MF109 Butlers

OTC RESIDUE MONITORING DRAFT REPORT October 2022 Treatment

Interim Report

May 2024

Report to:
Tassal Group Limited

Prepared by:
AQUENAL PTY LTD

AQUENAL

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Operational Summary

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Dates of fieldwork:

Round 1 - Sediment: 17/10/2022 Wild fish: 17-18/10/2022	Round 6 - Sediment: 20/2/2023	Round 11 - Sediment: 12/5/2023
Round 2 - Sediment: 28/10/2022	Round 7 - Sediment: 9/3/2023	Round 12 - Sediment: 19/7/2023
Round 3 - Sediment: 11/11/2022	Round 8 - Sediment: 22/3/2023	Round 13 - Sediment: 14/9/2023
Round 4 - Sediment: 10/12/2022	Round 9 - Sediment: 4/4/2023	Round 14 - Sediment: 20/11/2023
Round 5 - Wild fish: 4/1/2023	Round 10 - Sediment: 3/5/2023	Round 15 - Sediment: 25/1/2024

Weather	**17/10/2022**	**18/10/2022**	**28/10/2022**	**11/11/2022**	**10/12/2022**	**4/1/2023**
Wind	SSW 10 kts SSE 10-15 kts	N 5-15 kts	SW 10 kts	E 5 kts	N 0-5 kts E 5kts	SW 5 kts SE 10-15 kts
Sky	Cloudy	Cloudy	Cloudy	Cloudy	Partly cloudy	Partly cloudy
Rain	Light rain	Nil	Rain	Light rain	Nil	Nil
Sea	0 m	0 m	0 m	0 m	0 m	0 m
Current	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	20/2/2023	**9/3/2023**	**22/3/2023**	**4/4/2023**	**3/5/2023**	**12/5/2023**
Wind	S-SSE 10 kts	NW 10 kts	E 0-5 kts	NW 5-10 kts	Light & variable	W 10 kts
Sky	Cloudy	Cloudy	Partly cloudy	Cloudy	Cloudy	Cloudy
Rain	Light	Light rain	Nil	Nil	Light rain	Light shower
Sea	0 m	<0.5 m	<0.5 m	<0.5 m	Calm	<0.5 m
Current	Negligible	Surface from NW to SE	Negligible	Negligible	Negligible	Nil
	19/7/2023	**14/9/2023**	**20/11/2023**	**25/1/2024**		
Wind	WSW 5-10kts	W 5-10kts	E 10 kts	NW 10 kts		
Sky	Cloudy	Sunny	Cloudy	Cloudy		
Rain	Nil	Nil	Nil	Nil		
Sea	<0.5 m	<0.5 m	<0.5 m	<0.5 m		
Current	Nil	Nil	Nil	Nil		

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 9870/3). Sampling methodologies and analyses were in accordance with the monitoring requirements of the Environmental Protection Authority (EPA) *Antibiotic Residue Monitoring Schedule, Butlers MF109*.

The survey design provided by the EPA included sampling of sediment and wild fish for the purpose monitoring residue of the antibiotic Oxytetracycline (OTC), based on the treatment carried out between the 6th and 15th of October 2022 (see Appendix 2 for treatment details). The monitoring survey design included four sediment sampling events and two wild fish sampling events. The initial five sampling rounds were carried out on the 17th and 18th of October (Round 1), 28th of October (Round 2), 11th of November (Round 3), 10th of December 2022 (Round 4), and the 4th of January 2023 (Round 5). A sixth round of sampling (sediment only) was requested by EPA. Selected sediment sites (i.e. PB5, 35S, 100S) were subsequently sampled on the 20th of February 2023 (Round 6). Further follow up sediment monitoring was requested by EPA for Pen Bay sites and was undertaken between March 2023 and January 2024 (Rounds 7-15).

This report provides a summary of the sediment and wild fish sampling activities undertaken across the the 15 sampling rounds.

2 Methods

2.1 Sediment Sampling

2.1.1 Survey sites

The initial sediment survey design for residue monitoring included one pen bay site (PB5), six 'outside of lease' (transect) sites, and one ambient (reference) site (Figure 1). Each site was sampled during Rounds 1-4 of the survey, with a subset of sites sampled during Round 6 (one pen bay and two transect sites). Pen bay PB5 was sampled throughout the monitoring period, including the further follow up sampling requested by EPA in Rounds 7-15. The additional pen bay sites were sampled in Round 12 (PB1) and Rounds 12-15 (PB15; Figure 1). Sampling at pen bay sites was conducted as close to the pen edge as practical by securing the vessel to designated berthing locations on the pens. The pen grid incorporating these three positions was relocated after Round 3 and no pens were present at the sampling location from Round 4 onwards. The positions were subsequently sampled as close as practical to the original sampling location (or the previous pen bay location for positions PB1 and PB15) during Rounds 4-15.

'Outside of lease' sites were sampled in rounds 1-6 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.

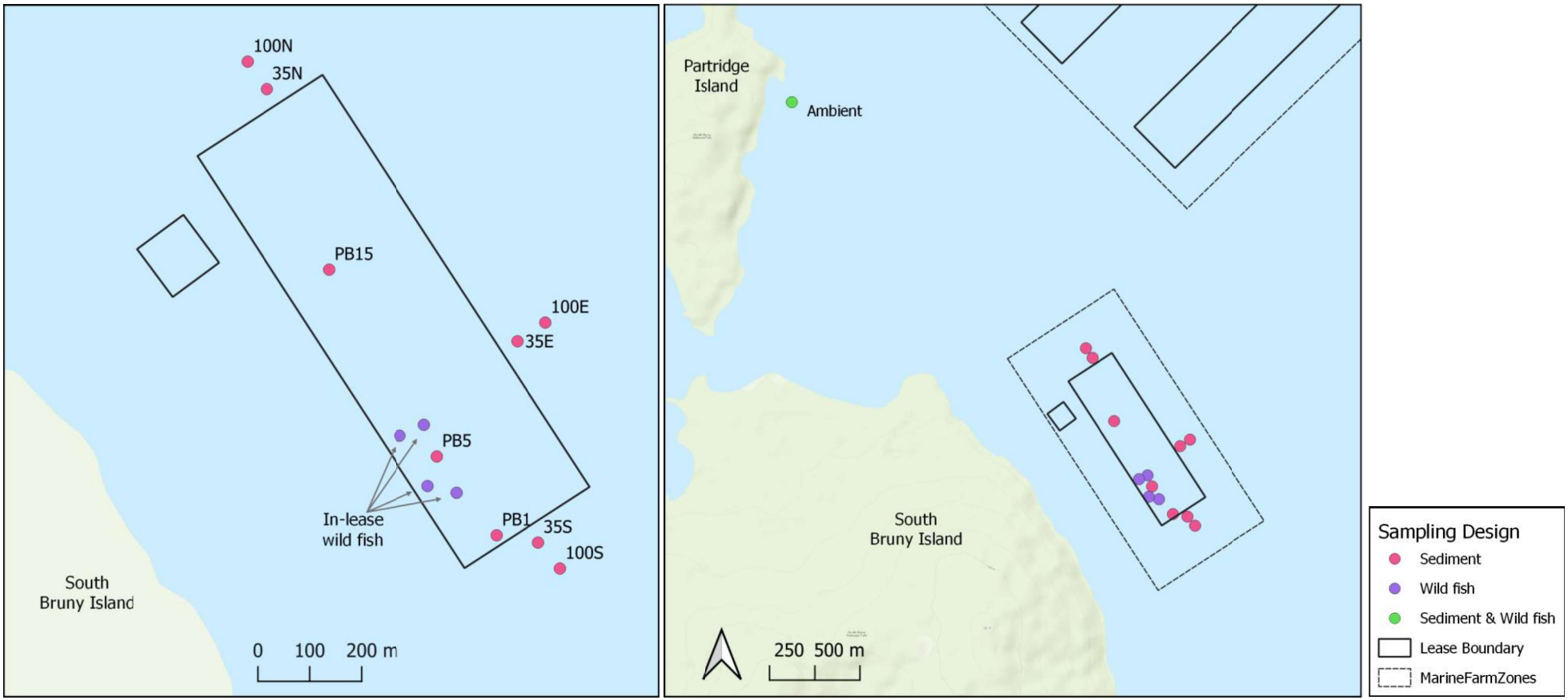

Figure 1: Location and survey maps – *MF109 Butlers* showing overview and location of sediment and wild fish sampling locations.

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. Cores sampled at sites furthermost from the lease area were processed first to prevent sample contamination.

(a) (b)

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

2.1.3 Sample analysis

Following processing of cores in the Aquenal laboratory, samples were refrigerated before delivery to 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 in survey Rounds 1-4 and analysis of bottom core sections was initially conducted for the pen bay site only (with the exception of Round 1). Following review of Round 1 results, the EPA requested analysis of selected archived samples. This included the replicate core (surface and bottom section samples) of the pen bay site, along with frozen archived replicate surface samples and previously un-analysed bottom section samples of selected transect sites from Round 1 (see Table 1). Pen bay site PB5 and a subset of transect sites were sampled and analysed in Round 6. In Rounds 7-15, sampling was restricted to pen bay sites. Site PB5 was sampled from Rounds 7-15, PB1 was sampled in Round 12, while PB15 was sampled in Rounds 12-15 (Table 2).

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 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 22129)* 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 across two sampling events (48 fish total).

In Round 1 of the survey, 12 fish were collected from the ambient location. For the in-lease location, only 10 fish were collected, despite considerable fishing effort (6 hours fishing spread over two days; 17-18/10/2022). The slightly lower number of fish collected at the in-lease location was due to apparent low fish abundance within the lease target area at the time of sampling. In Round 5 of the survey, 12 fish were collected at both the in-lease and ambient locations. The fish samples in both rounds were comprised entirely of the benthic species *Platycephalus bassensis* (sand flathead; see 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); except for pooled sample 8 (in-lease location) in the Round 1 survey which had only one individual. For processing, the tail of each fish was cut off posterior to the anus and bagged prior to investigation of gut contents (Figure 3b). 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 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 the majority of fish in Round 1 and all fish in Round 5 of the survey did not have evidence of pellets in their stomach contents, with naturally occurring dietary items prevalent (Figure 3c-d). In Round 1, evidence of pellets in wild fish samples was observed in 2 of the 10 in-lease specimens (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 flesh sample analysis by AST was on an 'as received' or 'wet' basis and OTC results were reported as mg/kgWMB.

Figure 3: Wild fish field sampling photos showing: (a) four pooled samples of sand flathead (*Platycephalus bassensis*), (b) processing of a specimen, and (c-d) an example of gut contents inspection.

3 Results and Discussion

3.1 Sediment Sampling

3.1.1 Pen bay sites

Results from the fourteen sediment sampling events (Rounds 1-4, Round 6 and Rounds 7-15) are summarised in Table 1 and Table 2. OTC concentrations were highest at the pen bay site (PB5), particularly for surface samples in Round 1 (75 mg/kg; Table 1). The archived replicate samples from PB5 during Round 1 were also analysed to further examine the OTC concentrations and results showed a similarly high concentration of 47 mg/kg in the surface sample (Table 1). Note that occasional feed pellets were evident in the surface sediment of the triplicate pen bay cores in the first two sampling events.

Surface sample OTC concentration for PB5 declined from 75 mg/kg in Round 1 to 1.1 mg/kg in Round 6 (Table 1). There was a slight increase in OTC concentrations observed between Round 6 and Round 7 (Table 1, Table 2, Figure 3). Between Rounds 7 and 11, surface sample OTC concentrations varied between 7.0 and 4.4 mg/kg (Table 2). OTC concentrations in surface samples declined to <1.0 mg/kg from survey Round 8 onwards, but persisted at low levels up to and including the Round 15 survey (Table 2, Figure 3). Additonal sampling was requested by EPA at PB1 and PB15 in survey Rounds 12-15. OTC concentration at PB1 was at a very low level in the Round 12 survey (i.e. <0.1 mg/kg; Table 2). At PB15, OTC concentrations were similar to PB5, with persistence of low OTC concentrations evident in Round 15 (Table 2).

Variation in OTC concentration between surveys is likely reflective of small-scale sampling variability and potential patchy distribution of OTC residue in surface sediments beneath medicated pen bays. It should be noted that the location of the pen bay sample site PB5 varied by +/-10 m across the survey period. This was due to movement of the cage (in Rounds 1-3) and the presence of underwater infrastructure in Rounds 4-15 following relocation of the grid.

OTC concentration was lower in the bottom section of core samples compared to the surface section for the vast majority of pen bay samples. The bottom OTC concentration

trends at pen bay site PB5 were generally consistent with patterns observed in surface sediment samples (Table 1, Table 2).

3.1.2 Transect and ambient sites

Throughout Rounds 1 to 6, surface sample OTC concentrations at the transect (outside of lease) sites were very low. Values ranged between 0.01 and 0.10 mg/kg at the three 35 m sites, and between 0.01 and 0.06 mg/kg at the three 100 m sites (Table 1). All transect sites recorded very low OTC concentrations in the last sampling event involving all sites (Round 4: between 0.01 and 0.06 mg/kg; Table 1)(Table 1). In accordance with the schedule (and as approved by EPA), sampling at transect sites was not required after the Round 6 survey. It is notable that sampling of the transect sites continued as part of a monitoring program for a separate OTC treatment carried out in February 2023, with OTC levels declining further in subsequent surveys at these sites (Aquenal 2023). OTC concentrations were equivalent or lower than the reporting limit for transect site samples collected in the March - May 2023 period, at the same transect sites used in the current (October 2022 medication) survey (Aquenal 2023).

There was no evidence of OTC residue in sediments sampled at the ambient site, with the sediment surface sample OTC concentrations below the limit of reporting (i.e. <0.01 mg/kg) in all applicable survey rounds (1-4; Table 1).

3.1.3 Summary

Residue monitoring showed that OTC concentrations in sediments were highest at pen bay site PB5, particularly in surface samples from Round 1. While there was an initial sharp decline following the Round 1 survey, low OTC concentrations persisted in sediment samples at PB5 and PB15 through to the final Round 15 sampling event. OTC concentrations were significantly lower at the transect sites (35 m and 100 m outside the lease edge) and remained very low throughout the survey, ranging between 0.01 mg/kg and 0.10 mg/kg (Table 1).

Persistence of OTC appears localised to the pen bay sites, with no evidence of OTC peristence beyond the lease area at the transect sites. Variation in OTC concentration between surveys at the pen bay sites is most likely reflective of sampling variability and potential patchy OTC concentrations beneath medicated pen bays.

Table 1: Results of OTC concentration analysis for sediment samples collected at *MF109 (Butlers)* during five sediment sampling events (Rounds 1-4 and 6). Antibiotic treatment was carried out from the 6th to the 15th of October 2022 and sediment sampling was undertaken between the 17th of October 2022 (Round 1) and the 20th of February 2023 (Round 6). Grey shaded cells indicate samples that were archived but not initially analysed (Rounds 1-4), or not required to be taken (Round 6). Note: OTC in sediment is reported by AST as mg/kgDMB (dry matter basis).

Location	Site	Round 1		Round 1 (additional archived analysis)		Round 2		Round 3		Round 4		Round 6		Depth sampled (m)
		OTC (mg/kg)												
		S	B	S	B	S	B	S	B	S	B	S	B	
Pen Bay	PB5	75	0.71	47	0.60	5.1	0.18	3.0	0.15	1.7	0.11	1.1	0.22	23.6
Transect	35N	0.05				0.02		0.02		0.01				22.3
	35E	0.02		0.06	<0.01	0.02		0.06		0.01				22.8
	35S	0.10		0.07	0.03	0.06		0.10		0.05		0.04		21.3
	100N	0.04				0.01		0.01		0.02				22.6
	100E	0.01				0.01		0.01		0.03				21.9
	100S	0.05		0.06		0.06		0.04		0.06		0.01		21.2
Ambient	Partridge Island	<0.01				<0.01		<0.01		<0.01				11.6

OTC treatment details	Round 1	Round 2	Round 3	Round 4	Round 6
Date sampled	17/10/2022	28/10/2022	11/11/2022	10/12/2022	20/02/2023
Days since treatment complete	2	13	27	56	128

Table 2: Results of OTC concentration analysis for sediment samples collected at *MF109 (Butlers)* pen bay sites PB5, PB1 and PB15 during sampling Rounds 7-15. Antibiotic treatment was carried out from the 6th to the 15th of October 2022 and sediment sampling was undertaken between the 9th of March 2023 (Round 7) and the 25th of January 2024 (Round 15). Grey shaded cells indicate samples that were not required to be taken during that round. Note: OTC in sediment is reported by AST as mg/kgDMB (dry matter basis).

Location	Site	Round 7		Round 8		Round 9		Round 10		Round 11		Round 12		Round 13		Round 14		Round 15	
		OTC (mg/kg)																	
		S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B
Pen Bay	PB5	7.0	1.1	6.3	1.5	5.8	0.72	4.1	0.64	4.4	1.7	0.18	0.04	0.16	0.03	0.34		0.35	
	PB1											0.06	0.04						
	PB15											0.38	0.11	0.13	0.12	0.16	0.11	0.18	0.25
OTC treatment details		**Round 7**		**Round 8**		**Round 9**		**Round 10**		**Round 11**		**Round 12**		**Round 13**		**Round 14**		**Round 15**	
Date sampled		9/03/2023		22/03/2023		4/04/2023		3/05/2023		12/05/2023		19/07/2023		14/09/2023		20/11/2023		25/01/2024	
Days since treatment complete		145		158		171		200		209		277		334		401		467	

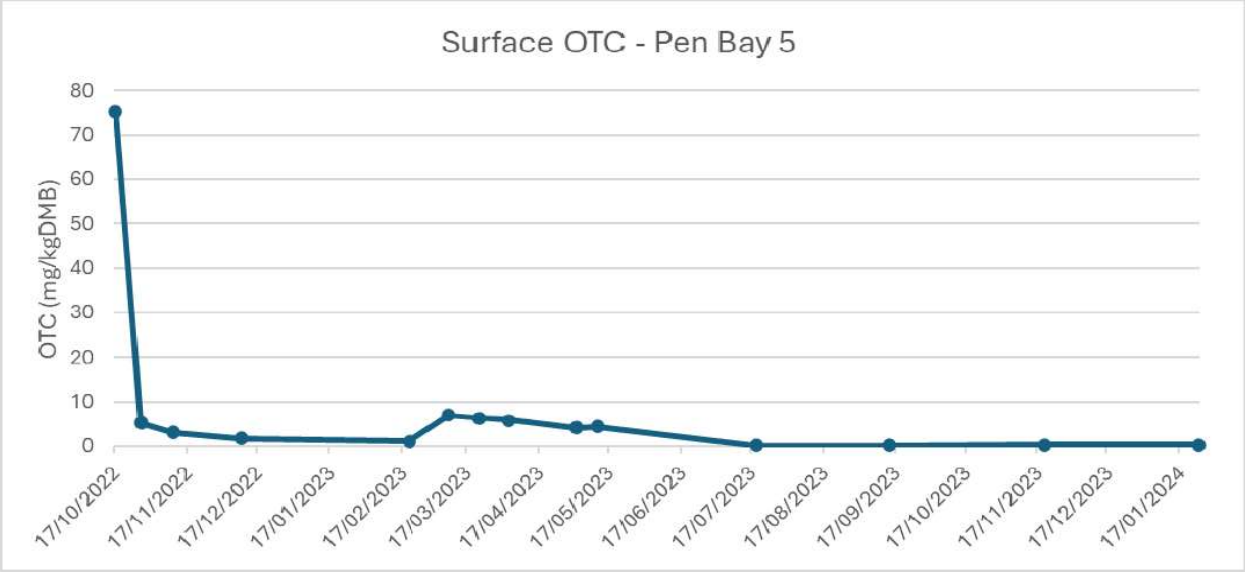

Figure 3: Time series illustrating OTC concentration at PB5 in surface samples, between Rounds 1 and 15, October 2022-January 2024.

3.2 Wild Fish Sampling

Results from wild fish sampling as part of the OTC residue survey are summarised in Table 3. In Round 1, OTC concentrations in wild fish were below the AST limit of reporting (i.e <0.01 mg/kg) for all ambient site samples (pooled samples 1-4; Table 3). Of the four in-lease wild fish samples, one sample (pooled sample 7) returned an OTC concentration equal to the limit of reporting (0.01 mg/kg; Table 3). This sample was comprised of the benthic species *Platycephalus bassensis* (sand flathead), and included one specimen with feed pellets present in the gut contents (see Appendix 4).

In Round 5 of the residue survey (the second wild fish sampling event) OTC concentration was below the limit of reporting (<0.01 mg/kg) for all samples collected, at both ambient and in-lease locations (pooled samples 9-16; Table 3).

At the completion of wild fish sampling, 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). Note that the result recorded in pooled sample 7 from Round 1 was twenty times below the maximum residue limit of OTC for fish, as specified in the Australia New Zealand Food Standard Code - Schedule 20[1], which sets a permitted residue of OTC at 0.2 mg/kg.

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

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

Table 3: 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 6th to the 15th of October 2022 and wild fish sampling was undertaken on the 17th and 18th of October 2022, and the 4th of January 2023. 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	<0.01
	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	17-18/10/2022		4/01/2023	
Days since treatment complete	2-3		81	

Reference

Aquenal (2023) MF109 Butlers OTC Residue Monitoring (February 2023 medication) FINAL REPORT Survey Rounds 1-5 June 2023 Report to: Tassal Group Limited, 22 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	17/10/2022	PB5	510594	5192884
			35N	510265	5193592
			35E	510749	5193106
			35S	510789	5192718
			100N	510228	5193645
			100E	510803	5193142
			100S	510831	5192668
			Ambient	508600	5195000
	Wild fish	17/10/2022	Ambient	508600	5195000
			In-lease (pooled sample 5)	510569	5192945
			SPM11089 ~5m to WNW of antenna	523981	5242071
		18/10/2022	In-lease (pooled sample 6)	510632	5192814
			In-lease (pooled sample 7,8)	510522	5192924
			SPM11089 ~5m to WNW of antenna	523982	5242070
Round 2	Sediment	28/10/2022	PB5	510593	5192885
			35N	510266	5193593
			35E	510750	5193106
			35S	510791	5192718
			100N	510227	5193645
			100E	510804	5193144
			100S	510829	5192667
			Ambient	508600	5195001
			SPM11089 ~5m to WNW of antenna	523983	5242072
Round 3	Sediment	11/11/2022	PB5	510602	5192877
			35N	510266	5193591
			35E	510748	5193107
			35S	510787	5192715
			100N	510228	5193646
			100E	510803	5193143
			100S	510829	5192667
			Ambient	508600	5194999
			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	10/12/2022	PB5	510602	5192878
			35N	510264	5193591
			35E	510748	5193105
			35S	510790	5192718
			100N	510227	5193643
			100E	510801	5193143
			100S	510830	5192667
			Ambient	508601	5195000
			SPM11089 ~5m to WNW of antenna	523983	5242070
Round 5	Wild fish	4/10/2023	Ambient	508601	5195000
			In-lease	510576	5192827
			SPM11089 ~5m to WNW of antenna	523982	5242071
Round 6	Sediment	20/02/2023	PB5	510594	5192884
			35S	510789	5192719
			100S	510830	5192668
			SPM11089 ~5m to WNW of antenna	523983	5242074
Round 7	Sediment	9/3/2023	PB5	510601	5192878
			SPM11089 ~5m to WNW of antenna	523984	5242074
Round 8	Sediment	22/3/2023	PB5	510604	5192882
			SPM9864 ~5m to N of antenna	497538	5221792
Round 9	Sediment	4/4/2023	PB5	510606	5192882
			SPM11089 ~5m to WNW of antenna	523982	5242072
Round 10	Sediment	3/5/2023	PB5	510606	5192883
			SPM11089 ~5m to WNW of antenna	523982	5242072
Round 11	Sediment	12/5/2023	PB5	510607	5192882
			SPM11089 ~5m to WNW of antenna	523984	5242073
Round 12	Sediment	19/7/2023	PB1	510709	5192732
			PB5	510607	5192883
			PB15	510385	5193244
			SPM11089 ~5m to WNW of antenna	523983	5242072
Round 13	Sediment	14/9/2023	PB5	510606	5192883
			PB15	510387	5193245
			SPM9864 ~5m to N of antenna	497537	5221789
Round 14	Sediment	20/11/2023	PB5	510604	5192882
			PB15	510385	5193245
			SPM11089 ~5m to WNW of antenna	523980	5242072
Round 15	Sediment	25/1/2024	PB5	510604	5192882
			PB15	510385	5193245
			SPM11089 ~5m to WNW of antenna	523983	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 (22-03-Tx)	Oxytetracycline	10	06/10/2022 – 15/10/2022	465

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 site	
Pooled sample 1	Pooled sample 2
Pooled sample 3	Pooled sample 4
In-lease site	
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 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	17/10/2022	1	1	105	33	Sand flathead	250	Empty	
	17/10/2022	2	1	150	41	Sand flathead	285	Empty	
	17/10/2022	3	1	110	35	Sand flathead	260	1 x isopod (Cirolanidae)	
	17/10/2022	4	2	490	100	Sand flathead	355	Fish bones	
	17/10/2022	5	2	120	35	Sand flathead	255	1 x isopod (Cirolanidae)	
	17/10/2022	6	2	95	25	Sand flathead	235	Decomposed fish	
	17/10/2022	7	3	150	40	Sand flathead	290	1 x isopod (Cirolanidae), decomposed fish	
	17/10/2022	8	3	50	15	Sand flathead	200	10 x amphipods	
	17/10/2022	9	3	115	30	Sand flathead	260	2 x pebble crabs, decomposed fish	
	17/10/2022	10	4	90	20	Sand flathead	250	2 x amphipods, 1 x gastropod	Regurgitated fish (blenny)
	17/10/2022	11	4	105	30	Sand flathead	250	Empty	
	17/10/2022	12	4	175	45	Sand flathead	300	Decomposed fish	
In-Lease	17/10/2022	13	5	140	35	Sand flathead	275	1 x crab, pellet	
	17/10/2022	14	5	85	25	Sand flathead	235	Decomposed fish	
	17/10/2022	15	5	75	25	Sand flathead	225	5 x amphipods	
	18/10/2022	16	6	70	20	Sand flathead	215	Decomposed fish, 1 x mysid, unknown brown substance	
	18/10/2022	17	6	125	33	Sand flathead	265	Empty	
	18/10/2022	18	6	56	16	Sand flathead	200	Empty	
	18/10/2022	19	7	100	28	Sand flathead	260	Pellets	
	18/10/2022	20	7	95	25	Sand flathead	235	Empty	
	18/10/2022	21	7	70	20	Sand flathead	215	Mussel	
	18/10/2022	22	8	106	28	Sand flathead	250	1 x decapod shrimp, 1 x amphipod, decomposed fish	
	NA	23	8	NA	NA	NA	NA	NA	
	NA	24	8	NA	NA	NA	NA	NA	

Appendix 4 (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		Fish species	Fish length (mm)	Gut contents	
				whole	tail				
Ambient	4/1/2023	25	9	95	24	Sand flathead	240	Empty	
	4/1/2023	26	9	57	17	Sand flathead	205	1 x shrimp, 1 x amphipod	
	4/1/2023	27	9	95	25	Sand flathead	240	3 x shrimp	
	4/1/2023	28	10	84	24	Sand flathead	235	3 x shrimp, 1 x decomposed fish	
	4/1/2023	29	10	66	19	Sand flathead	200	Empty	
	4/1/2023	30	10	110	31	Sand flathead	200	Empty	
	4/1/2023	31	11	62	18	Sand flathead	215	12 x amphipods	
	4/1/2023	32	11	65	18	Sand flathead	215	1 x decomposed fish, 6 x amphipods	
	4/1/2023	33	11	71	20	Sand flathead	220	1 x shrimp	
	4/1/2023	34	12	123	35	Sand flathead	265	Empty	
	4/1/2023	35	12	175	36	Sand flathead	270	1 x pebble crab	
	4/1/2023	36	12	310	87	Sand flathead	350	1 x decomposed fish	
In-Lease	4/1/2023	37	13	125	26	Sand flathead	255	10 x shrimp, 1 x squat lobster	
	4/1/2023	38	13	103	19	Sand flathead	250	1 x shrimp	
	4/1/2023	39	13	200	50	Sand flathead	300	2 x shrimp, 2 x squat lobster	
	4/1/2023	40	14	38	15	Sand flathead	185	Empty	
	4/1/2023	41	14	80	28	Sand flathead	215	1 x squat lobster	
	4/1/2023	42	14	48	18	Sand flathead	205	1 x squat lobster, 1 x decomposed fish	
	4/1/2023	43	15	99	25	Sand flathead	250	Empty	4 x regurgitated crab (*Petrolisthes elongatus*)
	4/1/2023	44	15	75	20	Sand flathead	220	Empty	
	4/1/2023	45	15	60	20	Sand flathead	200	Empty	
	4/1/2023	46	16	142	33	Sand flathead	270	3 x crabs	
	4/1/2023	47	16	140	39	Sand flathead	275	Empty	
	4/1/2023	48	16	188	45	Sand flathead	295	8 x squat lobsters	