

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

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 27th 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 specimen 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 imposex, 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.

Penis length - units @ 25x



GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: Aug. 9, 2005

Analyst: A.J. KORN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G166	<i>Clivella baltica</i> 1	1.0	♂	—	20 0.8	no visible vas deferens
"	" " 2	1.0	♀	0	—	
"	" " 3	0.9	♀	0	—	
"	" " 4	0.9	♂	—	1.0	
"	" " 5	0.9	♀	— 0	⊖	
"	" " 6	0.9	♀	— 0	⊖	
"	" " 7	0.8	♀	— 0	⊖	
"	" " 8	0.8	♀	— 0	⊖	
"	" " 9	0.6	♀	— 0	⊖	? may be immature
"	" " 10	0.9	♀	0		
"	" " 11	0.9	♀	—	0.5	yellow gonad
"	" " 12	0.9	♀	— 0	⊖	
"	" " 13	0.8	♀	— 0	⊖	
"	" " 14	0.9	♀	— 0	⊖	
"	" " 15	0.9	♀	— 0	⊖	
"	" " 16	0.8	♀	— 0	⊖	
"	" " 17	0.9	♀	— 0	⊖	
"	" " 18	0.6	?	—	—	(immature?) No penis, no gonad.
"	" " 19	0.8	♀	— 0	⊖	
"	" " 20	0.9	♀	⊖ 0	⊖	
"	" " 21	0.8	♀	0	⊖	
"	" " 22	0.7	♀	0	⊖	
"	" " 23	0.9	♀	0	⊖	prominent oviduct immature - no gonad no penis.
"	" " 24	0.6	?	—	⊖	
"	" " 25	0.7	♂	—	1.0	
"	" " 26	0.7	?	—	—	no gonad, no penis. immature. large clam in stomach!
"	" " 27	0.5	?	—		immature. from other gastropod

General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date:

Aug. 9, 2005

Analyst:

A. J. KOHN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G16b	<i>Astya's grausapota</i>	1.0	♂	—	10	
"	"	2	♂	—		
"	"	3	♀	0	—	
"	"	4	♂	—		
"	"	5	♀	0	—	
"	"	6	♂	—	6.6	
"	"	7	♂	—		
"	"	8	♂	—	5.8	
"	"	9	♂	—		
"	"	10	♀	0	—	
"	"	11	♀	0	—	
"	"	12	♂	—		
"	"	13	♀	0		
"	"	14	—	—	—	empty shell
"	"	15	♀	0	—	
"	"	16	♀	0	—	
"	"	17	♀	0	—	
"	"	18	♂	—		
"	"	19	♂			
"	"	20	♀			
"	"	21	♂	—		
"	"	22	♀	0	—	
"	"	23	♂	—		
"	"	24	♀			
"	"	25	♂	—		
"	"	26	♀	0	—	
"	"	27	♂	—		
"	"	28	—	—	—	empty shell

General Comments:



GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates Project no. 04-08-06-21

Date: Aug. 9, 2005 Analyst: A. J. KOHN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G16B	<i>Astytis gausapatana</i>					
		29	♀	0	—	
		30	♀	0	—	
		31	♂	—		
		32	♀	—		
		33	♂	—		
		34	♀	0		
		35	♂	—		
		36	♀	0		
		37	♀	0		
		38	—	—	—	empty shell
		39	—	—	—	empty shell
		40	♀	0	—	
		41	♀	0		
		42	♀♂	—		
		43	—	—	—	empty shell
		44	♂	—		
		45	♂	—		
		46	♂	—		
		47	♂			
		48	♂			
		49	♀	0	—	
		50	♀	0	—	
		51	♂	—	—	
		52	♀	0		
		53	♀	0	—	
		54	♂	—	—	
		55	♀?	0	—	no gonad? (mashed)

General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: Aug. 10, 2005

Analyst: A. J. KOHN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
617g	<i>Nassarius mendicis</i>	1.3	♀	2	3.3	No evidence of vas deferens
	1.6	♂	—	12.5	
	1.5	♂	—	11.7	
	<i>Astytis gausapatā</i>	1.0	♂	—		
		2 0.9	♀	0	—	
		3 1.0	♀	0	—	
		4 1.0	♂	1		
		5 1.0	♂	1		
		6 1.0	♀	0	—	
		7 1.0	♀	0	—	
		8 0.9	♀	0	—	
		9 1.0	♂	1		
		10 0.9	♀	0	—	
		11 0.9	♂	1		
		12 0.9	♀	0	—	
		13 0.9	♂	1		
		14 0.8	♀	0	—	
		15 0.7	♀	0	—	
		16 0.6	♂	1		

General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: 10 Aug. 2005 Analyzed 12 Aug. 2005

Analyst: A. J. Kolen

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G186	<i>Astyris gausapatai</i>	0.9	♂	1		
		2 1.0	♀	0		
		3 1.0	♀	0		
		4 1.0	♂	1		
		5 1.0	♀	0		
		6 0.9	♂	1		
		7 0.9	♂	1		
		8 0.9	♂	1		
		9 0.95	♀	0		
		10 1.0	♂	1		
		11 0.8	♂	1		
		12 0.9	♀	0		
		13 1.1	♀	0		
		14 1.0	♀	0		
		15 1.1	♀	0		
		16 1.1	♂	1		
		17 1.0	♂	1		
		18 1.0	♀	0		
		19 0.9	♂	1		
		20 0.9	♂	1		
		21 1.0	♀	0		
		22 1.0	♀	0		
		23 1.0	♂	1		
		24 1.0	♂	1		
		25 0.9	♂	1		
		26 0.9	♂	1		
		27 0.8	♂	1		
		28 0.9	♀	0		

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General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date:

10 Aug 2005. Analyzed 12 Aug. 2005 (#30) Analyst: A.J. Kohn
16 Aug. 2005 (31-56)

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G18b	<i>Astyrus gausapatii</i>	1.0	♀	0	—	
		0.9	♀	0	—	
		1.0	♀	0	—	
		1.0	♂	—	—	
		1.0	♀	0	—	
		0.95	♂	1	—	
		0.95	♀	0	—	
		0.8	♀	0	—	
		1.0	♂	1	—	
		0.85	♀	0	—	
		0.6	♂	1	—	
		0.9	♂	1	—	
		0.9	♂	1	—	
		1.0	♂	0	—	
		0.9	♂	1	—	
		0.9	♀	0	—	
		0.8	♂	1	—	
		1.0	♀	0	—	
		0.9	♀	0	—	
		0.8	♀	0	—	
		0.9	♀	0	—	
		0.8	♂	1	—	
		0.9	♀	0	—	
		1.0	♀	0	—	
		0.9	♀	0	—	
		0.9	♂	1	—	
		0.8	♂	1	—	
		1.0	♀	0	—	

General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: 8/11/2005

Analyst: A.S. KOHN, D.K. OYAMOTO

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G18b	<i>Nassarius mendicus</i> 1	1.5	♀	2	2.5	
	" " 2	1.4	♀	2	3.7	
	" " 3	1.45	♂	—	10.0	
	" " 4	1.4	♂	—	9.1	
"	<i>Polinices</i> sp.	0.7	♂		broken	} All <i>Polinices</i> sp. are juveniles with no gonad. ∴ immature
"	"	0.7	♂		1.0	
"	"	0.7	♂		2.9	
"	<i>Lacuna vineta</i>	0.9	♀	0	—	
	" "	0.5	—			not examined
"	<i>Polinices</i> sp.	0.7	♀	0		

General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: 10 Aug. 2005; Analyzed 12 Aug. 2005

Analyst: A.V. KENN

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G196	<i>Astyris gausapata</i>	1.0	♀	0		
		2 1.0	♂	1		
		3 1.0	♀	0		
		4 1.1	♂	1	6.4	
		5 1.0	♀	0		
		6 1.0	♂	1		
		7 1.0	♂	1		
		8 1.0	♀	0		
		9 1.1	♀	0		
		10 1.0	♀	0		
		11 0.9	♀	0		
		12 1.0	♀	0		
		13 1.0	♂	1		
		14 1.0	♀	0		
		15 1.0	♀	0		
		16 0.9	♀	0		
		17 1.0	♀	0		
		18 1.0	♂	1		
		19 1.0	♂	1		
		20 1.0	♂	1		
		21 1.0	♀	0		
		22 1.1	♀	0		
		23 1.0	♀	0		
		24 1.0	♂	1		
		25 .95	♂	1		
		26 1.05	♀	0		
		27 1.0	♀	0		
		28 1.0	♀	0		

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General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: 10 AUG. 2005. Analyzed 12 Aug. 2005 (1-30) Analyst: A. J. KORN

.. 16 AUG. 2005 (31-49)

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G196	<i>Astytis gausapata</i> ²⁵	1.0	♂	—		
"	"	1.0	♀	0		
	"	1.1	♂	—		
	"	1.0	♂	—		
	"	1.0	♀	0	—	
	"	1.0	♀	0	—	
	"	1.0	♂	—		
	"	1.0	♂	—		
	"	1.0	♂	—		
	"	1.0	♀	0	—	
	"	0.9	♂	—		
	"	1.0	♂	—		
	"	0.9	♀	0	—	
	"	1.0	♀	0	—	
	"	0.9	♀	0	—	
	"	1.0	♀	0	—	
	"	1.0	♀	0	—	
	"	1.0	♀	0	—	
	"	0.9	♀	0		
	"	0.8	♂	—		
	"	1.0	♀	0	—	

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General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI-Benthic Invertebrates

Project no. 04-08-06-21

Date: 11 Aug. 2005. Analyzed 12 Aug. 2005

Analyst: A. J. Kohn

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G20 b	<i>Turbonilla</i> sp. (red)		-			1 specimen
	<i>Turbonilla</i> sp. (white)		-			119 specimens
	<i>Potamococcus</i> sp.	0.8	♀?	0		No gonad; immature
	<i>Nassarius meridicus</i>	1.6	♂	-	12.5	
	<i>Astyris gausapata</i>	1.1	♂	-		
		2	1.0	♀	0	
		3	0.7	♂	-	
		4	1.1	♀	0	
		5	1.1	♀	0	
		6	1.0	♀	0	
		7	1.0	♂	-	
		8	0.95	♂	-	
		9	0.95	♀	0	
		10	1.0	♂	-	
		11	1.05	♂	-	
		12	1.1	♂	-	
		13	0.95	♂	-	
		14	1.0	♂	-	
		15	1.05	♂	-	
		16	0.9	♂	-	
		17	0.5	♂	-	
		18	0.8	♂	-	
		19	0.8	♂	-	
		20	0.85	♀	0	
		21	0.9	♂	-	
		22	1.0	♀	0	
		23	1.0	♂	-	
		24	0.9	♂	-	

General Comments:

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates

Project no. 04-08-06-21

Date: 11 Aug. 2005

Analyst: A.J. Kamp

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments
G 2016	<i>Astytis gausapa</i> Ta 25	0.9	♂	—		
cont.		26 0.7	♂	—		
		27 0.7	♀	0		
		28 0.9	♂	—		
		29 0.8	♀	0		
		30 0.6	♂	—		
		31 0.8	♂	—		
		32 0.9	♂	—		
		33 0.7	♀	0		
		34 0.7	♀	0		
		35 0.5	♀	0		
		36 0.6	♀	0		
		37 0.7	♀	0		
		38 0.6	♀	0		
		0.3	—	—		not examined (with other gastropods)

General Comments:

