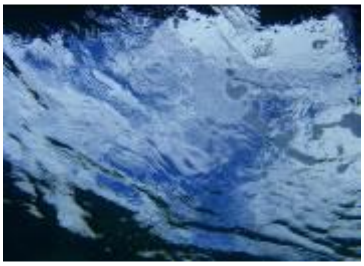
MF109 Butlers

OTC RESIDUE MONITORING
(February 2023 medication)
FINAL REPORT

Survey Rounds 1-5

June 2023

Report to:
Tassal Group Limited

Prepared by:
AQUENAL PTY LTD

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Operational Summary

Contractor: Aquenal Pty Ltd
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Client: Tassal Group Limited
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Field work: Aquenal Pty Ltd

Dates of fieldwork:

Round 1 – Sediment & wild fish: 9/3/23
Round 2 – Sediment: 22/3/23
Round 3 – Sediment: 4/4/23
Round 4 – Sediment: 3/5/23
Round 5 – Wild fish: 12/5/23

Weather	9/3/2023	22/3/2023	4/4/2023	3/5/2023	12/05/2023
Wind	NW 10 kts	E 0-5 kts	NW 5-10 kts	Light and variable	W 10 kts
Sky	Cloudy	Partly cloudy	Cloudy	Cloudy	Partly cloudy
Rain	Light rain	Nil	Nil	Light rain	Light rain
Sea	< 0.5 m	< 0.5 m	< 0.5 m	Calm	< 0.5 m
Current	Surface from NW to SE	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 *MF109 Butlers* marine farming lease (Environmental Licence 9870/2). 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. 109 Great Taylors Bay ('Butlers') - Environmental Licence No. 9870/2 – February 2023*, as amended by the EPA on the 9th of March.

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 25th of February and 9th of March 2023 (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 March and May 2023. This report provides a summary of the sediment and wildfish sampling events (referred to as Rounds 1-5 in this report), carried out on the 9th of March (Round 1), 22nd of March (Round 2), the 4th of April (Round 3), the 3rd of May (Round 4), and the 12th of May (Round 5) 2023.

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, due to timeframes and interstate logistics. Analysis was instead conducted by Analytical Services Tasmania. Analytical Services Tasmania (AST) have provided an acceptable methodology and have been previously approved by EPA for OTC analysis.

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 (PB2) was sampled at the edge of a selected medicated pen (Figure 1), and sampling was conducted as close to the pen edge as practical by securing the vessel to designated berthing locations on the pen. 'Outside of lease' sites were sampled along three transects, positioned at right angles to the marine farming lease boundary. Along each transect, sites were located at a distance of 35 m and 100 m from the lease boundary. The ambient site was located east of Partridge Island (Figure 1).

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

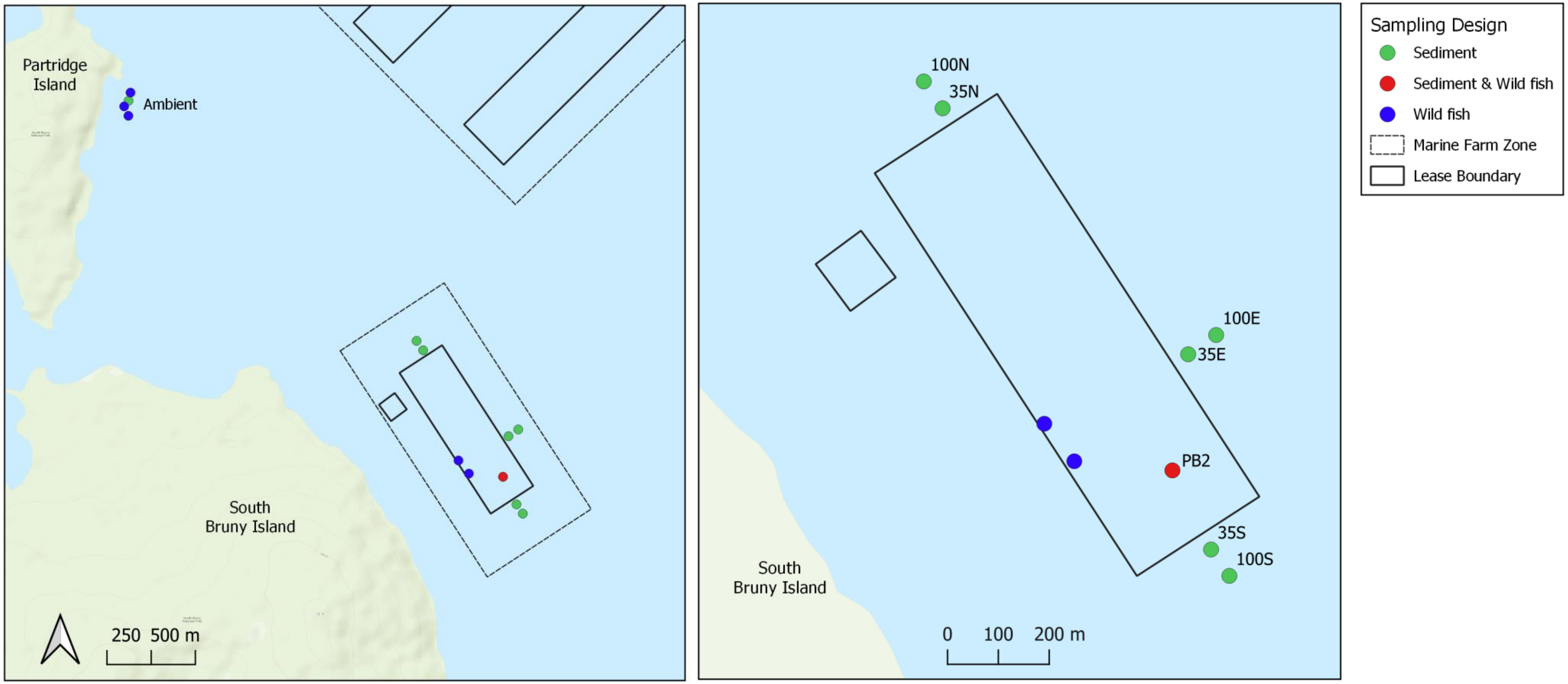

Figure 1: Location and survey maps – *MF109 Butlers* showing overview and location of sediment and wild fish sampling locations.

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, 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.

2.1.3 Sample analysis

Following processing of cores in the Aquenal laboratory, samples were refrigerated and delivered to Analytical Services Tasmania (AST) laboratory for analysis. Samples were analysed for Oxytetracycline (OTC) in sediment, with a limit of reporting (LOR) of 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 in Rounds 1-4 of the survey and analysis of bottom core sections was conducted for the pen bay site only.

(a) 

(b) 

Figure 2: Field photos showing: (a) Craib corer, and (b) sediment sample collected in Perspex core.

2.2 Wild Fish Sampling

2.2.1 Survey sites

The wild fish survey design for residue monitoring included ambient and in-lease locations for both Round 1 and Round 5 (Figure 1). For the in-lease sampling, wild fish were collected from locations within the central and southern part of the lease. Wild fish were also collected at the ambient site location (Partridge Island; 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 5 of the survey and conducted by hook and line fishing under an *Environmental Monitoring Permit (Number 23028)* issued by the Aquaculture Branch of the Department of Natural Resources and Environment Tasmania. The *Antibiotic Residue Monitoring Schedule* required 12 fish to be collected at both the in-lease and ambient locations, ideally including both pelagic and benthic species. In Round 1 of the survey, fish samples at the in-lease location were comprised of *Scomber australasicus* (Blue mackerel; pelagic), *Arripis trutta* (Australian salmon; pelagic) and *Platycephalus bassensis* (sand flathead; benthic). At the ambient (reference) location, *Thyrsites atun* (Barracouta; pelagic) and *P. bassensis* were sampled (see Appendix 4). In Round 5 of the survey, despite considerable fishing effort to sample both pelagic and benthic species, fish samples at both the in-lease and ambient locations were comprised entirely of the benthic species *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 3 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 3b-c). Tissue samples (tails) were retained in separate bags for each pooled sample, chilled immediately and then frozen.

Sampling was undertaken at the ambient site first, followed by the in-lease location. Details of each fish specimen was 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 more than half of the fish in Round 1 did not have evidence of pellets in their stomach contents. Similarly, in Round 5, none of the sampled fish had evidence of pellets in their stomach contents, with naturally occurring dietary items prevalent (Figure 3c-d; 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. Note that the flesh sample analysis by AST was on an ‘as received’ or ‘wet’ basis and the OTC results were reported as mg/kgWMB.


(a) (b) (c) (d)

Figure 3: Wild fish field sampling photos showing: (a) four pooled samples of three fish species (sand flathead, blue mackerel, and Australian salmon), (b) processing of a specimen, and (c-d) an example of gut contents inspection.

3 Results

3.1 Sediment Sampling

Results from Rounds 1-4 of sediment sampling are summarised in Table 1.

OTC concentrations were generally very low in all four survey events. OTC concentrations were slightly higher at the pen bay site (PB2, 0.03-0.05 mg/kg; Table 1) compared to the transect (outside of lease) sites (<0.01-0.02 mg/kg; Table 1). OTC concentrations in the bottom section of cores from the pen bay site were slightly lower than surface samples (Table 1).

For the majority of transect sites there was no evidence of OTC residues, with OTC concentrations across the four sampling rounds below the limit of reporting (LOR) for most samples (i.e. <0.01 mg/kg; Table 1).

There was no evidence of OTC residue in surface sediments sampled at the ambient site, with OTC concentrations below the LOR in all sample rounds (i.e. <0.01 mg/kg; Table 1).

Table 1: Results of OTC concentration analysis for sediment samples collected at *MF109 (Butlers)* during Rounds 1-4 of sampling. Antibiotic treatment was carried out from the 25th of February to the 9th of March 2023 and sediment sampling was undertaken on the 9th (Round 1) and 22nd (Round 2) of March, 4th of April (Round 3), and 3rd of May (Round 4) 2023. Coloured cells indicate OTC concentrations higher or equal to the limit of reporting (0.01 mg/kg), grey shaded cells indicate samples that were archived but not analysed. 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 range sampled (m)
		OTC (mg/kg)								
		S	B	S	B	S	B	S	B	
Pen Bay	PB2	0.04	0.02	0.05	0.01	0.04	0.02	0.03	0.02	22.7-23.2
Transect	35N	<0.01		<0.01		<0.01		<0.01		21.8-22.4
	35E	<0.01		<0.01		<0.01		0.01		22.0-22.7
	35S	0.02		0.01		<0.01		<0.01		20.6-21.1
	100N	<0.01		<0.01		<0.01		<0.01		22.0-22.6
	100E	<0.01		<0.01		<0.01		<0.01		21.3-21.8
	100S	<0.01		0.01		<0.01		<0.01		20.5-21.0
Ambient	Partridge Island	<0.01		<0.01		<0.01		<0.01		11.1-11.5

OTC treatment details	Round 1	Round 2	Round 3	Round 4
Date sampled	9/3/2023	22/3/2023	4/4/2023	3/5/2023
Days since treatment complete	0	13	26	55

3.2 Wild Fish Sampling

Results from wild fish sampling are summarised in Table 2.

In Round 1, OTC concentrations in wild fish were below the AST limit of reporting (LOR, i.e <0.01 mg/kg) for all ambient site samples (i.e. pooled samples 1-4; Table 2). Of the four in-lease wild fish samples, two samples comprised of the benthic species *Platycephalus bassensis* (sand flathead; pooled samples 7 and 8) returned OTC concentrations below the limit of reporting (<0.01 mg/kg; Table 2). The remaining two samples were comprised of pelagic species (pooled sample 5, blue mackerel; pooled sample 6, Australian salmon) and returned OTC concentrations of 0.96 mg/kg and 0.18 mg/kg, respectively (Table 2).

The result recorded in pooled sample 5 in Round 1 was relatively high and above the maximum residue limit of OTC for fish (0.2 mg/kg), as specified in the Australia New Zealand Food Standard Code - Schedule 20[1]. Given these results, Tassal requested a repeat AST test for this particular pooled sample to review for possible erroneous data. The repeat test results were consistent with the initial result as reported in Table 2. The result for pooled sample 5 is considered unusual given previous results from a large number of wild fish sampled over past OTC residue sampling events.

For the final sampling round (Round 5), OTC concentrations in all pooled fish samples collected from the lease area below the LOR (i.e. <0.01 mg/kg) and well below the Australia New Zealand Food Standard Code maximum residue limit for fish (i.e. 0.2 mg/kg). At the ambient site, three of the four pooled samples were also below the LOR. One sample from the ambient site, pooled sample 9, returned an OTC concentration slightly above the LOR (0.02 mg/kg).

The origin of OTC residue at the ambient location in Round 5 remains uncertain. *Platycephalus bassensis* are considered to be mainly sedentary (Dix et al. 1975, Jordan 2001, Tracey et al. 2011), although seasonal movement has been demonstrated for some individuals (Tracey et al. 2011). Ingestion of OTC from within the lease and movement to the control location is considered highly unlikely, given the distance to the lease (~2.5 km) and

[1] https://www.legislation.gov.au/Details/F2023C00001

lack of OTC residue detected in *P. bassensis* samples collected within the lease area during both Round 1 and Round 5 suveys.

Low level contamination of samples, either in the field, during processing, or in the AST laboratory are also a possible source of OTC for pooled sample 9. Each of these sources are considered very unlikely. For the field collection and processing, contamination is minimised by the sequence of sample collection and processing, as specified in the EPA schedule. Samples from the control location were collected and processed prior to the lease samples, minimising the risk of contamination. Similarly, contamination at the AST laboratory is considered very unlikely. Checks on the Liquid Chromatography Mass Spectrometry (LCMS) run sequence associated with pooled sample 9 were carried out and there were no signs of carryover between high and low samples (AST, personal communication).

The other possible OTC source could be background OTC from other anthropogenic sources (e.g. agriculture, wastewater), however, this remains unconfirmed.

In summary, the origin of the OTC residue at the ambient location in sampled Round 5 remains speculative. It is important to note the low level concentration involved (0.02 mg/kg), which is ten times lower than the relevant maximum residue limit of OTC for fish (0.2 mg/kg), as defined by Schedule 20 of the Australia New Zealand Food Standard Code.

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 *MF109 (Butlers)* during Round 1 and Round 5 of sampling. Antibiotic treatment was carried out from the 25th of February to the 9th of March and wild fish sampling was undertaken on the 9th of March and 12th of May 2023. Coloured cells indicate OTC concentrations higher or equal to the limit of reporting (0.01 mg/kg). Note: OTC in fish tissue is reported by AST as mg/kgWMB (wet matter basis).

Location	Round 1		Round 5	
	Pooled sample #	OTC (mg/kg)	Pooled sample #	OTC (mg/kg)
Ambient (Partridge Island)	1	<0.01	9	0.02
	2	<0.01	10	<0.01
	3	<0.01	11	<0.01
	4	<0.01	12	<0.01
In-Lease	5	0.96	13	<0.01
	6	0.18	14	<0.01
	7	<0.01	15	<0.01
	8	<0.01	16	<0.01
OTC treatment details				
Date sampled	9/3/2023		12/5/2023	
Days since treatment complete	0		64	

References

Dix, T. G., Martin A., Ayling G. M., Wilson K. C. and Ratkowsky, D. A. (1975). Sand flathead (*Platycephalus bassensis*), an indicator species for mercury pollution in Tasmanian waters. Marine Pollution Bulletin 6, 142–144.

Jordan, A. R. (2001). Reproductive biology, early life-history and settlement distribution of sand flathead (*Platycephalus bassensis*) in Tasmania. Marine and Freshwater Research 52, 589–601.

Tracey, S.R., K. Hartmann, K., E. Forbes, E., Semmens, J. and Lyle, J.M. (2011). Understanding movement patterns of key recreational fish species in south-east Tasmania, December 2011. Institute of Marine and Antarctic Studies, University of Tasmania, 69 pp.

Appendices

Appendix 1: Survey coordinates for Rounds 1-3 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	9/3/2023	PB2	510717	5192877
			35N	510265	5193591
			35E	510748	5193106
			35S	510793	5192721
			100N	510228	5193644
			100E	510803	5193144
			100S	510829	5192669
			Ambient (Partridge Island)	508599	5194999
	Wild fish	9/3/2023	Ambient (pooled sample 1)	508610	5195045
			Ambient (pooled sample 2,3,4)	508598	5194914
			In-lease (pooled sample 5,6,7)	510717	5192877
			In-lease (pooled sample 8)	510524	5192895
			SPM11089 ~5m to WNW of antenna	523984	5242074
Round 2	Sediment	22/3/2023	PB2	510717	5192878
			35N	510264	5193591
			35E	510748	5193106
			35S	510792	5192721
			100N	510228	5193644
			100E	510801	5193143
			100S	510830	5192667
			Ambient (Partridge Island)	508600	5194999
			SPM9864 ~5m to N of antenna	497538	5221792
Round 3	Sediment	4/4/2023	PB2	510718	5192877
			35N	510265	5193591
			35E	510747	5193107
			35S	510792	5192721
			100N	510226	5193644
			100E	510802	5193143
			100S	510831	5192668
			Ambient (Partridge Island)	508599	5195000
			SPM11089 ~5m to WNW of antenna	523982	5242072

Appendix 1 (continued): Survey coordinates for Rounds 4-5 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 4	Sediment	3/5/2023	PB2	510717	5192877
			35N	510264	5193592
			35E	510748	5193107
			35S	510794	5192722
			100N	510227	5193643
			100E	510802	5193144
			100S	510831	5192668
			Ambient (Partridge Island)	508600	5195000
			SPM11089 ~5m to WNW of antenna	523982	5242072
Round 5	Wild fish	12/5/2023	Ambient (pooled sample 9,10,11,12)	508574	5194967
			In-lease (pooled sample 13,14,15,16)	510465	5192969
			SPM11089 ~5m to WNW of antenna	523984	5242073

Appendix 2: Antibiotic oxytetracycline (OTC) treatment details for *MF109 Butlers* lease. Treatment was administered by feed.

Treatment	Type	Days	Dates prescribed	Active ingredient (kg)
Antibiotic	Oxytetracycline	13	25/02/2023 - 06/03/2023*	32.5

*Note that the treatment of position 2 was extended to a thirteen day treatment (concluding on the 9th March 2023) due to poor initial feeding rates.

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.

<table>
<tr><th colspan="2">Ambient site</th></tr>
<tr><td>
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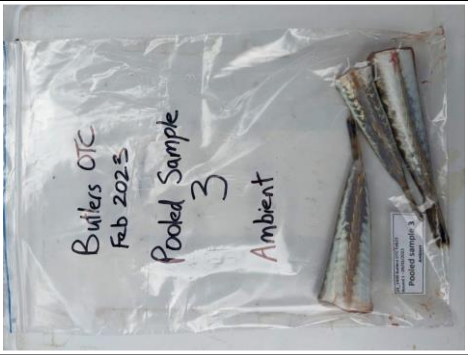
<tr><td>
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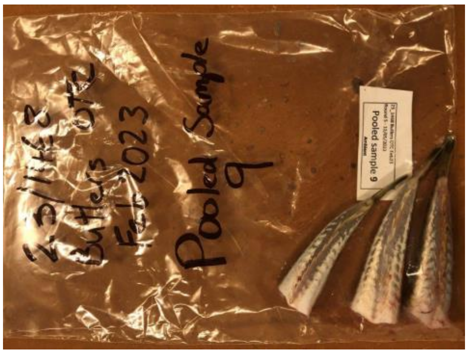
<tr><td>Pooled sample 3</td><td>Pooled sample 4</td></tr>
<tr><th colspan="2">In-lease site</th></tr>
<tr><td>
</td><td>
</td></tr>
<tr><td>Pooled sample 5</td><td>Pooled sample 6</td></tr>
<tr><td>
</td><td>
</td></tr>
<tr><td>Pooled sample 7</td><td>Pooled sample 8</td></tr>
</table>

Appendix 3 (continued): Pooled samples of wild fish specimens prepared for analysis from ambient and in-lease sites for Round 5 of the residue monitoring survey.

Ambient site



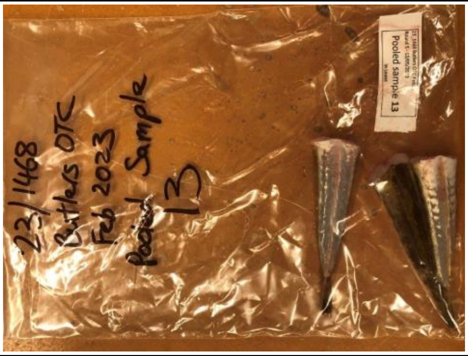
Pooled sample 9

Pooled sample 10

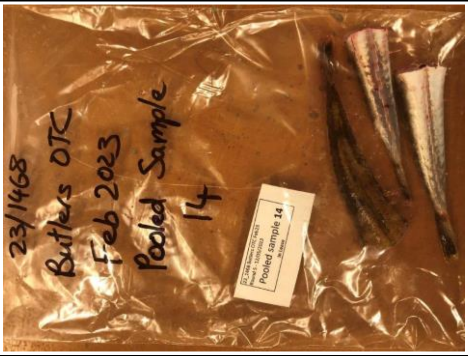
Pooled sample 11

Pooled sample 12

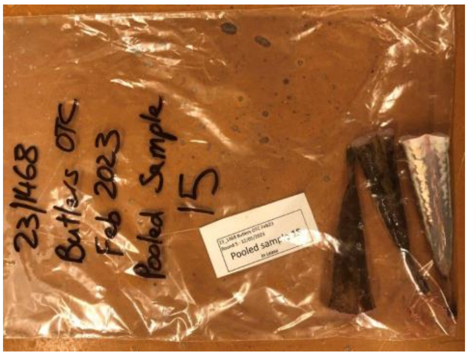
In-lease site



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 *Butlers MF109*.

Site	Sample date	Specimen #	Pooled sample #	Sample weight		Fish species	Fish length (mm)	Gut contents	
Category				whole	tail				
Ambient	9/3/2023	1	1	287	42	Barracouta	385	Empty	
	9/3/2023	2		234	43	Barracouta	390	Empty	
	9/3/2023	3		363	68	Barracouta	415	Empty	
	9/3/2023	4	2	78	19	Sand flathead	240	1 x shrimp	
	9/3/2023	5		118	31	Sand flathead	255	1 x crab, decomposed fish	
	9/3/2023	6		106	33	Sand flathead	250	1 x isopod, 1 x fire crabs, decomposed fish	
	9/3/2023	7	3	100	26	Sand flathead	250	1 x crab	
	9/3/2023	8		175	42	Sand flathead	300	3 x crabs, 2 x amphipods	
	9/3/2023	9		200	55	Sand flathead	300	Empty	
	9/3/2023	10	4	117	30	Sand flathead	250	Empty	
	9/3/2023	11		156	47	Sand flathead	270	1 x crab, 1 x crustacean	
	9/3/2023	12		233	60	Sand flathead	318	Empty	
In-Lease	9/3/2023	13	5	240	35	Blue mackerel	270	Pellets	Brown substance
	9/3/2023	14		280	34	Blue mackerel	285	Pellets	
	9/3/2023	15		247	34	Blue mackerel	275	Pellets, unknown orange substance	
	9/3/2023	16	6	222	27	Australian salmon	240	Empty	
	9/3/2023	17		470	64	Australian salmon	300	Pellets	
	9/3/2023	18		432	56	Australian salmon	300	Pellets, unknown yellow substance	
	9/3/2023	19	7	182	45	Sand flathead	300	2 x squat lobsters, 2 x fire crabs	
	9/3/2023	20		180	50	Sand flathead	290	2 x crabs (*Petrolisthes elongatus*)	
	9/3/2023	21		115	34	Sand flathead	260	Empty	
	9/3/2023	22	8	44	12	Sand flathead	190	1 x shrimp	Amphipods
	9/3/2023	23		68	16	Sand flathead	210	1 x shrimp	
	9/3/2023	24		51	17	Sand flathead	197	brown substance (suspected pellet), 1 x polychaete (Maldanidae)	

Appendix4 (continued): Round 5 wild fish sampling field data. Table adapted from Annexure 3 of the of Antibiotic Residue Monitoring Schedule *Butlers MF109*.

Site / Category	Sample date	Specimen #	Pooled sample #	Sample weight whole	Sample weight tail	Fish species	Fish length (mm)	Gut contents	
Ambient	12/5/2023	25	9	140	36	Sand flathead	265	Empty	1 x glyceridae
	12/5/2023	26		123	37	Sand flathead	255	1 x shrimp	
	12/5/2023	27		103	28	Sand flathead	245	2 x crabs	
	12/5/2023	28	10	173	49	Sand flathead	290	1x hermit crab	
	12/5/2023	29		160	43	Sand flathead	275	Empty	
	12/5/2023	30		132	34	Sand flathead	270	1 x squat lobster	
	12/5/2023	31	11	77	24	Sand flathead	230	1 x crab	
	12/5/2023	32		116	33	Sand flathead	250	Empty	
	12/5/2023	33		124	38	Sand flathead	265	1 x crab, 1 x isopod	
	12/5/2023	34	12	116	36	Sand flathead	255	1 x crab	
	12/5/2023	35		124	34	Sand flathead	260	1 x crab, 1 x squat lobster	
	12/5/2023	36		158	44	Sand flathead	280	1 x calamari	
In-Lease	12/5/2023	37	13	94	26	Sand flathead	240	Empty	9 x squat lobsters
	12/5/2023	38		96	31	Sand flathead	240	Decomposed biota	
	12/5/2023	39		82	27	Sand flathead	225	Empty	
	12/5/2023	40	14	166	42	Sand flathead	295	Empty	
	12/5/2023	41		98	26	Sand flathead	240	Empty	
	12/5/2023	42		116	34	Sand flathead	250	2 x squat lobsters	
	12/5/2023	43	15	106	29	Sand flathead	245	1 x polychaete, 1 x fire crab	
	12/5/2023	44		80	22	Sand flathead	220	2 x squat lobsters	
	12/5/2023	45		89	24	Sand flathead	225	1 x polychaete, 1 x decapod	
	12/5/2023	46	16	105	31	Sand flathead	245	Empty	
	12/5/2023	47		120	32	Sand flathead	250	3 x ghost shrimp	
	12/5/2023	48		134	34	Sand flathead	265	Decomposed fish	