDLOT ACTIVITY ............................................................................. 75

5.1. INTRODUCTION .............................................................................................................................................. 75

5.2. MATERIALS AND METHODS .......................................................................................................................... 77
5.2.1. Study sites .................................................................................................................................................. 77
5.2.2. Field collection sampling and identifying .................................................................................................. 77
5.2.3. Statistical procedure .................................................................................................................................. 77

5.3. RESULTS ......................................................................................................................................................... 79
5.3.1. Diversity indices ......................................................................................................................................... 79
5.3.2. Community composition ............................................................................................................................ 82
5.3.3. Invertebrate community response to water quality ................................................................................. 97

5.4. DISCUSSION .................................................................................................................................................. 100
5.4.1. Feedlot A .................................................................................................................................................. 100
5.4.2. Feedlot B .................................................................................................................................................. 101
5.4.3. Feedlot C .................................................................................................................................................. 102

5.5. CONCLUSION ................................................................................................................................................. 103

CHAPTER 6 ......................................................................................................................................................... 105
BIOMARKER RESPONSES IN *CLARIAS GARIEPINUS* FOLLOWING EXPOSURE TO TRENBOLONE ACETATE (TBA) AND DIETHYLSTILBESTROL (DES) .............................................. 105

6.1. INTRODUCTION ........................................................................................................... 105

6.1.1. Growth hormones ........................................................................................................ 106

6.1.2. Test organism ............................................................................................................... 106

6.1.3. Condition Factor (CF) ................................................................................................. 106

6.1.4. Cellular Energy Allocation .......................................................................................... 107

6.1.5. Metabolomics .............................................................................................................. 108

6.2. EXPERIMENTAL DESIGN AND METHODS APPLIED ............................................ 109

6.2.1. Chemicals .................................................................................................................... 109

   Hormones and exposures ................................................................................................... 109

   Cellular Energy Allocation (CEA) ..................................................................................... 109

   Metabolomics .................................................................................................................. 109

   Fish exposure and sampling ............................................................................................ 109

6.2.2. Conditioning factor and Somatic indices .................................................................. 110

6.2.3. CELLULAR ENERGY ALLOCATION ....................................................................... 111

   Energy available ............................................................................................................... 111

   Energy Consumed ............................................................................................................ 111

   Cellular Energy Allocation .............................................................................................. 111

   Cellular Energy Allocation data analysis ......................................................................... 112

6.2.4. Metabolomics ............................................................................................................ 112

   Blood plasma sampling ..................................................................................................... 112

   NMR metabolomics: Preparation of samples for $^1$H NMR ........................................ 112

   $^1$H NMR Spectroscopy .................................................................................................... 112

   Pre-processing of NMR data ........................................................................................... 113

   Multivariate analysis of NMR data .................................................................................. 113

6.3. RESULTS ....................................................................................................................... 114

6.3.1. Condition factor and somatic indices ......................................................................... 114
6.3.2. Cellular Energy Allocation (CEA) ................................................................. 116
6.3.3. Metabolomics ............................................................................................. 124

6.4. DISCUSSION ............................................................................................... 127
6.4.1. Diethylstilbestrol group ........................................................................ 127
6.4.2. Trenbolone acetate group ....................................................................... 129

6.5. CONCLUSION ............................................................................................. 130

CHAPTER 7 ........................................................................................................... 124

7. CONCLUSION AND RECOMMENDATIONS .................................................... 124

CHAPTER 8 ........................................................................................................... 127

8.1 REFERENCES ................................................................................................. 127
LIST OF TABLES

Chapter 3

Table 3.1: Site names, coordinates and altitude................................................................. 20
Table 3.2: Environmental features of area associated with Feedlot A (Mucina and Rutherford, 2006). .................................................................................................................. 21
Table 3.3: Environmental features of area associated with Feedlot B (Mucina and Rutherford, 2006). .................................................................................................................. 25
Table 3.4: Environmental features of area associated with Feedlot C (Mucina and Rutherford, 2006). .................................................................................................................. 27

Chapter 4

Table 4.1: Ecological state categories, categories, key colours and category descriptions presented within the biotic assessment (Kleynhans and Louw, 2007)................................. 36
Table 4.2: Biological Bands/ Ecological categories for interpreting SASS data (Dallas, 2007) ................................................................................................................................. 36
Table 4.3: Ecological state categories, key colours and category descriptions presented within this assessment for habitat.................................................................................. 36
Table 4.4: Water quality data including in situ variables and nutrients for upstream and downstream sites associated with each feedlot, during both the high flow and the low flow survey (November, 2007 and April 2008) ........................................................................... 43
Table 4.5: The 95th percentile values for historical data from the Suikerbosrand River Uitvlught monitoring station, from 1996-2000 (DWAF, 1996)............................................................... 44
Table 4.6: Target Water Quality Range (TWQR) for in situ and nutrient variables (DWAF, 1996).................................................................................................................................................. 44
Table 4.7: Metal concentrations (µg/l) for both upstream and downstream sites associated with feedlot activity for the low flow and the high flow survey................................................................................. 51
Table 4.8: The TWQR, CEV and AEV for metals assessed in this study (NA=Not Available). .............................................................................................................................................. 51
Table 4.9: IHI scores for the stream associated with the Feedlot A..................................... 55
Table 4.10: IHI scores for the stream associated with the Feedlot B.................................... 56
Table 4.11: IHI scores for the stream associated with the Feedlot C.................................... 57
Table 4.12: IHAS scores for stream associated with Feedlot A.......................................... 58
Table 4.13: IHAS scores for stream associated with Feedlot B. ................................. 59

Table 4.14: IHAS scores for stream associated with Feedlot C. ................................. 59

Table 4.15: Aquatic macro invertebrate data: SASS 5 Scores, ASPT’s and respective SASS 5 biotope scores for upstream and downstream of each feedlot, respectively for the low flow assessment. .......................................................... 61

Table 4.16: Aquatic macro invertebrate data: SASS 5 Scores, ASPT’s and respective SASS 5 biotope scores for upstream and downstream of each feedlot, respectively for the high flow assessment. .......................................................... 61

Table 4.17: Expected fish occurrence for all six sites assessed. Expected occurrence list includes alien species. Particular occurrence list excludes historical distribution as obtained from Skelton (2001). ................................................................. 63

Table 4.18 FAII scores and EC for upstream and downstream sites for the high flow survey. ........................................................................................................... 65

Table 4.19 FAII scores and EC for upstream and downstream sites for the high flow survey. ........................................................................................................... 65

Table 4.20: Summary table of chapter one, highlighting important observations for each site, as well as a summary of the EC of habitat, invertebrates and fish assessed at each site. ......................................................................................... 74

Chapter 5

Table 5.1: Results obtained from SIMPER analysis with a 90 % cut off for low contributions indicating the contribution of various macroinvertebrate families for the downstream site, associated with Feedlot A, to similarity within the macroinvertebrate groupings. ........... 82

Table 5.2: Results obtained from SIMPER analysis with a 90 % cut off for low contributions indicating the contribution of various macroinvertebrate families for the upstream and downstream sites, associated with Feedlot B, to similarity within the macroinvertebrate groupings. ................................................................................. 85

Table 5.3: Results obtained from SIMPER analysis with a 90 % cut off for low contributions indicating the contribution of various macroinvertebrate families for the low flow survey, associated with Feedlot C, to similarity within the macroinvertebrate groupings. ........... 87

Chapter 6

Table 6.1: Descriptive statistics indicating the significant (*) differences in Energy consumed (Ec) between control, TBA and DES group after an exposure period of five days........ 119

Table 6.2: Descriptive statistics indicating the significant (*) differences in lipid concentrations, Energy allocated (Ea), Energy consumed (Ec) as well as Cellular Energy Allocation between control, TBA and DES group after an exposure period of ten days. .................................................................................................................. 119
Table 6.3: Descriptive statistics indicating the significant (*) differences in lipid-protein concentrations, Energy allocated (Ea), Energy consumed (Ec) as well as Cellular Energy Allocation between control, TBA and DES group after an exposure period of fifteen days.
.................................................................................................................................................. 120

Table 6.4: $^1$H-NMR chemical shift regions in presat spectra of catfish bloom plasma responsible for dissimilarities between different exposure groups over five day exposure intervals. ........................................................................................................................................ 126
LIST OF FIGURES

Chapter 3

Figure 3.1: (A-D) Photos showing channel conditions and stream dimensions upstream from Feedlot A................................................................. 22

Figure 3.2: (A-D) Photos showing channel conditions and stream dimensions downstream from Feedlot A................................................................. 23

Figure 3.3: Photos showing channel conditions and stream dimensions upstream from Feedlot B......................................................................................... 25

Figure 3.4: Photos showing channel conditions and stream dimensions downstream from Feedlot B......................................................................................... 26

Figure 3.5: (A and B) Photos showing channel conditions and stream dimensions upstream from Feedlot C......................................................................................... 28

Figure 3.6: (A-D) Photos showing channel conditions and stream dimensions downstream from Feedlot C......................................................................................... 29

Chapter 4

Figure 4.1: PCA bi-plot of water quality variables showing (dis)similarities for upstream and downstream sites associated with Feedlot A during low flow and high flow surveys. K= Feedlot A.................................................................................................................. 46

Figure 4.2: PCA bi-plot of water quality variables showing (dis)similarities for upstream and downstream sites associated with Feedlot B during low flow and high flow surveys. T= Feedlot B.................................................................................................................. 47

Figure 4.3: PCA bi-plot of water quality variables showing (dis)similarities for upstream and downstream sites associated with Feedlot C during low flow and high flow surveys. B= Feedlot C.................................................................................................................. 48

Figure 4.4: Spider diagram representing the percentage grain size composition of both upstream and downstream sites associated with Feedlot A......................................................... 53

Figure 4.5: Spider diagram representing the percentage grain size composition of both upstream and downstream sites associated with Feedlot B......................................................... 53

Figure 4.6: Spider diagram representing the percentage grain size composition of both upstream and downstream sites associated with Feedlot C......................................................... 53

Figure 4.7: Spider diagram representing the percentage organic matter present at both upstream and downstream sites associated with Feedlot A......................................................... 54

Figure 4.8: Spider diagram representing the percentage organic matter present at both upstream and downstream sites associated with Feedlot B......................................................... 54
Figure 4.9: Spider diagram representing the percentage organic matter present at both upstream and downstream sites associated with Feedlot C.

Figure 4.10: Eye of *Labeo capensis*, sampled downstream from Feedlot A showing peripheral abnormality indicating internal bacterial infection.

Figure 4.11: Caudal fin of *Labeobarbus aeneus* sampled upstream from Feedlot A, indicating cyst infection.

Figure 4.12: *Labeobarbus aeneus* indicating symptoms of Costia or Trichodina, epidermal infection.

Chapter 5

Figure 5.1: Univariate diversity index values for macroinvertebrate indicating Margalef's species richness for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.2: Univariate diversity index values for macroinvertebrate indicating Total Species (S) for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.3: Univariate diversity index values for macroinvertebrate indicating Pielou's evenness (*J*') for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.4: Univariate diversity index values for macroinvertebrate indicating Shannon-Wiener diversity index (*H'*(logo)) for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.5: Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between upstream (U) and downstream (D) in relation to the macroinvertebrate community structure at each site for low flow (L); and high flow (H) survey for sites associated with Feedlot A (K).

Figure 5.6: Ranked species K-dominance plot for macroinvertebrate communities collected at upstream (U) and Downstream (D) sites associated with Feedlot A (K) during both high flow (H), and low flow (L) surveys.

Figure 5.7: Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between upstream (U) and downstream (D) in relation to the macroinvertebrate community structure at each site for low flow (L); and high flow (H) survey for sites associated with Feedlot B (T).

Figure 5.8: Ranked species K-dominance plot for macroinvertebrate communities collected at upstream (U) and Downstream (D) sites associated with Feedlot B (T) during both high flow (H), and low flow (L) surveys.

Figure 5.9: Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between upstream (U) and downstream (D) in relation to the macroinvertebrate community structure at each site for low flow (L); and high flow (H) survey for sites associated with Feedlot C (B).

Figure 5.10: Ranked species K-dominance plot for macroinvertebrate communities collected at upstream (U) and Downstream (D) sites associated with Feedlot C (B) during both high flow (H), and low flow (L) surveys.
Figure 5.11: Redundancy Analysis (RDA) indicating Site and Season ordination based on (a) invertebrate abundances and diversity and (b) water quality variables, for both upstream and downstream sites associated with Feedlot A over low and high flow field surveys. K=Feedlot A, D=Downstream, U=Upstream, H= High flow, L= Low flow

Figure 5.12: Redundancy Analysis (RDA) indicating Site and Season ordination based on (a) invertebrate abundances and diversity and (b) water quality variables, for both upstream and downstream sites associated with Feedlot B over low and high flow field surveys. T=Feedlot B, D=Downstream, U=Upstream, H= High flow, L= Low flow

Figure 5.13: Redundancy Analysis (RDA) indicating Site and Season ordination based on (a) invertebrate abundances and diversity and (b) water quality variables, for both upstream and downstream sites associated with Feedlot C feedlot over low and high flow field surveys. B=Feedlot C, D=Downstream, U=Upstream, H= High flow, L= Low flow

Chapter 6

Figure 6.1: Conditioning Factor of each exposure group, represented with standard error bars, over the 15 days exposure period, with specific 5 day interval values

Figure 6.2: HSI values of each exposure group, represented with standard error bars, over the 15 days exposure period, with specific 5 day interval values

Figure 6.3: GSI values of each exposure group, represented with standard error bars, over the 15 days exposure period, with specific 5 day interval values

Figure 6.5: Effects of five, ten and fifteen day exposure of TBA and DES on carbohydrate concentrations of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05

Figure 6.6: Effects of five, ten and fifteen day exposure of TBA and DES on protein concentrations of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05

Figure 6.7: Effects of five, ten and fifteen day exposure of TBA and DES on lipid concentrations of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05

Figure 6.8: Effects of five, ten and fifteen day exposure of TBA and DES on Energy allocation (Ea mJ/g) of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05

Figure 6.9: Effects of five, ten and fifteen day exposure of TBA and DES on Energy consumed (Ec mJ/g) of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05

Figure 6.10: Effects of five, ten and fifteen day exposure of TBA and DES on Cellular Energy Allocation (CEA mJ/g) of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05

Figure 6.11: Two-dimensional MDS indicating (dis)similarity between different exposure groups with reference to the CEA and CF relationship after five days of exposure
Figure 6.12: Two-dimensional MDS indicating (dis)similarity between different exposure groups with reference to the CEA and CF relationship after ten days of exposure. 123

Figure 6.13: Two-dimensional MDS indicating (dis)similarity between different exposure groups with reference to the CEA and CF relationship after fifteen days of exposure. 123

Figure 6.14: 300 MHz $^1$H-NMR presat spectra of catfish blood plasma with some identified metabolites indicated (Samuelsson et al., 2006). 124

Figure 6.15: Principle Component Analysis, indicating the (dis)similarity between control, DES and TBA exposure groups over five, ten and fifteen day intervals. The PCA accounts for 72 % of total variance observed when comparing the metabolic profiles of different exposure groups. 125
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I do not know much about gods; but I think that the river
Is a strong brown god - sullen, untamed and intractable,
Patient to some degree, at first recognised as a frontier;
Useful, untrustworthy, as a conveyor of commerce;
Then only a problem when confronting the builder of bridges.
The problem once solved, the brown god is almost forgotten
By the dwellers in cities – ever, however, implacable,
Keeping his seasons and rages, destroyer, reminder
Of what men choose to forget. Unhonoured, unpropitiated
By worshippers of machine, but waiting, watching and waiting.

T. S. Eliot, “The Dry Salvages”, from Four Quarters
Abstract

This study aims to assess the potential impacts of intense feedlot activity on the aquatic freshwater environment, with reference to three feedlots, ranging in production size and all situated in the upper Vaal catchment area. Field assessments were done over a high flow and low flow period, while controlled exposures were also done to quantify a potential stress reaction to growth hormone exposure (using *Clarias gariepinus* as test organism). It was ascertained that water quality variables contributing towards differences between upstream and downstream environmental conditions are NH$_4$ concentrations, pH, and conductivity. Lead concentrations were also periodically higher downstream from feedlot activity, in comparison with upstream. Taking the sediment assimilation potential of growth hormones into consideration, it was determined that Feedlot C showed the highest assimilation potential, while Feedlot A reflected the lowest. Alterations on family level invertebrate community structures indicated a categorical decline in abundances and species richness at sites situated downstream from feedlots. However, some clear seasonal influences were also observed. Further community and diversity analyses reflected alterations in invertebrate community structures that were not reflected in SASS 5 scores. With regards to the biomarkers applied in this study, it was noted that there was a significant ($p<0.05$) difference in the cellular energy allocation (CEA) between control and hormone exposed groups. The total amount of energy available (Ea) increased significantly for test organisms exposed to Diethylstilbestrol (DES), while there was a significant increase in energy consumption (Ec) of test organisms exposed to Trenbolone acetate (TBA). In addition to CEA, metabolic profiling of blood plasma was also performed, which indicated a definite ordination in metabolic constituents after fifteen days of exposure. This was established by subjecting the data to principal component analysis (PCA), which accounted for 83 % variance observed. The impacts and biotic responses identified in this study were contextualised with known literature on the effects of feedlot activity and growth hormone exposure on the aquatic environment. Finally, conclusions were drawn and recommendations made with regard to improving feedlot operational activities. The results obtained in this study contribute towards an integrated framework for the environmental management of feedlot activities.
Opsomming

Die studie poog om die potensiële impakte van hoë konsentrasie vleis produksie op varswater ekosisteme te kwantifiseer. Dit is gedoen met verwysing na drie strategies gekies voerkrale wat almal in die boonste gedeelte van die Vaalrivier opvangs gebied val. Beide hoog vloei en laag vloei veld assesserings is gedoen by liggings wat stroomop en stroomaf van voerkraal voorkom. ‘n Tweede aspek van die studie was om te bepaal wat die effek van ‘n groei hormoon blootstelling op toets organisme, *Clarias gariepinus*, is. In terme van water kwaliteit, is dit bepaal dat voerkraal aktiwiteite bydra tot hoër meetbare veranderlikes insluitende: pH en kondutiwiteit asook hoër NH₄ vlakke. Saam met hierdie veranderings bly dit ook of lood konsentrasies toeneem stroom af van voerkraal aktiwiteite. Die organiese en klei inhoud van sediment verkry by geassosierde water sisteme van elke voerkraal, versoek ‘n grondslag om die potensiële hormoon assimilering vir elke sisteem te bepaal. Met toepassing van die beginsel is dit duidelik dat die rivier sisteem geassosieer met Voerkraal C die hoogste hormoon assimilasie potensiaal toon terwyl, voerkrale A en B moontlik meer bio-beskikbare hormone kan huisves. ‘n Verdere poging van die studie was om variasie in makro-invertebraat gemeenskap strukture stroomop en stroomaf van voerkraal aktiwiteite te bepaal. Veranderings in gemeenskap strukture het ‘n afname in hoeveelhede asook spesie diversiteit stroomaf van voerkraal getoon. Daar is egter ook ‘n duidelike seisoenale variasie in gemeenskapstrukture waargeneem. Verdere gemeenskap and diversiteit analyse het veranderings in makro-invertebraat gemeenskap strukture aangetoon wat nie waargeneem kon word met die toepassing van die SASS 5 protokol nie. Die beheerde hormoon blootstelling en biomerkers wat toegepas was in die studie het beduidende (*p<0.05*) verskille in die energie reserves (CEA) getoon tussen die kontrole groep en die hormoon blootgestelde groepe. Daar word aangedui dat die totale hoeveelheid energie beskikbaar (Ea) toegeneem het vir organismes wat blootgestel was aan die hormoon Diethylstilbestrol (DES), terwyl daar ‘n beduidende toename in energie gebruik (Ec) was vir toets organismes wat bloot gestel was aan die sintetiese testosteroon, Trenbeloon asetaat (TBA). Metaboliese profiel bepaling was ook toegepas op bloedplasma van toets organismes en het ‘n duidelike groepering van bloed metaboliete getoon tussen blootstellings groepe. Die DES groep het ‘n toename in lipo-protiene getoon na 5 dae van blootstelling terwyl die TBA groep minder variasie in bloed metaboliete getoon het in vergelyking met die kontrole groep. Deur voerkraal geassosieerde impakte en biologiese stres reaksies wat in die studie gekwantifiseer word te kombineer met reeds bestaande literatuur is sekere gevolgtrekkings en voorstelle aangaande voerkraal bestuur en hormoon gebruik gemaak. Hierdie bevindinge kan toegepas word ten einde omgewings bestuur geassosieer met voerkrale te verbeter.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AEV</td>
<td>Acute Effect Value</td>
</tr>
<tr>
<td>ASPT</td>
<td>Average Score Per Taxa</td>
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<tr>
<td>BOD</td>
<td>Biological Oxygen Demand</td>
</tr>
<tr>
<td>CEA</td>
<td>Cellular Energy Allocation</td>
</tr>
<tr>
<td>CEV</td>
<td>Chronic Effect Value</td>
</tr>
<tr>
<td>CF</td>
<td>Condition Factor</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>DES</td>
<td>Diethylstilbestrol</td>
</tr>
<tr>
<td>DWAF</td>
<td>Department of Water Affairs and Forestry</td>
</tr>
<tr>
<td>Ea</td>
<td>Energy allocated</td>
</tr>
<tr>
<td>EC</td>
<td>Ecological Category</td>
</tr>
<tr>
<td>Ec</td>
<td>Energy consumed</td>
</tr>
<tr>
<td>EDC</td>
<td>Endocrine Disruptive Compounds</td>
</tr>
<tr>
<td>EDH</td>
<td>Endocrine Disruptive Hormones</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ETA</td>
<td>Electron Transport Activity</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAlI</td>
<td>Fish Assemblage Integrity Index</td>
</tr>
<tr>
<td>FRAI</td>
<td>Fish Response Assessment Index</td>
</tr>
<tr>
<td>GSI</td>
<td>Gonado-Somatic Index</td>
</tr>
<tr>
<td>GSM</td>
<td>Gravel, Sand and Mud</td>
</tr>
<tr>
<td>HIS</td>
<td>Hepatic-Somatic Index</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>Inductively Coupled Plasma Optical Emission Spectrometry</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
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<tr>
<td>IHAS</td>
<td>Invertebrate Habitat Assessment System</td>
</tr>
<tr>
<td>IHI</td>
<td>Index of Habitat Integrity</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Annual Precipitation</td>
</tr>
<tr>
<td>MAR</td>
<td>Mean Annual Rainfall</td>
</tr>
<tr>
<td>MDS</td>
<td>Multi Dimensional Scaling</td>
</tr>
<tr>
<td>MFD</td>
<td>Mean Frost days</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NTU</td>
<td>Nephelometric Turbidity Unit</td>
</tr>
<tr>
<td>PCA</td>
<td>Principle Component Analysis</td>
</tr>
<tr>
<td>PES</td>
<td>Present Ecological State</td>
</tr>
<tr>
<td>RDA</td>
<td>Redundancy Analysis</td>
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<tr>
<td>RHI</td>
<td>River Health Index</td>
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<tr>
<td>RHP</td>
<td>River Health Programme</td>
</tr>
<tr>
<td>S</td>
<td>Stones</td>
</tr>
<tr>
<td>SASS 5</td>
<td>South African Scoring System Version 5</td>
</tr>
<tr>
<td>TBA</td>
<td>Trenbolone acetate</td>
</tr>
<tr>
<td>TDS</td>
<td>Total Dissolved Solids</td>
</tr>
<tr>
<td>TWQR</td>
<td>Target Water Quality Range</td>
</tr>
<tr>
<td>Veg</td>
<td>Vegetation</td>
</tr>
<tr>
<td>WRC</td>
<td>Water Research Commission</td>
</tr>
</tbody>
</table>
## List of Notations used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>cm</td>
<td>centimetre: one hundredth of a metre</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>ha</td>
<td>hectare</td>
</tr>
<tr>
<td>kg</td>
<td>one thousand grams</td>
</tr>
<tr>
<td>L</td>
<td>Litre: one thousand millilitres, or one thousandth of a m$^3$</td>
</tr>
<tr>
<td>m</td>
<td>meter; one thousand millimetres</td>
</tr>
<tr>
<td>mg</td>
<td>milligram; one thousandth of a gram</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre; one thousandth of a litre</td>
</tr>
<tr>
<td>µg</td>
<td>microgram; a millionth of a gram</td>
</tr>
<tr>
<td>µl</td>
<td>microlitre; a millionth of a litre</td>
</tr>
<tr>
<td>M</td>
<td>molarity (moles of solute per unit volume of solution)</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar, $10^{-3}$ mol/dm or $10^{-3}$ mol/L</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram ($10^{-9}$)</td>
</tr>
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Chapter 1

Introduction

1.1. Study motivation

Aquatic ecosystems associated with feedlot activity are exposed to a number of potential impacts. Feedlots, by their very nature, need to be located near a natural drainage line accommodating a lotic system. Furthermore, the feedlot needs to be situated on a slight slope in order to manage cattle waste products. High density cattle feeding activity implies high volumes of water consumption and organic waste production. South Africans (in the order of 44 million) consume approximately 13.42 kg per capita of beef per year, with the total demand for red meat at 600 000 tons per annum, of which 580 000 tons are produced in local feedlots (SAFA, 2006). Feedlots are relatively small surface areas, with large numbers of grain-fed cattle. The average amount of grain fed per individual is 8.84 kg of total solids a day. The average steer is fed for 153 days with an average daily weight gain of 1.43 kg (Erickson et al., 2003). The production of weaners for the feedlot industry is the most frequent form of cattle farming. The total number of cattle in South Africa by the end of 2006 was estimated at 13.53 million, of which 80% is comprised of beef cattle while the remaining are dairy cattle (Insidepolitics, 2006). A range of impacts needs to be considered in order to determine the impact of feedlots on the aquatic environment.

Feedlot activity may alter water quality in a number of ways. Most commonly; nutrient enrichment, in the form of phosphates and nitrogen stemming from cattle manure and liquid waste, can be a major contributor towards nutrient overload in a particular aquatic system. Subsequent increases in pH and total dissolved solids, as well as increases in turbidity are also commonly associated with large scale cattle production operations (Tilman, 1999).

Organic waste discharge from livestock farms into streams is considered to be oxygen demanding (Bloxham, 1999) and this, in turn, may also be a source of bacterial contamination (Brenner and Mondok, 1995; Cooper and Lipe, 1992).

Formulated cattle diets often include metals such as selenium, copper, iron, manganese and zinc. Un-metabolised metals may find their way into the aquatic environment, where they can pose a toxic risk to aquatic biota, while increased turbidity and sedimentation are often associated with livestock production (Allan et al., 1997).
Another potential water quality impact of feedlot activities on freshwater systems is salinisation. Soils, in particular, are prone to salinisation, more so in drier areas that receive lower annual rainfall levels. However, freshwater systems are also susceptible to increased salt concentrations (Lemly, 1993).

Potential habitat alterations also need to be considered. Feedlot activity may infringe on the riparian vegetation or even be situated in the direct catchment areas. This will inevitably lead to alterations in riparian and in-stream habitat availability (Cooper, 1993). In addition to this, structural components of the associated aquatic system might be altered or even lost. For instance, an increase in hydrology due to a dysfunctional riparian zone will select for more flow-tolerant aquatic biota (Kleynhans, 1996). While a decrease in flow volumes, due to abstraction, will also alter aquatic biotic species composition.

An additional consideration is that of increased concentrations of pharmaceuticals entering the aquatic environment. Residual amounts of pharmaceuticals have been widely detected in surface water (Brown et al., 2007). An increase in human population has lead to increased rates of consumption of these chemicals and thus higher chronic concentrations in the environment. The use of pharmaceuticals is not limited to human consumption and a vast number of veterinary pharmaceuticals exist (Ervard et al., 1989).

Some of these veterinary pharmaceuticals entering the environment are growth hormones used in cattle feeding lots. A number of different growth hormones are commonly used in farming activities on a global scale (Raloff, 2002). The use of sex steroids in cattle farming has been a common practice since the 1950’s (Raun and Preston, 2002) when it was first introduced to a rapidly increasing agricultural market. Although most of these hormones have been banned in chicken farming for more than 40 years (Brown et al., 2007), they are still very much in use in cattle and dairy farming activities. Hormones are used by livestock producers to increase meat production as well as to increase the efficiency of converting feed energy into meat (SAFA, 2006). Five hormones are presently approved for use in beef production in South Africa under the Registrar Act 36 of 1947. They are estradiol, progesterone, testosterone, zeranol and trenbolone acetate (SAFA, 2006). It must be noted, however, that in 1988 the European Union (EU) prohibited the use of these hormones for growth promoting in farm animals in Europe (Brussels, 2002).

Having residual amounts of growth hormones available from cattle manure in rivers, have environmental and ecological implications (Schiffer et al., 2001). When manure from cattle feedlots enters the environment it poses the risk of contamination of surface and ground water (Schiffer et al., 2001). Water systems, in general, are predominantly susceptible to hormone residues (Schiffer et al., 2001). The need to assess the effects of chronic exposure...
to pharmaceuticals (including growth hormones) in the environment has become a research priority. Brown et al. (2007) developed a fish plasma model that estimates the risk of pharmaceutical products entering surface water systems. Schiffer et al. (2001) determined the residues and degradation of trenbolone acetate (TBA) in solid dung, liquid manure and soils. Their results showed that the fate of TBA should be investigated further concerning its potential endocrine-disrupting activity in agricultural ecosystems.

The research question is therefore principally concerned with addressing the effects of feedlot activity on associated freshwater systems and, secondary to that, what the effect of growth promoting hormones used in cattle feeding lots are on the freshwater biota that are exposed to definite residues of these hormones.

1.2. Research Hypothesis

The hypothesis for this study states that cattle feedlot activity alters the natural ecological condition and functioning of aquatic freshwater systems. Subsequently, it also states that the exposure of fish to growth-promoting pharmaceuticals (both androgenic and estrogenic) used in feedlot activity, stimulates a stress response at cellular and sub-cellular levels.

1.3. Aims

The main aims were:

- To determine whether activities associated with feedlots have an effect on the ecological state of adjacent natural aquatic ecosystems.
- To determine the sub-lethal effects of ecologically relevant pharmaceutical concentrations on fish using laboratory-based bioassays.

1.4. Specific research objectives

1. In order to elucidate the ecological state of aquatic systems, sites upstream and downstream of feedlot activity were assessed for the following parameters:
   a. changes in water quality (in situ, nutrient and metal concentrations),
   b. sediment composition (grain size and organic content),
   c. habitat characteristics (Index of Habitat Integrity –IHI and Invertebrate Habitat Assessment -IHAS),
   d. invertebrate community composition (South African Scoring System version 5 – SASS 5 index - and community structures), and
Chapter 1

e. fish composition (fish assemblage integrity index assessment - FAII).

2. The temporal influence of changing hydrological patterns on the ecological state was assessed by determining the above mentioned parameters during high and low flow surveys.

3. The third objective was to expose a number of fish to environmentally relevant concentrations of a synthetic testosterone (TBA) and synthetic estrogen (Diethylstilbestrol - DES). Sub-cellular stress responses were measured using the following biomarkers:
   a. metabolomics and
   b. Cellular Energy Allocation (CEA).

1.5. Dissertation outlay

The study approach followed is multidisciplinary. It incorporates biomarkers, invertebrate community structures, fish health assessment, water chemistry and sediment analysis, as well as exposures of specific species to growth hormones in a controlled environment. Each of these aspects is dealt with in separate chapters.

Chapter 2 presents a brief literature overview pertaining to the main components of this study.

Chapter 3 provides a detailed description of the study area, as well as the sites associated with each feedlot. In this chapter, a detailed description of habitat and local conditions will be given.

Chapter 4 is the first of three result chapters and deals with the differences in water quality, sediment analysis and present ecological state (PES) of upstream and downstream sites associated with each feedlot respectively.

Chapter 5 assessed invertebrate community structures of sites upstream and downstream from the feedlot activities. The assessment incorporates species richness and abundances in the form of diversity indices (Shannon-Weiner) and compares similarities between sites using the Bray-Curtis similarity coefficient. The relationship between invertebrate community composition and environmental driving variables (water quality) are studied.

In Chapter 6 the sub-lethal effects of the two most commonly used hormones at the feedlots are determined by exposing Clarias garpienus to TBA and DES and measuring two biomarkers namely, CEA and metabolomics, while chapter 7 provides a conclusion drawn from the major findings with recommendations.
Chapter 2

Literature Review and Components Addressed In This Study

2.1. Pharmaceuticals in the environment

A number of studies have been done on the possible implications of pharmaceuticals that enter the environment (Desbrow et al., 1998; Fent et al., 2006; Heberer, 2002; Hugget et al., 2003; Jobling et al., 2002; Kummerer, 2004; Larsson et al., 1999; Oaks et al., 2004). The literature ranges from human to veterinary products. However, Gane and Blaise (2005) found that there is still a lack of information on the physiological reaction of sentinel aquatic species that bioaccumulate any of these drugs.

2.1.1. Growth hormones used in meat production

Various types of growth hormones are commonly used in a range of farming activities on a global scale. The use of sex steroids in cattle farming has been common practice since the 1950’s. The total number of cattle in South Africa at the end of August 2006 was estimated at more than 13 million. More than two thirds of these animals are exposed to anabolic chemicals (SAFA, 2006). Little is known about the potential ecological effects of hormonally active substances associated with discharges from animal feeding activities (Durhan et al., 2002).

Trenbolone acetate is a synthetic anabolic steroid that is widely used in meat production. Referring to the literature, it is evident that three metabolites of TBA: 17 alpha-trenbolone, trendione and 17 beta-trenbolone occur predominantly in the aquatic environment (Ervard et al., 1989; Duran et al., 2002; Durhan et al., 2003; Ankley et al., 2003).

Diethylstilbestrol is an artificial non-steroidal estrogen, which is practically insoluble in water, but is persistent in water, soils and faeces (Shore et al., 1993; Zondek and Sulman, 1943). Both TBA and DES are used for the purpose of this study.
2.1.2. Bioaccumulation of the pharmaceuticals

At present there is still a lack of information regarding the bioaccumulation of veterinary products in freshwater fish. The quantities of pharmaceutical products entering natural environment are increasing daily (Boullfault and Willemart, 1983). Despite the large volumes of analytical data available, studies which assess the ecotoxicological potential of these compounds are scarce (Van der Ven et al., 2006). Until very recently, the use of such biomarkers were rather limited in their abilities and focussed mainly on individual gene expressions or a limited set of genes (Moens et al., 2006). Most available biomarkers focussed on estrogenic substances. The sub cellular effects of androgenic, xenobiotic and other endocrine disruptive chemicals are still largely unexplored (Moens et al., 2006). Furthermore; sentinel aquatic species, including fish have not yet been selected but studies have shown that there is a significant accumulation of certain antibiotics in the tissue of mussels and plants (Gane and Blaise, 2005). Orlando et al. (2004) observed that fathead minnows (Pimephales promelas), exposed to feedlot effluent, showed a significant alteration in their reproductive biology. Male fish showed a decrease in masculinity; having lower testicular testosterone synthesis, altered head morphometrics and smaller testes sizes. The Japanese medaka (Oryzias latipes) showed sexual differentiation when exposed to natural and synthetic growth hormones (Metcalfe et al., 1999).

2.1.3. Growth hormone residues in the environment

Two metabolites of TBA: 17 alpha- and 17 beta-trenbolone, are relatively stable in animal excreta and in the environment (Schiffer et al., 2001). These metabolites also bind with high affinities to fish androgen receptor(s) (Ankley et al., 2003; Bauer et al., 2000; Pottier et al., 1981; Wilson et al., 2004). In addition to this Durhan et al. (2003) also identified metabolites of TBA in androgenic runoff from beef feedlot activities. It was established that relatively high quantities of these metabolites are present in the runoff, while Ervard et al. (1989) studied the fate and residues of TBA in edible tissue from sheep and calves implanted with TBA and found that 17 beta-trenbolone is the prominent metabolite resonating in muscle tissue of cattle. Brown et al. (2007) demonstrated that these pharmaceuticals are capable of bioconcentrating in fish blood once they are released into the environment.

2.1.4. Effects at the individual level

A number of studies have been done on the effects of pharmaceutical residues in runoff on aquatic freshwater systems. Brown et al. (2007) developed a Fish Plasma Model that estimates the risk of pharmaceutical products entering surface water systems. Schiffer et al.
(2001) determined the residues and degradation of TBA in solid dung, liquid manure and soils. Their results show that the fate of TBA should be investigated further concerning its potential endocrine-disrupting activity in agricultural ecosystems.

Arcand-Hoy and Benson (1998) report that male fish exposed to estrogenic compounds show induced production of vitellogenin. However, the biological significance of elevated vitellogenin levels is still speculative. Gane and Blaise (2005) assessed the effects of pharmaceutical products (including veterinary products) on aquatic organisms. Their preliminary findings suggest that some aquatic species can accumulate certain drugs and that these drugs are likely to produce effects at designated target cells. The inevitable exposure of aquatic organisms to a wide variety of pollutants has increased with the addition of Endocrine Disruptive Chemicals (EDC's). The effects of these chemicals are poorly understood and often difficult to quantify. However, one of the effects of Endocrine Disruptive Hormones (EDH) is sexual abnormalities. A study done by Orlando et al. (2004) shows definite endocrine, morphological and anatomical abnormalities in fish exposed to growth promoting hormones.

In vivo studies with showed that androgenic growth hormones are highly potent in fish. They increase masculinity in females and decrease fecundity at water concentrations in the low nanogams per litre range (Ankley et al., 2003; Hewitt et al., 2003). More recently Holbeck et al. (2006) reported on the high endocrine disruption capabilities of both androgenic and estrogenic growth promoting hormones.

2.2. Water and sediment quality changes in relation to feedlot activity

2.2.1. In situ variables

In situ variables consist of pH, conductivity, oxygen content as well as temperature. These variables refer to a physical-chemical state of the water within the river. In situ variables also act as primary driving forces for the chemical composition of the applicable system. If, for example, the pH changes, it will alter the chemical species composition and the same principle applies for conductivity and temperature (Cooper and Lipe, 1992).

Natural background variation in temperature for any given system is a function of hydrological, climatological and geomorphological characteristics of that system (Dallas and Day, 2004). The position of the river in space and time, as well as the climatic conditions will
contribute towards the temperature measured for a specific site within the river. Other factors that also contribute towards thermal variation are topographic features, vegetation cover, channel form, water volume, water depth and turbidity (Dallas and Day, 2004).

Feedlot activity may alter water temperature in associated systems through stream regulation (Ward and Stanford, 1982) and changes in riparian vegetation (Rutherford et al., 1997). Stream regulation usually occurs in the form of dams (Pitchford and Visser, 1975), which are designed to accumulate water for consumption by cattle (approximately 20 litres of water per individual per day). Flood retention ponds are also employed to accumulate feedlot runoff before it re-enters an associated river system. Riparian vegetation removal allows more direct solar radiation to enter the water body thus increasing water temperature variation (Graynorth, 1979).

In addition to thermal variation, pH also needs to be considered. pH is a function of the dynamic equilibrium between hydrogen, hydroxyl, bicarbonate, and carbonate ions (Dallas and Day, 2004). For a more detailed discussion on the chemistry; with regard to pH, alkalinity and buffers; the reader is referred to Golterman et al. (1978). pH is defined as the negative log$_{10}$ of the hydrogen ion activity. Freshwater systems in South Africa are relatively well buffered and pH varies between six and eight (Dallas and Day, 2004).

pH variation needs to be considered when assessing the impact of feedlot activity on freshwater systems as alteration in pH will dictate chemical species dynamics. Ammonium ions, for instance, that are naturally associated with feedlot activity are not toxic at pH values under eight, however, pH values over eight gradually convert non toxic ammonium to more toxic un-ionized ammonia (Dallas and Day, 2004).

Another in situ variable contributing to the physico-chemical composition of a water body is the concentration of dissolved oxygen. With the exception of some facultative and obligate anaerobe invertebrate species, all other aquatic biota are dependent on the dissolved oxygen concentration within water. The variations in dissolved oxygen concentration are largely a function of temperature and air pressure (Dallas and Day, 2004). Secondary factors, such as channel width, hydrology, salinity, chemical oxygen demand (COD) and biological oxygen demand (BOD) will also alter the amount of dissolved oxygen in the water.

Feedlot activity, by its very nature, impacts on the salinity and, to a lesser extent, temperature of associated systems. The solubility of oxygen in water inversely relates to both temperature and salinity (Dallas and Day, 2004). Thus increased concentrations of salts (originating from cattle feeding activities) will decrease the amount of oxygen available for utilisation by aquatic organisms and this in turn selects for anaerobic organisms.
Chapter 2

An *in situ* variable also considered within the scope of this study is alteration in conductivity of water systems associated with feedlot activity. One of the better established indicators of general water quality is the total amount of material dissolved in the water (Dallas and Day, 2004). Typically, this is referred to as the conductivity of the water and can be regarded as a driving force behind the biological characteristics of an aquatic ecosystem. The greatest mass of total dissolved solids characteristically comprises of inorganic ions which include cations and anions. These ions together with organic ions, such as humic and fluvic acids, contribute towards the ability of the water to conduct electricity.

### 2.2.2. Nutrients

Both organic and inorganic nutrient enrichment was considered within the framework of this study to ascertain the possible implications of feedlot activity on the freshwater aquatic system. Nutrients refer to elements needed by primary producers for growth and reproduction. Subsequently, these can be divided into macro nutrients (carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sulphate, and silica) and various micro nutrients (Addiscott *et al.*, 1991).

Nitrogen and phosphorus are often responsible for nutrient enrichment of freshwater systems (Dallas and Day, 2004). Phosphorus is the limiting factor in primary production in freshwater systems (Hart *et al.*, 1992, Correl, 1998). However, Dodds and Welch, (2000) suggested that both nitrogen and phosphorus are limiting factors in primary production of freshwater systems. Nutrient enrichment is commonly referred to as eutrophication and is naturally associated with an imbalance in ecosystem dynamics, functionality and integrity.

Feedlot activity contributes to eutrophication as it increases phosphorus, phosphate ions, nitrogen and ammonium ions in the associated freshwater systems (Dallas and Day, 2004). Nitrogen and phosphorus may also occur in organic forms (i.e. proteins) but are used by plants in their inorganic form. Microbes convert organic nitrogen and phosphorus to their inorganic form (Wood, 2001).

### 2.2.3. Metals

Metal concentrations are naturally low in unpolluted water, for this reason metal contamination usually is associated with high toxicity potential. Unnatural sources of trace metals are typically: industrial effluent, agricultural runoff and acid mine drainage (Dallas and Day, 2004). Metal toxicity should always be interpreted in relation to other physico-chemical variables of the water. Nickel, for instance, has a species distribution dictated by pH with a higher pH relating to a smaller risk factor and lower toxicity, while a drop in pH contributes to
a higher bioavailability, and thus a higher toxicity. Metals that were analysed for the purpose of this study include: aluminium (Al), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn).

Aluminium is one of the more toxic trace metals and its solubility is strongly pH dependent. At higher pH measurements Al is present as soluble hydroxide complexes; which are not bioavailable. At intermediate pH values, it is sparingly soluble and occurs as hydroxy- and polyhydroxy- complexes while under acidic conditions it occurs as the soluble, bioavailable and toxic hexahydrate species (Dallas and Day, 2004). For a more detailed discussion on the toxic effects of Al exposure on biota the reader is referred to Chadwick and Whelan (1992). Prominent concentrations of available Al in water are toxic to a variety of organisms. There is uncertainty with regard to the form of bio-available Al as well as the mechanism of toxicity (DWAF, 1996). The toxic effects are correlated to variation in pH and Calcium (Ca) concentration. The pH may not only affect the chemistry of Al but may also determine how the organism responds to dissolved Al. In acidic waters, Al is generally more toxic over the pH range of 4.4 - 5.4, with maximum toxicity occurring about pH 5.0 - 5.2 (DWAF, 1996). It has been suggested that Al interferes with Ca metabolism, thus changing the functionality of the Ca regulating protein, calmodulin. Aluminium has been shown to interfere with ion exchange sites, in particular those involved with sodium homeostasis. This, in turn may lead to neuromuscular dysfunction (DWAF, 1996).

Cadmium is a trace metal classified as a “hazardous candidate”. It is easily absorbed by animals, in which it becomes concentrated by binding with a protein called metallothionein (Dallas and Day, 2004). It is known to inhibit bone repair mechanisms and to be teratogenic, mutagenic and carcinogenic (Dallas and Day, 2004; Eisler, 1985). Many plant and animal tissues contain Cd, but there is no evidence that Cd is biologically essential or beneficial. Cadmium is chemically similar to Zn and its physiological effects are often due to its replacement of Zn in some enzymes, thereby impairing enzyme activity. Cadmium is known to inhibit bone repair mechanisms. and bioavailable Cd may be accumulated by macrophytes, phytoplankton, zooplankton, invertebrates and fish (DWAF, 1996). Bioavailability is dependent on Cd speciation; for example, the free ion, Cd$^{2+}$, is readily taken up by aquatic plants, whereas organo-cadmium complexes are not absorbed. Lethal concentrations of Cd also vary, depending on the test animal, water hardness and temperature, and time of exposure. The level of bio-accumulation is dependent on the species and age of the organism. Cadmium bio-accumulates in the food chain due to its tendency to bind strongly to sulphhydril groups (DWAF, 1996).
Chromium is one of the least toxic of the trace metals at low concentrations. It, in fact, forms part of the glucose tolerance factors within most mammals (Dallas and Day, 2004). At higher concentrations, however, it is teratogenic, genotoxic, mutagenic, and carcinogenic (Dallas and Day, 2004; Eisler, 1986). Chromium exerts a toxic effect at different concentrations in different groups of aquatic organisms. Fish are the most resistant, and in some cases the toxicity of Cr(VI) is no greater than for Cr(III). A temporarily reduced growth phase has been reported for young fish at low Cr concentrations (DWAF, 1996). Invertebrates are more sensitive; with daphnia showing the greatest sensitivity to Cr. Green algae are also more sensitive than fish, whilst bacterial responses to Cr are variable.

Copper is a micronutrient and is involved in various redox reactions in cells. However, it can still be regarded as toxic at relatively low concentrations. The toxicity of Cu is reduced in the presence of Zn, molybdenum and sulphate as well as higher pH levels. At low pH values Cu become soluble, mobile and thus bioavailable. At higher pH levels, Cu precipitates out and is non-toxic. Dallas and Day (2004) state that, although Cu concentrations may be high in humic waters, they are bound to organic molecules and are unavailable as toxins. Copper is toxic at low concentrations in water and is known to cause neurological damage in mammals. Copper exerts its effect by forming stable co-ordinate bonds in proteins, where it functions as a catalyst in redox reactions. Metabolically, Cu interacts with zinc, molybdenum, arsenic and selenium. The effects of elevated Cu concentrations on aquatic organisms are also related to factors such as the duration of exposure and life stage of the organism. Studies have shown that species richness and species composition of invertebrate communities are changed as Cu concentrations increased (DWAF, 1996). Early life stages of organisms appear to be more sensitive than adults to Cu pollution. Nitrogen fixation by blue-green algae is reduced by the addition of trace amounts of Cu (DWAF, 1996).

Iron is an abundant micronutrient, and is important in a number of biological processes (oxygen transportation, catalases, cytochromes and peroxidases). Iron compounds are relatively easily oxidised and therefore high concentrations of reduced forms can result in oxygen depletion in the environment (Dallas and Day, 2004).

Lead is a common and toxic trace metal which readily accumulates in living tissue. Metabolically, Pb interacts with Fe and therefore interferes with haemoglobin synthesis. It also affects membrane permeability by displacing calcium at functional sites, and inhibits some of the enzymes involved in energy metabolism (DWAF, 1996). The toxicity of Pb is linked to the hardness, pH and salinity of the water (Merlini and Pozzi, 1977). Lead concentrations generally have greater background concentration in South Africa (Heath and Claassen, 1999). Lead tends to accumulate easily in aquatic biota and it is immobilised in
vertebrate bone structure where it might be remobilised during physiologically stressful episodes, such as sickness and old age (Dallas and Day, 2004). It has been shown that rainbow trout develop spinal deformities after exposure to Pb in soft water, while no deformities were evident in hard water (DWAF, 1996).

Manganese acts as an important micronutrient for vertebrates and plays a role in a number of physiological processes (Bervoets et al., 2001; Haux, 2001). An excess of Mn has been implicated in disturbances in the central nervous system by inhibition of dopamine formulation (Dallas, and Day, 2004).

Referring to Ni, it is estimated that half of the Ni present in surface water is in the ionic form, while the remaining half consists of stable organic complexes which are easily absorbed into clay particles (Dallas and Day, 2004). The toxicity of Ni shows an inverse correlation with pH values. At lower pH values (<6.5) Ni tends to be soluble and mobile, thus an increased toxicity can be expected (DWAF, 1996).

Zinc is an essential micronutrient as it is known to bind with metallothionein in mammals. Zinc is also associated with Cd in natural conditions (Dallas and Day, 2004). Harsh imbalances can cause death, while subsidiary imbalances contribute to reduced fitness. The lethal effect of Zn on fish is due to the formation of insoluble compounds in the mucus covering the gills. Sub-lethal concentrations at which toxic effects are evident depend on the concentration ratio of Zn to Cu, because Zn interferes with Cu absorption. Observed symptoms include depressed white blood cell-thrombocyte counts. Observed effects of prolonged exposure to sub-lethal concentrations of Zn in fish fry include oedema and liver necrosis (DWAF, 1996). Although invertebrate responses to Zn toxicity vary, molluscs are generally more resilient than are other organisms. Sub-lethal effects include reduced rates of shell growth, oxygen uptake and larval development. Algal photosynthesis can also be repressed by Zn exposure (DWAF, 1996).

### 2.2.4. Sediment composition

Soil mineral particles, associated with the river bed, can be grouped together into five broad classes, stones or gravel, coarse sand, fine sand and silt and clay (Ashman and Puri, 2002). These different partial sizes of soil samples were determined for sites located up- and downstream of rivers, which were associated with various feedlots. Percentage grain size and organic composition of soil samples are an important consideration in assessing the effects of feedlot activities on the aquatic environment.
Percentage grain size
Sediment is an important factor to consider in any toxicological study. Soil texture and structure contribute to the soil’s ability to accommodate the movement of nutrients and water (Asmen and Puri, 2002). Grain size analysis indicates the amount of small clay particles present at each site. Clay shows a high affinity for organic matter, which acts as storage for pollutants and toxins. Variation in grain size composition will also indicate alterations in hydrology.

Percentage organic matter
The amount of organic matter present in the soil will indicate the ability of the soil to assimilate a toxicant. Organic matter in soil also plays a very important role in soil formation as well as nutrient and gas cycling. Organic (humic) matter forms complexes with a number of trace metals, such as Ni and Cu, thereby immobilising the metals and lowering toxicity (Merlini and Pozzi, 1977).

2.3. Determining the effect of feedlot activity on the Present Ecological State
River Health Indices (RHI) have been used in PES determination since 1994 (DWAF, 2001). The main aim of the indices is to assess and categorise a current state for any given system that is linked to the national River Health Programme (RHP) (DWAF, 2001). The main objective for the RHP is to measure, assess and report on the ecological state of aquatic ecosystems.

The RHI provide a measure of ecological integrity based on community structures. In other words, any alteration in abiotic driving conditions (water quality) would reflect in the aquatic macroinvertebrate and fish community composition. Additional to that, supporting habitat assessment indices have also been developed.

River Health Indices typically implemented in the RHP, have been used in this study to ascertain the PES of both upstream and downstream sites associated with different cattle feedlot activities. By applying an upstream-downstream assessment, a source of possible pollution can be determined, indicating point source pollution, as well as the relative intensity thereof (Chutter, 1998).
This section deals with the components of the RHP in order to establish a PES. Firstly, some consideration is given to aquatic biota as indicators, and then a short introduction on the associated habitat assessments follows thereafter.

### 2.3.1. Aquatic biota as Indicators

Aquatic organisms, more so than any other organism, are in direct contact with their environment, leading to a greater exposure risk with any alteration in water quality. This characteristic of aquatic organisms renders them good indicators for alterations in environmental conditions (Dallas and Day, 2004; Plafkin et al., 2002). Aquatic macroinvertebrates and fish are often used as indicators of ecological status or health. Both of these indicators make use of the taxonomic composition of biotic systems. It should be noted, however, that a single system does not provide a complete quantitative analysis of all the organisms present in a river; rather each contributes toward assessing certain aspects of the integrity of the ecosystem (Dallas and Day, 2004).

Biotic indices, based on aquatic macroinvertebrates, are generally considered to be a useful tool for measuring ecosystem health (Chutter, 1972; Hellawell, 1986; Rosenberg and Resh, 1993). Macroinvertebrates have rapid lifecycles and are diverse in habitat preference. In addition to this, they are confined to relatively small and specific areas (far more so than fish, for instance). Many countries are now employing invertebrate indices as the primary measure of ecological state determination (Gerritsen et al., 2000). In South Africa the South African Scoring System (SASS 5) forms the backbone of the RHP (Dickens and Graham, 2002).

Biotic indices based on fish are less preferred, due to higher implementation costs; but are still powerful tools in assessing ecosystem health. Fish are relatively long lived and are good indicators of changes in river habitat. The Fish Assessment Integrity Index (FAII) encompasses the number of species found, their respective sensitivity to various forms of disturbances, preference to particular conditions and general health of the fish, to ascertain an overall expression of ecological health (DWAF, 2002). The result of the FAII is expressed as a ratio of observed conditions versus theoretical near-natural conditions.

### 2.3.2. Habitat assessments

The RHP also employs as an overall habitat assessment tool: the Index of Habitat Integrity (IHI). River habitats consist of in-stream and riparian habitat components and both are impacted by agricultural activities. The IHI provides a tool for assessing these habitat types. It incorporates factors and potential impacts, such as water abstraction, flow regulation, bed
and channel modification, removal of indigenous riparian vegetation as well as encroachment of alien vegetation, on pools, rapids, sandbanks, stones on the riverbed and vegetation fringing the water’s edge. Knowledge of the availability and quantity of habitats are central to an overall assessment of ecosystem health, since these are major determinants of whether a given system can sustain biota (DWAF, 1996).

In addition to the IHI and, incorporated into the SASS 5 protocol, is an Invertebrate Habitat Assessment Score (IHAS), which provides a quantitative and comparable description of habitat availability.

### 2.3.3. Aquatic macroinvertebrate community structures

In addition to the SASS 5 protocol used in this study; another, more sensitive, assessment was done in an effort to determine the effects of feedlot activity on aquatic ecosystem integrity. Macroinvertebrate community structure analyses in terms of diversity, community comparison and reduced assemblages were utilised for the purpose of this assessment. The use of alteration in invertebrate community structures as a measure of ecological stress have been used extensively in the past, for industrial, residential and agricultural impact (Chutter, 1998). Neumann and Dudgeon (2001) indicated that agricultural runoff does impact on stream benthos, with communities downstream from agricultural activities showing a decrease in species diversity. Berenzen et al. (2005) also demonstrated that invertebrate community structures change when exposed to runoff containing pesticides. A decrease in diversity and an increase in abundances of tolerant invertebrate families were noted. In another study, Probst et al. (2004) effectively linked land use variables with alterations observed in invertebrate community structures.

### 2.4. Using biomarker responses to determine the effects of exposure to growth promoting hormones

In order to establish the possible physiological implications of growth promoting hormone exposure at sub-cellular and cellular levels, two biomarkers were chosen. Firstly, CEA at cellular level (De Coen and Janssen, 1997) and secondly, metabolomics (Vaint et al., 2003) at a sub-cellular level were applied. It is expected that the exposure to growth hormones will impact on both CEA and metabolism of test organisms. Responses at molecular and cellular levels can be sensitive biomarkers of environmental exposure and biological effects (Stegeman et al., 1992). At present there is still a lack of literature referring to biomarkers
that indicate effects following exposure to pharmaceutical and veterinary products. The potential of using metabolomics as a functional tool in ecotoxicology has not been explored fully. Previous studies proved that CEA is a valuable tool in the assessment of the physiological stress in invertebrates following exposure to metal pollutants (Moolman et al., 2006) but it is yet to be tested on aquatic organisms that have been exposed to growth hormones.

2.4.1. Cellular Energy Allocation

The CEA methodology was developed as a biomarker technique to assess the effects of toxic stress on the energy budget of exposed organisms (De Coen and Janssen, 1997). It can be considered a short term biochemical assessment that combines the variation in the energy reserve (lipids, protein and sugar) with energy consumption (amount of oxygen consumed) to form an indicator of physiological stress. Organisms exposed to a stress-inducing environment show higher energy consumption per unit time than organisms that are not exposed to a stressful environment. Although the concept of CEA is relatively new (Verslycke and Janssen, 2001), the rate of energy consumption has been used extensively since 1975 when Owens and King (1975) measured the amount of oxygen consumed by marine zooplankton exposed to different compounds. De Coen and Janssen (1997) developed the new methodology to assess the energy budget of toxicant-stressed Daphnia magna. The ecological relevance of the CEA assay was established through positive correlations between sub-organism effects and population level effects (De Coen and Janssen, 1997). From these results, it was suggested that short-term CEA assay can be used to predict long-term effects at the population level.

Another study done by De Coen and Janssen (2003), confirmed the relationship between CEA and population characteristics. Verslycke and Janssen (2001) assessed the effects of abiotic environmental variables on Neomysis integer, using CEA as a biomarker. Smolders et al. (2003) performed a similar study, using CEA, and also linked cellular effects to higher levels of biological organisation. It is suggested that the energy budget at cellular level provides the fastest and most sensitive response. They concluded that energy budgets were a relevant means to extrapolate cellular effects to higher levels of biological organisation within the tested organisms.

It is evident that CEA has been used internationally with great success (De Coen and Janssen, 1997; Verslycke et al., 2007). However, the literature available on the use of this biomarker for determining the effects of growth hormones in the lotic aquatic environment is limited. A few studies show the potential of CEA as a biomarker on EDCs, as is evident in
Verslycke et al. (2007) whereby the estuarine mysid (N. integer) was exposed to a number of possible EDCs, including testosterone, and then assessed using the CEA assay. It was suggested that energy metabolism endpoints can be used to detect endocrine disruptive activity of chemicals after short-term exposure.

2.4.2. Metabolomics

Metabolomics can be considered the systematic study of the unique fingerprinting that cellular processes leave behind (Daviss, 2005; Nicholson et al., 1999). A metabolome, on the other hand, refers to the complete set of small molecular metabolites found within a biological sample (Oliver et al., 1998). Metabolites are the intermediates and end products of metabolism. A primary metabolite is directly involved with growth, development and reproduction, while secondary metabolites are not directly involved.

Metabolomics has been the subject of numerous reviews in recent years (Bino et al., 2004; Fernie et al., 2004; Fiehn, 2002; Goodacre et al., 2004; Lindon et al., 1999; Nicholson and Wilson., 1989; Weckwerth, 2003) and a volume on metabolic profiling was recently published (Harrigan and Goodacre, 2003). A large body of literature exists, discussing the applications of metabolomics in human toxicology (Griffen, 2003; Lindon et al., 1999; Nicholson et al., 1999; Robertson and Bulera, 2000; Schockor and Holmes, 2002).

Exposure to a variety of anthropogenic compounds has been shown to interfere with normal development, physiology and reproduction of organisms (Moens et al., 2006). The complex nature of the endocrine system makes it hard to establish the cause of possible stressors. As a result, a vast number of chemicals (approximately 86 000) that are used commercially, have not been adequately tested for possible endocrine disruption (Moens et al., 2006). There is, therefore, a need for a biomarker that is capable of indicating stress induced by the exposure to EDCs.

Metabolomics in its initial tactical phase has been in existence for quite some time. The chromatographic separation techniques that made the initial detection of metabolites possible were developed in the late 1960s (Pretricoin and Lance, 2003). Metabolomics began to develop further in 1970, when researchers realised that the chromatographic patterns of urine from vitamin B6-loaded subjects contain much more useful information (Robertson, 2005). The core suggestion was that the metabolites of body fluid are rich in information that reflect the functional status of a complex biological system (Robertson, 2005). In the last three years the value of metabolomics have been realised in the discipline of ecotoxicology, as a measure of toxicity assessment. To understand the complexity of a biological system, one has to take a closer look at the sub-cellular products of the system.
Bino et al. (2004) report that the current state of metabolomics allows the user to infer relevant associations between macromolecules, identify functional linkages between phenotypic expressions and construct models that quantitatively describe the dynamics of the biological system.
Chapter 3

Study Area and Site Description

3.1. Introduction

This chapter deals with study area, the river systems associated with each feedlot and the connection thereof to the greater catchment and drainage area. In addition to this, abiotic factors such as average precipitation, temperature and the general geology and soil types found in each study area is presented. Detailed descriptions of each sampling site are given, focussing on location, catchment conditions, land-use, channel condition, canopy cover and stream dimensions.

This study forms part of a larger study that aims to determine the overall effects of veterinary products used in cattle farming on freshwater systems. This includes rivers, wetlands and groundwater sources. Information was gathered for the 10 largest cattle feedlots out of the possible 47 registered in South Africa.

This information was represented on a GIS system and overlaid with other applicable variables such as, rivers, wetlands, geomorphology, underground water bodies and soil types. With this information at hand, sites most likely to be contaminated were selected. The Delmas, Bronkhorstspruit and Heidelberg areas in Gauteng and Mpumalanga were deemed to be at highest risk of contamination. Subsequently the following feedlots were selected: Feedlot A (Heidelberg Gauteng) with 85 000 cattle, Feedlot C (Bronkhorstspruit, Mpumalanga) with 45 000 cattle. A third feedlot of a smaller magnitude was added: Feedlot B (Potchefstroom, North-west Province) with 20 000 cattle. At each of the feedlots, an associated upstream and downstream site was selected (Table 3.1) indicates the coordinates as well as the altitude for each site. These sites were regarded as suitable if they were accessible and located in relatively close proximity to the feedlots. Upstream sites were selected as reference sites and results from downstream sites were compared relative to upstream sites. Upstream sites varied in location and were between 300 m to 1 km from the feed lots. This allows for a buffer zone for migrating fish and invertebrate species.
### 3.2. Feedlot A

#### 3.2.1. Introduction

Feedlot A is situated approximately 10 km outside of Heidelberg east of Johannesburg. It is a privately owned feedlot with the capacity to accommodate 85,000 cattle at any one time. The feedlot farm area is 2,500 ha in size with 48 ha utilised for the actual feeding (Vegter, 2006).

The greater area falls within the Soweto Highveld grassland biome and is characterised by an undulating landscape with deep red soils (Mucina and Rutherford, 2006). The area is water stressed as the potential evaporation rate far exceeds the average precipitation rate. The average temperature and the Mean Frost Days (MFD) also have to be considered in relation to the other feedlot areas, as this will influence changes in water temperature and thus also the physical-chemical properties of water bodies associated with each feedlot area (Table 3.2).

The feedlot itself is situated on a slight hill slope and at the bottom of the slope is the Suikerbosrand River flowing in a north-south direction. The Suikerbosrand River is joined by the Blesbokspruit on the north-western corner of the feedlot. Both of these rivers form part of the Upper Vaal water management area (WMA).

Both the Suikerbosrand River and the Blesbokspruit are historically seasonal systems but have subsequently been altered by heavy industrial and residential development. The more permanent nature and periods of high inundation has increased the velocity of the system. The previously meandering Suikerbosrand River is now much more canalised, resulting in remnant oxbow lakes.

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### Table 3.1: Site names, coordinates and altitude.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates</th>
<th>Altitude (m/sl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>South</td>
<td>East</td>
</tr>
<tr>
<td>Feedlot A Upstream</td>
<td>26° 37' 47.8&quot;</td>
<td>28° 17' 47.9&quot;</td>
</tr>
<tr>
<td>Feedlot A Downstream</td>
<td>26° 36' 44.9&quot;</td>
<td>28° 15' 09.2&quot;</td>
</tr>
<tr>
<td>Feedlot B Upstream</td>
<td>26° 56' 15.8&quot;</td>
<td>27° 03' 57.7&quot;</td>
</tr>
<tr>
<td>Feedlot B Downstream</td>
<td>26° 56' 11.1&quot;</td>
<td>27° 02' 03.7&quot;</td>
</tr>
<tr>
<td>Feedlot C Upstream</td>
<td>25° 55' 32.9&quot;</td>
<td>28° 35' 09.0&quot;</td>
</tr>
<tr>
<td>Feedlot C Downstream</td>
<td>25° 55' 48.8&quot;</td>
<td>28° 36' 48.7&quot;</td>
</tr>
</tbody>
</table>

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20 | P a g e
Table 3.2: Environmental features of area associated with Feedlot A (Mucina and Rutherford, 2006).

<table>
<thead>
<tr>
<th>Environmental features</th>
<th>Unit Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioregion</td>
<td>Soweto Highveld grassland</td>
</tr>
<tr>
<td>Ecoregion Level 1</td>
<td>Lower Highveld</td>
</tr>
<tr>
<td>Landscape features</td>
<td>Undulating landscape</td>
</tr>
<tr>
<td>Geology</td>
<td>Shale and Sandstone</td>
</tr>
<tr>
<td>Soils</td>
<td>Deep, reddish soils</td>
</tr>
<tr>
<td>MAP</td>
<td>662 mm</td>
</tr>
<tr>
<td>MAT</td>
<td>14.8 ºC</td>
</tr>
<tr>
<td>MFD</td>
<td>41 d</td>
</tr>
<tr>
<td>MAPE</td>
<td>2060 mm</td>
</tr>
<tr>
<td>MASMS</td>
<td>75 %</td>
</tr>
</tbody>
</table>

MAP: Mean Annual Precipitation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress.

3.2.2. Upstream site

The upstream site is situated south of Feedlot A. The site hosts diverse micro habitats, with velocity-depth classes ranging from fast shallow rapids to deeper slower flowing pools (Figure 3.1 A-D). The channel morphology consists predominantly of mixed bedrock and alluvial sedimentation, with sand, gravel, cobbles and boulders present. The canopy cover is patchy and most of the channel receives direct sunlight. For two weeks prior to sampling there was no significant rainfall event. The macro channel width is approximately 5 m to 10 m, while the active channel width is 2 m to 5 m wide, with banks on either side between 1 m and 3 m high.

Conditions of the local catchment are the product of upstream agricultural, mining, urban and industrial activities. Both alien macrophytes and alien vegetation was observed in-stream and in the riparian zone. The banks appeared to be relatively stable. However, clear signs of flash-flood erosion were visible on the left and right banks.
3.2.3. Downstream site

The downstream site stretches over 100 m in width of the Suikerbosrand River and is situated west of Feedlot A. The site has a greater macro channel width (> 15 m) resulting in lower flow velocity although, there are still a number of micro habitats available (Figure 3.2 A-D). The channel morphology is consistent with that of the upstream site and consists predominantly of mixed bedrock and alluvial sedimentation; however, there are more boulders and less gravel, sand and mud. Both banks are completely covered with *Phragmites australis*, thus no canopy cover exists.

For two weeks prior to the time of sampling there was no significant rainfall event. Due to the high level of inundation, both the macro channel and active channel width ranged between 10 m and 20 m. Banks on either side of the channel were less steep than that of the upper site and varied between 1 m and 2 m in height. Conditions of the local catchment are dictated by upstream agricultural activities (including those of Feedlot A). Little erosion
was observed at the site, however, the riparian zone was characterised by low vegetation diversity.

Figure 3.2: (A-D) Photos showing channel conditions and stream dimensions downstream from Feedlot 3.4 Feedlot B

3.3. Introduction

Feedlot B is situated between Potchefstroom and Scandinavia drift on the North-west and Free State Province border. It is smaller in comparison to the other two feedlots and has an estimated capacity of 20 000 cattle. Both feedlot A and Feedlot C were located in closer proximity to the associated river systems than Feedlot B. In addition to this, the associated river, which is the Vaal River, is also much greater in size and water throughput than the rivers connected to the other feedlots in question and thus has a much greater dilution factor. However, the Upper Vaal is also under great agricultural, mining and industrial stress.

The greater area associated with the study sites are classified under the Vaal- Vet Sandy grassland biome (Table 3. 3). The landscape consists mainly of undulating plains and hills,
with the dominant geology being aeolian and colluvial sand over sandstone and shale. The main soil types are Avalon, Westleigh and Clovelly forms. In comparison with the other feedlot study areas the associated bioregion receives less rainfall, with an annual precipitation rate of 530 mm. With the highest mean temperature of 16.4 °C this study area has the greatest precipitation deficit with a potential evaporation rate of 2423 mm per annum (Mucina and Rutherford, 2006).

3.3.1. Upstream site

The upstream site is situated on the left hand bank of the Vaal River, above the feedlot activity. The river flows past the feedlot from west to east. Even though the extent of inundation was relatively low, there was still a diverse micro habitat available (Figure 3.3 A-B). Flow velocities differed from fast-shallow to slow-deep. Sufficient substrate, providing fish refuge was available. Some canopy cover was noted; however it was provided by alien vegetation, but this is limited to the marginal shore zone.

The right hand bank reflected poor vegetation cover and erosion was clearly visible. The macro channel was greater than 100 m, while the active channel width was between 50 and 80 m wide. Banks on both sides are subjected to grazing and cutting activities and are, thus poorly covered. Water abstraction, presumably for the feedlot, was clearly visible. Some of the other impacts observed were the presence of alien macrophytes and alien vegetation encroachment into the riparian zone.
Table 3. 3: Environmental features of area associated with Feedlot B (Mucina and Rutherford, 2006).

<table>
<thead>
<tr>
<th>Environmental features</th>
<th>Unit Type</th>
</tr>
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<tbody>
<tr>
<td>Bioregion</td>
<td>Vaal-Vet Sandy grassland</td>
</tr>
<tr>
<td>Ecoregion Level 1</td>
<td>Lower Highveld</td>
</tr>
<tr>
<td>Landscape features</td>
<td>Undulating plains and hills</td>
</tr>
<tr>
<td>Geology</td>
<td>Aeolian and colluvial sand over sandstone and shale</td>
</tr>
<tr>
<td>Soils</td>
<td>Avalon, Westleigh and Clovelly</td>
</tr>
<tr>
<td>MAP</td>
<td>530 mm</td>
</tr>
<tr>
<td>MAT</td>
<td>16.4 °C</td>
</tr>
<tr>
<td>MFD</td>
<td>37 d</td>
</tr>
<tr>
<td>MAPE</td>
<td>2423 mm</td>
</tr>
<tr>
<td>MASMS</td>
<td>79 %</td>
</tr>
</tbody>
</table>

MAP: Mean Annual Precipitation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress.

Figure 3. 3: Photos showing channel conditions and stream dimensions upstream from Feedlot B.

3.3.2. Downstream site

The downstream site stretches over 80 m of the Vaal River and is situated on the left hand bank, below Feedlot B. The site generally has a smaller macro channel width than that of the upstream site (Figure 3.4 A-B). There are a number of micro habitats available. The channel morphology is consistent with that of the upstream site and consists predominantly of mixed bedrock consisting of boulders and cobbles, and alluvial sedimentation in the form of gravel,
sand and mud. The downstream site banks are well covered and buffered from agricultural activities. Banks on either side of the channel were less steep than that of the upstream site. Conditions of the local catchment are dictated by upstream feedlot activities as well as some additional agricultural activities.

Figure 3.4: Photos showing channel conditions and stream dimensions downstream from Feedlot B.

### 3.3. Feedlot C

#### 3.3.1. Introduction

Feedlot C is situated just outside of Bronkhorstspruit, north-east of Johannesburg, on the Gauteng-Mpumalanga border. Little technical information is available on the feedlot, however, it is estimated that it has a capacity of 45 000 cattle. The surrounding bioregion is classified as Rand Highveld grassland and is characterised by elevated ridges and undulating plains (Mucina and Rutherford, 2006). A generalisation of the geology reveals predominantly quartzite ridges with underlying sandstone and shale (Table 3.4).

The main soil types are shallow Glenrosa and Mispah forms. The average temperature and annual rainfall is slightly higher than the study area associated with Feedlot A. Nonetheless, the area also appears to be water stressed as the potential evaporation rate far exceeds the average rainfall (Mucina and Rutherford, 2006). The stream directly associated with Feedlot C is the Os Spruit. The spruit has its origin approximately 5 km to 10 km upstream from Feedlot C and probably receives its base flow from surrounding Highveld pan systems and, consequently, is highly seasonal and oligotrophic in nature. The Os spruit itself is a tributary of the Bronkhorstspruit Dam located within the Bronkhorstspruit nature reserve. Agricultural
activities are the main impacts on the Os Spruit, almost the entire direct catchment area connected with it is utilised for livestock farming, ranging from chicken farming to cattle production.

Table 3.4: Environmental features of area associated with Feedlot C (Mucina and Rutherford, 2006).

<table>
<thead>
<tr>
<th>Environmental features</th>
<th>Unit Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioregion</td>
<td>Vaal-Vet Sandy grassland</td>
</tr>
<tr>
<td>Ecoregion Level 1</td>
<td>Lower Highveld</td>
</tr>
<tr>
<td>Landscape features</td>
<td>Undulating plains and hills</td>
</tr>
<tr>
<td>Geology</td>
<td>Aeolian and colluvial sand over sandstone and shale</td>
</tr>
<tr>
<td>Soils</td>
<td>Avalon, Westleigh and Clovelly</td>
</tr>
<tr>
<td>MAP</td>
<td>530 mm</td>
</tr>
<tr>
<td>MAT</td>
<td>16.4 °C</td>
</tr>
<tr>
<td>MFD</td>
<td>37 d</td>
</tr>
<tr>
<td>MAPE</td>
<td>2423 mm</td>
</tr>
<tr>
<td>MASMS</td>
<td>79 %</td>
</tr>
</tbody>
</table>

MAP: Mean Annual Precipitation; MAT: Mean Annual Temperature; MFD: Mean Frost Days; MAPE: Mean Annual Potential Evaporation; MASMS: Mean Annual Soil Moisture Stress.

3.3.2. Upstream site

The upstream site encompasses approximately 80 m of the Os Spruit and is situated above Feedlot C. The condition of the local catchment is dictated by livestock production and other agricultural activities. The site hosts diverse micro habitats, ranging from fast-shallow rapids to deeper slower flowing pools, with average water depth of 0.5 m (Figure 3.5 A-B) The channel morphology consists of predominantly mixed bedrock and alluvial sedimentation, with an equal distribution of sand, gravel, cobble and boulders present. There is no canopy cover as the riparian zone is dominated by grasses and sedges. For two weeks prior to time of sampling there was no significant rainfall event. The macro channel width ranges between
Chapter 3

2 m and 5 m, while the active channel width is 1 m to 2 m wide, with the right hand bank slightly higher than the left hand bank. Banks on both sides are subjected to grazing and cutting activities and are thus poorly covered and prone to erosion. Some bed and channel modification is visible with a weir and a road crossing the site.

3.3.3. Downstream site

The downstream site includes approximately 100 m of the Os Spruit and is situated below Feedlot C. The only activity occurring between the upstream and the downstream site is feedlot activity associated with Feedlot C. The site is poor in habitat diversity and consists almost entirely of slow deep flowing water (Figure 3.6). An obvious decrease in water quantity was also visible, indicating possible water abstraction by the feedlot activity. It must be noted that no bedrock, stones or cobbles were present at the site.

The channel morphology consists predominantly of alluvial sedimentation dominated by gravel, sand and mud. Some canopy cover is provided by alien vegetation. The left hand bank indicated poor vegetation cover and erosion is clearly visible. For two weeks prior to time of sampling there was no significant rainfall event. The macro channel width ranges between 5 m and 10 m, while the active channel width is 3 m to 6 m wide. Banks on both sides are subjected to grazing and cutting activities and are thus poorly covered. Similar to the upstream site some bed and channel modification is visible with a weir and a road crossing the site.

Figure 3.5: (A and B) Photos showing channel conditions and stream dimensions upstream from Feedlot C.
Figure 3.6: (A-D) Photos showing channel conditions and stream dimensions downstream from Feedlot C.
Chapter 4

Determining the Present Ecological State (PES) of aquatic ecosystems that are influenced by feedlot activity

4.1. Introduction

This chapter deals with the effects of feedlot activity on the aquatic environment. It also attempts to quantify the effects of growth hormone residue on aquatic ecosystems. It is expected that feedlot activities will alter some abiotic driving forces, such as water quality and sediment composition. Furthermore, it will also directly alter habitat availability. The use of PES assessment indices were used to indicate a biotic response to the abiotic alterations caused by feedlot activities.

Water quality (physical, nutrients and metals), sediment analysis (percentage grain size and organic matter) as well as PES indices were employed to determine the possible impacts of feedlot activity on the aquatic environment. These variables are compared between upstream and downstream sites.

4.1.1. Water quality

Water quality was considered in two ways: the first being the actual impact of feedlot activity on the water quality of the system and the second, how these alterations can influence the environmental fate of hormones entering the system. Nutrients refer to elements needed by primary producers for growth and reproduction and are problematic in high concentrations as they can stimulate unwanted primary producer growth in aquatic systems. Subsequently these can be divided into macronutrients (carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sulphate, and silica) and micronutrients (Addiscott et al., 1991). Faecal matter from feedlot activity is high in nutrient and salt content and, if not managed correctly, can easily pollute the aquatic environment (Khan et al., 2008).

In addition to the nutrients, metals associated with feedlot activities were also assessed. The accumulation of heavy metals such as Cd, Pb and Hg in soils is a potential concern due to
the capability of these elements to negatively influence environmental health (McLaughlin et al., 2000). Cattle manure is a possible source of low concentrations of some heavy metals. For example, Pb concentrations of 1.6–8.6 mg/kg and Cd concentrations of 0.1–0.7 mg/kg have been reported in cattle manure (Bolan et al., 2004). Metals in livestock excreta may be derived from the animal diet, either deliberately or as a result of pollution. For example, some lighter metallic elements (such as As, Co, Cu, Mn, Se and Zn) may be added to livestock feeds as essential nutrients or to improve feed exchange efficiencies (Bolan et al., 2004). However, heavy metals are more likely to be derived from the ingestion of contaminated soil by the animal (Khan et al., 2008; Loganathan et al., 1999). Unnatural sources of trace metals are, typically, industrial effluent, agricultural runoff and acid mine drainage (Dallas and Day, 2004). Some metals are known for their endocrine disrupting capabilities. These include: Cd (Anoop et al., 2003); As (Gebel, 2000); Pb (Graeme and Pollack, 1998) and Mercury (Hg) (Dallas and Day, 2004). Other metals measured and compared between sites and feedlots include: Al, Cr, Cu, Fe, Mn, Ni and Zn.

The second consideration is the environmental fate of hormones, bearing in mind the alterations in water quality. The available information suggests that steroid hormones slowly degrade in manure, soil and water, but the exact mechanisms or factors controlling the rates are not yet fully understood (Khan et al., 2008). Unconjugated steroidal hormones are chemically stable, not volatile, have low water solubility and are hydrophobic (Hanselman et al., 2003; Layton et al., 2000). Their environmental fate from livestock manure depends upon both storage conditions and management of the manure (Lange et al., 2002). These results are consistent with field observations where testosterone was shown to reach groundwater, while estrogen remained bound to the upper crust of the soil (Shore and Shemesh, 2003). Both testosterone and estradiol have been measured in surface runoff from soils treated with animal manure (Finla-Moore et al., 2000).

4.1.2 Sediment composition

Soil consistency and arrangement relate back to the soil’s aptitude to contain the movement of nutrients and water (Asmen and Puri, 2002). Grain size analysis will indicate the amount of small clay particles present at each site. Clay, in turn, shows a high affinity for organic matter, which acts as storage for hormones (Khan et al., 2008). Thus the sediment structure of the soil will assist in predicting the environmental fate of hormones. Once released to soils, the environmental fate of steroid hormones depends upon the nature of the soil (Lange et al., 2002; Khan et al., 2008). In particular, particle size and organic components strongly affect adsorption and migration in soils. 17β-estradiol and estrogen have high absorption affinities to soils (Casey et al., 2004; Casey et al., 2005; Colucci and Topp, 2002). The
absorption affinity has been well correlated with mineral particle size and organic matter content (Khan et al., 2008).

The organic matter fraction present in soil plays a role in soil development as well as nutrient and gas cycling. Synthetic hormones, trenbolone and melengestrol-acetate behave similarly to testosterone, having an affinity to the organic fraction of soils, leading to a high retardation, but remaining nonetheless mobile in agricultural soils (Lange et al., 2002). Soil properties such as organic matter content, can have a major effect on the mobility of specific heavy metals (McLaughlin et al., 2000). Organic (humic) matter forms complexes with a number of trace metals, such as Ni and Cu, thus immobilising the metals and leading to a lower toxicity (Merlini and Pozzi, 1977).

4.1.3. Ecological Classification

Ecological classification refers to the determination and categorisation of the PES of various biophysical attributes of rivers compared to that of the reference conditions (Kleynhans and Louw, 2007). The purpose of ecological classification is to gain insight into the causes and sources of deviation of the PES of biophysical metrics (Kleynhans and Louw, 2007). This will provide information on possible implications of feedlot activities on associated freshwater systems. River Health Indices typically implemented in the RHP have been used in this study to ascertain the PES of both upstream and downstream sites associated with different cattle feedlot activities. By applying an upstream-downstream assessment, a location of possible pollution can be determined, indicating point source pollution, as well as the relative intensity thereof (Chutter, 1998).

Habitat assessment

The RHP employs an overall habitat assessment, the IHI, (Kleynhans, 1996). River habitats consist of in-stream and riparian components, both of which are impacted by agricultural activities. The IHI provides a tool for assessing these habitat types by incorporating factors and potential impacts. Knowledge of the availability and quantity of habitats are central to an overall assessment of ecosystem health, since these are major determinants of whether a given system can sustain biota (DWAF, 1996). In addition to the IHI and incorporated into the SASS 5 protocol is an Invertebrate Habitat Assessment Score (IHAS), (McMillan, 1998) which provides a quantitative and comparable description of habitat availability for aquatic invertebrates sampled.
South African Scoring System version 5

A number of authors have assessed the ability of macroinvertebrates to ascertain the state of freshwater systems (Chutter, 1972; Hellawell, 1986; Rosenberg and Resh, 1993). Macroinvertebrates have brief lifecycles and have adapted to various habitats. A number of countries are now utilising invertebrate indices as the principal measure of ecological state determination (Gerritsen et al., 2000). In South Africa the SASS 5 index plays an important role in the RHP (Dickens and Graham, 2002). Macro-invertebrates are exceptional indicators of water quality as they are exposed to their surrounding environment and show changes in community structure in response to the water quality. The SASS 5 protocol is a biotic index primarily alluding to water quality of a particular system, based on the inhabitant macroinvertebrate community structure. The index encompasses three basic components: the number of taxa observed at a particular location, the overall sensitivity of the invertebrates present and the estimated abundances of each (Dallas, 1997).

Fish Assemblage Integrity Index

The FAII is based on the fish species expected to be present in biological (fish habitat) segments which are sections of river with relatively uniform fish habitat (Kleynhans, 1999). Within this framework fish are categorised according to an intolerance index which takes into account trophic preferences and specialisation, habitat preferences and specialisation, requirement for flowing water during different life-stages and association with habitats with unchanged water quality.

Fish are relatively long lived and are good indicators of changes in river habitat. These changes can be explained by possible alterations in hydrology, river structure or chemical composition of water. The FAII encompasses the number of species found, their respective sensitivity to various forms of disturbances, preference to particular conditions and general health of the fish, to determine an expression of ecological health (Kleynhans, 1999). The use of FAII in the assessment of point source pollution is limited. Under normal conditions it is used in conjunction with other ecosystem assessment tools when attempting to determine the heath and function status of any particular system.
4.2. Materials and Methods

4.2.1. Field survey

With reference to Chapter 3, three feedlots were chosen for assessment. An upstream and downstream site was selected on a recogniscance visit in February 2007. Each site was surveyed during a low flow survey in November 2007 and a high flow survey in April 2008. Field assessments between upstream and downstream sites were done on the same day for each feedlot and in the absence of any significant rainfall event. All feedlots were surveyed in the same week for both the high flow and the low flow survey, thus limiting environmental variability of rainfall, pH, temperature and conductivity readings and ensuring accurate inter-site as well inter-feedlot comparisons.

4.2.2. Water quality

In situ variables and nutrients

The following surface water quality variables were measured on site: pH, conductivity, water temperature, dissolved oxygen and oxygen saturation using a Eutech Cyber Scan Series 600 portable water quality meter.

Water samples were collected on site in 500 ml polypropylene bottles and kept on ice until their return to the laboratory where they were frozen until analysis (DWAF, 1996). Water samples were thawed and each sample was analysed in triplicate for the following chemical variables: turbidity (NTU), Chloride (Cl), Phosphate (PO₄), Sulphate (SO₄), Ammonium (NH₄), Nitrite (NO₂), and Nitrate (NO₃) as well as COD. Analysis was conducted using a Merck Spectroquant Spectrophotometer. Standard sample preparation techniques were followed before measuring the concentration of each nutrient stated above (DWAF, 1996).

Metals

Suspended Al, Cd, Cr, Cu, Fe, Ni, Zn and Pb concentrations were ascertained by filtering 250 ml of sample through pre-weighed 0.45 µm filter paper. The filter paper was dried to a constant mass (at 60 °C), reweighed to determine the mass of suspended matter and acid digested using 33% HNO₃ solution. A 0.05 ml aliquot was converted to 10 ml using ultrapure MQ water, and metal concentrations were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) techniques (DWAF, 1996). The filtered water sample was acidified with 0.05 ml HNO₃ and dissolved metal concentrations were measured.
using ICP-OES techniques. Total metal concentrations were determined by analysing unfiltered water samples.

4.2.3.  Sediment composition

Surface (top 5 cm) sediment was collected in the middle of streams associated with each site and subjected to the following analyses (Ashman and Puri, 2002):

- percentage grain size
- organic content

The following methods were used:

The percentage grain size was determined by drying sediment to a constant mass at 60 °C. A known mass of sample was sieved through a series of Endecott sieves using an Endicott shaker for ten minutes. A series of 4000 µm, 2000 µm, 500 µm, 212 µm and 53 µm sieves were used. The mass of sediment retained in each sieve was determined and calculated as a percentage of the whole sample (Ashman and Puri, 2002).

The organic content of the sediments was analysed in triplicate. Dried sediment was added to porcelain crucibles and weighed. The crucibles were placed in an incinerator at 600 ºC for six hours and left to cool down for 24 hours. After incinerating the sediment was weighed again to determine the percentage organic content (Ashman and Puri, 2002).

4.2.4.  Ecological classification and PES determination

The implementation of the following standard biomonitoring protocols for aquatic macroinvertebrates, fish and habitat were undertaken in the field surveys:

- Habitat – IHAS (McMillan, 1998) and IHI (Kleynhans, 1996).
- Aquatic macroinvertebrates – SASS 5 (Dickens & Graham, 2002), utilising eco-region and biological bands to perform the ecological classification (Dallas, 2007)
- Fish – FAII (Kleynhans, 1999)

Results of the biomonitoring data are expressed in terms of Ecological Categories (EC) ranging from Natural (Category A) to Critically Modified (Category E or F) (Table 4.1). For the purpose of classification, the interpretation of SASS 5 scores were based on the Lower Highveld eco-region classification bands (Dallas, 2007), (Table 4.1).
Table 4.1: Ecological categories, key colours and category descriptions presented within the biotic assessment (Kleynhans and Louw, 2007).

<table>
<thead>
<tr>
<th>Category</th>
<th>Category description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Very good</td>
</tr>
<tr>
<td>B</td>
<td>Good</td>
</tr>
<tr>
<td>C</td>
<td>Moderate</td>
</tr>
<tr>
<td>D</td>
<td>Poor</td>
</tr>
<tr>
<td>E</td>
<td>Very poor</td>
</tr>
<tr>
<td>F</td>
<td>Critical</td>
</tr>
</tbody>
</table>

Table 4.2: Biological Bands/ Ecological categories for interpreting SASS 5 data (Dallas, 2007)

<table>
<thead>
<tr>
<th>Biological Band</th>
<th>Ecological Category Name</th>
<th>Description</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Natural</td>
<td>Unmodified natural</td>
<td>Blue</td>
</tr>
<tr>
<td>B</td>
<td>Good</td>
<td>Largely natural with few modifications</td>
<td>Green</td>
</tr>
<tr>
<td>C</td>
<td>Fair</td>
<td>Moderately modified</td>
<td>Yellow</td>
</tr>
<tr>
<td>D</td>
<td>Poor</td>
<td>Largely modified</td>
<td>Red</td>
</tr>
<tr>
<td>E</td>
<td>Seriously modified</td>
<td>Seriously modified</td>
<td>Purple</td>
</tr>
<tr>
<td>F</td>
<td>Critically Modified</td>
<td>Extremely modified</td>
<td>Black</td>
</tr>
</tbody>
</table>

The habitat integrity for both in-stream and riparian habitats are classified into one of six classes, ranging from unmodified (Category A), to critically modified (Category F), (Table 4.3).

Table 4.3: Ecological state categories, key colours and category descriptions presented within this assessment for FAII, IHAS and IHI.

<table>
<thead>
<tr>
<th>Category</th>
<th>Category description</th>
<th>Integrity Score (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Natural</td>
<td>90-100</td>
</tr>
<tr>
<td>B</td>
<td>Largely Natural</td>
<td>80-89</td>
</tr>
<tr>
<td>C</td>
<td>Moderately Modified</td>
<td>60-79</td>
</tr>
<tr>
<td>D</td>
<td>Largely Modified</td>
<td>40-59</td>
</tr>
<tr>
<td>E</td>
<td>Seriously Modified</td>
<td>20-39</td>
</tr>
<tr>
<td>F</td>
<td>Critically Modified</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>
Habitat assessment

The IHI (Kleynhans, 1999) is applied on a site specific basis and aims to assess the number and severity of anthropogenic disturbances on a river and the changes they potentially cause on the habitat integrity of the system. These disturbances include abiotic and biotic factors. Aspects considered in the assessment comprise those in-stream and riparian zone perturbations regarded as primary causes of degradation of a river ecosystem. The severity of each of these impacts is assessed, using scores as a measure of impact.

A habitat assessment was conducted on site using IHAS) Version 2 (McMillan, 1998) to enrich macroinvertebrate data obtained. IHAS measures the availability and integrity of each biotope (stones, vegetation and gravel, sand and mud) as well as physical stream integrity.

Invertebrate assessment

The SASS 5 index is a qualitative rapid biological assessment method used to evaluate the impacts of changes in water quality using aquatic macro-invertebrates as indicator organisms.

The SASS 5 methodology (Dickens & Graham, 2002) was used at the selected sites to assess the macroinvertebrate community integrity. Identification of the organisms was done to family level (Davies and Day, 1998; Dickens and Graham, 2002; Gerber and Gabriel, 2002). While Dallas (1997) was used for interpretation of results obtained.

Sample collection was done using a standardised invertebrate collection net (as stipulated by the RHP – 1 000 µm mesh with a 300 x 300 mm square opening. All of the available biotopes were sampled as described by Dickens & Graham (2002). The biotopes were divided into stones (S), vegetation (Veg) and gravel, sand and mud (GSM). These biotopes were sampled and assessed separately and are described below:

- **Marginal vegetation (MVeG).** This is defined as the overhanging or emergent flora growing on the edge of the river, both in current (MvegIC) and out of current (MvegOOC). Sampling was performed by holding the net perpendicular to the vegetation (half in and half out of the water) and sweeping back and forth in the vegetation (± 2 m of vegetation).

- **Submerged vegetation (AqVeg).** This vegetation is totally submerged and includes filamentous algae and the roots of floating aquatic flora. This biotope is sampled by moving the net vigorously amongst the vegetation for an area of approximately one square meter.

- **GSM biotopes.** (1) Gravel is made up of small stones (2 mm up to 3 cm). Sampling was performed in the same method as described for sand above. Sand
includes sandbanks within the river, small patches of sand in hollows at the side of
the river or sand between the stones at the side of the river. This biotope is
sampled by stirring the substrate through shuffling of the feet for half a minute,
whilst the net is continuously swept over the disturbed area. (2) Mud consists of
very fine particles of sediment. Mud usually settles to the bottom in still or slow
flowing areas of the river. Sampling was performed in the same method to that for
sand.

- **Hand picking and visual observation.** Before and after disturbing the site,
approximately 1 minute of “hand-picking” for specimens that may have been
missed by the sampling procedures was carried out.

The endpoint of an ecosystem assessment is a value expressed either in the form of
measurements (data collected) or as a summarised value in the form of index values. The
index values generated for this study include biotope scores (and their respective ASPT),
total scores, number of taxa and average score per taxon (ASPT).

**Fish assemblage assessment**

A desktop review pertaining to distribution (Skelton, 2001) and habitat preference of fish
(Kleynhans, 2007) was done to ascertain what fish species may find potential refuge in
associated systems. Fish were sampled at each site by means of electro-shocking for
approximately 45 min. Location, as well as the natures of the streams did not make it
possible to apply other means of sampling i.e. seine nets.

The species intolerance ratings used in the calculation of the FAII was taken from Kleynhans
(1999). Four components are taken into account in estimating the intolerance of the relevant
fish species, namely habitat preferences and specialisation (HS), food preference and
specialisation (TS), requirements for flowing water during different life-stages (FW) and
water quality requirements (WQ). Each of these aspects are scored for a species according
to low requirement/specialisation (rating=1), moderate requirement/ specialisation (rating=3)
and high requirement/specialisation (rating=5).

The percentage of fish with externally evident disease or other abnormalities are used to
score general fish condition. The following procedure was used to score the health of
individual species:

- Frequency of affected fish >5%, score = 1

- Frequency of affected fish 2 - 5%, score = 3

- Frequency of affected fish <2%, score = 5
Chapter 4

The expected health for a species living under undisturbed conditions is assumed to be unimpaired and would receive a score of 5.

The expected index score [FAII (exp.)] per segment:

- \( \text{FAII (exp.)} = \sum (T \times H) \)

Where: \( T \) = Tolerance rating for individual species

- \( H \) = Expected health rating for individual species.

The observed index score [FAII (obs)] is calculated on a similar basis but is based on the information collected during the survey:

- \( \text{FAII (obs)} = \sum (T \times H) \).

The observed fish assemblage index score for a segment is expressed as a percentage of the expected total FAII score to arrive at a relative FAII rating:

- \( \frac{\text{FAII (obs)}}{\text{FAII (exp.)}} \times 100 \)

4.2.4. Statistical analysis

Primer Version 6 statistical suite was used on water quality data for sample sites at high and low flow. Temperature data was excluded after an initial statistical analysis due to the contribution of the variation in temperature between seasons to skewness in the data. Selected water quality data variables (Conductivity, pH, NO\(_2\), NO\(_3\), COD, NH\(_3\), and NH\(_4\)) were Log (V) transformed due to the skewness in data indicated by the scatter plot for variable pairs (Draftsman Plot). Principal Component Analysis (PCA) was carried out on normalised data.
4.3. Results

4.3.1. Water quality

Water quality variables are divided into \textit{in situ} variables, nutrients and metals for each site respectively. The descriptions are designed in such a manner that both inter-site and inter-feedlot comparisons can be made at the same time incorporating temporal influences:

\textit{In situ variables comparison}

Table 4.4 shows the \textit{in situ} and nutrient variables for the low flow and the high flow survey. Comparisons between values measured at each site during this study and the 95\textsuperscript{th} percentiles of historical data monitored at nearby monitoring stations (Table 4.5) were made (DWAF, 2000). The results were related to the Targeted Water Quality Range (DWAF, 1996) for aquatic ecosystems (Table 4.6).

The concentration of available oxygen at Feedlot A downstream was higher than Feedlot A upstream, i.e. 7.81 mg/l in the low flow survey and 4.4 mg/l in the high flow survey. Feedlot A shows \( O_2 \) saturation levels below that of the Targeted Water Quality Requirement (TWQR), (DWAF, 1996). A similar trend is visible when referring to conductivity, which is higher downstream (1882 \( \mu \)S/cm, 724 \( \mu \)S/cm) than upstream (549 \( \mu \)S/cm, 207 \( \mu \)S/cm) for both seasons sampled, but less so in the high flow survey. In addition to this, pH for the downstream site is also higher for both sampling efforts, upstream is 7.24 and 5.95 respectively, while downstream pH is, 8.68 and 7.5 respectively.

\textit{In situ} variables for Feedlot B showed less variability between sites and between flow surveys. Concentration of oxygen was similar for the upstream and downstream site, with the amount of oxygen available being marginally higher in the downstream site during both high flow and low flow field assessments. The conductivity during the low flow survey was higher at Feedlot B downstream (1786 \( \mu \)S/cm) compared to upstream sites (720 \( \mu \)S/cm) during the same sampling time. In the high flow survey the difference decreased slightly but downstream the conductivity was still higher (409 \( \mu \)S/cm) than upstream (211 \( \mu \)S/cm).

The \textit{in situ} variables for Feedlot C differ when compared to the other two feedlots. Oxygen availability is higher for the upstream site for both low flow and high flow surveys (9.79 mg/l and 4.6 mg/l respectively) compared with downstream site (7.29 mg/l and 3.2 mg/l respectively). During the low flow survey the upstream conductivity was higher (728 \( \mu \)S/cm) than the downstream site conductivity (395 \( \mu \)S/cm). This, however, changed for the high flow survey where the upstream site conductivity was measured at 211 \( \mu \)S/cm and downstream
was measured at 409 µS/cm. pH measurements were highest for both upstream low flow survey (8.45) and for upstream high flow survey (8.10).

**Nutrient variable: Feedlot A**

Nutrient concentrations in water samples associated with Feedlot A do show distinct differences between the upstream and the downstream sites. The Cl concentrations for upstream sites during both survey times were lower (4.36 mg/l and 1.5 mg/l respectively), than downstream sites (10.5 mg/l and 22 mg/l respectively). Chemical oxygen demand was the highest at the downstream site during high flow field survey and lowest at the upstream site during the high flow survey. On both occasions the COD was higher downstream from the feedlot than upstream. During the low flow survey there was little difference between PO₄ when comparing the upstream site with that of the downstream site. However, the PO₄ for the downstream site during the high flow survey is higher (1.87 mg/l) compared to upstream site (0.18 mg/l) during the same surveying period. Phosphate levels downstream for Feedlot A, during the high flow survey show concentrations far greater than that of the 95th percentile value (Table 4.5).

A similar trend was observed when comparing SO₄ concentrations measured at Feedlot A. A smaller difference was detected during the low flow survey with the upstream site at 52 mg/l and the downstream site at 34 mg/l. The high flow situation differs, with the downstream site at 306 mg/l and the upstream site at 10 mg/l. The SO₄ concentration for the downstream site associated with Feedlot A is higher than the TWQR (Table 4.6) during the high flow survey. The NH₄ concentrations are similar for both sites during the low flow survey (0.09 and 0.08 mg/l respectively) but exponentially higher for the downstream site (0.16 mg/l) during the high flow survey. The NH₄ concentrations for both upstream and downstream sites associated with Feedlot A during both low flow and high flow surveys are above TWQR (Table 4.6). The NO₂ concentrations are higher at the downstream site (0.14 mg/l and 0.20 mg/l) for both low flow and high flow surveys compared to upstream sites (0.07 mg/l and 0.02 mg/l). Turbidity is lowest at the downstream site (2.5 NTU) during the low flow survey and highest at the upstream site (7 NTU) during the high flow survey. The NO₃ concentrations do not show the same trend as NO₂. NO₃ concentrations were highest during the low flow survey for both upstream (9.2 mg/l) and downstream sites (3.85 mg/l), while being much lower during the high flow survey.

**Nutrient variables: Feedlot B**

The nutrient concentrations for Feedlot B indicate a downward trend for most of the nutrients, with differences more closely related to seasonality than feedlot activity. Chloride concentrations for Feedlot B were highest for the upstream site during the low flow survey
(11 mg/l). However, they were similar for both sites during the high flow survey (7.5 mg/l and 7.25 mg/l respectively). Chemical oxygen demand was highest for the upstream site during low flow and high flow surveys. While PO$_4$ was lower for the downstream site for both low flow (0.92 mg/l) and high flow (0.51 mg/l) survey, compared to the upstream site during the low flow (1.57 mg/l) and high flow (0.81 mg/l) survey. Phosphate concentrations for both upstream and downstream sites are higher than the 95$^{th}$ percentile values obtained from a nearby monitoring station (Table 4.5).

Sulphate was highest for the site located upstream from the feedlot during the low flow survey (26 mg/l) and also high for the upstream and downstream site during the high flow survey (16 mg/l). Ammonium concentration were higher for the upstream site during both low flow (0.05 mg/l) and high flow surveys (0.07 mg/l) compared to the downstream sites during the low flow (0.03 mg/l) and high flow survey (0.05 mg/l). Nitrate levels showed a similar trend, being higher for the upstream site during the low flow survey (4.3 mg/l) and the high flow (2.28 mg/l) when compared to the downstream site during the low flow and high flow surveys at 0.65 mg/l and 1.8 mg/l respectively.

There was little or no variations in NO$_2$ concentration measured at upstream and downstream sites, furthermore there were also any differences between low flow and high flow surveys. Turbidity for both upstream and downstream was the same during the low flow survey and lower for both sites during the high flow survey.
Table 4.4: Water quality data including *in situ* variables and nutrients for upstream and downstream sites associated with each feedlot, during both the high flow and the low flow survey (November, 2007 and April 2008)

<table>
<thead>
<tr>
<th></th>
<th>O₂ (mg/l)</th>
<th>O₂ (%)</th>
<th>Temperature °C</th>
<th>Conductivity (µS/cm)</th>
<th>pH</th>
<th>NH₄ (mg/l)</th>
<th>Cl (mg/l)</th>
<th>COD (O₂/mg/l)</th>
<th>NO₃ (mg/l)</th>
<th>NO₂ (mg/l)</th>
<th>PO₄ (mg/l)</th>
<th>SO₄ (mg/l)</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)UL</td>
<td>5.64</td>
<td>62.60*</td>
<td>20.60</td>
<td>549.</td>
<td>7.24</td>
<td>0.09*</td>
<td>4.36</td>
<td>2.22</td>
<td>9.20</td>
<td>0.07</td>
<td>1.13</td>
<td>52.00</td>
<td>6.00</td>
</tr>
<tr>
<td>(A)DL</td>
<td>7.81</td>
<td>92.70</td>
<td>22.40</td>
<td>1,882*</td>
<td>8.68</td>
<td>0.08*</td>
<td>10.50</td>
<td>5.29</td>
<td>3.85</td>
<td>0.14</td>
<td>1.11</td>
<td>34.00</td>
<td>2.50</td>
</tr>
<tr>
<td>(A)UH</td>
<td>2.20</td>
<td>27.00*</td>
<td>15.30</td>
<td>207.00</td>
<td>5.95</td>
<td>0.03*</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>0.02</td>
<td>0.18</td>
<td>10.00</td>
<td>7.00</td>
</tr>
<tr>
<td>(A)DH</td>
<td>4.40</td>
<td>53.00*</td>
<td>17.20</td>
<td>724.00</td>
<td>7.50</td>
<td>0.16*</td>
<td>22.00</td>
<td>8.00</td>
<td>1.75</td>
<td>0.20</td>
<td>1.87</td>
<td>306.00*</td>
<td>5.00</td>
</tr>
<tr>
<td>(B)UL</td>
<td>8.35</td>
<td>99.60</td>
<td>23.50</td>
<td>720.</td>
<td>8.45</td>
<td>0.05*</td>
<td>11.00</td>
<td>1.50</td>
<td>4.30</td>
<td>0.02</td>
<td>1.57</td>
<td>26.00</td>
<td>4.50</td>
</tr>
<tr>
<td>(B)DL</td>
<td>9.75</td>
<td>116.50</td>
<td>23.60</td>
<td>1,786*</td>
<td>9.16*</td>
<td>0.03*</td>
<td>6.50</td>
<td>0.00</td>
<td>0.65</td>
<td>0.01</td>
<td>0.92</td>
<td>19.00</td>
<td>4.50</td>
</tr>
<tr>
<td>(B)UH</td>
<td>8.48</td>
<td>92.51</td>
<td>15.80</td>
<td>794.30</td>
<td>9.00</td>
<td>0.07*</td>
<td>7.50</td>
<td>7.75</td>
<td>2.28</td>
<td>0.02</td>
<td>0.81</td>
<td>16.00</td>
<td>4.25</td>
</tr>
<tr>
<td>(B)DH</td>
<td>9.46</td>
<td>96.10</td>
<td>15.50</td>
<td>806.60</td>
<td>9.28</td>
<td>0.05*</td>
<td>7.25</td>
<td>0.75</td>
<td>1.80</td>
<td>0.02</td>
<td>0.51</td>
<td>16.00</td>
<td>3.75</td>
</tr>
<tr>
<td>(C)UL</td>
<td>9.79</td>
<td>129.00</td>
<td>28.20</td>
<td>728.</td>
<td>9.25*</td>
<td>0.03*</td>
<td>4.00</td>
<td>2.01</td>
<td>2.05</td>
<td>0.01</td>
<td>0.04</td>
<td>15.67</td>
<td>5.50</td>
</tr>
<tr>
<td>(C)DL</td>
<td>7.29</td>
<td>88.90</td>
<td>26.00</td>
<td>395.</td>
<td>8.10</td>
<td>0.05*</td>
<td>6.00</td>
<td>3.03</td>
<td>3.50</td>
<td>0.04</td>
<td>0.02</td>
<td>8.67</td>
<td>2.00</td>
</tr>
<tr>
<td>(C)UH</td>
<td>9.14</td>
<td>110.76</td>
<td>14.50</td>
<td>211.00</td>
<td>7.09</td>
<td>0.10*</td>
<td>4.00</td>
<td>14.00</td>
<td>0.25</td>
<td>0.02</td>
<td>0.05</td>
<td>6.00</td>
<td>4.00</td>
</tr>
<tr>
<td>(C)DH</td>
<td>8.38</td>
<td>92.50</td>
<td>14.60</td>
<td>409.00</td>
<td>7.50</td>
<td>0.07*</td>
<td>8.00</td>
<td>1.50</td>
<td>2.95</td>
<td>0.02</td>
<td>0.09</td>
<td>13.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

A/UL: Feedlot A Upstream Low flow; (A)DL: Feedlot A Downstream Low flow; (B)UL: Feedlot B Upstream Low flow; (B)DL: Feedlot B Downstream Low flow; (C)UL: Feedlot C Upstream Low flow; (C)DL: Feedlot C Downstream. (*)= Values exceeding TWQR or higher than natural background levels.
Table 4.5: The 95th percentile values for historical data from the Suikerbosrand River Uitvlught monitoring station, from 1996-2000 (DWAF, 2000)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>NH4 (mg/l)</th>
<th>Cl (mg/l)</th>
<th>NO3 (mg/l)</th>
<th>PO4 (mg/l)</th>
<th>SO4 (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2H004Q1 (Suikerbosrand)</td>
<td>8.5</td>
<td>0.26</td>
<td>199.14</td>
<td>0.97</td>
<td>0.322</td>
<td>1061.17</td>
</tr>
<tr>
<td>C1H017Q01 (Vaal River)</td>
<td>8.64</td>
<td>0.091</td>
<td>28.68</td>
<td>0.786</td>
<td>0.09</td>
<td>45.57</td>
</tr>
</tbody>
</table>

Table 4.6: Target Water Quality Range (TWQR) for in situ and nutrient variables (DWAF, 1996)

<table>
<thead>
<tr>
<th></th>
<th>O2 (%)</th>
<th>pH</th>
<th>NH4 (mg/l)</th>
<th>Cl (mg/l)</th>
<th>COD (O2/mg/l)</th>
<th>NO3 (mg/l)</th>
<th>NO2 (mg/l)</th>
<th>PO4 (mg/l)</th>
<th>SO4 (mg/l)</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWQR</td>
<td>80-120</td>
<td>&lt;5 % of background pH</td>
<td>0.007</td>
<td>0-100</td>
<td>Not available</td>
<td>0-100</td>
<td>0-10</td>
<td>&lt;15 % of background concentration</td>
<td>0-200</td>
<td>&lt;15 from normal cycle of water body</td>
</tr>
</tbody>
</table>
**Nutrient variables: Feedlot C**

Nutrient concentrations at Feedlot C showed some fundamental differences between upstream and downstream sites, as well as differences in concentration between low flow and high flow surveys. The Cl concentrations were highest at the downstream site during the high flow survey (8 mg/l) and lowest at the upstream site during the low flow survey (4 mg/l). Chemical oxygen demand was much higher at the upstream site during the high flow survey. While PO$_4$ concentrations were similar for all sites during low flow and high flow surveys.

The SO$_4$ concentration, during the low flow survey was highest at the upstream site (15.67 mg/l) and during the high flow survey highest at the downstream site (13 mg/l). Ammonium concentrations showed a seasonal trend, being higher upstream (0.1 mg/l) and downstream (0.07 mg/l) during the high flow survey, compared to the 0.03 mg/l and 0.05 mg/l for upstream and downstream sites, respectively, during the low flow survey. Ammonium concentrations at the upstream site associated with Feedlot C during the low flow survey were far above the TWQR (Table 4.6). Nitrate concentrations increased downstream from Feedlot C with both downstream surveys yielding higher values than the upstream site. However, the NO$_3$ concentrations also showed a general decrease in concentration during the high flow survey. Nitrite concentrations showed little or no change between sites or between seasons. Turbidity decreases downstream form Feedlot C, as the upstream site showed a higher turbidity during low flow (5.5 NTU) and high flow (4 NTU) surveys.

Figure 4.1 presents the PCA ordination for nutrients and *in situ* variables for the sites associated with Feedlot A. Both high flow and low flow data sets are represented in this diagram. The two dimensional PCA bi-plot describes 96.1 % of the variation in the data. 89.6 % of the variation is represented in the first axis while the rest (6.6 %) is represented by the second axis. Regardless of seemingly higher concentrations of nutrients present at Feedlot A downstream during the high flow survey, the PCA results indicates seasonal similarities in the data sets. Conductivity contributes the most towards the low flow similarities between upstream and downstream sites, while SO$_4$, Cl and PO$_4$ contribute towards the dissimilarities between Feedlot A downstream during the high flow survey. A site ordination is also evident, regardless of season. Both low flow and high flow data sets for the upstream site group together and the similar grouping can be seen for the downstream site, although to a lesser extent.
Figure 4.1: PCA bi-plot of water quality variables showing (dis)similarities for upstream and downstream sites associated with Feedlot A during low flow and high flow surveys. K= Feedlot A

Figure 4.2 shows a similar PCA bi-plot for sites associated with Feedlot B, also including low flow and high flow data sets. The two dimensional bi-plot accounts for 98.5 % of the variation in the data of which 66.9 % is represented on the first axis, while 31.5 % is represented on the second axis. Greater similarities can be observed between seasons, with high flow sites grouping together and low flow sites grouping together based on similar turbidity, SO$_4$ and PO$_4$ concentrations. Similarly, there is also a site ordination. Downstream sites are principally driven by a higher conductivity, O$_2$ and pH values, while NO$_3$ and COD contribute more to the similarity of the upstream sites.
Figure 4.2: PCA bi-plot of water quality variables showing (dis)similarities for upstream and downstream sites associated with Feedlot B during low flow and high flow surveys. T= Feedlot B

The PCA for sites associated with Feedlot C (Figure 4.3) indicate similarities in season and site ordination. The two dimensional bi-plot accounts for 84% of the variation in the data where the first axis represents 49.2% of the variation while the rest (34.8%) is represented by the second axis. A strong seasonal similarity can be observed, especially during the high flow survey. Sites, during the high flow survey, show similarities in NH$_4$ and PO$_4$ concentrations. Chemical oxygen demand, Cl and NO$_3$ contribute towards the low flow ordination. Despite the seasonal grouping of sites, an upstream downstream grouping can also be observed. Driving similarities of the downstream site, during both surveys, are NO$_2$ and NH$_4$, while turbidity and oxygen concentration contribute to the similarities of the upstream site associated with Feedlot C.
Figure 4.3: PCA bi-plot of water quality variables showing (dis)similarities for upstream and downstream sites associated with Feedlot C during low flow and high flow surveys. B = Feedlot C

**Metals**

The data obtained show inter-site comparison as well as a comparison between feedlots. The data represented reflects the dissolved values ascertained for each site during each survey (Table 4.7). However, it must be noted that suspended metals will still be available to filter-feeders and cannot be overlooked. Together with TWQR, Chronic Effect values (CEV) and Acute Effect Values (AEV) are also represented in Table 4.8.

Aluminium concentrations obtained for all feedlots, showed no apparent difference between upstream and downstream sites. They also did not show any correlation with seasonal variation. The Al concentrations were the highest at Feedlot C downstream (1001 µg/l) during the low flow survey. Feedlot C also showed, on average, a higher concentration of Al when compared to that of Feedlot A and Feedlot B. Both Feedlot A and Feedlot B reflected highest concentrations of Al at the downstream sites (50.5 µg/l and 332.5 µg/l respectively)
Chapter 4

associated with each, but again with no seasonal preference. Al concentrations, at all the sites and during both low flow and high flow survey, indicated concentrations far above the TWQR (Table 4.8). Both Feedlot A downstream and Feedlot C downstream had Al levels exceeding the AEV (in excesses of 150 µg/l).

Cadmium concentrations, much like the Al concentrations, did not show consistent differences between upstream and downstream sites associated with each feedlot. However, Feedlot A and Feedlot B, on average had a higher Cd concentration than Feedlot C. Feedlot C showed a decline in Cd concentration during the high flow survey for both upstream and downstream sites at 33.5 µg/l and 28.5 µg/l respectively. The highest Cd concentration was detected at Feedlot C (101.5 µg/l) at the downstream site during the low flow survey. Cd levels for all sites were above the TWQR and also far exceeded the AEC (Table 4.8).

Chromium did not show noticeable differences between upstream and downstream sites. However, it did show a seasonal correlation, with Cr concentrations being higher at all three feedlots during the high flow survey. Feedlot C showed the highest Cr levels, measured at the downstream site during the high flow survey (210.5 µg/l), while Feedlot C upstream site showed the lowest Cr levels during the low flow survey (173 µg/l). Chromium concentrations for all sites and during both low flow and high flow surveys were above TWQR, while sites associated with Feedlot C showed Cr levels higher than the AEV (in excess of 200 µg/l) (Table 4.8).

Copper concentrations ranged between 172.5 µg/l and 220 µg/l, with the highest levels being upstream from Feedlot B, during the low flow survey. Feedlot C on average had lower levels of Cu compared to Feedlot A or Feedlot B. No site or season differences between Cu concentrations were observed. Cu concentrations far exceeded TWQR as well as AEV (>7.5 µg/l) (Table 4.8).

On average sites associated with Feedlot A showed higher levels of Fe than that of Feedlot C or Feedlot B. Iron levels were lowest for all three feedlots at the downstream sites during the low flow survey, which suggests a seasonal trend. This trend repeats itself during the high flow survey, where Fe levels for both upstream and downstream sites were higher on average when compared to the low flow Fe levels. TWQR for Fe indicated variations of smaller than 10 % when compared to background concentrations. No background concentration could be established; therefore Fe concentrations cannot be compared.

Manganese levels for Feedlot A were higher upstream than downstream during both low flow and high flow surveys. Feedlot C indicated higher Mn levels during the high flow survey at both upstream and downstream sites. Manganese concentrations obtained from sites
associated with Feedlot B did not show any differences between upstream and downstream sites. However, Mn levels for Feedlot B were lower during the high flow survey than the low flow survey. The Mn concentration measured at all the sites were below the TWQR (Table 4.8).

Feedlot C downstream, during the low flow survey obtained the highest Ni concentrations (Table 4.7) (167 µg/l). Feedlot A also showed elevated levels of Ni during the low flow survey for both upstream and downstream sites, while Ni concentrations for Feedlot B were higher during the low flow assessment for both upstream and downstream sites. Nickel did not show distinct differences between sites or between seasons. Nickel concentrations for all the sites were below TWQR (Table 4.8).

Zinc levels (Table 4.7) were very similar for all the feedlots. Feedlot A and Feedlot B showed slightly higher concentrations of Zn, particularly during the high flow survey, while Feedlot C showed slightly elevated Zn levels during the low flow survey. Zinc concentrations for all the sites were much higher than TWQR and the AEV (>36 µg/l) (Table 4.8).
Table 4.7: Metal concentrations (µg/l) for both upstream and downstream sites associated with feedlot activity for the low flow and the high flow survey.

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe µg/l</th>
<th>Mn µg/l</th>
<th>Ni µg/l</th>
<th>Zn µg/l</th>
<th>Pb µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)UL</td>
<td>130.00</td>
<td>96.00</td>
<td>178.50</td>
<td>210.50</td>
<td>31.00</td>
<td>34.00</td>
<td>77.00</td>
<td>176.50</td>
<td>340.50</td>
</tr>
<tr>
<td>(A)DL</td>
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<td>189.00</td>
<td>207.00</td>
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<td>14.00</td>
<td>48.50</td>
<td>159.00</td>
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<td>(A)UH</td>
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<td>191.50</td>
<td>206.00</td>
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<td>15.50</td>
<td>29.50</td>
<td>175.00</td>
<td>190.00</td>
</tr>
<tr>
<td>(A)DH</td>
<td>332.50</td>
<td>93.00</td>
<td>172.50</td>
<td>209.50</td>
<td>53.00</td>
<td>11.50</td>
<td>30.00</td>
<td>177.50</td>
<td>532.00</td>
</tr>
<tr>
<td>(B)UL</td>
<td>49.50</td>
<td>91.50</td>
<td>181.00</td>
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<td>39.50</td>
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<td>93.50</td>
<td>181.00</td>
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<td>38.50</td>
<td>23.00</td>
<td>187.00</td>
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<td>190.50</td>
<td>207.00</td>
<td>5.00</td>
<td>42.50</td>
<td>22.00</td>
<td>158.50</td>
<td>217.50</td>
</tr>
<tr>
<td>(B)DH</td>
<td>35.50</td>
<td>91.00</td>
<td>188.50</td>
<td>205.00</td>
<td>4.50</td>
<td>42.00</td>
<td>28.00</td>
<td>165.50</td>
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<td>95.50</td>
<td>173.00</td>
<td>208.00</td>
<td>14.00</td>
<td>15.50</td>
<td>91.00</td>
<td>159.50</td>
<td>819.00</td>
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<td>167.00</td>
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<td>100.50</td>
<td>4.50</td>
<td>142.00</td>
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<td>(C)DH</td>
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<td>28.50</td>
<td>210.50</td>
<td>172.50</td>
<td>13.50</td>
<td>105.50</td>
<td>13.00</td>
<td>129.00</td>
<td>901.50</td>
</tr>
</tbody>
</table>

(A)UL: Feedlot A Upstream Low flow; (A)DL: Feedlot A Downstream Low flow; (B)UL: Feedlot B Upstream Low flow; (B)DL: Feedlot B Downstream Low flow; (C)UL: Feedlot C Upstream Low flow; (C)DL: Feedlot C Downstream Low flow.

Table 4.8: The TWQR, CEV and AEV for metals assessed in this study (NA=Not Available), (DWAF, 1996).

<table>
<thead>
<tr>
<th></th>
<th>Al µg/l</th>
<th>Cd µg/l</th>
<th>Cr µg/l</th>
<th>Cu µg/l</th>
<th>Fe µg/l</th>
<th>Mn µg/l</th>
<th>Ni µg/l</th>
<th>Zn µg/l</th>
<th>Pb µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWQR</td>
<td>10</td>
<td>0.35</td>
<td>7</td>
<td>1.2</td>
<td>&lt;10 variation on background concentration</td>
<td>180</td>
<td>0-1000</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Chronic Effect Value (CEV)</td>
<td>20</td>
<td>0.7</td>
<td>14</td>
<td>2.4</td>
<td>NA</td>
<td>370</td>
<td>NA</td>
<td>3.6</td>
<td>2</td>
</tr>
<tr>
<td>Acute Effect Value (AEV)</td>
<td>150</td>
<td>10</td>
<td>200</td>
<td>7.5</td>
<td>NA</td>
<td>1300</td>
<td>NA</td>
<td>36</td>
<td>13</td>
</tr>
</tbody>
</table>
4.3.2. Sediment composition

Grain size composition

Figure 4.4 to Figure 4.6 show spider diagrams, which indicate the percentage grain size for each feedlot during low flow and high flow field assessments. Figure 4.4, shows the grain size composition of sites associated with Feedlot A. During the low flow survey the grain size composition was sandier, while during the high flow survey, it became slightly silt-like in composition. Neither upstream nor downstream sites associated with Feedlot A consisted of high clay content. However, Feedlot A downstream during the high flow survey consisted of more than 40 % silty soil.

The soil composition of Feedlot B (Figure 4.5) showed a similar texture to that of Feedlot A. Feedlot B upstream low flow had the highest silt content, while Feedlot B downstream high flow showed a coarser soil texture. Overall soil texture for both upstream and downstream sites associated with Feedlot B showed a loamy sand grain size composition.

Figure 4.6 shows the grain size composition for Feedlot C. Feedlot C upstream low flow showed a silty soil composition while the downstream site during the same survey was composed of a less coarse grain size. Feedlot C downstream high flow showed a soil composition that was consistent with a silty clay loam soil, with more than 60 % of the grain size composition being smaller than 53 µm.

Organic contents

Feedlot A upstream showed a higher organic content during both low flow and high flow surveys, when compared to the downstream site during the same surveying times. The organic content for soils associated with Feedlot A upstream site was almost 3 % during low flow and high flow surveys, compared to a much lower 1 % at the downstream site (Figure 4.7).

Soil organic content for Feedlot B was consistently higher for the downstream sites, during the low flow and high flow surveys. Overall, Feedlot B, on average, had less organic matter per unit soil than Feedlot A (Figure 4.8).

The organic content of Feedlot C was highest during the low flow survey, for both upstream and downstream sites. Feedlot C downstream showed the highest percentage organic matter present in soil at 8 % (Figure 4.9).
Figure 4.4: Spider diagram representing the percentage grain size composition of both upstream and downstream sites associated with Feedlot A.

Figure 4.5: Spider diagram representing the percentage grain size composition of both upstream and downstream sites associated with Feedlot B.

Figure 4.6: Spider diagram representing the percentage grain size composition of both upstream and downstream sites associated with Feedlot C.

Key to abbreviations:

K: Feedlot A
T: Feedlot B
B: Feedlot C
U: Upstream
D: downstream
L: Low flow
H: High flow
Chapter 4

Figure 4.7: Spider diagram representing the percentage organic matter present at both upstream and downstream sites associated with Feedlot A.

Figure 4.8: Spider diagram representing the percentage organic matter present at both upstream and downstream sites associated with Feedlot B.

Figure 4.9: Spider diagram representing the percentage organic matter present at both upstream and downstream sites associated with Feedlot C.

Key to abbreviations:
- K: Feedlot A
- T: Feedlot B
- B: Feedlot C
- U: Upstream
- D: downstream
- L: Low flow
- H: High flow
4.3. Ecological Classification (EC) and Present Ecological State (PES) determination

The results obtained for the PES includes habitat assessment indices, aquatic macroinvertebrate assessment as well as fish assemblage integrity assessment.

4.3.1. Habitat assessments

Index of Habitat Integrity
The IHI rates impacts on a scale between 0 (none) to 25 (extreme). It subdivides factors that impact on in-stream habitat and riparian habitat. When comparing the habitat integrity of upstream and downstream sites of each feedlot, differences were evident (Table 4.9) and Table 4.10). Both sites of Feedlot A and Feedlot B fell within a C-class. The downstream site associated with Feedlot C showed more of a decline in habitat integrity and was classed in a D-class.

With regards to Feedlot A, both upstream and downstream sites were categorised in a C-class, which relates to moderately modified, with clear community modification and impacts visible. The upstream site however, did score slightly higher than the downstream site with an IHI score of 68 % compared to the 62.8 % of the downstream site (Table 4.9). The habitat integrity for both upstream and downstream sites associated with Feedlot B fell within a C-class (Table 4.9) and the upstream site scored an IHI score of 73.6 % while the downstream obtained a score of 75.8 % (Table 4.10).
### Table 4.9: IHI scores for the stream associated with the Feedlot A.

<table>
<thead>
<tr>
<th>IHI</th>
<th>Feedlot A</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary IHI</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>Secondary IHI</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Riparian Zone Habitat Integrity</td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>Total (425)</td>
<td></td>
<td>136</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td>68%</td>
</tr>
<tr>
<td>Category</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

### Table 4.10: IHI scores for the stream associated with the Feedlot B.

<table>
<thead>
<tr>
<th>IHI</th>
<th>Feedlot B</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary IHI</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Secondary IHI</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Riparian Zone Habitat Integrity</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Total (425)</td>
<td></td>
<td>112</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td>73.6%</td>
</tr>
<tr>
<td>Category</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

### Table 4.11: IHI scores for the stream associated with the Feedlot C.

<table>
<thead>
<tr>
<th>IHI</th>
<th>Feedlot C</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary IHI</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Secondary IHI</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Riparian Zone Habitat Integrity</td>
<td></td>
<td>51</td>
</tr>
<tr>
<td>Total (425)</td>
<td></td>
<td>103</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td>75.8%</td>
</tr>
<tr>
<td>Category</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>
Feedlot C indicated differences between the upstream and downstream sites in terms of habitat integrity (Table 4.11). The upstream site associated with Feedlot C scored 78% on the IHI and fell within a C-class. The downstream site, however, fell within D-class (58.6%) and this relates to a poor, largely modified and unacceptable impacted state.

**Table 4.11: IHI scores for the stream associated with the Feedlot C.**

<table>
<thead>
<tr>
<th>Feedlot C</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary IHI</td>
<td>43</td>
</tr>
<tr>
<td>Secondary IHI</td>
<td>0</td>
</tr>
<tr>
<td>Riparian Zone Habitat Integrity</td>
<td>50</td>
</tr>
<tr>
<td>Total (425)</td>
<td>93</td>
</tr>
<tr>
<td>Percentage</td>
<td>78%</td>
</tr>
<tr>
<td>Category</td>
<td>C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary IHI</td>
</tr>
<tr>
<td>Secondary IHI</td>
</tr>
<tr>
<td>Riparian Zone Habitat Integrity</td>
</tr>
<tr>
<td>Total (425)</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Category</td>
</tr>
</tbody>
</table>

**Invertebrate Habitat Assessment Score**

The IHAS is a measure of the SASS biotopes sampled. Biotopes sampled include stones, vegetation and other biotopes.

All available habitats were sampled, including stones in and out of current, vegetation in and out of current, gravel between rocks, as well as sand and mud in the deeper
pools. Habitat integrity in terms of availability and the general state of the habitat that was available, for both upstream and downstream sites of Feedlot A fell with a C-class. This class relates to moderately modified (Table 4.12).

<table>
<thead>
<tr>
<th>IHAS</th>
<th>Feedlot A</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stone in-current score</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Vegetation</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Other habitats</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Stream Condition</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>IHAS Score</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>State category</td>
<td>C</td>
</tr>
</tbody>
</table>

Upstream

<table>
<thead>
<tr>
<th>IHAS</th>
<th>Feedlot A</th>
<th>Downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stone in-current score</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Vegetation</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Other habitats</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Stream Condition</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>IHAS Score</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>State category</td>
<td>C</td>
</tr>
</tbody>
</table>

Feedlot B is located adjacent to a larger river system (Vaal River). Both the upstream and the downstream sites related to this feedlot fell within a similar ecological category (Table 4.13). A number of different habitat biotopes were present at both sites, with no obvious differences between them. Both the upstream and the downstream sites were classified in a B-class which relates to a largely natural state.
### Table 4.13: IHAS scores for stream associated with Feedlot B.

<table>
<thead>
<tr>
<th>Feedlot B</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHAS</td>
<td></td>
</tr>
<tr>
<td>Stone in-current score</td>
<td>20</td>
</tr>
<tr>
<td>Vegetation</td>
<td>15</td>
</tr>
<tr>
<td>Other habitats</td>
<td>18</td>
</tr>
<tr>
<td>Stream Condition</td>
<td>32</td>
</tr>
<tr>
<td>IHAS Score</td>
<td>85</td>
</tr>
<tr>
<td>State category</td>
<td><strong>B</strong></td>
</tr>
</tbody>
</table>

### Table 4.14: IHAS scores for stream associated with Feedlot C.

<table>
<thead>
<tr>
<th>Feedlot C</th>
<th>Upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHAS</td>
<td></td>
</tr>
<tr>
<td>Stone in-current score</td>
<td>19</td>
</tr>
<tr>
<td>Vegetation</td>
<td>14</td>
</tr>
<tr>
<td>Other habitats</td>
<td>19</td>
</tr>
<tr>
<td>Stream Condition</td>
<td>24</td>
</tr>
<tr>
<td>IHAS Score</td>
<td>76</td>
</tr>
<tr>
<td>State category</td>
<td><strong>C</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IHAS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stone in-current score</td>
<td>0</td>
</tr>
<tr>
<td>Vegetation</td>
<td>13</td>
</tr>
<tr>
<td>Other habitats</td>
<td>10</td>
</tr>
<tr>
<td>Stream Condition</td>
<td>23</td>
</tr>
<tr>
<td>IHAS Score</td>
<td>46</td>
</tr>
<tr>
<td>State category</td>
<td><strong>D</strong></td>
</tr>
</tbody>
</table>
Feedlot C (Table 4.14) was the only feedlot that showed clear differences in habitat availability for invertebrate colonisation. The upstream site associated with Feedlot C fell with a C ecological category, which relates to moderately modified state of habitat. The downstream site associated with Feedlot C fell in a D-category, which relates to a seriously modified state.

**Invertebrate assessment**

Both upstream and downstream sites associated with Feedlot A were classified in a D-class during the low flow survey (Table 4.15). The SASS 5 and ASPT scores for the upstream site were 49.4 and 3.8 respectively and 54.6 and 4.2 for the downstream site respectively. During the high flow survey invertebrate community structures showed a general increase in sensitivity, with the upstream site falling in an A-class and the downstream site in a B-class (Table 4.16).

The upstream site showed higher species diversity, with 16 invertebrate families sampled while only 10 were sampled downstream from Feedlot A. During the high flow survey the cumulative sensitivity also decreased from 105.6 upstream to 51 downstream.

Feedlot B reflected a higher taxon diversity and sensitivity at the site located upstream from the feedlot. During the low flow survey (Table 4.15) the invertebrate composition fell within an A-class, relating to a healthy site, while during the high flow survey (Table 4.16), it was grouped in a B-class. Although the downstream site was also grouped in a B-class for both surveys, it had a decreased diversity and a lower ASPT score than the upstream site.

There was an obvious difference in ASPT scores between upstream and downstream sites associated with Feedlot C (Table 4.15 and Table 4.16). The ASPT score for the upstream site increased from 4.4 during the low flow survey to 5.4 during the high flow survey, while the ASPT scores for the downstream site decreased from 4.6 during the low flow survey to 4.1 during the high flow survey.
### Table 4.15: Aquatic macro invertebrate data: SASS 5 Scores, ASPT’s and respective SASS 5 biotope scores for upstream and downstream of each feedlot, respectively for the low flow assessment.

<table>
<thead>
<tr>
<th>Feedlot</th>
<th>Site</th>
<th>SASS score</th>
<th>No of Taxa</th>
<th>ASPT</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedlot A</td>
<td>Upstream</td>
<td>49.4</td>
<td>13</td>
<td>3.8</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>54.6</td>
<td>13</td>
<td>4.2</td>
<td>D</td>
</tr>
<tr>
<td>Feedlot B</td>
<td>Upstream</td>
<td>140</td>
<td>25</td>
<td>5.6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>76.5</td>
<td>15</td>
<td>5.1</td>
<td>B</td>
</tr>
<tr>
<td>Feedlot C</td>
<td>Upstream</td>
<td>83.6</td>
<td>19</td>
<td>4.4</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>73.6</td>
<td>16</td>
<td>4.6</td>
<td>C</td>
</tr>
</tbody>
</table>

ASPT: Average Score Per Taxa; EC: Ecological Category; GSM – Gravel Sand and Mud habitat;

### Table 4.16: Aquatic macro invertebrate data: SASS 5 Scores, ASPT’s and respective SASS 5 biotope scores for upstream and downstream of each feedlot, respectively for the high flow assessment.

<table>
<thead>
<tr>
<th>Feedlot</th>
<th>Site</th>
<th>SASS score</th>
<th>No of Taxa</th>
<th>ASPT</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedlot A</td>
<td>Upstream</td>
<td>105.6</td>
<td>16</td>
<td>6.6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>51</td>
<td>10</td>
<td>5.1</td>
<td>B</td>
</tr>
<tr>
<td>Feedlot B</td>
<td>Upstream</td>
<td>117.6</td>
<td>21</td>
<td>5.6</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>96.9</td>
<td>19</td>
<td>5.1</td>
<td>B</td>
</tr>
<tr>
<td>Feedlot C</td>
<td>Upstream</td>
<td>145.8</td>
<td>27</td>
<td>5.4</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>41</td>
<td>10</td>
<td>4.1</td>
<td>E/F</td>
</tr>
</tbody>
</table>

ASPT: Average Score Per Taxa; EC: Ecological Category; GSM: Gravel Sand and Mud habitat;
**Fish assemblage assessment**

Fish species that potentially occur in the systems associated with the feedlots assessed are represented in (Table 4.17). Both *Labeobarbus kimberleyensis* and *Austroglanus sclateri* have conservation status (Kleynhans, 1999). A number of exotic fish species also share the same distribution range, including: *Gambusia affinis*, *Lepomis macrochirus* and *Micropterus salmoides* (Kleynhans, 1999).

Fish assemblage integrity classes for all sites surveyed during the low flow survey are shown for the low flow survey (Table 4.18) and for the high flow survey (Table 4.19). No apparent differences are visible, however, it must be noted that sampling efforts involved a time constraint. Accordingly, electro-shocking was applied at all sites for the same amount of time in order to obtain comparable data.

Feedlot A upstream scored 44.2 % on the FAII during the low flow survey and 47.5 % during the high flow survey, while the downstream site scored 37.2 % during the low flow survey and 39.8 % during the high flow survey (Table 4.18 and Table 4.19). The sites surveyed fell within a D/E-class, which relates to a seriously modified state. The same fish species were sampled during both surveys, however, a decline in fish condition and health was observed during the low flow survey (Figure 4.10, Figure 4.11 and Figure 4.12).

Feedlot B upstream scored 37 % on the FAII during the low flow survey and 32.6 % during the high flow survey (Table 4.18 and Table 4.19) The fish species sampled with the highest occurrence during the low flow survey was *Pseudocrenilabrus philander* (15 sampled), while *Labeo capensis* were more abundant during the high flow survey (13 sampled). FAII scores for the downstream site were 40.4 % during the low flow survey and 34.9 % during the high flow survey, therefore also falling within a D/E class.

FAII scores for Feedlot C were the lowest of all the feedlots sampled (Table 4.18 and Table 4.19). The low flow survey score was 25.3 %, while the high flow survey score was 22.0 %. Only four species of fish were sampled at the upstream site: *Tilapia sparrmanii*, *Pseudocrenilabrus philander*, *Labeobarbus aeneus* and *Clarias gariepinus*. *P. philander* had the highest abundance with 19 individuals sampled during the low flow survey and 10 sampled during the high flow survey.
Table 4.17: Expected fish occurrence for all six sites assessed. Expected occurrence list includes alien species. Particular occurrence list excludes historical distribution as obtained from Skelton (2001). Ex: exotic or alien.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Austroglanus sclateri</em> (Boulenger, 1901)</td>
<td>Rock Catfish</td>
</tr>
<tr>
<td><em>Labeobarbus aeneus</em> (Burchell, 1822)</td>
<td>Smallmouth Yellowfish</td>
</tr>
<tr>
<td><em>Barbus anoplus</em> (Weber, 1897)</td>
<td>Chubbyhead Barb</td>
</tr>
<tr>
<td><em>Labeobarbus kimberleyensis</em> (Gilchrist and Thompson, 1913)</td>
<td>Largemouth Yellowfish</td>
</tr>
<tr>
<td><em>Barbus neefi</em> (Greenwood, 1962)</td>
<td>Sidespot Barb</td>
</tr>
<tr>
<td><em>Barbus pallidus</em> (Smith, 1841)</td>
<td>Goldie Barb</td>
</tr>
<tr>
<td><em>Barbus trimaculatus</em> (Peters, 1852)</td>
<td>Threespot Barb</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em> (Burchell, 1822)</td>
<td>Sharptooth Catfish</td>
</tr>
<tr>
<td><em>Gambusia affinis</em> (Baird and Girard, 1853)</td>
<td>Mosquito fish (Ex)</td>
</tr>
<tr>
<td><em>Labeo capensis</em> (Smith, 1841)</td>
<td>Orange River Labeo</td>
</tr>
<tr>
<td><em>Lepomis macrochirus</em> (Rafinesque, 1819)</td>
<td>Bluegill Sunfish (Ex)</td>
</tr>
<tr>
<td><em>Labeo umbratus</em> (Smith, 1841)</td>
<td>Moggel</td>
</tr>
<tr>
<td><em>Micropterus salmoides</em> (Lacepède, 1802)</td>
<td>Largemouth Bass (Ex)</td>
</tr>
<tr>
<td><em>Pseudocrenilabrus philander</em> (Weber, 1897)</td>
<td>Southern Mouthbrooder</td>
</tr>
<tr>
<td><em>Tilapia sparrmanii</em> (Smith, 1840)</td>
<td>Banded Tilapia</td>
</tr>
</tbody>
</table>
The downstream site associated with Feedlot C scored 11.0 % during the low flow survey and 9.5 % during the high flow survey, thus relating to a highly impacted and unacceptable state. Only three species of fish were sampled: *T. sparrmanii*, *P. philander* and *C. gariepinus*.

Figure 4.10: Eye of *Labeo capensis*, sampled downstream from Feedlot A showing peripheral abnormality indicating internal bacterial infection.

Figure 4.11: Caudal fin of *Labeobarbus aeneus* sampled upstream from Feedlot A, indicating cyst infection.

Figure 4.12: *Labeobarbus aeneus* indicating symptoms of *Costia* or *Trichodina*, epidermal infection.
Table 4.18 FAII scores and EC for upstream and downstream sites for the high flow survey.

<table>
<thead>
<tr>
<th>Site</th>
<th>Relative Fish Assemblage</th>
<th>Fish Assemblage Integrity Ecological Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)UH</td>
<td>47.5 %</td>
<td>D</td>
</tr>
<tr>
<td>(A)DH</td>
<td>39.8 %</td>
<td>E</td>
</tr>
<tr>
<td>(B)UH</td>
<td>32.6 %</td>
<td>E</td>
</tr>
<tr>
<td>(B)DH</td>
<td>34.9 %</td>
<td>E</td>
</tr>
<tr>
<td>(C)UH</td>
<td>22 %</td>
<td>E</td>
</tr>
<tr>
<td>(C)DH</td>
<td>9.5 %</td>
<td>F</td>
</tr>
</tbody>
</table>

(A)UH: Feedlot A Upstream High flow; (A)DH: Feedlot A Downstream High flow; (B)UH: Feedlot B Upstream High flow; (B)DH: Feedlot B Downstream High flow; (C)UH: Feedlot C Upstream High flow; (C)DH: Feedlot C Downstream High flow

Table 4.19 FAII scores and EC for upstream and downstream sites for the low flow survey.

<table>
<thead>
<tr>
<th>Site</th>
<th>Relative Fish Assemblage</th>
<th>Fish Assemblage Integrity Ecological Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)UL</td>
<td>44.2 %</td>
<td>D</td>
</tr>
<tr>
<td>(A)DL</td>
<td>37.2 %</td>
<td>E</td>
</tr>
<tr>
<td>(B)UL</td>
<td>37 %</td>
<td>E</td>
</tr>
<tr>
<td>(B)DL</td>
<td>40.4 %</td>
<td>D</td>
</tr>
<tr>
<td>(C)UL</td>
<td>25.3 %</td>
<td>E</td>
</tr>
<tr>
<td>(C)DL</td>
<td>11 %</td>
<td>F</td>
</tr>
</tbody>
</table>

(A)UL: Feedlot A Upstream Low flow; (A)DL: Feedlot A Downstream Low flow; (B)UL: Feedlot B Upstream Low flow; (B)DL: Feedlot B Downstream Low flow; (C)UL: Feedlot C Upstream Low flow; (C)DL: Feedlot C Downstream Low flow
4.4. Discussion

4.4.1. Water quality

Feedlot activities increase the conductivity and pH of downstream sites associated with them (Table 4.4). Higher pH values might be explained by the high NH$_4$ values measured at all three feedlots. Ammonia enters the systems through runoff from feedlot activities. Upon entering the system it reacts with water to form ammonium hydroxide, which dissociates into less toxic ammonium ions and hydroxyl ions (VanLoon and Duffy, 2000). This, in turn, increases the pH value of the water (Dallas and Day, 2004) and the concentration of NH$_3$ in relation to that of NH$_4$ is dependent on water pH. At higher pH values the concentration of toxic NH$_3$ increases (Schubauer-Berigan et al., 1995). These findings are consistent with those of Wright (2007) who also identified NH$_4$ from cattle feedlot operations as a major contributor of Nitrogen to the associated water systems. Potential consequences related to exceeding threshold concentrations of Nitrogen includes NO$_3$ contaminations of drinking water, eutrophication of surface water bodies resulting in harmful algal blooms and alteration in primary production structure (Ndegwa et al., 2008).

Higher conductivity levels observed (Table 4.4) can be explained by an influx of urinal salts. Erickson et al. (2003) quantified the volumes of cations associated with the average feedlot steer’s urine to be: Ca (7.8 kg), K (9.2 kg), Na (1.8 kg) and Mg (2.7 kg) during the total standing period. Higher conductivity levels contribute towards water hardness (VanLoon and Duffy, 2000), and can result in an alteration in natural community structures, as this will select for organisms with a higher tolerance to saline conditions (Dallas and Day, 2004). However, higher conductivity in water can protect aquatic organisms from pollutants such as heavy metals. Voyer and McGovern (1991) found that water with higher conductivities suppressed the toxic effect of Cd. The opposite reaction is reflected in a study done by Monserrat et al. (1991), which indicted elevated sensitivities in aquatic organisms to biocides in the presence of water with higher conductivity values.

Metal concentrations (with the exception of Pb) do not indicate seasonal or site preference and could therefore not be attributed to feedlot activity (Table 4.7). However, some metals in livestock excreta may be derived from the animal diet (Khan et al., 2008). For example, As, Co, Cu, Mn, Se and Zn may be added to livestock feeds as essential nutrients or to improve feed conversion efficiencies (Bolan et al., 2004). However, heavy metals are more likely to be derived from the
ingestion of contaminated soils by the animals (Khan et al., 2008). Soils can be a significant source of Cd and Pb (Loganathan et al., 1999) and this notion has been verified by McBride and Spiers (2001) who indicated that dairy cattle manure exhibited contamination with Pb via soil ingestion. The Pb concentration is intermittently higher downstream of feedlot activity when compared to upstream sites. And, unlike organic pollutants, metals persist indefinitely; changing only in speciation and thus mobility, partitioning and toxicity (Nolan et al., 2003).

It is also important to note that biological processing of manure does not alter the concentrations of most metals (Fares et al., 2005). In addition to this, soil properties such as organic matter content, grain size, mineralogy, salinity and pH greatly affect the mobility of Pb (McLaughlin, 2000). In a particular study, based on nutrient loading rates, it was estimated that land application of manures such as beef effluent, dairy slurry or composted cattle manure produced in England and Wales could result in typical field concentrations of approximately 0.002 kg/ha of Cd and around 0.04 kg/ha of Pb (Nicholson et al., 1999). For example sudden addition of Ca\textsuperscript{2+} to soils may result in the possible leeching of Pb from soils into the aquatic environment (Voegelin et al., 2003). With the exception of Feedlot C upstream, during the low flow survey, Pb concentrations (Table 4.7) showed a definite site partiality, with both Feedlot A and Feedlot B downstream site indicating elevated levels of Pb during both low flow and high flow survey. Downstream from Feedlot C showed a similar trend but only for the high flow survey. Lead levels at all the sites far exceed the TWQR (DWAF, 1996) for aquatic ecosystems as well as the AEV (>13 µg/l) (Table 4.8).

With the exception of Mn, Ni and Fe; metal concentrations are at levels which warrant concern as they are much higher than the TWQR for aquatic ecosystems (Table 4.7). Cumulative mining, agriculture and industrial activities, in the associated catchments of each feedlot, are likely to be predominantly responsible for elevated metals concentrations in water. However, feedlot activity may impact on the speciation and toxicity of these metals.

### 4.4.2. Sediment composition

Sediment is an important factor to consider in any ecotoxicological study. Soil texture and structure relate to the soil’s ability to accommodate the movement of nutrients and water (Asmen and Puri, 2003). Grain size analysis indicates the amount of small clay particles present at each site. Clay, in turn, shows high affinity for organic
matter, which act as storage for pollutants and toxicants. The higher the clay content of the sediment, the greater the potential for organic retention, thus increasing the absorption potential of the soil (Khan et al., 2008). Variation in grain size composition will also indicate alterations in hydrology.

The fate of excreted hormones depends upon a number of possible pathways, one of which depends on the nature of the sediment present. Hormones may be retained in soil or transported to surface or ground water systems (Khan et al., 2008). Manure and effluent application rates are macro-nutrient based. For example, one approach that has been used to predict hormone loading rates in fields receiving dairy waste has been to determine the mass ratio of specific hormones to N and P concentrations (Raman et al., 2001). From this it is clear that grain size and organic content of the sediment strongly affects the movement of growth hormones. Estrogen and estrogen-mimicking compounds indicate high sorption affinities to sediment (Casey et al., 2005; Collucci and Top, 2002). The sorption potential has been well correlated with sediment grain size and organic content.

Testosterone appears to behave differently, with lower soil sorption affinity only weakly correlating with soil grain size and organic matter content (Casey et al., 2004). This notion is verified by Shore and Shemesh (2003) who observed testosterone leeching into groundwater, while estrogen remained bound to the upper crust of the soil. However, both testosterone and estrogen have been noted in surface run-off from soils treated with animal manure (Finlay-Moore et al., 2000).

Synthetic hormone, TBA appears to behave similarly to testosterone, having a significant affinity to the organic fraction of sediment, leading to a high retardation, but remaining nonetheless mobile in agricultural soils (Khan et al., 2008; Lange et al., 2002). Schiffer et al. (2001) verified this, indicated that TBA remained measurable in soil up to eight weeks after application.

Taking this into consideration, it was determined that downstream sites associated with Feedlot A and Feedlot B have very little hormone assimilation potential (Figure 4.7 to Figure 4.9). Hormones entering these systems will stay mobile and bioavailable, thus increasing the risk of contaminating aquatic biota. However, sediment obtained from the downstream site associated with Feedlot C consisted of higher clay content (Figure 4.6) and organic matter (Figure 4.9), thus decreasing the mobility of hormones. This approach implies that the utilisation of estrogen or estrogen-mimicking growth hormones in meat production may lead to an overall lower risk of ground and surface water contamination.
4.4.3. Present Ecological State

Habitat integrity associated with feedlots

The in-stream habitat integrity for the upstream site at Feedlot A was largely compromised by water quality (Table 4.9). The presence of alien macrophytes also contributed to a decline in habitat integrity. While a decrease in natural vegetation in the riparian zone contributed considerably to the demise of riparian habitat integrity. Feedlot A downstream scored a IHI score similar to the upstream site. However, water abstraction was evident, thus leading to hydrological alteration of the in-stream habitat. The presence of alien invasive species and decreases in natural vegetation were the main drivers for the decrease in riparian habitat integrity.

The habitat integrity of Feedlot B for both sites were impacted by the same factors (Table 4.10) namely, water abstraction, water quality, presence of alien flora, while the riparian zone for both sites indicated a decrease in indigenous vegetation and clear signs of alien vegetation encroachment.

Feedlot C showed a distinct difference between the habitat integrity of its upstream and downstream sites (Table 4.11). This difference is important to note, as invertebrate responses measured may not be due simply to water quality, but also habitat integrity. Factors that influence the habitat integrity of the upstream site are channel modification in the form of a weir crossing the site and bed alteration. The downstream site was impacted by possible water abstraction that led to a very low degree of inundation at the time of sampling. A very slow flowing downstream site caused poor water quality and in addition to this, a decrease in indigenous riparian vegetation also contributed to the demise in overall habitat integrity of this site.

South African Scoring Systems version 5

Aquatic macro-invertebrates have been used extensively to assess the biological integrity of river ecosystems more commonly than any other biological group (O'Keefe et al., 1992). The reason for this is that they are relatively sedentary and allow the detection of local disturbances. The methodology is relatively simple and since the communities are heterogeneous and several taxa are usually represented, response to environmental impacts is usually detectable in terms of the community as a whole.
SASS 5 is one of the numerous biotic indices that have been developed, which allocates numerical scores to specific “indicator” organisms at a particular taxonomic level (Bornman et al., 2007). A number of other factors also need to be considered when interpreting SASS 5 scores, the most important of which is habitat quality, quantity and diversity (Dickens and Graham, 2002). Chutter (1998) argues that ASPT is more reliable measure of the health of a good system than the SASS 5 score. The average water depth and flow rates were higher during the high flow survey, and therefore natural temporal variations within ASPT scores are expected. Relatively large differences were observed in the ASPT scores for sites associated with Feedlot A during the low flow and high flow surveys (Table 4.15 and Table 4.16).

Increases in more sensitive taxa were observed for the upstream site during the high flow survey. Individuals belonging to families such as Leptophlebiidae, Tricorythidae as well as more than three species of Hydropsychidae were noted. However, these families were absent from the downstream site during the same sampling period. The downstream site indicated a strong reduction in diversity compared to the upstream site during the high flow period. This was not the case during the low flow survey with similar ASPT scores obtained for both the upstream and downstream sites associated with Feedlot A. This indicates a possible decrease in water quality downstream from the feedlot during the high flow survey. This view is verified when referring to the decrease water quality measured at the downstream site during the high flow survey (Table 4.4) It must also be noted that high numbers of Simuliidae were sampled at the downstream site during both high flow and low flow surveys. High density Simuliids in the absence of Beataidae in water bodies is usually indicative of organic pollution (William and Feltmate, 1992)

Feedlot B is situated adjacent to a bigger river system and reflects similar IHAS scores for both upstream and downstream sites (Table 4.13). The larger river provides a greater dilution factor and can therefore cope with greater feedlot runoff before observing a stress response in invertebrate communities. Contradictory to the SASS 5 and ASPT scores obtained for Feedlot A, Feedlot B reflected greater differences in these scores during the low flow survey between upstream and downstream sites (Table 4.15). A substantial decrease in sensitive invertebrates as well as a decrease in diversity was observed downstream from feedlot operations during the low flow survey. These differences dissipated during the high flow survey and only a slight difference in ASPT scores was obtained during the high flow survey (Table 4.16).
However, high abundances of the order Diptera was noted at the downstream site during both low flow and high flow surveys, including individuals from Chironomidae, Muscidae and Simuliidae. William and Feltmare (1992) reported that mayflies, caddisflies and stoneflies are usually succeeded by more tolerant midgets in impacted systems.

Where habitat is poor, a low SASS 5 score can not be attributed to poor water quality (Bornman et al., 2007). The site situated downstream from Feedlot C indicates low IHAS scores (Table 4.14), subsequently the poor ASPT scores (Table 4.15 and Table 4.16) observed for this site may be attributed to lack of available habitat. Nonetheless, when comparing the ASPT scores obtained for the downstream site between low flow and high flow surveys, it is evident that there is a decrease in the number of taxa as well as SASS 5 scores during the high flow survey. The opposite is true for the upstream site, which yielded 27 taxa during the high flow survey and only 19 during the low flow survey. Feedlot C shows a similar trend to that of Feedlot A, suggesting a greater impact during the high flow survey. Furthermore, the demise in habitat integrity is also (at least partially) caused by the nearby feedlot activity.

**Fish Assemblage Integrity Index**

With regards to FAII scores obtained for sites associated with Feedlot A (Table 4.18 and Table 4.19), *Barbus anoplus*, *Labeobarbus aeneus* and *Labeo capensis* show a moderate tolerance for decrease in water quality (Kleynhans, 1996) while *Pseudocrenilabrus philander*, *Tilapia sparrmanii*, *Labeo umbratus* and *Clarias gariepinus* all have a high tolerance to decreases in water quality (Kleynhans, 1996). None of the potentially occurring, more sensitive species (i.e. *Labeo kimberleyensis* and *Barbus neefi*) were sampled. It must be noted that there was a decline in FAII scores for the downstream site at Feedlot A for both the high flow and the low flow survey (Table 4.18 and Table 4.19).

Feedlot B upstream also scored similar FAII scores between sampling surveys (Table 4.18 and Table 4.19). *Pseudocrenilabrus philander* have a higher occurrence during the low flow survey while *L. capensis* were more abundant during the high flow survey. This is expected as *P. philander* shows a higher tolerance for decrease in flow velocity (Kleynhans, 1996). The downstream site indicated similar FAII scores to that of the upstream site (Table 4.18 and Table 4.19).

FAII scores associated with Feedlot C are the lowest of all the feedlots sampled (Table 4.18 and Table 4.19). However the high flow survey scores appear to be
marginally better than those of the low flow survey for both the upstream and downstream sites. A lack of connectivity due to upstream and downstream weirs and habitat (especially at the downstream site) is most likely the reason for this. Another possible explanation for the low FAII scores obtained for this feedlot is the fact that the associated river system is situated near the top if its catchment area. Anthropogenic exposure is thus limited. However, higher altitude lotic systems are subjected to higher variations in flow rates and inundation (Davies and Day, 1998). The overall connectivity of the system is also not very good, which limits upstream migration of fish. Only four species of fish were sampled at the upstream site: *T. sparrmanii*, *P. philander*, *L. aeneus* and *C. gariepinus*. *Pseudocrenilabrus philander* had the highest abundance during the low flow survey, which is similar to that of Feedlot B. The downstream yielded only three species of fish: *T. sparrmanii*, *P. philander* and *C. gariepinus*; all of which are tolerant to decreases water quality and flow velocity (Kleynhans, 1999).

FAII scores obtained for all the systems assessed were very low and this is indicative of a demise in ecological integrity, of the systems assessed, at large. The application of FAII in determining point source pollution should be done with caution. Both Feedlot A and Feedlot C did show a downward trend in FAII scores, however this was not the case for Feedlot C. Fish are highly mobile and can migrate upstream or downstream from point-source pollution, complicating comparisons between upstream and downstream sites. This notion is consistent with that of Kleynhans (2007), who states that the FAII is not considered suitable for the assessment of streams with naturally low fish species richness. Consequently, the FAII cannot be considered highly responsive to changes in biological integrity of the upper segments of some rivers. Furthermore, it can also be stated that the FAII only provides an indication of the overall biological integrity in segments of rivers (Kleynhans, 2007). This information is suitable for a synoptic level of assessment. It is for this reason that the FAII must not be regarded as providing a final answer with regard to the biological integrity of a river and should always be interpreted in combination with other ecosystem assessment tools.
4.5. Chapter Summary and Conclusion

Feedlot activities periodically increase the conductivity and pH of downstream sites associated with them. Feedlot A activities impacted the most on the aquatic ecosystem. The NH$_4$ concentrations at all the sites are at levels higher than the TWQR (Table 4.20). Metal concentrations (with the exception of Pb) do not indicate seasonal or site preference and could therefore not be attributed to feedlot activity. The Pb concentration, however, is intermittently higher downstream of feedlot activity when compared to upstream sites. With the exception of Mn, Ni and Fe, all metal concentrations are at levels of concern and much higher than the TWQR for aquatic ecosystems (Table 4.20).

With regards to sediment grain size composition and organic matter, it can be concluded that downstream sites associated with Feedlot A and Feedlot B have very little hormone assimilation potential. However, sediment obtained from the downstream site associated with Feedlot C consists of higher clay and organic matter, thus decreasing the mobility and bioavailability of estrogenic hormones.

Habitat assessment indicated similar habitat integrity and habitat availability, for sites associated with Feedlot A and Feedlot B. This however is not the case for Feedlot C, where both habitat integrity and habitat diversity are compromised (Table 4.20).

SASS 5 scores did indicate impacts at downstream sites with lower diversities and lower sensitivity scores obtained for these sites. The ability of SASS 5 to reflect impacts was best seen during the high flow survey. Further interpretation is needed to ascertain which macroinvertebrates are prominent downstream and which water quality variables are responsible for driving those species (Table 4.20).

The FAII did show alteration in fish assemblage structures between the upstream sites and downstream sites of Feedlot A and Feedlot C, respectively. However, FAII scores for all sites and over both surveys were alarmingly low (Table 4.20). General catchment utilisation adds cumulative and synergistic impacts causing habitat alteration and a decrease in biotic diversity.
Table 4.20: Summary table of chapter one, highlighting important observations for each site, as well as a summary of the EC of habitat, invertebrates and fish assessed at each site.

<table>
<thead>
<tr>
<th>Feedlot</th>
<th>Water quality</th>
<th>Sediment</th>
<th>Habitat</th>
<th>Invertebrates</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In situ</td>
<td>Nutrients</td>
<td>Metals</td>
<td>AVG % Clay</td>
<td>IHI</td>
</tr>
<tr>
<td>(A)U</td>
<td>O₂ (H, L) under TWQR</td>
<td>NH₄ (H, L) above TWQR</td>
<td>All metals except Fe, Mn, Ni above TWQR</td>
<td>6.74</td>
<td>2.79</td>
</tr>
<tr>
<td>(A)D</td>
<td>Conductivity (L) over, O₂ (H, L) above TWQR</td>
<td>NH₄ (H, L); SO₄ (H), above TWQR</td>
<td>All metals except Fe, Mn, Ni above TWQR</td>
<td>6.39</td>
<td>0.96</td>
</tr>
<tr>
<td>(B)U</td>
<td>pH (H, L) above TWQR</td>
<td>NH₄ (H, L) above TWQR</td>
<td>All metals except Fe, Mn, Ni above TWQR</td>
<td>12.12</td>
<td>0.67</td>
</tr>
<tr>
<td>(B)D</td>
<td>Conductivity (L); pH (H, L) above TWQR</td>
<td>NH₄ (H, L) above TWQR</td>
<td>All metals except Fe, Mn, Ni above TWQR</td>
<td>0.74</td>
<td>0.73</td>
</tr>
<tr>
<td>(C)U</td>
<td>pH (L) above TWQR</td>
<td>NH₄ (H;L) above TWQR</td>
<td>All metals except Al (L), Fe, Mn, Ni above TWQR</td>
<td>12.26</td>
<td>3.51</td>
</tr>
<tr>
<td>(C)D</td>
<td>NH₄ (H;L) above TWQR</td>
<td>All metals except Fe, Mn, Ni above TWQR</td>
<td>46.80</td>
<td>5.19</td>
<td>B</td>
</tr>
</tbody>
</table>

(A)U: Feedlot A Upstream; (A)D: Feedlot A Downstream; (B)U: Feedlot B Upstream; (B)D: Feedlot B Downstream; (C)U: Feedlot C Upstream; (C)D: Feedlot C Downstream. TWQR: Target Water Quality Range; L: Low flow; H: High flow.
Chapter 5

Alteration to Aquatic Macroinvertebrate Community Structures With Reference to Feedlot Activity

5.1. Introduction

Feedlots usually consist of large numbers of cattle on relatively small areas. Runoff from feedlot activity introduces soil, salts, organic matter, manure, metals, as well as hormone metabolites to associated aquatic systems (Cooper, 1993; Whitehurst, 1991). In addition to this, aquatic biota also need to adapt to alterations in habitat conditions (Hilsenhoff, 1982; Plafkin et al. 1989). Landscape-level investigations of invertebrate communities show that species diversity, abundance and composition are largely dependent on the environmental variables to which the communities are exposed (Wally, 1998; Ruse, 2000; Probst et al. 2004).

Sedimentation associated with feedlot activity is an important abiotic driver of invertebrate community responses (Zweig and Rabeni, 2001) and an increase in sedimentation causes a reduction in habitat integrity. It has been indicated that even small increases in sediment deposition may alter invertebrate community compositions (Lenat and Crawford, 1994; Quinn et al., 1997) and cause changes in dominance from the Ephemeroptera, Plecoptera and Trichoptera taxa to Oligochaetes. These habitat alterations are difficult to quantify as sedimentation is generally accompanied by other impacts on freshwater ecosystems (Zweig and Rabeni, 2001).

In addition to sedimentation, feedlots also introduce large volumes of salts into associated systems (Erickson, 2003). This might occur directly (through runoff) or indirectly (through manure application to agricultural fields) (Dodds and Welch, 2000). An Australian study indicated salinity as one of the main contributors to changes in macroinvertebrate community compositions (Kay et al., 2001). The study showed that invertebrate communities, exposed to saline conditions, reflected uniform community structures, consisting of species with a high tolerance to saline conditions.
The effects of organic matter, which originate from feedlot activities and enter the associated aquatic system, contributing to nutrient enrichment, have not been well documented (Wang et al., 2007). The potential effect of a nutrient increase caused by feedlot activity may be an alteration in the primary production structure. Studies that have been undertaken in this regard indicate that an influx of nutrients may influence light availability and temperature and this may augment in-stream primary production, resulting in changes in the trophic structure of benthic communities (Johnson et al., 1997; Sponseller et al., 2001). Controlled experiments on nutrient enrichment have indicated that macroinvertebrate and periphyton abundance escalated with increased nutrient availability (Dudley et al., 1986). Changes in the makeup and abundance of periphyton algae have also been found to reduce macroinvertebrate drift (Kerans, 1996). Increased periphyton abundances can also affect sensitive macroinvertebrates by depleting oxygen through nocturnal respiration (Wang et al., 2001).

In order to measure alterations in invertebrate community structures; diversity indices are employed to determine alteration in taxon richness. Diversity indices are mathematical expressions of three components of community structure, namely: richness, evenness and abundance and can be used to describe alteration in community structure relative to water quality (Dallas and Day, 2004; Kramer, 2006). The earlier forms of these indices expressed the species richness of a community as the number of species relative to the total number of individuals observed (Warren, 1971). A modification of this method is the Shannon-Weiner index which also takes into account the number of individuals for every species or taxa observed (Hawkes, 1979). The Shannon-Weiner index (Krebs, 1999) was used as a diversity index in this study.

A macroinvertebrate community comparison index, namely, the Bray-Curtis index (Bray and Curtis, 1957) was also used in this study. This index was identified as feasible because it includes species abundances, utilises transformed data, index values are not clumped and there is a linear response in changes in specie numbers and abundances (Reynoldson and Metcalfe-Smith, 1992). Essentially, similarity indices compare community assemblages at different sites (Boyle et al., 1990). These methods are informative and less reliant on sampling intensity, but require realistically comparable sites, sampling and analytical methods (Dallas and Day, 2004).

Principal ordination techniques were used on species assemblages to identify community changes in space and time, as well as the physical processes responsible for that particular ordination. During the last decade PCAs have been successfully applied to the study of the effects of environmental conditions on species assemblages (Berenzen et al. 2005; Borcard
et al. 1992; Laroque et al. 2001; Ter Braak and Wiertz, 1994) and are thus incorporated into this study.

This chapter aims to assess the feedlot-related runoff contamination that contributes to differentiation in the macroinvertebrate communities, by comparing upstream sites with downstream sites associated with each feedlot.

5.2. Materials and Methods

5.2.1. Study sites

Refer to Chapter two for a detailed description on the study areas and site selection.

5.2.2. Field collection sampling and identifying

A standardised invertebrate collection net (1 mm mesh with a 300 x 300 mm square opening) was used for the collection of the aquatic macroinvertebrates. All of the available biotopes were sampled as described by Dickens & Graham (2002). The biotopes were divided into stones (S), vegetation (Veg) and gravel, sand and mud (GSM). Before and after disturbing the site, approximately one minute of “hand-picking” was carried out for specimens that may have been missed by the sampling procedures. Samples for each site were fixated in 10 % formaldehyde and stained with Rose Bengal. Samples were then washed and stored in 70 % ethanol before family level identification was done and abundances determined for each site and biotope. Abundance data collected from the implementation of SASS 5 protocol at each site for high and low flow was subjected to statistical analyses described below.

5.2.3. Statistical procedure

The macroinvertebrate community structure was assessed with univariate and multivariate statistical analyses. Univariate diversity and evenness indices were used to describe macroinvertebrate species-abundance relations using Primer version 6. Univariate analyses undertaken was Margalef’s index (d) (Margalef, 1951), which is a measure of the number of species present for a given number of individuals, the Shannon-Wiener diversity index (H’) (Wilhm and Dorris, 1968), and Pielou’s evenness index (J’) (Pielou, 1997).

Multivariate statistical procedures were used to assess changes in macroinvertebrate communities between sites using Primer version 6. Bray-Curtis similarity matrices were
constructed from log transformed macroinvertebrate family abundance data recorded for each site at high and low flow surveys. Similarity matrices were subjected to group averaged hierarchical clustering (CLUSTER) to summarise patterns in species composition. Factors were assigned to Bray-Curtis resemblance matrices based on groupings from the CLUSTER analysis.

A One way Analysis of Similarity Percentages (SIMPER) based on species contribution was used to identify the families of macroinvertebrates that primarily provided discrimination between sample clusters. K-dominance plots were included to indicate sites that have an increased dominance of taxa relative to the other samples and flow periods.

For the purpose of visually illustrating the relationship between macroinvertebrate community data and water quality, the data were subjected to Redundancy Analysis (RDA) using Canoco version 4.5. Redundancy Analysis was carried out on log transformed data and the significance of RDA axes was tested using unrestricted Monte Carlo permutation testing (499 permutations, \( p = 0.05 \)). Redundancy Analysis is an ordination technique that uses best fit values from multiple linear regression between variables, and includes a second axis (in this case, habitat) (Ter Braak and Smilauer, 2002).
5.3. Results

5.3.1. Diversity indices

Figure 5.1 and Figure 5.2 reflect the Margalef’s species richness \((d)\) of the feedlots in relation to each other as well as for both high flow and low flow surveys. Sites associated with Feedlot A have, on average, lower species richness than the other two feedlots. It is also evident that the site situated downstream from Feedlot A reflected the lowest species diversity for both low flow \((2.9)\) and high flow surveys \((2.7)\), compared to the higher species richness of the upstream site during the low flow survey \((3.9)\) and high flow survey \((4.19)\).

Feedlot B is situated between Feedlot A and Feedlot C in terms of Margalef’s species richness (Figure 5.1 and Figure 5.2) and follows a similar trend to that of Feedlot A. It obtained its highest species richness score \((d)\) at the upstream site during the low flow survey \((5.26)\) and during the same surveying time it obtained a much lower species richness score for the downstream site \((3.74)\). However, during the high flow survey there was a slight correction in species richness of the downstream site \((4.2)\), compared to that of the upstream site \((4.8)\).

Sites associated with Feedlot C had the highest Margalef’s species richness scores (Figure 5.1 and Figure 5.2), with the upstream site scoring \(5.52\) and \(5.6\) during both low flow and high flow surveys, respectively. The downstream site comprised of slightly lower species richness during the low flow survey \((5.1)\), but showed a considerable decline during the high flow survey \((3.2)\).

Both downstream sites associated with Feedlot A and Feedlot C during the high flow survey revealed a decline in species richness. All downstream sites consisted of lower species richness when compared to upstream sites, regardless of season. Feedlot B indicated a slight improvement with regards to species richness during the high flow survey at the downstream site, in contrast with the downstream sites associated with Feedlot A and Feedlot C that showed a decline in species richness during the high flow survey, while the upstream sites associated with these feedlots reflected their highest species richness obtained.

Figure 5.3 indicates Pielou’s evenness index \((J')\), which takes into consideration the total number of species sampled and the extent to which the total abundance is spread equally amongst the observed species. A higher score will, therefore indicate a more even distribution while a lower score will indicate a more skewed distribution. With this in mind, sites reflecting a lower evenness are Feedlot A upstream, during the high flow survey, with a
J’ score of 7.6, as well as Feedlot B downstream during the high flow survey with a J’ score of 0.77. The site situated downstream from Feedlot C, recorded the highest J’ score of 0.9 during the high flow survey. The extent to which the abundances are spread over the number of species was very similar for all sites during both low flow and high flow surveys.

The Shannon-Wiener diversity index (H’) incorporates both the Margalef’s species richness (d) and Pielou’s evenness index (J’) to reflect diversity obtained for each feedlot (Figure 5.4). From this diagram, it is clear that sites associated with Feedlot A scored the lowest on the diversity index, with both upstream and downstream sites scoring below 2.56. The downstream site associated with Feedlot A reflects the lowest diversity (2.0).

Feedlot B reflected a higher diversity score at the upstream site, regardless of season, when compared to the downstream site, which scored 2.5 during the low flow survey and 2.4 during the high flow survey.

Sites associated with Feedlot C feedlot generally produced higher diversity. The upstream site did not indicate much variation in diversity between seasons, scoring 2.82 during the low flow survey and 2.89 during the high flow survey. The same was observed for the downstream site which scored 2.66 during the low flow survey and 2.48 during the high flow survey.

Both upstream sites, associated with Feedlot A and Feedlot B, indicated a reduction in diversity during the high flow survey, while diversity at Feedlot C increased slightly. There was a general decrease in diversity at sites situated downstream from feedlot activity, however, seasonal factors also contribute towards this decrease in diversity.
Chapter 5

Figure 5.1: Univariate diversity index values for macroinvertebrate indicating Margalef’s species richness for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.2: Univariate diversity index values for macroinvertebrate indicating Total Species (S) for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.3: Univariate diversity index values for macroinvertebrate indicating Pielou’s evenness (J') for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.

Figure 5.4: Univariate diversity index values for macroinvertebrate indicating Shannon-Wiener diversity index (H' (logo)) for all three feedlots for upstream (U) and downstream (D) site, during low flow (L) and high flow (H) surveys.
5.3.2. Community composition

Figure 5.5 represents a cluster analysis of Bray-Curtis resemblance matrices for the invertebrate community structure sampled at the sites of with Feedlot A. It is evident that the downstream site had the greatest similarity (less than significant \( p<0.05 \)) in community structure when comparing low flow data to that obtained for the high flow survey. The average similarity for the grouping of downstream sites during both low flow and high flow survey was 59.03 %. The main driving factors behind this similarity consisted predominantly of *Hydropsychidae* (30.69 %), *Simuliidae* (20.11 %), *Baetidae* (15.36 %) and *Chironomidae* (12.8 %) (Table 5.1). The upstream site associated with Feedlot A, sampled during the low flow survey, differed from the downstream site, with an average dissimilarity of 51.45 %. The main contributors towards this indifference were *Hydropsychidae* (20.62 %), *Baetidae* (15.51 %), *Corbiculidae* (11.59 %) and *Corixidae* (8.61 %). While the invertebrate community structure associated with the upstream site during the low flow survey differed from that of the high flow survey with 58.13 % (Figure 5.5). The invertebrate families primarily responsible for this difference are *Hydropsychidae* (25.91 %), *Simuliidae* (24.88 %) and *Baetidae* (10.31 %).

Seasonal variation and disturbance is reflected in the cumulative dominance plot for Feedlot A (Figure 5.6), where the high flow survey indicated a single family dominance of 48 % for the upstream site and 52 % for the downstream site. During the low flow survey, only 28 % of the individuals sampled belonged to the same family for the downstream site and 32 % for the upstream site.

Table 5.1: Results obtained from SIMPER analysis with a 90 % cut off for low contributions indicating the contribution of various macroinvertebrate families for the downstream site, associated with Feedlot A, to similarity within the macroinvertebrate groupings.

<table>
<thead>
<tr>
<th>Site</th>
<th>Family</th>
<th>Contr (%)</th>
<th>Cumulative Contribution (%)</th>
<th>Average Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td><em>(A)DL and (A)DH</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hydropsychidae</em></td>
<td>30.69</td>
<td>30.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Simuliidae</em></td>
<td>20.11</td>
<td>50.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Baetidae</em></td>
<td>15.36</td>
<td>66.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chironomidae</em></td>
<td>12.8</td>
<td>78.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oligochaeta</em></td>
<td>6.61</td>
<td>85.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Elmidae</em></td>
<td>5.12</td>
<td>90.69</td>
<td></td>
</tr>
</tbody>
</table>

*(A)DL: Feedlot A Downstream Low flow; (A)DH: Feedlot A Downstream High flow. Contr: Contribution*
Figure 5.5: Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between upstream (U) and downstream (D) in relation to the macroinvertebrate community structure at each site for low flow (L); and high flow (H) survey for sites associated with Feedlot A (K).

Figure 5.6: Ranked species K-dominance plot for macroinvertebrate communities collected at upstream (U) and Downstream (D) sites associated with Feedlot A (K) during both high flow (H), and low flow (L) surveys.
Chapter 5

The cluster diagram for Feedlot B indicates a site ordination rather than a seasonal grouping (Figure 5.7). Invertebrate community structures for the upstream site were on average 66.24% (Table 5.2) similar when comparing the low flow survey with that of the high flow survey. Invertebrate families responsible for this similarity were *Hydropsychidae* (22.04%), *Baetidae* (15.1), *Simuliidae* (11.15%) and *Chironomidae* (10.77%). The downstream site also grouped together, and had an average of 69.26% (Table 5.2). Similarity between low flow and high flow surveys. *Simuliidae* (30.87%), *Hydropsychidae* (19.88) as well as *Baetidae* (8.91 %) contributed largely to the observed similarity for the downstream site. The cumulative dominance plot for Feedlot B showed the highest single family dominance (67%) for the site located downstream from Feedlot B during the high flow survey, while the same site reflected the lowest single species dominance (38 %) during the low flow survey (Figure 5.8). The upstream site did not indicate large single family dominance differences between season, with 39 % during the high flow survey and 43 % during the low flow survey.
Table 5.2: Results obtained from SIMPER analysis with a 90 % cut off for low contributions indicating the contribution of various macroinvertebrate families for the upstream and downstream sites, associated with Feedlot B, to similarity within the macroinvertebrate groupings.

<table>
<thead>
<tr>
<th>Site</th>
<th>Family</th>
<th>Contr. (%)</th>
<th>Cumulative Contribution (%)</th>
<th>Average Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B)UL and (B)UH</td>
<td>Hydropsychidae</td>
<td>22.04</td>
<td>22.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baetidae</td>
<td>15.10</td>
<td>37.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Simuliidae</td>
<td>11.15</td>
<td>48.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chironomidae</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Tricorythidae</td>
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</tr>
<tr>
<td></td>
<td>Leptophlebiidae</td>
<td>3.34</td>
<td>72.02</td>
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<tr>
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<td>Oligochaeta</td>
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<td>75.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physidae</td>
<td>2.72</td>
<td>77.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corbiculidae</td>
<td>2.55</td>
<td>80.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conixidae</td>
<td>2.36</td>
<td>82.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elmidae</td>
<td>2.36</td>
<td>85.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atyidae</td>
<td>2.15</td>
<td>87.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caenidae</td>
<td>2.15</td>
<td>89.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gyrinidae</td>
<td>2.15</td>
<td>91.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>66.24</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B)DL and (B)DH</td>
<td>Simuliidae</td>
<td>30.87</td>
<td>30.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydropsychidae</td>
<td>19.88</td>
<td>50.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baetidae</td>
<td>8.91</td>
<td>59.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oligochaeta</td>
<td>6.17</td>
<td>65.83</td>
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</tr>
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<td>Muscidae</td>
<td>6.04</td>
<td>71.88</td>
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</tr>
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<td></td>
<td>Chironomidae</td>
<td>4.79</td>
<td>76.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physidae</td>
<td>4.43</td>
<td>81.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hirudinea</td>
<td>3.19</td>
<td>84.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corbiculidae</td>
<td>2.94</td>
<td>87.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tricorythidae</td>
<td>2.68</td>
<td>89.9</td>
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</tr>
<tr>
<td></td>
<td>Lymnaeidae</td>
<td>2.38</td>
<td>92.28</td>
<td></td>
</tr>
</tbody>
</table>

(B)UL: Feedlot B Upstream Low flow; (B)UH: Feedlot B Upstream High flow; (B)DL: Feedlot B Downstream Low flow; (B)DH: Feedlot B Downstream High flow. Contr: Contribution
Figure 5.7: Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between upstream (U) and downstream (D) in relation to the macroinvertebrate community structure at each site for low flow (L); and high flow (H) survey for sites associated with Feedlot B (T).

Figure 5.8: Ranked species K-dominance plot for macroinvertebrate communities collected at upstream (U) and downstream (D) sites associated with Feedlot B (T) during both high flow (H), and low flow (L) surveys.
Chapter 5

With reference to Feedlot C, a seasonal ordination is visible (Figure 5.9) for the low flow survey, with the upstream and the downstream site showing an average similarity of 63.97 % (Table 5.3). This similarity predominantly constitutes of Chironomidae (20.87 %), Corixidae (20.01 %) and Notonectidae (11.89 %). During the high flow survey the upstream site associated with Feedlot C indicated an average dissimilarity of 64.39 % when compared to Group 1. Hydropsychidae (13.19 %), Corixidae (10.59 %) and Baetidae (10.49 %) contributed largely to this dissimilarity. During the high flow survey the site situated downstream from Feedlot C differed from upstream site (both low and high flow) and the downstream site (low flow), on average, with 71.41 %. Macroinvertebrates contributing to this dissimilarity included Chironomidae (17.15 %), Corixidae (15.90 %), Corbiculidae (9.02 %) and Notonectidae (6.41 %)

The cumulative dominance plot for Feedlot C verifies the seasonal trend observed through the cluster diagram (Figure 5.10). However, the single dominant family decreased during the high flow, indicating increased environmental stress during the low flow survey for both the upstream site with almost 50 % of all individuals sampled, belonging to the same family as well as the downstream site, with a single family dominance of 40 %. The downstream site, during the high flow survey indicated the lowest cumulative dominance (19 %), while the upstream site during the same survey time was slightly higher at 30 % cumulative dominance.

| Table 5.3: Results obtained from SIMPER analysis with a 90 % cut off for low contributions indicating the contribution of various macroinvertebrate families for the low flow survey, associated with Feedlot C, to similarity within the macroinvertebrate groupings. |
|---|---|---|---|
| Site | Family | Contribution (%) | Cumulative Contribution (%) | Average Similarity (%) |
| Group 1 | (C)UL and (C)DL | Chironomidae | 22.87 | 22.87 | 63.97 |
| | | Corixidae | 20.01 | 42.88 | |
| | | Notonectidae | 11.89 | 54.77 | |
| | | Oligochaeta | 6.67 | 61.44 | |
| | | Dytiscidae | 5.83 | 67.27 | |
| | | Baetidae | 3.96 | 71.23 | |
| | | Ancylidae | 2.56 | 73.79 | |
| | | Gyrinidae | 2.56 | 76.34 | |
| | | Hydracarina | 2.56 | 78.9 | |
| | | Physidae | 2.56 | 81.46 | |
| | | Planaria | 2.56 | 84.02 | |
| | | Caenidae | 2.29 | 86.3 | |
| | | Hirudinea | 2.29 | 88.59 | |
| | | Belostomatidae | 1.98 | 90.57 | |

(C)UL: Feedlot C Upstream Low flow; (C)DL: Feedlot C Downstream Low flow.
Figure 5.9: Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between upstream (U) and downstream (D) in relation to the macroinvertebrate community structure at each site for low flow (L); and high flow (H) survey for sites associated with Feedlot C (B).

Figure 5.10: Ranked species K-dominance plot for macroinvertebrate communities collected at upstream (U) and Downstream (D) sites associated with Feedlot C (B) during both high flow (H), and low flow (L) surveys.
5.3.3. Invertebrate community response to water quality

The axes of the RDA for Feedlot A (Figure 5.11) account for 80.3% of the variance in the data and 46.3% of this is represented on the first axis while the other 34% is represented on the second axis. The RDA indicates a site ordination based on invertebrate diversity and abundances as well as the water quality variables, influencing that ordination. A clear site grouping is observed, with both downstream sites grouping together as well as both upstream sites. The invertebrates responsible for the ordination of the downstream site were Physidae, Ancylidae, Planaria and Corbiculidae. The downstream site, during the high flow survey, showed elevated levels of nutrients and pH, relative to the upstream site which was predominantly influenced by turbidity. There was also a greater difference between the macroinvertebrate diversity and abundances of the upstream site during low flow and high flow assessment, indicating a seasonal influence.

![Figure 5.11: Redundancy Analysis (RDA) indicating Site and Season ordination based on (a) invertebrate abundances and diversity and (b) water quality variables, for both upstream and downstream sites associated with Feedlot A over low and high flow field surveys. K=Feedlot A, D=Downstream, U=Upstream, H= High flow, L= Low flow.](image-url)
Figure 5.12 represents a RDA for Feedlot B and accounts for 81.7 % of total variance with 45.1 % represented on the first axis. A site ordination is apparent, with similar water quality parameters and invertebrate community structures contributing to the ordination of the downstream site and upstream site, respectively. The downstream site groups below the x-axis, while the upstream site groups above it. pH, oxygen, conductivity and turbidity are the driving factors at the downstream site during both high flow and low flow surveys. Phosphate, Cl\(^-\) and SO\(_4\) are more prominent at the upstream site during the low flow, while COD, NH\(_4\) and NO\(_3\) contribute toward the similarity between upstream sites, regardless of season. The downstream site macroinvertebrate community structure was composed predominantly of Simuliidae, Muscidae and Corbiculidae. Notwithstanding the clear site ordination, seasonal similarities are also observed with the high flow data grouping on the lefts of the y-axis and the low flow data ordination on the right of the y-axis.

Figure 5.12: Redundancy Analysis (RDA) indicating Site and Season ordination based on (a) invertebrate abundances and diversity and (b) water quality variables, for both upstream and downstream sites associated with Feedlot B over low and high flow field surveys. T=Feedlot B, D=Downstream, U=Upstream, H= High flow, L= Low flow.

The RDA for Feedlot C (Figure 5.13) represents 86 % of total variance, 51.2 % of which is on the first axis while 34.8 % is represented on the second axis. The RDA indicates a
definite macroinvertebrate community structure difference between upstream and downstream sites associated with Feedlot C. Furthermore, the differences are dissimilar with regard to seasonality and site ordination.

The water quality driving forces associated with this invertebrate composition for the upstream site were $O_2$, Turbidity, $SO_4$, pH and COD, while the downstream site was characterised by higher $NO_2$ and $NO_3$ concentrations. The downstream site ordinate above the x-axis and the invertebrates driving this ordination included *Nepidae* and *Lymnaeidae* during the low flow survey and *Chlorolestidae* and *Gomphidae* during the high flow survey.

The upstream site ordinate below the x-axis, but did, however, show a strong seasonal response in invertebrate community structures. The high flow survey indicated higher $PO_4$ and $NH_4$ values for both upstream and downstream sites, with higher abundances of *Gyrinidae* and *Coenagrionidae* also present at these sites during the high flow survey. Water quality variables present in greater quantities during the high low flow survey at both upstream and downstream sites included higher COD, pH, Cl, and $SO_4$ values.

![Figure 5.13: Redundancy Analysis (RDA) indicating Site and Season ordination based on (a) invertebrate abundances and diversity and (b) water quality variables, for both upstream and downstream sites associated with Feedlot C feedlot over low and high flow field surveys. B=Feedlot C, D=Downstream, U=Upstream, H= High flow, L= Low flow.](image.png)
Chapter 5

5.4. Discussion

5.4.1. Feedlot A

With reference to the diversity trend observed for Feedlot A (Figure 5.4), a large decline is observed for the downstream site during the high flow survey. The same survey yielded a much larger diversity upstream from the feedlot, indicating a decrease in water quality downstream from the feedlot during the high flow survey. This was also reflected in the water quality data for the high flow survey, with invertebrate community structure responding to higher dissolved solids and nutrient levels (Figure 5.11). Runoff from agricultural fields is known to cause changes in abiotic conditions in particular, increases in nutrient load (Cooper and Lipe, 1992; Higler and Repko, 1981) as well as in sediment loads (Kuhnle, 1992). In addition to increased concentrations of nutrients and sediment, mobilisation of hormones will also increase with flow rates and the presence of organic matter (Casey et al., 2004; Casey et al., 2005). Chapter three established no differences in habitat integrity and availability; therefore these factors will not contribute to a decrease in invertebrate diversity. Another consideration is a general decline in species richness (Figure 5.2) and abundances observed downstream of Feedlot A activity, for both the low and the high flow survey, implying a community stress response to the actual feedlot activity.

With regards to the community structures and similarity observed for upstream and downstream sites associated with Feedlot A, higher numbers of more tolerant individuals belonging to the Simuliidae, Hydropsychidae, Chironomidae and Oligochaeta families were observed (Table 5.1). Invertebrate community structure typically responds to organic enrichment and alteration in water quality with an increase in tolerant taxa and a decrease in more sensitive taxa (Dallas and Day, 2004; Seager and Abrahams, 1990).

Furthermore, polluted sites are typically characterised by smaller diversities and higher abundances (Dallas and Day, 2004). In this manner the cumulative dominance plot acts as a measure of disturbance (Figure 5.6). It appears if Feedlot A activity is impacting on the diversity of invertebrates, with less diversity obtained downstream. However, season also plays an important role in quantities of invertebrates present, with a much higher single family dominance during the high flow survey obtained.

The downstream site during the high flow survey reflected the highest single taxon dominance, verifying the diversity and cluster analysis, indicating a higher disturbance for this site during the high flow survey (Figure 5.6).
5.4.2. Feedlot B

Sites associated with Feedlot B, indicated a diversity trend similar to that of the sites associated with Feedlot A (Figure 5.1 to Figure 5.4), with a general decline in diversity at the downstream sites when compared to the upstream site, for both the high flow and the low flow survey. It is also worth mentioning that invertebrate abundances for sites connected to Feedlot B reflected a more even distribution, according to Pielou’s evenness (Figure 5.3).

In addition to the diversity index scores, the Bray-Curtis cluster analysis also indicated a site ordination for this feedlot (Figure 5.7), with invertebrate community structures grouping together for each site, regardless of season. The downstream site indicated a greater similarity during seasons, with Simuliidae contributing mainly to the similarity (Table 5.2). The upstream site also indicated similarities between surveys however; this similarity was predominantly driven by Hydropsychidae and Baetidae. The downstream site indicated more disturbance during the high flow survey, with almost 70% of all individuals sampled belonging to the same taxa (Simuliidae) (Figure 5.8). Other invertebrates observed in higher quantities at the downstream site during both surveys included: Muscidae and Oligochaeta.

The extent the impacts of feedlot activity may also depend on the river zone where it occurs (Dallas and Day, 2004). Biota of oligotrophic zones will be more sensitive to organic enrichment and higher sediment loads when compared to mesotrophic, depositional zones (Davies and Day, 1998). Both the upstream and the downstream community composition include dominant species, such as Hydropsychidae, Chironomidae, Oligochaeta and Hirudinea, all of which are typically associated with organic and nutrient enriched systems (Dallas and Day, 2004). However, the excessive numbers of Simuliidae, Muscidae and Oligochaeta present downstream of the feedlot were indicative of a biological stress response. All of these species indicate a high tolerance to decreased oxygen concentrations, higher dissolved solids and variation in pH (Hellawell, 1986) (Figure 5.12).

Determining the abiotic drivers responsible for the alteration in invertebrate community structures are speculative, as there were no observable differences in habitat availability and water quality (with the exception of higher pH and conductivity values (Figure 5.12) between upstream and downstream sites. Another factor, which is potentially responsible for the restrained similarity in invertebrate community structures downstream of feedlot activity may be the duration of discharge and organic content of the effluent (Dallas and Day, 2004). Continuous low level discharges will lead to slow alteration in pH and dissolved solids contents (Lloyd and Swift, 1976), which may enable aquatic biota to acclimate to the situation (Dallas and Day, 2004). Episodic events carrying higher volumes of organic matter, salts and nutrients, will trigger a greater stress response (Dallas and Day, 2004). This,
however, does not appear to be the case with Feedlot B as much similarity was observed when comparing the low flow survey with that of the high flow survey (Figure 5.12).

### 5.4.3. Feedlot C

Sites associated with Feedlot C reflected a similar trend to that of Feedlot A and Feedlot B, with the downstream sites indicating lower Shannon-Wiener diversity scores when compared to the upstream site, for both the high flow and the low flow survey (Figure 5.4). However, there was a substantial drop in diversity during the high flow survey, with a large drop in Margalef’s species richness score (Figure 5.2) as well as a very uneven abundance distribution (Figure 5.3) observed for the downstream site. Natural variation in diversity is not uncommon during seasons (Chutter, 1998). However, this does not explain the higher diversity score observed for the upstream site during the high flow survey, when compared to the low flow survey, while the downstream site indicates a much lower diversity score during the high flow survey when compared to the low flow survey. Lower diversity scores were expected downstream from Feedlot C activity, as the habitat availability was reduced. Nevertheless, one would expect a consistent lower diversity score relative to the upstream site during both high and low flow surveys. The sudden and unrelated drop in diversity observed downstream of the feedlot is possibly a response to feedlot activity.

The cluster analysis indicates a definite seasonal response in biotic community composition (Figure 5.9). Invertebrate community structures during the low flow survey are almost 70% similar, with the high flow survey indicating greater dissimilarity. In terms of single taxa dominance (Figure 5.10), the low flow survey indicates the community stress measured, with 50% of invertebrates sampled at the downstream site belonging to the same family and 40% of invertebrates sampled at the upstream site also belonging to the same family.

The invertebrate stress responses observed at sites associated with Feedlot C are predominantly driven by habitat availability and seasonal changes. Factors such as stream width and discharge (Wally et al., 1998), nutrients, substrate, pH and velocity (Ruse, 2000) have repeatedly been identified as influential with regard to the composition of invertebrate communities. Feedlot C is situated next to the Os Spruit, which is located in the upper reaches of its catchment area, making it all the more susceptible to seasonal alteration such as active channel width, extent of inundation and hydrological variation. Because no obvious differences were observed for water quality parameters upstream and downstream from the feedlot (Figure 5.12), it is possible that feedlot activity is contributing to the alterations in habitat availability. Large volumes of water abstraction to provide cattle with drinking water,
as well as increase runoff rates due to direct catchment alterations, will contribute to alterations in flow and substrate conditions (Probst et al., 2004).

5.5. Conclusion

In this study it was illustrated that feedlot activity does contribute to the alteration in the Shannon-Wiener diversity and Bray-Curtis similarity coefficient when comparing downstream sites with upstream sites. The intermediate stretch of river between upstream and downstream sites did not receive industrial or urban runoff, consequently the only source of organic and hormonal substances were the actual feedlot activities and taking into account “natural” seasonal variation within invertebrate community structures, upstream and downstream ordination was observed (with reference to the RDA).

The Shannon-Wiener diversity scores were periodically lower downstream of feedlots, but were observed to be the lowest during the high flow survey, indicating an additional seasonal response.

Sites associated with both Feedlot A and Feedlot B, both grouped together in terms of community composition, regardless of seasonal variation, while community structures observed for sites associated with Feedlot C indicated a strong seasonal similarity. However, the feedlot activity’s contribution to the differences in habitat integrity and availability, between the upstream and downstream site, must be considered.

In addition to diversity and similarity, cumulative dominance can also be use as a relative disturbance indicator. All sites reflecting the highest single taxon diversity are located downstream from feedlots and can thus be considered to have an increased exposure to impacts of feedlot activities.

Furthermore, it can be concluded that both the invertebrate diversity and community analysis do indicate a stress response not perceived by SASS 5. Referring to chapter three, the SASS 5 scores indicated similar scores for sites connected to Feedlot A during the low flow survey, as well as sites associated with Feedlot B during the high flow survey. In this chapter, clear differences in diversity and invertebrate community composition were obtained for both upstream and downstream sites.
Chapter 6

Biomarker responses in *Clarias gariepinus* following exposure to Trenbolone Acetate (TBA) and Diethylstilbestrol (DES)

6.1. Introduction

Biological markers can act as sensitive and ecologically relevant measures of environmental conditions (Adams and Greeley, 2000). In essence the biomarker approach is a proven bioassessment method that uses responses of sentinel species to act as indicators of stress as well as indicators of early warning alteration in environmental conditions (Schlenk et al., 1996).

In the previous chapters a detailed assessment was done on the possible environmental and feedlot-associated variables impacting on exposed aquatic biota. In this chapter, an assessment of possible stress response to the exposure of growth hormones used in cattle production is presented. In addition to monitoring the general condition (McKenzie et al., 2006) of the test organisms over a chronic exposure period, somatic indices were also employed. Two biomarkers, CEA (De Coen and Janssen, 1997) and metabolomics (Samuelsson et al., 2006), were used to assess alteration in the energy metabolism of exposed organisms.

This integrated approach measures a range of variables over different levels of organisation, from sub-cellular (metabolomics), cellular (CEA), organ (HIS) and (GSI) to organism level (CF). When properly designed and implemented, both of these biomarkers can identify causal mechanisms between environmental stressors and population- and community-level effects (De Coen and Janssen, 2003; Nicholson et al., 1999). This in turn will form the basis from which remedial and management actions, in terms of aquatic health and feedlot activities, are implemented.
6.1.1. Growth hormones

Trenbolone acetate, or 17 β-hydroxyestra-4,9,11-triene-3-one, is a synthetic steroid with anabolic properties and is eight to ten times as potent as testosterone (Boulffault and Willemart, 1983). In cattle production; TBA, alone or in combination with 17 β-oestradiol, is used to improve weight gain and efficient feed metabolism. Trenbolone acetate is administered by subcutaneous implantation in the ear. The dosage ranges between 40 and 300 mg per animal and is usually expressed in ratio with the weight of the animal (JECFA-WHO, 1988). After entering the system of the animal, TBA is rapidly hydrolysed to its active free form 17 β-trenbolone. In cattle the 17 Ω-epimer is the major metabolite occurring in the excreta (European Commission, 1999).

Diethylstilbestrol is a synthetic non-steroidal estrogen. It has a molecular weight of 268.4 and occurs as small plates from benzene or as a white crystalline powder (Halling-Sorensen et al., 1997). Diethylstilbestrol is practically insoluble in water, but is persistent in standing water, soils and faeces (Shore et al., 1993; Zondek and Sulman, 1943). Coats et al. (1976) determined LC₅₀ values for Daphnia magna and Gambusia affinis to DES exposures at 10 mg/l and >1 mg/l respectively. The use of DES in meat production was first applied in 1947 and it was approved by the United Stated Food and Drug Administrator in 1957 (Raun and Preston, 2002). It was in active use until 1972 when it was discovered to be a carcinogen.

6.1.2. Test organism

*Clarias gariepinus*, or commonly known as the Sharptooth catfish belongs to the family Clariidae. Clarrids are African and Asian fish that are well known for their hardiness and ability to breathe air (Skelton, 2001). The common Sharptooth catfish is widely distributed, which is one of the main objectives when selecting a test organism. *Clarias gariepinus* is noted in the historical distribution list of all river systems assessed in this study and is therefore considered to be an appropriate test subject.

6.1.3. Condition Factor (CF)

The conditioning factor measures the general condition of the test organism and is an indicator of general fish health (Bervoets and Blust, 2003). Although the conditioning factor is usually more applicable to field specimens, it was included in this study to ascertain a baseline value for specimens of which the historical and nutritional information were known.

Another factor to consider is that the stress reaction observed for growth hormone exposure might not be a conventional reaction. Possible increases in condition are expected at
environmentally relevant concentrations. Although increased growth rate might be associated with more efficient metabolism, Clarrids that are transgenic for growth hormones, and show higher growth rates, exhibit higher routine metabolic rates (Cook et al., 2000; McKenzie et al., 2006; Stevans et al., 1998). Furthermore, little is known about how feeding and growing fish utilise major nutrient groups (lipids, proteins and carbohydrates) as sustenance (Wood, 2001).

The CF values ascertained in this study will serve as a relative measure of variation between control group and growth hormone-group exposures. In addition to the CF, other indices utilised include the HSI as well as the GSI.

### 6.1.4. Cellular Energy Allocation

Responses at molecular and cellular levels can be used as markers of biological effects to environmental exposure (Stegeman et al., 1992). One of the recently developed markers takes into account the energy metabolism of organisms. The energy metabolism is under hormonal control and is thus susceptible to endocrine disruption. Changes in the energy metabolism will influence life characteristics such as growth and reproduction on the organism level (Verslycke and Janssen, 2001). This will be amplified at a later stage and the results will be observed in the total fertility of the exposed population. Organisms exposed to suboptimal environments acquire a cost of dealing with stress in terms of metabolic resources. The total amount of energy available for maintenance, growth and reproduction can be calculated, based on biochemical analysis of the energy budget (Orlando et al., 2004). This analysis is called CEA.

The method of CEA was developed as a biomarker technique to assess the effects of toxic stress on the energy budget of exposed organisms (De Coen and Janssen, 1997). It can be considered a short term biochemical assessment that combines the variation in the energy reserve (lipids, protein and sugar) with energy consumption (amount of oxygen consumed) to form an indicator of physiological stress. Organisms exposed to a stress-inducing environment show higher energy consumption per unit time than organisms that are not exposed to a stressful environment.

In this chapter, an in-depth assessment of CEA is used to evaluate the effects of growth hormone exposure on the metabolic balance of test organisms (De Coen and Janssen, 1997). Using this approach, energy reserves available (Ea) and energy consumed (Ec) are quantified biochemically and integrated into a general stress indicator. Energy consumption is estimated by measuring the electron transport activity (ETA) at the mitochondrial level,
while the energy reserves available for metabolism are assessed by measuring the total lipid, protein and sugar content of the test organism.

The CEA methodology was used to determine the energy allocation of *C. gariepinus* exposed to environmentally relevant concentrations of TBA and DES over a fifteen day chronic exposure, with five-day dissection intervals. The CEA values were then compared to trends in condition factor and somatic indices, in order to connect effects on cellular level to higher levels of organisation.

### 6.1.5. Metabolomics

Metabolomics is a term used for the study of endogenous metabolite profiles in biological samples (Samuelsson *et al.*, 2006). Metabolomics can bridge the gap between environmental variation and functional physiological responses (Beecher, 2002). It is also virtually specie-independent, making it suitable for broad ecotoxicological investigation (Samuelsson *et al.*, 2006). A number of studies have been done on the application of metabolomics in the field of ecotoxicology (Bundy *et al.*, 2002; Griffin, 2003; Rosenblum *et al.*, 2005; Stantiford *et al.*, 2005; Vaint *et al.*, 2003). Additionally, a few studies have utilised metabolomics to identify stress responses in aquatic organism due to hormone exposure (Bino *et al.*, 2004; De Graaf and Behar, 2003; Lindon *et al.*, 1999; Samuelsson *et al.*, 2006).

Metabolomics can be applied to any biological fluid (Nicholson *et al.*, 1999; Lindon *et al.*, 2000) and, in some cases intact tissue (Ching Yu *et al.*, 2006) and tissue extract (Coen *et al.*, 2003). For the purpose of this study, blood plasma was used as it is a complex mixture of lipoprotein particles, low molecular weight metabolites, and electrolytes (De Graaf and Behar, 2003). Two main methods of metabolomic analysis are commonly used namely: Nuclear Magnetic Resonance (NMR) spectroscopy (Lindon *et al.*, 2000) and Mass Spectrometry (MS) (Plumb *et al.*, 2002). NMR analysis has been widely used to study blood plasma of animals, including fish (Nicholson and Wilson, 1989; Samuelsson *et al.*, 2006) and was therefore employed in this study.

The effects of growth hormones on fish are well studied (Arcand-Hoy *et al.*, 1998; Brown *et al.*, 2007; Gagne and Blaise, 2005; Lai *et al.*, 2002; Samuelsson *et al.*, 2006) and constitute a good model system to assess the use of Metabolomics in determining a growth hormone stress response. In order to evaluate the applicability of this biomarker in fish ecotoxicology, *C. gariepinus* were exposed to environmental relevant concentrations of synthetic testosterone and estrogen. The subsequent plasma profile of endogenous metabolites was investigated using one dimensional NMR analysis.
6.2. Experimental design and methods applied

6.2.1. Chemicals

**Hormones and exposures**

Trenbolone acetate and DES was obtained from Industrial Analytical Distributors(Pty). A dosing solution for TBA was prepared by making 1 L stock solution with a concentration of 12 µg/l. Using DMSO (>0.02 % of stock solution) as a solvent. The DES was prepared by making a 1 L stock solution with a concentration of 0.23 µg/l, also using DMSO as a solvent.

**Cellular Energy Allocation (CEA)**

The chemicals used in the homogenising buffer included Tris HCl (0.1 M), Triton X-100 (0.2 %), Poly-Vinyl Pyrrolidone (15 %) and MgSO₄, all of which were obtained from Merck chemicals. The chemicals that were used in protein determination included a stock solution of BSA (0.001 g), as well as Bradford reagent, also obtained from Merck chemicals. CFAS was purchased from Sigma-Aldrich and used in the carbohydrate determination. The stock solution for lipid determination comprised of Tripalmitin (0.006 g) and chloroform (1 ml). Other chemicals used in sample preparation were Methanol (500 ml), H₂SO₄, NADH (0.00025 mM), NAD(P)H (1.7 mM) and INT (p-Iodonitrotetrazium violet) (8 mM) obtained from Sigma-Aldrich (De Coen and Janssen, 1997).

**Metabolomics**

Blood plasma samples of 400 µl were mixed with 200 µl D₂O obtained from Sigma-Aldrich in preparation for NMR analysis.

**Fish exposure and sampling**

Fifteen sub-adult fish per hormone exposure group and fifteen control fish were transferred to individual 1000 L flow through systems, with an approximate flow rate of 100 ml/min. The fish were allowed to acclimate to these conditions for a period of seven days before the experiment was initiated. The water temperature was kept constant at 25 ºC. Other variables such as pH, conductivity, and dissolved oxygen were monitored daily. Fish were fed Tetra-Fin non-degradable pellets every second day pre- and during exposure periods. Because the dose response curve for TBA and DES is not known, fish were exposed for periods of five days up to a maximum of 15 days.

Exposure concentrations were based on environmentally relevant concentrations. As most concentrations were below instrument detection limit, exposures were carried out at detectable limit + 20 % which relates back to:
• Trenbolone acetate = 12.00 µg/l
• Diethylstilbestrol = 0.24 µg/l

Following exposure periods of five, ten and fifteen days, the body measurements (total length, standard length, total weight, gutted weight, gonad and liver weight) were recorded and each specimen was sacrificed by severing the spinal cord anterior to the dorsal fin. Liver and gonads were dissected using sterile scalpel blades and sterile dissection tools. A macroscopic examination of major visceral organs was performed to identify any possible nodules, cysts, growths, inflammation and discolouration of the organs. The organs were removed and the liver weight was established as well as gonad weight and length for the somatic indices.

### 6.2.2. Conditioning factor and Somatic indices

The CF used in this study is a factor of both total body mass as well as total body length and is expressed in the following equation (McKenzie et al., 2006):

\[
CF = 100 \times \left(\frac{\text{Fish mass}}{\text{Fish length}^3}\right)
\]

A value of (1) indicates a good heath status, while values smaller than one reflects a demise in fish condition. Interpretations were made relative to control group CF values.

In addition to CF somatic indices were also used. These indices relate the organ mass to body mass and can indicate possible hypertrophy following contaminant exposure. For the purposes of this study, the somatic indices for the liver (Hepato-somatic index or HSI) and gonads (Gonado-somatic index or GSI) were utilised.

The HSI is calculated by dividing the total liver mass by the body mass and is expressed in percentage by the following calculation:

\[
\text{HSI} (%) = \left(\frac{\text{liver mass (g)}}{\text{Body mass (g)}}\right) \times 100
\]

The GSI was calculated by dividing the total gonad mass by the total body mass and is expressed as a percentage in the following calculation:

\[
\text{GSI} (%) = \left(\frac{\text{Combined gonad mass (g)}}{\text{Body mass (g)}}\right) \times 100
\]
6.2.3. Cellular Energy Allocation

Energy available
Total lipids were extracted using the method described by Bligh and Dyer, (1959). Liver samples of 0.2 g were homogenised in 600 µl homogenising buffer after which spectrofotometric grade chloroform and 500 µl methanol were added. After centrifuging the samples, the top phase was removed and 500 µl H₂SO₄ added to 100 µl lipid extract and charred for 15 min at 200 ºC. The total lipid content was determined by measuring the absorbance at 405 nm using tripalmitin as a standard. To determine the total protein and carbohydrate content, fish livers were homogenised in 600 µl homogenizing buffer after which 15 % trichloroacetic acid (TCA) was added and incubated at 20 ºC for 10 min. After centrifugation, the resulted pellet was washed with 5 % TCA. Both supernatant fractions were combined and used for the total carbohydrate analysis. The remaining pellet was resuspended in NaOH, incubated at 60 ºC for 30 min and neutralised with HCl. Total protein content was then determined using Bradford’s reagent. The absorbance was measured at 595 nm using bovine serum albumin as a standard. As a third fraction of the Ea parameter, the total carbohydrate content of the supernatant fraction was quantified by adding 5 % phenol and H₂SO₄. After 30 min incubation at 25 ºC, the absorbance was measured at 630 nm, using carbohydrate as a standard (De Coen and Janssen, 1997).

Energy Consumed
The electron transport activity was measured, according to the protocol suggested by King and Packard (1975) with major modifications as described below. The fish livers, 45 samples, were homogenised on ice using a motor-driven Teflon pestle in homogenising buffer (0.1 M Tris-HCl pH 8.5, 15 % (w/v) Poly-Vinyl Pyrrolidone, 153 µM MgSO₄ of 0.2 % (w/v) Triton X-100). After centrifugation (4 ºC, 3000 g for 10 min), 50 µl from each extract were added to 150 µl buffered substrate solution (BSS; 0.13 M Tris HCl, 0.3 % (w/v) Triton X-100, pH 8.5) and 50 µl NAD(P)H solution (1.7 mM NADH and 250 M NADPH). The reaction was carretised by adding 100 µl INT (p-IodoNitroTetrazolium; 8 mM) and the absorbance measured kinetically at 20 ºC for 10 min.

Cellular Energy Allocation
The different energy reserve fractions (Ea) for the individual organisms were transformed into energetic equivalents using the energy of combustion: 17500 mJ/mg glycogen, 24000 mJ/mg protein and 39500 mJ/mg lipid. The cellular respiration rate (Ec) was determined, using the ETS data, based on the theoretical stoichiometrical relationship that for each 2 µmol of formazan formed, 1 µmol of O₂ was consumed in the ETS system. The quantity of oxygen consumed per fish liver unit was transformed into energetic equivalents using the
specific oxyenthalpic equivalents for an average lipid, protein and carbohydrate mixture of 484 kJ/mol O₂. The Ea value was calculated by integrating the change in the different energy reserve fractions over the 15 day exposure period. Similarly, the Ec value was obtained by integrating the change in energy consumption over the exposure period. Subsequently, the total net energy budget was calculated (De Coen and Janssen, 1997).

**Cellular Energy Allocation data analysis**

Graph Pad Prism version 4 was used to create bar graphs representing mean plus standard error bars. For descriptive statistics and in order to compare means, to test for significant differences in CEA data between exposure groups, a one-way ANOVA was performed using SPSS version 15. Post hoc tests, using the Scheffe of Dunnett’s T3 multiple comparison tests were performed where appropriate. The selection of the post hoc test was based on whether the data displayed homogeneity of variance. This was determined using Levene’s test. Significant differences were regarded at $p < 0.05$. The degree of similarity in the CEA and CF data between hormones and exposure periods were determined using Bray-Curtis similarity coefficients and then ordinating the data using multidimensional scaling techniques (MDS). Similarity matrices were subjected to group averaged hierarchical clustering and ordinated by MDS to summarise patterns between exposure groups. Permutation-based hypothesis testing using one-way Analysis of Similarity (ANOSIM) was undertaken to determine the extent of the differences between exposure groups.

**6.2.4. Metabolomics**

**Blood plasma sampling**

The effect of the growth stimulants on metabolomics was studied by employing the technique described by Vaint et al. (2003). Prior to the fish being sacrificed, blood was collected from the caudal vessel using ice-cooled syringes and blood tubes pre-treated with EDTA. The plasma was separated immediately by centrifugation at 6000 rpm for 3 min and samples were snap frozen in liquid nitrogen and stored at –80 °C until analysis.

**NMR metabolomics: Preparation of samples for $^1$H NMR**

Plasma samples were analysed on a group basis. Plasma (400 µL) and D₂O (200 µL) were mixed and transferred to 5 mm NMR micro-tubes. Samples were analysed by $^1$H-NMR spectroscopy within two hours of sample preparation. Buffering the plasma sample did not reduce variability and was therefore not applied (Duran et al., 2003).

**$^1$H NMR Spectroscopy**

$^1$H-NMR spectra were measured at 300 MHz on a Varian Inova 300 MHz spectrometer, equipped with 300 MHz cold probe. Tuning and gradient shimming was performed for each
sample and $^1$H-NMR chemical shifts were referenced internally to the methyl doublet of valine at 1.042 ppm. All spectra were Fourier transformed, followed by 1st order phasing and drift correction. $^1$H-NMR spectra of fish were required at 25 °C with a standard sequence using a 90 ° pulse and a relaxation period of 2.0 s. Suppression of the large water resonance was achieved by pre-saturation during 1.5 s of the relaxation delay and determining the saturation frequency for each individual sample (preset spectra) (Duran et al, 2003).

**Pre-processing of NMR data**

$^1$H-NMR spectra were integrated and divided into chemical shift buckets using a custom-written programme. The total sum of all bucket integrals in each spectrum was set at 90, encompassing 0.04 ppm.

**Multivariate analysis of NMR data**

Data were imported into Canoco multivariate statistical programme. Principal Component Analysis (PCA), (Duran et al., 2003) was performed to create an overview of trends, groupings and outliers in the data.
6.3. Results

6.3.1. Condition factor and somatic indices

Figure 6.1 reflects the CF of the different exposure groups over time. The trends observed did not indicate any significant difference between exposure groups. However, both DES and TBA exposures resulted in a decrease in CF after 10 days of exposure. Diethylstilbestrol caused a CF decrease from 0.48 after five days of exposure to 0.45 after ten days of exposure, while a similar trend was observed for TBA, which reflected a decline in CF from 0.46 after five days, to 0.39 after ten days of exposure. The test organisms of both hormone exposures indicated an increase in CF after fifteen days of exposure.

The results for the Somatic indices are reflected in Figure 6.2 (HSI) and Figure 6.3 (GSI). Somatic indices calculate the organ mass relevant to the body mass and can indicate possible hypertrophy or atrophy after hormone exposure. In terms of the HSI the control group indicated hypotrophy of the liver after fifteen days of exposure, but remained similar for the first ten days of exposure. The DES exposure group indicated a steady increase in liver size relative to body mass over the extent of the exposure period. After five days of exposure the HSI value for the DES group was 0.48 this increased over the following ten days of exposure to 0.5. The TBA exposure group reflected an excessive increase in HSI values after just five days of exposure (5.4) but decreased after ten days of exposure to 4.6. The HSI reflected slight hypertrophy after fifteen days of exposure with a HSI value of 4.9.

The GSI reflects the relationship between combined gonad mass and total body weight. This index must be applied with caution as additional environmental factors, such as time of year, temperature and sexual developmental stages of fish, will impact on the GSI values. Both the hormone exposure groups indicate gonadal atrophy after ten days of exposure. The DES exposure group GSI value dropped from 0.28 after five days to 0.24 after ten days of exposure, but increased after fifteen days of exposure to 0.37. Similarly the TBA exposure group reflects excessive hypotrophy of the gonads after five days (0.41) of exposure followed by a decrease in gonad size after ten days (0.31) and then reflecting another increase after fifteen days (0.41) of exposure.
Figure 6.1: Conditioning Factor of each exposure group, represented with standard error bars, over the 15 days exposure period, with specific 5 day interval values.

Figure 6.2: HSI values of each exposure group, represented with standard error bars, over the 15 days exposure period, with specific 5 day interval values.

Figure 6.3: GSI values of each exposure group, represented with standard error bars, over the 15 days exposure period, with specific 5 day interval values.
Chapter 6

6.3.2. Cellular Energy Allocation (CEA)

The experimental design was structured in such a way that a possible stress response can be observed after a given time of exposure. The CEA data sets reflect this data obtained for the different exposure groups over five day dissection intervals. Figure 6.5 to Figure 6.7 shows the trends in variation of carbohydrate, protein and lipid concentrations within liver tissue of test organisms.

Energy availability in the form of carbohydrates present was constant for the control group over the extent of the fifteen day exposure period. A slight increase in energy available from carbohydrate was observable after five days of exposure (200 000 mJ/g) to 238 000 mJ/g after fifteen days of exposure. This, however, was not the case for the DES exposure group, which showed an initial increase in liver carbohydrate levels (250 000 mJ/g) after five days of exposure, followed by a decrease (120 000 mJ/g) after ten days of exposure and an increase to 200 000 mJ/g after fifteen days of exposure. The TBA exposure group reflected little variation in carbohydrate levels over the extent of the exposure period. A decrease was observed after ten days of exposure from 190 000 mJ/g to 160 000 mJ/g, but returned to “normal” (with reference to the control group). No significant differences ($p < 0.05$) in carbohydrate concentrations were observed between exposure groups. However, variation within carbohydrate concentrations between groups may explain the overall significant difference observed in amount of energy consumed (Ec) after five days of exposure (Table 6.1).

The amount of energy available or consumed in the form of protein is indicated in Figure 6.6. A considerable increase in protein concentrations were observed for the DES group during the extent of the exposure period. Initially, the DES group reflected the lowest protein concentration when compared to the control and TBA group after five days of exposure (800 000 mJ/g) and this changed over the next ten days of exposure, increasing to more than 870 000 mJ/g after fifteen days of exposure. A contradictory trend is observed for the TBA exposure group, which indicated an overall decrease in protein concentration over the extent of fifteen day exposure time. The TBA protein levels decreased from 840 000 mJ/g over five days to 820 000 mJ/g after fifteen days of exposure. The trend observed for the TBA group mimics the trend observed for the control group. It must be noted that after fifteen days of exposure, a significant difference ($p < 0.05$) was observed between protein levels of DES group and the control group as well as those of the TBA group (Table 6.3).

With regard to the lipid concentrations measured for the different exposure groups, it was observed that after ten days of exposure there was a (Table 6.2) difference between the lipid
levels of the DES group and TBA group, with the DES group at 225 000 mJ/g and the TBA group at less than 150 000 mJ/g. This difference (Table 6.3) increased over fifteen days of exposure with the DES group mounting to almost 400 000 mJ/g and the TBA group at higher than 150 000 mJ/g.

The abovementioned results were used to establish the amount of energy available, in terms of carbohydrate, protein and lipid concentrations, between exposure groups. From this, the energy available (Ea), energy consumed (Ec) and overall Cellular Energy Allocation (CEA) was calculated (Figure 6.8 to Figure 6.10). Significant differences as well as the temporal intervals thereof are reflected in Table 6.1 to Table 6.3. After a ten day exposure period there were considerable differences between the Ea values of the DES group and the TBA group, with the DES group reflecting more energy available than the TBA exposure group. Once again, this difference increases in significance (p<0.005), after fifteen days of exposure, with the DES group showing higher Ea values than that of the TBA exposure group.

An inverse relationship between exposure groups are observed when comparing the energy consumed values (Ec). The TBA exposure group reflected a constant increase in Ec values over the exposure period, while the DES group decreased its energy consumption over ten days of exposure, after which it reflected an increase again. Overall, a consequential difference exists between the Ec values of different exposure groups after simply five days of exposure (Table 6.1). The control group consumed far less energy than that of the DES and TBA exposure groups.

From the Ea and Ec values, the total CEA values were calculated (Figure 5.9), indicating a significant difference in the amount of energy allocated for growth and reproduction between exposure groups. This important difference was observed after ten days (Table 6.2) and after fifteen days (Table 6.3) of exposure, between the DES and the TBA groups, with the DES exposure group showing an increase in CEA values over the exposure period.
Figure 6.5: Effects of five, ten and fifteen day exposure of TBA and DES on carbohydrate concentrations of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05.

Figure 6.6: Effects of five, ten and fifteen day exposure of TBA and DES on protein concentrations of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05.

Figure 6.7: Effects of five, ten and fifteen day exposure of TBA and DES on lipid concentrations of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05.
Table 6.1: Descriptive statistics indicating the significant (*) differences in Energy consumed (Ec) between control, TBA and DES group after an exposure period of five days.

<table>
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<tr>
<th>Dependent Variable</th>
<th>Test</th>
<th>(I) F1</th>
<th>(J) F1</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec</td>
<td>Schffe</td>
<td>Control</td>
<td>DES</td>
<td>-5823.92609(*)</td>
<td>1 499.11825</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TBA</td>
<td>-9150.24018(*)</td>
<td>1 499.11825</td>
<td>0.000</td>
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</tbody>
</table>

(*) Indicates significant differences in values. DES= Diethylstilbestrol; TBA= Trenbolone acetate; Ec=Energy consumed

Table 6.2: Descriptive statistics indicating the significant (*) differences in lipid concentrations, Energy allocated (Ea), Energy consumed (Ec) as well as Cellular Energy Allocation between control, TBA and DES group after an exposure period of ten days.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Test</th>
<th>(I) F1</th>
<th>(J) F1</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
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</tr>
</thead>
<tbody>
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<td>Dunnett T3</td>
<td>DES</td>
<td>Control</td>
<td>173 339.16667</td>
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<td></td>
<td></td>
<td></td>
<td>TBA</td>
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<td>Ea</td>
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<td>DES</td>
<td>Control</td>
<td>112 535.26228</td>
<td>407 439.52461</td>
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<td></td>
<td></td>
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<td>0.033</td>
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<td>Ec</td>
<td>Schffe</td>
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<td>821330.57693(*)</td>
<td>204 154.91728</td>
<td>0.034</td>
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</table>

(*) Indicates significant differences in values. DES= Diethylstilbestrol; TBA= Trenbolone acetate; Ea= Energy allocated; Ec= Energy Consumed; CEA= Cellular Energy Allocation.
Table 6.3: Descriptive statistics indicating the significant (*) differences in lipid-protein concentrations, Energy allocated (Ea), Energy consumed (Ec) as well as Cellular Energy Allocation between control, TBA and DES group after an exposure period of fifteen days.

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<th>(J) F1</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
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<tr>
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</tbody>
</table>

(*) Indicates significant differences in values. DES= Diethylstilbestrol; TBA= Trenbolone acetate; Ea= Energy allocated; Ec= Energy Consumed; CEA= Cellular Energy Allocation.
Figure 6.8: Effects of five, ten and fifteen day exposure of TBA and DES on Energy allocation (Ea mJ/g) of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05.

Figure 6.9: Effects of five, ten and fifteen day exposure of TBA and DES on Energy consumed (Ec mJ/g) of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05.

Figure 6.10: Effects of five, ten and fifteen day exposure of TBA and DES on Cellular Energy Allocation (CEA mJ/g) of test organisms. Error bars represent standard error on the mean with n=5; significance are measured at p<0.05.
In order to establish a possible link between CEA values and CF of exposed organisms, a MDS ordination was done to determine the intrinsic ordination between groups with CEA and CF being the driving factors of Bray-Curtis similarity coefficient (indicated in Figure 6.11 to Figure 6.13). The MDS models were constructed over the same time intervals as the CEA analysis in the above named section (five, ten and fifteen days). Although some ordinations were observed, no significant relationship exists between CEA and CF after fifteen days exposure period.

After a five day exposure period the MDS indicated a grouping, reflecting greater similarities between the CEA and CF relationship of the control and TBA group; while the DES group showed more outliers on the periphery of the graph. However, some of the TBA exposed organism’s ordinated with the DES group.

After ten days of exposure, there appeared to be no distinct difference between the CF and CEA relationship between exposure groups. Some DES exposed organisms showed a strong similarity to certain control fish, while the remainder of the exposed organisms ordinated together on the left of the graph. Some extreme outliers included individuals of the control group and the TBA group.

After fifteen days of exposure clear trends in the CF and CEA relationships between groups became apparent, with the DES group indicating a strong similarity (>98 %) and grouping together on the left side of the graph. The TBA group and the control group, ordinate together with a similarity of 98 %, however one TBA exposed individual reflected as an outlier. It is also important to note the strong similarity (99 %) between individuals of the control group.
Figure 6.11: Two-dimensional MDS indicating (dis)similarity between different exposure groups with reference to the CEA and CF relationship after five days of exposure.

Figure 6.12: Two-dimensional MDS indicating (dis)similarity between different exposure groups with reference to the CEA and CF relationship after ten days of exposure.

Figure 6.13: Two-dimensional MDS indicating (dis)similarity between different exposure groups with reference to the CEA and CF relationship after fifteen days of exposure.
6.3.3. **Metabolomics**

One dimensional $^1$H-NMR metabolic profiling was performed for each individual in the different exposure groups. Figure 6.14 represents a presat metabolic profile of a fish blood plasma sample out of the control group. The spectrum ranges from 0.5 ppm to 5.5 ppm and reflects major metabolites identified. Integrals in the region 1.30- 1.34 ppm shows lactate, 2.54-2.58 ppm represents citrate, and while 2.10-2.18 and 2.34-2.38 ppm reflects glutamate and 3.38-3.94 ppm represents carbohydrate and glycogen.

![Figure 6.14: 300 MHz $^1$H-NMR presat spectra of catfish blood plasma with some identified metabolites indicated (Samuelsson et al., 2006).](image)

After dividing the ppm scale into 90 bins of 0.04 ppm and calculating the surface area of each metabolic chemical shift (as described in section 6.2), a PCA was performed, indicating the dissimilarities in the metabolic profile between different exposure groups, after five, ten and fifteen days of exposure (Figure 6.15). The first axis of the PCA reflects 50 % of the variance observed in the data set, while the second axis explains another 22 % of the variance.

After five days of exposure to the synthetic estrogen, DES, fish showed an alteration in metabolic structure when compared to that of the control group (Figure 6.15). The main metabolites responsible for the ordination of the five day DES group are represented in Table 6.4 and include: vitellogenin (1.22 ppm) and hydroxybutyrate (1.26 ppm). After the same period of exposure the TBA group also reflected dissimilarities with regards to metabolic profile in relation to the control and DES groups. Furthermore, it was observed that this dissimilarity was greatest between the DES group after five days and the DES group after ten days of exposure. Metabolites
responsible for the placing of the TBA group after five days includes: carbohydrate (3.66 ppm) and melonate (3.33 ppm) (Table 6.4).

Figure 6.15: Principle Component Analysis, indicating the (dis)similarity between control, DES and TBA exposure groups over five, ten and fifteen day intervals. The PCA accounts for 72 % of total variance observed when comparing the metabolic profiles of different exposure groups.

After ten days of exposure, the DES group still ordinate with great dissimilarity in relation to the control group, however, this ordination also differed from the previous DES five day exposure. A sudden decrease in vitellogenin (1.22 ppm) and an increase in carbohydrate metabolites (3.42 - 3.74 ppm) were responsible for the outlying ordination of the DES group after ten days of exposure (Table 6.4). Fish exposed to the synthetic testosterone (TBA) reflected a “normalisation” in blood metabolites in relation to the control group after ten days of exposure. However, slight increases in metabolites such as choline and carbohydrate and a decrease in phenylalanine levels still contributed to the ordination of the TBA group after ten days of exposure.

After fifteen days of exposure, the metabolic composition in the DES exposed group returned to “normal” and grouped in close proximity to the control group. The difference in the metabolic structure of the DES group between ten and fifteen days of exposure was characterised by a further drop in vitellogenin and an increase in carbohydrate and melonate levels. The differences in terms of metabolic composition
Chapter 6

of blood plasma of the TBA group between ten and fifteen days were very small. After fifteen days of exposure to TBA a slight decrease in fructose, hydroxybutyrate and choline levels was observed.

Table 6.4: \(^1\)H-NMR chemical shift regions in presat spectra of catfish blood plasma responsible for dissimilarities between different exposure groups over five day exposure intervals. An increase or decrease in metabolites are indicated by the contribution factor where ↑: increase concentration and ↓: decrease concentration.

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Exposure period (days)</th>
<th>Chemical shift (ppm)</th>
<th>Contribution factor</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>3.66</td>
<td>↑</td>
<td>Carbohydrate</td>
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<tr>
<td></td>
<td></td>
<td>3.33</td>
<td>↑</td>
<td>Melonate</td>
</tr>
<tr>
<td>DES</td>
<td>5</td>
<td>1.22</td>
<td>↑</td>
<td>Vitellogenin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.26</td>
<td>↑</td>
<td>Hydroxybutyrate</td>
</tr>
<tr>
<td>TBA</td>
<td>5</td>
<td>3.33</td>
<td>↑</td>
<td>Melonate</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Carbohydrate</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>3.62</td>
<td>↑</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.26</td>
<td>↑</td>
<td>Phenylalanine</td>
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<tr>
<td></td>
<td></td>
<td>1.18</td>
<td>↓</td>
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</tr>
<tr>
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<td></td>
<td>1.21</td>
<td>↓</td>
<td>Hydroxybutyrate</td>
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<td>1.26</td>
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<td>Hydroxybutyrate</td>
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<tr>
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<td></td>
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<td>3.18</td>
<td>↓</td>
<td>Choline</td>
</tr>
</tbody>
</table>

DES= Diethylstilbestrol; TBA= Trenbolone acetate
Chapter 6

6.4. Discussion

6.4.1. Diethylstilbestrol group

The CF is believed to be a good indicator of general fish health (Bagenal and Tesh, 1978; Bolger and Connolly, 1989) however; its applicability in a controlled environment is questionable (Bervoets and Blust, 2003). Nonetheless, it serves as a measure of comparison for hormone exposed groups in relation to that of the control group. Taking the CF calculated for each exposure group over the extent of the experiment into consideration, a general decrease is observed in the exposed groups after ten days of exposure (Figure 6.1). This trend is also observed with the HSI values, reflecting an exaggerated relationship with the control group after ten days of exposure (Figure 6.2). Some form of “normalisation” in the CF is observed after fifteen days of exposure, reflecting similar relationships with the five day exposure period (Figure 6.1).

Some emphasis should be placed on the increase in CF of test organisms exposed to DES, with the CF values of this group frequently higher than the control group (Figure 6.1). To explain this, the nature of the CF index as well as that of synthetic estrogen should be examined. CF is a function of body length, condition and tissue energy concentrations, thereby expressing the general fitness of the test organism (Bolger and Connolly, 1989; Bervoets et al., 2001). Any increase in lipid, carbohydrate or protein concentration will therefore correlate positively with the CF value calculated for any test organism. Estrogenic compounds, by their very nature and design, cause subcutaneous and peripheral organ fat deposition (Raun and Preston, 2002) and this is the trend observed in the group of test organisms exposed to the synthetic estrogen (DES).

The CEA verifies this notion, reflecting much more energy availability (from lipids) in the groups exposed to DES (Figure 6.7). In addition to higher lipid concentrations in the DES group, higher protein levels (Figure 6.6) were also observed over ten and fifteen days of exposure. This will further contribute to the higher CF allocated to organisms exposed to DES. This approach effectively links CEA and CF values between organisms (Figure 6.13). These results to some extent contradict a study done by Verlycke et al. (2007), in which a decrease in CEA values was observed for the estuarine Mysid integer exposed to sub-lethal concentrations of nonylphenol (an estrogen agonist). However, DES is a non-steroidal growth hormone and it is not clear how it will differ in structure and function when compared to xeno-estrogenic
nonylphenol. In addition to this, the test organisms used also differ, further contributing towards explaining the contradicting results.

A further verification of the CEA values obtained for the DES group is reflected in the metabolomics (Figure 6.15). The metabolic profile ordination reflected an increase of lipoproteins and possible vitellogenin after five days of exposure (Table 6.4), however a decrease in these metabolites was observed over the following five days of exposure (Table 6.4). An increase in vitellogenin levels is expected in test organisms exposed to estrogenic compounds (Coats et al., 1976; Holbeck et al., 2006). Metabolites in the form of lipo-proteins are the major contributors toward the differences in metabolic profiles between the DES and control group and this is consistent with other studies that indicate the pronounced effect of estrogenic compounds, synthesising lipoproteins (Rotchell and Ostrander, 2003; Thomas-Jones et al., 2003; Holbeck et al., 2006). The possible presence of vitellogenin in the DES exposed group may indicate endocrine disruption as 75% of the test organisms were male and should not possess above-detectable concentrations of vitellogenin. In male fish the vitellogenin gene is normally silent but exposure to exogenous estrogens will facilitate expression (Flouriot et al., 1993; Jobling et al., 1995).

Other metabolites of importance after an exposure period of five days are hydroxybutyrate (Table 6.4), which is a ketone and a by-product of fat hydrolysis (Samuelsson et al., 2006). Hydroxybutyrate can also serve as an energy source in the absence of cellular carbohydrates (Samuelsson et al., 2006). This notion is verified by the PCA (Figure 6.15), indicating a decrease in carbohydrate and glycogen levels after ten days of exposure and is also reflected in the carbohydrate standard error graph (Figure 6.5).

The sub-cellular response of test organisms to DES appeared to be at its peak after five days of exposure. After ten days a decrease in lipoproteins was observed, with a further decrease in carbohydrate levels, followed by a prompt correction in metabolic composition after fifteen days of exposure (Figure 6.15). This observation highlights two important points of DES exposure: firstly, a dose-response trend was observed, reflecting a metabolic response after five and ten days of exposure. Secondly, the metabolic variation between the DES group was greater than that of the TBA group (Figure 6.15).
6.4.2. Trenbolone acetate group.

Trenbalone Acetate differs completely in form and function, compared to DES (Ankley et al., 2003). A complete difference in response is thus expected from test organisms. A comparison of the CF of the TBA group to that of the control group indicated a non-significant decrease in CF values after fifteen days of exposure. Ankley et al. (2003) observed an induction of male secondary sexual characteristics and decreased fecundity after 21 days of exposure to 27 ng/l of trenbolone in adult female fathead minnow. Furthermore, a significant increase in HSI values is observed after five days of exposure (Figure 6.2), indicating a toxic stress response. Mounting evidence also implicates gonadal steroids in somatic growth regulation (Knoebel, 1999) as chronic exposure to dihydro-testosterone enhanced somatic growth (Borski and Tsai, 1996). In contrast to an expected increase in CF values (which was not observed in this study), Orlando et al., 2004 observed lower GSI values were obtained for fish downstream of feedlot activities. The androgenic ability of TBA is well known (Orlando et al., 2004), however the GSI values obtained for this study should be interpreted with caution as there appeared to be some difference in sexual maturity of the test organisms (Figure 6.3).

The slight decrease in CF caused by the exposure of test organism to TBA is more perceptibly reflected in the CEA values and this is especially apparent after fifteen days of exposure (Figure 6.13). After further analyses of the overall energy availability and energy consumed, the following observations are apparent: the TBA group reflected a decrease in the amount of cellular energy available when compared to the control group. The main constituent of this demise in Ea is a decrease in the lipid content of the blood plasma (Figure 6.7). This observation relates the use of TBA and other synthetic testosterones in meat production. Trenbolone metabolism causes a decrease in fatty acids and an increase in amino acid synthesis within the exposed organism (ZoBell et al., 2000). The protein content of the TBA group was on average higher than that of the control group (Figure 6.6) however, the ability of TBA to assist in amino acid production is limited by the dietary protein intake. The protein intake of the test organisms was limited as they were fed a diet with low protein content.

The CEA values for the TBA group were significantly lower than those of the control group (Table 6.1 to Table 6.3), however, the amount of cellular energy available in the TBA group was consistent throughout the exposure period (fifteen days) and indicates far less variation than the DES group. In addition to this, the differences in
the CEA values between the TBA and the control group are smaller than the differences between the DES and control group. Energy consumption in the TBA group was significantly ($p<0.05$) affected (at 12 µg/l), resulting in lower energy availability in these test organisms. This observation verifies the conclusions drawn by Verlsycke and Janssen (2002), who observed the same trend in exposed estuarine mysids to testosterone.

With regards to the metabolomics, increases in melonate and carbohydrate were observed after five days of exposure to TBA. Melonate inhibits cellular respiration (Samuelsson et al., 2006) and the higher quantities present in the TBA group might explain the decrease in CF values observed after ten days of exposure (Figure 6.1). This, however, is speculative and requires further verification. A decrease in melonate is observed after ten days of exposure with a slight increase in choline levels (Table 6.4).

The overall metabolic profile of TBA exposed organisms reflects with great similarity that of the control group, especially after ten and fifteen days of exposure (Figure 6.14). Trenbolone acetate is an androgenic substance for which its ability to act as an endocrine disruptor has been observed and reported on by Ankley et al. (2003). In this study, the ability of the biomarkers, CEA and metabolomics are shown in assessing a stress response to TBA exposure. Both the CEA and the metabolomics analyses indicated a smaller stress response observed in test organisms exposed to TBA. This implies that in terms of feedlot management the utilisation of TBA should be considered above DES.

### 6.5. Conclusion

The potential effects of growth hormone exposure on fish in the aquatic freshwater environment were assessed in this chapter. Firstly, the CF assessment after fifteen days of exposure did not indicate any significant alteration on organism level. However, this is not to say that given enough time sub-cellular and cellular responses will eventually be expressed on higher levels of cellular organisation. Other factors to consider, with reference to the somatic indices, are the limitations in active functions, for example the CF used in this study is a function of body mass in relation to standard body length and expressed with a constant. Therefore, it does not take natural inter-specie variation, possible age or sexual differences into account. However, even within the limitations of the CF, some alterations were observed. On closer inspection these differences could be correlated (to some extent but with no
significance) to energy availability as expressed by the lipids and the proteins. This was especially true for the DES group.

Secondly, the CEA was capable of effectively indicating a stress response between exposed organisms. Definite differences in energy allocations were measured for both the TBA and the DES exposed group. The intensity and nature of this response is of importance, as it indicates which of the hormones used is of greater concern. Keeping this in mind, the significant differences between the DES group and the control group were greater than the differences between the TBA group and the control group. This initially might seem confusing, as the CEA indicated a “positive” increase in energy availability from organisms exposed to DES. The nature of this “positive” increase in energy availability is speculative as the increase in lipo-proteins observed might be the presence of vitellogenin. This will then be considered endocrine disrupting; a concept also associated with the exposure to synthetic estrogens (Samuelsson et al., 2006). What is important is that, even though the TBA exposed group showed less energy available for growth and reproduction the variation difference when compared to the control group was smaller than that of the DES group.

Thirdly, the application of metabolomics as a functional biomarker was done with some success. The use of this biomarker indicated two important points: firstly, it succeeded in determining a dose response observation. This was also aided by the experimental design whereby fish were exposed to a single concentration over set intervals. From this, it is clear that the exposure to DES caused an increased response both after five and ten days of exposure. This was not the case with the TBA group, which reflected a slight sub-cellular response after five days of exposure. Secondly, the application of metabolomics aided in determining which metabolites are responsible for the sub-cellular response observed between exposed organisms. This in turn verified and explained observations made in the application of the CEA biomarker. For example, it was shown that the higher energy availability after five days of exposure to DES was due to higher lipoprotein levels.

Taking all of the above into account, the exposure concentration of the different hormones with DES at 0.23 µg/l and TBA at 12.0 µg/l as well as the reaction measured in this study, it was shown that even though the exposure concentrations were lower for DES than TBA, the results still indicate that DES is more potent than TBA. This notion can contribute to feedlot management, when decisions are made, regarding the type of hormones to be used.
Chapter 7

7. Conclusion and Recommendations

South Africa’s freshwater resources are under increasing threat, not just by relentless industrial and residential development, but also by agricultural expansion. The increasing demand in red meat production has resulted in an exponential growth in cattle production. With regards to pharmaceutical products entering the environment, the emphasis was historically on human medication and personal health-care products. However, these substances are subjected to some form of water purification. By contrast, the agricultural sector which is also a significant user of veterinary pharmaceuticals, has no such treatment and compounds are deposited straight into associated soils and water systems through manure and urine.

This study was principally concerned with determining the effects of high density cattle feedlot activity on the ecological state of associated natural aquatic ecosystems as well as determining the sublethal effects of ecologically relevant pharmaceutical concentrations on fish using laboratory-based bioassays.

It was determined that NH$_4$ at all the sites are at levels higher than the TWQR. This is of particular concern as higher pH values increase the quantity and toxicity of NH$_3$. In addition to this, high NH$_4$ concentrations caused by feedlot activity also contributed to higher conductivity and pH of downstream sites associated with them.

Several approaches have been recommended and evaluated for reducing NH$_3$ emissions from excreted animal manure (Ndewga et al., 2008):

1. Reducing nitrogen excretion through dietary manipulation.
2. Reducing volatile NH$_3$ in the manure to stop NH$_3$ loss.
3. Segregating urine from faeces to reduce contact between urease and urine.
4. When urine-faeces segregation is not an option, urease inhibition can also be used to reduce or eliminate the hydrolysis of urea into NH$_3$.
5. Methods for reducing the more volatile NH$_3$ in manure include the reduction of pH, which shifts the equilibrium in favour of NH$_4$ over NH$_3$.
6. The use of other chemical additives that bind ammonium-N and the use of biological nitrification to convert NH$_4$ into non-volatile N-species.
7. Manure collection facility design and appropriate management are also essential for abating NH$_3$ emissions.
Other water quality parameters contributing significantly to variation in data include: NTU, COD and NO$_3$. Metal concentration with the exception of Pb, does not indicate seasonal or site preference and is consequently, not the result of feedlot activity. Lead concentration, however, is higher downstream from feedlot activity than upstream. With the exception of Mn, Ni and Fe, all metal concentrations are at levels of concern and much higher than the TWQR for aquatic ecosystems.

Some consideration should be given to improve retention ponds used in feedlot runoff management. It is recommended that horizontal subsurface constructed wetlands (Vymazal, 2008) should be employed for smaller feedlot operations. This will also contribute to metal retention as metals tent to accumulate in wetland sediment and taken up by certain vegetation types, for example *Phragmites australis* (Dallas and Day, 2004).

In addition to water quality it was also illustrated that feedlot activity does contribute to the alteration in invertebrate communities. The subtle increases of sedimentation, water abstraction and nutrient influxes associated with feedlot activities, are stimulating this observed alteration. The Shannon-Wiener diversity scores were typically lower downstream of feedlots, but were observed to be the lowest during the high flow survey, indicating an additional seasonal response. All sites that reflected the highest single taxon dominance were located downstream from feedlots, and can be considered to be at an increased risk of exposure to the impacts of feedlot activities. Furthermore, it can be concluded that both the invertebrate diversity and community analysis does indicate a stress response, not perceived by SASS 5.

Sediment composition was shown to be important as it facilitates the movement of hormones in the environment. Sediment from sites with a higher organic fraction as well as smaller particle size showed higher sorption potential for hormones. However, estrogens and estrogen-mimicking compounds indicated a higher affinity for sediment and are not as mobile as testosterone and testosterone-mimicking compounds.

This notion implies that the use of estrogens pose a smaller environmental risk. However this cannot be viewed in isolation. The biomarker responses obtained during this study suggest that DES (synthetic estrogen) is more potent at smaller concentrations than TBA (synthetic testosterone).

These combining factors then suggest an adaptive management approach with regards to feedlot activity. The backbone of adaptive management is monitoring.
Relatively easy changes can be made to the way feedlot properties are adaptively managed (Fisher and Scott, 2008):

1. Two stage retention ponds should be of a capacity to cater for the herd size. In many cases the ponds are old and of small capacity and the number of cattle have increased.
2. The ponds should be routinely maintained and the sludge applied in a manner designed to minimise mobilisation.
3. Annual monitoring of associated river systems using an upstream downstream approach will indicate effective management and will provide a base for decision making.

Finally, the use of biological and thermo-chemical conversion in cattle production is recommended to decrease both the overall impacts of feedlot activities on the freshwater environment as well as the potential risk of increase concentrations of pharmaceuticals in the environment. Waste-to-bioenergy treatments can provide livestock operators with multiple value-added, renewable energy products (Cantrell et al., 2008). While biological production of methanol and hydrogen are in the early research stages, anaerobic digestion is an established method of generating methane gas. The thermo-chemical conversion processes can convert feedlot waste into gaseous fuels, combustible oils and charcoal, while at the same time breaking down pharmaceuticals and converting nutrients into non-volatile species.

A possible South African approach should include the development of manure processing plants central to mass cattle production areas (which is concentrated in Gauteng and the Free State provinces of South Africa). This will allow for a constant and sustainable influx of manure that can be utilised in thermo-chemical bioenergy production.

With regards to the use of growth hormones and other veterinary pharmaceuticals, they should be applied with great caution and should be accompanied by an adaptive management system. Untreated animal manure cannot be applied as compost as hormone metabolites are stable in the environment and have enduring half-lives.

The impacts and biotic responses identified in this study do contribute towards a better understanding of the environmental implications feedlot operational activities. The results obtained in this study contribute towards an integrated framework for the environmental management of feedlot activities.
Chapter 8

8.1 References


Chapter 8


Chapter 8


Chapter 8


Hilsenhoff W. L. (1982). *Using a biotic index to evaluate water quality in streams*. USA: Department of Natural resources.

Chapter 8


Chapter 8


Chapter 8


Chapter 8


Chapter 8


Chapter 8


Chapter 8


Chapter 8


