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# Thiaminase activity in native freshwater mussels

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ABSTRACT

Thiamine (vitamin $B_1$) deficiency in the Great Lakes has been attributed to elevated levels of thiaminase I enzyme activity in invasive prey species; however, few studies have investigated thiaminase activity in native prey species. Some of the highest levels of thiaminase activity have been measured in invasive dreissenid mussels with little understanding of background levels contributed by native freshwater mussels (Bivalvia: Unionidae). In this study, thiaminase activity was measured in two freshwater mussel species, *Elliptio complanata* and *Strophitus undulatus*, from the Delaware and Susquehanna River drainage basins located in north eastern United States. Thiaminase activity was also measured in gravid and non-gravid *S. undulatus*. Average thiaminase activity differed significantly between species (7.2 and 42.4 μmol/g/min, for *E. complanata* and *S. undulatus* respectively) with no differences observed between drainage basins. Gravid *S. undulatus* had significantly lower thiaminase activity (28.0 μmol/g/min) than non-gravid mussels (42.4 μmol/g/min). Our results suggest that a suite of factors may regulate thiaminase activity in freshwater mussels and that native freshwater mussel thiaminase activity is within the range observed for invasive dreissenids. These results add to our understanding of the complexities in identifying the ecological conditions that set the stage for thiamine deficiency.

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## Introduction

Thiamine deficiency, the lack of sufficient levels of the essential nutrient thiamine (vitamin B1), has been shown to have dramatic organismal and ecosystem-level consequences. The enzymatic degradation of thiamine by thiaminase I (enzyme number 2.5.1.2; Webb, 1992), hereafter "thiaminase", is just one of several mechanisms by which thiamine deficiency can occur. This enzyme inactivates thiamine by acting as a catalyst for a base substitution reaction where the thiazole portion of a thiamine molecule is replaced by one of several nucleophiles (Fujita, 1954; Honeyfield et al., 2010; Lienhard, 1970). Thiaminase has been documented in a variety of taxa ranging from plants and bacteria (Evans, 1975; Fujita, 1954; Honeyfield et al., 2002) to higher order vertebrates including fish (Honeyfield et al., 2008; Nishimune et al., 2008; Wistbacka et al., 2002).

Elevated levels of thiaminase have been associated with the declines in native salmonid abundance and recruitment failure in the Great Lakes. Here, thiamine deficiency has been primarily attributed to consumption of non-native prey fish which are high in thiaminase (Fitzsimons, 1995; Fitzsimons et al., 1999; Tillitt et al., 2005), resulting in direct mortality of both fry and adult fish (Blazer and Brown, 2005; Brown et al., 2005a; McDonald et al., 1998). Additionally, numerous secondary effects associated with thiamine deficiency including decreased growth, impaired vision, reduced predator avoidance, and prey capture as well as immune dysfunction and disruption of spawning migration have been documented (Brown et al., 2005a, 2005b; Carvalho et al., 2009; Fitzsimons et al., 2005, 2009; Honeyfield et al., 2012; Ketola et al., 2005; Ottinger et al., 2012).

Recent research suggests that thiamine deficiency within the Great Lakes may also be attributed to both the consumption of, and food web alterations from, other non-native species, specifically dreissenid mussels (*Dreissena bugensis* and *D. polymorpha*). These invasive mussels have 5–100 times more thiaminase activity than has been observed in many Great Lakes fishes (Tillitt et al., 2009), suggesting that these mussels may be an important source of thiaminase in the regions they have invaded. Dreissenid mussels have decimated ecologically important native freshwater mussel (Bivalvia: Unionidae, hereafter "freshwater mussels") populations throughout much of the United States (Ricciardi et al., 1998). While research has begun to document the ecosystem impacts of replacing native freshwater mussels with dreissenid mussels (Ricciardi et al., 1998; Strayer and Malcom, 2007), their potential effects on food webs through thiaminase activity have not been investigated. In fact, reports on thiaminase activity in native freshwater mussels are scarce worldwide (see Fujita, 1954; Puzach et al., 1984; Reddi, 1950; Reddi et al., 1948), and no data have been reported on thiaminase activity in mussels in the United States where freshwater mussel diversity is highest (Lydeard and Mayden, 1995; Williams et al., 1993). Therefore the purpose of this study was to document thiaminase activity in two native freshwater mussels and compare it to literature-reported activity in invasive dreissenids (Tillitt et al., 2009). We assessed thiaminase levels in two native species of freshwater mussels (*Elliptio complanata* and *Strophitus undulatus*) and determined if levels varied by species, drainage basin, and reproductive status.

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## Material and methods

### *Species and collection locations*

*E. complanata* and *S. undulatus* are common and abundant species found throughout the Atlantic Slope, with *S. undulatus* extending into the Interior Basin (Nedeau, 2008). Mussels were collected by hand (depth < 0.5 m) from sites devoid of dreissenids from two separate drainage basins (Susquehanna and Delaware River drainage basins) located in the Northeastern United States between June (average water temperature: 23.3 °C; range: 18.8–27.4 °C) and September (average water temperature: 20.3 °C; range: 17.4–22.7 °C) of 2012. Within the Susquehanna drainage basin, mussels were collected from Pine Creek, Tioga County, PA and Buffalo Creek, Union County, PA; within the Delaware River, mussels were collected from the mainstem river near Hankins, Sullivan County, NY and near Callicoon, Wayne County, NY (Fig. 1). Five individuals were collected at each of the 4 sites for *E. complanata*. Because *S. undulatus* is less abundant than *E. complanata* at these sites (although still the second most abundant species), *S. undulatus* was only collected from one site in each of the drainage basins (Fig. 1). Five additional gravid female *S. undulatus* were collected from Pine Creek. Mussels were determined to be gravid by visual inspection (females exhibited swollen gill marsupium). Non-gravid mussels collected for analysis were not sexed but assumed to represent equal male:female sex ratio. Live mussels were transported in aerated coolers to the US Geological Survey Northern Appalachian Research Branch in Wellsboro, PA.

### *Tissue preparation*

All tissue from live mussels was removed from shells, immediately frozen, and stored at −80 °C until assayed for thiaminase activity. Frozen mussel tissue was homogenized on dry ice as described by Zajicek et al. (2005) and residual dry ice was sublimated for at least 24 h at −80 °C prior to thiaminase analysis.

### *Thiaminase I analysis*

Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Soltec Ventures and all other buffered reagents, salts, and chemicals were obtained from Sigma-Aldrich at the highest purity offered. All buffers and reagents were prepared immediately prior to use. Thiaminase activity was measured using the colorimetric thiaminase I assay of Honeyfield et al. (2010); however, reaction volumes were scaled down to quantify changes in optical density of 4NTP on a microplate spectrophotometer (BioTek®, PowerWave 340) rather than a standard UV–vis spectrophotometer. Briefly, sample processing was as follows. Sample masses of approximately 0.3 g were first homogenized in five volumes of chilled buffer. The sample was then vortexed, centrifuged, and the supernatants transferred into 2-ml Pierce centrifuge columns (~30 μm pore size, Thermo Fisher Scientific Inc.) to remove any residual particulate matter. Then 6 μl of processed, filtered supernatant was placed in each of four replicate wells of the microplate (NUNC, 96 well, Thermo Fisher Scientific Inc.) containing 194 μl of cocktail A (with thiamine) and 4 wells containing 194 μl cocktail B (no

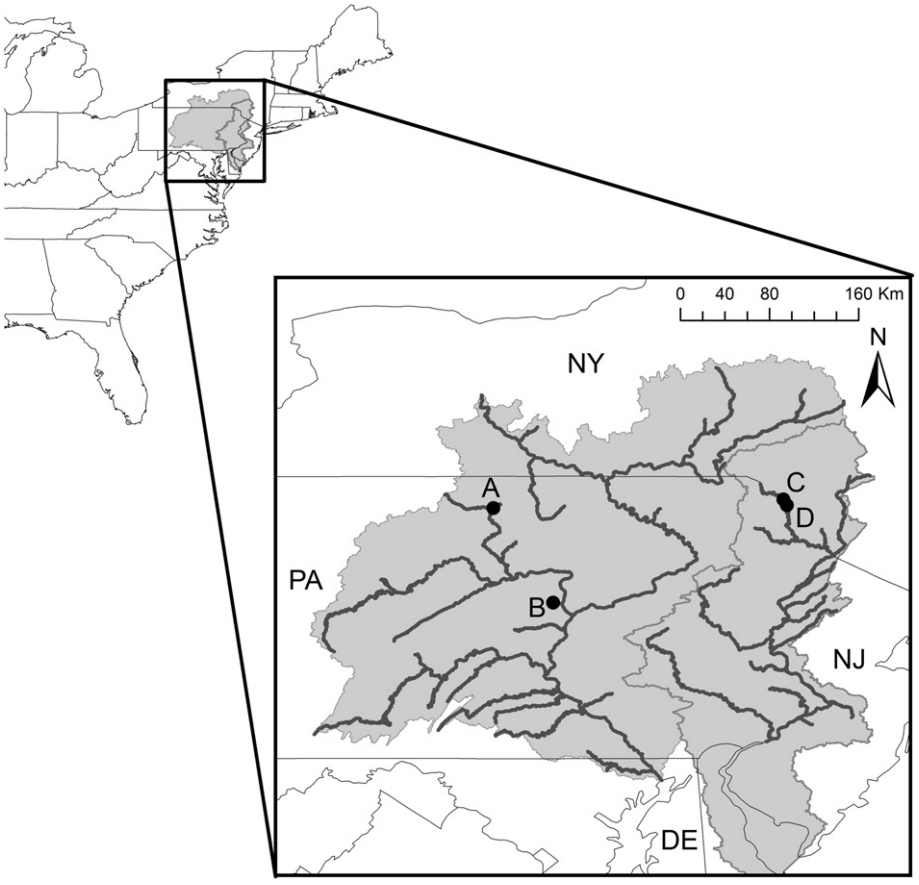


**Fig. 1.** Freshwater mussel collection locations in (A) Pine Creek, Tioga County, PA and (B) Buffalo Creek, Union County, PA within the Susquehanna River drainage basin and (C) near Hankins, Sullivan County, NY and (D) Callicoon, Wayne County, NY within the Delaware River drainage basin. *Elliptio complanata* was collected from all four collection locations, while *Strophitus undulatus* was collected from (A) and (C) during 2012. Both gravid and non-gravid *S. undulatus* were collected from (A). Drainage basins are shaded light gray; rivers are dark gray lines.