# APPENDIX B. DR. KOHN'S IMPOSEX REPORT AND DATA FORMS



2005 Gastropod imposex study results

## 2005 Gastropod Imposex Study: Report on Results

Sta. G16b. Coll. Aug. 8, analyzed Aug. 9, 2005.

Protocol. The protocol required alteration for the analysis of *Olivella baetica*. Removing the shell and examining the animal directly proved unsatisfactory, because of the large quantities of mucus the animals secreted. I therefore euthanized the animals by placing them in boiling water for two minutes. They were then transferred to cold water, and then to 70% alcohol to denature the mucus, prior to examination.

Olivella baetica. None of the 26 specimens appear to be affected by imposex, but this result is not completely unambiguous. Nineteen of the specimens were clearly females; they had white to beige gonads overlying the digestive gland, some had very well developed yellow glandular oviducts, and none had any evidence of a penis. Four specimens are considered to be males. All possessed a penis, but in all cases it was small, 0.8-1.0 mm. At first I considered these imposex females, because of the short penis, but they had dark yellow gonads and lacked glandular oviducts. I consider them more likely to be immature males, although they are about the same length as the apparently mature females. Three and probably four specimens had no evident gonad, oviduct, or penis; these are best considered immature and of indeterminate gender. I conclude that the sex ratio in this sample is strongly biased to females (18/22 or 82% of those whose sex could be determined), and females appear to mature at a smaller size than males. Four specimens of shell length 0.6-0.7 cm, and a 27<sup>th</sup> specimen (0.5 cm), from the jar labeled "other gastropods" lacked gonads and were judged to be immature and not determinable as to sex. Two additional, even smaller specimens in the "other gastropods" jar were not examined. The largest specimens of Olivella baetica were only half the typical adult size of 2 cm, so no large adults were available for comparison. Some specimens had small penises, but these were judged to be immature males, as their gonads differed in color and consistency from those of females.

*Astyris gausapata*. Fifty-eight specimens of shell lengths 0.6-1.0 cm were examined; five of the shells were empty. Because the smallest specimens was definitely a male, two specimens (0.6-0.7 mm) that lacked gonads or penises, are considered females. The sex ratio was 1:1 (26 males, 27 females). None of the females showed any symptoms of imposex.

Other gastropods. The one *Nassarius mendicus* specimen (1.4 cm) was a male. One specimen of *Natica* sp. (0.65 cm shell length) was sexually immature. The following gastropods were present but not examined for imposex: *Kurtziella plumbea*, *Turbonilla* spp. (8), and *Melanella* sp(p.) (3). In *K. plumbea* and *Melanella*, the sexes are separate, but the specimens were too small to examine. *Turbonilla* spp. are hermaphroditic.

Sta. G17b. Coll. Aug. 9, analyzed Aug. 10, 2005.

*Astyris gausapata*. Sixteen specimens of shell lengths 0.55-1.0 cm were examined; 7 were male and 9 were female. The smallest specimen was a male. The sex ratio was 9 males, 7 females). None of the females showed any symptoms of imposex.

*Nassarius mendicus.* Two of the three specimens were male (1.5 and 1.6 cm). Their penis lengths were 11.7 and 12 mm. The female (1.3 cm) had stage two imposes, with a rather long penis (3.3 mm) (RPS index = 2.0) but no evidence of a vas deferens.

Other gastropods. Other gastropods from Sta. G17b included four *Acteocina* sp. cf. *A. cerealis*, 3 *Turbonilla* sp., and one *Odostomia* sp. These small gastropods were not examined for imposex; all are hermaphroditic.

Sta. G17b. Coll. Aug. 10, analyzed Aug. 11, 2005.

Other gastropods. One *Turbonilla* sp. and one empty shell of same.

Sta. G18b. Coll. Aug. 10, analyzed Aug. 11, 2005.

*Nassarius mendicus.* Four specimens, two males and two stage 2 imposex females. Male penis lengths averaged 9.6 mm and female penis lengths averaged 3.1 mm; RPS index = 3.4.

Other gastropods. 54 *Turbonilla* (2 spp.), 4 *Polinices* sp., all immature (all 0.7 cm in maximum dimension); 3 with short (1.0-2.4 mm) penises are concluded to be immature males, one without penis is concluded to be an immature female. None had gonads. 2 *Acteon (Rictaxis) punctocaelata* Carpenter, 1 *Acteocina* sp. cf. *A. cerealis*, 2 *Lacuna vincta* (Montagu).

Sta. G18b. Coll. Aug. 10, analyzed Aug. 12 and 16, 2005.

*Astyris gausapata*. Fifty-eight of the >100 specimens were analyzed, in order to examine 30 females as requested; 28 were males. Shell lengths were 0.8-1.0 cm. None of the females showed any symptoms of imposex.

Sta. G19b. Coll. Aug. 10, analyzed Aug. 11, 2005.

Nassarius mendicus. Two specimens, both stage 2 imposex females.

Other gastropods. Three specimens of two *Turbonilla* spp.

Sta. G19b. Coll. Aug. 10, analyzed Aug. 12, 2005.

Astyris gausapata. Forty-nine of the >100 specimens were analyzed, in order to examine 30 females as requested; 19 were males. Shell lengths were 0.8-1.1 cm. None of the females showed any symptoms of imposex.

Sta. G19b. Coll. Aug. 11, analyzed Aug. 12, 2005.

Other gastropods. Thirteen specimens of two Turbonilla spp., and one empty shell.

Sta. G20b. Coll. Aug. 11, analyzed Aug. 12, 2005.

*Astyris gausapata*. Thirty-eight specimens of shell lengths 0.5-1.1 cm were examined; one specimen 0.3 cm long was not examined. Of those examined, 22 were male and 16 were female. None of the females showed any symptoms of imposex.

Nassarius mendicus. Only one specimen, a male, was present.

Other gastropods. One specimen of *Turbonilla* sp. with red shell, and 119 specimens of a second *Turbonilla* sp. with white shell. One specimen of *Polinices* sp. 0.8 cm in maximum dimension was examined; it was immature. Three *Natica* sp. 0.5-0.6 cm, 2 *Acteon* (*Rictaxis*) *punctocaelata* Carpenter, 2 *Acteocina* sp. cf. *A. cerealis*, and 7 *Odostomia* sp. (?) were not analyzed. The *Natica* specimens were too small to examine, and those of the latter three genera are hermaphroditic.

Sta. G21b. Coll. Aug. 9, analyzed Aug. 10, 2005.

*Astyris gausapata.* Twelve specimens of shell lengths 0.7-1.2 cm were examined; 6 were male and 6 were female. None of the females showed any symptoms of imposex.

Other gastropods. Other gastropods from Sta. G21b included one *Acteocina* sp. cf. *A. cerealis*, 3 *Turbonilla* sp., and one *Natica* sp. These gastropods were too small to be examined for imposex, and *Acteocina* and *Turbonilla* are hermaphroditic.

Summary of imposex in Nassarius mendicus

*Nassarius mendicus* was the only gastropod that showed imposex symptoms. All females collected were affected, and all were graded at Stage 2, specifically Stage 2a, 'larger penis with a penis duct,' following the scheme of Oehlmann, Stroben and Fioroni (1991). A penis was present in all (lengths are given in Table 1), but none had an evident vas deferens.

In all other gastropods that have been studied, stage 2 imposex does not interfere with female reproduction. It is thus most likely that the level of imposex observed in *N. mendicus* in the LDW does not prevent reproduction.

Table 1. Measured penis lengths of male and female *N. mendicus* are listed. For all samples combined the RPS index = 1.8.

Station	Male penis lengths (mm)	Female penis lengths (mm)
G16b	(not measured)	
G17b	12.5, 11.7	3.3
G18b	10.0, 9.1	2.5, 3.7
G19b		2.5, 2.6
G20b	12.5	

### Summary of examination of other gastropods

Most of the other gastropods collected belonged to the Subclass Heterobranchia, specifically the genera *Turbonilla, Odostomia, Acteon*, and *Acteocina*. In addition to being very small, members of these genera are normally hermaphroditic, and hence not subject to detectable imposex.

The other members of the Subclass Prosobranchia examined were all very small, immature, or both. The largest moon snails (*Polinices* sp. and *Natica* sp.) were examined. None had evidence of imposex, but all appeared to be immature, as they lacked visible gonads.

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Date:

Project Name: Lower Duwarnish Waterway RI- Benthic Invertebrates AUG. 9, 2005

Analyst A.J. KORN

Project no. 04-08-06-21

Sample ID	Gastro	pod Specie	s	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
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		-	25	0.7	8		1.0	
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**	• '	4	27.	0.5	?			From other 92stropod"

**General Comments:** 

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2005 Gastropod Imposex Study July 28, 2005 Page 24

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Date:

Project Name: Lower Duwarnish Waterway RI- Benthic Invertebrates Aug. 9,2005

Project no. 04-08-06-21

Analyst: A. T. KottN

Sample ID	Gastro	pod Specie	es	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
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2005 Gastropod Imposex Study July 28, 2005 Page 24

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Wind Ward

Date:

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates AUG. 9. 2005

Project no. 04-08-06-21

Analyst: A. J. KONN

Sample ID	Gastropod Species	Sheli Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
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	41	0.75	\$	0		·
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	55.	0.65	\$?	<b>O</b> .		no gonad? (Mashed)

General Comments:



2005 Gastropod Imposex Study July 28, 2005 Page 24

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roject Name: _	Lower Duwamish Waterway RI-Be	nthic Inverteb	ates	Proje	ectno. 04-08-0	06-21
ate: _	Ava, 8, 2005 (C	-/(.) ma(.)		Ar	nalyst: 🛛 📈	V. KONN
Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G1610	Astyrin gausapata SE.	1.0	ð Ø 1			no gonad; no glando
	57. 58.	1.0	₽ ₽	47-0		may be immistore
	Nassarius mendicus	14	5	••		- <u>_</u>
	Nautican sp.	0.65	4:			female or immature
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2005 Gastropod Imposex Study July 28, 2005 Page 24

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Wind Ward

Date:

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates AUG. 10, 2005

Project no. 04-08-06-21

Analyst: A. J. KOHN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
6172	Nassavius upudicus	1.3	$\mathcal{Q}$	2	3.3	No evilence of van
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		0.9	2	0		
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	7	1.0	9	0	<b>.</b>	
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General Comments:

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2005 Gastropod Imposex Study July 28, 2005 Page 24

Wind Ward

Date:

Project Name: Lower Duwarnish Waterway RI- Benthic Invertebrates 10 AUG. 2005 Analyzed 12 Aug. 2005 Analyst: A. J.Kolin

Project no. 04-08-06-21

Shell Penis Height Length Sample ID **Gastropod Species** Sex Imposex Stage (mm) Comments (cm) 3 G186 <u>Astyris gausapatai</u> 0.9 ₽ 0 1.0 2 Ŷ 1.0 0 3 8 ų 1.0 \_\_\_\_\_ q 5 1.0 Q 07 0.9 ۲  $\sim$ 0.9 8 7 8 8 0.9 Ŷ 9 0.95 0 on 13 1.0 0 11 0.8 -2  $\mathcal{O}$ 0.9 12 2 1.1 Э 13 2 1.0 0 14 9 0 15 1.1 07 1.5 \_\_\_\_ 16 07 17 \_\_\_\_ 1.0 \$ 18 1.0 0 ₹ 19 0,9 OF -----20 0.9 Ŷ O 21 1.0 9 Ò 22 1.0 0 Z.3 -----1,0 3 24 1.0 3 zS 0.9 ₹ 0.9 z۵ 077 0.8 -27 9 27 0.9 0

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2005 Gastropod Imposex Study July 28, 2005 FINAL Page 24

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ct Name:	Lower Duwamish Waterway RI-Be	Proj	ect no. <u>04-08-06</u>	-21		
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	0	! 16	. 2005 (31-56	)		
ample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	
185	Astyris gausapatal	1.0	9	0		
	30	0.9	2	0		

oumpic to	Castropod opecies	(cm)	JEA	mithosex stade	(uuu)	Comments
G18b	Astyris gaisapatal	1.0	9	0		
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2005 Gastropod Imposex Study July 28, 2005 Page 24

Wind Ward

Date:

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates 16 AUG. 2005

Project no. 04-08-06-21

Analyst: A.J.KOHN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
GIRR	Ant . a cz	5 6	ব			an a
6186-	ASTYVA GAUSAPATA ST	0,7	0	~		
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General Comments:	

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2005 Gastropod Imposex Study July 28, 2005 Page 24

Wind Ward

Project Name: Lower Duwarnish Waterway RI- Benthic Invertebrates 8/11/2005

Project no. 04-08-06-21

Date:

Analyst: A.J. KOHN, D.K. OKAMOTO

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G18b	Nassarius mendicus !	1.5	4	2	2.5	
	2	1.4	\$	<b></b>	3.7-	
	3	1.45	07	<del>.</del>	10.0	
	4	1.4	8	. <u> </u>	9.1	
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2005 Gastropod Imposex Study July 28, 2005 Page 24

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Date:

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Sample ID	Gas	stropod S	pecies	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
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			7	1.0	on			
			8	1.0	9	0		
			9	1.7	₽	0		
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			11	0.9	5	0		
	L.		12	1.0	\$	0		
			13	(.0	3			
			14	1.0	9	0		
			15	1.0	Ŷ	0		
			16	0,9	1	0		
			17	1.0	5	0		
			18	1.0	07			
			19	1.0	67	•==		
			20	1.0	07			
	-		21	1.0	P F	0		
			22	1.1	9	0		
			23	1.0	12	0		
			24	1.0	3			
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General Comments:

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Lower Duwamish Waterway Group

2005 Gastropod Imposex Study July 28, 2005 Page 24

Wind Ward

Project no. \_04-08-06-21

Project Name:	Lower Duwamish Waterway R	I- Benthic Invertebrates
Date:	10 AUG. 2005.	Analyzed 12

Analyzed 12 Ave. 2005 (1-30) Analyst H.J. KORIN

Sample IDGastropod SpeciesShell Height (cm)SexImposex StagePenis Length (mm)Comments $G 19 h$ As tyris $ga 0 sapata7a 03  aaaaa  aaaa  aaaa  aaaaa aaaaa aaaaa aa$
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1292

General Comments:

# Lower Duwamish Waterway Group

2005 Gastropod Imposex Study July 28, 2005 FINAL Page 24

Wind Ward

Date:

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates 8/11/2005

Project no. 04-08-06-21

Analyst: A.J. John

Sample ID	Gastropod Species		Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
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General Comments:



2005 Gastropod Imposex Study July 28, 2005 Page 24

FINAL

Wind Ward

Project Name: Lower Duwarnish Waterway RI- Benthic Invertebrates

Date:

11 AUG. 2005. Anslyzed 12 AUG. 2005 Analyst: A. V. KONN

Project no. 04-08-06-21

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
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	Turbon; ((a sp.(d)) (white	) )				119 specimens
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	12	1.1	07		-	
	13	0.95	07	<u> </u>		
	14	1.0	07	••••••		
	15	1.05	07	-		
	16	09	07			
	17	0.5	07			
	18	0.8	07			
	19	0.8	0-7			
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	23	1.0	07	·····		
	24	0.9	3			

#### General Comments:

Lower Duwamish Waterway Group

2005 Gastropod Imposex Study July 28, 2005 Page 24

FINAL

Wind Ward

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date:

\_11 Aug. 2005

Analyst: A.J. Kome

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General Comments:

# Lower Duwamish Waterway Group

2005 Gastropod Imposex Study July 28, 2005 Page 24

FINAL

Wind Ward

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates Date:

Aug. 10,2005

Project no. 04-08-06-21

Analyst: A.J. KOHN, D.K. OKAMOTO

				Shell Height	na mana dalaman		Penis Length	
Sample ID	Gast	ropod Sp	ecies	(cm)	Sex	Imposex Stage	(mm)	Comments
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General Comments:

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2005 Gastropod Imposex Study July 28, 2005 Page 24