

## Comparison of bacterial and parasitic infection between upper Klamath lake and out-migrant (Fish Evaluation Station, FES) 0<sup>+</sup> suckers and chubs.

### Summary:

- Total sample size for sucker juveniles was 36. A majority of suckers died soon after capture and initial sorting indicating a low tolerance to stress. Histological examination did not corroborate disease signs to this response.
- While not the focus of this survey, the majority of chubs > 100mm in the FES catch had multiple lesions (clinical columnaris) and were moribund. It suggests that weak moribund fish were drawn into FES and may act as a disease reservoir in the region above the intake screens.
- Hepatocytes in both suckers and chubs had normal morphology and showed no signs of algal toxin response.
- While commonly observed on gills, the ciliate parasite, *Trichodina sp.*, was not associated with lamellar inflammation (hyperplasia) or necrosis, and could be viewed as a commensal organism for these fish populations.
- *Lernaea* infection was common to FES and lake captured suckers. External and internal parasites were common to both chubs and suckers.
- Higher numbers of bacteria were isolated from chub collected at FES compared to the lake during both sample dates. Signs of peritonitis were more prevalent in FES chubs.
- *A change in collection method for 0+chub on August 15 likely reduced sample bias and allowed for chi-square and odds ratio testing of Lernaea infection between FES and lake collection.*
  - ***FES chub were 1.46x more likely to be infected than lake cohorts. While having a low P- value (0.062), chubs with high bacteria counts were 1.45x more common at the FES than the lake collection.***
  - ***This preliminary data suggests that moribund fish are more likely to enter the FES than the general lake population. If this trend is correct, the use of FES catch disease incidence would likely overestimate disease in the lake populations.***
- Future questions:
  - Does the extensive FHM population enhance a multi-host fish parasite clade that affect suckers (*Myxobolus sp.*, *Lernaea* copepod, trematodes, cestodes, and nematodes)?
  - What is the health profile of fish in the FES forebay and does it increase transmission of bacteria and external parasites to fish moving through it?

Background: Primary questions as the basis of the effort.

1. Does systemic bacterial and/or external parasite infection during August result in obvious disease impacts on 0+ chub and suckers in Upper Klamath Lake?
2. Does the health status of out-migrant 0+ suckers, captured at the FES in August, represent the 0+ population in the southern portion of Upper Klamath Lake?
  - a. Can the FES be used to survey the lake population for health and condition?

Objectives:

- In August 2017, collect age 0+ suckers and chub in the south UKL (multiple site composite sample) and from the FES within the same time period.
  - Perform microbiological and histology evaluation of samples
  - Repeat this sample effort (permit sample maximum 50 suckers).
- If sample collection bias is minimized, compare (Chi-square) 0+ chub sample groups (same week) for high prevalence lesions and infections.

Methods:

*Fish samples* – At the A-canal Fish Evaluation Station (FES), a 20 fish subsample of 0+ chubs (> 40mm TL) from each 20 minute sample set between 20:00 and 22:00 was collected for external evaluation, microbiological sampling, and histology on August 1 and 15, 2017 (Aug01 by hand, Aug15 by 5mm bar grader) . Any 0+ sucker collected at this time was also sampled (maximum of 20 per sample site/event). Chub samples were not identified to species but likely were composed of two species in Upper Klamath lake (*Gila bicolor* tui chub, *Gila coerulea* blue chub). Several 0+ fathead minnows (*Pimephales promelas*) were included in the histology sample by error (late night focus issue). Similarly, juvenile suckers (0+) were not identified to species (*Deltistes luxatus*, Lost River sucker, *Chasmistes brevirostris*, Shortnose sucker). Up to eight trap nets were fished for 8-9 hours on the night of collection, the catch sorted for 0+ suckers and chub (8/1 by hand, 8/15 by 5mm bar grader), transported to the FES for sample collection (Figure 1). External features were recorded for all fish (total length, lesions, external parasites), bacterial sample collected from the kidney of all suckers (Brain Heart Infusion agar, BHIA) and subsample of chubs (Ampicillin Dextrin Agar, ADA), whole fish fixed in Davidson's fixative (all live suckers and 11-16 chub/site), and 5-chub pools of viscera collected for virology assay.

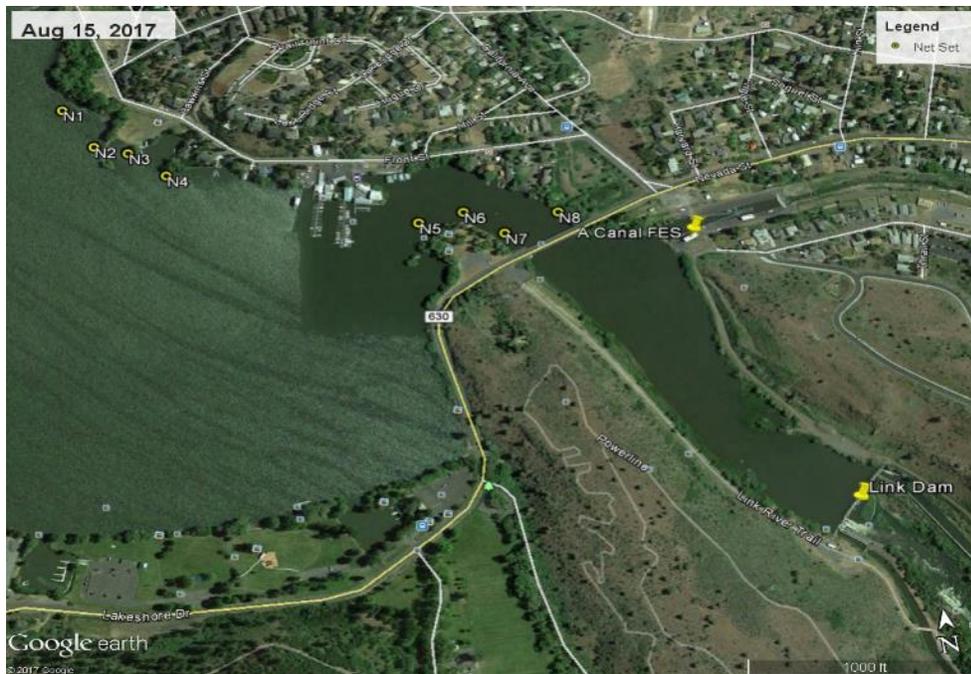


Figure 1. Sample sites for lake and FES collection (J. Ross, USBR).

**Bacterial assays** - Samples were incubated for 48h at 22°C and colonies tabulated. Preliminary bio-chemical tests were conducted on 1-3 individual colonies from each BHIA (sucker) plate consisting of  $\geq 10$  isolated colonies. We screened for gram-negative, oxidase-positive, non-spore-forming, rod-shaped, motile bacteria indicative of *Aeromonas* spp. Some presumptive *Aeromonas* spp isolates were run through the Biolog (Hayward CA) carbohydrate metabolism microbial identification system to identify them to species. Ampicillin Dextrin Agar (ADA, Hardy Diagnostics, CRTIERION™ Cat.no. C7581 with 10mg/L Ampicillin) is a selective media for *Aeromonas* spp. bacteria. Aeromonid bacteria yield faintly yellow to yellowish-green colonies. Biochemical tests were performed on a subset of isolates to differentiate Aeromonids from other bacteria.

**Virology**- A triangle portion of chub (including gill- anterior kidney, muscle) was pooled (5p) and screened for cytopathic effect on EPC, CHSE24(15°C) , and FHM (25°C) cell lines for 18 – 21 days. Detailed procedures and laboratory protocols can be found in The National Wild Fish Health Survey Procedures Manual.

**Histology** – Gill, viscera and kidney were dissected from fixed juvenile suckers and processed for 5µm H&E sections. Whole juvenile chub were decalcified in Rapid Bone Decalcifier for 2h and sectioned at a shallow and midline sagittal plane for H&E sections. On August 15, sucker livers were also fixed in Karnovsky's EM fixative to archive in case examination of light microscopy specimens showed abnormal hepatocytes.

**August 1 (20:00-22:00) Case 17-85,86,87,88**

Catches from the FES were predominately small cyprinids (< 40mm Tui chub, blue chub, and fathead minnows) and required hand sorting to obtain 0+ suckers and the subsample of 0+ chubs (> 40mm). This method of sample selection introduced an unknown level of bias. Similarly, trap net collections in the south lake were sorted for species (juvenile chub and sucker) by hand. The larger fork length of the lake chub sample may be related to this sorting method. Prior to sampling, 45% (5 of 11) of FES suckers and all 2 lake captures had died in the 20 – 60 min period after initial capture (Table 1). It is unclear if infectious disease was a significant mortality factor. While the majority of the suckers had *Lernaea* (69%, 9/13) and *Aeromonas* sp. (54%, 6/11) infections, few showed obvious clinical signs of disease such as ulcers or histological evidence of organ dysfunction due to infection.

Water quality was similar at both collection sites and typical of algal bloom conditions:

Lake (Link River Dam Gauge 4214011211480900)	25.9°C, 11.8mg/L DO, pH 9.6
FES	26.1°C, 12.1mg/L DO, pH 10.0

External observations (Table 1):

*Suckers - Lernaea* infection was common to both collection groups. Five of 11 suckers from the FES group died prior to necropsy and both lake samples were also dead. It is unclear whether the 55% prevalence of petechiae (minute hemorrhage) on the ventral surface of FES sucker could be related to their high prevalence of bacterial infection (Table 3) or collection trauma. Total length of the 2 sample groups were similar (mean 60 – 62mm).

*Chub* - Few chubs died prior to necropsy. *Lernaea* infection was observed in a lower percentage (3-11%) of chub compared to suckers. FES chub showed a higher prevalence of petechiae and trauma signs than the lake group. The large plerocercoid stage of *Ligula intestinalis* was observed in the peritoneal cavity of 2 fish. FES chub were smaller (mean 44mm) than those selected from the lake (mean 60mm) collection.

Table 1. External observations.

	TL(mm)	total sampled	Live	Dead	Lernaea	blackspot	petechia	trauma	Ligula
FES Chub	44 (4)	40	36	2	4	1 (3%)	5	9	2
POI					11%	3%	13%	24%	5%
Lake Chub	60(12)	37	36	1	1	0	2	1	0
POI					3%	0	5%	3%	0
FES Sucker	60 (7)	11	6	5	7	2	6	1	0
POI					64%	18%	55%	9%	0%
Lake Sucker	62 (1)	2	0	2	2	0	0	0	0
POI					100%	0%	0%	0%	0%

Histology (Table2)

*Suckers* - Lake samples were dead prior to fixation and post-mortem changes precluded interpretation of gill condition. Despite the presence of *Trichodina* (18%), FES sucker gill condition was considered normal. Presporogonic myxozoans were observed in kidney tubules of suckers from the lake and FES. No lesions were associated with this infection. Two fish had mild level of hyaline deposits in kidney tubules. Two of 6 sections, with view of the peritoneum, showed signs of peritonitis. No obvious bacteria or parasite were associated with the inflamed regions. Hepatocytes were normal in both groups (one mortality had eosinophilic cytoplasm droplets which could be an artifact).

*Chub* – *Trichodina* infestation of the gill was common (64%) to both lake and FES collection groups but was not associated with hyperplasia. Other gill parasites include monogenetic and digenetic trematodes. One gill section had presumptive epitheliocystis cysts. Intestine helminth parasites (trematode, cestode, and nematode) were observed in both groups (20 and 31%) but no lesions were associated with the infections. Presumptive *Myxobolus* sp. cysts were seen in 3 lake chub kidneys. Peritonitis was observed in the FES group (36%). Metacercaria (black spot) were seen in muscle tissue cysts from 11% and 18% of both groups (Fig. 4). Livers were normal.

Table 2. Prevalence of histological observations of juvenile chub and suckers collected in the south lake (Lake) or Fish Evaluation Station (FES) on August 1. Data includes multifocal gill lamellae hyperplasia (Gill L-HP3), ciliate external parasite *Trichodina* sp., myxozoan gill cysts (Myxoz.), other non myxozoan gill parasites, kidney parasites, hyaline droplets within tubule epithelial cells, hepatocyte abnormalities or parasites (abn/parasite), intestine abnormalities or parasites, inflammatory cells within the peritoneum (peritonitis), metacercaria cyst in muscle, and metacercaria within eye (diplostomid).

	N	Gill L-HP3	<i>Trichodina</i>	Myxoz. Cysts	other gill parasites	Kidney parasite	Tubule hyaline deposits	Liver abn/parasite	Intestine abn/parasite	peritonitis	muscle cyst - metacercaria	eye diplostomid
FES Chub	16	0/12*	9/14	4/14	2/14	0/15	1/15	0/16	5/16	5/14	2/15	1/16
POI		0	64%	29%	14%	0%	7%	0%	31%	36%	13%	0.1%
Lake Chub	11	0/11	7/11	0/11	2/11	3/11	1/11	0/11	2/10	0/10	2/11	0/11
POI		0%	64%	0%	18%	27%	9%	0%	20%	0%	18%	0%
FES Sucker	11	0/11	2/11	0/11	0/11	3/11	2/11	0/11 **	1/9	2/6	1/11	ND
POI		0%	18%	0%	0%	27%	18%	0%	11%	33%	9%	
Lake Sucker	2	ND	0/2	0/2	0/2	1/2	0/2	0/2	1/2	0/1	0/2	ND
POI			0%	0%	0%	50%	0%	0%	50%	0%	0%	

\*\* mortality - fixation artifact and hepatocyte eosinophilic deposits

ND not done as tissue was missing or compromised



Microbiological samples:

*Suckers* -Mixed bacterial isolates were isolated from most of the suckers (91% FES, 1 of 2 Lake) including Aeromonids. Isolates were not identified to species.

Table 3. Bacteria isolation data from suckers (August1).

	total sample	fish with growth	Gram positive rod	Gram positive coc	Aeromonid
FES Sucker	11	10/11	3/11	7/11	6/11
POI		91%	27%	64%	55%
Lake Sucker	2	1/2	1/2	0/2	0/2
POI		50%	50%	0	0

*Chub*- Bacteria were isolated from 50 – 63% of the sample group (Table 4) with the majority of isolates being presumptive *Aeromonas* bacteria (yellow colonies on the ADA media). Four of these presumptive *Aeromonas* isolates were tested in the Biolog biochemical system with following results: two were not identified to species but were in the *Aeromonas* genera, *Aeromonas caviae*, and *A. sobria*. A subjective threshold for significant infection level was set at  $\geq 5$  colonies with FES chubs having a higher prevalence (60%) of these significant infections than the lake chub (20%). No virus was isolated from either the FES or lake sample groups (8- five fish pools).

Table 4. Bacteria isolation data from chubs (August1).

	total sample	fish with grow	>5 colonies	Aeromonid	ADA non-yello
FES Chub	24	15	9	15	7
POI		63%	60%	63%	29%
Lake Chub	10	5	1	4	3
POI		50%	20%	40%	30%

**August 15 20:00- 23:00h (Cases 17-92 to 95)**

A 5mm fish sorting grate was used for both FES and lake captured fish. It was successful in excluding fish smaller than 40mm and allowed for several “grab sample” netting of subsamples from the size sorted collection to obtain the 60 fish sample (0+chubs > 40) without overt bias. The best design incorporated a 5mm metal grate into the bottom of plastic tote. At FES, the first 20 juvenile suckers of the 70 captured that night were sampled.

Water quality on this date was similar at both collection sites and typical of algal bloom conditions:

Lake (Link River Dam Gauge 4214011211480900) 21.8°C, 10.1 mg/L, DO, pH 10.3  
 FES 22.3°C, 11.5 mg/L, DO, pH 10.4

External observations (Table5)

*Suckers - Lernaea* infection prevalence was high (55 and 100%) but was not significantly different between the 2 collection groups similar (Chi-square, P=1.000). Five of 20 suckers from the FES group died prior to necropsy and one of the 3 lake samples were also dead. Fewer fish in either sample group showed external lesions (petechiae, trauma signs) compared to the August 1 collection. Mean total length of the FES group was 65mm while Lake suckers had a mean TL of 71mm.

*Chub* – The prevalence of *Lernaea* infection was significantly lower in lake chub (7%) than FES (37%),(Chi-square=9.744, 1d.f.,P=0.002). The odds ratio of *Lernaea* occurrence at FES was 1.46X higher than from the lake ((22+38)/(3+38)). Overt blackspot (metacercaria cysts in dermis was seen in 10-12% of the 2 sample groups however, only FES chub were observed with columnaris lesions (5%) and internal helminth infection (*Ligula* and nematodes). Similar to the suckers, FES chub mean TL (46mm) was smaller than lake chub (57mm).

Table 5 Mean total length (SD), number of live and dead fish at time of necropsy, external observations of *Lernaea*, blackspot (metacercaria), presumptive columnaris lesion, petechial, signs of trauma, internal helminths in peritoneum (*Ligula* plerocercoid and nematodes).

	TL(mm)	total sampled	Live	Dead	Lernaea	blackspot	columnaris lesion	petechia	trauma	Ligula	viscera nematode
FES Chub	46(6)	60	60	0	22	6	3*	1	3	1	3
POI				0%	37%	10%	5%	2%	5%	2%	5%
Lake Chub	57(8)	41	41	0	3	5	0	1	2	0	0
POI				0%	7%	12%	0%	2%	5%	0%	0%
FES Sucker	65(5)	20	15	5	11	2	0	2	1	0	0
POI				25%	55%	10%	0%	10%	5%	0%	0%
Lake Sucker	71(10)	3	2	1	3	0	1	1	1	0	0
POI				2%	100%	0%	33%	33%	33%	0%	0%

\* gram stain of 1 suspect lesion contained filamentous gram negative rods = "presumptive *F. columnaris*"

**Histology (Table6)**

*Suckers* - Both sample groups had few parasites or abnormality observations in their tissues. One exception to this trend was a high prevalence of hyaline deposits in kidney tubes (58%) and metacercaria cysts (“blackspot, 42%) in FES sample group. It is unclear what the cause(s) of the observed hyaline deposit in FES suckers. Hyaline deposits are proteins from the tubular filtrate and can indicate diuresis (increased urine formation). Factors associated with diuresis include alkaline conditions and elevated ammonia. This condition has been observed in both juvenile suckers from lake exposures and laboratory experiments. It is assumed that capture stress (time of handling to necropsy) was similar in both groups (Lake = up to hours in net with 1h post-collection to sampling, FES = up to 25 minutes in livebox with necropsy occurring 10 – 30 minutes post collection). Liver was normal in both groups.

*Chub* – In comparison to lake chub, FES chub had higher prevalence of peritonitis, metacercaria (blackspot, Fig. 4), eye diplostomid metacercaria (Fig 2.) and (similar to suckers) kidney tubule hyaline deposits (33%). *Trichodina* infection was common to both groups and was not associated with lamellar hyperplasia. Other gill parasites observed included myxozoan cysts (presumptive *Myxobolus* sp., Fig. 1), monogene flatworms, and metacercaria cysts. *Lernaea* holdfast was associated with inflammation (Fig.3).

Table 6. . Prevalence of histological observations of juvenile chub and suckers collected in the south lake (Lake) or Fish Evaluation Station (FES) on August 1. Data includes multifocal gill lamellae hyperplasia (Gill L-HP3), ciliate external parasite *Trichodina* sp., myxozoan gill cysts (Myxoz.), other non myxozoan gill parasites, kidney parasites, hyaline droplets within tubule epithelial cells , hepatocyte abnormalities or parasites (abn/parasite), intestine abnormalities or parasites, inflammatory cells within the peritoneum (peritonitis), metacercaria cyst in muscle, and metacercaria within eye (diplostomid).

	N	Gill L-HP3	<i>Trichodina</i>	Myxoz. Cysts	other gill parasites	Kidney parasite	Tubule hyaline deposits	Liver abn/parasite	Intestine abn/parasite	peritonitis	muscle cyst - metacercaria	eye diplostomid
FES Chub	12	0	7	2	2	0	4	0	1	5	3	2
POI		0	58%	17%	17%	0%	<b>33%</b>	0%	8%	42%	25%	17%
Lake Chub	12	4	2	4	1	0	0	0	0	2	1	0
POI		33%	17%	33%	8%	0%	0%	0%	0%	17%	8%	0%
FES Sucker	16		1	0	0	0	7	0	1	0	5	ND
POI		0%	8%	0%	0%	0%	<b>58%</b>	0%	8%	0%	<b>42%</b>	
Lake Sucker	3	0	0	0	0	0	1	0	0	0	1	ND
POI		0%	0%	0%	0%	0%	8%	0%	0%	0%	8%	

**Microbiological samples (Table 7 and 8)**

*Suckers* - Bacterial prevalence was similar in both groups (50 and 67%) with both aeromonad and gram positive bacteria (mixed colonies) present. The four presumptive aeromonad isolates from FES suckers were analyzed by Biolog and resulted in the following identifications: *Aeromonas popoffi*, *A. sobria*, *A. jandaei*, and one isolate without species identification with closest match being the genus *Aeromonas*.

Table 7. Bacteria isolation from juvenile suckers collected at FES and south lake on August 15.

	total sample	samples with bacteria	Gram positive rod	Gram positive cocci	Aeromonid
FES Sucker	16	8	4	1	4
POI		50%	25%	6%	25%
Lake Sucker	3	2	1	0	1
POI		67%	33%	0%	33%

*Chub*- Bacteria were isolated in 29 and 60% of the sample group kidneys (Table 8) with the majority of isolates being presumptive *Aeromonas* bacteria (yellow colonies on the ADA media). Twice as many FES than Lake chub had high bacteria counts. Fisher’s exact test, on the proportion of samples with greater than 5 colonies between FES and Lake groups, was not significant (P=0.062). No virus was isolated from either the FES or Lake sample group (12 five fish pools).

Table 8 Bacteria isolation on ADA media from juvenile chubs collected at FES and south lake on August 15.

	total sample	samples with bacteria	>5 colonies	Aeromonid	ADA non-yellow
FES Chub	30	18	11	16	4
POI		60%	37%	53%	13%
Lake Chub	24	7	3	6	7
POI		29%	13%	25%	29%

Figure 1 Presumptive *Myxobolus sp.* cyst in chub gill.

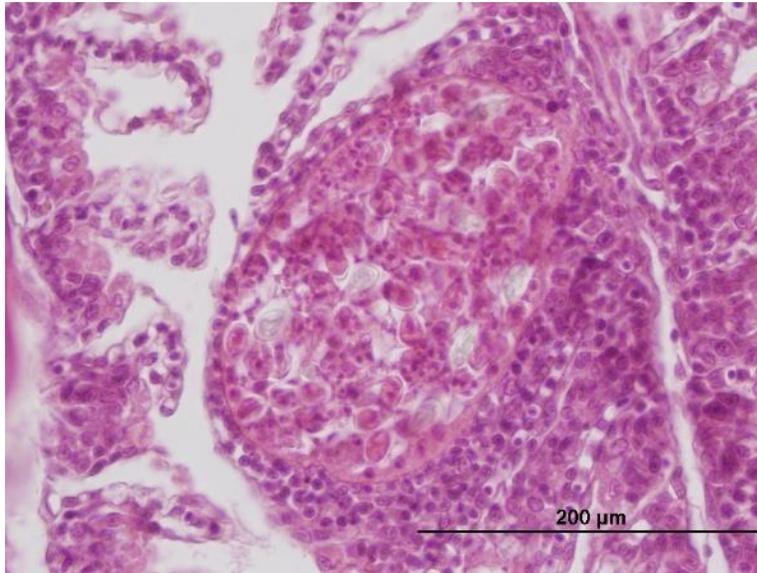


Figure 2. Presumptive diplostomid metacercaria in chub eye.



Figure 3. Low and high magnification of adult *Lernaea* (see arrows) attached to chub. Note inflammation associated with holdfast in higher magnification micrograph.

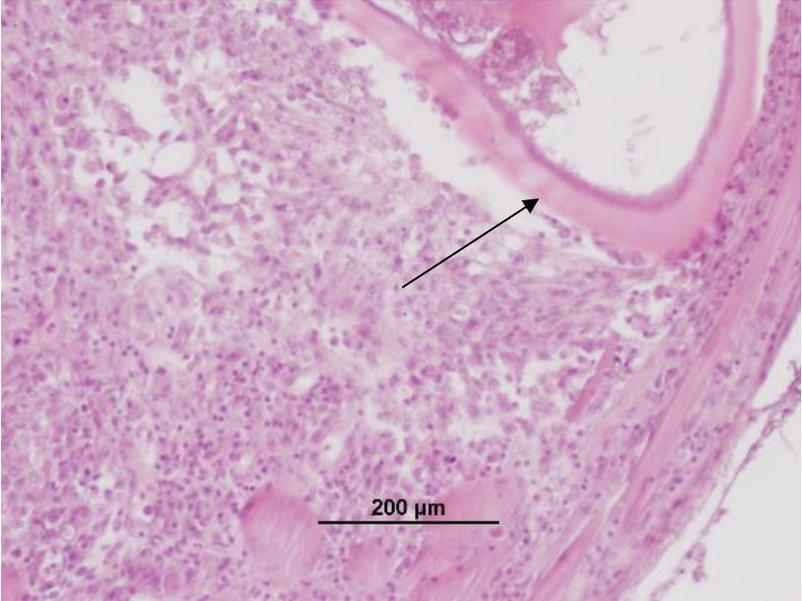
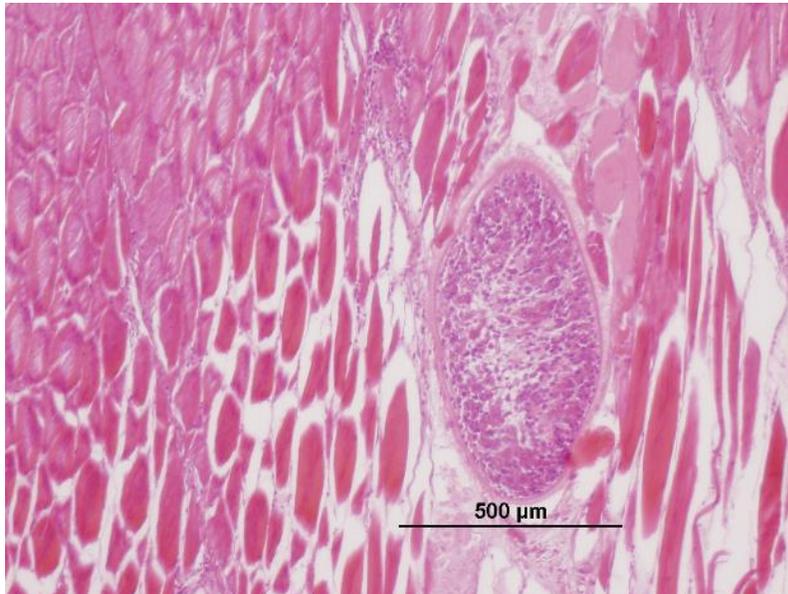


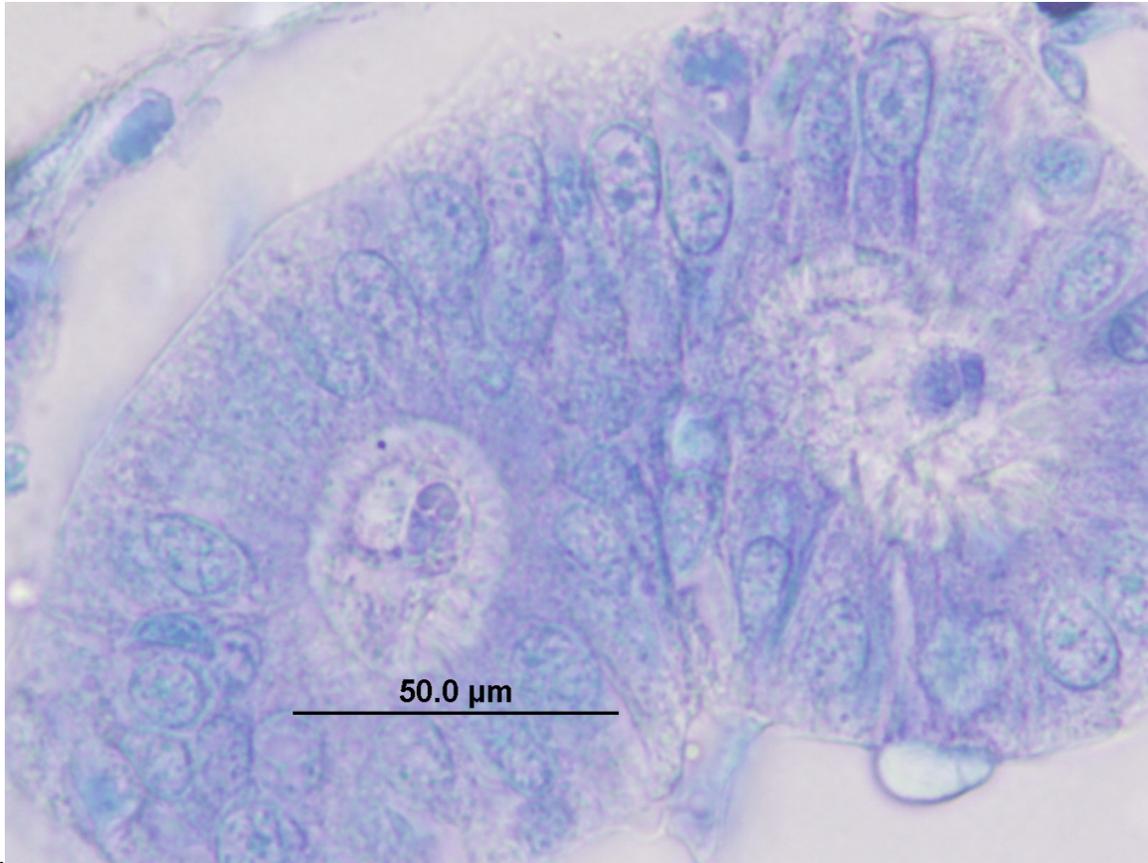
Figure 4. Blackspot metacercaria in chub muscle.



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FES sucker kidney myxozoan – presumptive *Parvicapsula* spore