Lobster Early Life-Stages:

Health Research Programme



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Final Report:
Atlantic Lobster Sustainability Foundation

In most animal populations, the younger life-stages are the most crucial and they often represent 'bottlenecks' in terms of survival. This project helped establish the framework for baseline information on larval health to be used for on-going health monitoring programmes.

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Background & Rationale

In most animal populations, the younger life-stages are the most crucial and they often represent 'bottlenecks' in terms of survival. Very little is known when it comes to larval and early post-larval lobster health. This proposed research programme will include a health baseline determination that will help build an on-going health monitoring programme. Finally, the results generated through this programme could be used to help design a suitable and appropriate pre-release health assessment protocol for Stages IV.

Assessing lobster larval health at multiple production stages in different hatcheries allowed us to look for disease(s) that may be causal or contributing factors for mortalities that routinely occur in hatcheries. With the recent interest in lobster enhancement initiatives, it is important to ensure that any hatchery-raised lobster being released into the wild is healthy, thereby protecting wild stocks from potential diseases that would be 'hatchery-based'.

Project Description

"Lobster Early Life-Stages Health Research Programme"

The project included the collection of healthy lobster eggs and Stages I thru IV. These samples were fixed and used to build a histological database of lobster larvae and eggs of normal tissues. We collected samples from three different batches representing three different 'production' times: an early summer batch, a mid summer batch and a late summer batch. Each batch consisted of samples from the same group of lobsters, from the egg stage to the time of release, that were followed over time within the hatchery.

Each batch had approximately 100 eggs and 100 Stage I, II, III and IV larvae collected and preserved in fixative. Of these, we examined with light microscopy approximately 50 eggs, 40-50 Stage I, 40-50 Stage II, 40-50 Stage II and 40-50 Stage IV. The remaining are stored as reference material.

Below are the major findings from the diagnostic work, while the three different histopathology reports along with any ancillary test results are presented as Appendices A-C.

Major Findings

1. **Surface fouling**

   A biofilm on the shell surface is not unexpected. It was noted that the amount of material increased as the larvae matured. Whether or not this is causing a problem for the larvae is unknown. It may be worth investigating ways to decrease the amount of biofilm buildup if it appears to be associated with overall survival in the tanks.

2. **Protozoal infection**

A protozoan organism was identified as part of the surface 'fouling' microbial community on stage 3 and 4 larvae in all three batches to varying degrees. The organism was also noted inside some stage 4 larvae (infected) where it did cause an inflammatory reaction by the lobster. In batch one (labelled 'crash batch') this organism was noted in ~ 20% of stage 4 larvae. Attempts to identify the organism using transmission electron microscopy and DNA isolation have been of limited success. While some possibilities have been ruled out by negative findings, no definitive identification can be made.

As the organism was found in all batches at low levels but at higher levels in the crash batch, it could be an opportunistic pathogen where an underlying problem or weakness in the larvae allowed the organism to overwhelm normally resistant larvae. It would be helpful to determine the source of the organism. Possibilities include: i) that it is part of the normal flora of the intake water or, ii) possibly introduced into the system e.g., via frozen feed.

3. **Cuticular erosions**

These were generally mild and random affecting low numbers of larvae. This could be secondary to trauma while in the rearing tanks, which could include sampling/net injuries. Whether or not this differs greatly from lesions that could occur in the wild is not known.

4. **Poor fixation of internal tissues in stage 4 larvae**

This was a common problem in the larger larvae which decreased the usefulness of these tissues. It is presumed that this is due to poor penetration of the fixative inside the larvae. Cutting the dorsal membrane behind the thoracic carapace may help increase fixative penetration.

Conclusions & Recommendations

This work constituted only our first attempt at describing normal and potentially abnormal lobster larvae histology. Therefore, this is the start of the baseline determination. Additional histology assessments should be conducted to better differentiate between normal and abnormal.

The principal goal of this project was to establish a histology baseline of normal larval lobster tissues. We examined approximately 150 lobster eggs and 350 larvae ranging from stage I to IV, Although some poor fixation of tissues which impaired somewhat our ability to accurately examine some of the larger stages, we were able to describe normal histological findings while also reporting on the presence of an unidentified fusiform protozoan. Among other, we noted the presence of a biofilm on the surface of the larvae that seemed to increase as the lobster matured, while some random and mild cuticular erosion was seen in a low number of larvae.

A secondary objective was to identify potential pathogens of lobster early life cycle stages. We were able to identify a protozoan organism as part of the surface 'fouling' microbial community on stage 3 and 4 larvae in all three batches to varying degrees. The organism was also noted inside some stage 4 larvae (infected) where it did cause an inflammatory reaction by the lobster. Attempts to identify the organism using transmission electron microscopy and DNA isolation have been of limited success. As the organism was found in all batches at low levels but at higher levels in the crash batch, it could be an opportunistic pathogen where an underlying problem or weakness in the larvae allowed the organism to overwhelm normally resistant larvae.

It would be helpful to determine the source of the protozoan organisms. If these are part of the normal flora, then it is likely that they represent opportunistic pathogens, taking advantage of larvae already weakened due to some other cause. It would be important to determine if these protozoans are normally found on wild lobster larvae, as this could have an impact in terms of pre-release assessment if these protozoans are not normally found in the environment. Other potential sources of introduction could be with the feed, or other infected aquatic species within the research facilities/public aquarium.

Finally, we intended on using the information generated from this project to help draft a health monitoring programme specific to lobster hatcheries, including a pre-release assessment. We must be able to determine what is normal flora and what is unusual or problematic. It would be appropriate to continue this larval health sampling for a minimum of 2 years; this would help determine what is normal and expected and abnormal and unexpected in a hatchery setting. Nevertheless, the information collected in this project represents a significant building block in establishing a baseline histology for larval lobsters. Once the baseline data are more complete, a pre-release assessment and an ongoing lobster health management programme for lobster hatcheries could be designed and implemented. We recommend that other hatcheries be approached to become co-contributors in the establishment of a general health management programme.

APPENDIX A: Batch ONE - Report

Case Number:	U17650-09	**Site Collected**:	Shippagan, NB
Date:	September 9, 2009	**Submitted by**:	Homarus Inc.

History:
Lobster Early Life-Stages Health Research Programme: Batch One (June 2009). Regular health check. One tank did have increased mortalities at stage 3 larvae ("crash tank").

Final Diagnoses:

1) Gill fouling includes a fusiform protozoa in some cases ~ mild to moderate, first noted at stage 3 larvae
2) Gill inflammation ~ mild to moderate, subacute to chronic, first noted in stage 3 larvae
3) Fusiform protozoal infection with associated inflammation ~ mild to moderate noted predominantly in the 'crash' batch
4) Gastric epithelial cell karyorhexis, moderate, focal

Addendum - Examination of additional fixed larvae (September 23/09)
5) Cuticular inflammation with epithelial pyknosis and karyorhexis, erosion and pigment deposition - mild, multifocal stages 2 - 4. This included ulceration of the eye in a few larvae
6) Antennal gland tubule lumens contain hemocyte aggregates and, in one case cocci, in a few stage 3 and 4 larvae

Comments:

1) The degree of fouling is not severe, but may be impacting overall larval health and could be worth investigating further. Minor surface fouling also noted on some eggs. A pink , hyaline, angular material is present with fouling agents - possible food material?

2) No etiologic agent was associated with these lesions. A transient systemic bacterial infection with bacteria being trapped in the gill vasculature (filter effect) would be a possibility.

3) Protozoa found inside some larvae are accompanied by an inflammatory response indicating that these are true ante-mortem infections and not simply post-mortem invaders. The organism may represent an opportunistic pathogen as it was limited to the surface of other larvae. Why the organism could invade some larvae and not others remains unclear. Transmission electron microscopy results did not reveal any distinguishing characteristics. The working hypothesis is that these are Gregarine life stages. PCR testing on the fixed tissues will attempt to confirm this (results pending). Low numbers of these organisms were also noted on the exterior of the gills of some of the stage 2 & 4 larvae.

4) This was noted in only one of the 'crash batch' stage 3 larvae examined. It is an interesting finding. It may represent necrosis secondary to regional hypoxia or other insult. Also to be considered, would be the effect of a viral agent (this pattern has been observed in shrimp with some viral infections). As only the single larvae was affected, further testing (e.g. electron microscopy on the paraffin block) may not be rewarding.

ADDENDUM Sept 23/09:

5) This indicates that the cuticle has been damaged in some way (the exact cause is not apparent here) e.g., traumatic, infectious, toxic (?). No infectious agents were observed associated with the lesions.

6) This likely reflects either a time when bacteria were transiently in the circulation and subsequently filtered and removed by the antennal gland or, an ascending (entry via the urinary pore at the base of the antenna) infection.

Main Gross Findings:

None. Eggs and larvae submitted in fixative.

Main Histologic Findings:

Egg: lobster 1; 2 June 2009; tank 2-1

- No visible lesions.

Stage 1, lobster 1; tank 6-4; 2 June 2009

- Mild vacuolation in some muscle fibres, diffuse (Significance?).

Stage 2; lobster 1; 8 June 2009

- Gill fouling ~ mild, multifocal.
- Gill inflammation ~ focal, moderate hemocyte accumulation obstructing the gill filament with a small amount of pigment deposition with no organisms visible.
- Muscle fibre fragmentation ~ mild, focal, no inflammation.

Stage 3; lobster 1; 12 June 2009 tank 6-4

- Some larvae show moderate to marked autolysis. There is also melanisation and hemocyte aggregates in multiple areas, including gills, indicating inflammation occurred prior to the larval death
- Protozoal fusiform (~8-10 µm in longest axis)'tachyzoite' forms are noted in one larva in the circulatory space in one larva with autolytic changes (which could be an ante- or post-mortem 'infection').

Stage 3; crash batch 1; tank 6-4

- Mild to moderate surface fouling is present.
- Many larvae are severely autolysed with concurrent bacterial overgrowth.
- Protozoal fusiform organisms are found in ~20% of examined larvae. Some are in high numbers in the autolytic larvae. Protozoa surrounded by a hemocyte aggregate, with or without some pigment deposition.
- Also noted in one larvae is a locally extensive area of epithelial cell karyorhexis in the stomach (interspersed with normal nuclei). Similar cells were noted in very low numbers at an area of inflammation in a separate larva.

Stage 4; lobster 1; 16 June 2009

- Gill inflammation (≥3/10 larvae) ~ multifocal, moderate, occlusive with mixed hemocytes, organising and some pigment deposition. Karyorhexis noted. No organisms visible in lesions although there are some of the fusiform protozoal forms present as fouling agents on one larvae (organisms not noted inside the inflammatory lesions).

Ancillary Test Results:

Wet fixed tissue from the 'crash batch' were processed for transmission electron microscopy. Elliptical (~4 µm up to 10 µm) organisms lacking a cell wall or significant identifying structures such apical complex, cilia, or flagellae were identified in both intra- and extracellular locations. Organisms were found in myocytes and subcuticular epithelial cells. Organisms were found singly and in small groupings of upt to 10 to 13 organisms. Empty vacuoles were also found in these groupings possibly representing areas where the organisms had been. The organisms had one large central nucleus with an electron dense nucleolus (occasionally quite large). In a few instances, longitudinal division was suggested by the presence of an internal double membrane extending the length of the organism.

Pending Results:

PCR analysis to confirm suspicion of organism as a Gregarine life stage.

APPENDIX B: Batch TWO - Report

Case Number:	U25178-09	**Site Collected**:	Shippagan, NB
Date:	September 26, 2009	**Submitted by**:	Homarus Inc.

History:
Lobster Early Life-Stages Health Research Programme: Batch Two (July 2009). Regular health check. No increased mortalities reported.

Final Diagnoses:

1) Gill and surface fouling in eggs, stage 3 and 4 larvae, mild to moderate
2) Muscle fibre degeneration (stages 1 - 3), mild, multifocal
3) Poor fixation of hepatopancreas (stage 3 and 4)
4) Gill inflammation with pigment (melanin) deposition, mild, focal to multifocal in Stage 3 and 4 larvae
5) A small amount of a pink, hyaline, angular material is present with surface fouling agents - possible food material?

Addendum - Examination of additional fixed larvae (September 23/09)
6) Protozoal infection (stage IV larvae), mild multifocal

Comments:
1) The degree of surface fouling is relatively mild, however it could be worth investigating possible ways to decrease it.

2) There are changes in some of the muscle cells consistent with cell injury/death. This could be due to a multitude of causes - similar changes are seen in vertebrate muscle cells secondary to hypoxia or nutritional deficiencies e.g., selenium and vitamin E.

3) The breakdown of the hepatopancreas tissues in a few of these larvae was not accompanied by any inflammation. Poor penetration of the fixative into this deeper tissue, which is very rich in digestive enzymes, is the suspected cause. It may be necessary to experiment with alternate methods to fix these larger larvae which would allow for better fixative penetration.

ADDENDUM: Sept 23/09
6) Examination of additional fixed larvae showed the 'fusiform agent' identified in batch 1 to be in the surface fouling layer of a few of the stage 3 larvae. The agent was also found in inflammatory lesions in the gills of some stage 4 larvae.

Main Gross Findings:
None. Eggs and larvae submitted in fixative.

Main Histologic Findings:
Egg, Lobster 2
- Rarely, an egg with a small area of karyorhectic cells (possibly epithelial origin) is noted. No associated inflammation.

Stage 1, Lobster 2
- A few larvae show localised areas of myocyte degeneration evidenced by hypereosinophilic fibres, pyknotic nuclei, and fragmented (finely granular) myofibres.

Stage 2, Lobster 2

- Myocyte degeneration (hypereosinophilic cytoplasm, finely fragmented cytoplasm, pyknotic nuclei) noted as multifocal, mild, without associated inflammation.

Stage 3; Lobster 2

- Gill and surface fouling ~ mild to moderate.
- Myocyte degeneration ~ mild, infrequent.
- Hepatopancreas deterioration ~ poor cellular cohesion, no inflammation. Poor fixation suspected.

Stage 4; Lobster 2

- Gill fouling ~ mild to moderatel
- Gill inflammation ~ mild to moderate, multifocal, occluding and organising with a primarily semigranular hemocyte infiltrate, mild pigmentation, mild karyorhexis with no visible organisms in the lesions.
- Hepatopancreas deterioration ~ poor cellular cohesion, more prominent in the deeper sections. Poor fixation suspected.

Ancillary Test Results:
None ordered.

APPENDIX C: Batch THREE - Report

Case Number:	U25178-09	**Site Collected**:	Shippagan, NB
Date:	February 22, 2010	**Submitted by**:	Homarus Inc.

History:
Lobster Early Life Stages Health Research Programme: Batch Three (September 2009). Regular health check. No increased mortalities reported.

Final Diagnoses:

1) Protozoal surface fouling, mild to moderate, Stage 3 larvae (A & B)
2) Fusiform protozoal infection, mild, focal two Stage 4 larvae (A & B)
3) Hepatopancreas tubule dilation and attenuation, moderate, one Stage 4 larva (B)
4) Myositis, mild, multifocal, with intralesional helminth larvae, in two Stage 4 larvae (one each in B & C)
5) Hepatopancreatitis, mild, multifocal to diffuse, non-septic, in five Stage 4 larvae (A & C)

Comments:

1) A moderate degree of surface fouling is noted on the Stage 3 larvae as in *Batches One* and *Two.* It may be worthwhile investigating water quality at this stage/time.

2) The protozoal infection is still evident in Stage 4 larvae. The lesions are much milder than in the 'crash batch' (S3L1) specimens and fewer larvae are affected. We are still awaiting the PCR results on the earlier samples.

3) The cause of the hepatopancreas tubule dilation in one larva is not known. No infective, inflammatory or degenerative process was identified in the sections.

4) A few Stage 4 larvae had small foci of inflammation in the muscle tissue which appears to be in response to a 'worm' (likely a migrating larval stage of a helminth). Whether this represents a normal or aberrant migration is unknown - identification of the organism would be required to determine this.

5) The hepatopancreas in some of the Stage 4 larvae show evidence of inflammation ('activated' fixed phagocytes and/or increased hemocytes). One possibility is that this represents reaction of this tissue to bacteria entering the larvae from the gastrointestinal tract; however, no infective agent was identified in the sections examined. Approximately 13% of the larvae examined were affected. Some dead larvae with secondary bacterial overgrowth were noted in the Stage 4 specimens. Was there above average mortality in these tanks?

Main Gross Findings:
None.

Main Histologic Findings:
Eggs (n = 40):
- Mild surface foulingl

S1L3A (n = 33):
- Small amounts of ingesta noted in gastrointestinal tracts. Lipid vacuoles evident in hepatopancreas.
- Myocyte degeneration represented by increased cytoplasmic eosinophilia, rounded fibres, and mild fragmentation (x 2 larvae).

- Hemocyte nodule, focal, moderate, no visible organisms (nvo) in perihepatopancreas location (x 1 larva).

S1L3B (n = 36):
- Small amounts of ingesta noted in gastrointestinal tracts. Lipid vacuoles evident in hepatopancreas.
- Myocyte degeneration, focal, mild (x 1 larva).

S2L3A (n = 34):
- Marked vacuolar distension of all tissues of all larvae (presumptive freezing/fixation artifact). No indication of inflammation or necrosis in samples.

S2L3B (n = 32):
- Marked vacuolar distension of all tissues of all larvae (presumptive freezing/fixation artifact). No indication of inflammation or necrosis in samples.

S3L3A (n = 26):
- Protozoal surface fouling - diffuse, mild to moderate.
- Poor fixation affecting the hepatopancreas of ~ 50% of larvae.
- One larvae with a mixed hemocyte organising nodule in the hepatopancreas with a central foreign body (possible migrating larval form of a parasitic worm?).
- Cuticle erosion with pigmentation and inflammation, very mild, multifocal, rarely, bacteria noted within the lesion.
- Perihepatopancreatic hemocyte nodule, mild, focal, no visible agent (x 1 larva).

S3L3B (n = 28):
- Protozoal surface fouling - mild to moderate.
- Poor fixation in ~ 33% of larvae.
- Antennal gland - small, melanising, hemocyte nodule with +/- visible organisms in the tubule lumen (x 2 larvae).
- Dead larva with secondary bacterial invasion (x 1).
- One larva with a mixed hemocyte nodule in the circulatory space, nvo.
- Cuticular erosion with pigmentation, mild, multifocal, nvo (x 2 larvae).
- Myositis, focal, mild, non-melanising with karyorhexis, nvo.

S4L3A (n = 14):
- Poor fixation (x 1).
- Protozoal surface fouling - absent to moderate.
- Gill - one larva with mild, multifocal, small areas of melanisation and hemocyte aggregation and karyorhexis associated with small fusiform protozoal agents (as described in *Batch One and Two* larvae).
- Hepatopancreas - possible mild increase in hemocytes in periarteriolar region; increased vacuolation of the fixed phagocytes, nvo.
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S4L3B (n = 15):
- Poor fixation x 2.
- Protozoal surface fouling - mild.
- One larvae with marked dilation of hepatopancreas tubule and attenuation of epithelial cells.
- Myositis mild to moderate, focal, organising hemocyte aggregate with small amounts of karyorhexis noted (nvo) ; fusiform protozoal organisms (as described in *Batch One and Two* larvae) noted on external cuticle surface in close proximity to this lesion; same larva has increased prominence of fixed phagocytes in the hepatopancreas.
- Aggregate of organising hemocytes in the antennal gland tubule lumen with small amount of melanisation (nvo) (x 1 larva).

- Hepatopancreas - focal area of necrosis with inflammation and intralesional presumed helminth larva (organism not identifiable in section).

S4L3C (n = 15):
- Protozoal surface fouling - mild.
- Hepatopancreas - mildly increased cellularity in the periarteriolar region with some areas of karyorhexis and pyknosis (mild) and, increased vacuolation of fixed phagocytes, nvo (x 2 larvae). One larva with a focal area of necrosis and inflammation (nvo).
- Myositis - mild, focal, with intralesional presumed helminth larva (x 1 larva).
- Aggregate of organising hemocytes in the antennal gland tubule lumen with small amount of melanisation (nvo) (x 1 larva).

Ancillary Test Results:
None.