JULY 15, 2016

LOBSTER BLOOD PROTEIN AND MOULT CYCLE ANALYSIS 2016

GULF NOVA SCOTIA FLEET PLANNING BOARD

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Project Background

The health of lobsters for shipping and holding purposes is affected by their stage in the moult cycle. Knowing and even predicting the moult cycle stage of lobsters is therefore desirable for harvesters, buyers, and processors, in order to produce high quality, high price, lobster for the marketplace.

The moult cycle itself can be affected by multiple parameters, such as water temperature, photoperiod, diet, health and other environmental and ecosystem factors. It takes approximately 2 months for a lobster to return to high quality conditions (full meat and hard shell) after moulting (Retzlaff, et al. n.d.). Data on blood protein levels, moult stage, and shell hardness help us to understand the overall quality of lobsters for the purpose of holding and shipping.

There are many factors that can affect the overall health of a lobster, including biological, environmental, and human handling factors. This must be kept in mind when determining the health of the animal and interpreting data.

This project adds to the data and analysis that has been collected in previous years (Aquatic Science and Health Services, 2015) for the Gulf of Nova Scotia lobster fishery and builds upon the long-term data set.

Quality Assessment: The Brix Index and Moult Cycle Analysis

Brix refractometers[1] are used to measure the amount of sugar in a liquid sample; however, the Brix index (provided by the refractometer) is also highly correlated to lobster blood protein levels. The Brix index is now used by scientists and industry to assess lobster health and make decisions on holding, processing, and shipping lobster (Wang and McGaw, 2014; PRWeb, 2013).

A hard shell lobster that is full of meat will generally have a high protein level (i.e. greater than 8), while a soft shell lobster that is not full of meat will have a lower blood protein level. Additionally, a healthy, strong lobster will generally have a high blood protein level and a weaker lobster will generally have a low blood protein level (Wang and McGaw, 2014). Some live lobster dealers will only hold or ship lobsters (long term) with a Brix level of 10 or higher; some may use a Brix level of 8 depending on their standards (PRWeb, 2013).

Timing of the moult is most affected by water temperature. Likewise, lobsters at deeper depths are said to moult later than lobsters at shallow depths, due likely to the cooler temperatures in deeper water. Other factors can have some affect on moult timing as well, for example, smaller lobsters (under 82.5 mm) were found to moult later and less uniformly than larger (over 82.5 mm) lobsters in LFA 33 and 34, and the sex of lobsters may also influence when they moult (Retzlaff, et al. n.d.).

The stage of moult cycle is said to have the greatest effect on a lobster's blood protein level. When lobsters are preparing to moult they may lose about 30-60% of the tissue mass in their claws (Skinner, 1966 as observed in land crab), or perhaps even more (PARL, nd). This is necessary because the largest cross-sectional area of the largest part of the claw is about 10X that of the base, which it has to squeeze through during moulting (Spees et al., 2003). Shrinking of the claw, namely through dehydration, causes the lobster's blood protein levels to increase and concentrate. After moulting, when the lobster has a very soft shell, it will take in water to expand the new shell before it hardens. The lobster will be up to

[1] we use the ATAGO Pal-1 Digital Pocket Refractometer

15-20% larger than before moulting and its weight will increase by up to 40-50% (SLGO, nd). This rehydration will cause the blood protein level to decrease sharply (blood is diluted) (Figure 1). Muscle will slowly replace the water inside the shell, filling the shell with meat and raising the blood protein level, yielding a high quality lobster about 2 months after moulting (Retzlaff, et al. n.d.).

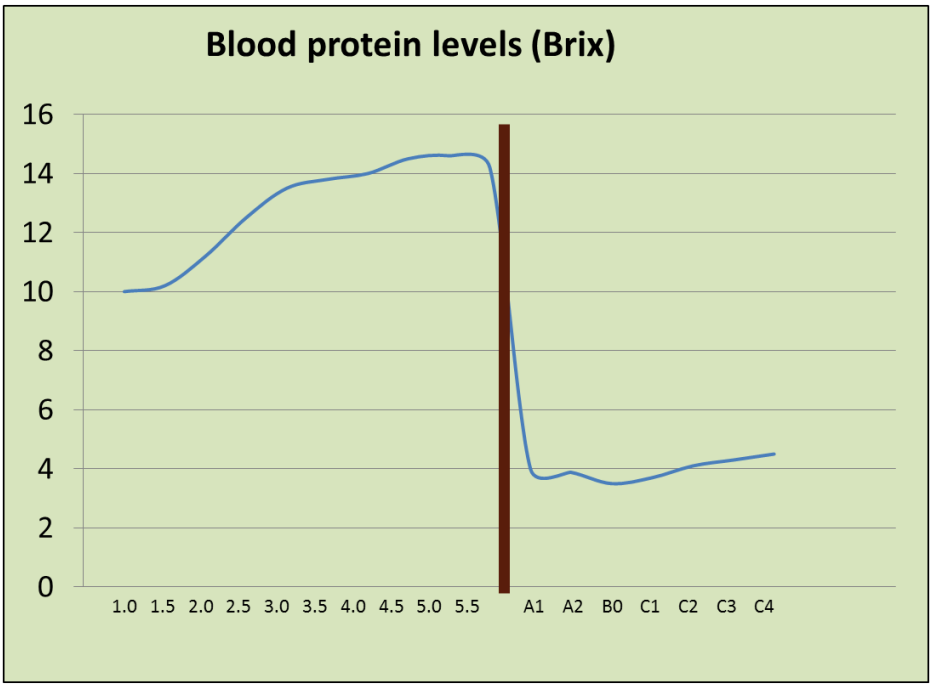

Figure 1. A normal lobster blood protein curve, over time. The sharp drop happens at the time of moulting when the lobster dilutes its blood to expand in size after moulting (Aquatic Science and Health Services, 2015).

Data Analysis and Results

Data was collected throughout the 2016 lobster season in LFAs 26 A and 26 B. A total of 4532 lobsters were sampled. Information on sex, size (carapace length), blood protein levels (Brix index), shell hardness, premoult stage, presence of cement glands in pleopods of females, presence of deformed pleopods, and other notable features. The data was collected from 6 sampling locations, during 51 sampling trips. All sites were sampled between May 1 and June 30, 2016, except for Cheticamp, which was sampled between May 6 and July 6, 2016 (Figure 2).

This section on Data Analysis and Results will review the findings from this year's sampling program, and it will be compared to last year's findings in the Interpretation section.

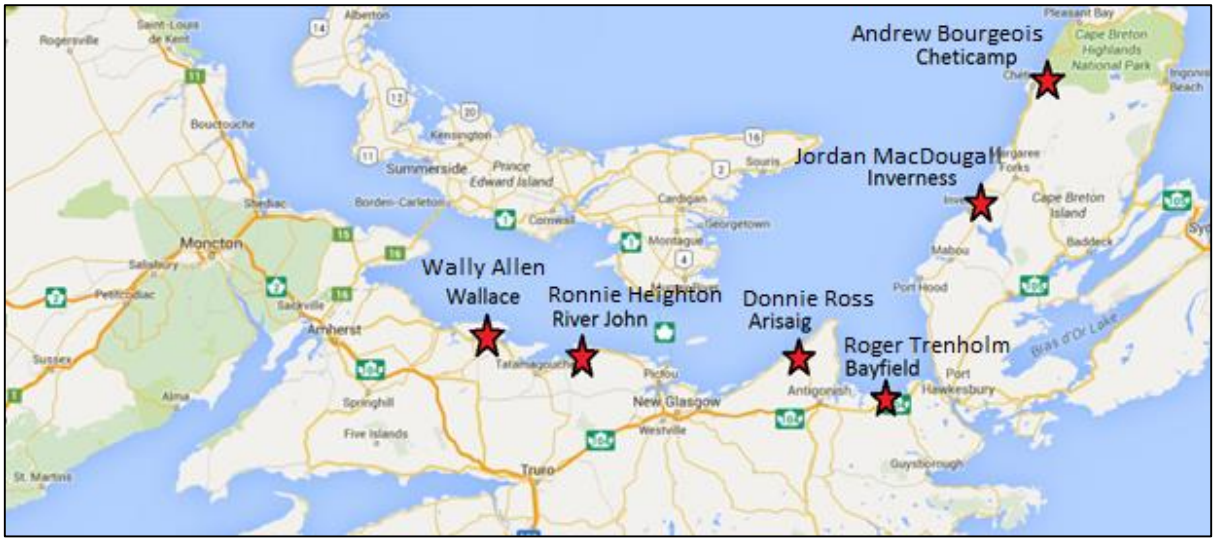

Figure 2. Sampling locations and participating harvesters, marked with stars.

Blood Protein Levels (Brix)

The protein levels over time were analysed for approximately 100 lobsters per sampling trip in all areas. Sometimes fewer lobsters were sampled because of low catches (i.e. the participating harvester did not catch 100 lobsters that day). According to the protein levels, shell hardness, and moult cycle analysis, the lobsters did not moult during the sampling period. However, high average Brix levels were recorded, particularly in River John, during week 8, and may be correlated with the water temperatures or indicate the spike in protein levels just before moulting (Figure 3).

When comparing blood protein levels between ports, River John had significantly higher levels than Cheticamp ($p<0.0001$). There was a significant difference ($p<0.0001$) between Wallace and River John blood protein levels, even though they are geographically near each other.

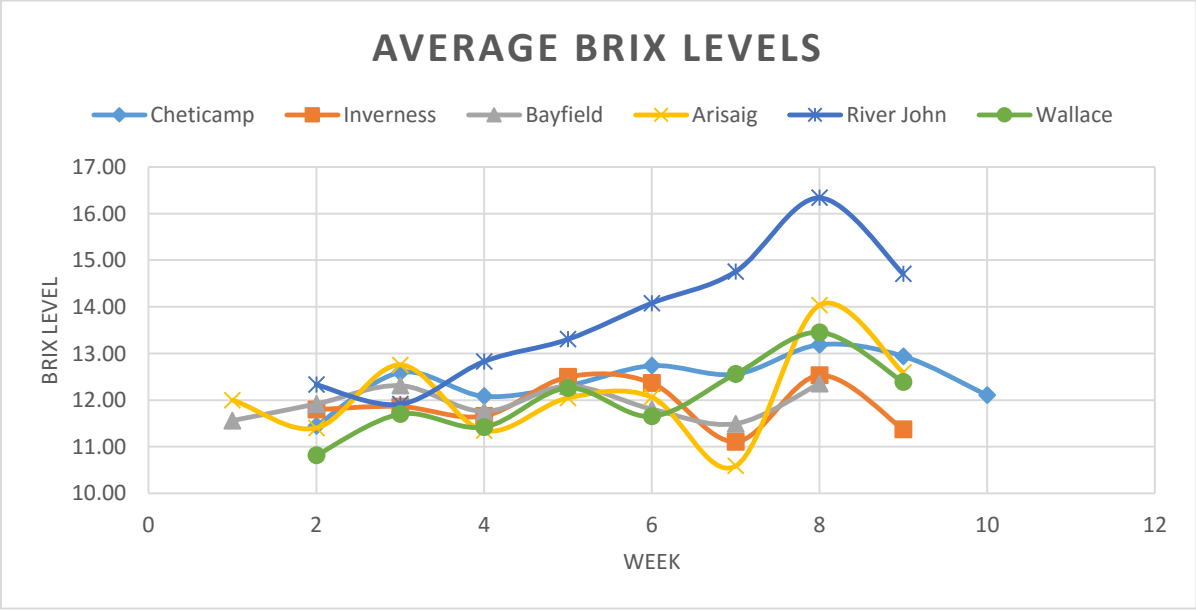

Figure 3. Average Brix levels by week for each sampling location.

Moult Stage

The lobster moult cycle has five different stages, A-E, which are based on physiological and carapace changes (Waddy et al., 1995). Stage D is the premoult cycle which lasts for about 24-75% of the entire moult cycle, and it is divided into five sub-stages in the literature. However, we group together the five sub-stages and use three sub-stages for the purpose of this project, which are described in Table 1, and pictures are provided in Appendix B. The premoult cycle was assessed through this sampling program by examining the tips of the lobster pleopods under a light microscope[2].

Table 1. The three sub-stages of Stage D, the premoult cycle.

Stage 0	Post/intermoult
Stage 1-2.5	Passive premoult
Stage 3 – 5.5	Active premoult – the lobster has committed to moult

Figures 5 - 10 below show the distribution of lobsters in each stage of the premoult cycle for each sampling location. Lobsters in the active premoult were first observed in week 3 (May 15-21, 2016) of sampling in Bayfield (11%), Arisaig (3%), River John (4%), and Wallace (6%). In Inverness, lobsters were first observed in active premoult in week 6 (June 5-11, 1%) and in Cheticamp they were observed in week 7 (June 12-18, 2%). Although there is variation between weeks in the sampling, the moult stage results seem to indicate that lobsters in River John and Wallace areas may be the first to moult while lobsters in Inverness and Cheticamp would be the last to moult.

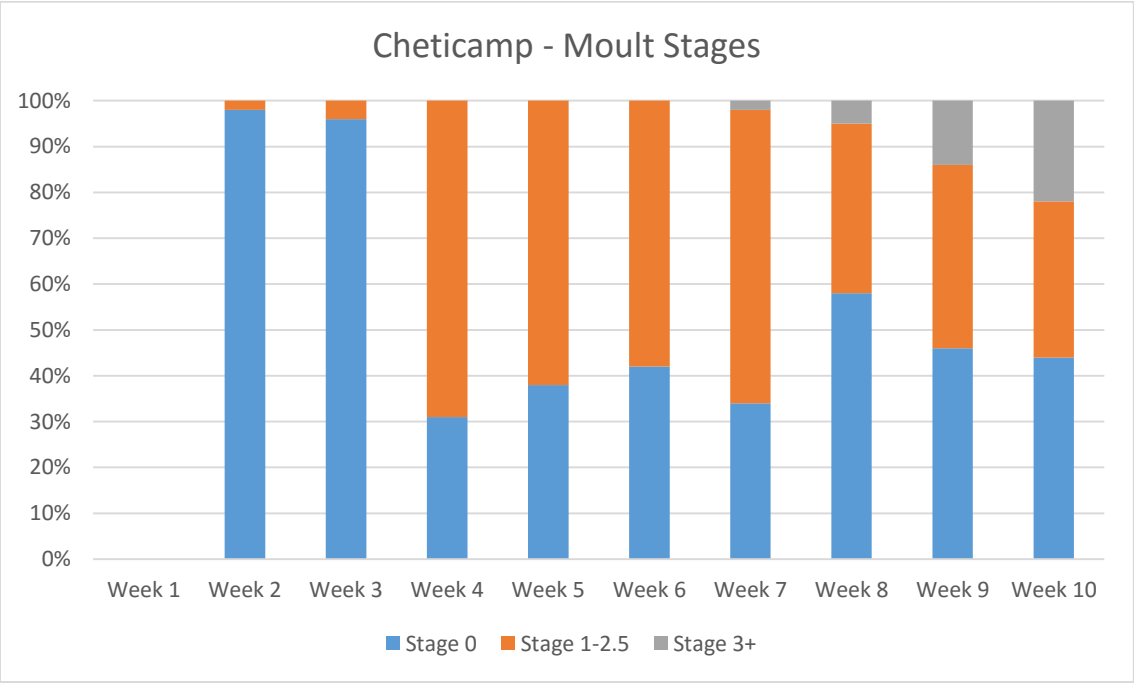

Figure 5. Moult stages by week for Cheticamp.

[2] Omano trinocular, fitted with an OptixCam camera

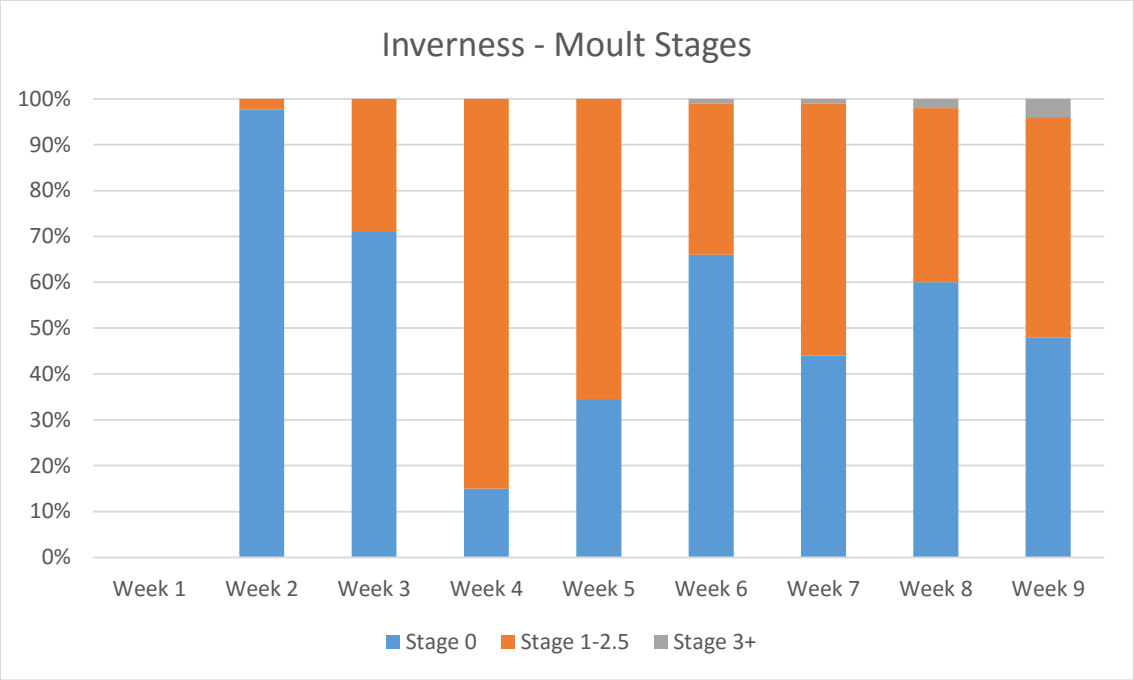

Figure 6. Moult stages by week for Inverness.

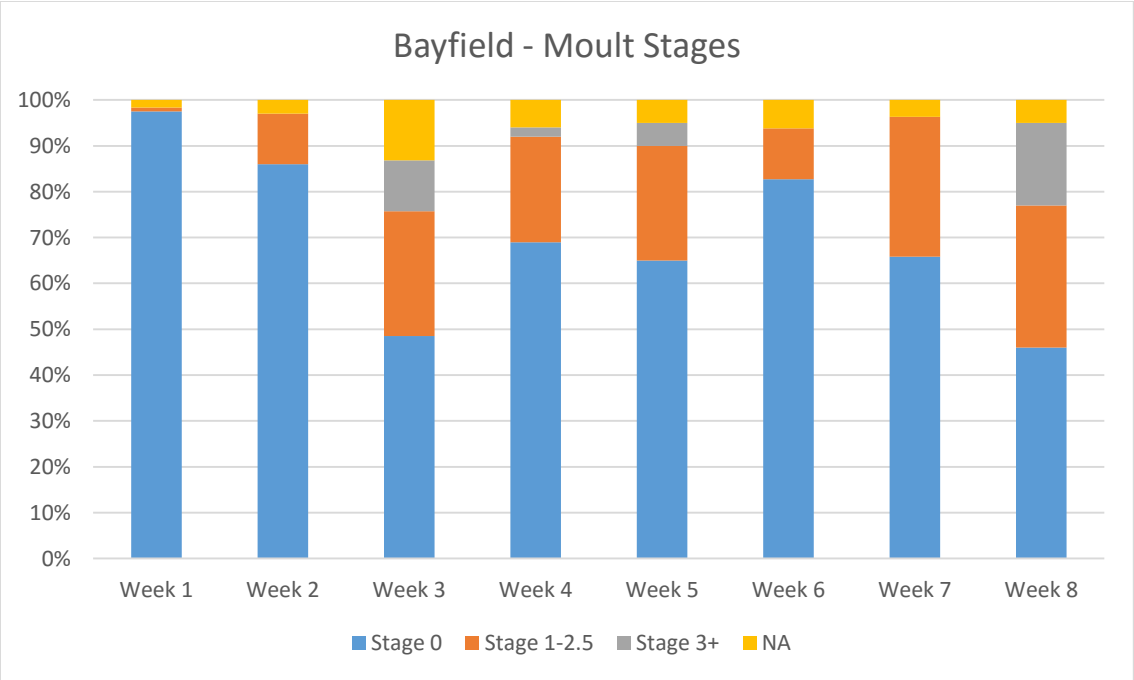

Figure 7. Moult stages by week for Bayfield. NA represents lobsters from the sample that were not staged due to deformities or presence of ciliates/parasite eggs.

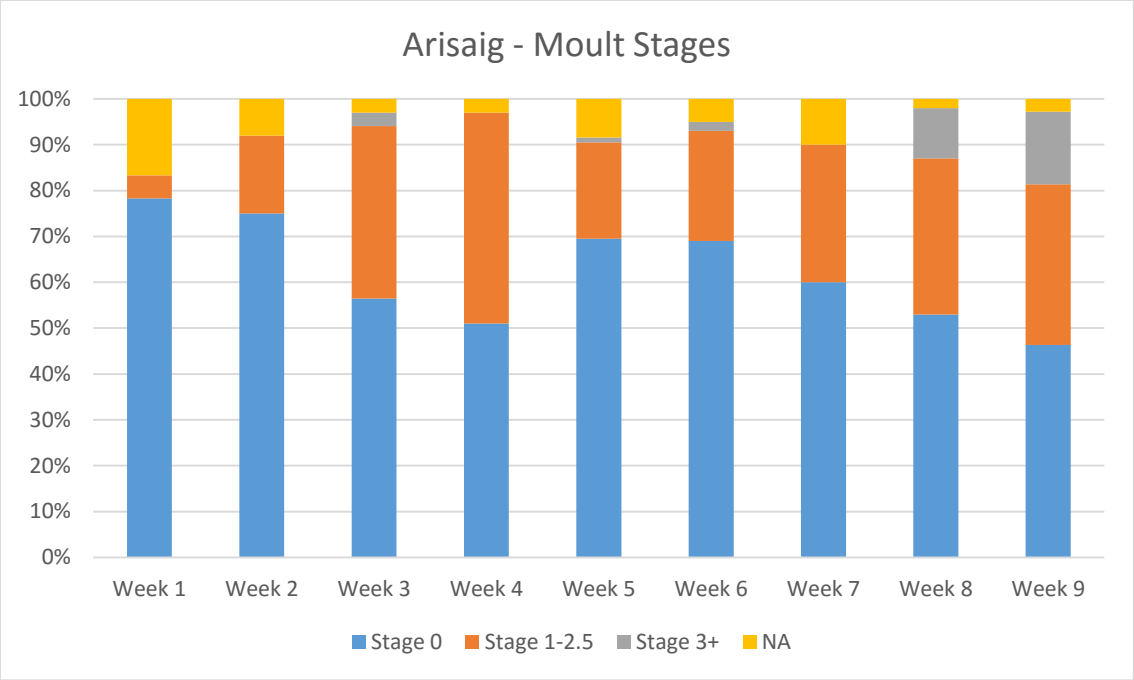

Figure 8. Moult stages by week for Arisaig. NA represents lobsters from the sample that were not staged due to deformities or presence of ciliates/parasite eggs.

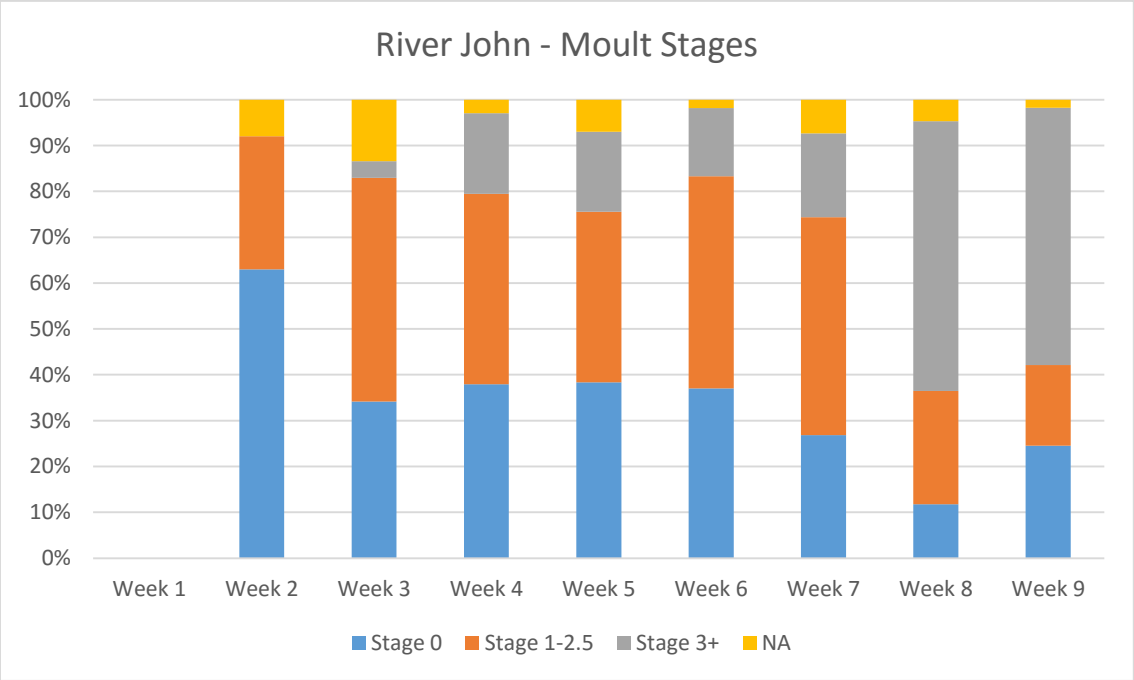

Figure 9. Moult stages by week for River John. NA represents lobsters from the sample that were not staged due to deformities or presence of ciliates/parasite eggs.

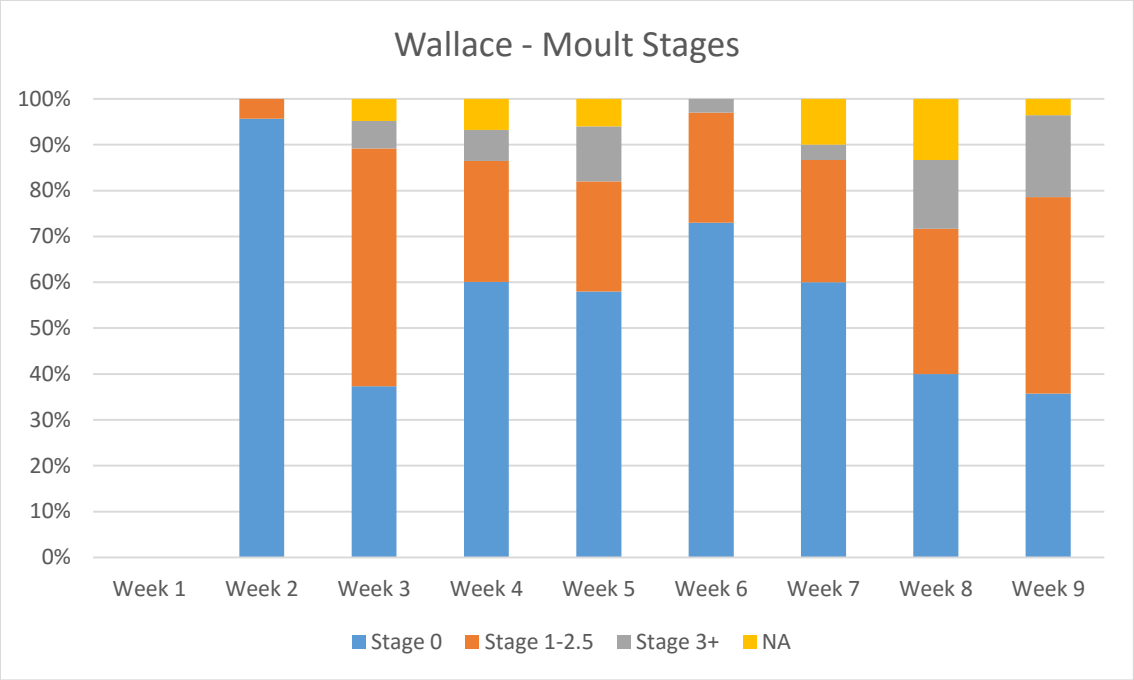

Figure 10. Moult stages by week for Wallace. NA represents lobsters from the sample that were not staged due to deformities or presence of ciliates/parasite eggs.

Shell Hardness

Data on shell hardness was collected through gently squeezing the carapace of the lobster and subjectively determining if the lobster had a soft, medium, or hard shell (Table 2). Almost all lobsters were hard shell during the time of sampling, with only few lobsters observed with a medium shell (total of 20 out of 4532 lobsters sampled), over the entire season.

Cement Glands

Cement glands have a cycle that follows ovary development. It is believed that cement glands secrete a substance that "cements" the eggs to the abdomen of female lobsters. Sexually mature female lobsters will exhibit cement glands, even if they are not carrying eggs. There are four stages of cement gland development, determined by the amount of the pleopod that is covered by glands.

Presence of cement glands was recorded for this study (regardless of the stage of the cement glands). Presence of cement glands in females tended to increase over the season for most sampling sites, with 67% (River John) - 93% (Cheticamp) of females having cements glands in week 8 (June 19-25). The steady increase in cement glands is very obvious for sampling sites in LFA 26 B, but not nearly as obvious for sites in LFA 26 A, where cement glands remained relatively more stable throughout the season. Appendix A shows the proportions of female lobsters with and without cement glands for each sampling location, during each week.

Table 2. Descriptive statistics from the 2016 sampling season. Week 1: May 1-7, Week 2: May 8-14, Week 3: May 15-21, Week 4: May 22-28, Week 5: May 29-June 4, Week 6: June 5-11, Week 7: June 12-18, Week 8: June 19-25, Week 9: June 26-July 2, Week 10: July 3-9.

Port	Week	Sex		N	CL (mm)		Protein level (Brix)		Shell hardness		
		male	female		mean	std. dev.	mean	std. dev.	soft	medium	hard
Cheticamp	2	61	39	100	87.98	3.97	11.45	1.72	0	0	100
	3	56	44	100	88.75	5.20	12.59	1.92	0	0	100
	4	49	51	100	88.82	4.69	12.08	2.15	0	0	100
	5	53	47	100	87.34	3.46	12.31	1.65	0	0	100
	6	47	53	100	88.69	4.75	12.74	1.82	0	0	100
	7	58	41	100	89.67	4.64	12.56	1.66	0	0	100
	8	44	56	100	90.32	5.54	13.18	1.56	0	0	100
	9	47	53	100	88.26	4.18	12.94	1.64	0	1	99
	10	51	49	100	90.06	5.09	12.11	1.60	0	2	98
Inverness	2	65	19	84	87.08	4.15	11.80	1.37	0	1	83
	3	43	57	100	86.71	4.41	11.86	1.72	0	0	100
	4	54	46	100	88.36	7.60	11.66	1.90	0	0	100
	5	36	63	99	88.18	5.65	12.49	1.43	0	0	99
	6	25	75	100	88.05	7.05	12.37	1.85	0	0	100
	7	51	49	100	89.56	6.64	11.11	1.65	0	0	100
	8	19	31	50	89.72	7.14	12.53	1.76	0	0	50
	9	52	48	100	93.37	9.13	11.37	1.37	0	0	100
Bayfield	1	77	43	120	86.81	5.35	11.56	2.26	0	1	119
	2	66	34	100	86.71	5.55	11.92	2.47	0	0	100
	3	68	32	100	89.44	7.48	12.31	2.63	0	0	100
	4	51	49	100	86.93	5.72	11.76	2.16	0	1	99
	5	57	43	100	89.30	7.45	12.30	2.06	0	0	100
	6	44	37	81	87.78	7.50	11.81	2.05	0	0	81
	7	49	33	82	89.46	6.92	11.49	1.90	0	1	81
	8	58	42	100	89.95	9.69	12.35	2.15	0	3	97
Arisaig	1	81	39	120	86.69	4.69	12.00	1.91	0	1	119
	2	61	39	100	87.32	6.01	11.40	1.87	0	1	99
	3	60	40	100	86.48	4.97	12.76	7.39	0	0	100
	4	66	34	100	88.90	8.29	11.35	2.30	0	0	100
	5	53	42	95	87.99	7.84	12.05	1.87	0	0	95
	6	55	45	100	88.30	5.91	12.06	1.86	0	1	99
	7	59	41	100	92.77	10.43	10.59	2.25	0	2	98
	8	49	51	100	91.23	10.89	14.03	1.89	0	1	99
	9	45	30	75	90.56	13.36	12.59	2.24	0	0	75
River John	2	87	13	100	83.25	7.13	12.33	1.99	0	0	100
	3	63	19	82	85.07	7.90	11.91	2.05	0	0	82
	4	64	17	81	85.26	8.05	12.83	2.32	0	0	81
	5	70	16	86	85.48	8.20	13.30	2.60	0	0	86
	6	42	12	54	86.69	9.35	14.08	2.10	0	0	54
	7	62	20	82	87.30	9.20	14.75	2.50	0	0	82
	8	68	18	86	84.90	8.31	16.34	2.30	0	0	86
	9	41	16	57	85.37	8.25	14.71	2.45	0	0	57
Wallace	2	66	26	92	87.47	14.04	10.82	1.74	0	0	92
	3	46	37	83	83.63	7.27	11.70	2.01	0	1	82
	4	30	35	65	85.54	8.65	11.42	2.04	0	0	65
	5	53	47	100	83.03	5.01	12.26	1.97	0	1	99
	6	43	47	90	94.36	5.85	11.65	2.05	0	0	90
	7	36	24	60	86.77	13.96	12.56	1.93	0	0	60
	8	34	26	60	93.53	17.80	13.45	2.34	0	2	58
	9	21	7	28	95.61	19.94	12.39	2.44	0	0	28

Ciliates and Deformed Pleopods

The presence of ciliates and deformed pleopods was recorded. However, there was some discrepancy in the sampling methods between the field technicians and no ciliates were recorded for LFA 26 B sampling sites.

No ciliates were recorded for the LFA 26 A sites (Bayfield, Arisaig, River John, and Wallace). However, low numbers of "parasite eggs" (Figure 4) were recorded by the field technician, and the prevalence of those was 2% in Bayfield and Wallace, 3% in River John, and 5% in Arisaig. No "parasite eggs" were recorded for LFA 26 B.

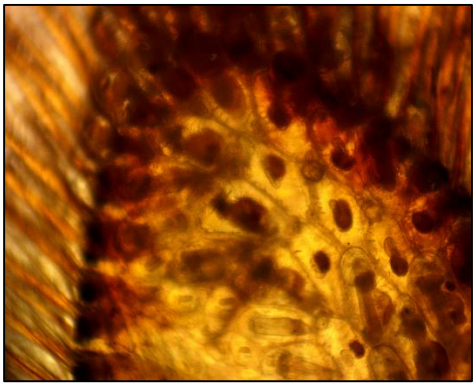

Figure 4. "Parasite eggs" inside of the pleopod.

The number of deformed pleopods was also very low, with 2% recorded in Arisaig, Bayfield, and River John, and 3% recorded in Wallace. The prevalence of deformed pleopods was 0.2% in Cheticamp and 0.6% in Inverness.

Interpretation 2015-2016

In 2015, data was collected from 4 sites: Port Hood, Bayfield, Arisaig, and River John. A total of 3103 lobsters were sampled. Information was collected on sex, size (carapace length), blood protein levels (Brix index), shell hardness, premoult stage, presence of cement glands in pleopods of females, presence of deformed pleopods, and other notable features. The data was collected during 29 sampling trips between May 13 and July 2, 2015 (Aquatic Science and Health Services, 2015).

In 2016, a total of 4532 lobsters were sampled. The data was collected from 6 sampling locations: Cheticamp, Inverness, Bayfield, Arisaig, River John, and Wallace. There were 51 sampling trips in total between May 1 and June 30, 2016, except for Cheticamp, which was sampled between May 6 and July 6, 2016.

Based on the data that was collected in 2016, the lobsters in LFA 26 A and B did not moult during the sampling season – this was evident as there were no soft shell lobsters recorded and no extreme drop in blood protein levels that is seen after moulting. This is good, and consistent with last year's study and other studies done in the area (Aquatic Science and Health Services, 2015, www.lobstermoult.ca). The lobsters therefore, are not likely to moult for at least another 2 weeks after the end of the sampling period (Aquatic Science and Health Services, 2015).

The average overall Brix levels for 2016 appeared to be slightly higher for all areas, compared to last year (Figure 5).

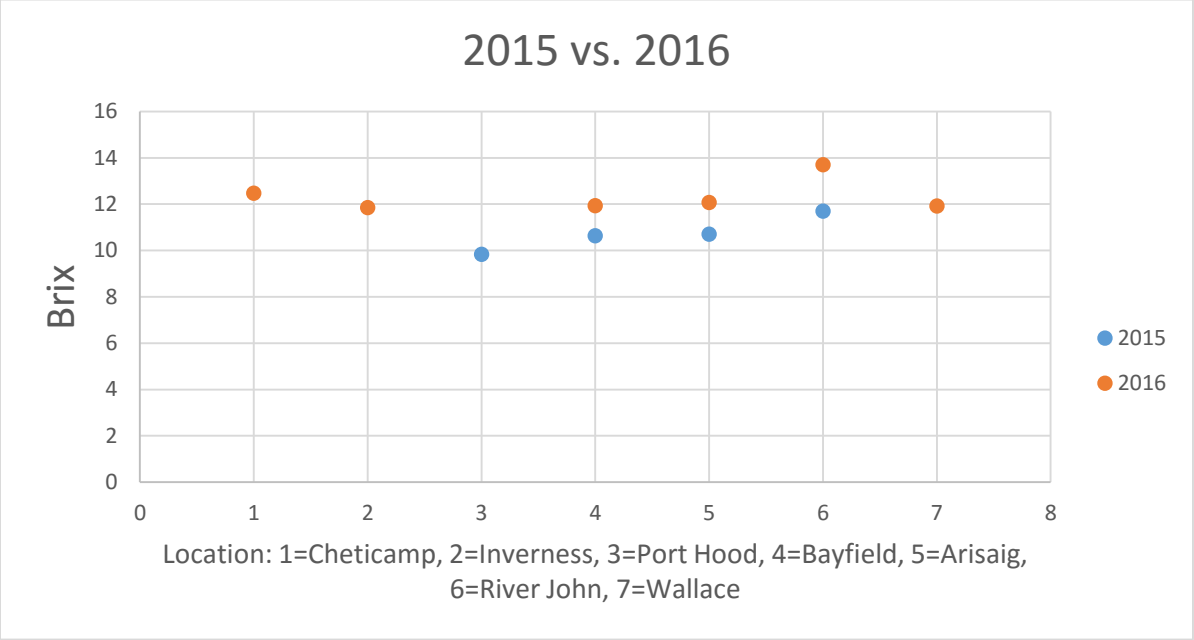

Figure 5. Average overall Brix levels for each sampling location in 2015 and 2016.

The 2016 results show very few lobsters (a total of 20 out of 4532 lobsters sampled) with a shell of medium hardness. This is much lower than 2015 when high numbers of medium shell lobsters were recorded, particularly in the first week of sampling (i.e. 44.4% in Arisaig and 48.5% in Bayfield). However, shell hardness is semi-subjective and findings can vary depending on who is doing the sampling. Nonetheless, it appears that this year's lobsters had reached hard shell state before last year's lobsters.

Presence of cement glands increased steadily over the 2016 season for LFA 26 B, which appears to be similar to all four of the sampling sites from 2015. However, the four sites in LFA 26 A that were sampled this year did not show an obvious increase in cement glands over the season, and prevalence of cement glands remained relatively high throughout the entire season (i.e. in Bayfield, 88% of female lobsters had cement glands in the first week and 90% had them in the eighth week). Compared to last year, the combined presence of cement glands was lower at the end of the season this year.

Presence of "parasite eggs" (ciliates) and deformed pleopods was very low in 2016. These findings were similar to last year. The presence of ciliates is not considered to be disease-causing, and the significance of them is unknown and may require further study.

Temperature loggers were provided by DFO for this project. They are retrieved in the Fall, and the data is not currently available. However, temperature is a major factor influencing the moult cycle and protein levels, and is a critical piece of information that should be included in analyses such as this. Once the data on temperature becomes available it will be compared to the finding presented here and interpreted.

This year's sampling marks the second consecutive year of data collection for this area by the Gulf of Nova Scotia Fleet Planning Board. Last year there were four sampling sites (River John, Arisaig, Bayfield,

and Port Hood), and this year six sites were sampled (Wallace, River John, Arisaig, Bayfield, Inverness, and Cheticamp). Each study represents a “snapshot” of that year, and results may vary from year-to-year. Long term data in this case is extremely valuable, in order to compare yearly results and build an understanding of the moult cycle and lobster quality in the Gulf of Nova Scotia. Blood protein analysis has been done in 26 B North for the past few years and they are now gaining a better understanding of the population in that area.

Acknowledgements

Funding to complete this project was provided by the Gulf Nova Scotia Fleet Planning Board, and Service Canada grants. DFO provided temperature loggers to be used in collaboration with this project. The field work was performed by two university students, Michael Brenan (St. FX University) and Douglas Cameron (University of Moncton). Training was provided to the students and the Science Coordinator (Andrea Flynn) by the Marine Research Centre at the University of Sainte-Anne (Michelle Theriault and Aleasha Boudreau). Harvesters who participated in the program by volunteering their lobsters to be sampled include Andrew Bourgeois (Cheticamp), Jordan MacDougall (Inverness), Roger Trenholm (Bayfield), Donnie Ross (Arisaig), Ronnie Heighton (River John), and Wallace Allen (Wallace).

Glossary

Blood: Lobster blood is the fluid that circulates throughout the body of the lobster, carrying gases (i.e. oxygen), nutrients, cellular waste, hormones, defence molecules, and hemocytes. The primary protein in the blood is the copper-rich hemocyanin (60-90% of total blood protein). Lobster blood volume is highest immediately after molt (about 55% of total weight), and decreases to about 30% by stage C of the molt cycle (Factor, 1995). Synonyms for lobster blood include: hemolymph, serum.

Cement glands: glands in the pleopods of mature female lobsters which are believe to secrete a sticky substance that “glues” the eggs to the lobsters’ abdomen once they are extruded.

Ciliates: parasite found in lobsters that can result in death of the lobster. An outbreak of ciliates was observed in at a holding facility in Nova Scotia, and it is commonly referred to as “bumper car disease”.

Deformed pleopod: deformation of the pleopod, not apparently due to an injury or mishandling.

LFA 26 A: mainland sites in the Gulf of Nova Scotia, including Wallace, River John, Arisaig, and Bayfield.

LFA 26 B: Cape Breton sites in the Gulf of Nova Scotia, including Inverness and Cheticamp. LFA 26 B include the North and South sub-management areas.

Moult: the process of lobster growth whereby a lobster sheds its shell.

Photoperiod: day length

Pleopod: the pleopod is an appendage attached to the abdomen of a lobster. They are used for swimming and females use them to attach eggs to their abdomen. Synonyms: swimmerets.

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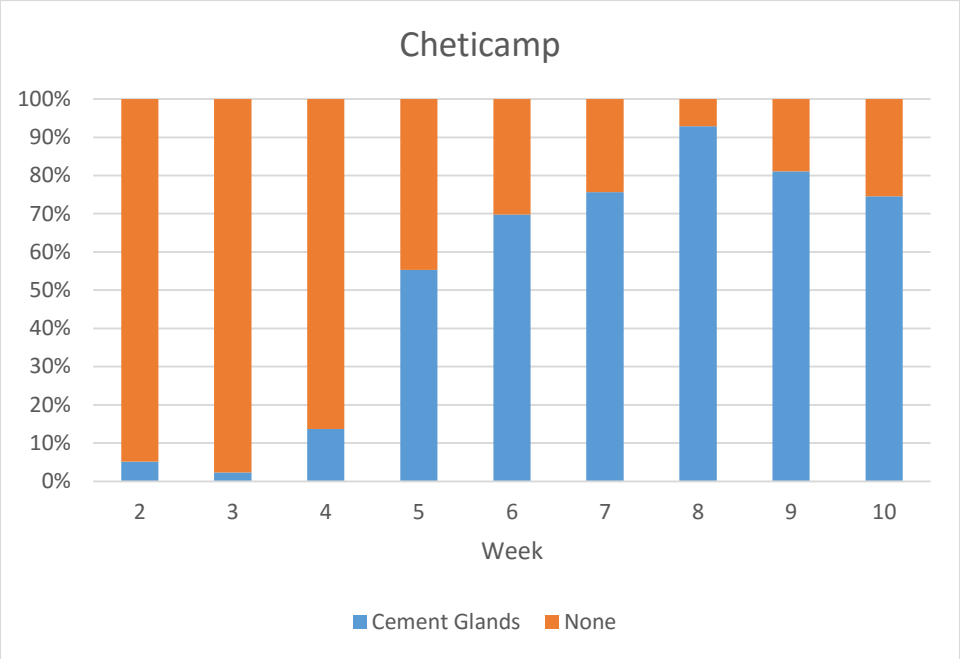
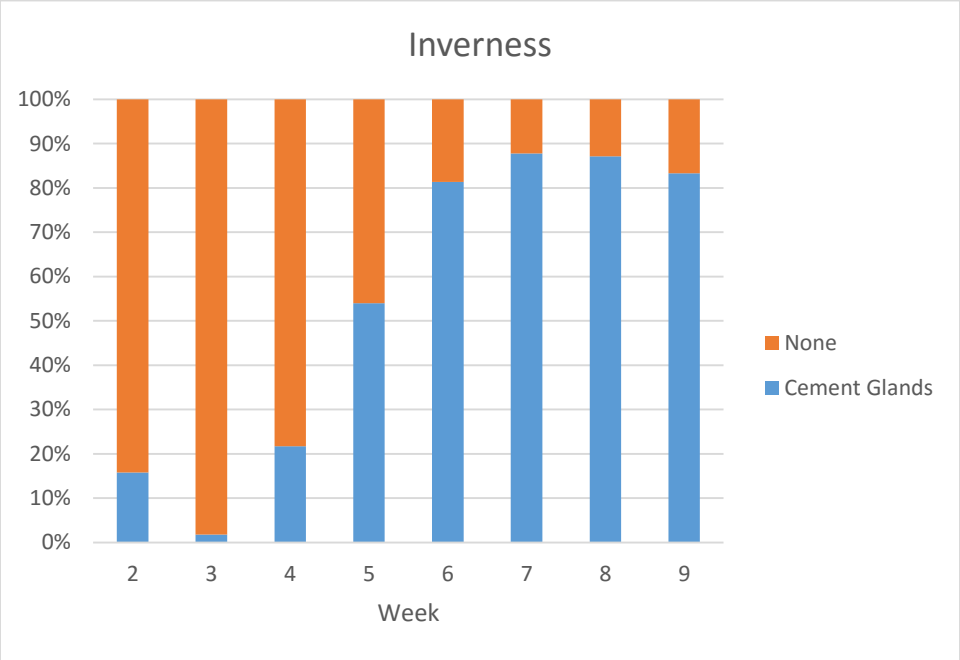
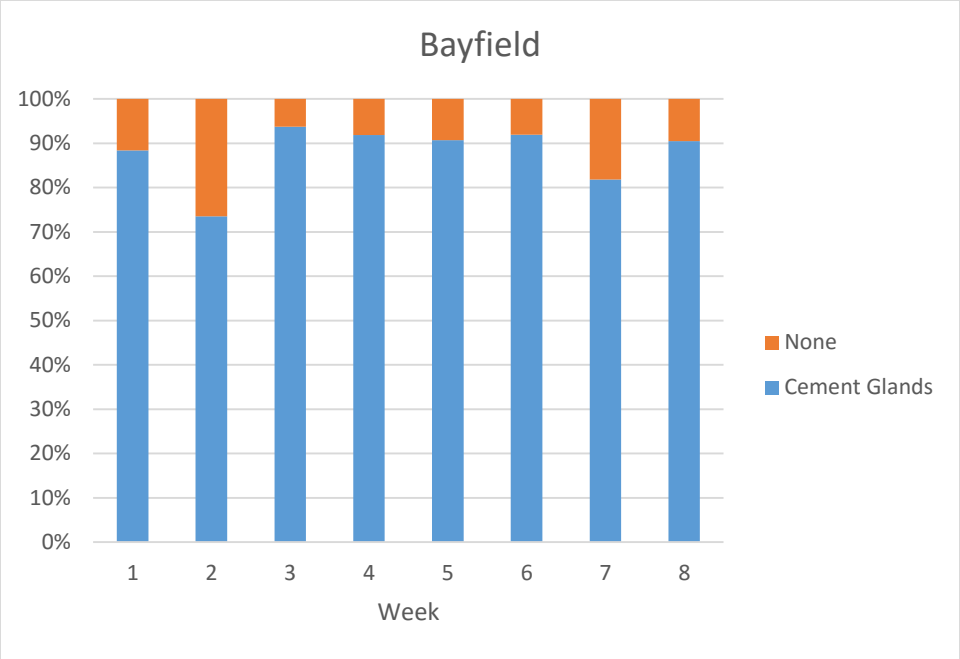
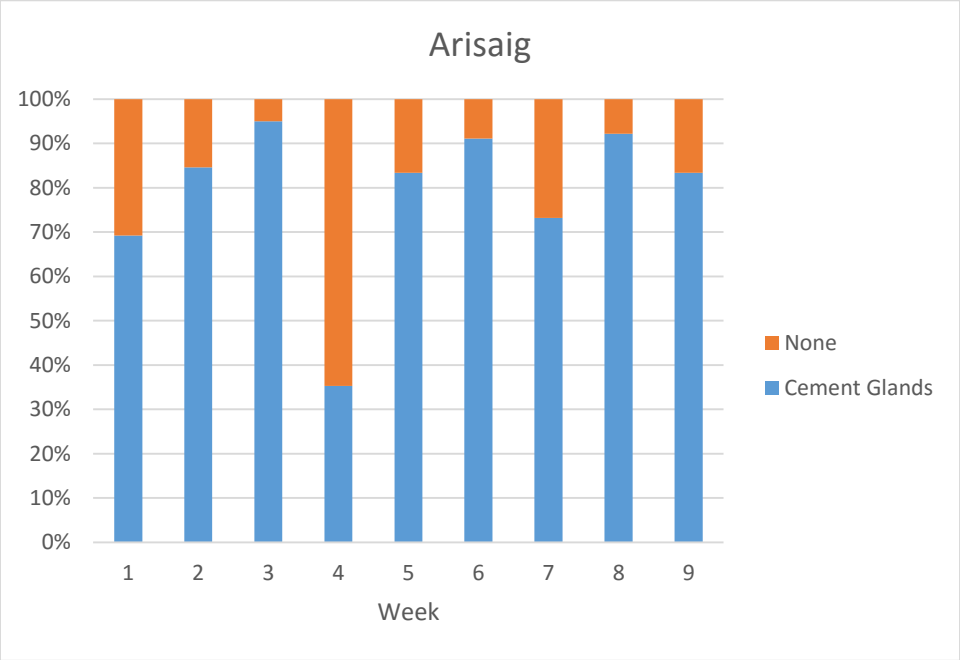
Appendix A

Proportion of female lobsters with and without cement glands for all 2016 sampling sites, by week.






Bayfield
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
3
4
5
6
7
8
Week
None
Cement Glands


Arisaig
100%
90%
80%
70%
60%
50%
40%
30%
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10%
0%
1
2
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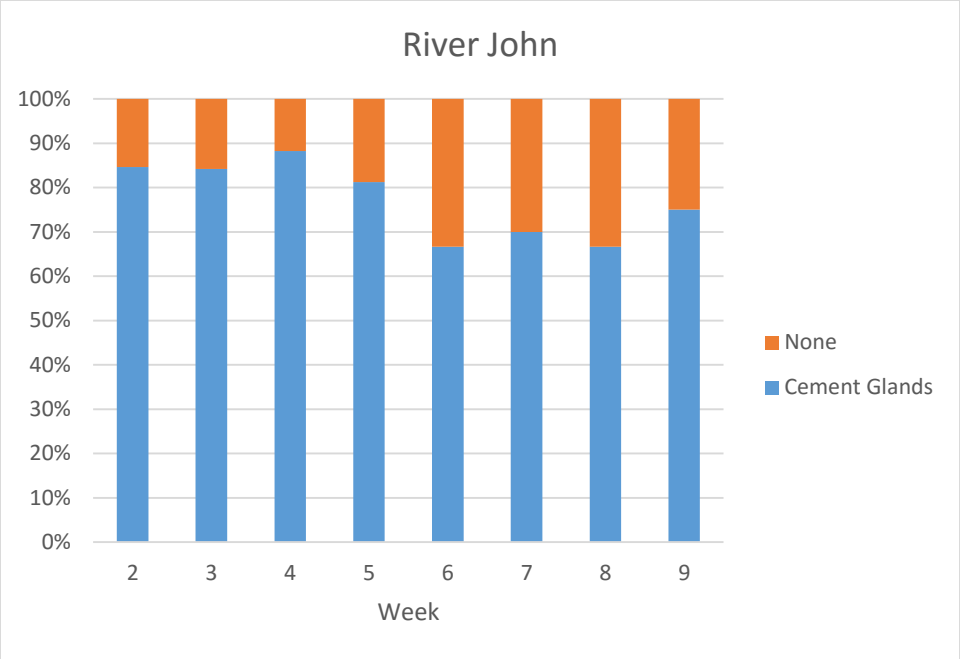
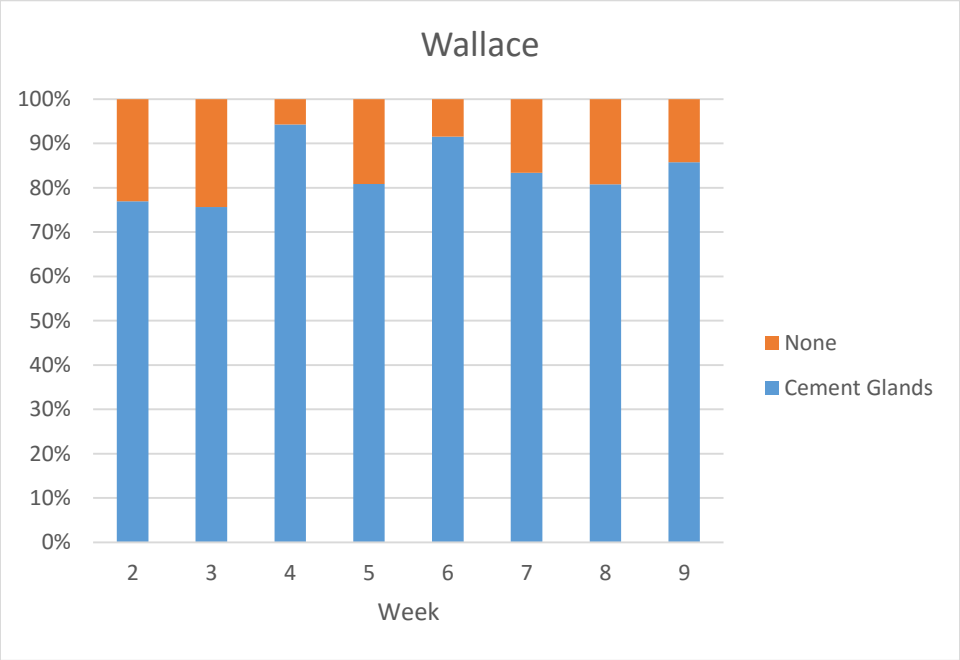
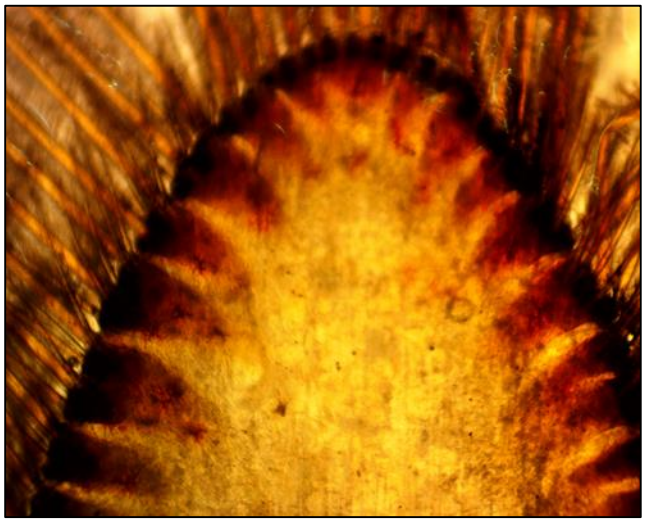
6
7
8
9
Week
None
Cement Glands


River John
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2
3
4
5
6
7
8
9
Week
None
Cement Glands

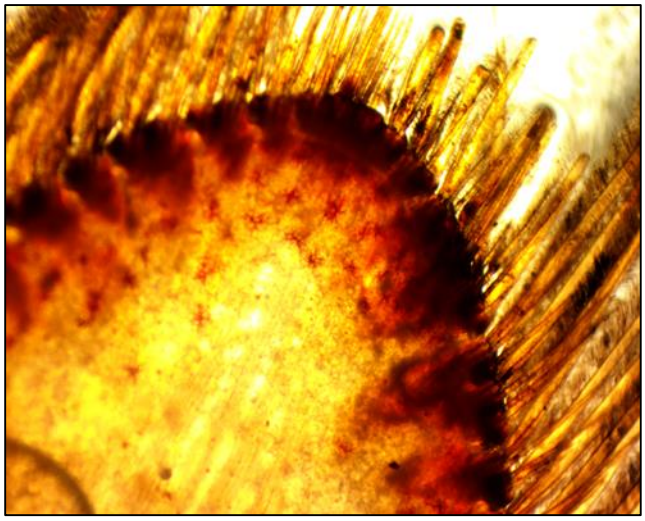

Wallace
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2
3
4
5
6
7
8
9
Week
None
Cement Glands

Appendix B

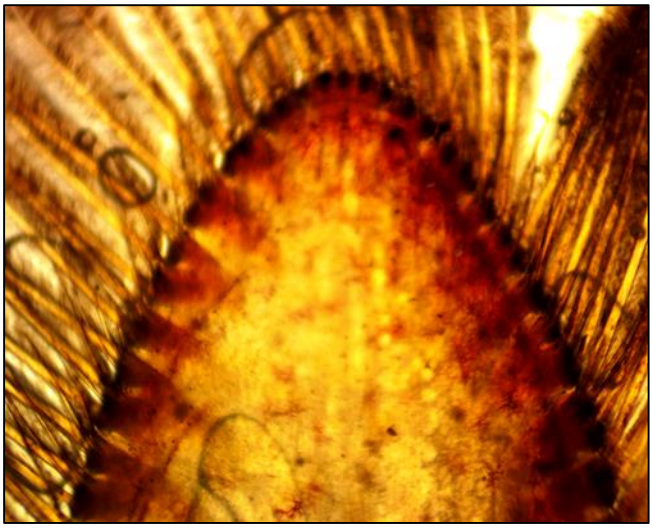
Pictures of various stages in the premoult cycle. Photographed using an Omano trinocular microscppe, fitted with an OptixCam camera, 2016 sampling.



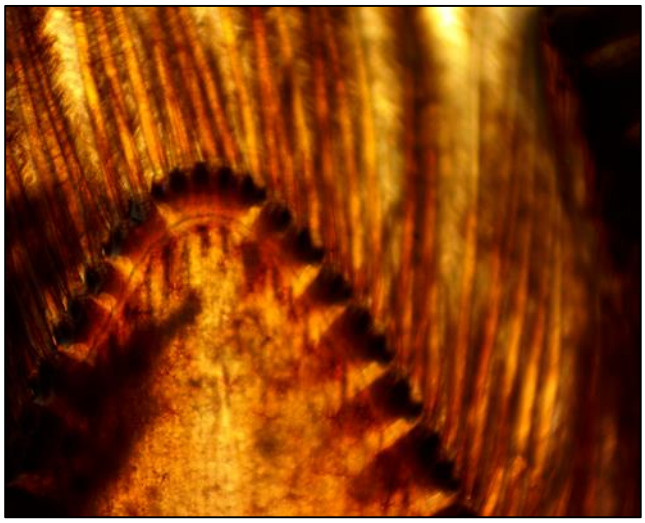
Stage 0, no line is visible.



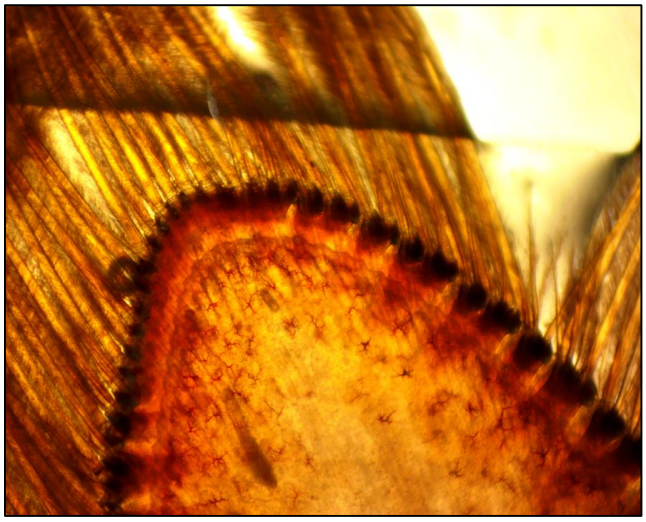
Stage 1-1.5. The line is becoming visible at the tip of the pleopod.



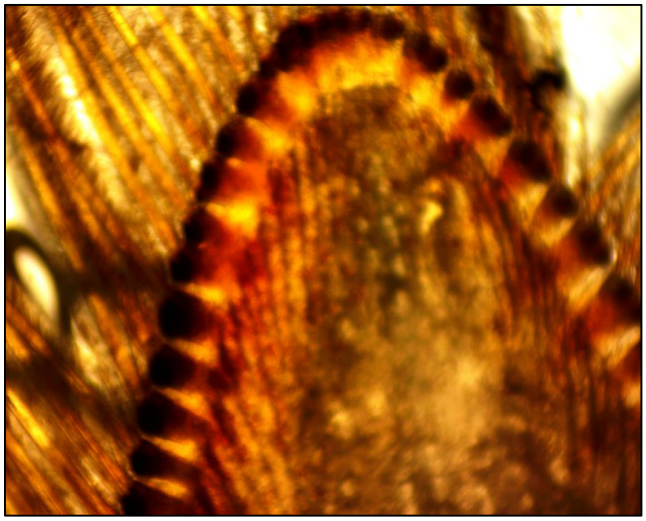
Stage 2-2.5. The line is becoming even more clear, and is pulling down and away from the tip of the pleopod, forming a “double-bordered” area at the tip.



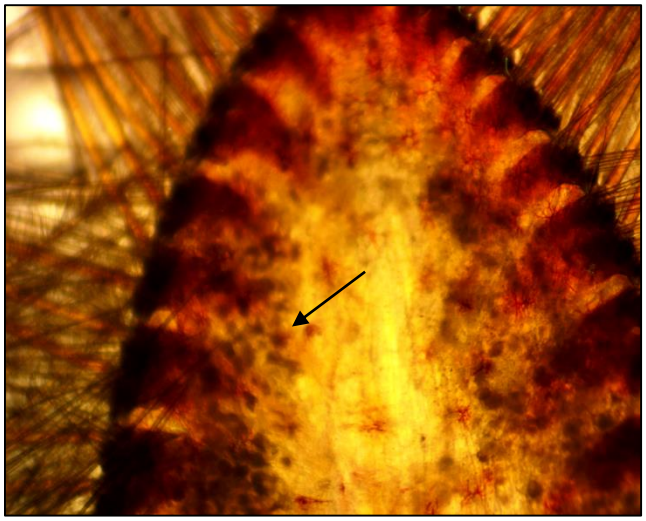
Stage 3-3.5. The line is very clear now, has pulled away from the outside of the pleopod and is beginning to look “wavy”. New setae are also becoming visible.



Stage 4-4.5. The wavy line is clearly visible here, as well as the developing shafts of new setae, but the proximal ends are not yet blunt.



Stage 5. Shafts of new setae are very clear and their proximal ends are becoming blunt.



Cement glands in a female lobster's pleopod (the small black dots, indicated by arrow).