Ecotoxicology and Environmental Safety 142 (2017) 222–229

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Quantification of heat shock protein 70 and acetylcholinesterase over a time course suggests environmental adaptation in a foundational molluscan species

Andrew Ravaschiere, Caroline Cutler, Kristi Edleson, Zachery Halem, Henry Magun, Fred Meckler, Rachel Cox*

*Lisman Laboratories, Riverdale Country School, 5250 Fieldston Rd., Bronx, NY 10471, USA*

ARTICLE INFO

*Keywords:*
Acetylcholine esterase
Dissolved oxygen
Ecological services
Urban estuary
Heat shock protein 70
Mollusc

ABSTRACT

Waterways in urban areas often act as repositories for sewage, industrial waste, and environmental contaminants. In response, inhabitants of these watersheds undergo physiological adaptations specific to their respective environments. Effects of these stressors can be assayed by quantification of various well-documented biomarkers in sentinel species such as the Atlantic Ribbed mussel, *Geukensia demissa*, a native to the Bronx River Estuary, Bronx, NY, USA. Heat shock protein 70 (Hsp70) is a universally expressed biomarker for an array of environmental stressors including toxins and low dissolved oxygen. To better understand the mechanisms by which organisms tolerate their contaminated environments, we monitored the constitutive and heat shock-induced levels of two proteins: Hsp70 and acetylcholinesterase (AChE) in natural populations of *G. demissa* from differentially impacted sites: the Bronx River and Greenwich Cove estuaries. We show that *G. demissa* from the Bronx River exhibits a higher level of constitutive Hsp70, and launches a more rapid and robust heat shock response than does its Greenwich Cove counterpart. In addition, AChE levels are recovered more quickly in Bronx River mussels. Based on response pattern investigations from heat stress as well as constitutive expression, we suggest that the Hsp70/AChE chaperone/client relationship exemplifies the unique adaptive mechanisms utilized by organisms in order to tolerate environmentally impacted habitats. Results from this study offer important insights from an ecological perspective into the molecular and cellular basis of stress response and provide valuable information regarding adaptation to the increased demands of challenging environments.

## 1. Introduction

Urbanization impacts the well-being of estuarine ecosystems more severely than any other form of human activity (Limburg et al., 2005; Chin et al., 2013). Changes in patterns of land use and water consumption stemming from human industrialization continually introduce harmful contaminants into nearby watersheds (Van Dolah et al., 2008; Astaraie-Imani et al., 2012). As urban communities become the most predominant form of human dwellings, it is important to understand the consequences of our actions on urban ecosystems and watersheds (Sanderson and Labruna, 2005). The two problems addressed in this article concern the extent to which animals tolerate rapidly urbanizing ecosystems and the means by which stress-tolerant organisms adapt to their changing environments.

The Bronx River originates at small tributaries near the Kensico dam in Westchester NY and enters the city of New York after 12 miles, where it flows through densely populated and industrialized neighborhoods. The Bronx River drains into the East River on the west end of the Long Island Sound where it becomes tidally influenced and more estuarine. Throughout the nineteenth and twentieth centuries, combined sewage outflow and industrial waste continued to contaminate this urban watershed with pathogens (Crimmens, 2002), chemical toxins (Litten et al., 2007) and sewage (Crimmens, 2002; Kriesberg and Larson, 2010; Enecio and Krakauer, 2014). The Bronx River Estuary experiences relatively low dissolved oxygen (DO) levels (as shown in Table 1 relative to our “clean test site”, Greenwich Cove, in Connecticut). These environmental factors may impact organisms living in the river. The Bronx River Estruary's native molluscan species *Geukensia demissa* (*G. demissa*) exhibits endocrine disruption and stunted growth (Halem et al., 2014). In addition, the foundational species *Spartina alterniflora* demonstrates elevated levels of the stress protein heat shock protein 70 (Hsp70), which is indicative of an adaptation to environmental stress

* Corresponding author.
*E-mail address:* rcox@riverdale.edu (R. Cox).

http://dx.doi.org/10.1016/j.ecoenv.2017.04.003
Received 27 November 2016; Received in revised form 14 March 2017; Accepted 3 April 2017

**Table 1**
Average levels of dissolved oxygen and water temperature over a 7-year survey, and pH over a 5-year survey of the Bronx River and Greenwich Cove.

| Estuary Site | Dissolved Oxygen (mg/L) | Temperature (°C) | pH |
|---|---|---|---|
| Bronx River | 4.64 ± 1.22 | 22.8 ± 1.08 | 7.28 ± 0.05 |
| Greenwich Cove | 9.88 ± 1.83 | 23.9 ± 0.24 | 7.89 ± 0.24 |

All water collections (1–3 each year) occurred in June and July, around low tide. Data are presented as mean ± SD. $p < 0.0001$ for dissolved oxygen concentrations. $p = 0.0063$ for pH values. Revised from Halem et al. (2014).

(Decarlo et al., 2017).

With reference to comparisons made between the Bronx River Estuary, Bronx, NY and Greenwich Cove in Greenwich, CT, both of these watersheds are estuaries in the northeast region of the United States, roughly on the same latitude. The distance between these two collection points is 45.5 km (28.3 mi). A clear and important distinction is that the Bronx River runs through the city of New York, with concomitant exposure to urbanization, pollution, and contamination since the early 1900s. In contrast, Greenwich Cove is in a suburban area that has not experienced the same high level exposure to industrial contamination and sewage dumping. Low dissolved oxygen levels as those recorded in the Bronx River, (Table 1) approach hypoxic conditions that make life sustaining metabolic requirements very difficult if not impossible to meet (Diaz, 2001).

Despite extensive anthropogenic pollution, the Bronx River has remained a relatively resilient aquatic community (Rachlin, 2007). To better understand the cellular mechanisms utilized by stress-tolerant species, we compared the Hsp70 protein levels of two natural populations of ribbed mussels (*G. demissa*) under field conditions. One population originates from the Bronx River Estuary and one from Greenwich Cove, Connecticut, a relatively un-impacted site that has not undergone environmental disruption to the same degree.

As sessile estuarian filter feeders, mussels are well-established as sentinel organisms for biomonitoring environmental contamination (Goldberg, 1986). Specifically, *G. demissa* is well documented as a keystone bio-indicator species for the assessment of water quality (Bergen et al., 2001; Galimany et al., 2014; Giarratano et al., 2014; Halem et al., 2014). *G. demissa* reside in a wide range of geographical environments, from the Gulf of Lawrence, Canada to Florida, in mostly intertidal habitats, and experience a broad spectrum of salinities. *G. demissa* play an important role in the environmental flow of nitrogen (Abbott, 1974; Castagna and Chanley, 1973; Galimany et al., 2014; Jordan and Valiela, 1982).

Heat shock protein 70 (Hsp70) is a chaperone protein (Nover et al., 1996) that is biosynthesized both constitutively and in response to multiple stress factors in order to prevent or reverse protein denaturation, release stores of destroyed proteins, and guide the degradation of misfolded proteins (Bozaykut et al., 2014). The Hsp70 family is universally conserved, suggesting its relevance across evolutionarily diverse species (Gupta et al., 2010). Constitutive expression of Hsp70 has been maintained over at least 2.5 million years of evolution (Carpenter and Hofmann, 2000), perhaps in part because induction of Hsp70 synthesis facilitates the survival of organisms living under chronic environmental stress (Sanders et al., 1991). Hsp70 quantification is thus utilized as a consistent biomarker for environmental stress (Köhler et al., 1992; De Pomerai, 1996; Nadeau et al., 2001).

The enzyme acetylcholinesterase (AChE) breaks down the neurotransmitter acetylcholine, and plays a central role in neurotransmission. AChE responds to a variety of environmental triggers such as algal toxins (Cadavid, 2003; Lehtonen et al., 2003) and metal and pesticide exposure (Kopecka-Pilarczyk, 2010). In organisms exposed to carbamate and organophosphorus pesticides, AChE activity is inhibited (Singh and Agarwal, 1982) and such decreases are associated with several types of neurological disorders (Shinotoh et al., 2000). Due to its relevance with regard to environmental stress, AChE is an appropriate protein to study under the protection of Hsp70 acting as a potential chaperone.

The main aim of this research is to better understand the tolerance mechanisms utilized by these estuarine species. We measured both Hsp70 and AChE levels constitutively as well as after acute heat stress in two *G. demissa* field populations. Using AChE as a potential cognate protein under protection of Hsp70 chaperone, we propose that constitutive Hsp70 synthesis may be considered as a biochemical exaptation (Gould and Vrba, 1982) that allows mussels to survive under the pressure of chronic and multiple environmental stress factors (Koban et al., 1991; Sanders et al., 1991; Bednarek et al., 2016). This work provides empirical evidence for what appears to be a rapidly growing understanding that changing ecological conditions drive swift evolutionary change (Alberti, 2015). In addition, from the perspective of ecosystem services, which focus on the societal benefits offered by threatened species, we describe adaptive mechanisms in a highly tolerant foundational species (Gascon et al., 2015). We hope that these findings will better inform precautionary principles driving decisions that affect our urban waterways.

## 2. Materials and methods

### 2.1. Sample collection

Over the course of a three year study, *Geukensia demissa* specimens were collected between June 20 and July 10, at low tide, from Harding Lagoon, Bronx River Estuary, Bronx, NY, USA (40° 48′ 35.563″ N, 73° 51′ 40.893″ W), and Todd's Point, Greenwich Cove, Connecticut, USA (41° 0′ 31.296″ N, 73° 34′ 18.042″ W), see Fig. 1. Each collection resulted in twenty-four mussels overall. Organisms were transported to the laboratory in native water. Transport times varied from 30 to 90 min, field to laboratory. All samples, kept in native waters, were allowed to equilibrate at 24 °C for 24 h.

### 2.2. Heat shock treatment, time course, and dissection

Half of the mussels from each site were incubated at 24 °C for three hours. The remaining mussels were incubated in their respective site's waters at 37 °C for 2.5 h. After treatment, half of the mussels from the control group (24 °C) and heat shock group (37 °C) from each site (Bronx River Estuary and Greenwich Cove) equilibrated at 24 °C for 2.5 h before dissection. The other mussels equilibrated after heat shock at 24 °C for 27.0 h before dissection.

### 2.3. Protein extraction and concentration determination

Gills were pooled based on their site, treatment (heat shock vs. control), and equilibration time. Tissues were homogenized and lysed (Tissue PE, G-Biosciences #786181 and Halt PI #78425) in a 1:101 ratio (parts-to-whole). Gills were ground with a pestle and mortar before being further homogenized electronically. Samples were centrifuged for 20 min at 4 °C at 13,000 rpm. Protein containing supernatants were collected and concentrations were determined according to the Pierce BCA Protein Assay Kit (Thermo Scientific, #189966, Rockford, IL).

### 2.4. Determination of Hsp70 and AChE levels

Hsp 70 and AChE protein levels in *G. demissa* gills were determined by Western blot analysis. Gill homogenates were run on a 10% gel (30 µg) and transferred to PVDF membranes, which were Ponceau stained to visualize protein transfer. Membranes were blocked (Superblock, Thermo-scientific, # 37535, Rockford, IL) overnight at 4 °C. Blots were incubated in rabbit Hsp70 polyclonal antibody (1:2,000 Enzo SPA-812, Plymouth Meeting, PA) and in rabbit AChE polyclonal