

# Long-Term Changes in Imposex Frequency in File Dogwinkles, *Nucella lima* G., and Tributyltin Concentrations in Bay Mussels, *Mytilus trossulus* G.

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**Abstract** We quantified imposex in file dogwinkles (*Nucella lima*) and tributyltin (TBT) contamination in bay mussels (*Mytilus trossulus*) from a number of sites in Auke Bay, Alaska, previously studied in the late 1980s. Imposex occurrence and TBT contamination have generally declined in spatial extent and severity over time. However, high levels of TBT contamination (0.069 µg TBT/g wet tissue wt.) and imposex (100% of females affected) are still present near a large boat docking facility in the bay and deserve continued attention because of the importance of this bay to the local economy and fisheries.

**Keywords** *Nucella lima* · *Mytilus trossulus* · Imposex · Tributyltin

Many marinas and ports throughout the world have been contaminated by tributyltin (TBT), a tin-containing antifouling compound used in boat paints to prevent hull colonization by invertebrates. TBT is an endocrine disruptor and has caused female prosobranch snails to develop male sexual characteristics, a condition known as imposex. Unfortunately, even extremely low concentrations of TBT can lead to imposex in prosobranch snails, and severe imposex can prevent females from releasing egg capsules, impairing recruitment and causing local population decline

(Bryan et al. 1986). TBT can also negatively impact marine bivalve aquaculture efforts (Alzieu et al. 1986).

Following a recent ban on TBT in many parts of the world, researchers have tracked changes in TBT concentrations and prosobranch snail imposex and demography. In some cases, TBT levels, imposex severity, and imposex frequency have declined and snail populations appear to have recovered (Evans et al. 1996; Gibson and Wilson 2003). However, TBT contamination and imposex continue to afflict snail populations near some major shipping lanes and ports frequented by large ships (>25 m) in many parts of the world (Gibson and Wilson 2003; Miller et al. 1999; Morton and Blackmore 2001; Reitsema et al. 2002; Smith 1996; Sousa et al. 2003; Stickle and Zhang 2003).

The first studies to demonstrate the link between TBT and imposex in the file dogwinkle (*Nucella lima*), and to document TBT impacts on file dogwinkle populations, were conducted two decades ago at our study sites in Auke Bay, Alaska (Short et al. 1989; Stickle et al. 1990). Experimental and observational studies showed that TBT induces imposex in this dogwinkle, and TBT levels in bay mussels (*Mytilus trossulus*) and imposex frequency in dogwinkles were positively correlated at sites throughout Auke Bay. Furthermore, imposex and TBT levels increased with proximity to Auke Bay Marina, and all female snails exhibited imposex at the four sites nearest Auke Bay Marina where they could be collected. Dogwinkles could not be found in the intertidal zone near the heavily contaminated harbor but were present in similar habitat throughout less contaminated portions of Auke Bay, suggesting that local extinction may have occurred near the harbor as a result of TBT contamination. In the last two decades, Auke Bay has become increasingly important to local fisheries, including local salmon charter businesses, newly created chinook (*Oncorhynchus tshawytscha*)

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aquaculture efforts, and a recently built delivery center for a local seafood company.

We quantified imposex in file dogwinkles and TBT contamination in bay mussels from sites where the relationships between these organisms and this contaminant were originally quantified and described. We re-sampled some of the sites studied 18 years before (Short et al. 1989) to determine whether TBT concentrations and imposex have been reduced in Auke Bay. We tested the hypotheses that the frequency of imposex in dogwinkles and TBT levels in bay mussels are now lower than in the past.

## Materials and Methods

Auke Bay is a small bay near Juneau, Alaska, that contains several islands, an extensive rocky intertidal zone, and two boat docking facilities (Fig. 1). Auke Bay Marina is primarily a small (<25 m), recreational boat docking facility. In contrast, Auke Nu Cove has docking facilities for large (>25 m), private vessels and Alaska state ferries.

In June and July, 2005, dogwinkles were collected from seven of the eight sites previously sampled by (Short et al. 1989; Fig. 1). The proportion of imposex-affected females and the Relative Penis Size index (RPS) for each sample was calculated following standard methods (Gibbs et al. 1987).

In fall 2006, we collected mussels from nine of the 11 sites previously studied by Short et al. (1989) (Fig. 1). Upon collection, mussels were rinsed with 18 MΩ water and kept frozen at −20°C for up to 6 weeks prior to TBT analysis.

Mussel tissues from each site were combined and homogenized. Three determinations of TBT in the homogenate from each site were made. The extraction and analysis of tissue was based on the method of Short and Sharp (1989). Approximately 15 g of pooled mussel homogenate from a given site was stirred with 40 mL of high purity, concentrated hydrochloric acid (Optima) for 3 h at room temperature. The digest was extracted with 2 × 20 mL portions of reagent grade hexanes (Baker), and the combined hexane extract washed with 2 × 20 mL of 3% NaOH(aq) (Chempure). The hexane extract was then evaporated to dryness under a stream of nitrogen at 25°C. The residue was dissolved in a solution of 0.1 mL concentrated nitric acid (Omnitrace) and 4.9 mL of glacial acetic acid (Seastar; double-distilled) and analyzed for total tin content on a Solaar M series (Thermo Electron) graphite furnace atomic absorption (GFAA) spectrometer with Zeeman background correction. 15 μL of sample was coinjected with 5 μL of matrix modifier solution (Pd/Mg(NO<sub>3</sub>)<sub>2</sub>) into pyrolyzed graphite tubes and the absorption measured at  $\lambda = 286.3$  nm. The argon purge gas flow

was replaced with 5% hydrogen/argon during the ashing step and stopped during the atomization stage. The heating conditions were as follows: dry, 100°C for 10 s, 120°C for 30 s; ash, 800°C for 20 s; atomization, 2,300°C for 3 s; and cleaning, 2,500°C for 3 s. Peak height comparison to a series of standard solutions along with a correction for percent recovery was used for signal quantification. The sample detection limit (mean value of the reagent blank plus five times the standard deviation) was 0.01 μg Sn/g wet tissue.

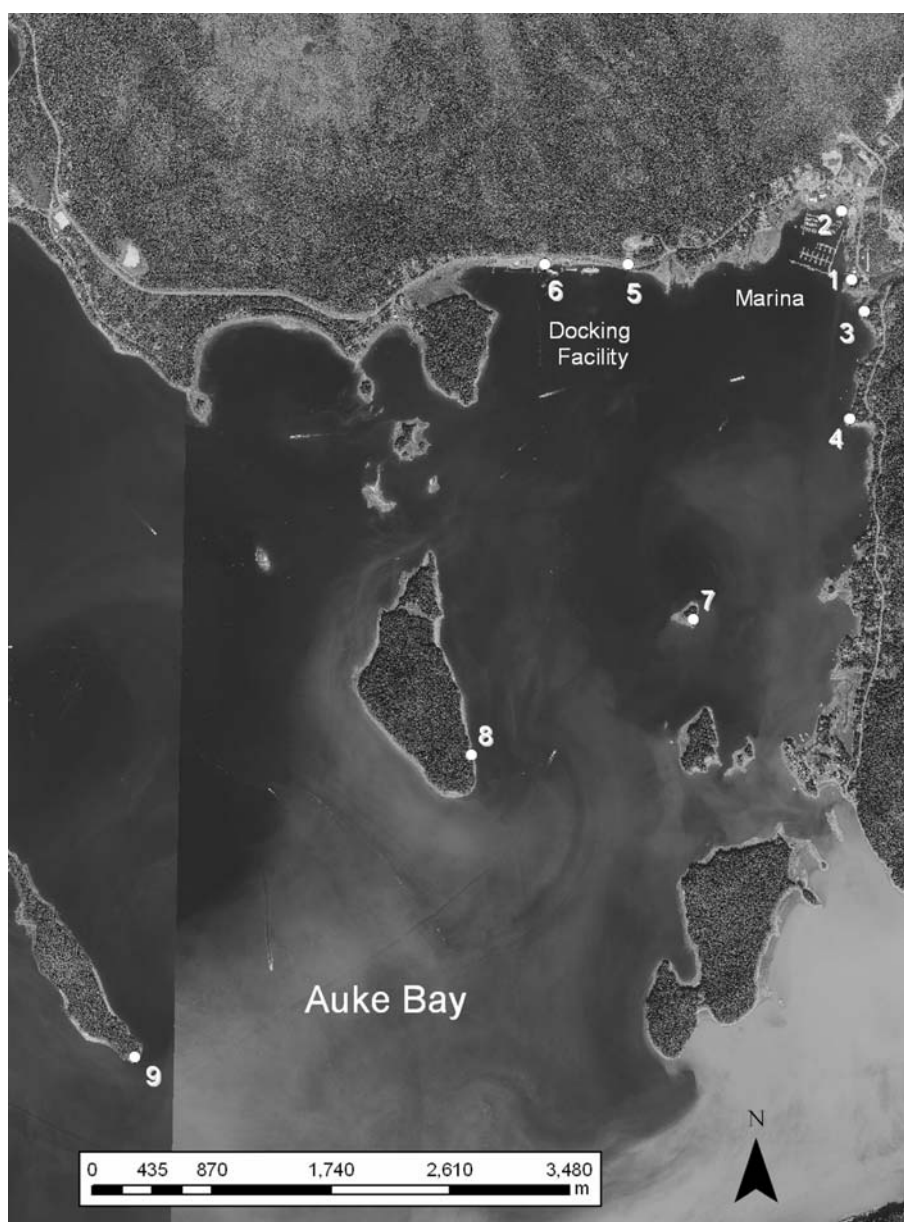
A known amount of tributyltin chloride (Aldrich) was added to two additional 15 g aliquots from two of the mussel homogenates and the results used to calculate percent recovery. The average recovery was 17.53%, which is lower than in previous studies (Short et al. 1989). The ppb Sn value derived from the GFAA signal for each sample was multiplied by the sample volume (0.005 L), divided by the fractional recovery (0.1753) and then divided by the grams of tissue to give the corrected μg Sn/g wet tissue. The triplicate values for each site were averaged and are reported with the associated standard error (Fig. 2c).

Wilcoxon signed ranks tests were used to determine whether RPS and the proportion of imposex-affected females had decreased significantly in Auke Bay from the mid-1980's to the present. Because we had an a priori expectation of decreased impacts of TBT based on studies conducted elsewhere (Evans et al. 1996; Gibson and Wilson 2003), we used a one-tailed test value for each statistical test. Only sites where dogwinkle samples were collected in the late 1980s and 2005 were used in statistical tests. Changes in TBT concentration were not tested statistically due to an extremely small number of sites ( $n = 5$ ) where TBT was detected in 2006.

## Results and Discussion

The extent and severity of imposex in dogwinkles of Auke Bay has decreased over time. The proportion of imposex-affected females ( $p < 0.05$ ,  $W = 21$ ,  $n = 7$ ) and RPS ( $p < 0.05$ ,  $W = 21$ ,  $n = 7$ ) were lower in 2005 than 1987 (Fig. 2a, b). However, in the present study, imposex was detected at two (sites 5 and 6) of the seven sites where it was previously reported. All females were affected by imposex at site 6 (Fig. 2a), near the Auke Nu Cove docking facility, and these females had the highest RPS of any site (13%; Fig. 2b). No imposex was detected at sites 3 and 4, near Auke Bay Marina. No snails were detected at sites 1 and 2 in 1987 or recent collections. It is unclear why snails have failed to recolonize sites 1 and 2, but this is probably related to their lack of a planktonic life stage and long, cold winters that limit adult dispersal at this latitude.

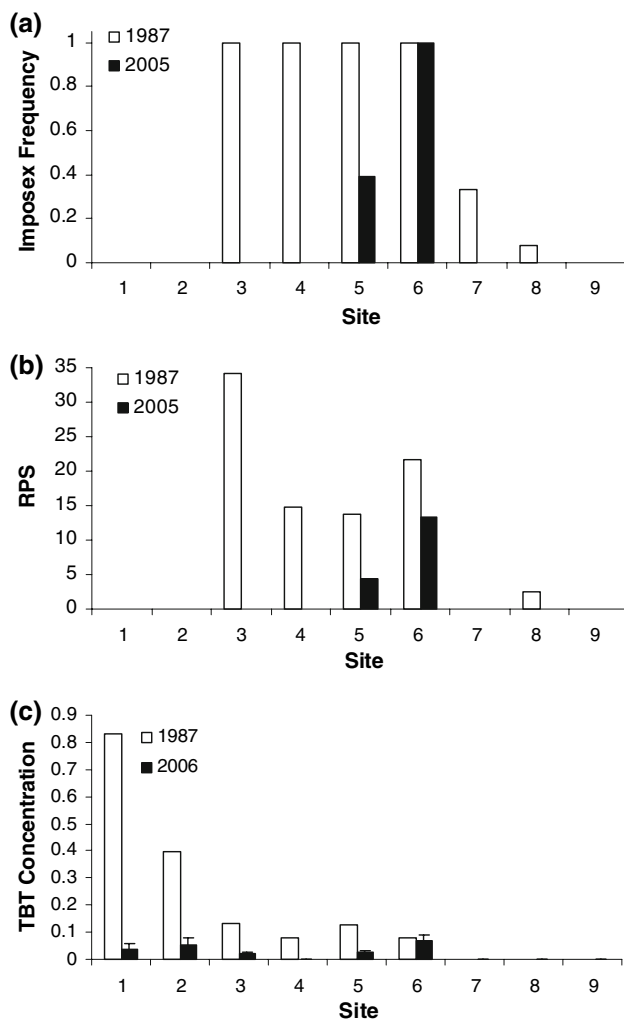
**Fig. 1** Locations of study sites in Auke Bay, Alaska



The extent, severity, and distribution of TBT contamination in mussels of Auke Bay has also changed over time (Fig. 2c). TBT contamination was uniformly lower in 2006 across all sites where it was detected in 1987. The highest level of TBT contamination detected in the present study ( $0.069 \mu\text{g TBT/g wet tissue wt.}$ ) was an order of magnitude lower than the highest levels found previously ( $0.883 \mu\text{g/g wet tissue wt.}$ ; Fig. 2c). Sites 1 and 2, near Auke Bay Marina, were previously the most contaminated sites, but now show the most dramatic reductions in TBT levels (Fig. 2c). In agreement with the dogwinkle imposex results, site 6 had the highest TBT levels in 2006, and the smallest reduction in TBT contamination since 1987, of any site in Auke Bay. Based upon bioconcentration factors

calculated previously for mussels, we would expect ambient TBT concentrations of  $0.016 \mu\text{g TBT/L water}$  at site 6. Using bioconcentration factors for dogwinkles found in Auke Bay (Stickle et al. 1990), we would predict  $0.070\text{--}0.046 \mu\text{g TBT/g wet wt}$  in dogwinkles. This is above the threshold necessary for imposex induction (Short et al. 1989).

The observed patterns are consistent with those for closely related species in many parts of the world where TBT and imposex levels have decreased following TBT bans (Evans et al. 1996; Gibson and Wilson 2003; Miller et al. 1999; Morton and Blackmore 2001; Reitsema et al. 2002; Smith 1996; Sousa et al. 2003; Stickle and Zhang 2003). The continued presence of TBT contamination and



**Fig. 2** Historic and recent data for **a** imposex frequency, **b** RPS in *Nucella* snails, and **c** tributyltin contamination ( $\mu\text{g Sn/g}$  wet tissue) in *Mytilus* mussels at sites sampled in Auke Bay, Alaska. Mean and SE are shown for 2006 samples

large proportions of imposex-affected dogwinkles at sites 5 and 6, near the Auke Nu Cove large boat docking facilities, are worthy of concern and continued monitoring. However, these results are also consistent with results from other studies, where persistent impacts of TBT are found near areas with heavy amounts of large vessel traffic (e.g., Stickle and Zhang 2003). Clearly, the overall concentrations, biological impacts, and threats posed by TBT in Auke Bay are less now than nearly two decades ago, but deserve continued attention because of the importance of this area to local fisheries.

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