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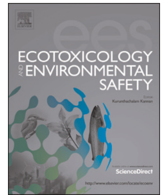

Sublethal exposure to azamethiphos causes neurotoxicity, altered energy allocation and high mortality during simulated live transport in American lobster

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ABSTRACT

In the Bay of Fundy, New Brunswick, sea lice outbreaks in caged salmon are treated with pesticides including Salmosan®, applied as bath treatments and then released into the surrounding seawater. The effect of chronic exposure to low concentrations of this pesticide on neighboring lobster populations is a concern. Adult male lobsters were exposed to 61 ng L^{-1} of azamethiphos (a.i. in Salmosan® formulation) continuously for 10 days. In addition to the direct effects of pesticide exposure, effects on the ability to cope with shipping conditions and the persistence of the effects after a 24 h depuration period in clean seawater were assessed. Indicators of stress and hypoxia (serum total proteins, hemocyanin and lactate), oxidative damage (protein carbonyls in gills and serum) and altered energy allocation (hepatosomatic and gonadosomatic indices, hepatopancreas lipids) were assessed in addition to neurotoxicity (cholinesterase activity in muscle). Directly after exposure, azamethiphos-treated lobsters had inhibition of muscle cholinesterase, reduced gonadosomatic index and enhanced hepatosomatic index and hepatopancreas lipid content. All these responses persisted after 24-h depuration, increasing the risk of cumulative impacts with further exposure to chemical or non-chemical stressors. In both control and treated lobsters exposed to simulated shipment conditions, concentrations of protein and lactate in serum, and protein carbonyls in gills increased. However, mortality rate was higher in azamethiphos-treated lobsters (33 ± 14%) than in controls (2.6 ± 4%). Shipment and azamethiphos had cumulative impacts on serum proteins. Both direct effects on neurological function and energy allocation and indirect effect on ability to cope with shipping stress could have significant impacts on lobster population and/or fisheries.

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1. Introduction

In the last decades, salmon aquaculture industry is rapidly expanding in the coastal zones of the Bay Fundy, New Brunswick, Canada, in areas used for lobster fisheries. Since 1994, widespread infestation of salmon with the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus*, have led to treatment of salmon cages with a variety of compounds including hydrogen peroxide, cypermethrin, deltamethrin and azamethiphos. Salmosan® (active ingredient-azamethiphos), an organophosphate pesticide formulation, has been applied to salmon cages in the Bay of Fundy as bath treatments (100 μg L^{-1} azamethiphos for 30–60 min) and released directly into surrounding seawater after treatment (Burridge et al., 2014). This insecticide targets crustacean species such as sea lice, but its effect on non-target crustaceans, such as the American lobster, *Homarus americanus*, living near aquaculture sites is not clearly understood.

Azamethiphos is neurotoxic, causing inhibition of acetylcholinesterase (AChE) activity. Concentrations equivalent to 10% of the recommended treatment concentrations are lethal for lobsters. The 48-h LC50 of azametipho s varied from 0.61 to 3.24 μg L^{-1} for adult American lobster exposed continuously at various times of the year at temperatures varying 2 °C to 14.6 °C (Burridge et al., 1999, 2005). Lobsters were most sensitive during the spawning and molting season, in the summer and early fall. Repeated 1-h biweekly aqueous exposures to azamethiphos (10 μg L^{-1}, at 13 °C) impacted survival and reduced spawning incidence in the spring in pre-ovigerous female American lobsters (Burridge et al., 2008). The LC50 (95% CI) was estimated to be 0.216 (0.157–0.273) μg L^{-1} for adult lobsters exposed to Salmosan® continuously for 10 days, at temperatures ranging from 11 to 14 °C (Burridge, 2013). At one particular aquaculture site, there may be

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up to three treatments per day for several consecutive days. Treatments could occur during the same period in different aquaculture facilities located in the same area resulting in repeated daily pulse exposure to pesticides. Thus, the effect of chronic exposure to low concentrations of this pesticide on neighboring lobster populations remained a concern (Burridge et al., 2014).

Another question was of possible sub-lethal cumulative impacts of pesticides and other stressors on lobster's survival after capture in the commercial fishery. American lobsters are transported live around the world for commercial purposes. During this shipping, lobsters are maintained in cool and humid conditions but out of water. Thus, they are subjected to multiple stressors including emersion and air exposure, hypoxia, temperature changes, handling and interactions with other lobsters (Lorenzon et al., 2007). Exposure to sublethal concentrations (75% LC50) of the organophosphate pesticide, chlorpyrifos caused hyperactivity and increased oxygen consumption in the freshwater crab *Barytelphusa guerini* (Srivastava et al., 2013). In addition to causing AChE inhibition and altered neuromuscular function (Fulton and Key, 2001), organophosphate pesticides can also cause oxidative stress (Negro et al., 2011), gill damage (Lignot et al., 1997) and metabolic disturbances (Tu et al., 2010). Exposure to organophosphate pesticides prior to shipment therefore have the potential to impair the ability of lobster to cope with subsequent stressors and increase mortality during shipment, resulting in economic loss.

The objective of this study was to assess sublethal effects in adult lobsters exposed continuously for 10 days to low concentrations of Salmosan®. Indicators of stress and hypoxia (serum total proteins, hemocyanin and lactate), oxidative damage (protein carbonyls in gills and serum) and altered energy allocation (hepatosomatic and gonadosomatic indices, hepatopancreas lipids) were evaluated in addition to the classical biomarker of organophosphate neurotoxic activity, AChE activity in muscle. The persistence of these effects after a 24 h period in clean seawater, and the impact of prior pesticide exposure on the ability of adult lobster to cope with live transport, were also assessed. To our knowledge, this is the first study to assess the effects of contaminant exposure on the capacity of decapod crustacea to cope with commercial live transport.

2. Materials and methods

2.1. Source of pesticide

Salmosan® was purchased from Aqua Vet Services International (Old Ridge, NB, Canada). Salmosan® is a wetable powder containing 47.5% (w/w) of azamethiphos (S-6-chloro-2,3-dihydro-2-oxo-1,3-oxazolo[4,5-b] pyridin-3-ylmethyl) O,O-dimethyl phosphorothioate), an organophosphate pesticide.

2.2. Lobsters

Post-molt mature male American lobsters (570 ± 75 g) were obtained from the commercial catch of fishermen at Eastport (Maine, USA) in September of 2012. They were held communally with shelter in flowing seawater (30 ppt, 15 ± 1 °C). A photoperiod of approximately 12 h light:12 h dark (Eastern standard time) was maintained. The water was aerated throughout each test. Lobsters were acclimated to the experimental tanks for 2 weeks prior to experiment. Prior to and during the exposure period, the lobsters were fed three times a week a diet of fresh clams and fresh-frozen shrimp and herring. Lobsters were not fed during simulated shipment or depuration. The tanks were cleaned daily to remove uneaten food and waste.

2.3. Experimental design

The lobsters ($N = 194$) were randomly distributed into 6 (3 m $\times$ 1.2 m, 0.25 m depth) fiberglass tanks (32–33 lobsters per tank). These tanks were distributed into 3 photoperiod-controlled rooms, with one control and one Salmosan® – treated tank in each room (three replicates per treatment). Each treated tank was given an initial spike of 110,000 ng of azamethiphos to reach target concentration quickly (square-wave addition of pesticide). Water and toxicant flows were maintained respectively at approximately 5 L min^{-1} and toxicant solution flow at approximately 1 ml min^{-1} and were monitored daily. Exposure concentrations calculated from measured concentration in the stock solution (340,000 ng L^{-1}) and measured flow rates were 72 ± 12, 82 ± 7 and 81 ± 11 ng azamethiphos L^{-1} in the treated tanks from Rooms No 1, 2 and 3 respectively (means $\pm$ SD, 10 days). Lobsters were exposed to Salmosan® continuously for 10 days (September 10th to September 20th). Water samples were collected from each tank for chemical analysis on September 10th, daily from September 11th to September 17th and again on September 19th.

At the end of the exposure period, 10 lobsters were randomly sampled in each tank (E: Post-exposure sampling). Live lobsters remaining in each tank were randomly divided into two groups put into two plastic crates: one of 12 and one of 9–11 lobsters. For each replicate, 12 control and 12 treated lobsters (differentiated by colored elastic bands on their claws) were mixed and packed with damp seaweed in an ice chest (33 cm $\times$ 41 cm $\times$ 23 cm, 0.031 m^3) to simulate commercial live transportation. These lobsters were kept in a cold room at 7 °C for approximately 24 h before sampling (S: post-shipment sampling). The last group of lobsters (N 10–11 per replicate) was transferred to a clean tank with flowing seawater and aeration at 15 ± 10 °C (within an approximate emersion sorting and transport time of 1 h). Control lobsters were put into one tank and treated lobster in another. In each tank, lobsters from different replicates were separated with plastic crates. These lobsters were kept in the depuration tanks for approximately 24 h until sampling (D: post-depuration sampling).

2.4. Sampling and morphometric measurements

Molt stage was assessed by examining carapace and setal development of pleopods (Waddy et al., 1995). Total body mass and carapace length were measured. A sample of hemolymph was collected from the dorsal abdominal artery into a 5 ml syringe with an 18 gauge needle, frozen in liquid nitrogen and stored at −80 °C. Hepatopancreas and gonads were dissected and weighed. Condition factor ($CF = 10^3 \cdot$ body mass $\cdot$ carapace length^{-3}), the gonadosomatic index ($GSI = 10^6 \cdot$ gonad mass $\cdot$ carapace length^{-3}) and the hepatosomatic index ($HSI = 10^5 \cdot$ hepatopancreas mass $\cdot$ carapace length^{-3}) were calculated. Hepatopancreas tissue was frozen at −20 °C for lipid analyzes. A section of tail muscle was minced, frozen in liquid nitrogen and stored at −80 °C for cholinesterase (ChE) analysis.

2.5. Hepatopancreas lipid, water and protein content

Hepatopancreas samples were homogenized with a Brinkmann Polytron Homogenizer (Kinematica Inc., Bohemia, NY, USA). Percentages of water and lipid in hepatopancreas were measured by microwave drying with nuclear magnetic resonance (SmarTrac II™, CEM Corporation, Matthews, NC, USA) (Keeton et al., 2003). Quality controls included one blank, a certified reference material (LUTS-1, Non Defatted Lobster Hepatopancreas, NRC, Ottawa, ONT, Canada) and duplicate samples in each series of 20 analyzes. For protein measurements, 60–80 mg of the homogenate was mixed with 1 ml phosphate buffered saline (0.01 M, pH 7.4, 1.4 mM

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