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Imposex in Carnivorous Marine  
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## ABSTRACT

The phenomenon of imposex, the growth of male sex organs in female snails, has been observed only in the past twenty years. The cause of the growth of a penis and a vas deferens has been determined to be continued exposure to tributyltin in the water in which the snails live. The neogastropods used in this research are whelks of the type *Searlesia dira* and *Nucella lamellosa*. This paper examined imposex in and around Vancouver Island, British Columbia, Canada, and of the thirty-four sites tested, only three locations showed no evidence of imposex. All sites tested in high boat traffic areas (within one kilometer of a harbor or marina) or sites near centers of human population showed a one hundred percent incidence of imposex. Even the high current area of the Race Rocks Ecological Reserve was contaminated by TBT since 100% of the snails were affected there as well. It can be seen from this evidence that all of the intercoastal water of the Strait of Georgia, Strait of Juan de Fuca, and Puget Sound are affected by TBT and that actions must be taken to halt the spread of this contamination.

# TABLE OF CONTENTS

ABSTRACT	1
INTRODUCTION	2
The Problem	2
Purpose of the Study	2
Hypotheses	3
Assumptions	3
Definition of Terms	4
REVIEW OF LITERATURE AND RELATED RESEARCH	5
Previous Studies of Imposex	5
Table #1 (Ellis and Saavedra Alvarez Study, 1987-1989)	7
Figure #1 (Map of locations studied)	8
Neogastropods	9
RESEARCH DESIGN AND PROCEDURES	10
Method	10
Method of Analysis	11
ANALYSIS OF DATA	13
Results	13
Analysis of Data	14
CONCLUSIONS, IMPLICATIONS, RECOMMENDATIONS	17
Conclusions	17
Implications	17
Suggestions for Future Study	18
REFERENCES CITED	20

# INTRODUCTION

## THE PROBLEM

Imposex was a fairly rare phenomenon until the development of antifouling paints containing tributyltin. Imposex, male sex characteristics imposed upon the female, was first observed in carnivorous marine snails in 1970 in Plymouth Sound, Britain. Since then, it has been observed worldwide in all marine environments with a high incidence of boat traffic. It was first observed in British Columbia in 1987. It has been evidenced that the cause of such pseudohermaphroditism (the growth of a penis and a vas deferens) is induced by continued exposure of organotin compounds generally found in antifouling paint. Imposex in whelks is a biological indicator of TBT contamination, and as mariculture is important in British Columbia, an accurate bioindicator is important and must be implemented. This paper intends to examine imposex in various carnivorous whelks at locations in the Strait of Juan de Fuca, both from data gathered by the author and by previously researched data.

## PURPOSE OF THE STUDY

The purpose of this extended essay is to determine whether Race Rocks Ecological Reserve, at the east end of the Strait of Juan de Fuca, is as contaminated by TBT as other areas around Vancouver Island as indicated by the presence of imposex in carnivorous snails.

## HYPOTHESES

For statistical purposes, all hypotheses are presented in null form.

1. There will be no difference in percent of snails exhibiting the phenomenon of imposex from one species to another at the same location.
2. There will be no difference in percent of snails exhibiting the phenomenon of imposex in one species at 34 different locations around Vancouver Island.
3. The Race Rocks Ecological Reserve will be uncontaminated by TBT and therefore there will be no incidence of imposex in the snails living there.

## ASSUMPTIONS

It is hoped that these assumptions are true. Nevertheless, it is important that they must be stated.

1. The method of sampling was accurate and complete.
2. The number of samples is sufficient for statistical analysis.
3. The researcher was unbiased.

## DEFINITION OF TERMS

imposex - pseudohermaphroditism; male genitalia appearing in females, mainly characterized by the formation of a penis (often deformed and smaller than that of males) and a vas deferens.

tributyltin - TBT; a contaminant found in boat and ship antifouling paint. Demonstrated to cause imposex in neogastropods.

*Nucella lamellosa* - a neogastropod commonly found on the coast of British Columbia. Common name Wrinkled Purple Frilled Dogwinkle.

Phylum Mollusca / Class Gastropoda / Subclass Prosobranchia / Order Neogastropoda / Superfamily Muricacea / Family Thaisidae

*Searlesia dira* - a neogastropod commonly found on the coast of British Columbia. Common name Dire Whelk. Phylum Mollusca / Class Gastropoda / Subclass Prosobranchia / Order Neogastropoda / Superfamily Buccinacea / Family Buccinadae

high boat traffic areas - areas less than one kilometer away from a marina or other harbor

moderate / low boat traffic areas - areas more than one kilometer away from a marina or other harbor

# REVIEW OF LITERATURE AND RELATED RESEARCH

## PREVIOUS STUDIES OF IMPOSEX

In 1980, a phenomenon was observed in the American mud snail, *Nassarius obsoletus*, in which female snails, which were not usually hermaphroditic, were growing a penis and a vas deferens. This pseudohermaphroditism was not proven to decrease reproductive capability, nor reduce population of the species. Smith (1980) found strong evidence that this was induced by exposure to TBT, leaching from antifouling paints<sup>1</sup>.

Imposex was observed in 1970 by Blaber, who observed the decrease of the population of dog-whelks, *Nucella lapillus*, in Plymouth Sound, Britain<sup>2</sup>. Since then, it has also been concluded that exposure to TBT is the cause, and that, unlike *N. obsoletus*, *N. lapillus* reproductive capabilities are affected by imposex. By 1986, the population had significantly decreased, and both the incidence and intensity of imposex had greatly increased in high boat traffic areas<sup>3</sup>.

In 1986, Bryan divided imposex in *N. lapillus* into three stages, "early",

1

Smith, B.S. (1980) "Tributyltin compounds induce male characteristics on female mud snails *Nassarius obsoletus* = *lyanassa obsoleta*," Journal of Applied Toxicology, 1, 141-144.

2

Blaber, S.J.M. (1970) "The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus*" Proceedings of the Malacological Society of London, 39, 231-233.

3

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., and Burt G.R. (1986) "The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin from antifouling paints." Journal of the Marine Biological Association of the United Kingdom, 66, 611-640.

"intermediate", and "late". The early stage is characterized by the formation of a small penis and a vas deferens. The vas deferens is formed in two phases, and the second phase blocks the genital papilla surrounding the female opening. The penis begins as a protuberance behind the right tentacle, which is the same location as in males. By the intermediate stage, the vas deferens is in its second phase of growth and the penis is approaching the size of that of a normal male. The late phase causes complete sterility, and is often lethal, because of the massive growth and subsequent rupture of the vas deferens. The populations of *N. lapillus* have been observed to have a majority of males, which is accounted for by the premature death of females caused by late stage imposex.<sup>4</sup>

In other species, imposex is not always fatal, but still is very common. As reported in 1990, by Ellis, imposex occurs in 9 out of 10 shoreline species of whelks on the Pacific coast of Canada, and in a further 4 out of 4 in South East Asia. A total of 45 species (34 identified by Jenner in 1979, plus the eleven new ones found by Ellis) worldwide has been shown to have a significant frequency of imposex.<sup>5</sup>

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4

Bryan, G.W. et. al. (1986) pp. 631-640.

5

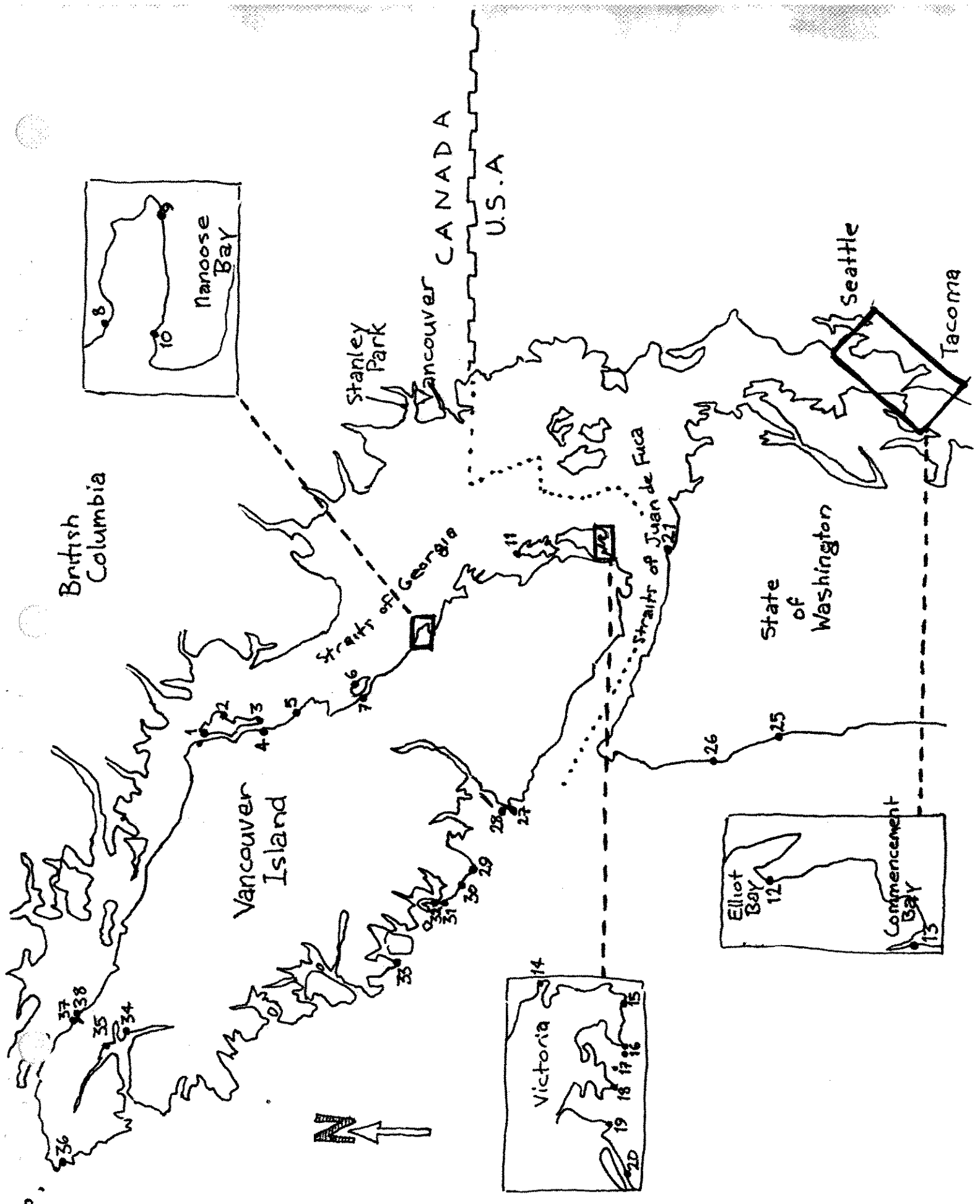
Ellis, D.V. and Pattina, L.A. (1990)



TABLE #1

## ELLIS &amp; SAAVEDRA ALVAREZ STUDY OF NEOGASTROPOD IMPOSEX 1987-89

LOCATION	BOAT TRAFFIC	IMPOSEX FREQUENCY	
<u>Strait of Georgia</u>			
Quadra Island			
1. Granite Bay	mod/low	<u>S. dira</u>	50%
2. Bold Point	mod/low	<u>N. lamellosa</u>	100%
3. Dogfish Bay	mod/low	<u>S. dira</u>	62.5%
4. Campbell River	high	<u>N. lamellosa</u>	100%
		<u>S. dira</u>	100%
5. Miracle Beach	mod/low	<u>N. lamellosa</u>	33%
Denman Island			
6. Filongley P.P.	mod/low	<u>N. lamellosa</u>	60%
7. Ferry Terminal	high	<u>N. lamellosa</u>	100%
Nanoose Bay			
8. Dolphin Beach	high	<u>N. lamellosa</u>	100%
9. Richard Pt.	high	<u>N. lamellosa</u>	100%
10. Head of bay	high	<u>N. lamellosa</u>	100%
11. Saltspring Isl.	mod/low	<u>N. lamellosa</u>	100%
		<u>S. dira</u>	85%
<u>Puget Sound</u>			
12. Seattle	high	<u>N. lamellosa</u>	100%
13. Tacoma	high	<u>N. lamellosa</u>	100%
<u>Juan de Fuca Strait</u>			
Victoria			
14. Ten Mile Pt.	high	<u>N. lamellosa</u>	100%
15. Clover Pt.	high	<u>N. lamellosa</u>	100%
16. Breakwater	high	<u>N. lamellosa</u>	100%
17. Harrison Isl.	high	<u>N. lamellosa</u>	100%
18. Saxe Pt. Park	high	<u>N. lamellosa</u>	100%
		<u>S. dira</u>	100%
19. Ft. Rodd Hill Pk.	high	<u>S. dira</u>	100%
20. Esquimalt Lag.	high	<u>N. lamellosa</u>	100%
Western Washington Coast			
21. Port Angeles	high	<u>N. lamellosa</u>	100%
25. Ruby Beach	mod/low	<u>N. lamellosa</u>	32%
26. La Push	mod/low	<u>N. lamellosa</u>	36%
<u>West Vancouver Island</u>			
Bamfield			
27. Grappler Inlet	high	<u>S. dira</u>	100%
29. Ucluelet Beach	mod/low	<u>S. dira</u>	43%
30. Wichaninnish	mod/low	<u>N. lamellosa</u>	0%
Tofino			
31. Chesterman Bch.	mod/low	<u>N. lamellosa</u>	100%
33. Hot Springs Cv.	mod/low	<u>N. lamellosa</u>	56%
34. Quatsino Narrows	mod/low	<u>S. dira</u>	0%
35. Coal Harbour	high	<u>N. lamellosa</u>	100%
36. St. Josef Bay	mod/low	<u>N. lamellosa</u>	0%
Port Hardy			
37. Indian reserve	high	<u>N. lamellosa</u>	100%
		<u>S. dira</u>	100%
38. Ferry terminal	high	<u>N. lamellosa</u>	100%
		<u>S. dira</u>	100%



British Columbia

CANADA  
U.S.A

State of Washington

Seattle

Tacoma

Stanley Park

Vancouver

Vancouver Island

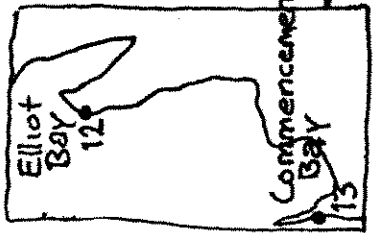
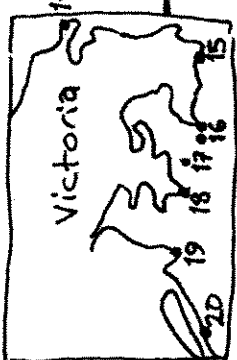
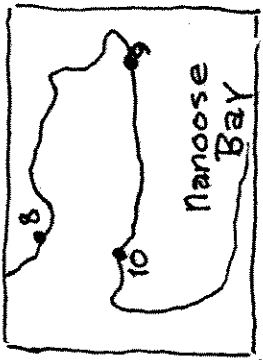
Strait of Georgia

Strait of Juan de Fuca

Victoria

Elliot Bay

Commencement Bay



## NEOGASTROPODS

The snails used in this study are shelled gastropods of the phylum Mollusca. Gastropods have a foot made mostly of muscle for locomotion and a tongue-like radula for scraping and burrowing into food, and tentacles which are sensory organs. The shell is secreted by the mantle, which provides the shelter for most of the organs. The subclass Prosobranchia (gills forward) is so called because they exhibit "torsion", that is, a complete rotation of the location of the visceral mass on the body so the mantle cavity, anus, and respiratory organs which were originally at the posterior of the animal, come to lie next to the head. In the order Neogastropoda (sometimes called Pectiniranchia [Stenoglossa]) have single respiratory organs, and a narrow radula. They possess a long siphon and proboscis, and an osphradium, which is a sensory organ aiding in the location of prey. Neogastropods are carnivorous, with a usual diet of macroscopic species such as limpets, chitons, barnacles, and other snails. They are usually located in the intertidal zone and are abundant along many shorelines.<sup>6</sup>

# RESEARCH DESIGN AND PROCEDURES

## METHOD

Snails were collected from both above and below the tidal level. Hand collection by walking along the coast and SCUBA diving for underwater specimens comprise the method of collection. Because of the relative scarcity of some species, few samples were found.

I went to the University of Victoria, to the lab of Dr. Ellis, where he and his colleague Ms. Saavedra Alvarez instructed me in the proper method of determining imposex frequency. They demonstrated the imposex measurement protocol, designed by Ellis and Pattsina. In the lab, samples were measured for length and width with calipers. Using a bench vise, the shell was cracked and peeled away. The specimen was placed in an alcohol solution to kill and preserve it. Then, its sex was determined. Female snails were identified by the presence of bright orange capsule and sperm ingesting glands. Male snails were seen to exhibit a much darker, browner gland. The penis in the males was large (half the length of the body) and easy to find. The penis in females exhibiting imposex was in the same location and that of the males, under the mantle, close to and behind the right tentacle. It was very small in comparison the male's and often deformed. (See Figure #1) The length of the penis was recorded, using a stereoscope and a millimeter scale.

Only adult snails were evaluated. Adults are snails that have stopped growing, and can be identified by the formation of "teeth" inside the lip of the shell. Only snails with no signs of visible parasites or deformation were evaluated.

Once enough data were gathered, percentage frequency of imposex was determined. Also, relative penis size was computed, in accordance with the protocol. RPS is determined to standardize against different average sizes of snails in different populations and differences in population penis sizes.

$$\text{RPS} = \frac{(\text{mean female penis length})^3}{(\text{mean male penis length})^3} \times 100$$

There are two types of RPS: Population RPS and Incidence RPS. Population RPS is calculated using all females in the population, regardless of occurrence of imposex. Incidence RPS uses only affected females, which, unless 100% frequency occurs, raises the RPS. The three indices: frequency, population RPS, and incidence RPS provide levels of sensitivity suitable for all environments.<sup>7</sup>

## METHOD OF ANALYSIS

The evaluation for hypothesis #1 is a chi-square test. This is to determine whether any difference in percent of species exhibiting imposex at the same location was significant. It must be remembered that due to the small sample size, considerable deviations from the expected results are not necessarily significant. The formula for chi-square is

$$\chi^2 = \sum_{i=1}^c \frac{(o_i - e_i)^2}{e_i}$$

where  $e_i$  = number expected

$o_i$  = number observed

$c$  = number of classes

The analysis for the second hypothesis involves mapping all locations tested and determining the frequency of imposex in each species separately according to its distance from populations centers and high boat traffic areas.

The third hypothesis involved considering all factors which influence the frequency of imposex and to see if the unique location of Race Rocks had any effect on these factors, and therefore the imposex frequency.

## ANALYSIS OF DATA

### RESULTS

One hundred percent of female whelks I examined exhibited the phenomenon of imposex. Table #2 documents the frequency of *Searlesia dira* and *Nucella lamellosa* at the Race Rocks Ecological Reserve, in the Strait of Juan de Fuca. (See Figure #1)

TABLE #2

Dire Whelk	<i>Searlesia dira</i>		location: Race Rocks			
sample number	shell length	shell width	penis length	M/F	imposex?	
1	37	14	9.5	M	---	
2	36	14	10	M	---	
3	35	15	3	F	yes	
4	38	16	2	F	yes	
5	41	17	12	M	---	
6	34	16	2	F	yes	
7	37	17	3	F	yes	

Wrinkled Purple Frilled Dogwinkle	<i>Nucella lamellosa</i>		location: Race Rocks			
sample number	shell length	shell width	penis length	M/F	imposex?	
1	37	15	2	F	yes	
2	34	14	9	M	---	
3	39	14	10	M	---	
4	40	17	3	F	yes	
5	37	14	2.5	F	yes	
6	41	16	9	M	---	
7	38	15	2	F	yes	
8	38	14	2	F	yes	
9	35	14	10	M	---	
10	37	15	2	F	yes	
11	40	17	9	M	---	
12	34	16	8	M	---	
13	40	16	3	F	yes	
14	38	16	10	M	---	
15	40	16	8	M	---	
16	34	14	9	M	---	
17	35	15	2	F	yes	
18	39	16	3	F	yes	
19	37	15	2	F	yes	
20	38	17	3	F	yes	

## ANALYSIS OF DATA

Specific techniques were planned to analyse each hypothesis. However, because all the data obtained for the location tested was 100% frequency of imposex, all statistical techniques become moot; it is unnecessary and useless to compute significant deviation since all figures are identical.

### HYPOTHESIS #1

There will be no difference in percent of snails exhibiting the phenomenon of imposex from one species to another at the same location.

It can be seen from Table #? that at every location (including Race Rocks) where two different species were tested at the same location, the imposex frequency is identical, and is in fact 100%. Therefore the null hypothesis is confirmed: there is no difference in imposex frequency of different species at the same location.

This confirms the evidence that imposex is definitely caused by foreign substance in the water of the habitat of the snails. If two species at the same location had different imposex frequencies, it could mean two things. Either the species are affected differently by the factor which causes imposex, or the factor which causes imposex is something other than a foreign substance in the water. However, since both species at all locations have the same frequency of imposex, it can be seen that the factors causing imposex affect both species in the same manner.

### HYPOTHESIS #2

There will be no difference in percent of snails exhibiting the phenomenon of imposex in one species at 34 different locations around Vancouver Island.



This hypothesis can be analysed in summary by examining Table #1 and Figure #1 simultaneously. All snails living in high boat traffic and near population centers displayed imposex. This includes location numbers 4, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 27, 35, 37 and 38. Some locations which were neither near population centers or in high boat traffic areas still displayed a 100% rate of imposex: 2, 11, and 31. Some locations in moderate to low boat traffic displayed a significant amount of snails with imposex : 1, 3, 5, 6, 11, 25, 26, 29 and 33. Only three locations tested in British Columbia had no evidence of imposex : 30, 34, and 36. Such figures are self-explanatory; the small amount of unaffected snails is obvious. The fact that snails which live in moderate to low boat traffic areas and away from dense human population are still affected is also significant. (E.g. Quadra Island.) It can be seen that there is a difference in the percentage of snails exhibiting imposex at the 34 sites tested, so that Hypothesis #2 has been refuted. This makes sense because the amount of TBT in the water varies greatly from location to location, and this variation accounts for the variation in imposex frequency.

### HYPOTHESIS #3

The Race Rocks Ecological Reserve will be uncontaminated by TBT and therefore there will be no incidence of imposex in the snails living there.

Every snail tested at Race Rocks demonstrated the phenomenon of imposex. It had been hoped that because of the unique situation of Race Rocks, the snails would be unaffected. Race Rocks is in a high current zone (hence the name "Race"). Every day flood tides bring nutrient-rich upwelling water from the Pacific Ocean, and the ebb tides carry water from the Strait of Georgia and water from the interior of British Columbia. It seems that even this transition

area between the inner coastal waters and the open ocean has been contaminated by TBT. It seems likely that the TBT is carried to Race Rocks on the ebb tide, from the higher boat traffic inner coastal areas.

# CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS

## CONCLUSIONS

One hundred percent of neogastropods examined at the Race Rocks Ecological Reserve exhibited the phenomenon of imposex. Because the location is in a high boat traffic area, this was to be expected. Because the ebb tides bring water from coastal rivers and tidal marshes as well as the Strait of Georgia and Puget Sound, and flood tides bring nutrient-rich water from the Pacific Ocean, the area is exposed to water from many sources which could easily be TBT contaminated. Even areas which have moderate to low boat traffic and are far from human population centers still have snails which are affected by imposex and therefore have high TBT contamination. The results obtained for this extended essay are also being used by Dr. Ellis in his continuing research on imposex, and will be added to the data file on the Race Rocks Ecological Reserve.

## IMPLICATIONS

45 species worldwide display a significant frequency of imposex. Since it has been proven that TBT is the cause of this, it is seen that TBT contamination is an environmental problem of global proportions. The contamination is present primarily in high boat traffic areas; however, the bioindicator snails show that in moderate to low traffic areas, high amounts of TBT are still present. (E.g. Saltspring Island, Nanoose Bay, Strait of Georgia.)

Neogastropods have proved to be biological indicators for the presence of TBT contamination. Other organisms are affected by this contamination as well. Oysters and other molluscs which have economic as well as ecological significance are known to be damaged by TBT. Even farmed salmon can bioaccumulate TBT.

This is of direct concern to humans, because "it is undesirable that a toxin causing growth and reproductive deformities should enter human foods."

If organotin contamination is the sole cause of imposex in snails, as it seems to be, action must be taken immediately to prevent increase in the leaching of TBT from antifouling paints into the ocean. The existing control procedures which allow these paints on small boats do not seem to be working effectively. New laws must be made and enforced which control the sale and use of these paints, and a viable alternative to TBT based painted must be found. If there are other causes of imposex, these should be investigated and controlled as soon as possible.

### SUGGESTIONS FOR FURTHER STUDY

Imposex is a phenomenon that has so far only been observed in neogastropods. These species could be studied on a much larger scale in locations already given a cursory examination to find if any high boat traffic areas have unaffected snails, and if there are any, examine why these have not been affected.

The same species of snails also could be researched at different times of the year to see if there is seasonal variation of imposex presence, and if there is, determine why this occurs.

The populations of species already examined could be researched over a long period of time to determine if imposex is lethal over larger spans of time. Even if the phenomenon does not raise the mortality rate, the population size may be affected because the females are not able to reproduce.

Other snails could be examined to determine if imposex occurs in any other gastropods, such as non-carnivorous snails occupying a similar ecological niche.

Other biological indicators could be researched at the same locations to confirm that TBT is the sole cause of imposex. These studies would further knowledge about bioaccumulation as well as effects of TBT on other animals.

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