MF110 Soldiers Point

OTC RESIDUE MONITORING
January 2024 treatment

FINAL REPORT

April 2024

Report to:
Tassal Group Limited




Prepared by:
AQUENAL PTY LTD

AQUENAL

Contents

Operational Summary

Contractor: Aquenal Pty Ltd
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Field work: Aquenal

Dates of fieldwork:
Round 1 – Sediment & wild fish: 23/01/2024
Round 2 – Sediment: 31/01/2024
Round 3 – Sediment: 15/02/2024
Round 4 – Sediment & wild fish: 15/03/2024

Weather	23/01/2024	31/01/2024	15/02/2024	15/03/2024
Wind	Light N	0 kts SW	Light NW 10-15 kts W	5-10 kts SW
Sky	Cloudy	Cloudy	Sunny	Cloudy
Rain	Nil	Nil	Nil	Nil
Sea	<0.5 m	0.5 m	<0.5 m	0.5 m
Current	Negligible	Negligible	Negligible	Negligible

Laboratory Analysis:
Analytical Services Tasmania
New Town Laboratory
18 St Johns Avenue
New Town, Tasmania 7008

Seabed and wild fish sampling was undertaken by Aquenal. Positioning for seabed sampling was undertaken using a Garmin GPS plotter with 19x NMEA 2000 position antenna. This system provides real-time GPS positions accurate to ~2 m. Positions were referenced to a State Permanent Mark (SPM) prior to commencement of fieldwork.

1 Introduction and Scope

An Environmental Antibiotic Residue Monitoring Survey was undertaken in the vicinity of *MF110 Soldiers Point* marine farming lease (Environmental Licence 9900/3). Sampling methodologies and analyses were in accordance with the monitoring requirements stipulated by the Environmental Protection Authority (EPA) in the *Antibiotic Residue Monitoring Schedule – Marine Farming Lease No. 110 Soldiers Point – Environmental Licence No. 9900/3 – January 2024.*

The survey design provided by the EPA included sampling of sediment and wild fish for the purpose of monitoring residue of the antibiotic Oxytetracycline (OTC), based on the treatment carried out between the 9th and 18th January 2024 (see Appendix 2 for treatment details). The monitoring survey design included four sediment sampling events and two wild fish sampling events, to be undertaken between January and March 2024. This report provides a summary of the four rounds of sampling (referred to as Rounds 1-4 in this report), carried out on the 23rd January[1] (Round 1 sediment and wild fish), 31st January (Round 2 sediment), 15th February (Round 3 sediment), and 15th March (Round 4 sediment and wild fish) 2024.

It should be noted that the *Antibiotic Residue Monitoring Schedule* specifies that "sample analysis or measurement must be conducted by a laboratory or testing facility accredited by the National Association of Testing Authorities (NATA), or a laboratory or testing facility approved in writing by the Director, for the specified test". Analysis via a NATA certified test was not practical for this survey. Analysis was instead conducted by Analytical Services Tasmania. Analytical Services Tasmania (AST) have provided an acceptable methodology and have been previously approved by the EPA for OTC analysis.

[1] The monitoring schedule specified 19th January as the Round 1 sampling date, however due to weather and operational constraints sampling was delayed until 23rd January 2024, as approved by the EPA (23/01/2024).

2 Methods

2.1 Sediment Sampling

2.1.1 Survey sites

The sediment survey design for residue monitoring included one pen bay site, six 'outside of lease' (transect) sites, and one ambient (reference) site (Figure 1). The pen bay site (PB6) was sampled at the edge of a selected medicated pen (Figure 1). In the first survey round, sampling was conducted as close to the pen edge as practical by securing the vessel to a designated berthing location on the pen. Subsequent sampling was conducted as close to the original position as possible, tying up to the pen bay edge. 'Outside of lease' sites were sampled along two transects, positioned on west and north-west axes, outwards from the the marine farming lease boundary. Along the west and north-west transects, sites were located at a distance of 35 m, 100 m and 500 m from the lease boundary. The ambient site was located south of *Soldiers Point* marine farming lease, at the 'M5/B6' BEMP (broadscale environmental monitoring program) site, as referred to in Environmental Licence 9900/3 (Figure 1).

All sediment sites were sampled in Rounds 1-3. In accordance with the sampling schedule, sediment sites that returned results below the threshold limit of 0.1 mg/kg for two consecutive sampling events were discontinued (as approved by the EPA prior to Round 4 sampling, 22nd February 2024). Hence, in Round 4, sediment sampling was completed at the pen bay (PB6) and ambient (M5/B6) sites.

Sites were specified by the EPA and sampling coordinates are provided in Appendix 1.

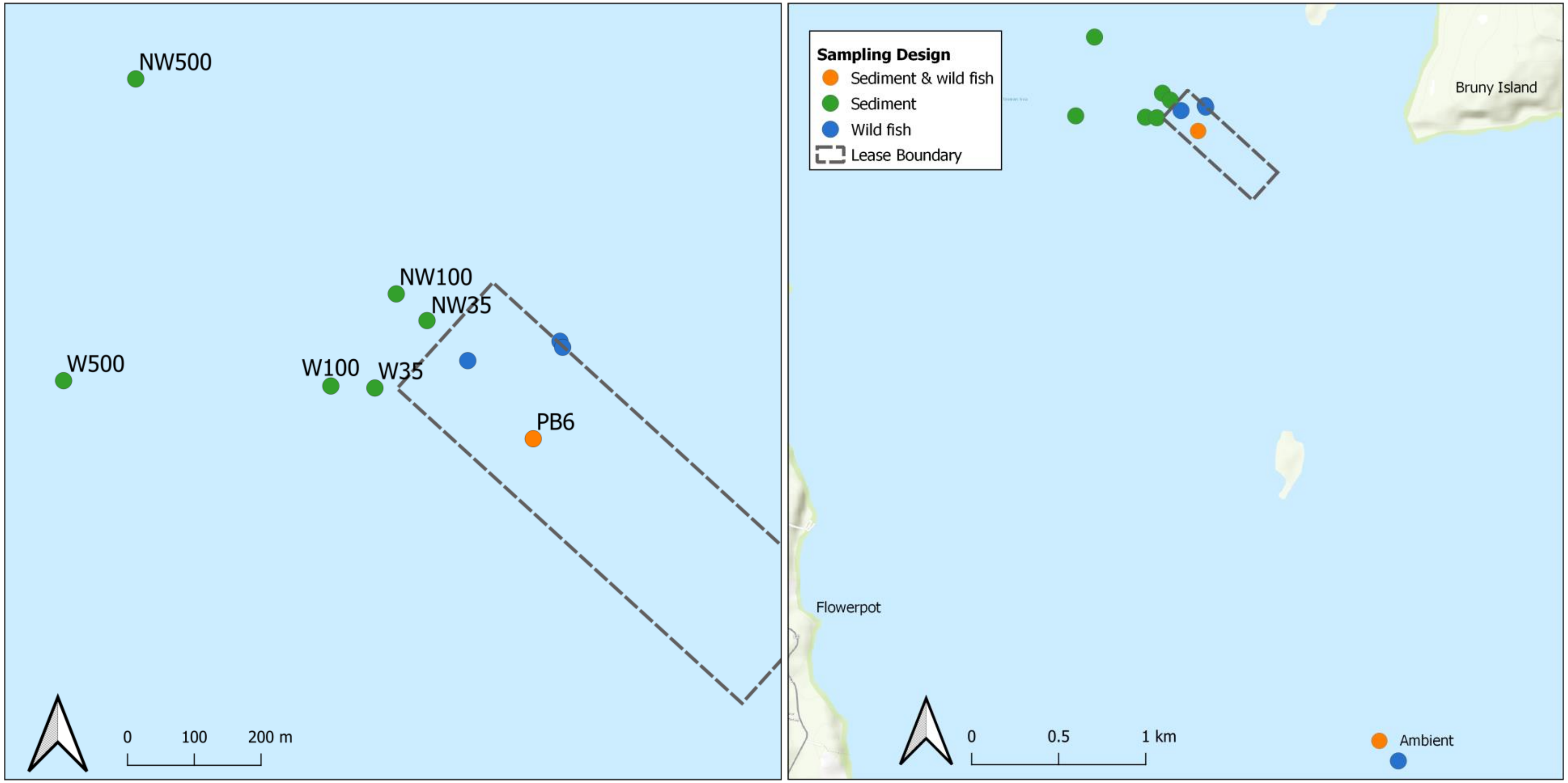


Figure 1: Location and survey maps – *MF110 Soldiers Point,* showing overview and location of sediment and wild fish sampling locations.

2.1.2 Sample collection and core processing

At each sample site, a Craib corer was used to collect sediment cores. Core samples (75 mm minimum length) were collected in clean Perspex cores (Figure 2). The Craib corer was thoroughly cleaned and rinsed with seawater between each site. Samples were taken from the sites furthermost from the lease area first, to minimise the risk of sample contamination (i.e. ambient site, 500 m, 100 m, 35 m and then pen bay site). Triplicate cores were taken at each site.

In the Aquenal laboratory, samples were split into surface (top 25 mm) and bottom (bottom 50 mm) sections and composited according to the EPA schedule. For the duplicate composited samples, the top 25 mm from two replicate cores were combined into one jar. Similarly, the bottom 50 mm from the same two cores were combined into a single jar. The third replicate core sample was separated into the 25 mm and 50 mm sections, with samples retained in frozen storage in the event that further analysis was required. Cores sampled at sites furthermost from the lease area were processed first to prevent sample contamination.

2.1.3 Sample analysis

Following processing of cores in the Aquenal laboratory, samples were refridgerated prior to delivery to the Analytical Services Tasmania (AST) laboratory for analysis. Samples were analysed for Oxytetracycline (OTC) in sediment, and the limit of reporting (LOR) was 0.01 mg/kg. AST analysed sediments on a dry mass basis and OTC results were reported as mg/kgDMB. Analysis of surface core sections was conducted for all sites while analysis of bottom core sections was restricted to the pen bay site.

(a)  (b) 

Figure 2: (a) Craib corer in field, and (b) example sediment sample triplicates collected in Perspex cores.

2.2 Wild Fish Sampling

2.2.1 Survey sites

The wild fish survey design for residue monitoring included ambient and in-lease locations (Figure 1). For the in-lease sampling, wild fish were collected from locations within the northern part of the lease. Wild fish were also collected at the ambient site location (M5/B6; Figure 1). Fish were sampled within a radius of ~50 m from the recorded GPS location.

Sampling coordinates are provided in Appendix 1.

2.2.2 Sample collection and processing

Wild fish sampling was undertaken in Round 1 and Round 4 of the survey and conducted by hook and line fishing under an *Environmental Monitoring Permit (Number 24005)* issued by the Aquaculture Branch of the Department of Natural Resources and Environment Tasmania. The *Antibiotic Residue Monitoring Schedule* required twelve fish to be collected at both the in-lease and ambient locations during each wild fish sampling round, ideally including both pelagic and benthic species. In both Round 1 and Round 4 of the survey, fish samples at the in-lease location were comprised of *Platycephalus bassensis* (sand flathead; benthic), and *Scomber australasicus* (blue mackerel; pelagic), and at the ambient location fish samples were comprised entirely of *P. bassensis* (Appendix 4).

At each location (in-lease and ambient) the fish were divided into 4 samples for analysis, with each pooled sample comprised of three individuals (e.g. Figure 3a). For processing, the tail of each fish was cut off posterior to the anus and bagged prior to investigation of gut contents (Figure 3c-d). Tissue samples (tails) were retained in separate bags for each pooled sample, chilled immediately and then frozen upon return to the laboratory.

Sampling was undertaken at the ambient site first, followed by the in-lease location. Details of each fish specimen were recorded in the field. All sampling gear and preparation equipment used in the field was thoroughly cleaned during sampling and processing. Photos of fish specimens prepared for analysis are presented in Appendix 3 and field data for wild fish sampling is included in Appendix 4.

Note that the EPA schedule specified the requirement for feed pellets to be present in analysed samples from the in-lease location. This requirement was not always practical as evidence of pellets in specimen stomach contents was observed in only three instances during Round 1 (in-lease specimens; pooled samples 6, 8), and six instances in Round 4 (in-lease specimens; pooled samples 13, 15, 16). Naturally occurring dietary items were prevalent in both the in-lease and ambient fish samples (Figure 3d; Appendix 4).

2.2.3 Sample analysis

Following processing of fish samples in the field and Aquenal laboratory, samples were delivered to the Analytical Services Tasmania (AST) laboratory for analysis. Samples were analysed for Oxytetracycline (OTC) in fish tissue, and the limit of reporting (LOR) was 0.01 mg/kg. Flesh sample analysis conducted by AST was on an 'as received' or 'wet' basis and the OTC results were reported as mg/kgWMB.

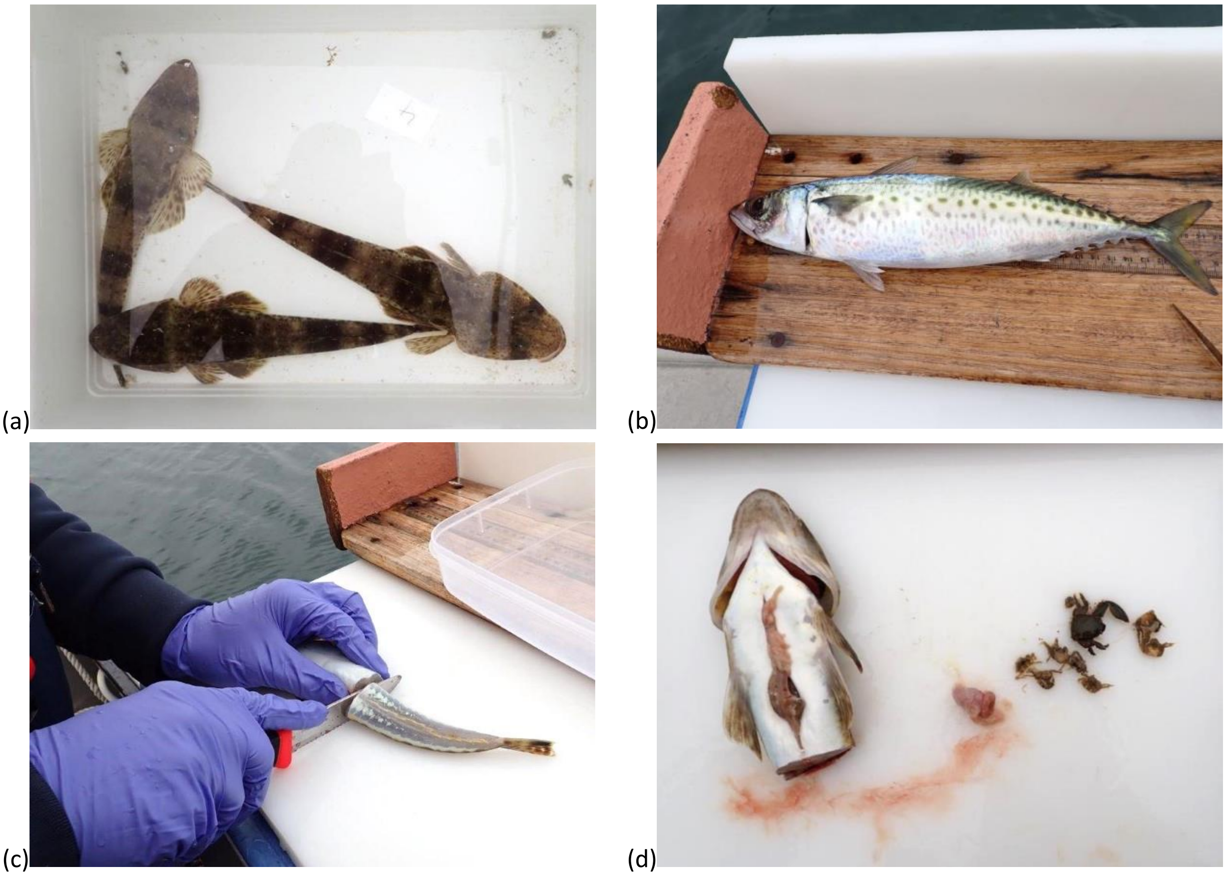


Figure 3: Wild fish field sampling photos showing: (a) one pooled sample of three wild fish specimens, (b,c) processing of a specimen, and (d) an example of gut contents inspection.

3 Results

3.1 Sediment Sampling

Results of sediment sampling are summarised in Table 1.

OTC concentrations were relatively low across all four sediment sampling events (Rounds 1-4) of the monitoring survey. OTC concentrations were slightly higher at the pen bay site (PB6) in Round 1 (0.73 mg/kg surface sample; 0.13 mg/kg bottom sample) compared to the transect (outside of lease) and ambient sites (Table 1). At PB6, OTC concentrations declined in subsequent sampling rounds, with surface sample results of 0.20 mg/kg recorded in Round 2, 0.08 mg/kg in Round 3, and 0.03 mg/kg in Round 4 (Table 1). The PB6 bottom samples also declined incrementally from 0.13 mg/kg in Round 2, to a concentration below the limit of reporting (LOR; <0.01 mg/kg) in Round 4 (Table 1).

At the outside of lease sites in Round 1, the north-west transect recorded very low OTC concentrations of 0.01 mg/kg at the 35 m (NW35) and 100 m (NW100) sites (Table 1). For the remaining outside of lease sites, including the 500 m north-west site (NW500) and the entire west transect (W35, W100, W500), there was no evidence of OTC residue in Round 1, with OTC concentrations below the LOR (<0.01 mg/kg).

In Rounds 2 and 3, there was no evidence of OTC residue in surface sediments sampled at all transect (outside of lease) sites, with OTC concentrations below the LOR (<0.01 mg/kg) in both rounds (Table 1). Outside of lease transect sites were omitted from sampling in Round 4.

There was no evidence of OTC residue in surface sediments sampled at the ambient site (M5/B6), with OTC concentrations below the LOR (<0.01 mg/kg) in all sampling events (Rounds 1-4; Table 1).

In summary, OTC concentrations were generally low across the monitoring survey. OTC concentrations were higher at the pen bay site (PB6) compared to outside of lease sites, but declined to very low levels by the final sampling event.

Table 1: Results of OTC concentration analysis for sediment samples collected at *MF110 (Soldiers Point)* during Rounds 1-4 of sampling. Antibiotic treatment was carried out from the 9th to 18th January 2024 and sediment sampling was undertaken on 23rd (Round 1) and 31st (Round 2) January, 15th February (Round 3), and 15th March (Round 4) 2024. S = surface, B= bottom sample. Note: OTC in sediment is reported by AST as mg/kgDMB (dry matter basis).

Location	Site	Round 1		Round 2		Round 3		Round 4		Depth sampled (m)
		OTC (mg/kg)								
		S	B	S	B	S	B	S	B	
Pen Bay	PB6	0.73	0.13	0.20	0.13	0.08	0.03	0.03	<0.01	21.8
Transect	NW35	0.01		<0.01		<0.01				22.6
	W35	<0.01		<0.01		<0.01				19.3
	NW100	0.01		<0.01		<0.01				25.8
	W100	<0.01		<0.01		<0.01				19.6
	NW500	<0.01		<0.01		<0.01				30.9
	W500	<0.01		<0.01		<0.01				15.0
Ambient	M5/B6	0.01		<0.01		<0.01		<0.01		14.6
OTC treatment details		**Round 1**		**Round 2**		**Round 3**		**Round 4**		
Date sampled		23/01/2024		31/01/2024		15/02/2024		15/03/2024		
Days since treatment complete		5		13		28		57		

3.2 Wild Fish Sampling

Results from wild fish sampling are summarised in Table 2.

OTC concentrations in pooled fish samples collected from the in-lease and ambient locations were below the limit of reporting (LOR; <0.01 mg/kg; Table 2) in both wild fish sampling events (i.e. Round 1 and Round 4). There was no evidence of environmental accumulation of OTC residue in fish tissue based on the LOR used in this survey (0.01 mg/kg). OTC concentrations were well below the relevant maximum residue limit of OTC for fish (0.2 mg/kg), as defined by the Australian Food Standards[2].

Wild fish specimen descriptions and images are provided in Appendices 3 and 4.

Table 2: Results of OTC concentration analysis for wild fish samples collected at *MF110 (Soldiers Point)* during Round 1 and Round 4 of sampling. Antibiotic treatment was carried out from the 9th to 18th January 2024 and wild fish sampling was undertaken on 23rd January (Round 1) and 15th March (Round 4) 2024. Note: OTC in fish tissue is reported by AST as mg/kgWMB (wet matter basis).

Location	Round 1		Round 4	
	Pooled sample #	OTC (mg/kg)	Pooled sample #	OTC (mg/kg)
Ambient (MP4)	1	<0.01	9	<0.01
	2	<0.01	10	<0.01
	3	<0.01	11	<0.01
	4	<0.01	12	<0.01
In-Lease	5	<0.01	13	<0.01
	6	<0.01	14	<0.01
	7	<0.01	15	<0.01
	8	<0.01	16	<0.01
OTC treatment details				
Date sampled	23/01/2024		15/03/2024	
Days since treatment complete	5		57	

[2] https://www.legislation.gov.au/F2015L00468

4 Appendices

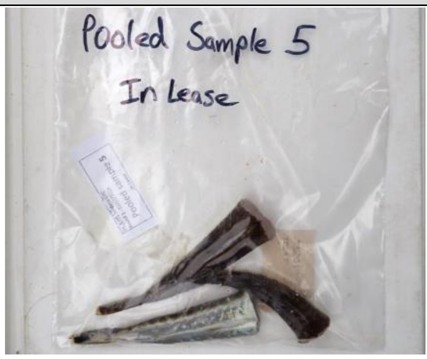
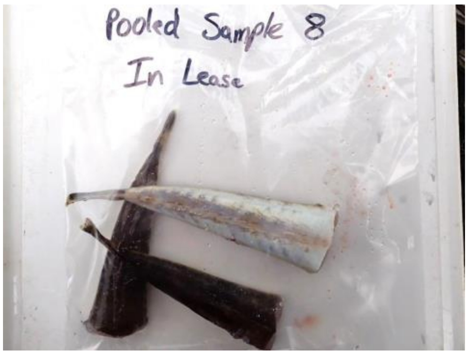
Appendix 1: Survey coordinates for Rounds 1-4 of the residue monitoring survey including sediment and wild fish sampling, based on the Mapping Grid of Australia Zone 55 (Datum GDA94).

Sampling Round	Sampling Method	Date	Site	Easting	Northing
Round 1	Sediment	23/01/2024	PB6	523085	5219135
			NW35	522926	5219312
			W35	522848	5219211
			NW100	522880	5219352
			W100	522782	5219214
			NW500	522490	5219674
			W500	522382	5219222
			M5/B6 (Ambient)	524126	5215636
	Wild fish	23/01/2024	Ambient (pooled sample 1-4)	524126	5215636
			In-lease (pooled sample 5-6)	522987	5219252
			In-lease (pooled sample 7-8)	523125	5219281
			SPM 11089 ~5m WNW of antenna	523983	5242072
Round 2	Sediment	31/01/2024	PB6	523084	5219135
			NW35	522927	5219312
			W35	522847	5219211
			NW100	522881	5219352
			W100	522783	5219215
			NW500	522490	5219675
			W500	522384	5219223
			M5/B6 (Ambient)	524127	5215637
			SPM 11089 ~5m WNW of antenna	523982	5242072
Round 3	Sediment	15/02/2024	PB6	523088	5219134
			NW35	522926	5219313
			W35	522848	5219212
			NW100	522881	5219353
			W100	522784	5219213
			NW500	522490	5219675
			W500	522383	5219222
			M5/B6 (Ambient)	524125	5215638
			SPM 11089 ~5m WNW of antenna	523983	5242069
Round 4	Sediment	15/03/2024	PB6	523075	5219133
			M5/B6 (Ambient)	524125	5215636
Round 4	Wild fish	15/03/2024	Ambient (pooled sample 9-10)	524125	5215636
			Ambient (pooled sample 11-12)	524235	5215521
			In-lease (pooled sample 13-14)	523129	5219272
			In-lease (pooled sample 15-16)	523075	5219133
			SPM 11089 ~5m WNW of antenna	523982	5242070

Appendix 2: Antibiotic oxytetracycline (OTC) treatment details for *MF110 Soldiers Point* lease. Treatment was administered by feed.

Treatment	Type	Days	Dates prescribed	Active ingredient (kg)
Antibiotic	Oxytetracycline	10	9th January 2024 to 18th January 2024	180

Appendix 3: Pooled samples of wild fish specimens prepared for analysis from ambient and in-lease sites for Round 1 of the residue monitoring survey.

Ambient location	
	
Pooled sample 1	Pooled sample 2
	
Pooled sample 3	Pooled sample 4
In-lease location	
	
Pooled sample 5	Pooled sample 6
	
Pooled sample 7	Pooled sample 8

Appendix 3 (continued): Pooled samples of wild fish specimens prepared for analysis from ambient and in-lease sites for Round 4 of the residue monitoring survey.

Ambient location

Pooled sample 9

Pooled sample 10

Pooled sample 11

Pooled sample 12

In-lease location

Pooled sample 13

Pooled sample 14

Pooled sample 15

Pooled sample 16

Appendix 4: Round 1 wild fish sampling field data. Table adapted from Annexure 3 of the of Antibiotic Residue Monitoring Schedule *MF110 Soldiers Point*.

<table>
<tr><th>Site</th><th rowspan="2">Sample date</th><th rowspan="2">Specimen #</th><th rowspan="2">Pooled sample #</th><th colspan="2">Sample weight</th><th rowspan="2">Fish species</th><th rowspan="2">Fish length (mm)</th><th rowspan="2" colspan="2">Gut contents</th></tr>
<tr><th>Category</th><th>whole</th><th>tail</th></tr>
<tr><td rowspan="12">Ambient</td><td>23/01/2024</td><td>1</td><td rowspan="3">1</td><td>95</td><td>30</td><td>Sand flathead</td><td>249</td><td colspan="2">Empty</td></tr>
<tr><td>23/01/2024</td><td>2</td><td>103</td><td>40</td><td>Sand flathead</td><td>256</td><td colspan="2">1x scallop</td></tr>
<tr><td>23/01/2024</td><td>3</td><td>99</td><td>32</td><td>Sand flathead</td><td>240</td><td colspan="2">1x shrimp</td></tr>
<tr><td>23/01/2024</td><td>4</td><td rowspan="3">2</td><td>165</td><td>47</td><td>Sand flathead</td><td>282</td><td colspan="2">Decomposed fish</td></tr>
<tr><td>23/01/2024</td><td>5</td><td>130</td><td>41</td><td>Sand flathead</td><td>261</td><td colspan="2">Decomposed biotic substance</td></tr>
<tr><td>23/01/2024</td><td>6</td><td>111</td><td>32</td><td>Sand flathead</td><td>257</td><td colspan="2">Decomposed fish</td></tr>
<tr><td>23/01/2024</td><td>7</td><td rowspan="3">3</td><td>125</td><td>35</td><td>Sand flathead</td><td>270</td><td colspan="2">Empty</td></tr>
<tr><td>23/01/2024</td><td>8</td><td>102</td><td>27</td><td>Sand flathead</td><td>247</td><td colspan="2">1x shrimp</td></tr>
<tr><td>23/01/2024</td><td>9</td><td>84</td><td>26</td><td>Sand flathead</td><td>244</td><td colspan="2">1x shrimp</td></tr>
<tr><td>23/01/2024</td><td>10</td><td rowspan="3">4</td><td>182</td><td>50</td><td>Sand flathead</td><td>310</td><td colspan="2">4x isopods</td></tr>
<tr><td>23/01/2024</td><td>11</td><td>125</td><td>34</td><td>Sand flathead</td><td>270</td><td colspan="2">1x fish</td></tr>
<tr><td>23/01/2024</td><td>12</td><td>143</td><td>43</td><td>Sand flathead</td><td>280</td><td colspan="2">1x decomposed fish, 1x isopod</td></tr>
<tr><td rowspan="12">In-Lease</td><td>23/01/2024</td><td>13</td><td rowspan="3">5</td><td>116</td><td>33</td><td>Sand flathead</td><td>262</td><td colspan="2">3x decomposed fish</td></tr>
<tr><td>23/01/2024</td><td>14</td><td>102</td><td>30</td><td>Sand flathead</td><td>257</td><td colspan="2">Decomposed biotic substance</td></tr>
<tr><td>23/01/2024</td><td>15</td><td>58</td><td>17</td><td>Sand flathead</td><td>215</td><td colspan="2">2x crabs</td></tr>
<tr><td>23/01/2024</td><td>16</td><td rowspan="3">6</td><td>106</td><td>35</td><td>Sand flathead</td><td>254</td><td>1x pellet</td><td rowspan="3">Regurgitated: 2x pellets, 7x crabs</td></tr>
<tr><td>23/01/2024</td><td>17</td><td>128</td><td>42</td><td>Sand flathead</td><td>265</td><td>8x crabs</td></tr>
<tr><td>23/01/2024</td><td>18</td><td>125</td><td>34</td><td>Sand flathead</td><td>258</td><td>Empty</td></tr>
<tr><td>23/01/2024</td><td>19</td><td rowspan="3">7</td><td>90</td><td>12</td><td>Blue mackerel</td><td>217</td><td colspan="2">Decomposed brown substance</td></tr>
<tr><td>23/01/2024</td><td>20</td><td>358</td><td>52</td><td>Blue mackerel</td><td>327</td><td colspan="2">Decomposed fish, decomposed brown substance (suspected pellet)</td></tr>
<tr><td>23/01/2024</td><td>21</td><td>345</td><td>47</td><td>Blue mackerel</td><td>335</td><td colspan="2">Empty</td></tr>
<tr><td>23/01/2024</td><td>22</td><td rowspan="3">8</td><td>159</td><td>48</td><td>Sand flathead</td><td>285</td><td colspan="2">2x isopods</td></tr>
<tr><td>23/01/2024</td><td>23</td><td>166</td><td>46</td><td>Sand flathead</td><td>285</td><td colspan="2">7x crabs</td></tr>
<tr><td>23/01/2024</td><td>24</td><td>300</td><td>78</td><td>Sand flathead</td><td>345</td><td colspan="2">Pellets</td></tr>
</table>

Appendix 4 (continued): Round 4 wild fish sampling field data. Table adapted from Annexure 3 of the of Antibiotic Residue Monitoring Schedule *MF110 Soldiers Point*.

Site / Category	Sample date	Specimen #	Pooled sample #	Sample weight whole	Sample weight tail	Fish species	Fish length (mm)	Gut contents
Ambient	15/03/2024	25	9	88	24	Sand flathead	235	1x octopus
	15/03/2024	26		86	21	Sand flathead	240	2x crabs
	15/03/2024	27		128	34	Sand flathead	255	1x decomposed fish
	15/03/2024	28	10	90	22	Sand flathead	245	2x shrimp
	15/03/2024	29		96	27	Sand flathead	240	Empty
	15/03/2024	30		126	32	Sand flathead	270	Decomposed fish
	15/03/2024	31	11	128	33	Sand flathead	260	Empty
	15/03/2024	32		88	26	Sand flathead	240	Small fish
	15/03/2024	33		109	31	Sand flathead	250	1x crab
	15/03/2024	34	12	82	23	Sand flathead	235	1x decomposed fish
	15/03/2024	35		125	38	Sand flathead	265	Empty
	15/03/2024	36		235	58	Sand flathead	320	Empty
In-Lease	15/03/2024	37	13	44	14	Sand flathead	185	1x decomposed fish, 1x amphipod
	15/03/2024	38		34	12	Sand flathead	165	1x decomposed fish
	15/03/2024	39		110	37	Sand flathead	240	2x pellets, 1x shrimp
	15/03/2024	40	14	70	26	Sand flathead	215	1x decomposed fish, 1x crustacean
	15/03/2024	41		168	55	Sand flathead	290	1x fish, 1x crab
	15/03/2024	42		222	73	Sand flathead	305	Empty
	15/03/2024	43	15	170	23	Blue mackerel	260	1x pellet (mushed)
	15/03/2024	44		183	38	Blue mackerel	270	1x pellet (mushed)
	15/03/2024	45		143	28	Blue mackerel	250	Pellets
	15/03/2024	46	16	145	20	Blue mackerel	250	Pellets
	15/03/2024	47		160	34	Blue mackerel	250	Decomposed substance
	15/03/2024	48		155	36	Blue mackerel	255	Pellets