Connecticut Department of
ENERGY &
ENVIRONMENTAL
PROTECTION

STATE OF CONNECTICUT
DEPARTMENT OF ENERGY AND ENVIRONMENTAL PROTECTION

Public Hearing – February 25, 2013
Environment Committee

Testimony Submitted by Commissioner Dan Esty
Presented By Deputy Commissioner Susan Whalen

Raised House Bill No. 6438 - AN ACT RESTRICTING THE USE OF METHOPRENE AND RESMETHRIN

Thank you for the opportunity to present testimony regarding Raised House Bill No. 6438 - An Act Restricting The Use Of Methoprene And Resmethrin. The Department of Energy and Environmental Protection (DEEP) welcomes the opportunity to offer the following testimony.

In 2012, DEEP scientists discovered small amounts of methoprene and resmethrin in the tissues of lobsters. (See attached map and data.) As a result of that discovery, testing of lobsters from Long Island Sound and other New England near-shore waters for levels of methoprene, resmethrin and three other pesticides is currently being done at the University of Connecticut with results due back to DEEP by July 31.

During 2012, the Agency collected 90 lobsters from throughout Long Island Sound (Eastern, Central, Western, and Narrows) and 40 lobsters from near shore Maine, Cape Cod Bay (MA), Nantucket Sound (MA), and Narragansett Bay (RI). These lobsters are being tested to provide a sound scientific basis for legislative and/or agency decisions regarding the role of pesticides in lobster health and for human health advice in consuming lobster meat. All of these lobsters have been examined by pathologists at the University of Connecticut and tissue samples have been removed and prepared for toxicology testing at the UCONN Center for Environmental Sciences and Engineering to determine levels of methoprene, resmethrin, and three other commonly used pyrethroid pesticides (permethrin, bifenthrin, cyhalothrin). Results due to DEEP by the end of July will include data and analyses of the levels of pesticides, disease (parasitic and bacterial infections, etc.) and effects on the immune system found in the lobsters. Decisions on legislation and further research should not be made until these results are thoroughly reviewed and considered.

Raised Bill 6438 would further restrict the use of methoprene by prohibiting application to storm drains or water conveyance structures in the coastal zone and by limiting application in wetlands to instances where surveillance of larvae indicate an increasing threat of mosquito-borne disease. Methoprene serves as an important mosquito control agent. Together with bacterial larvicides, methoprene is used as part of an Integrated Pest Management (IPM) plan. Bacterial agents are often ineffective on mosquito larvae in stagnant water containing high levels of organic matter and methoprene is needed to

treat these areas. Application of methoprene in stagnant waters is often done as a preventative measure that cannot be linked to disease surveillance that same year. Used in this manner, methoprene is an important tool for helping to prevent serious disease outbreak.

Previous data and modeling results have led us to conclude that there is little to no probability of achieving concentration levels sufficient to cause lobster mortalities in the Sound given the small amount of the pesticide that is applied[1,2,3]. We continue to believe this to be true even though some lobsters collected in fall 2011 were found to have detectable levels of methoprene and resmethrin in organ tissue. Rather, DEEP believes that the available data support the conclusion that elevated bottom water temperatures are a more likely causative agent for recent lobster mortalities[4,5,6,7,8]. To our knowledge, concentration levels of methoprene sufficient to cause lethal or sublethal effects on lobsters have never been measured in LIS[1,2,3,8]. Hydrodynamic modeling results support the conclusion that concentrations sufficient to cause adult lobster mortality never occurred, even during the 1999 lobster die-off event; whereas, high bottom water temperatures above the thermal stress threshold for lobster (69° F) are recorded each year with increasing frequency and duration[1,2,3,4,5,6,7,8].

The proposed bill also aims to further restrict the use of resmethrin. Resmethrin is used for public health mosquito control programs to kill adult mosquitoes. It is not applied to storm drains or waterways. Rather, it is applied as a fine mist that breaks down rapidly in sunlight. The work being done at the University of Connecticut also includes a laboratory study to investigate the biological processing of resmethrin in lobster tissues.

In summary, this bill further restricts the use of two effective mosquito control agents used by the state, municipalities and private parties, and adds additional administrative responsibility to the agency (record keeping, reporting, pilot program on retail sale). Significant research has failed to make a connection between the die-offs of lobsters and the use of mosquito pesticides[1,2,3,4,5,6,7,8,9]. DEEP suggests that decisions placing further restrictions on the use of methoprene and resmethrin ought to be delayed until the results of current research are available.

Thank you for the opportunity to present testimony on this proposal. If you should require any additional information, please contact DEEP's legislative liaison, Robert LaFrance at 424-3401 or Robert.LaFrance@ct.gov

LITERATURE CITED

[1]Landeck Miller R.E., Wands J.R., Chytalo K.N. & D'Amico R.A. 2005. Application of water quality modeling technology to investigate the mortality of lobsters (*Homarus americanus*) in western Long Island Sound during the summer of 1999. *J Shell Res* 24:859-864.

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[4]Atlantic States Marine Fisheries Commission (ASMFC). 2010. Recruitment Failure in the Southern New England Lobster Stock. Technical Committee report to the American Lobster Management Board of the Atlantic States Marine Fisheries Commission.

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[8]Zulkosky, A.M., Ruggieri, J.P., Terracciano, S.A., Bronawell, B.J. & McElroy, A.E. 2005. Acute toxicity of resmethrin, malathion and methoprene to larval and juvenile American lobsters (*Homarus americanus*) and analysis of pesticide levels in surface waters after Scourge™,Anvil™ and Altosid™ application. *J Shell Res* 24:795-804.

[9]Butler M., Ginsberg H.S., LeBrun R.A. & Gettman A. 2010. Evaluation of nontarget effects of methoprene applied to catch basins for mosquito control. *J Vector Ecol* 35: 372–384.

Health Assessment Monitoring of American Lobster in Long Island Sound

This study was designed to develop a laboratory assessment and monitoring strategy to document the health of lobster from Long Island Sound. This involved seasonal collections of lobster, physical and laboratory health assessments and toxicology testing. A second component of this study involves a laboratory experiment designed to better understand the persistence of resmethrin in lobster hepatopancreas.

I. Lobster Health Monitoring Program

Sample Collection

CT DEEP staff conducted routine sea-sampling trips aboard the vessels of cooperating commercial lobstermen in CT. Commercial lobster fishery sea-sampling trips were scheduled seasonally to be proportional to the average landings from 2001-2004 and were equally divided among the three basins of the Sound (eastern, central, western); (Figure 1.1). Ten lobsters (five male, five female) from each basin (east, central, western and the Narrows (south of Stamford, CT)) were randomly selected during these routine sampling trips to undergo health assessment (Table 1.1). When possible, animals at or above the current minimum legal length (3 3/8" or 85.7 mm carapace length) were collected for analyses. When this was not possible, recruits, or animals within one molt group, ranging from 79.7 mm to 85.6 mm were selected.

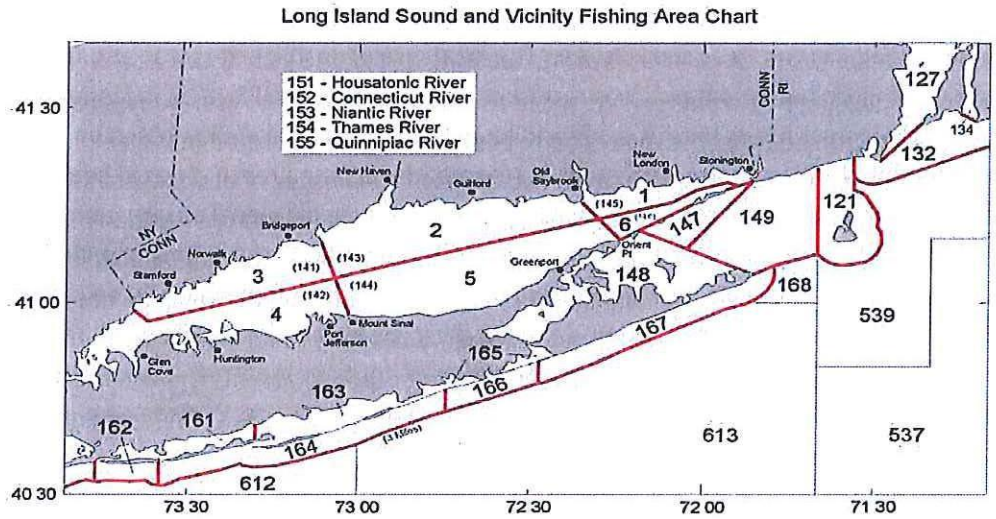

Figure 1.1 Logbook reporting areas for Connecticut commercial catch data. Eastern basin = reporting areas 1, 6, and 147; Central basin = areas 2 and 5; Western basin = 3 and 4 and Narrows = waters south of Stamford, CT.

Basin	Jul/Aug	Sep/Oct	Dec/Jan	Total By Basin
Eastern	10	0	10	20
Central	10	0	0	10
Western	10	10	10	30
Narrows	10	10	10	30

Table 1.1. Sample size by basin and collection period.

Biological Data

Carapace length for all lobsters were measured to the nearest 0.1 mm. Additional data recorded included sex; shell hardness; relative fullness of egg mass (<1/4 complement, 1/4, 1/2, 3/4, full); developmental stage of eggs (green, brown, tan); damage observations to determine cull rates and incidence of damage to claws, carapace, abdomen (tail) and walking legs; incidence of shell fouling organisms and incidence and extent of shell disease (0, 1-10%, 11-50%, >50% of shell surface covered). Care was taken to identify wounds caused by mechanical action so they are not identified incorrectly as shell disease. The incidence of dead lobsters was also recorded. The location of individual trap trawls was recorded using a handheld GPS. Also, in response to the health assessment needs for this study and to allow statistical correlation between environmental and various health metrics, two additional measures of physical health with regard to physical response to handling were assessed while individual observations were made. The first described claw posturing: 1) absent = no response, claws hang limp and lifeless to full defensive spread; 2) weak = some response to handling, would not inflict pain on handler; 3) strong = full, active response to stimuli and handling, claws raised and defensive. The second described physical strength of the muscular abdomen (or tail): 1) absent = no contraction, no response to handling; 2) weak = some contraction, limited response; 3) strong = energetic contraction, desire to 'upright' position while being held.

Additional data to be associated with collection events will include water temperature, dissolved oxygen and salinity levels (tested directly or by proxy from LISWQMP, LISTS or other source).

Atlantic Coast Sampling

Ten lobsters from four sites outside of Long Island Sound weree purchased from local lobstermen and delivered to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL), UCONN, Storrs, CT. These four sites included near shore coast of Maine, Cape Cod Bay Massachusetts, Buzzards Bay Massachusetts and near shore Rhode Island. All forty lobsters will have the hepatopancreas removed and two pools, each comprised of equally weighted samples of the hepatopancreas from five lobsters, will be created. Initially, these pooled samples of

hepatopancreas from each location will be submitted for toxicological testing to determine the presence of methoprene, resmethrin, permethrin, bifenthrin and cyhalothrin. Pending the results, individual hepatopancreas samples can be submitted for toxicological testing if necessary. In addition, for each Atlantic Coast submission, claw and tail muscle from each of 5 lobsters will be equally represented in a pool, i.e.two pools from each submission, and processed for toxicological testing to determine the presence of methoprene, resmethrin, permethrin, bifenthrin and cyhalothrin.

Laboratory Health Assessment

All lobsters collected were held on ice, with a sea water soaked burlap barrier, overnight and delivered to the CVMDL, UCONN the following day.

Necropsy

Each lobster will be necropsied, which includes the collection and storage of hemolymph, aerobic bacterial culture of hemolymph, gross examination of tissues, tissue collection for toxicology testing, tissue collection for histopathologic examination, trimming (Table 2.1), embedding of tissues in paraffin wax and histologic sectioning and microscopic evaluation.

Block	Tissue(s)
A	hepatopancreas
B	tail muscle, claw muscle, heart
C	antennal gland, gonad
D	nerve cord, intestine, gill
E	Shell (with and/or without lesion)

Table 2.1 Trimming protocol for American lobster tissues

Histopathology

Each lobster will undergo histopathologic testing. This includes sectioning and staining of paraffin embedded tissues (see Table 2.1) and pathologist's interpretation of microscopic pathologic changes. One non-routine histochemical stain is included in this routine work-up (if necessary). Histopathologic assessment of additional tissues will be performed at the discretion of the pathologist after gross examination; histopathologic examination may be performed on selected other tissues based on the results of toxicologic testing and after discussion with all parties involved.

Toxicology

Samples of lobster submitted for toxicological analysis will be comprised of hepatopancreas from individuals. Additional testing for human health consumption is described in section II below. Each sample will be submitted to the UCONN Center for Environmental Sciences and Engineering (UCONN CESE). Each of these samples will be tested for the presence of resmethrin,

permethrin, bifenthrin, cyhalothrin and methoprene using a gas chromatography/ tandem mass spectrometer (GC/MS/MS).

Blood chemistry

Each lobster will have hemolymph submitted to the Atlantic Veterinary College Lobster Research Center. Each sample will be analyzed for calcium, magnesium, sodium, potassium, protein and glucose content.

Disease/Infection

Histologic sections of hepatopancreas, tail muscle, claw muscle, heart, antennal gland, gonad, ventral nerve cord, gill, intestine and shell will be examined microscopically to survey for lesions and agents of disease. After assessing the nature and distribution of lesions from the sample population of lobsters, additional interpretations focused on categorizing levels of infection or disease will be conducted. The presence or absence of the following diseases or conditions can be determined by the aforementioned diagnostic tests;

a. Paramoebiasis
b. Calcinosis
c. Bacterial infections – *Vibrio; Aerococcus* (causative agent of Gaffkemia)
d. Other parasitic infections – *Hermatodinium* (bumper car disease, occurs under holding conditions)

Immune System Function : Phagocytosis

Phagocytosis will be evaluated as previously described (De Guise et al. 1995) with some variations. 500 µL of hemolymph will be mixed with 2.5 mL 3.8% (0.129M) buffered sodium citrate (pH 6.1) in Vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey) to prevent coagulation without significantly modifying pH. Hemocytes will be incubated in their hemolymph at room temperature (20-25°C). One µm diameter fluorescent latex beads (Molecular probes, Eugene, OR) will be diluted 1:10 in PBS and 5 µl of the bead mixture will be added for every 200 µl of hemolymph. After a 1 hour incubation in the dark, 200 µl of each cell suspension will be analyzed by flow cytometry. The fluorescence of approximately 10,000 hemocytes will be evaluated with a FACScan (Becton Dickinson, Mountain View, CA) flow cytometer. Phagocytosis will be evaluated as the proportion of hemocytes that have phagocytized 1 or more, 2 or more, and 3 or more beads.

II. Testing for Human Health Consumption

Sixty lobsters collected during the July through October 2012 timeframe will undergo additional toxicological testing to address any questions related to human health consumption. This timeframe was selected because it is the period when the greatest amounts of pesticide are likely applied.

A total of 60 lobsters were collected (Table 1.1) for the monitoring component of this study. In addition to the routine hepatopancreas submittal, composite samples of claw and abdominal muscle tissue from each of the 60 individuals will be submitted for toxicological analyses to determine the presence of resmethrin, methoprene, permethrin, bifenthrin and cyhalothin (CT Department of Public Health (CT DPH) 09/14/2012).

All results from these tests will be provided to the CT DPH to evaluate the need to modify, if necessary, the current health consumption advisory on lobster from LIS.

III. Paired Laboratory Toxicology Testing

As a cross-check on pesticide concentrations determined by state-of-the-art techniques used in the UCONN CESE analysis, a subsample of frozen hepatopancreas tissue will be sent to the Michigan State Center for Integrative Toxicology (MSCIT) for independent toxicological analysis. In a blind test, MSCIT will independently determine concentrations of the five subject pesticides: resmethrin, methoprene, permethrin, bifenthrin and cyhalothrin. Twelve samples incorporating the full range of pesticide levels detected are envisioned, including: the three highest, three medium and three lowest concentrations found among all samples analyzed, as well as three negative samples. UCONN CVMDL will ship samples to the MSCIT after receipt of the last toxicological test results performed by UCONN CESE. MSCIT will submit all test results for these samples to the UCONN CVMDL where they will be included in the database which was created to house individual test results for each lobster submitted.

IV. Biological Processing of Resmethrin

UCONN will perform a study (with previously used designs in exposure studies) to more precisely assess tissue concentrations following similar exposure (and health effects). This study will address questions regarding bioaccumulation and depuration in order to shed light on the potential health consequences associated with the tissue concentrations (21.3 – 71.7 ppb) recently measured in Long Island Sound lobsters.

Bioaccumulation of Resmethrin

In order to assess if resmethrin bioaccumulates, researchers will quantify hepatopancreas concentrations following repeat (once weekly) exposure for up to 1 month. Lobsters will be exposed to 0 (control), 0.1, 0.5, 1 and 5 µg/L once a week, and sampled after 1, 2, 3 and 4 weeks, as previously performed in laboratory exposures to determine effects on the immune system (we previously tested 0.01, 0.1 and 1 µg/L, without detectable tissue concentrations, using a less sensitive method, and propose to add 0.5 and 5µg/L). Researchers will expose 9 lobsters per resmethrin concentration, with 3 lobsters in each of 3 tanks, as previously described. Following euthanasia, the hepatopancreas will be collected from all lobsters and samples pooled from the 3 lobsters in each tank (3 pools of 3 lobsters per concentration) for pesticides analyses using a highly sensitive gas chromatography/ tandem mass spectrometer (GC/MS/MS).

Depuration of Resmethrin

In order to assess if lobsters can depurate resmethrin, researchers will assess hepatopancreas concentrations over time following a single exposure to the lowest concentration that results in measurable concentrations following a 1 week exposure. Lobsters will be exposed once and sampled after 1, 2, 3 and 4-week post-exposure. Researchers will expose 9 lobsters per time point, with 3 lobsters in each of 3 tanks, as previously described. Following euthanasia, the hepatopancreas will be collected from all lobsters and samples pooled from the 3 lobsters in each tank (3 pools of 3 lobsters per concentration) for pesticides analyses (as in 1) at each time point.

V. Budget

	UCONN STUDY COMPONENTS						
Components Include:	**Necropsies**	**Phagocytosis**	**Atlantic Coast Samples**	**Paired Toxicology Testing**	**Human Health Consumption Testing**	**Resmethrin Lab Study**	**UCONN Study Total**
Complete Study w 90 LIS lobsters & 40 Atlantic Coast **lobsters submitted to date** minus Opportunistic Sampling	$ 35,214.00	$ 3,742.00	$ 15,120.00	$ 3,120.00	$ 12,600.00	$ 28,482.00	**$ 98,278.00**

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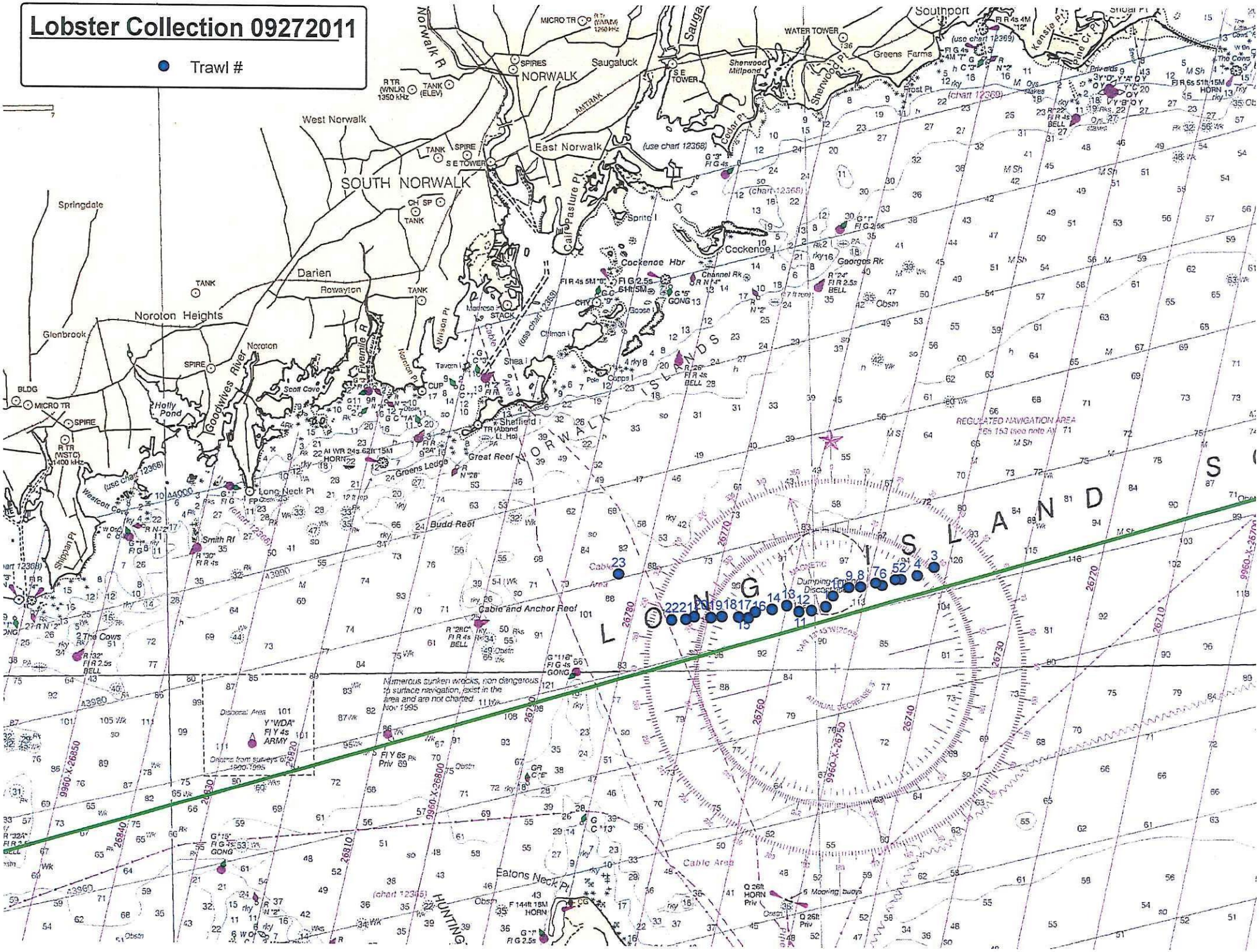
CT DEEP - Marine Fisheries
Order # 120080
Matrix: Lobster Tissue
Contact: David Simpson
Report Date: 5/18/12
Reported by: C. Perkins

Instrument				GC/MS/MS	GC/MS/MS	GC/MS/MS
Units	WET WEIGHT			ng/g	ng/g	ng/g
Prep date				5/11/12	5/11/12	5/11/12
Analysis date				5/11/12	5/11/12	5/11/12
CESE ID	Field ID	Collected	Recieved	Malathion	Methoprene	Resmethrin
120080-001	control-hepato	N/A	04/10/12	ND	ND	21.3
120080-002	control-gonad	N/A	04/10/12	ND	ND	ND
120080-003	male-testes	N/A	04/10/12	ND	ND	ND
120080-004	male-hepato	N/A	04/10/12	ND	ND	ND
120080-005	female-hepato-5-8	N/A	04/10/12	ND	264.4	71.7
120080-006	female-ovary-5-8	N/A	04/10/12	ND	ND	ND
120080-007	female-ovary-1-4	N/A	04/10/12	ND	ND	35.4
120080-008	female-hepato-1-4	N/A	04/10/12	ND	ND	40.2
			Reporting Limit	5.0	5.0	5.0

Lobster Collection 09272011

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