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Tributyltin Contamination and Imposex in Alaska Harbors

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Abstract We quantified imposex in file dogwinkles (*Nucella lima*) and tributyltin (TBT) contamination in bay mussels (*Mytilus trossulus*) from 10 harbors and nearby control sites throughout Alaska. We found evidence of TBT contamination in mussels from four harbors (29–54 ng TBT/g wet tissue wt). Two of these harbors now show reduced TBT contamination relative to levels found in 1987. We were able to find and collect dogwinkles from seven sites. Of these, all three dogwinkle samples from harbor sites exhibited imposex, with 36%–87.5% females affected per site. In total, six of the 10 harbors had some evidence of TBT contamination.

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

Alaska has some of the world's most important fisheries and is generally thought to have some of the most pristine nearshore marine ecosystems in the world. Despite the importance of marine resources to Alaska, an extensive coastline, and seasonally high levels of shipping traffic, relative little coastal contaminants research has been conducted in Alaska. Rapid reductions in Arctic sea ice leading to reduced ice cover and renewed interest in offshore Arctic oil deposits (Gautier et al. 2011) have led to a realization that industrial maritime shipping will soon increase in Alaskan waters (Stephenson et al. 2011). Consequently there is a need to assess current contaminant levels in Alaska coastal areas.

A prominent example of our lack of knowledge about contaminant levels in Alaska is the antifouling paint compound tributyltin (TBT). TBT levels have been quantified in only a few places in Alaska, despite its previous widespread use, its high toxicity, its links to imposex and other developmental problems in a variety of invertebrates at low concentrations (Alzieu et al. 1986; Vos et al. 2000), and its possible contribution to infectious disease mortality in marine mammals (Murata et al. 2008). Imposex is the development of male sexual characteristics in females, and can lead to population decline when severe (Alzieu et al. 1986; Bryan et al. 1986). Following an international ban on the use of TBT in antifouling paints, evidence has been mixed for continuing effects of TBT on prosobranch snail imposex and demography in different parts of the world. In some places, imposex severity and extent has declined (Evans et al. 1996; Gibson and Wilson 2003). In other locations, particularly those with heavy ship traffic, TBT contamination and imposex remain a persistent problem (Gibson and Wilson 2003; Miller et al. 1999; Morton and Blackmore 2001; Stickle and Zhang 2003). This is likely due to unequal enforcement of marine regulations and the vagaries associated with ship registration and use in different parts of the world.

TBT was detected in Alaska over two decades ago (Short et al. 1989; Short and Sharp 1989), but no wide scale study was ever conducted. We measured TBT concentrations in bay mussels (*Mytilus trossulus*) and quantified imposex in file dogwinkles (*Nucella lima*) from Alaskan harbors and paired control sites just outside these harbors.

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Both of these intertidal species are distributed across the entire range of this study from Southeast Alaska to the Aleutian Islands. This provided an assessment of the current TBT threats to Alaska nearshore areas that are the most likely candidates for TBT contamination due to the potential continued use of TBT by some seagoing vessels. Because Alaska is a relatively remote location, TBT paints have long been banned in Alaska, and we have documented a decline in TBT in one southeast Alaska location (Tallmon and Hoferkamp 2009) we expected to see little evidence of TBT and imposex in Alaskan harbors.

Materials and Methods

Mussel and dogwinkle samples were collected from the intertidal zone of harbors and paired, nearby control sites in 10 locations throughout southeast Alaska and the Gulf of Alaska (Table 1). Whenever possible, at least 30 g wet weight of mussels and 50 dogwinkles were collected at each sample site. Each sample of mussels or dogwinkles was placed into a one gallon Ziplock© bag and placed in a cooler on blue ice until frozen. All samples were kept in a −20°C freezer until analyzed for TBT (mussels) or imposex (dogwinkles).

It was not always possible to collect both mussels and dogwinkles from each sampling site. Although mussels were found and collected from all harbor sites and control sites, the much less abundant and patchily distributed dogwinkles could not be found at many sites. Dogwinkles were collected only from the control sites in Cordova and Seward. In Dutch Harbor and Juneau (Auke Nu) dogwinkle and mussel samples were collected from both the harbor and control sites. In Homer, mussels were collected from the harbor interior and dogwinkles were sampled at the harbor mouth, at sites located several hundred meters apart. Dogwinkles were not found at either control or harbor sites in Kodiak, Ketchikan, Valdez, Sitka, or Skagway. The exact location of each sample collection site was recorded with a handheld GPS unit (Table 1).

Mussel samples were analyzed for TBT content by TestAmerica Analytical Testing Corporation (Tacoma, WA). These samples were analyzed using a standard proprietary TestAmerica protocol, SOP No. TA-MS-0346, Rev. 7. The TBT data were log transformed and a paired, one-sided paired t-test assuming unequal variances was used to determine whether TBT levels are elevated in harbors relative to control sites.

The proportion of imposex-affected females and the Relative Penis Size index (RPSI) for each sample was

Table 1 Collection sites in Alaska for bay mussels (*Mytilus trossulus*) tissue samples and dogwinkles (*Nucella lima*)

Site name	Site number[a]	Description	Collection date	Location		Dogwinkles[b]
				North	West	
Dutch Harbor	1H	Dutch Harbor	26 May 09	53.9033	−166.5113	Yes
	1C	Dutch Control	26 May 09	53.8774	−166.5158	Yes
Kodiak	2H	Kodiak Harbor	26 Apr. 09	57.7870	−152.4078	No
	2C	Kodiak Control	26 Apr. 09	57.7739	−152.4559	No
Homer	3H	Homer Harbor	08 May 09	59.6052	−151.4303	Yes
	3C	Homer Control	09 May 09	59.6661	−151.7004	No
Seward	4H	Seward Harbor	08 May 09	60.1155	−149.4345	No
	4C	Seward Control	08 May 09	60.0882	−149.4432	Yes
Valdez	5H	Valdez Harbor	01 May 08	61.1254	−146.3998	No
	5C	Valdez Control	02 May 08	61.1260	−146.3398	No
Cordova	6H	Cordova Harbor	08 May 09	60.5474	−145.7655	No
	6C	Cordova Control	08 May 09	60.5800	−145.7178	Yes
Skagway	7H	Skagway Harbor	13 Jul. 07	59.4489	−135.3219	No
	7C	Skagway Control	13 Jul. 07	59.4794	−135.3475	No
Juneau	8H	Juneau Harbor	29 May 09	58.3822	−134.6796	Yes
	8C	Juneau Control	29 May 09	58.3723	−134.7267	Yes
Sitka	9H	Sitka Harbor	29 Aug. 07	57.0507	−135.3267	No
	9C	Sitka Control	29 Aug. 07	57.0448	−135.3116	No
Ketchikan	10H	Ketchikan Harbor	20 Apr. 08	55.3508	−131.6816	No
	10C	Ketchikan Control	21 Apr. 08	55.5121	−131.7256	No

[a] Indicates site numbers of harbor (H) and paired control sites (C) located outside harbors

[b] Indicates whether dogwinkles were found

calculated according to the method described by Stickle and Zhang (2003). RPSI is simply the ratio of mean penis length of females cubed over mean male penis length cubed, multiplied by 100. Because only two locations provided paired (harbor and control) samples of dogwinkles, we did not test whether imposex was elevated in harbor sites.

Results and Discussion

We collected bay mussels from paired harbor and control sites in ten communities throughout Alaska (Table 1). We found evidence of TBT contamination in mussels from 4 of 10 harbors and none of the 10 control sites (Fig. 1a). The range of TBT at contaminated sites varied from 29 ng TBT/g wet wt. in the Seward harbor to 54 ng TBT/g wet wt. in Kodiak harbor (parts per billion). The difference in TBT contamination between paired harbor and control sites is statistically significant ($p = 0.015$, $t = 2.57$, $df = 9$).

We collected dogwinkles from the subset of seven sites at which they were detected. Four of these were control sites and three were harbor sites. None of the control site samples had detectable levels of imposex, whereas all three of the harbor sites had imposex-affected females (Fig. 1b). The percent of *N. lima* displaying imposex varied between 36% and 87.5%. However, the RPSI was fairly low and varied between 0.15 and 1.17 (Fig. 1c). The largest percent of imposex-affected females was found in Dutch Harbor (87.5%), but these also had a moderate RPSI (0.81). In contrast, the highest RPSI (1.17) and lowest percent of affected females (36.0%) was found in the Juneau harbor. Forty-three percent of the Homer harbor dogwinkles exhibited imposex, but their RPSI was low (0.15). In these cases, the percent of imposex affected female dogwinkles and the severity of imposex (as indicated by RPSI) did not match closely.

There is only one site, the Homer harbor, at which TBT contamination in mussels was detected and dogwinkles could be found. The TBT contamination level in Homer harbor is a middling value among those four harbor sites at which contamination was detected and the level of imposex in Homer harbor is also moderate relative to Juneau and Dutch Harbor. In the Dutch Harbor and Juneau harbor sites, imposex affects dogwinkles, and is extreme in the case of Dutch Harbor, but no TBT contamination is detectable in our co-located mussel samples. There is evidence of TBT contamination in Kodiak, Seward, and Sitka harbors, but no imposex data are available because no dogwinkles could be found and collected from these harbors.

The overall pattern suggests harbor contamination by TBT is widespread in Alaska harbors, despite long-term bans on the use of this substance in this state and a more recent global ban. Studies conducted in Juneau (Auke Bay) and Kodiak in the 1980s revealed TBT contamination in these harbors (Short and Sharp 1989; Short et al. 1989). In a recent study of Juneau only, we found evidence of TBT contamination and imposex in the Juneau harbor (Tallmon and Hoferkamp 2009).

The failure to detect TBT contamination in mussels at two (Dutch Harbor, Juneau) of the three harbor sites at which imposex was detected in dogwinkles is counter-intuitive. Only in the Homer harbor sites were both imposex and TBT contamination found. However, the lab results suggest TBT-contaminated mussel samples were just above detection limits for the methods used. Consequently, there may well be TBT contamination present at levels just below detection limits in several harbors that still has biological effects. It has been suggested that TBT can have morphological impacts at just a few parts per trillion (Alzieu et al. 1986), which is below our detection limits. Therefore, it seems likely that there may be very small, undetectable concentrations of TBT causing imposex in *Nucella* in Dutch Harbor and Juneau that we cannot detect with our mussel analyses.

On the positive side, the Juneau and Kodiak harbor data also suggest there may be a long-term trend of decreasing contamination in Alaska. In Kodiak, TBT concentrations in mussels sampled at the same harbor location have gone down from 800 ppb (or ng/g) in 1987 (Short et al. 1989, #292) to 54 ppb in 2009 (Fig 1a). At three sites within 1 km of one another in Juneau, TBT concentrations have gone down from a high of 150 ppb in 1987 near the Auke Bay marina (Short et al. 1989), to 69 ppb in 2006 (Tallmon and Hoferkamp 2009), and below detection limits in 2009 (Table 1). Similarly, imposex levels in nearby dogwinkles appear to have decreased over the past two decades. In addition, no direct or indirect evidence of TBT contamination was found outside any of the harbors in control sites.

TBT contamination in mussels or its biological effects on dogwinkles are detectable in six Alaskan harbors located throughout the state. At two of these two sites, TBT may be at low enough concentrations to escape detection in mussel tissues, but still high enough that dogwinkles are impacted by TBT. Despite international and state bans on the use of TBT in antifouling paints, several Alaska communities still have a TBT contamination issue to address. Our data provide an initial assessment of the extent and severity of TBT contamination, and should be incorporated into a long-term marine contaminants monitoring program for the state. This monitoring will be increasingly important as shipping and related economic activities in Alaska increase in the near future.

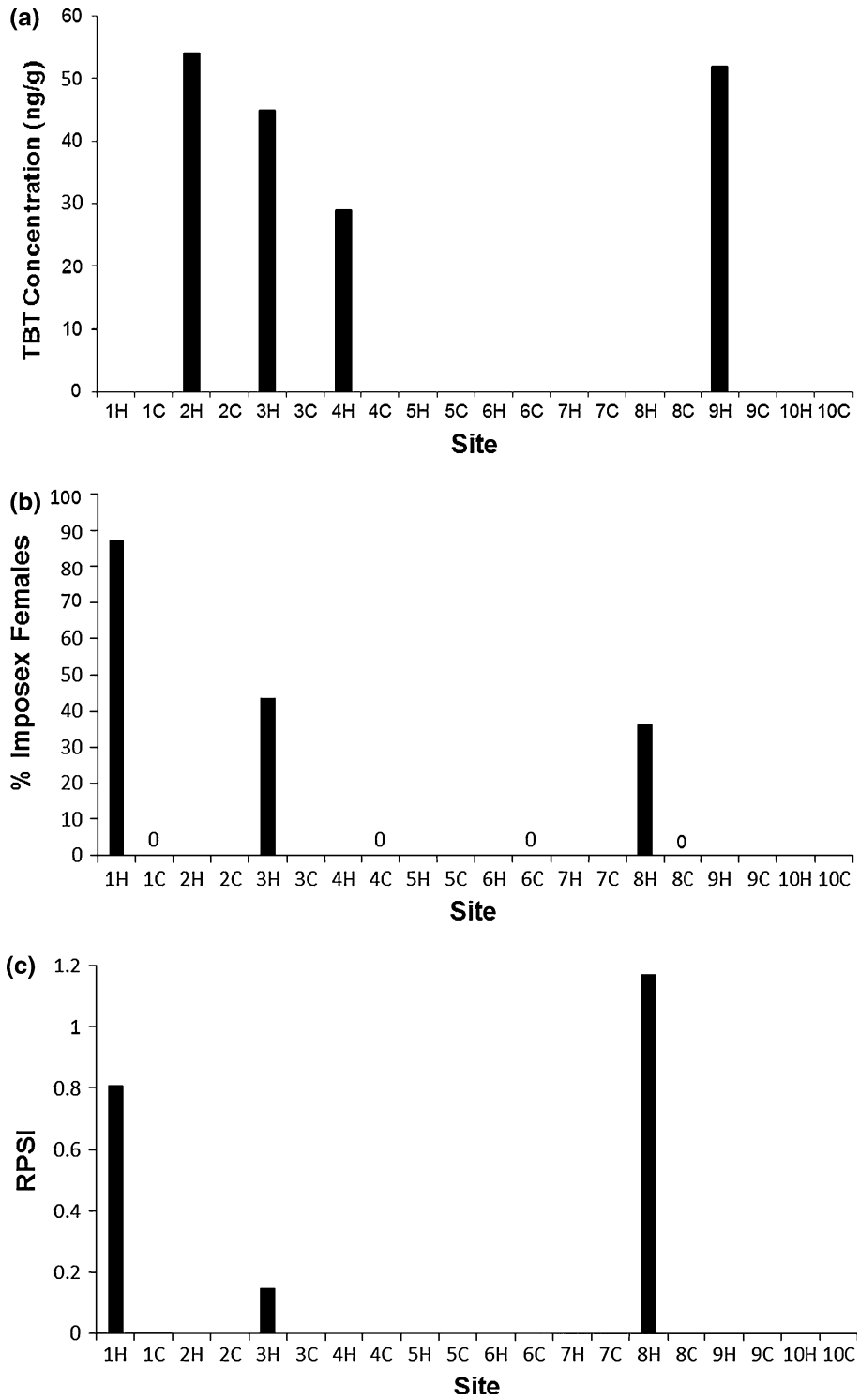

Fig. 1 TBT concentrations in *Mytilus* mussels (**a**), along with *% Nucella* snail females exhibiting imposex (**b**) and RPSI (**c**) at harbor (H) and control (C) sites in 10 Alaskan communities

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