Analysis of Carotenoids in Maine Sea Urchins - 2009

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Abstract

Wild green sea urchins from various locations along the Maine coast were collected and their roe evaluated for the carotenoid, canthaxanthin, using a HPLC method. Canthaxanthin was detected in urchins collected under active and recently active salmon pens at levels above Japan's MRL. The closer sea urchins were to a salmon pen, the more likely they were to have detectable canthaxanthin levels. However, the results reported here are too variable to determine a useful minimum distance from pens for sea urchin harvest that would ensure canthaxanthin levels lower than Japan's MRL. The HPLC method was improved over the method used the previous year.

Introduction

Canthaxanthin is one of over 700 naturally occurring carotenoids, pigments that are usually red, orange, or yellow in color (Tsushima, 2007), found in egg yolks, flowers, flamingos, shrimp, salmon, carrots, the gonads and guts of sea urchins, and many other plants and animals. The carotenoids most commonly found in sea urchins are echinones and carotenes. Canthaxanthin is one of the predominant carotenoids in the roe of some sea urchin species, but has not been reported in the green sea urchin species commercially harvested in Maine, *Strongylocentrotus droebachiensis* (Müller) (Tsushima, 2007). It is not clear from the literature whether it does not occur naturally in this species, or just that it has not been evaluated (Tsushima, 2007), but it is not one of the major carotenoids of this species (Griffiths and Perrott, 1976).

Canthaxanthin is approved for use as a food color additive by the U.S. Food and Drug Administration (FDA). The legal limitation for canthaxanthin in food in the U.S. is 30 mg/lb. It is also allowed in salmonid feed at levels not to exceed 80mg per kg (US FDA 21 CFR 73.75). The maximum allowable level in most fish and shellfish, including sea urchins, set by the Japan Ministry of Health, Labor, and Welfare, Department of Food Safety, is 0.1 ppm MRL (Maximum Residue Level) (Japan MHW Notification, No. 370, 1959, amendment No.499 2005).

The green sea urchin has been harvested commercially in Maine since at least 1929, but the industry grew rapidly in the late 1980s when markets for processed roe (the gonad of both sexes) developed in Japan. Japan consumes more than 80% of the world's sea urchin production. The only species harvested in Maine is the green sea urchin, *S. droebachiensis* (Andrew et al, 2002).

Most of Maine's sea urchins are processed in Maine and most of the processed roe is shipped to Japan (Jim Wadsworth, sea urchin buyer, personal communication). Maine landings during the 2007-08 annual fall-winter fishery were 2.9 million lbs (1,338 mt) (whole) valued at $4.9 million to the harvesters (Maine DMR, preliminary data). About 65-80% of Maine urchin landings come from the Washington County area (Figure 1), which also supports several Atlantic salmon (*Salmo salar*) farms. For more information about the Maine sea urchin fishery, visit http://www.maine.gov/dmr/rm/seaurchin/, and for information about aquaculture in Maine, visit http://www.maine.gov/dmr/aquaculture/.

During the spring of 2007, the Maine Sea Urchin Zone Council (SUZC) discussed an unconfirmed report that a shipment of sea urchins exported to Japan had been refused because the urchin roe had unacceptably high levels of canthaxanthin (Maine SUZC, June 14, 2007). Allegedly, the urchins had been harvested from under salmon pens in Cobscook Bay (Figure 1). Sebastian Belle, director of the Maine Aquaculture Association, confirmed that canthaxanthin is used as a nutritional supplement in salmon feed in Maine, and meets US FDA requirements (Maine SUZC, June 14, 2007). In addition to providing pigmentation, carotenoids function as vitamin A precursors, and improve growth, survival, and reproduction in salmonid fish (Anderson, 2000; Torrissen and Christiansen, 1995).

Later that summer, a letter was received by a Maine processor from a major seafood wholesaler in Tokyo (Appendix 2-3), urging the Maine industry to refrain from harvesting sea urchins under salmon pens. In October 2007, the Maine DMR commissioner mailed a copy of this letter to all licensed Maine sea urchin harvesters and buyers, and also urged all Maine sea urchin divers to refrain from harvesting sea urchins under salmon pens (Appendix 1).

At its October 2007 meeting, the SUZC recommended that funds from the Maine Sea Urchin Research Fund be used for testing sea urchins from under salmon aquaculture pens for canthaxanthin (Maine SUZC, October 11, 2007).

In November 2007, sea urchins were collected under three active salmon pens in Cobscook Bay, and from various distances up to 120m away from the pens. The sea urchins were tested at the University of Maine Food Chemical Safety Laboratory, with the following results (Perkins, 2008):

- Most samples were made up of 10 sea urchins. Canthaxanthin was detected, in 10 out of the 11 samples tested, using the Japanese HPLC testing method with slight modification. A total of 102 sea urchins were tested, and canthaxanthin was detected in 51 of them.
- Concentrations, when detected, were relatively low (5-8 ppm), but higher than the Japanese MRL of 0.1 ppm.
- There was no significant relationship between the distance from the salmon pen and the incidence or concentration of canthaxanthin. That is, levels were just as high some distance away as they were under the pens.
- The minimum level of quantification for canthaxanthin at the Maine lab was 1.0 ppm, not as sensitive as reportedly used in Japan (0.1 ppm).

It was also concluded that there were no adequate control samples, and that sea urchins further away from salmon pens should have been tested – only one sample of 4 urchins was collected more than about 60 m from a pen. There was no way to determine whether the source of the canthaxanthin in the sea urchins was from salmon feed, or occurred naturally. Also, gender and gonadal development stage were not identified in the November 2007 samples, but there is evidence in the scientific literature that these can influence carotenoid level (Griffiths and Perrott, 1976; Matsuno and Tsushima, 2001; Borisovets el al, 2002; Lawrence et al, 2004).

At its November 2008 meeting, the SUZC recommended that further testing be done (Maine SUZC, November 20, 2008).

The purpose of this testing would be:

- To further investigate distance effects, if any
- To determine whether there was a gender effect
- To determine whether there were detectable natural, background canthaxanthin levels in sea urchin roe, by analyzing sea urchins collected far from any salmon pens
- To determine whether this is just a "Cobscook Bay problem"
- To improve the sensitivity of the test, to more closely approximate, or surpass, the Japanese testing sensitivity

Methods

Sample collection: From December 2008 to February 2009, sea urchins were collected by divers from various locations along the Maine coast, including the Johnson Cove salmon pen site sampled in Cobscook Bay in 2007. Collection data are summarized in Table 1, and locations are mapped in Figure 1.

Cobscook Bay area: None of the three pens sampled in 2007 were still active in the winter of 2008-09, having been harvested in July, 2008 (about seven months earlier) and left fallow. In fact, there were no active pens in Cobscook Bay during the winter of 2008-09. One sample of about twenty urchins was collected from under the Johnson Cove pen on February 11, 2009, and twenty more from about 100m away from the edge of the pen, in approximately the same locations as 2007 samples, by DMR divers (see map, Figure 2). A sample of seaweed (*Fucus vesiculosus*) that some of the urchins were clumped around was also collected under the pen. The Johnson Cove site was selected because it had the highest canthaxanthin levels in the 2007 analysis, and it had the most sea urchins present, of the three sites sampled in 2007.

Another sample of at least twenty urchins was collected on the same day in Eastport, south of the breakwater, about 1000m from the nearest recently active salmon pen at Prince Cove (Figure 2).

Machias Bay area: Also on February 11, 2009, samples of two or three sea urchins each were collected by a commercial sea urchin diver at four sites in the Machias Bay area. Two samples were collected under active salmon pens near Cross Island (designated MACH CI and MACH CW2, Figure 3), one was about half a mile southwest of these pens (Cross Is SW), and the forth was over 6 miles east of Cutler near Moose Cove (Figure 1).

Mount Desert Island area: On January 6, 2009 a sample of about 20 urchins was collected from a commercial urchin diver, who said he had been fishing in Frenchman Bay (Figure 1 and 4), which is about 19 miles away from the nearest salmon pen at Black Island, near Swan Island (indicated as SWAN BI in Figure 4).

Boothbay Harbor area: On December 19, 2008, and January 5, 2009, samples of about 20 urchins each were collected at Pemaquid Point, Bristol, and near the Trevett bridge, Boothbay,

(Figure 1). These sites are about 65 and 69 miles, respectively, from the nearest salmon pen, which is in the Mount Desert area (Black Island).

All samples were returned in coolers to the DMR laboratory in Boothbay Harbor, and frozen whole.

Roe extraction: During the middle of February the sea urchins were thawed, weighed, and measured for diameter. The gonads were extracted, weighed, and sexed until five males and five females had been identified from each sample. These ten were used, the rest discarded, unless there were fewer than five, in which case all urchins were used. In two cases (from Eastport), two or more gonads of the same sex from the same sample of urchins were combined to make a single gonad sample of at least 5g. Otherwise, the gonad from each urchin was analyzed separately. Samples were placed in numbered glass vials and then refrozen and transported to the University of Maine Food Safety Laboratory for canthaxanthin and astaxanthin analysis. The one seaweed sample (*Fucus vesiculosus*) from Johnson Cove was also analyzed.

Laboratory analysis: Analysis for canthaxanthin was carried out using a HPLC (high performance liquid chromatography) method (Appendix 3) modified from the Japanese method described by the Japan Ministry of Health, Labor, and Welfare, Department of Food Safety at http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/zanryu3/2-042.html, using a newer HPLC system than that used in 2008. Because the laboratory was also doing work with astaxanthin, also used in commercial salmon feeds, it was decided to analyze the urchins for this carotenoid as well.

Mean canthaxanthin and astaxanthin levels were calculated for each site; a value of 0 ppm was assigned to those samples where the carotenoid was not detected for this calculation. Logs (base 10) of the mean canthaxanthin level +1 were plotted against the logs of the distance in meters +1 of the site from the nearest active or recently active salmon pen. A simple linear correlation coefficient was calculated (Zar, 1999).

Canthaxanthin levels for each urchin were also plotted against gonad weight, by sex, for each site with detectable canthaxanthin levels.

Results

Seventy-one sea urchins, and one seaweed sample, were collected and analyzed. See Table 1 for sample locations and dates, and Table 2 for canthaxanthin and astaxanthin levels detected.

Minimum levels of quantification (LOQ) for astaxanthin and canthaxanthin were 0.05 ppm. The Japan method followed for this study lists the detection limit for canthaxanthin as 0.1 ppm. Detection levels for this work are 10 to 20 times lower (more sensitive) than for the work we reported in 2008. We were able to achieve greater detection sensitivity for the carotenoids by using a new HPLC system and by modifying the Japan method slightly for better resolution.

Development stage: Walker et al (2001) describe four developmental stages for sea urchin gonads:

- Inter-gametogenesis and nutritive phagocyte phagocytosis
- Pre-gametogenesis and nutritive phagocyte renewal
- Gametogenesis and nutritive phagocyte utilization
- End of gametogenesis, nutritive phagocyte exhaustion and spawning

The sea urchins sampled during this project were probably in the third stage – gametogenesis and nutritive phagocyte utilization – but no histological determination was made.

Cobscook Bay area: Canthaxanthin levels in sea urchins collected under the Johnson Cove pen ranged from 1.58-8.09 ppm, all above the Japan MRL of 0.1 ppm, with a mean of 3.51 ppm. This was lower than the mean level of 6.75 ppm detected in samples collected in the fall of 2007, when the salmon pens were stocked (Perkins, 2008). Canthaxanthin was not detected in the seaweed sample taken from under the pen. At the site about 100m southwest of the pen, levels were lower – not detectable in three urchins and ranging from 0.71-5.72 ppm in the other seven – for a mean of 1.61 ppm. At the Eastport site, levels were even lower – not detectable in four urchins, below Japan's MRL of 0.1 ppm in five, and 0.45 ppm in one – for a mean of 0.07 ppm.

Machias Bay area: Canthaxanthin levels in all six sea urchins collected under the two salmon pens in Machias Bay were low and similar, but above Japan's MRL – with means of 0.47 and 0.51 ppm for the two pens. Canthaxanthin was not detected in the three urchins sampled over half a mile (~1200 m) southwest of any pens. Over six miles (10 km) east of Cutler (Moose Cove), it was not detected in one urchin, and detected at a very low level (0.08 ppm) in the other, for a mean of 0.04 ppm. The nearest active pens to this site were actually in Lubec, over 9 miles away. Lease sites in Cutler Harbor had not been active since August 2004.

Mount Desert Island area: Canthaxanthin was not detected in seven of the ten sea urchins analyzed, but was detected at low levels (0.22-0.93 ppm) in the other three, for a mean value of 0.17 ppm, slightly above Japan's MRL. Unfortunately, there is uncertainty about the exact location of harvest for this sample.

Boothbay Harbor area: Canthaxanthin was not detected in any of the ten sea urchins collected at Pemaquid Point, and only detected in one of ten near the Trevett bridge at a low level (0.14 ppm).

Statewide: When mean canthaxanthin level was plotted against distance from the nearest active or recently active pen, for all samples, using log scales, there was a significant negative linear correlation (r^2=0.47, r = -0.68, $p<0.05$) statewide (Figure 5).

Conclusions

To our knowledge, except for our earlier work (Perkins, 2008), this is the first time canthaxanthin has been analyzed and results reported for this sea urchin species.

There was a significant correlation between canthaxanthin level and proximity to the nearest active or recently active salmon pen statewide. This suggests that salmon feed is a source of elevated levels of canthaxanthin in sea urchin tissues. However, the results reported here are too variable to determine a useful minimum distance from pens for sea urchin harvest that would ensure canthaxanthin levels lower than Japan's MRL. The detection of canthaxanthin in two sea urchins (one at Trevett, one at Cutler) some miles from salmon farms indicates the possibility of another, perhaps naturally occurring, source.

Canthaxanthin was detected in sea urchins from under the Johnson Cove pen 7 months after the last salmon feed was applied, suggesting that canthaxanthin was persistent in the sea urchin tissue or in the marine environment under the pen during that period.

There was no obvious relationship between canthaxanthin level and gender or gonad weight (Figure 6), although no statistical tests were performed.

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Table 1. Sea urchin sample collection data.

Date	Location	Depth	Latitude	Longitude	No. of urchins collected	Distance from salmon pen (m)
	Boothbay Harbor area					
12/19/2008	Trevett Bridge	10-15 ft	43° 52.887'	69° 40.276'	20	111,045
1/5/2009	Pemaquid Point	10-25 ft	43° 52.100'	69° 31.032'	20	104,607
	Mount Desert Island area					
1/6/2009	Frenchman's Bay, probably Bar Harbor Shore, maybe Otter Pt	20 ft			20	30,578
	Cobscook Bay area					
2/11/2009	Eastport	10 ft	44° 54.202'	66° 58.979'	20	977
2/11/2009	Johnson Cove, lease COB JK, under pen	25-35 ft	44° 55.863'	67° 00.756'	20	0
2/11/2009	Johnson Cove, 100+ meters SW from pen	15-20 ft	44° 55.863'	67° 00.756'	20	100
	Machias Bay area					
2/11/2009	Bag # 1, Cross Island, north side, lease MACH CI, under pens		44° 36.9'	67° 18.9'	3	0
2/11/2009	Bag # 2, Cross Island, southwest side, ½ mile from pens		44° 36.4'	67° 19.3'	3	1,287
2/11/2009	Bag # 3, Near Towers, lease MACH CW2, under pens		44° 37'	67° 16'	3	0
2/11/2009	Bag # 4, 6.38 mi east of Cutler, Moose Cove, away from pens		44° 43.7'	67° 05.7'	2	15,289

Table 2. Results for each sea urchin analyzed from the Cobscook Bay area.
"nd" = not detected, "Asta" = Astaxanthin, "Cantha" = Canthaxanthin

Johnson Cove, Under Pen							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
1	88.04	61	17.06	M	19%	0.27	1.69
2	77.33	60	12.30	M	16%	0.41	3.78
3	60.71	53	11.40	M	19%	0.21	3.04
4	58.75	53	11.24	F	19%	0.33	1.73
5	98.63	65	26.25	F	27%	0.23	3.28
6	71.50	59	18.80	F	26%	0.25	1.58
7	97.78	66	20.81	M	21%	0.43	6.36
8	84.41	61	10.94	F	13%	0.46	3.17
9	89.77	62	8.80	M	10%	0.38	8.09
10	81.55	61	15.11	F	19%	0.40	2.33
means	80.85	60	15.27		19%	0.34	3.51
Rockweed (*Fucus vesiculosus*) from Johnson Cove, Under Pen							
Vial Number						Asta. (ppm)	Cantha. (ppm)
72						nd (<0.05)	nd (<0.05)

Johnson Cove, Away from Pen (100+ meters SW)							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
11	52.60	52	5.39	M	10%	0.20	0.71
12	68.97	59	5.54	F	8%	nd (<0.05)	nd (<0.05)
13	47.99	50	14.95	F	31%	0.58	5.20
14	61.41	54	12.14	M	20%	0.25	1.54
15	55.78	56	5.78	M	10%	nd (<0.05)	nd (<0.05)
16	60.74	54	8.34	M	14%	0.56	3.74
17	57.25	56	6.46	M	11%	nd (<0.05)	nd (<0.05)
18	52.14	51	9.83	F	19%	0.20	0.74
19	54.20	52	15.32	F	28%	0.38	2.16
20	46.19	49	11.06	F	24%	0.14	2.01
means	55.73	53	9.48		18%	0.23	1.61
Eastport							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
51	78.38	64	4.15	M	5%	0.22	0.45
52	70.96	58	5.05	M	7%	0.23	0.07
53	77.20	61	5.20	F	7%	nd (<0.05)	nd (<0.05)
54	68.52	59	7.48	F	11%	nd (<0.05)	nd (<0.05)
55	68.85	62	2.00	F	3%	nd (<0.05)	nd (<0.05)
	81.30	63	2.94	F	4%		
56	50.95	55	5.38	F	11%	nd (<0.05)	0.05
57	81.54	62	7.36	F	9%	0.11	0.06
58	84.80	64	5.17	M	6%	0.19	0.06
59	77.26	60	5.79	M	7%	0.46	0.05
60	45.53	52	1.95	M	4%	0.22	nd (<0.05)
	59.26	58	1.76	M	3%		
	53.04	52	1.01	M	2%		
	65.22	57	3.78	M	6%		
means	68.77	59	4.22		6%	0.14	0.07

Table 3. Results for each sea urchin analyzed from the Machias Bay and Mount Desert Island areas. "nd" = not detected, "Asta" = Astaxanthin, "Cantha" = Canthaxanthin

Machias Bay, MACH CW2, West of Cutler, Under Pen							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
61	148.68	71	20.50	F	14%	0.30	0.47
62	138.08	73	17.38	M	13%	nd (<0.05)	0.72
63	140.63	73	20.71	M	15%	0.54	0.24
means	142.46	72	19.53		14%	0.28	0.47

Machias Bay, MACH CI, Cross Island, North Side, Under Pen							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
64	121.57	69	28.12	M	23%	0.14	0.66
65	119.80	70	19.91	M	17%	0.33	0.58
66	112.24	65	21.52	F	19%	0.15	0.28
means	117.87	68	23.18		20%	0.21	0.51

Machias Bay, Cross Island, ½ mile (SW) Away from Pens							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
69	121.72	68	27.25	M	22%	0.29	nd (<0.05)
70	101.60	65	17.34	M	17%	nd (<0.05)	nd (<0.05)
71	98.94	65	27.85	F	28%	nd (<0.05)	nd (<0.05)
means	107.42	66	24.15		23%	0.10	nd (<0.05)

6 mi East of Cutler, near Moose Cove, Away from Pens							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
67	137.17	71	25.35	M	18%	0.25	0.08
68	100.22	66	29.83	F	30%	0.16	nd (<0.05)
means	118.70	69	27.59		24%	0.21	0.04

Frenchman Bay							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt (g)	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
41	90.34	60	20.27	M	22%	0.28	0.22
42	79.63	59	8.46	M	11%	nd (<0.05)	nd (<0.05)
43	107.53	71	17.98	F	17%	nd (<0.05)	nd (<0.05)
44	88.15	65	12.74	M	14%	0.50	0.93
45	82.38	67	12.73	M	15%	0.68	0.58
46	103.35	65	19.10	F	18%	0.13	nd (<0.05)
47	83.81	62	12.51	F	15%	nd (<0.05)	nd (<0.05)
48	103.02	69	9.14	F	9%	nd (<0.05)	nd (<0.05)
49	98.60	65	15.93	F	16%	0.10	nd (<0.05)
50	91.03	65	15.54	M	17%	0.34	nd (<0.05)
means	92.78	65	14.44		16%	0.20	0.17

Table 4. Results for each sea urchin analyzed from the Boothbay Harbor area. “nd” = not detected, “Asta” = Astaxanthin, “Cantha” = Canthaxanthin

Pemaquid Point, South Bristol							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
21	106.28	65	17.30	M	16%	nd (<0.05)	nd (<0.05)
22	96.57	62	11.89	M	12%	nd (<0.05)	nd (<0.05)
23	121.29	69	24.93	M	21%	nd (<0.05)	nd (<0.05)
24	109.80	68	20.26	F	18%	nd (<0.05)	nd (<0.05)
25	108.86	64	15.81	M	15%	nd (<0.05)	nd (<0.05)
26	114.44	67	18.85	F	16%	nd (<0.05)	nd (<0.05)
27	91.69	63	20.21	M	22%	nd (<0.05)	nd (<0.05)
28	97.47	65	17.19	F	18%	nd (<0.05)	nd (<0.05)
29	71.27	60	17.17	F	24%	nd (<0.05)	nd (<0.05)
30	102.70	63	9.37	F	9%	nd (<0.05)	nd (<0.05)
means	102.04	65	17.30		17%	nd (<0.05)	nd (<0.05)

Trevett Bridge, Barters Island, Boothbay Harbor							
Vial Number	Whole Wt (g)	Diam (mm)	Gonad Wt	Sex	% Roe	Asta. (ppm)	Cantha. (ppm)
31	139.50	70	18.73	M	13%	nd (<0.05)	nd (<0.05)
32	130.22	67	23.83	M	18%	nd (<0.05)	nd (<0.05)
33	141.61	72	25.77	M	18%	nd (<0.05)	nd (<0.05)
34	124.32	70	27.28	M	22%	nd (<0.05)	nd (<0.05)
35	190.56	75	33.17	M	17%	0.14	0.14
36	188.18	76	26.97	F	14%	nd (<0.05)	nd (<0.05)
37	106.84	70	12.76	F	12%	nd (<0.05)	nd (<0.05)
38	279.42	92	69.25	F	25%	nd (<0.05)	nd (<0.05)
39	179.61	77	37.14	F	21%	nd (<0.05)	nd (<0.05)
40	91.54	61	19.61	F	21%	nd (<0.05)	nd (<0.05)
means	157.18	73	29.45		18%	0.01	0.01

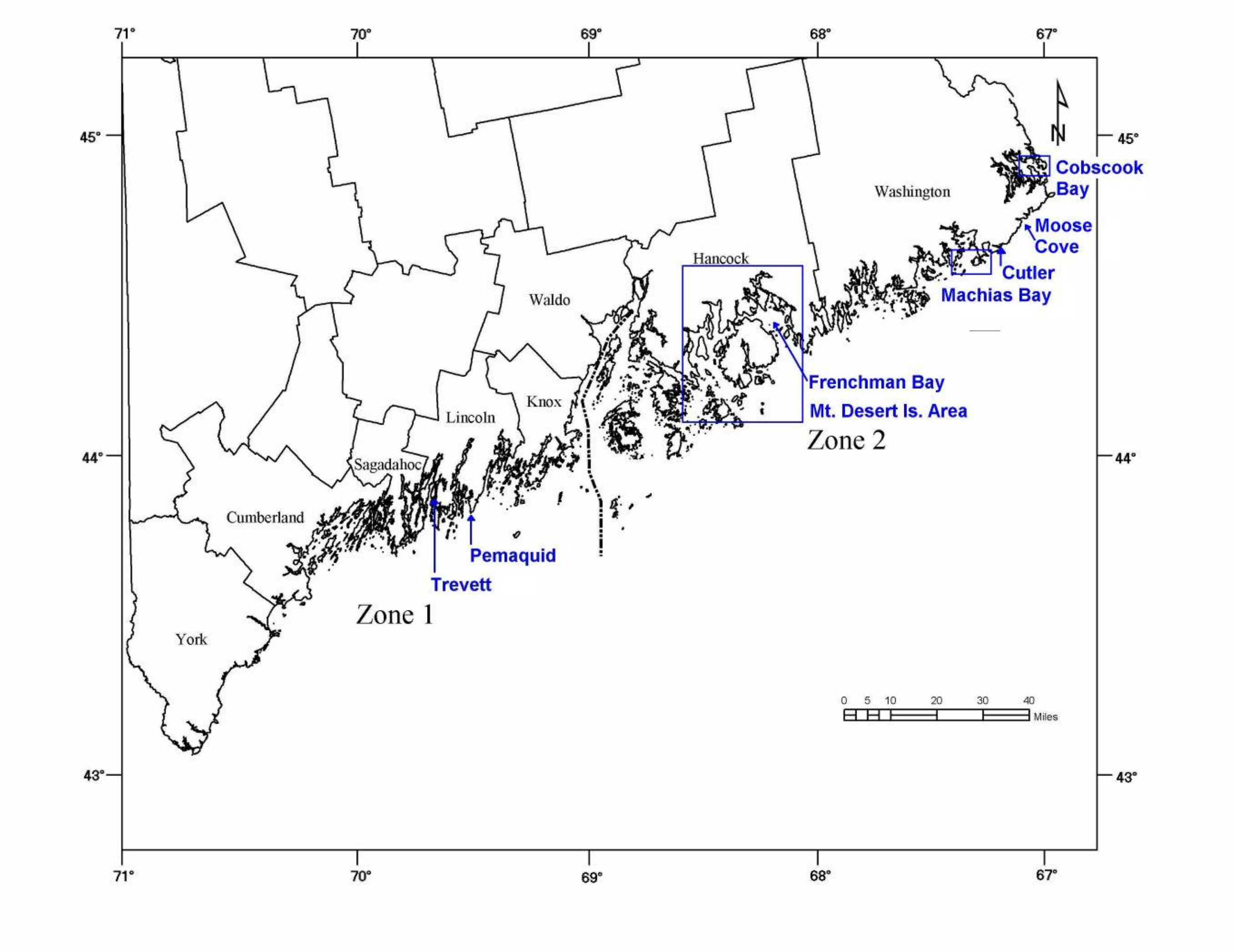

Figure 1. The Maine coast with coastal counties, sea urchin management zones, and sea urchin sample location areas (blue).

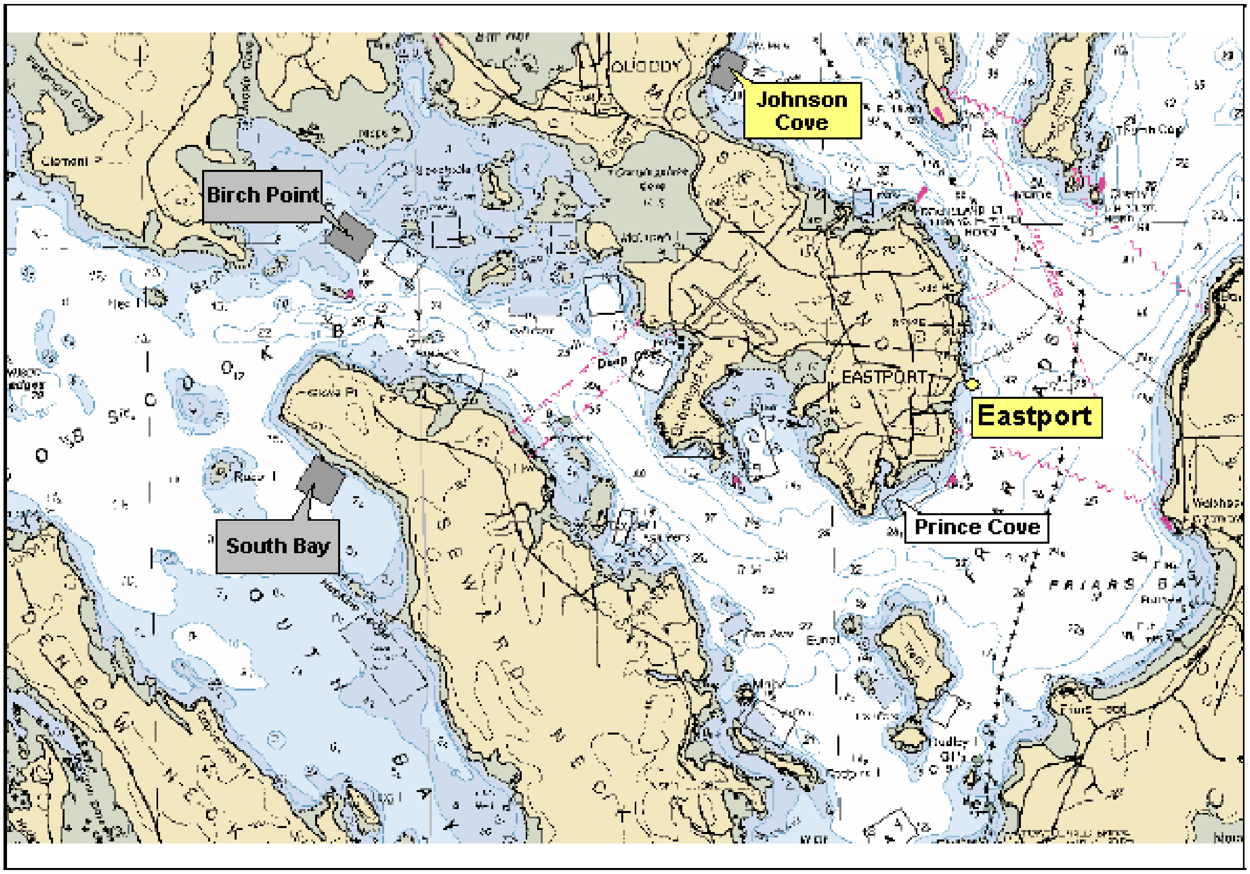

Figure 2. Sea urchin sampling locations in the Cobscook Bay area. Yellow areas were sampled in 2009. Johnson Cove and gray areas were sampled in 2007. Other rectangles are other salmon lease sites.

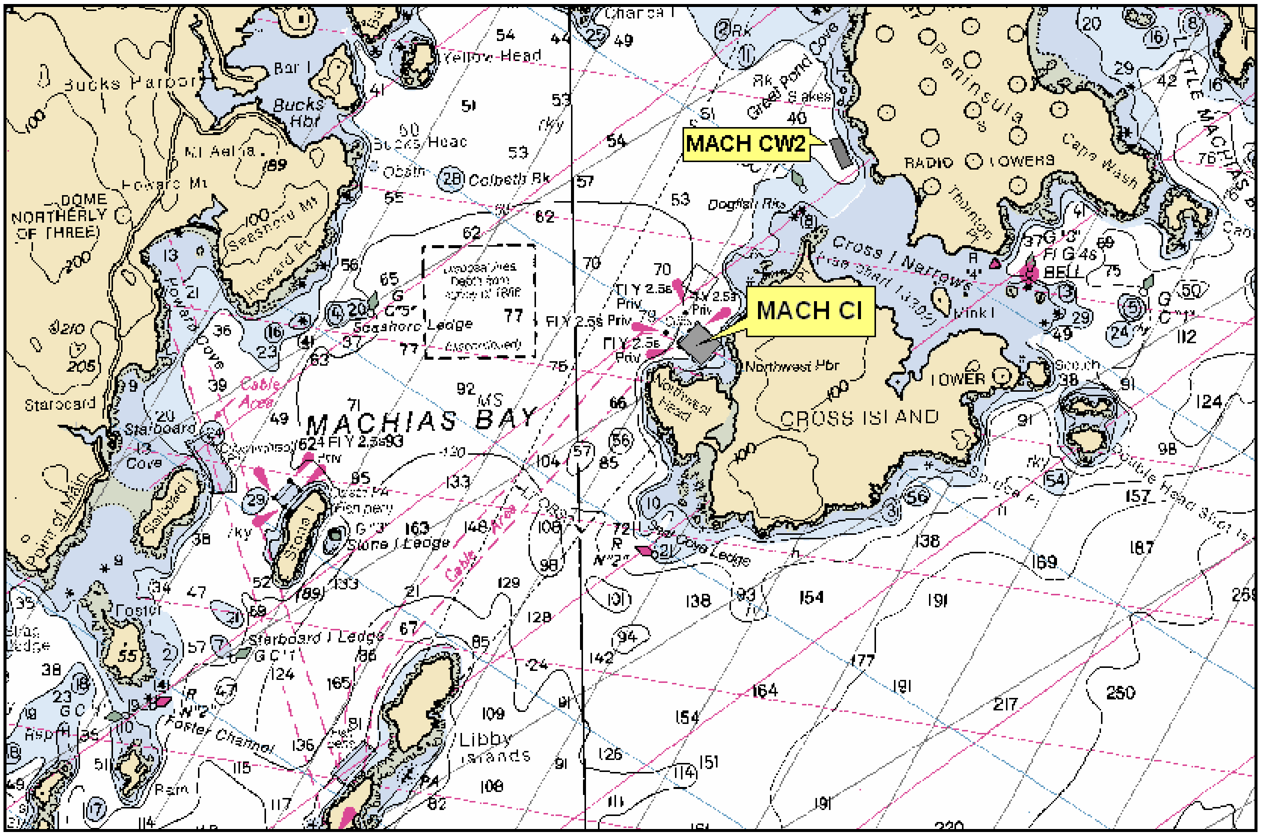

Figure 3. Sea urchin sampling locations in the Machias Bay area.

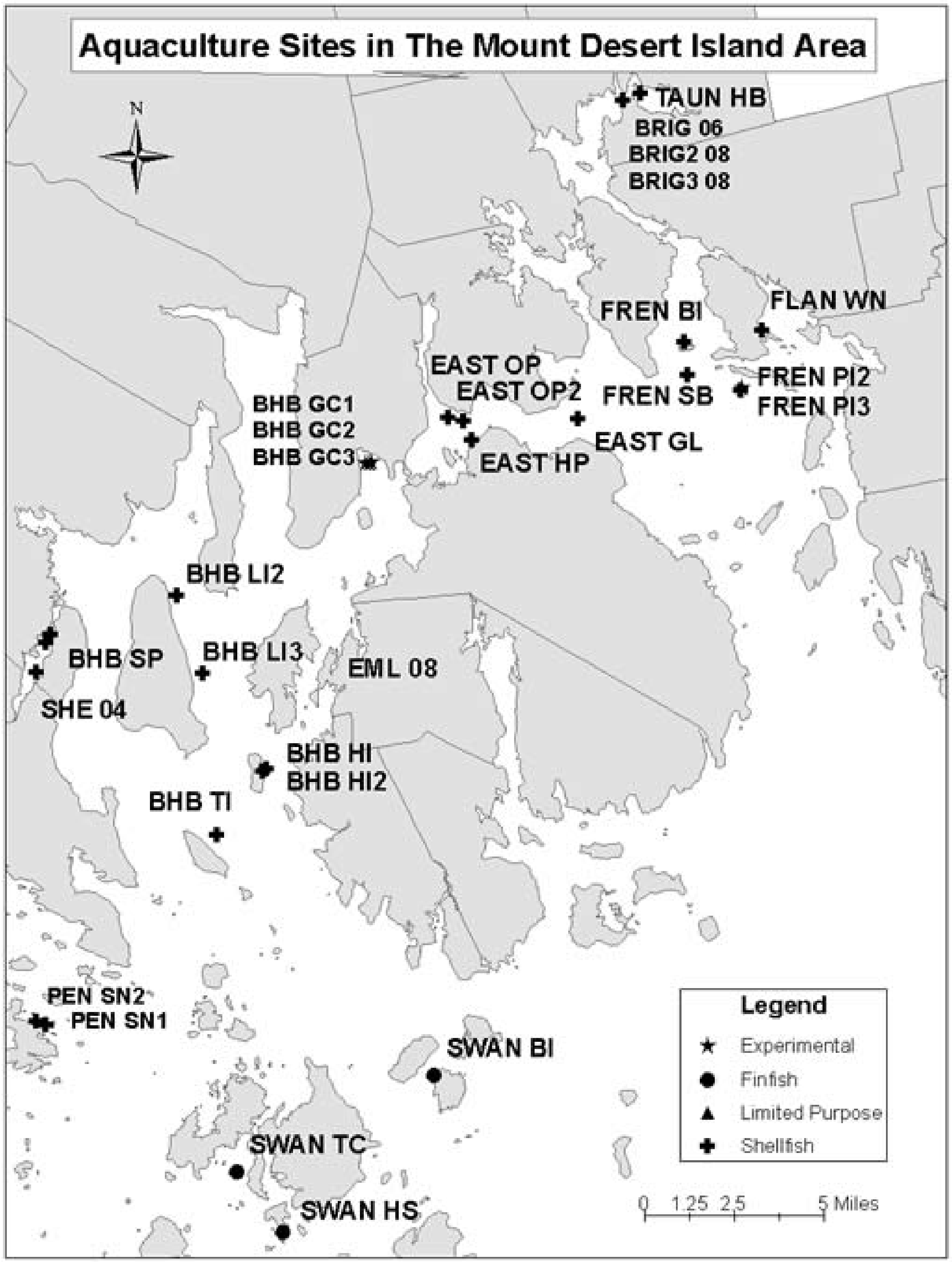

Figure 4. The Mount Desert Island area. Frenchman Bay is in the upper right. Note that the only salmon aquaculture sites are around Swan Island; the others are shellfish (mostly blue mussel) sites.

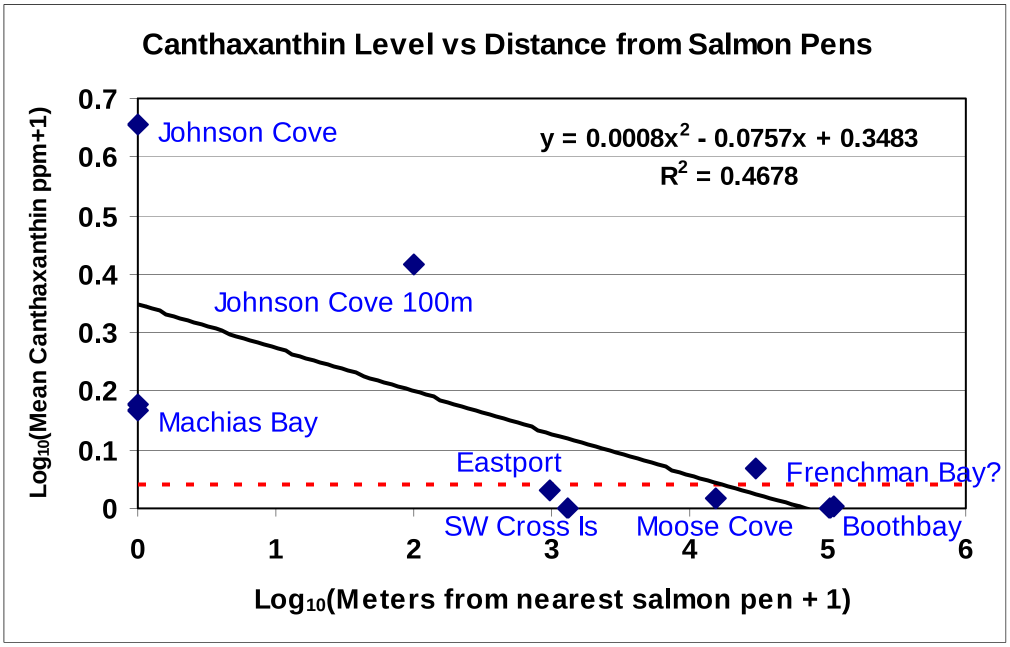

Figure 5. Mean canthaxanthin level vs distance from the nearest salmon pen. Red dotted line is Japan's MRL (0.1 ppm).

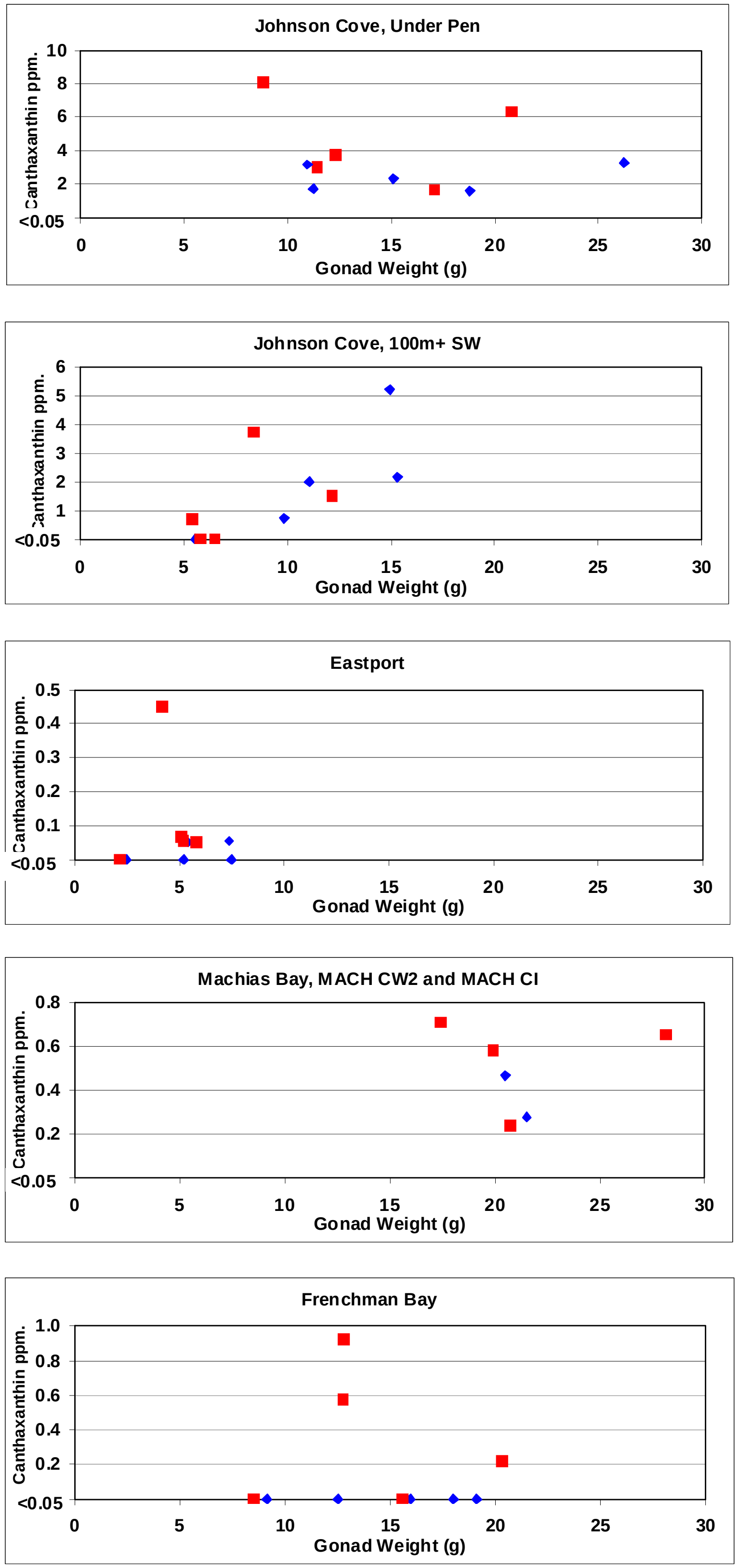

Figure 6. Canthaxanthin levels from individual urchins by gonad weight, with sex (■Male ♦Female).

Appendix 1. Letter from the DMR commissioner, mailed to all licensed members of the Maine sea urchin industry, October, 2007.

STATE OF MAINE
DEPARTMENT OF
MARINE RESOURCES
21 STATE HOUSE STATION
AUGUSTA, MAINE
04333-0021

JOHN ELIAS BALDACCI
GOVERNOR

GEORGE D. LAPOINTE
COMMISSIONER

To Maine Sea Urchin Harvesters, Tenders, Buyers, and Processors

The Maine Sea Urchin Zone Council, at its last two meetings, has been discussing the issue of Maine urchins being marketed in Japan with discoloration and an "off" taste. It is believed that these urchins are harvested under salmon aquaculture pens in eastern Maine and are picking up a compound that is used as a dye in the salmon aquaculture industry. Urchin industry members expressed a strong concern that the continued sale of these urchins could jeopardize Maine's position in the critical Japanese market.

Last spring, a shipment of sea urchin roe from Maine was tested by the Japan Ministry of Health, and found to have unacceptably high levels of canthaxanthin (by Japanese standards). Canthaxanthin is an FDA-approved additive used in feed for animals including cultured salmon. It is likely that these sea urchins were harvested from under salmon pens in Washington County.

Within the urchin industry, there is information Japanese will stop buying Maine sea urchins if high levels of canthaxanthin are found in them again. The enclosed, translated letter was received this summer by a Maine processor, and confirms that Japan will continue to test Maine urchins for canthaxanthin.

As we continue to learn more about this situation, we urge all Maine sea urchin divers to refrain from harvesting sea urchins under salmon pens. Draggers are not allowed to do so already. As we all work to better understand this issue, it is not worth risking damage to the Maine sea urchin fishery.

Please contact Sarah Cotnoir or the Sea Urchin Zone Council if you have any questions.

Sincerely,

George D. Lapointe
Commissioner

Appendix 2a. Letter received by a Maine sea urchin processor from Tokyo, summer 2007, translated by Atchan Tamaki, and mailed to all licensed members of the Maine industry in October, 2007.

To All Involved in the State of Maine Sea Urchin Industry

In the past years, we have enjoyed the high quality sea urchin products that you have exported to the Japanese markets. Thank you very much for consistently exporting high quality seafood products.

Now Japanese F.D.A. has introduced a list of unacceptable levels of food additives that includes canthaxanthin. The Japanese FDA will inspect all sea urchin products exported to Japan. If this additive, canthaxanthin is present in the seafood, it must be at the level below 0.1 ppm. Otherwise, the products will not be accepted for sale in the Japanese market

Currently, the U.S. FDA has approved the use of additive, canthaxanthin in farm raised salmon industry. This additive gives salmon orange color. But the additive, canthaxanthin is not acceptable in Japanese markets on the level at or above 0.1 ppm in any imported products.

The Japanese FDA believes that the additive, canthaxanthin is added to sea urchin roe voluntarily by the seafood processors. Japanese FDA doesn't have any data how much canthaxathin the wild sea urchin contains in the salmon pens. Therefore, the seafood processors are required to comply with the Japanese F.D.A. guidelines and not to harvest sea urchins underneath Salmon pens.

For all reason, we advise if you are harvesting sea urchins under the farm salmon pens, you are taking extremely high risk of harvesting contaminated sea urchin.

Tokyo Tsukiji Auction Market

Appendix 2b. The same letter translated by Minoru Kanaiwa.

Dear Maine sea urchin's participators

Thank you for continuing to provide high quality sea urchin. We will report the information from Dept. of Food Safety, Ministry of Health, Labor and Welfare which is showing the probability to make your guys a big disbenefit, because of "positive list" in force.

If Dept. of Food Safety will find canthaxanthin which is accepted in U.S.A. and Canada in imported sea urchin from Maine under import inspection, we must result that the canthaxanthin is accretive when Maine sea urchin was processed because Japanese side does not have any information about canthaxanthin of wild sea urchin in North America.
If the content amount is larger than 0.1 upm [Minoru's comment: they don't write the density] which is depend on the limitation of the "positive list", we cannot get the permission to sell it in Japan.

To turn over this explanation [? maybe, about hard to get permission], you need to provide the report and information which is showing the wild sea urchin in North America is including canthaxanthin, originally.

However after forcing this "positive list" rule, many problem has been found, the process of turning over will be very slow. In such a circumstance, you need to recognize there is very big risk in which you will export the sea urchin which has big probability of including canthaxanthin because it was caught under salmon pens, we advise to make self management to the catching area of sea-urchin.

Daito Gyorui Corporation
5-2-1 Tukiji, Chuou-Ku, Tokyo JAPAN

Appendix 3. Laboratory methodology (modified from Japan Ministry of Health, Labour, and Welfare, Department of Food Safety)

A. Sample Preparation
   1. Homogenize 5.0 g of sample (roe) with 30 mL acetonitrile, 20 mL n-hexane and 10 g sodium sulfate.
   2. Centrifuge homogenate for 5 min at 3000 rpm.
   3. Transfer acetonitrile and hexane fractions to a 125 mL separatory funnel collect the acetonitrile (lower) fraction.
   4. Add another 20 mL acetonitrile and the residual hexane fraction to the centrifuge pellet and re-homogenize.
   5. Centrifuge mixture again, at 3000 rpm and discard hexane fraction.
   6. Mix the two acetonitrile fractions together and add 10 mL n-propanol.
   7. Concentrate the resulting solution to 5 mL under nitrogen at 40 degrees C using a TurboVap (Zymark, Inc., Hopkinton, MA).
   8. Bring final evaporated sample to 10 mL (volumetric flask) with HPLC grade methanol.

B. Preparation of Standard Curve
   1. Prepare separate stock solutions of canthaxanthin (99%, Chromadex, CA) and astaxanthin (99%, Acros Organics, Morris Plains, NJ) by diluting 1.0 mg canthaxanthin to 10 mL and 25 mg astaxanthin to 50 mL with N,N-dimethylformamide.
   2. Mixed working standards ranging from 0.5 – 50 ppm are prepared by stepwise dilution with methanol.

C. HPLC Analysis

   1. HPLC System: Agilent 1100 with autosampler, diode array detector, gradient pump and Chemstation software.
   2. Column: YMC carotenoid, 5 micron particle size, 4.6 x 250 mm.
   3. Column Temp: 30 degrees C
   4. Mobile phase: 83% HPLC grade methanol, 17% HPLC grade hexane
   5. Flow rate: 0.8 mL/min.
   6. Wavelength: 470 nm
   7. Injection Volume: 25 ul
   8. Elution time(s): astaxanthin – 4.7 min, canthaxanthin – 6.3 min.