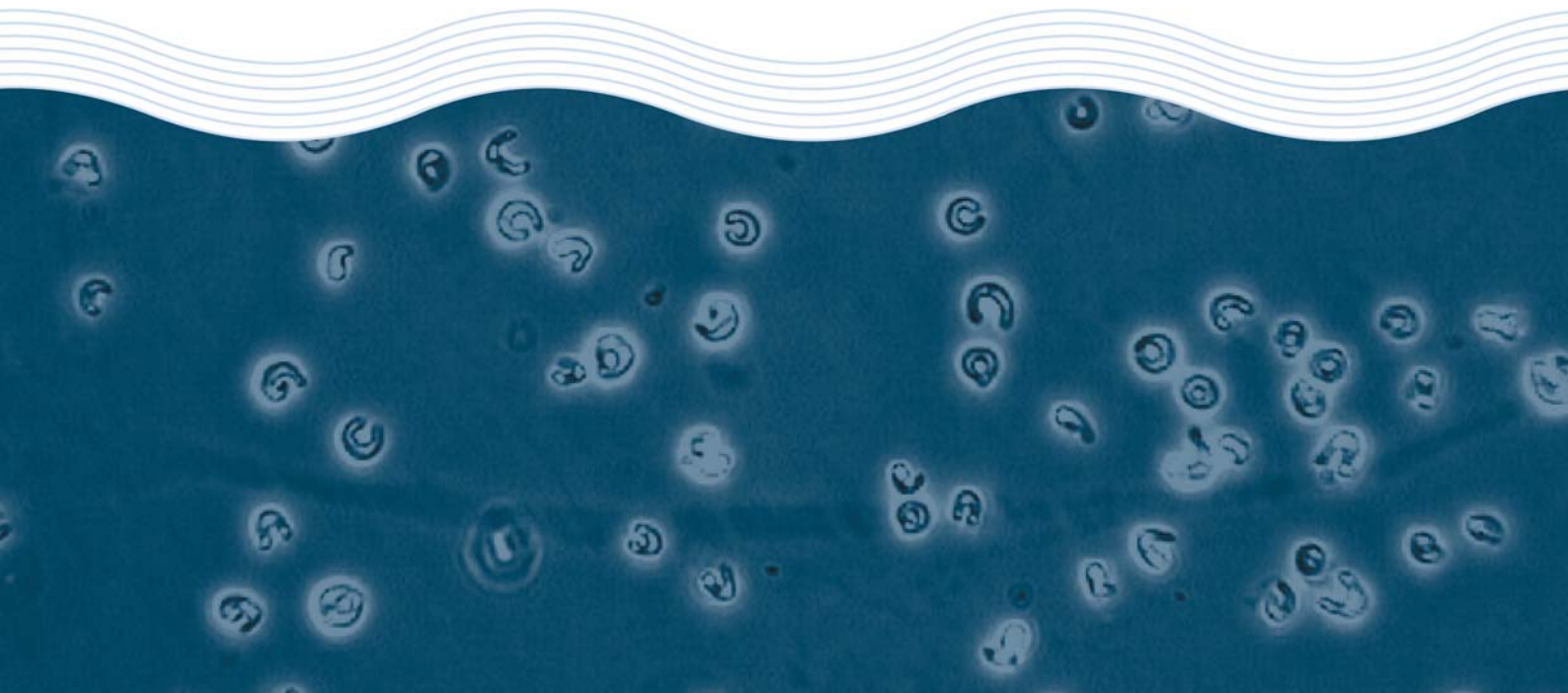


**Toxicity Assessment of a  
Brazilian Pulp Mill Sample to  
Gametophytes of the Kelp  
*Ecklonia radiata***

**Gunns Limited**

**Test Report**

**December 2009**



**Toxicity Assessment of a  
Brazilian Pulp Mill Sample to  
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*Ecklonia radiata***

**Gunns Limited**

**Test Report**

**December 2009**

## Toxicity Test Report: TR0490/1

(page 1 of 2)

<b>Client:</b>	Gunns Ltd PO Box 572 Launceston TAS 7250	<b>ESA Job #:</b>	PR0490
<b>Attention:</b>	Mr Lawson Harding	<b>Date Sampled:</b>	17 November 2009
<b>Client Ref:</b>	Not Supplied	<b>Date Received:</b>	19 November 2009
		<b>Sampled By:</b>	Client
		<b>ESA Quote #:</b>	PL0490_q01

<b>Lab ID No.:</b>	<b>Sample Name:</b>	<b>Sample Description:</b>
3891Q	Brazil	Aqueous sample, pH 8.5, conductivity 2950 $\mu$ S/cm, total ammonia <2.0mg/L. Sample received at 7-8°C in apparent good condition

<b>Test Performed:</b>	14 day macroalgal growth test using <i>Ecklonia radiata</i>
<b>Test Protocol:</b>	ESA SOP 116 (ESA 2009), based on Bidwell <i>et al.</i> (1998) and Burrige <i>et al.</i> (1999)
<b>Deviations from Protocol:</b>	Test extended from 72 h to 14 days to encompass growth endpoint.
<b>Comments on Solution Preparation:</b>	The sample was adjusted to a salinity of 35 $\pm$ 1‰ with GP2 artificial sea salts prior to testing. The sample was serially diluted with filtered seawater (FSW) to achieve the test concentrations. A FSW control and an artificial seawater (ASW) control were tested concurrently with the sample.
<b>Source of Test Organisms:</b>	Field collected from Mercury Passage, TAS
<b>Test Initiated:</b>	19 November 2009 at 1330 h

Sample 3891Q: <i>Brazil</i>		<i>Vacant</i>	<i>Vacant</i>
Concentration (%)	Gametophyte Length, $\mu$ m (Mean $\pm$ SD)		
FSW Control	26.0 $\pm$ 2.5		
ASW Control	24.7 $\pm$ 2.3		
1.6	24.8 $\pm$ 2.3		
3.1	25.3 $\pm$ 1.0		
6.3	25.0 $\pm$ 1.3		
12.5	25.0 $\pm$ 0.8		
25	25.1 $\pm$ 1.0		
50	25.5 $\pm$ 1.1		
100	23.8 $\pm$ 1.2		
<b>72-hr EC10 = &gt;100%</b>			
<b>72-hr EC50 = &gt;100%</b>			
<b>NOEC = 100%</b>			
<b>LOEC = &gt;100%</b>			

**Toxicity Test Report: TR0490/1**

(page 2 of 2)

QA/QC Parameter	Criterion	This Test	Criterion met?
Reference Toxicant within cusum chart limits	114.4-1627.0µg Cu/L	263.8µg Cu/L	Yes



Test Report Authorised by:

Dr Rick Krasso, Director on 20 January 2010

Results are based on the samples in the condition as received by ESA. This document shall not be reproduced except in full.

**Citations:**

Bidwell, J. R., Wheeler, K. W., & Burrige, T. R. (1998). Toxicant effects on the zoospore stage of the marine maroalga *Ecklonia radiata* (Phaeophyta:Laminariales). *Marine Ecology Progress Series*. Vol 163 , 259-265.

Burrige, T. R., Karistanios, M., & Bidwell, J. (1999). The use of aquatic macrophyte ecotoxicological assays in monitoring coastal effluent discharges in southern Australia. *Marine Pollution Bulletin*. Vol 39 , 1-12.

ESA (2009) *SOP 116 – Macroalgal Germination Success Test*. Issue No. 9. Ecotox Services Australasia, Sydney NSW

# Chain-of-Custody Documentation

# Sample Receipt Notification

**Attention** : Lawson Harding

**Client** : Gunns Limited  
PO Box 572  
Launceston TAS 7250

**Email** : Lawson.harding@gunns.com.au  
**Telephone** : (03) 6335 5446  
**Facsimile** : (03) 6335 5498

**Date** : 19/11/2009

**Re** : Receipt of Sample for Toxicity Testing

**Pages** : 2

**ESA Project** : PR0490

For Review

Additional Documentation Required - Please Respond

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## **Sample Delivery Details**

**Completed Chain of Custody accompanied samples:** YES  
**Samples received in apparent good condition and correctly bottled:** YES  
**Security seals on sample bottles and esky intact:** YES

**Date samples received** : 19/11/2009  
**Time samples received** : 9:15  
**No. of samples received** : 1  
**Sample matrix** : aqueous  
**Sample temperature** : 6-10°C

**Comments** : Includes 5 x 2L Brazil sample (ESA ID# 3891Q)

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## **Contact Details**

Customer Services Officer : Tina Micevska  
Telephone : 61 2 9420 9481  
Facsimile : 61 2 9420 9484  
Email : tmicevska@ecotox.com.au

Please contact customer services officer for all queries or issues regarding samples

**Note that the chain-of-custody provides definitive information on the tests to be performed**

---

## **Ecotox Services Australia**

ABN 45 094 714 904  
Unit 27, 2 Chaplin Drive  
Lane Cove NSW 2066 Australia

Phone : 61 2 9420 9481  
Fax : 61 2 9420 9484  
Email : info@ecotox.com.au

# Chain-of-Custody / Service Request Form

Customer: *Guas Ltd.*

Ship To: *ESA*

Contact Name: *Kaaso's Handling*

Attention: *Dr. Rick Kaasoi*

Phone: *61 407 827 958* Email: *kaaso@handling.com.au*

(please provide an email address for sample receipt notification)

Sampled by: *Kaaso's Handling*

Sample Date (day/month/year)	Sample Time	Sample Name  (exactly as written on the sample vessel)	Sample Method  (eg. Grab, composite etc.)	Number and Volume of Containers  (eg 2 x 1L)	Tests Requested  (See reverse for guidance)			Comments / Instructions
<i>17/11/09</i>		<i>Brazil 1,2,3,4,5</i>	<i>Grab</i>	<i>5 x 2L</i>				<p><b>Note that testing will be delayed if an incomplete chain of custody is received</b></p> <ul style="list-style-type: none"> <li>• Additional treatment of samples (i.e. spiking)</li> <li>• Sub-contracted services (i.e. chemical analyses)</li> <li>• Dilutions required (if different than 100% down to 6.25%)</li> <li>• Sample holding time restriction (if applicable)</li> <li>• Sample used for litigation (if applicable)</li> </ul> <p>Note: An MSDS must be attached if Available</p> <p>ESA Project Number: PR</p> <p><i>As per SAP &amp; as Requested by L. Harding</i> <i>Ref Quoted by PL 0499/101</i></p>

1) Released By: <i>[Signature]</i>	Date: <i>19/11/09</i>	2) Received By: <i>[Signature]</i>	Date: <i>19/11/09</i>
Of: <i>Guas Ltd.</i>	Time: <i>09:15</i>	Of: <i>ESA</i>	Time: <i>0915</i>
3) Released By:	Date:	4) Received By:	Date:
Of:	Time:	Of:	Time:

Note that the chain-of-custody documentation will provide definitive information on the tests to be performed.

**Statistical Printouts for the kelp  
*Ecklonia radiata* 14-d  
Gametophyte Growth Test**

**Macroalgal Growth Test-Gametophyte Length**

Start Date:	19/11/2009 13:30	Test ID:	PR0490/22	Sample ID:	Brazil
End Date:	3/12/2009 13:30	Lab ID:	3891Q	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:		Test Species:	ER-Ecklonia radiata
Comments:					

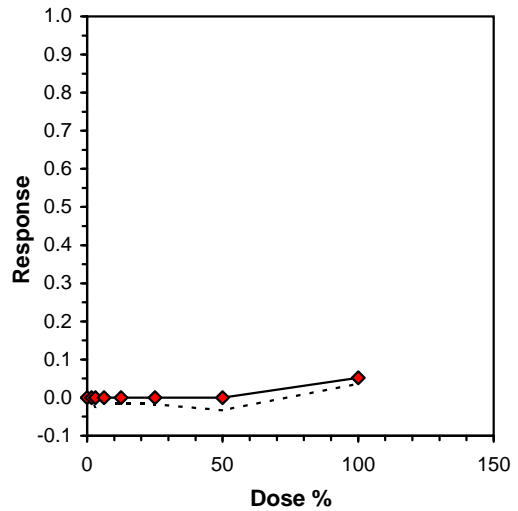
Conc-%	1	2	3	4
FSW Control	28.764	26.313	26.013	22.711
ASW Control	23.739	23.900	23.033	27.987
1.6	22.824	23.538	24.929	28.062
3.1	23.873	26.135	25.856	25.194
6.3	26.506	23.614	25.728	24.180
12.5	24.828	25.659	25.690	23.918
25	23.705	25.745	25.722	25.129
50	24.234	26.076	24.879	26.716
100	24.289	23.315	25.057	22.371

Conc-%	Transform: Untransformed							t-Stat	1-Tailed Critical	MSD	Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N				Mean	N-Mean
FSW Control	25.950	1.0521	25.950	22.711	28.764	9.581	4					
ASW Control	24.665	1.0000	24.665	23.033	27.987	9.109	4	*			25.050	1.0000
1.6	24.838	1.0070	24.838	22.824	28.062	9.341	4	-0.166	2.480	2.593	25.050	1.0000
3.1	25.265	1.0243	25.265	23.873	26.135	3.990	4	-0.574	2.480	2.593	25.050	1.0000
6.3	25.007	1.0139	25.007	23.614	26.506	5.361	4	-0.327	2.480	2.593	25.050	1.0000
12.5	25.024	1.0146	25.024	23.918	25.690	3.350	4	-0.343	2.480	2.593	25.050	1.0000
25	25.075	1.0166	25.075	23.705	25.745	3.816	4	-0.393	2.480	2.593	25.050	1.0000
50	25.476	1.0329	25.476	24.234	26.716	4.416	4	-0.776	2.480	2.593	25.050	1.0000
100	23.758	0.9632	23.758	22.371	25.057	4.914	4	0.867	2.480	2.593	23.758	0.9484

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.924651	0.93	0.775485	0.632122
Bartlett's Test indicates equal variances (p = 0.54)	5.996903	18.47531		
The control means are not significantly different (p = 0.47)	0.767238	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	2.592961	0.105128	1.076946	2.186345	0.830531	7, 24
Treatments vs ASW Control										

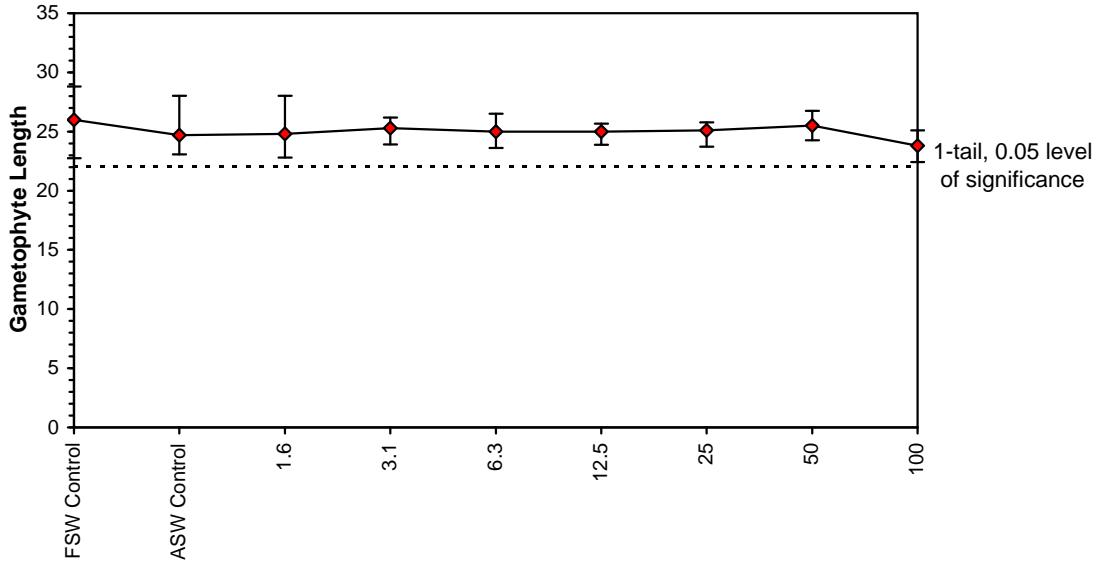
Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)	Skew
IC05	98.473			
IC10	>100			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			



**Macroalgal Growth Test-Gametophyte Length**

Start Date: 19/11/2009 13:30 Test ID: PR0490/22 Sample ID: Brazil  
End Date: 3/12/2009 13:30 Lab ID: 3891Q Sample Type: AQ-Aqueous  
Sample Date: Protocol: Test Species: ER-Ecklonia radiata  
Comments:

**Dose-Response Plot**



**Macroalgal Growth Test-Gametophyte Length**

Start Date:	19/11/2009 13:30	Test ID:	PR0490/22	Sample ID:	Brazil
End Date:	3/12/2009 13:30	Lab ID:	3891Q	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:		Test Species:	ER-Ecklonia radiata
Comments:					

**Auxiliary Data Summary**

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	Length um	25.95	22.71	28.76	2.49	6.08	4
ASW Control		24.66	23.03	27.99	2.25	6.08	4
1.6		24.84	22.82	28.06	2.32	6.13	4
3.1		25.26	23.87	26.14	1.01	3.97	4
6.3		25.01	23.61	26.51	1.34	4.63	4
12.5		25.02	23.92	25.69	0.84	3.66	4
25		25.08	23.71	25.75	0.96	3.90	4
50		25.48	24.23	26.72	1.12	4.16	4
100		23.76	22.37	25.06	1.17	4.55	4
FSW Control		pH	8.00	8.00	8.00	0.00	0.00
ASW Control	8.00		8.00	8.00	0.00	0.00	1
1.6	8.00		8.00	8.00	0.00	0.00	1
3.1	8.00		8.00	8.00	0.00	0.00	1
6.3	7.90		7.90	7.90	0.00	0.00	1
12.5	7.90		7.90	7.90	0.00	0.00	1
25	7.80		7.80	7.80	0.00	0.00	1
50	8.00		8.00	8.00	0.00	0.00	1
100	8.00		8.00	8.00	0.00	0.00	1
FSW Control	Salinity ppt		34.40	34.40	34.40	0.00	0.00
ASW Control		34.40	34.40	34.40	0.00	0.00	1
1.6		34.50	34.50	34.50	0.00	0.00	1
3.1		34.50	34.50	34.50	0.00	0.00	1
6.3		34.50	34.50	34.50	0.00	0.00	1
12.5		34.70	34.70	34.70	0.00	0.00	1
25		35.00	35.00	35.00	0.00	0.00	1
50		34.20	34.20	34.20	0.00	0.00	1
100		34.40	34.40	34.40	0.00	0.00	1
FSW Control		DO % sat	96.70	96.70	96.70	0.00	0.00
ASW Control	96.90		96.90	96.90	0.00	0.00	1
1.6	96.70		96.70	96.70	0.00	0.00	1
3.1	96.60		96.60	96.60	0.00	0.00	1
6.3	96.90		96.90	96.90	0.00	0.00	1
12.5	98.70		98.70	98.70	0.00	0.00	1
25	101.50		101.50	101.50	0.00	0.00	1
50	96.50		96.50	96.50	0.00	0.00	1
100	96.20		96.20	96.20	0.00	0.00	1