GILL SCORE GUIDE
AMOEBIC GILL DISEASE (AGD) MANAGEMENT TRAINING DOCUMENT

RICHARD TAYLOR[1], CHRISTINE HUYNH[2], DAVID CAMERON[2], BRAD EVANS[2], MATHEW COOK[1], GORDON RITCHIE[3]

1. CSIRO AGRICULTURE AND FOOD, 2. TASSAL OPERATIONS PTY LTD, 3. MARINE HARVEST ASA

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FOREWORD

Amoebic gill disease (AGD) has been the main disease affecting farmed Atlantic salmon in Tasmania (Australia) since the mid-1980's. The disease occurs year round and adds significantly to the cost of production because fish need to be regularly treated with fresh water throughout the marine growing cycle.

AGD is proactively managed by regularly sub-sampling each caged population and inspecting the gills of anaesthetised fish for gross AGD signs (characteristic white mucoid spots and patches on the gill surface). This 'Gill Score' is a 0 (clear) to 5 (extensive lesions) scale and is assessed across all 16 hemibranch surfaces (i.e both sides of all 8 gill arches), it is a conservative scale that is designed to pick up and manage AGD from the onset of the disease. As gill score progresses, the risk to the fish increases from reduced feed intake, to increased stress and eventual death if not treated. Monitoring of the frequency of gill scores in a population and the average gill score ('Gill Index') over time allows the farmer to make informed stock management decisions to minimise adverse impact to the animals and to ensure that farm resources can be balanced to treatment requirements in a cost effective manner. A central concept of the gill score is that it is simple and consistent so that producers can compare AGD development trends between farms.

Since 2011, AGD has become a major health concern in Northern Europe (particularly Ireland, Norway and Scotland) and has been reported in Chile and Canada. Without prior experience in managing the disease, European producers were quick to adopt the Tasmanian gill score method as described in Taylor et al. (2009). However, the interpretation of the score has varied between European users because the initial visual guides were based upon single hemibranch photographs. Some farmers now use a 'worst arch' score, while others score each arch and then average the score for each fish, thus complicating essential communication of disease management between farmers. These different interpretations risk understating the gill score in the early stages of development and represent increased risk to the fish. It is in the interests of all producers to standardise AGD monitoring and communication.

With a need to standardise gill score training across the Marine Harvest organisation, the company requested that a simple AGD training guide be developed by CSIRO and Tassal to document the gill score method. The central element of this guide is that fish are first externally photographed showing the gill arches being turned over as they would be during gill checking. Because it is not possible to show the entire gill surface this way, the gills are thereafter laid out to enable both surfaces to be photographed in a fresh state.

This booklet is released for reference to the Atlantic salmon industry and fish health professionals.

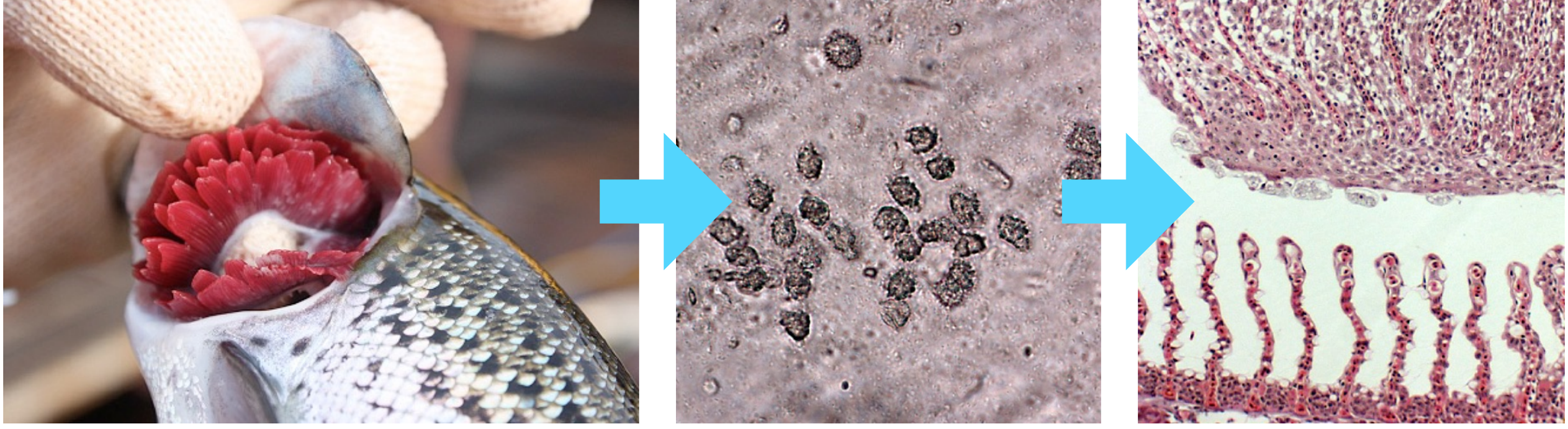
Richard Taylor
CSIRO
November 2016

AMOEBIC GILL DISEASE

1.1 ABOUT AMOEBIC GILL DISEASE

1.2 RISK FACTORS

1.3 MANAGING AMOEBIC GILL DISEASE RISK FACTORS



1.1 ABOUT AMOEBIC GILL DISEASE

- Amoebic Gill Disease (AGD) is caused by attachment of marine amoeba, *Paramoeba perurans* to the gill.
- AGD is a host tissue response (reaction to presence of the parasite) resulting in hyperplasia/hypertrophy of the gill epithelium, lamellar fusion and mucus production.
- AGD has been the main disease issue affecting Tasmanian salmon farms since the 1980's.
- AGD is reduced or controlled in brackish conditions, as such, freshwater bathing has been effectively used to control infections in Tasmania.
- Hydrogen peroxide treatment has been developed in Norway and Scotland with effect, but this does not promote a healing response in heavy infections. Risk of toxicity to fish increases at higher water temperatures.
- If not managed effectively, AGD can lead to mortalities of between 50% and 85% of an infected population.
- AGD will impact fish performance well before mortality occurs.

1.2 AMOEBIC GILL DISEASE RISK FACTORS

INFECTIVE LOAD

- High levels of *P. perurans* in the water column lead to rapid AGD infection in fish.
- AGD mortalities should be removed from cages regularly.

STRESS

- Immunosuppression is known to occur following acute and chronic stress.
- Immuno-supressed salmon are known to succumb to AGD infection more rapidly than healthy salmon.

SALINITY

- High salinity (> 32ppt) and periods of low rainfall correlate to AGD outbreaks.

TEMPERATURE

- AGD infection occurs more rapidly at higher temperatures.
- Amoeba reproduction is more efficient at higher temperatures.
- Salmon are more stressed at elevated temperatures, leading to suppressed immune response.
- In Norway, warmer winters are believed to be an added risk factor.

SITE CHARACTERISTICS AND SEASON

- Surrounding biomass and AGD infection will impact on the rate of infection in nearby cages.

1.3 MANAGING AMOEBIC GILL DISEASE RISK FACTORS

BE AWARE OF RISK FACTORS

- Modify AGD surveillance to ensure frequency is appropriate to the risk profile.
- Modify treatment strategies to ensure impacted pens can be treated prior to the onset of mortality.

MANAGE HOST STRESS

- Limit stocking density in pens and regions.
- Maintain net hygiene levels to reduce fish stress.
- Limit handling of fish during periods of elevated temperature or gill score.

These approaches will help to minimise disease impacts, and the rate of infection.

MONITORING AMOEBIC GILL DISEASE

2.1 PCR

2.2 GROSS GILL SCORE

2.3 GILL SCORE SAMPLING METHOD

2.4 GILL SCORE SAMPLING FREQUENCY

2.5 AGD GILL SCORE OF ALL 16 GILL SURFACES

2.6 RECORDING GILL SCORE AND CALCULATING INDEX

2.7 CONFIRMING AMOEBA PRESENCE

2.1 PCR

PROS

- Confirms presence of *P. perurans.*
- Early detection prior to development of gross lesions – useful on sites with infrequent or seasonal AGD.
- Supports early treatment decisions – particularly early peroxide bath intervention.
- Confirms gill score assessment, useful for staff training.
- Quantified measure, relates to gill score.
- Molecular sampling supports investigation of range of gill pathogens.
- Useful measure of treatment efficacy.

CONS

- Generally 2-3 day turnaround, does not support real-time decisions.
- Relatively expensive, limited sample size.
- Not warranted on sites with continuous AGD issues.

Depending on strategy, PCR should be used to inform early treatment intervention or to signal the onset of regular gill score measures.

2.2 GROSS GILL SCORE

PROS

- Real-time.
- Non-destructive.
- Low cost.
- Supports treatment decisions.
- Functional measure – reflects risk to stock, feed conversion, handling stress and mortality.
- Proven tool for breeding selection – lower gill score equates to fewer treatments.

CONS

- Presumptive - does not confirm presence of *Paramoebas.*
- Subjective.
- Added work.

Data storage, decision tools and communication are central to effective AGD management.

Application of Gill Score information depends on company strategies.

Refine strategy with ongoing gillscore / PCR/histology / performance information.

2.3 GILL SCORE SAMPLING METHOD

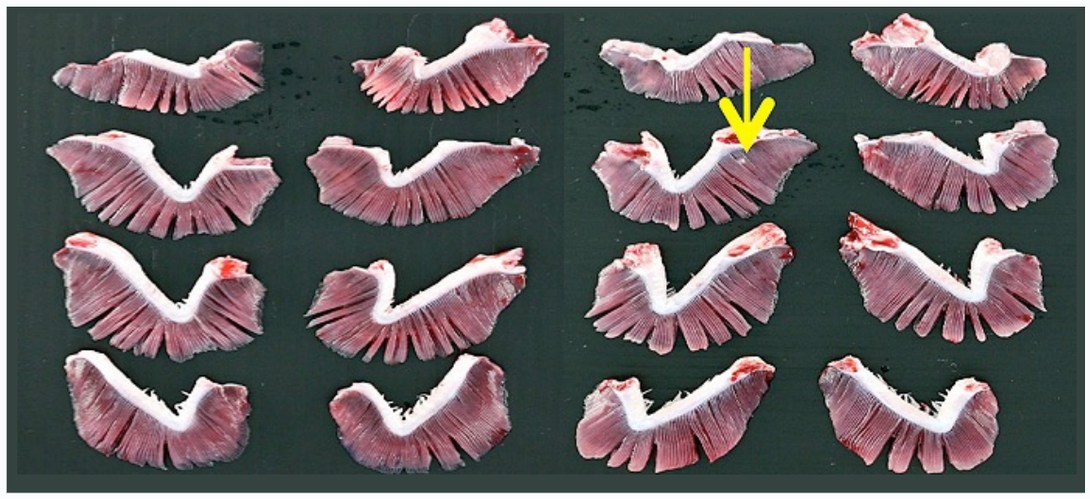
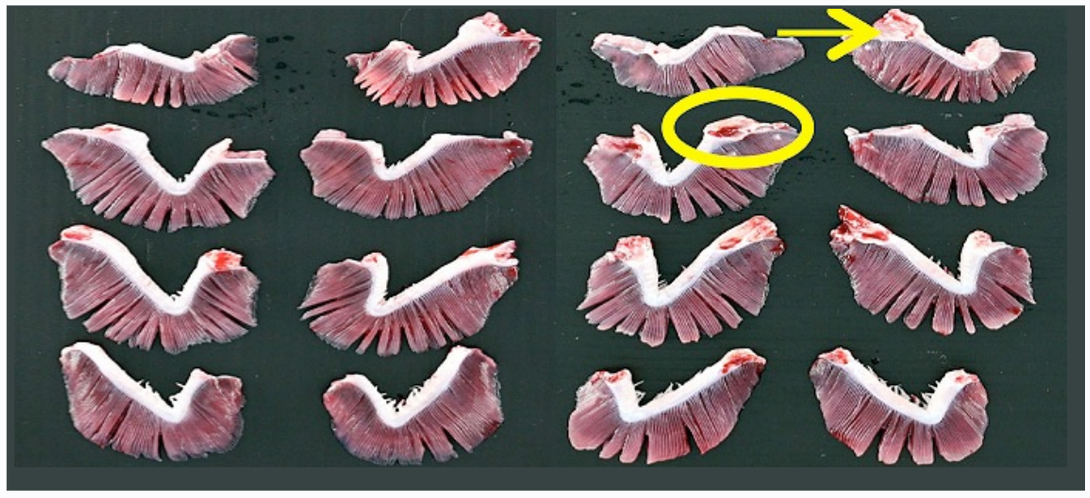
- Sampling from fish cages is never truly random but error can be minimised by good sampling technique and adequate sample size.
- Develop methods to sample the main population, don't focus on fish around the edges.
- Take a large sample (200+ fish) using a large seine or box-net.
- Raise the seine to 'mix' the fish, subsample from this.
- Transfer fish to anaesthetic with aeration or circulation.
- Avoid direct oxygenation (lesions are harder to see on bright gills).
- Examine fish in bright natural light (direct sun can be difficult).
- Open the gills fully so you can look into the corners between gill arches.
- Change the orientation of the fish to study the 3D structure of lesions.
- Test lesion consistency with your finger – AGD will 'thin' or 'move' when rubbed.
- Regularly cross check your score with other experienced operators.
- Return fish to an oxygenated recovery bin/recovery net.

Sample size should be a minimum of 40 fish per cage to ensure repeatability of measures.

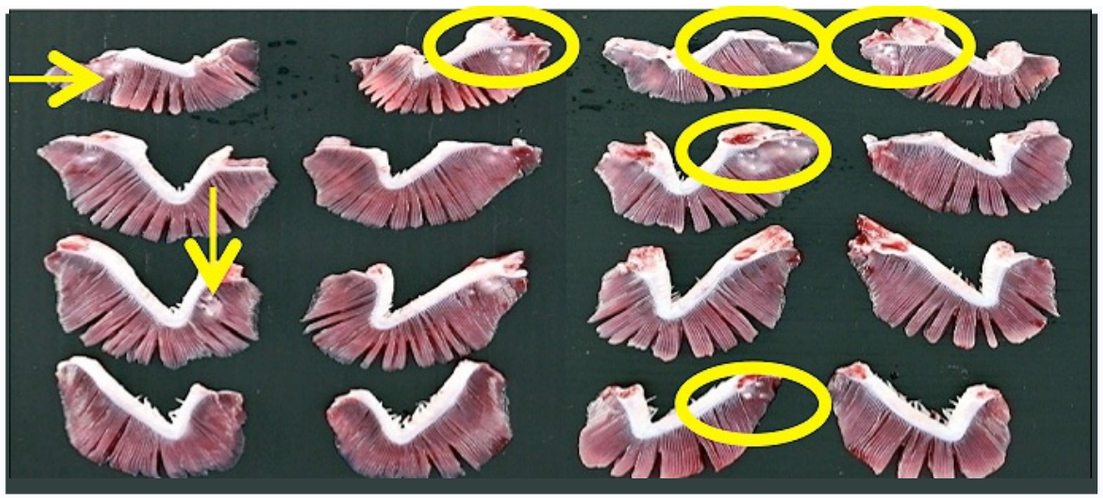
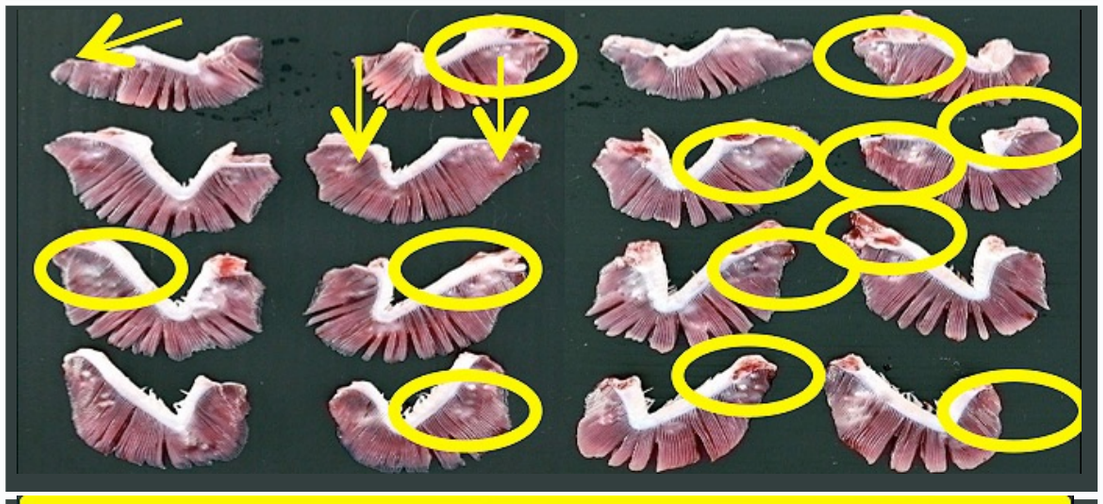
2.4 GILL SCORE SAMPLING FREQUENCY

- Devise and adapt strategy according to likely risk (site, season, etc.).
- All cages should be checked regularly.
- Following smolt transfer, AGD monitoring should begin after 14 days in regions of known AGD.
- Continue to check on 14 day cycle.
- If necessary increase frequency as fish approach treatment threshold.
- Assessment of the population gill score at treatment is particularly useful.
- For 10-14 days post treatment, scarring and mucus may be present.
- Advisable not to check inside of 14 days unless an issue was experienced during treatment.
- Check mortalities for gill lesions.
- Frequency of checks can be reduced in larger fish as water temperatures decline.

2.5 AGD GILL SCORE OF ALL 16 GILL SURFACES

CLEAR	**0**	No sign of infection and healthy red colour	
VERY LIGHT	**1**	1 white spot, light scarring or undefined necrotic streaking	
LIGHT	**2**	2-3 spots/small mucus patch	

AGD GILL SCORE OF ALL 16 GILL SURFACES (CONTINUED)

MODERATE	**3**	Established thickened mucus patch or spot groupings up to 20% of gill area	
ADVANCED	**4**	Established lesions covering up to 50% of gill area	
HEAVY	**5**	Extensive lesions covering most of the gill surface (50%+)	

2.6 RECORDING GILL SCORE AND CALCULATING INDEX

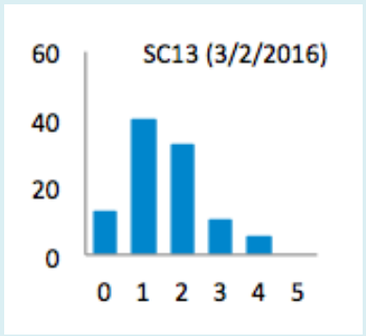
A

Date	Cage
3/02/2016	SC13

Fish	AGD score	Comments
1	0	
2	1	
3	2	
4	1	
5	3	
6	0	
7	2	
8	3	swab #1
9	1	
10	2	
11	2	
12	1	
13	4	

B

Score	Number	Frequency%
0	5	12.5%
1	16	40.0%
2	13	32.5%
3	4	10.0%
4	2	5.0%
5	0	0.0%
Total	40	



C

$$\text{Gill index} = \frac{(0x5)+(1x16)+(2x13)+(3x4)+(4x2)+(5x0)}{40} = 1.55$$

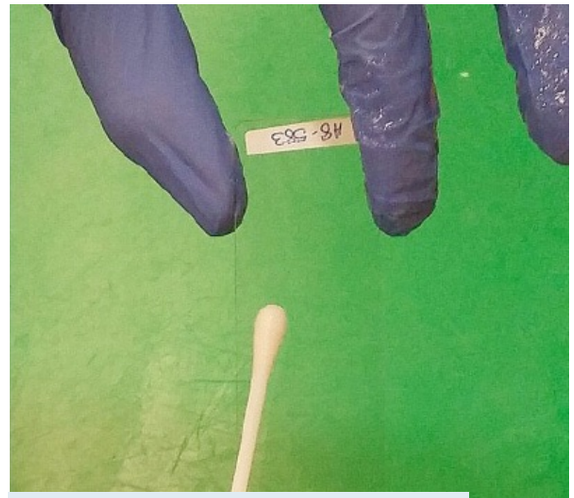
2.7 CONFIRMING AMOEBA PRESENCE

Gill score is presumptive – if in doubt, confirm presence of amoebas.

Options are:

1. Wet mount of gill smear (real-time decision).
2. qPCR (may take a few days, confirms *P. perurans* presence.
3. Histology (confirms amoebas in association with gill lesions).

1. Swab lesions

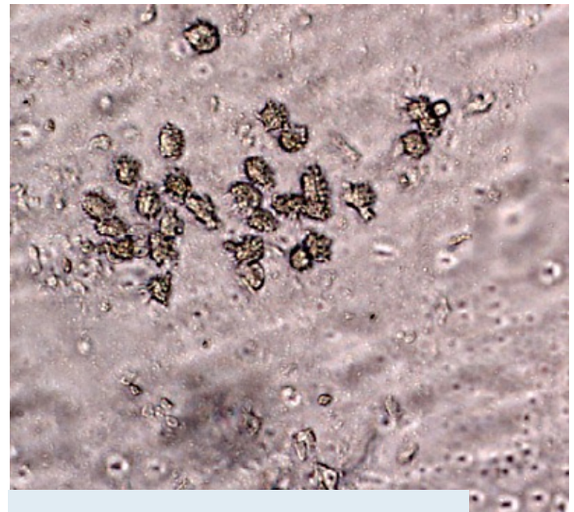


2. Transfer to slide

3. Add Seawater and smear

4. Add cover slip

5. Examine sample



6. 10x to 20x

USING THE GUIDE

3.1 USING THE GILL SCORE

3.2 FOLLOW INDEX AND DISTRIBUTION

3.3 ORIENTATION OF GILL IMAGES (EXTERNAL)

3.4 ORIENTATION OF GILL IMAGES

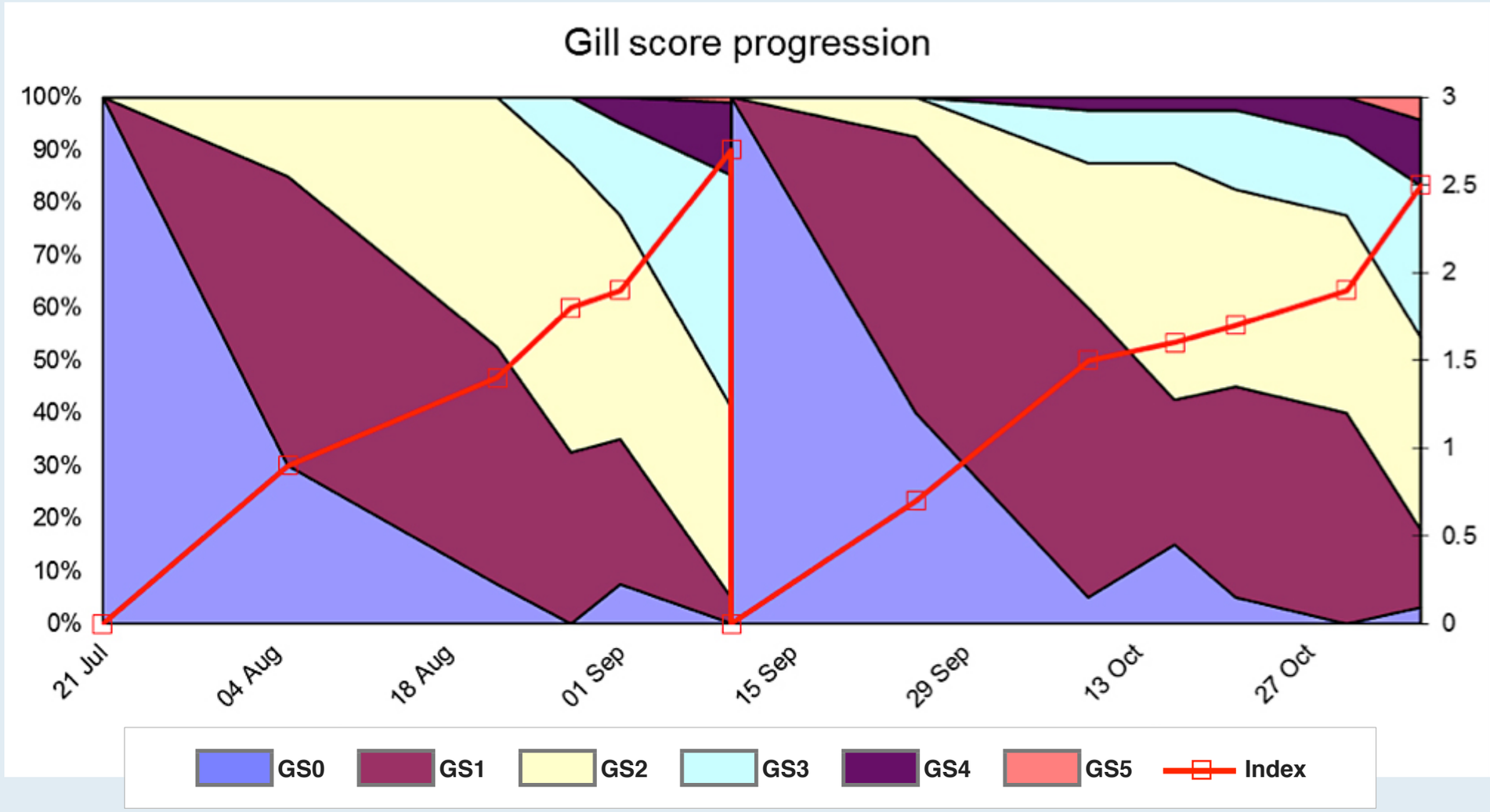
3.1 USING THE GILL SCORE

- Record frequency per gill score by cage.
- Follow trend of average gill score (gill index) development, treat at an agreed gill index threshold.
- Be aware of gill score distribution.
- Handling losses are more likely with gill score 4-5 fish, this risk is amplified in H2O2 treatment.
- Treatment decision also depends upon other health or environmental issues that may compromise fish.

Comment	Gill Score	Days	GS0	GS1	GS2	GS3	GS4	GS5		Index
Input	21/07/2008	0	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100%	0.0
	5/08/2008	15	30.0%	55.0%	15.0%	0.0%	0.0%	0.0%	100%	0.9
	22/08/2008	32	7.5%	45.0%	47.5%	0.0%	0.0%	0.0%	100%	1.4
	28/08/2008	38	0.0%	32.5%	55.0%	12.5%	0.0%	0.0%	100%	1.8
	1/09/2008	42	7.5%	27.5%	42.5%	17.5%	5.0%	0.0%	100%	1.9
AGD1	**10/09/2008**	**51**	**0.1%**	**4.9%**	**36.1%**	**44.0%**	**13.8%**	**1.0%**	**100%**	**2.7**
(bathed)	10/09/2008	0	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100%	0.0
	25/09/2008	15	40.0%	52.5%	7.5%	0.0%	0.0%	0.0%	100%	0.7
	9/10/2008	29	5.0%	55.0%	27.5%	10.0%	2.5%	0.0%	100%	1.5
	16/10/2008	36	15.0%	27.5%	45.0%	10.0%	2.5%	0.0%	100%	1.6
	21/10/2008	41	5.0%	40.0%	37.5%	15.0%	2.5%	0.0%	100%	1.7
	30/10/2008	50	0.0%	40.0%	37.5%	15.0%	7.5%	0.0%	100%	1.9
AGD2	**5/11/2008**	**56**	**3.1%**	**14.8%**	**36.5%**	**28.8%**	**12.5%**	**4.3%**	**100%**	**2.5**

Table: This table tracks the frequency distribution of each gill score and the progression of gill index through regular farm checks. Upon treatment, the population is considered to return to 100% gill score 0 for clarity.

3.2 FOLLOW INDEX AND DISTRIBUTION



Gill score progression: This chart is an example of how the progression of AGD index, and the proportion of each gill score can be tracked with regular farm checks. Upon treatment, the population is considered to return to 100% gill score 0 for clarity.

3.3 ORIENTATION OF GILL IMAGES (EXTERNAL)

10 images, not possible to see all surfaces or in corners.

(i = inner (proximal) gill surface, o = outer (distal) gill surface)

L1o

L1i - L2o

L2i - L3o

L3i - L4o

L4i

R1o

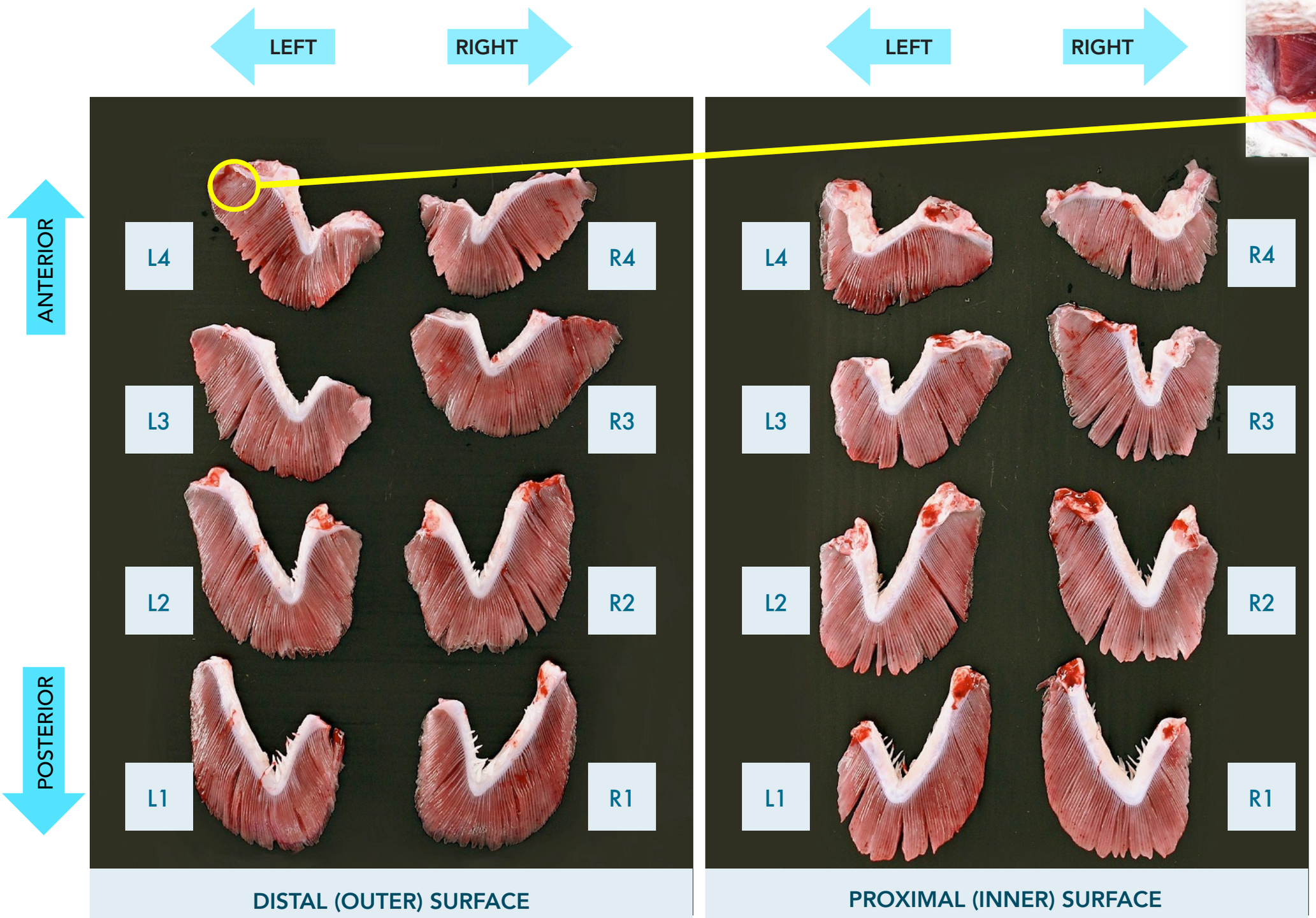
R1i - R2o

R2i - R3o

R3i - R4o

R4i

3.4 ORIENTATION OF GILL IMAGES

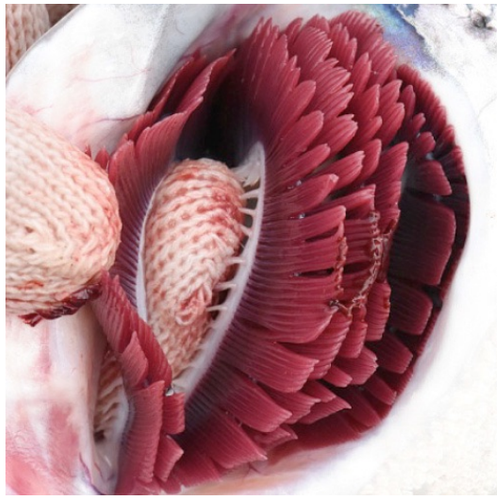
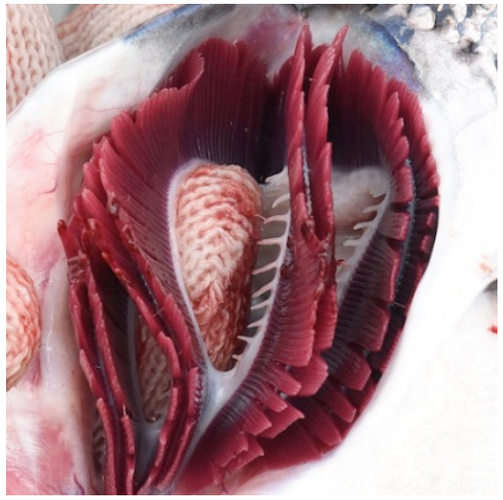


DISTAL (OUTER) SURFACE

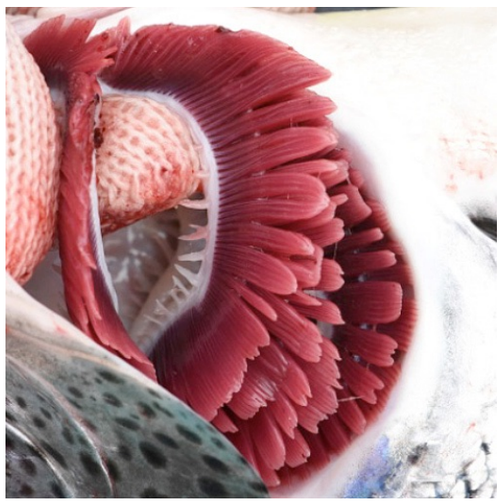
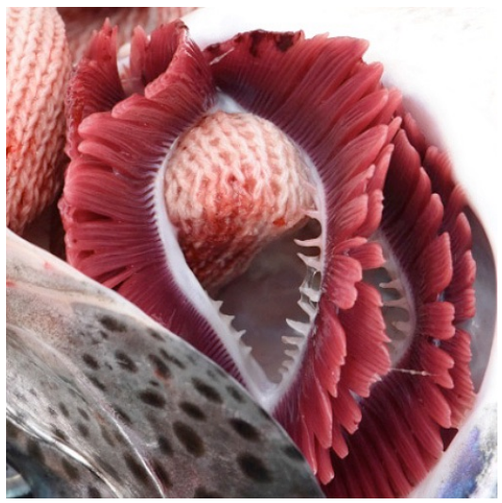
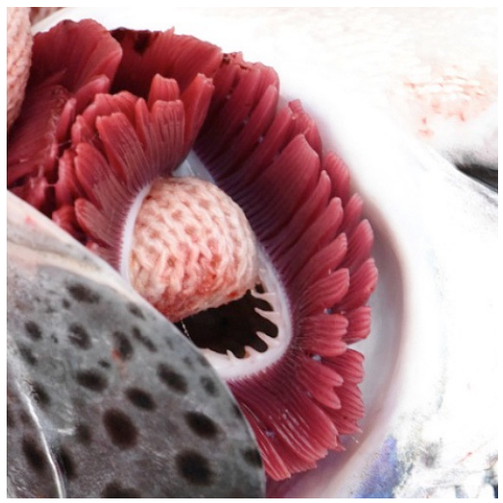
PROXIMAL (INNER) SURFACE

GILL SCORE IMAGE GUIDE

4.1 GILL SCORE 0

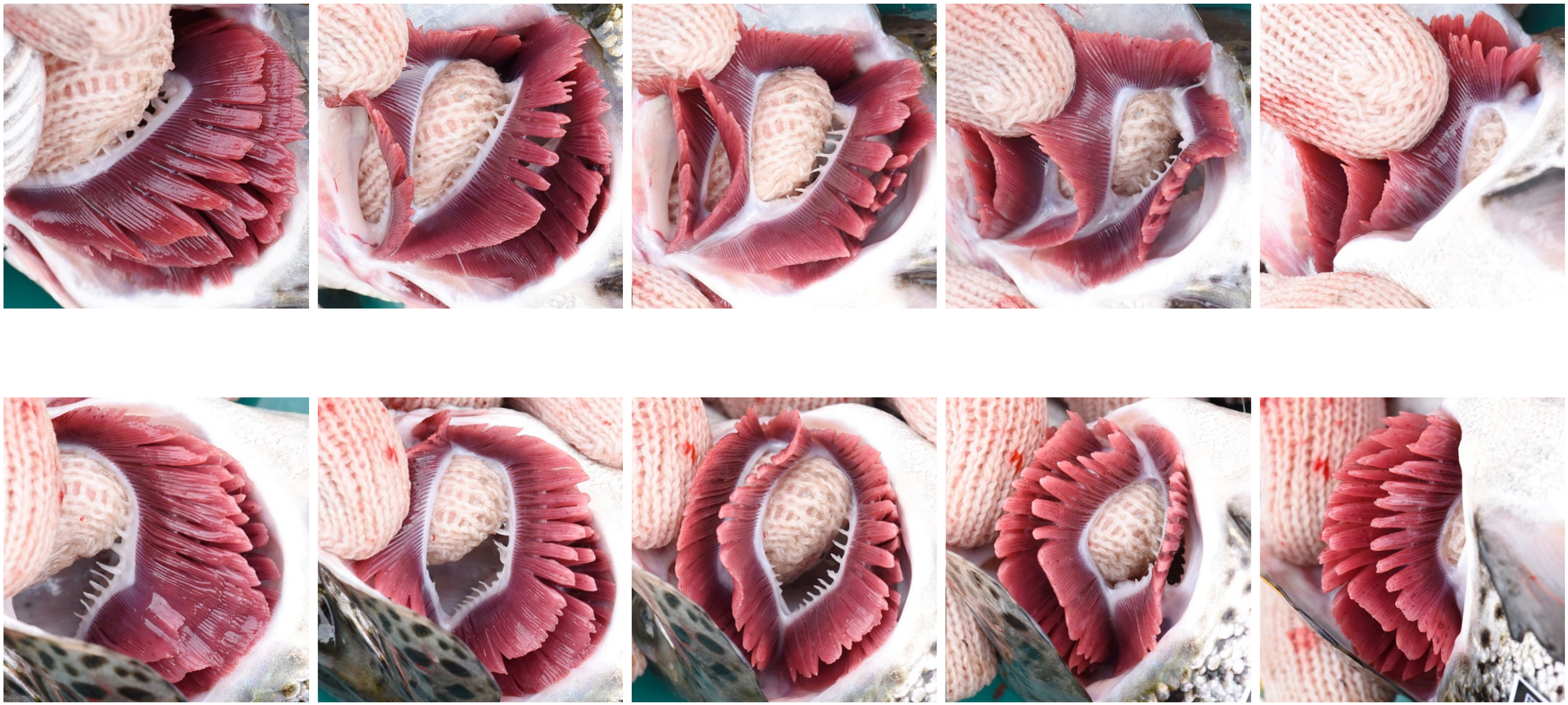



Gill Score = 0

Note: Healthy red gills, no gross sign of infection.

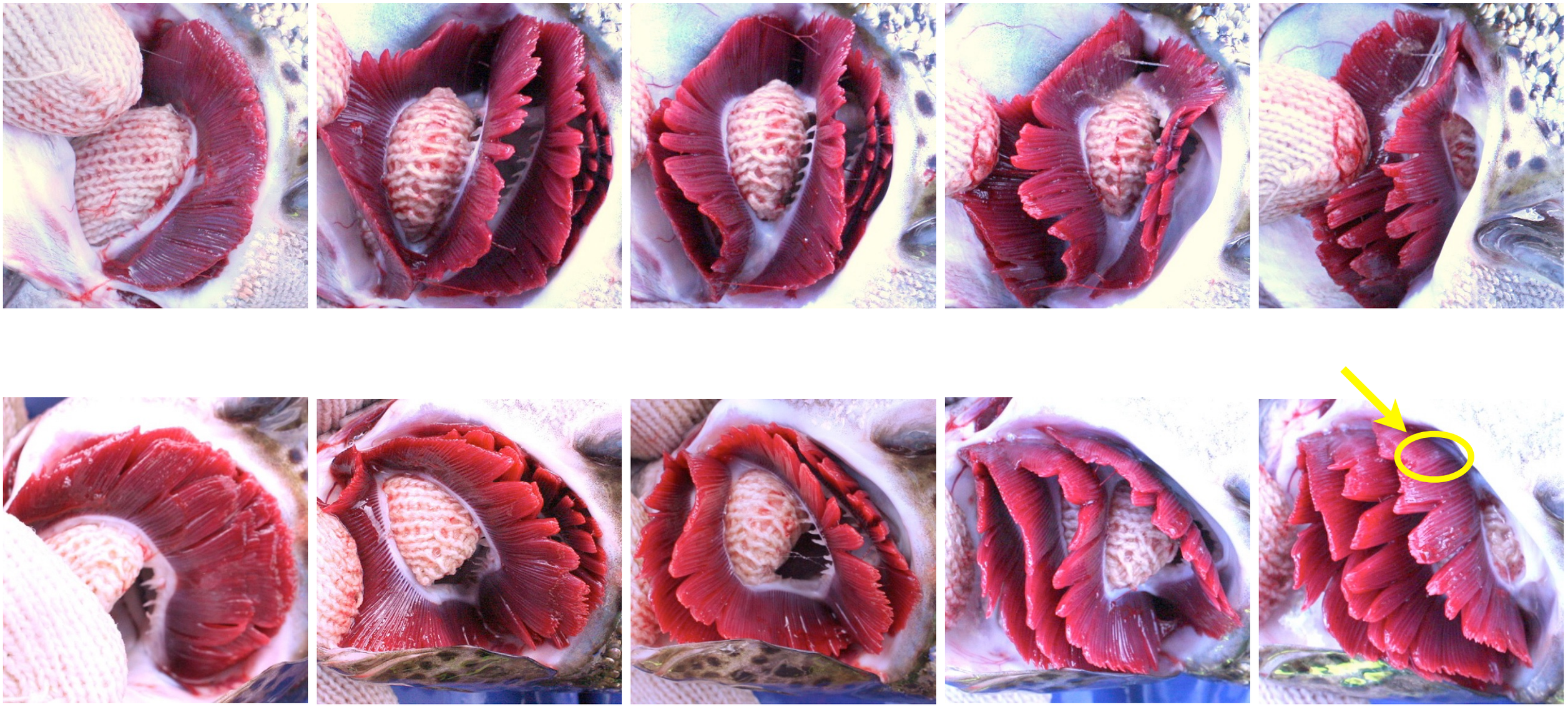
4.2 GILL SCORE 1



Gill Score = 1

Note: One white spot, light scarring or undefined streaking (circled on R4 outer). Some damage on L4 outer.

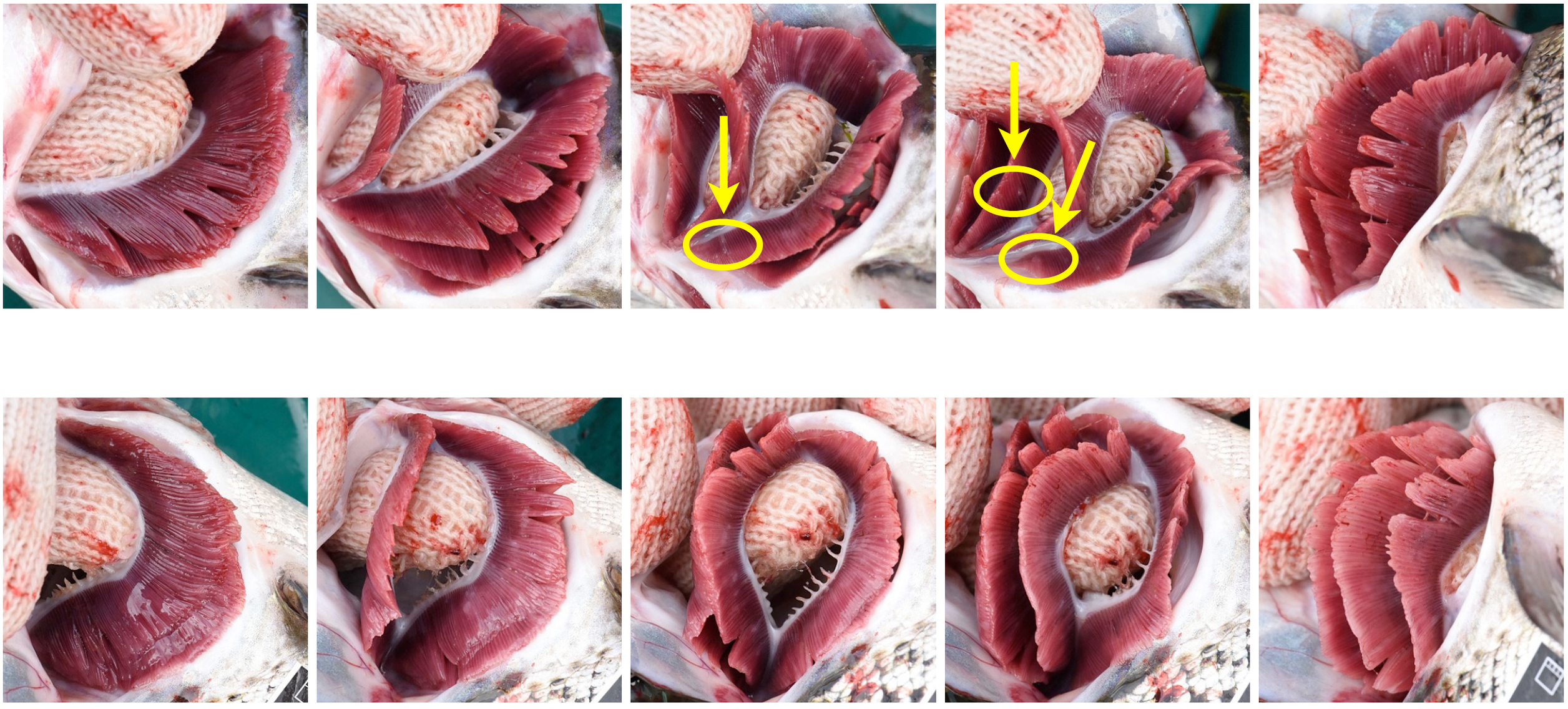
4.3 GILL SCORE 2



Gill Score = 2

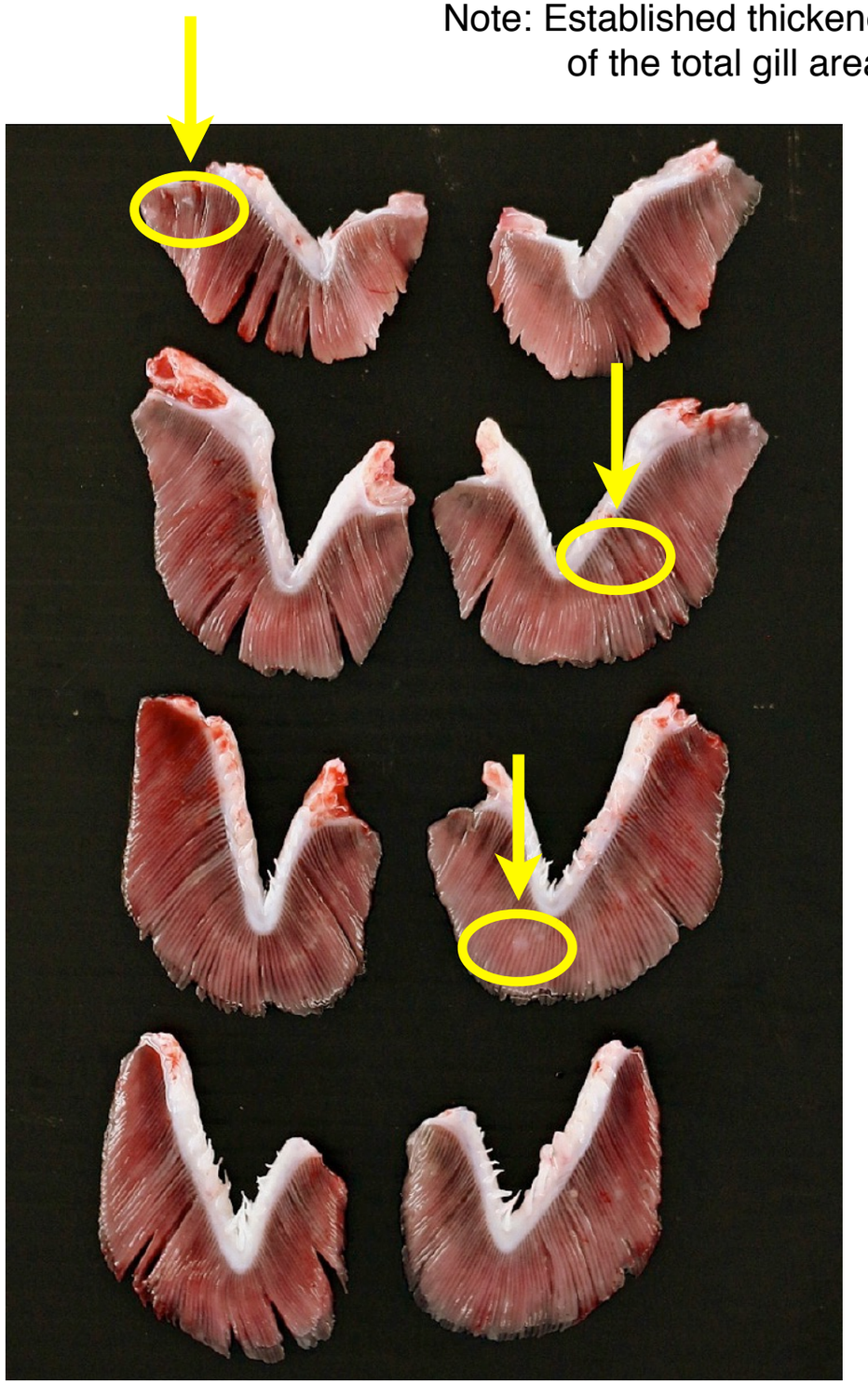
Note: One white spot, light scarring or undefined streaking (circled on R4 outer). Some damage on L4 outer.

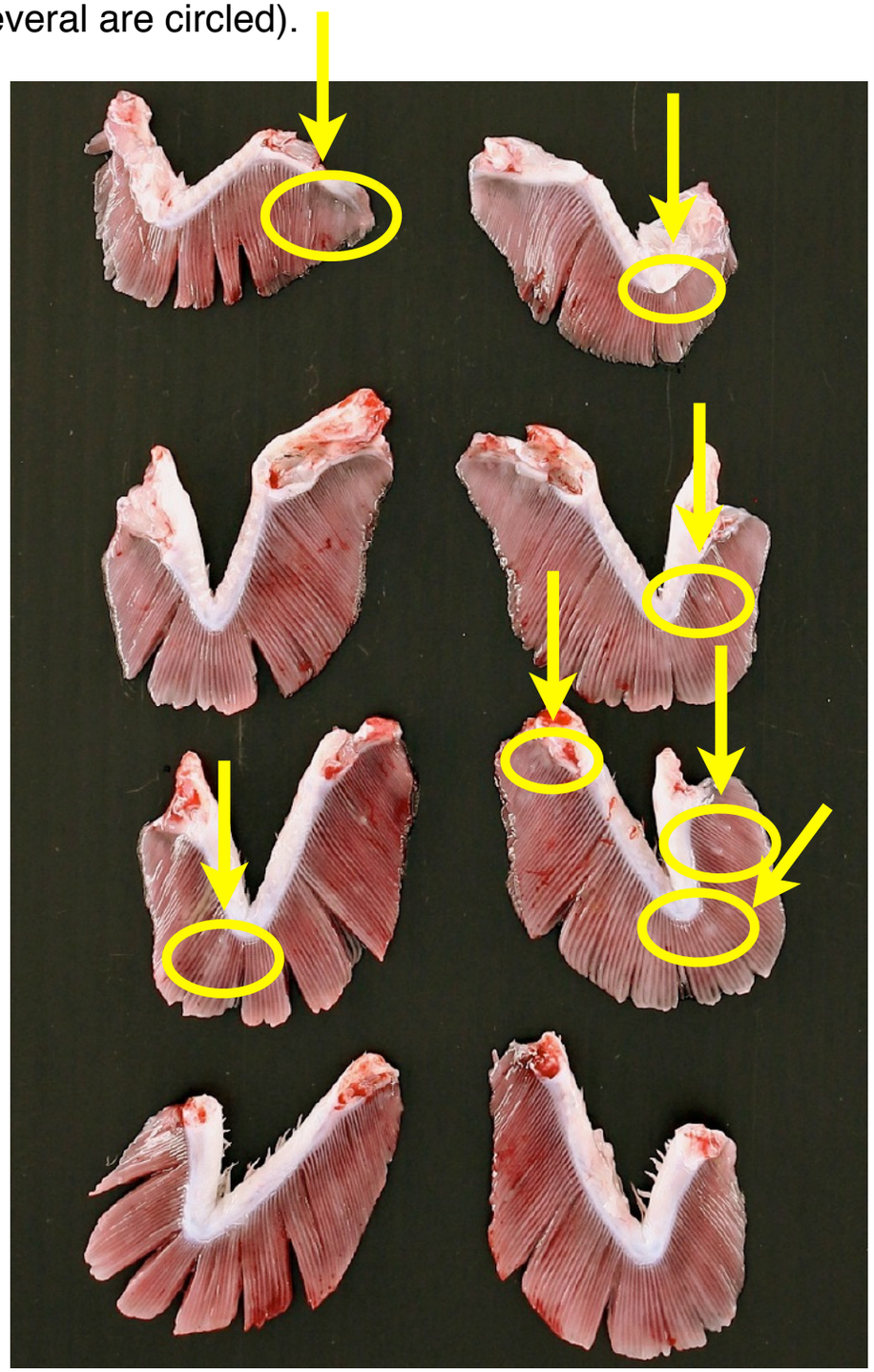
4.4 GILL SCORE 3



Gill Score = 3

Note: Established thickened patches or spots on <20% of the total gill area (several are circled).





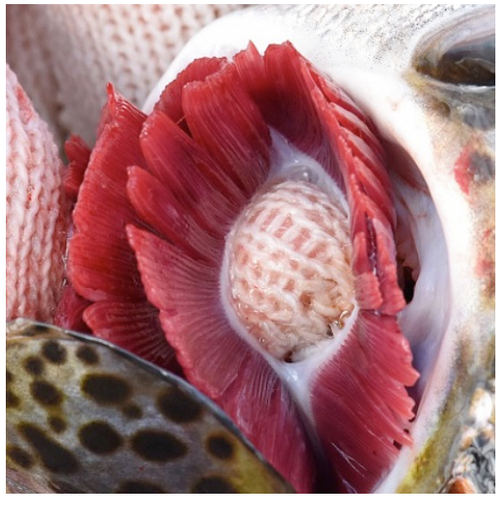
4.5 GILL SCORE 4





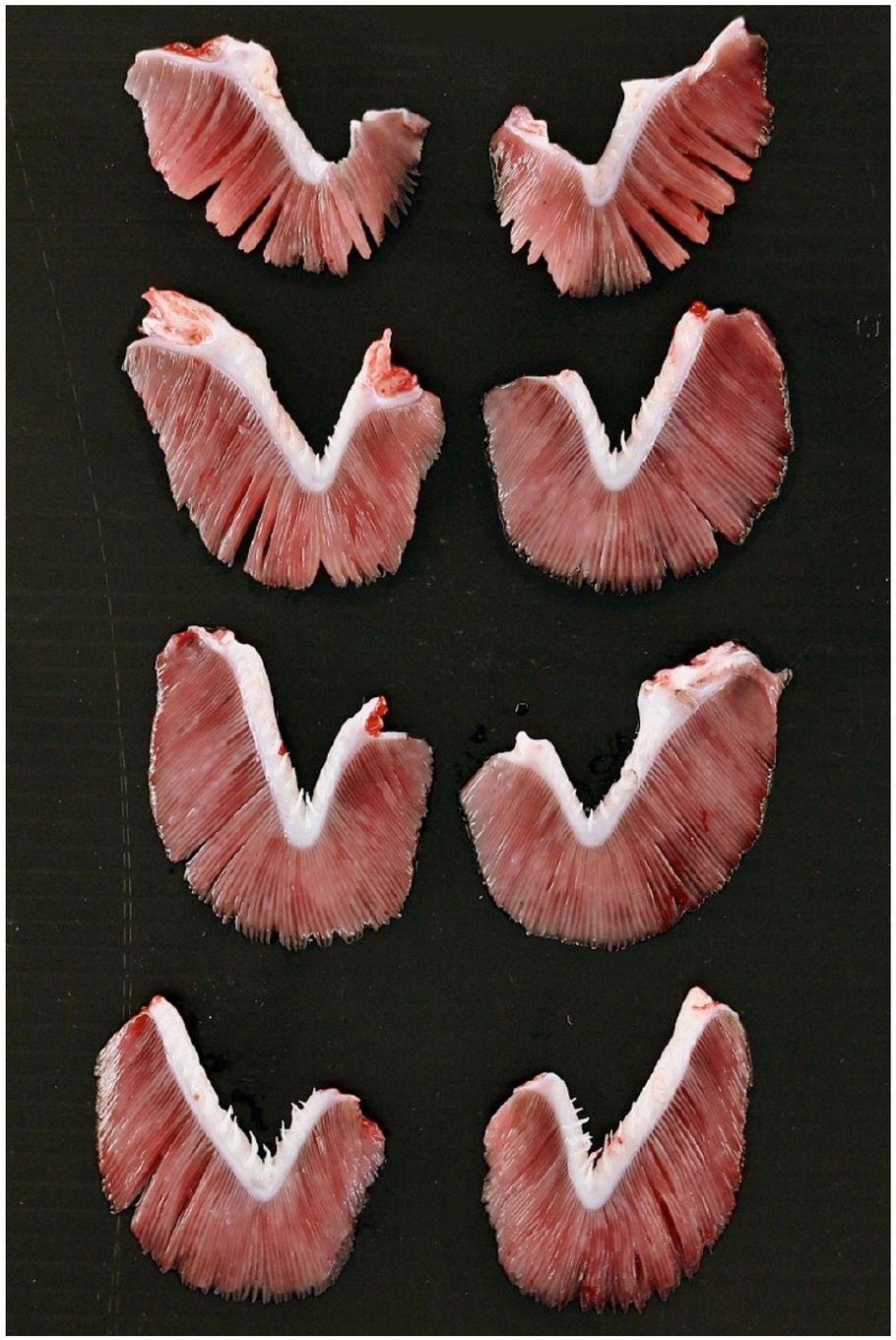







Gill Score = 4

Note: Lesions on up to 50% of the total gill area.



4.6 GILL SCORE 5










Gill Score = 5

Note: Lesions covering the majority of the gill area.

OTHER LESIONS

- AGD is not the only cause of gill lesions.
- Other types of lesions (e.g. necrosis, scarring) can be quantified grossly using separate scoring systems.
- If in doubt, the type of damage should be checked by histology. Test for the presence of *P. perurans* by PCR.
- AGD lesions will generally change when rubbed with your finger.

Focal gill necrosis.

- AGD gill score should be recorded separately this is the only meaningful score to manage AGD.
- Non-AGD pathology may affect treatment decisions.
- Do not add scores from differing aetiologies.
- Be consistent in your training and recording.

Fish	Gill score	Necrosis score
1	0	0
2	0	0
3	1	3
4	2	1
5	0	0
6	3	5
7	2	4
8	4	

Example recording of separate AGD and necrosis scores, these should not be combined as a single score.

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