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Gill Health Workshop
February 9 - 10, 2017
Campbell River, BC

2018-2

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Cover photo: Histological section of healthy Atlantic salmon gill filaments and lamellae (credit: Hamish Roger)

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GILL HEALTH WORKSHOP

Feb 9th & 10th, 2017

Campbell River BC

ABSTRACT

By understanding the fundamental structure and function of the gill, culturists and fish health practitioners view a window of individual and herd health. This workshop reviewed the structure and function of the gill architecture, physiological operation, changes in the parr-smolt transition, and the indicators of optimal animal health.

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INTRODUCTION TO THE GILL HEALTH WORKSHOP

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The gills of fish serve a multiplicity of functions that support the overall health of the organism. They are the active interface between the external and internal environments of the fish. Gills not only serve as barriers to particle movement between environments, they also serve as the main organ indicator of whole animal health that is visible to the fish culturist. By understanding the fundamental structure and function of the gill, culturists and fish health practitioners view a window of individual and herd health. This workshop reviewed the structure and function of the gill architecture, physiological operation, changes in the parr-smolt transition, and the indicators of optimal animal health. Over both days, the assemblies reviewed the local existing and emergent pathogens and effectors now present in BC fresh and salt waters. Local and international experts discussed the indicators of optimal gill health, illustrated the state of the art for care and prevention of pathogens and harmful agents, identified knowledge gaps, and recommend research opportunities for further work.

The workshop objectives were:

1. To review the current knowledge of: gill structure and function in freshwater and seawater; observation, detection and prevention of gill disorders in order to mitigate losses to production; and steps to promote optimal health.

2. At the end of the workshop there was a clear understanding of the future needs for research, identification of the key participants, and a pathway forward to investigate and eliminate the knowledge gaps identified. Research opportunities were identified and reported.

Goals

The workshop fit within the goals of the ACRDP as described below:

- **Improve the competitiveness and sustainability of the Canadian aquaculture industry:** by learning about gill structure and function,

current issues, detection, and treatment options, workshop participants took away information to help better inform their decisions and guide their practices in their production facilities, thus increasing their competitiveness and sustainability in the industry.

- **Increase collaborative research between the department and industry:** One of the goals of the workshop was to identify knowledge gaps and future directions for research in gill health. With key speakers and participants attending from both DFO and industry, as well as a moderated panel discussion each day, the opportunity to present ideas for new collaborations existed.
- **Facilitate the process of technology transfer and knowledge mobilization:** The workshop report documented details provided over the two days of technical information and moderated discussions. This report was shared and publicized with the intent to increase gill-health-related knowledge within the industry and to identify knowledge gaps and future directions for research.
- **Increase scientific capacity of the Canadian aquaculture industry for essential aquaculture research and development:** With a broader audience of both freshwater and seawater culturists and fish health practitioners, a greater opportunity existed for new discussions and ideas to be exchanged. The inclusion of international perspectives on gill health and identification of knowledge gaps and new research opportunities contributed to fostering new research collaborations, thereby supporting ACRDP's key goal of increasing the scientific capacity of the Canadian aquaculture industry.

Objectives: The ACRDP's primary objective is to serve to increase the level of collaborative research and development activities between the aquaculture industry and Fisheries and Oceans Canada. The gill health workshop included moderated panel discussions to help prioritize the needs of industry, which created opportunities for government researchers to assist industry through collaborations.

Priorities: The workshop included information about disease detection and surveillance, causative agents affecting the gills, and health management practices – therefore, it fit with the ACRDP's objective of Optimal Fish Health. The workshop also touched on ecological/environmental conditions and impacts on aquaculture under the Environmental Performance objective.

The workshop also fitted well with the 2016-17 National Research Priorities for Marine Finfish - Health Management, Management and Control of Pests and Pathogens, and Environmental Impacts - from the environment to aquaculture.

THE MULTIFUNCTIONAL FISH GILL: PLASTICITY AND COMPROMISE

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Introduction

The fish gill is truly a multifunctional organ. It serves as the dominant site for gas exchange, ion regulation, acid-base regulation, and nitrogenous waste excretion. The gill is therefore critical to vital processes such as metabolism, growth, and exercise, as well as dealing with environmental challenges such as low oxygen, elevated carbon dioxide and ammonia, and changes to salinity and temperature.

The gill's role in these multiple functions is relatively simple: move gases and ions between the blood and water. As described in this brief review, the effectiveness of the gill in moving gases and ions is tightly linked to its structural organization. Many of these structural elements are plastic and can be altered to enhance specific functions in response to changing internal and environmental conditions. These changes can occur over acute, acclimatory, and evolutionary timescales. However, alterations that enhance the movement of gases can sometimes compromise the movement of ions and vice versa. Overall, gill structure is largely tailored to meet the demands of the local environment and can therefore provide a window into the current physiological state or health of the fish.

We begin this article with a brief description of gill morphology and then follow with abbreviated summaries of how this morphology and its plasticity relate to gill function in gas exchange and ion regulation. A comprehensive review of gill function is beyond the scope of this short article, and interested readers are encouraged to explore other excellent sources for more detailed information (Cameron, 1989; Evans et al., 2005; McCormick et al., 2012; Wilson & Laurent, 2002).

Simplified gill morphology

The gill is the primary interface between the internal and external environments of the fish. In very simple terms, it is a collection of blood vessels covered with a specialized epithelium in contact with the water. Fish possess two gill baskets, with one located under the protection of each operculum. Each gill basket is divided into four thick cartilaginous arches that provide structural support for the organ (Figure 1; modified from Evans et al., 2005). Blood flows from the arches into numerous laterally projecting vessels called filaments. Filaments are more delicate than the arches, with an epithelium often 1 to 2 cells thick (Figure 1). From the filaments, blood flows into laterally projecting sheets called lamellae. Lamellae are even more delicate than filaments, with an epithelium often 1 cell thick (Figure 1c). From lamellae, blood flows back through the filaments to the arches and out to the rest of the body.

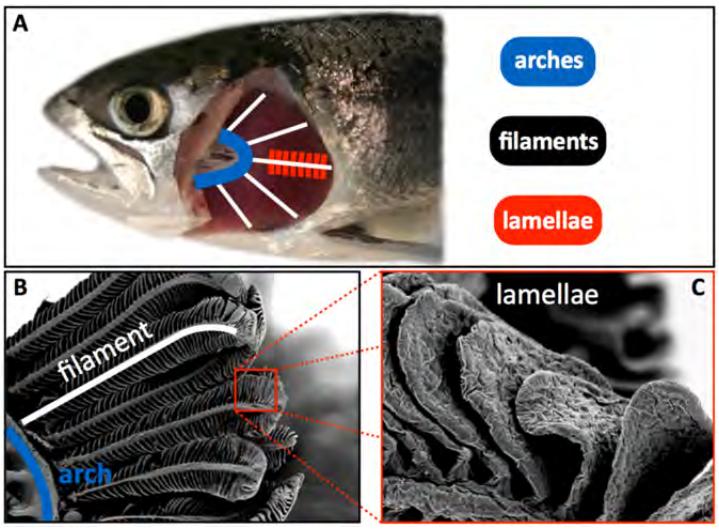

Figure 1
(A) Schematic of gill morphology depicting arches, filaments and lamellae. (B) SEM image of a single gill arch with filaments and lamellae (modified from Evans et al., 2005), and (C) closeup SEM image of lamellae (modified from Evans et al., 2005).

The filaments and lamellae are the primary sites of gas exchange and ion regulation, and are thus the focus of this article. As shown in Figure 2, these structures are largely made up of 4 main cell types: goblet cells, pillar cells, ionocytes, and pavement cells. Goblet cells are scattered throughout the gill and secrete the protective mucus layer that covers the gill epithelium. Pillar cells act as pillars in the lamellar sheets, helping to keep the sides of lamellae from collapsing in on one another. Pillar cells also have contractile properties that can be used to control blood flow through lamellae. Ionocytes contain specialized transporters and channels used for ion transport. They are larger cells and contain many mitochondria to fuel the active process of ion transport. Ionocytes make up ~10% of the epithelium and can be found on both filaments and lamellae. Pavement cells are thin cells typically associated with gas exchange but may serve an accessory function in ion transport as well. Pavements cells make up ~90% of the epithelium.

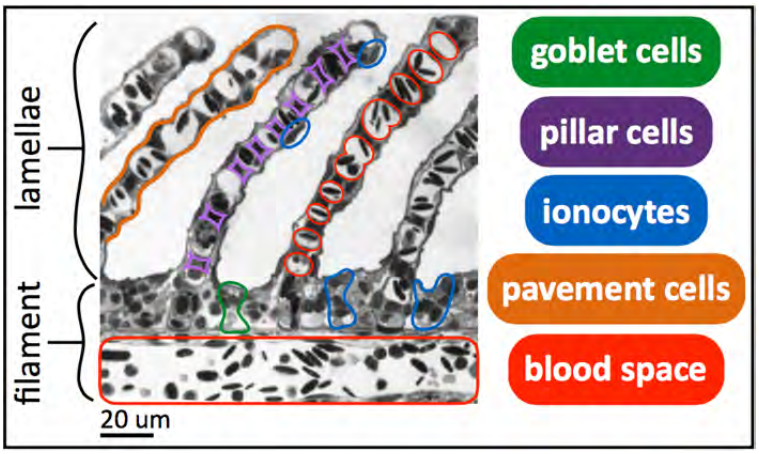

Figure 2
Light micrograph of filament and lamellae depicting primary cell types (modified from Evans et al., 2005).

Gas exchange

Gases like oxygen and carbon dioxide can diffuse freely across the gills between the blood and water. This diffusion can be described with a modified Fick equation (Equation 1), where F is the rate of diffusion or movement of gas, A is gill area, T is gill thickness, ΔP is the partial pressure gradient of the gas between the blood and water, and D is a coefficient of diffusion specific to the properties of the gas being described.

$$F = (A \bullet \Delta P \bullet D) / T \qquad \text{equation 1}$$

Based on this equation, if the gill is to be an effective organ for gas exchange, it would have a large area, a thin epithelium, and be arranged to enhance the partial pressure gradients of gases between the blood and water. Gill morphology is organized in exactly this way. For example, a 10-g Atlantic salmon has an approximate body surface area of 280 mm^2, but the tightly packed filaments and lamellar folds provide a total gill surface area of approximately 6000 mm^2 (Figure 3a; modified from Wells & Pinder, 1996). Closer examination reveals that lamellae possess more than five times the area of filaments. Lamellae also prove to be the thinnest structures with an epithelial thickness five-fold thinner than filaments and more than thirty-five times thinner than the skin of the outer body surface (Figure 3b).

With our Fick equation, we can use the values from our 10-g Atlantic salmon to quantify exactly how much more diffusive capacity the lamellae possess relative to the filaments (A/T). Dividing our difference in area (5) by our difference in thickness (1/5) reveals that the diffusive capacity of the lamellae is twenty-five times greater than that of the filaments. In other words, the lamellae in a 10-g Atlantic salmon account for over 96% of total gill diffusive capacity. Thus, the lamellae are clearly the dominant gill structure for gas exchange.

Area and thickness of the gill epithelium are also very plastic, able to alter gill diffusive capacity to meet changing demands of gas exchange over different timescales. For example, only about 60% of total gill area is perfused with blood in a resting salmon (Booth, 1978). The pillar cells of the lamellae restrict blood flow to only as much area as is needed for adequate gas exchange. During exercise, however, gill perfusion can be increased to 100% of the total area in

seconds to accommodate the increased demand for oxygen uptake (Randall, 1981).

Structural changes in gill morphology to alter diffusive capacity can also occur over longer timescales when exposed chronically to different environmental conditions. For example, when the scaleless carp is chronically exposed to low environmental oxygen (0.3 mg/L), epithelial cells begin disappearing from the spaces between lamellae on the filaments (Figure 4; modified from Matey et al., 2008). This exposes more lamellar area and reduces filament thickness. In as little as 24 hours, lamellar area increases by more than 50% while total gill diffusion distance is halved. This results in a ~3-fold increase in diffusive capacity. Scaleless carp are extreme examples of gill plasticity, but similar structural changes do also occur in salmon exposed chronically to different environmental conditions.

We also see changes in gill morphology over evolutionary timescales as different fishes are shaped by the demands of their specific life histories and environments. For example, an adult Atlantic salmon has approximately 30 cm^2 of gill area for every gram of body mass (Hughes, 1966). This value is much greater in athletic fishes like mackerel, which have approximately 100 cm^2 of gill area per gram of body mass to accommodate the elevated demands for oxygen uptake associated with their more-active lifestyle. The reverse is true for less-active couch-potato fish like the toadfish, which have only 15 cm^2 of gill area per gram of body mass.

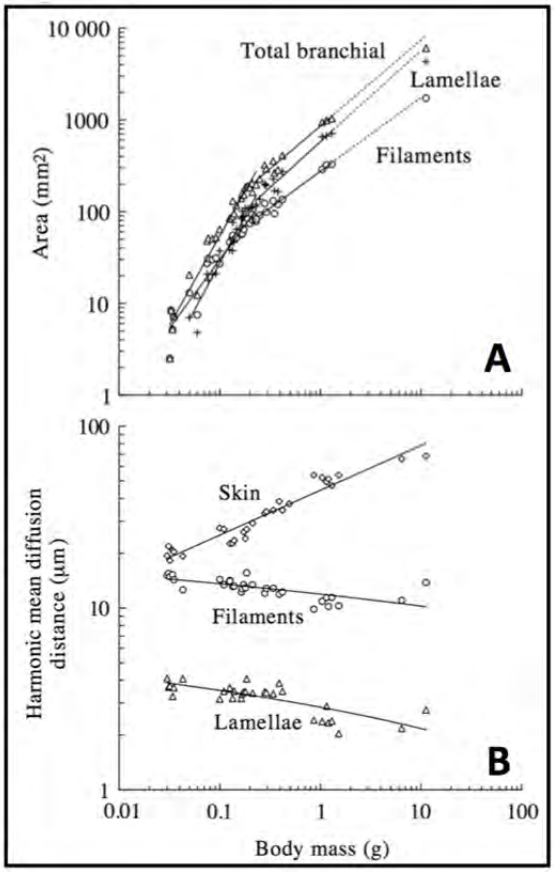

Figure 3
(A) Total gill, filament and lamellar area (mm^2) as a function of body mass in Atlantic salmon (modified from Wells & Pinder, 1996). (B) Harmonic mean diffusion distance (μm) of skin, filaments and lamellae as a function of body mass in Atlantic salmon (modified from Wells & Pinder, 1996).

Lastly, gill morphology is also arranged to maintain favourable partial pressure gradients of gases between the water and blood over the majority of gill surface area. This is achieved by having water and blood flow in opposite directions to one another at the gill and ensures a more complete transfer of gases between water and blood (Figure 5; modified from Evans et al., 2005). For example, water that first enters the opercular cavity to flow across the gill has a high partial pressure of oxygen (i.e. high % saturation). Blood enters the gill at the opposite end with a very low partial pressure of oxygen (i.e. low % saturation). As water flows along the gill, the partial pressure of oxygen in the water decreases as oxygen diffuses into the blood down the local partial-pressure gradient. Conversely, blood flowing through the gill from the opposite end shows an increase in the partial pressure of oxygen as oxygen diffuses in

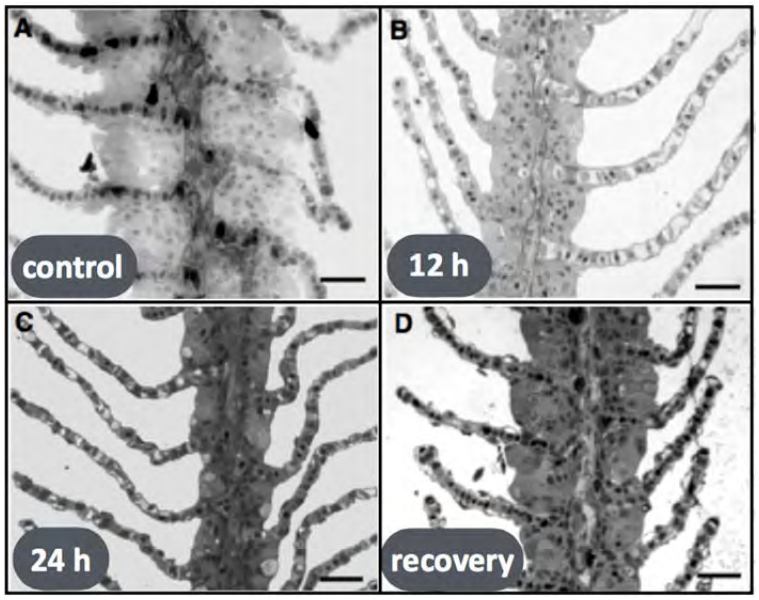

Figure 4
Light micrograph of filament and lamellae of scaleless carp in (A) normoxia (control), (B) 12 hours of hypoxia (0.3 mg O_2/L), (C) 24 hours of hypoxia and (D) 12 hours of recovery in normoxia (modified from Matey et al., 2008; scale bar = 20 µm).

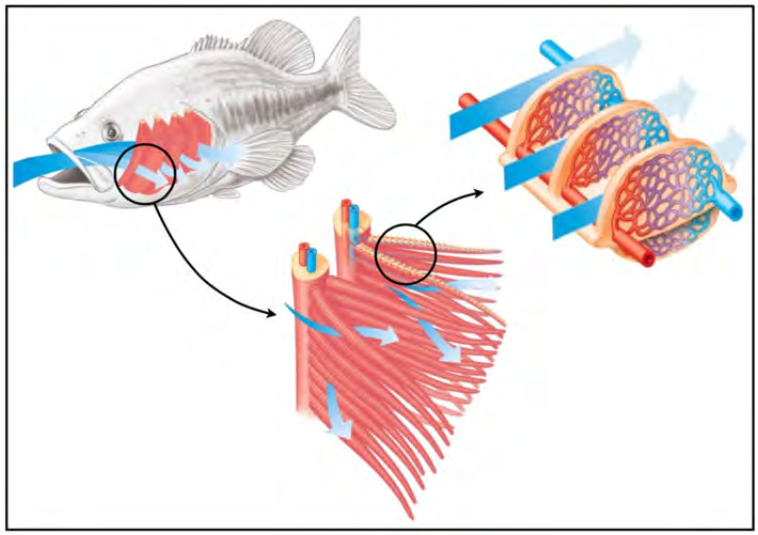

Figure 5
Schematic of counter-current water/blood flow in the generalized fish gill (modified from Evans et al, 2005).

from the water down the same local gradient. This counter-current arrangement ensures that a positive partial-pressure gradient between the water and blood is maintained along the entire length of the gill, even when the partial pressure of oxygen in the water becomes very low further along the gill (Figure 6a). This results in a more complete transfer of oxygen from the water to the blood. Studies estimate that approximately 80% of oxygen from the inspired water can be transferred to the blood in a counter-current arrangement. This arrangement is vastly superior to concurrent flow (Figure 6b; modified from Evans et al., 2005), where the partial pressures in water and blood simply equilibrate at their midpoint, resulting in a less than 50% transfer.

Ion regulation

Salmon maintain the concentration of ions in their blood at very specific levels for proper organism function and survival. The total concentration of ions dissolved in the plasma of the blood is referred to as plasma osmolarity. In freshwater, salmon typically maintain plasma osmolarity at approximately 300 mOsm, which is much more concentrated than the surrounding water osmolarity of approximately 1 mOsm. One drawback associated with having a very large, thin, and permeable gill epithelium for gas exchange is that the gill is also permeable to water and ions. As a result, salmon in freshwater constantly lose ions and gain water across their gills down the osmotic gradient. In other words, enhancement of the gill's gas exchange capacity also incurs an ionic/osmotic burden. This phenomenon is

termed the osmorespiratory compromise (Gonzalez & McDonald, 1992). To combat this passive osmotic disturbance, salmon in freshwater actively take up ions at the gills (such as Na+ and Cl-) and produce dilute urine to excrete excess water. In seawater, the situation is reversed. Salmon in seawater typically maintain plasma osmolarity at approximately 330 mOsm, which is much less concentrated than the surrounding water osmolarity of approximately 1000 mOsm. Thus, salmon are constantly losing water and gaining ions across the gills down the osmotic gradient in seawater. To combat this passive disturbance, salmon in seawater actively excrete excess ions across the gills and drink to actively take up water across the gut.

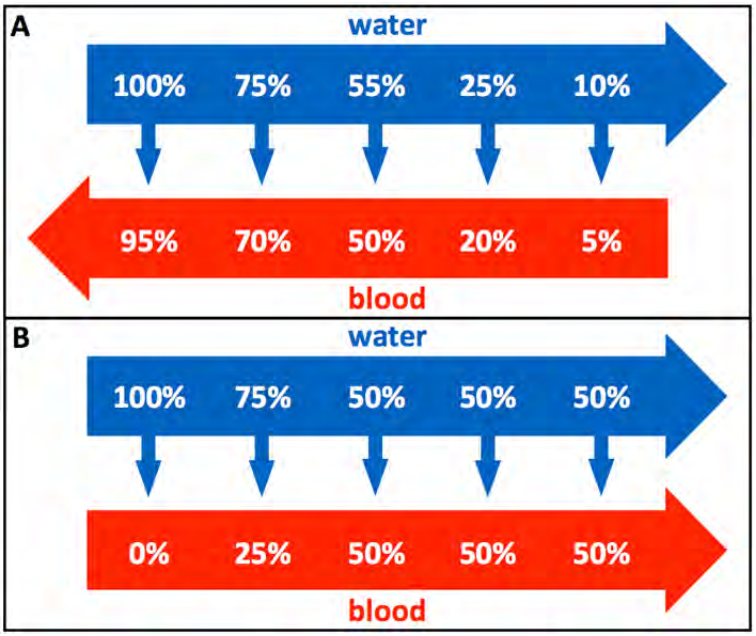

Figure 6
Schematic of gas exchange efficiency between blood/water in (A) counter-current and (B) concurrent flow arrangements.

In both freshwater and seawater scenarios, active ion uptake (FW) and ion excretion (SW) at the gills is driven by ionocytes. As mentioned earlier, ionocytes are larger cells packed with mitochondria, ion transporters, and ion channels. The mitochondria provide energy for the active processes required by transporters and channels to move ions against their electrochemical gradients. There are many different types of ionocytes that contain different combinations of transporters and channels, especially in freshwater. For example, zebrafish are currently believed to have at least five different types of ionocytes involved in freshwater ion regulation (Figure 7; modified from Hwang et al., 2011). Describing all pathways for different ionocytes is beyond the scope of this short article, and interested readers are referred to Hwang et al. (2011) for a more detailed review. However, one transporter worth mentioning here is Na+,K+-ATPase (NKA). NKA is present in nearly all ionocyte cell types and is the major energy consuming component that drives ion regulation in both freshwater and seawater. NKA is located basolaterally on the blood side of ionocytes and serves to establish the electrochemical gradients that drive nearly all ion movement at the gills. This includes ion uptake in freshwater and ion excretion in seawater.

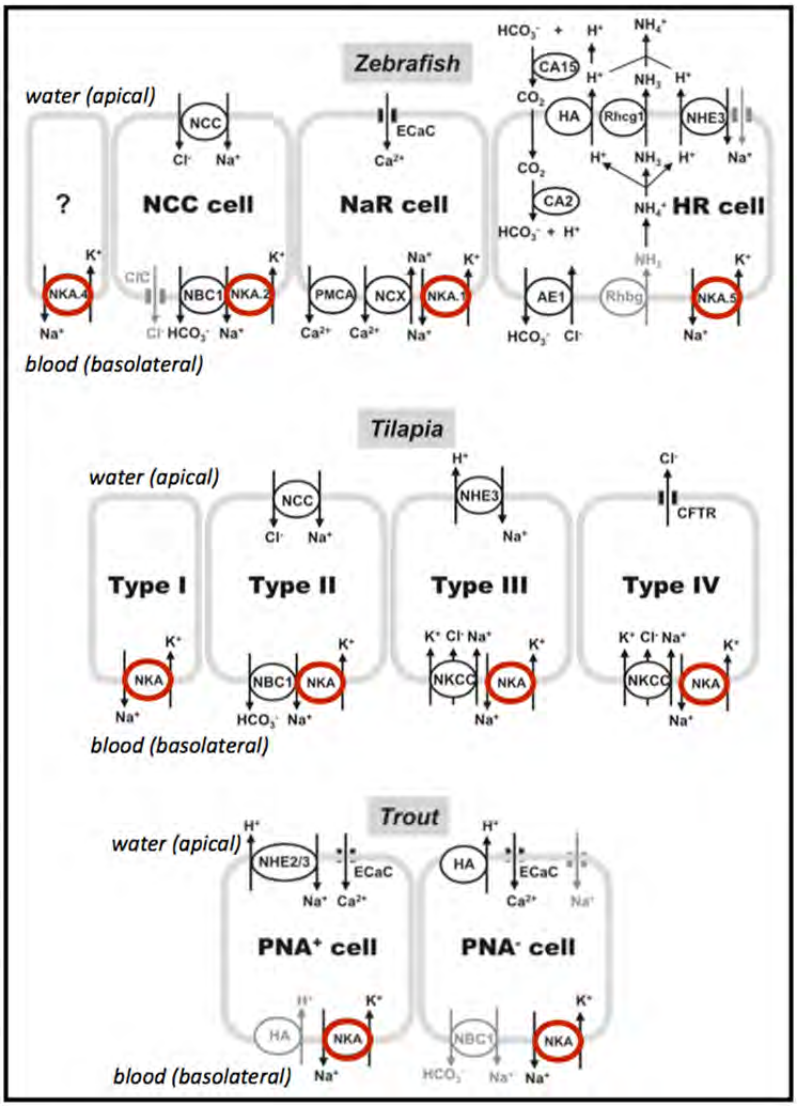

Figure 7
Schematic of various ionocyte cell types hypothesized for zebrafish, tilapia, and trout in freshwater. Gill NKA is located basolaterally in all cell types and highlighted in red (modified from Hwang et al., 2011).

Much like gas exchange, the gill exhibits extensive plasticity with respect to ion regulation. For example, salmon change ionocyte cell type when moving from freshwater to seawater during smoltification, to accommodate the switch from ion uptake to ion excretion (Hiroi & McCormick, 2012). Gills also exhibit plasticity in response to more subtle changes in water ion composition. For example, rainbow trout transferred from ion-rich freshwater (~600 uM Na+) to ion-poor freshwater (~60 uM Na+) undergo dramatic changes in gill morphology (Greco et al., 1995). After two weeks in the ion-poor water, trout increased the size and number of ionocytes at the gill to maintain routine levels of ion uptake (Figure 8; modified from Greco et al., 1995). Because the ionocytes are relatively large cells, their proliferation and expansion resulted in a doubling of the average gill thickness (Figure 8; modified from Greco et al., 1995). According to our Fick equation, this would cut gill diffusive capacity in half. This is yet another example of the osmorespiratory compromise, but this time alterations at the gill to enhance ion regulation compromise the capacity for gas exchange. In this particular study, rainbow trout increased water ventilation rate by ~30% to maintain routine levels of oxygen uptake despite the reduction in gill diffusive capacity associated with ionocyte proliferation.

Summary

The fish gill is a multifunctional organ that serves as the dominant site for gas exchange, ion regulation, acid-base regulation, and nitrogenous waste excretion. Gill morphology is arranged for the effective movement of gases and ions between the blood and water in order to carry out these vital biological processes. This morphology is incredibly plastic and can be altered to enhance different functions in response to changing environmental demands over acute, acclamatory, and evolutionary timescales. However, alterations that enhance gas exchange can compromise ion regulation, and alterations that enhance ion regulation can compromise gas exchange. Gill structure is clearly tailored to meet the combined demands of the local environment and can therefore provide a window into the current physiological state or health of the fish.

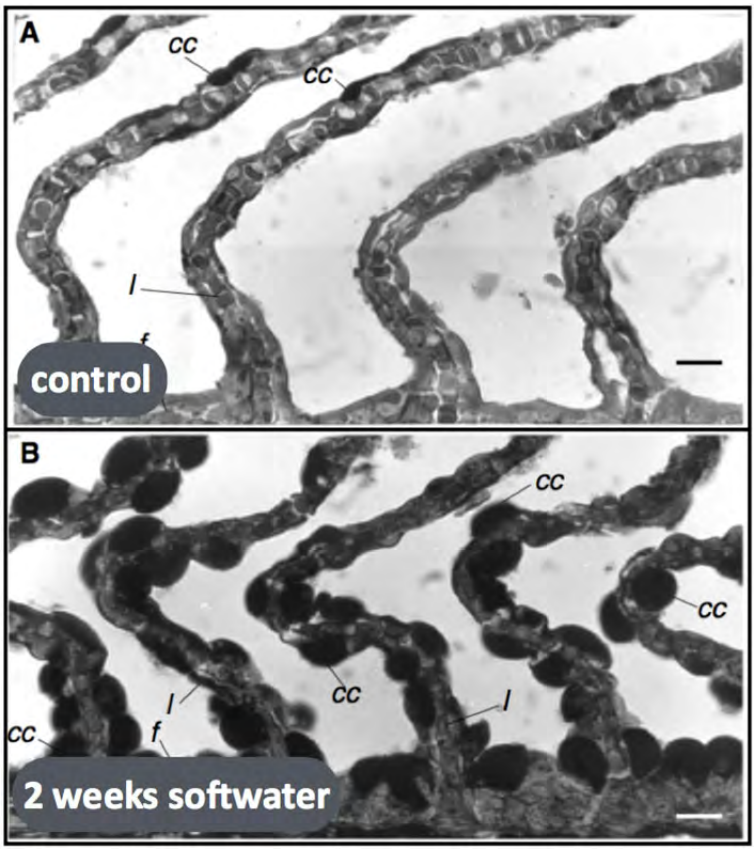

Figure 8
Light micrographs of trout gills (f = filament, l = lamella) in (A) control conditions and (B) following two weeks of exposure to ion-poor water (modified from Greco et al., 1995). Ionocytes (labeled CC) are larger and more numerous in ion-poor water.

Although only oxygen uptake and the regulation of sodium and chloride are discussed in this article, these same principles of gas exchange and ion regulation apply generally to the movements of the majority of gases (O_2, CO_2, NH_3) and ions (Na^+, Cl^-, H^+, HCO3-, Ca^{2+}, etc.) at the gill for other processes like acid-base regulation and nitrogenous waste excretion. This article is a good introduction to gill function but greatly simplifies very detailed and comprehensive areas of ongoing research in fish physiology. Interested readers are encouraged to explore the many excellent sources listed herein for more in-depth explanations.

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SMOLTVISION-A NEW WELFARE INDICATOR

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Abstract

SmoltVision is the first commercially available test able to differentiate between saltwater and freshwater ATPase isoforms via real-time RT-PCR. This assay was field tested in Norway from 2015 to 2016 prior to its commercialization in early 2017. Additionally, further field trials were undertaken within Canada in early 2017. The Norwegian field trial highlighted includes a SmoltVision and ATPase comparison where a population of Atlantic salmon was subjected to formalin treatments for a Costia infection. SmoltVision was able to identify "false" smoltification and served as a better predictive tool for seawater tolerance. In Canada, SmoltVision was trialed alongside ATPase and blood chlorides in two similar S1 Atlantic salmon populations held at different flow-through hatcheries at different water temperatures. Unlike the chlorides and ATPase, SmoltVision was able to provide assurance of progression through the smoltification process and served as a welfare indicator by picking up on the slowed rate of smoltification in the group of fish subjected to suboptimal water temperatures.

Introduction

Na+, K+ ATPase (total NKA) activity has long been used as a proxy for seawater readiness in juvenile salmonids. For some time it has been known that inadequate smoltification negatively affects marine survival, growth, and disease susceptibility (McCormick, 2013). Research has linked normal progression through the smoltification process with increasing total NKA activity (Zaugg & McClain, 1970; McCormick et al., 2009a). As a result, total NKA has been widely accepted as the "gold standard" of smoltification-assessment methodology globally within commercial salmon farming (McCormick, 2013). PHARMAQ Analytiq, a Bergen-based ISO-accredited fish-diagnostics laboratory, has since

performed this assay on several hundred thousand fish within Norway. Over this same time period, a trend towards increased size at time of seawater transfer has also been observed within the Norwegian salmon farming industry. Despite this focus on seawater tolerance, however, the Norwegian salmon farming industry is still experiencing considerable post-saltwater transfer mortality and stock productivity losses, some of which have been linked to suboptimal time of sea transfer (Bleie & Skrudland, 2014).

Distinct isoforms of the α catalytic subunit of the NKA enzyme within the chloride cells of salmonids have been identified and characterized (Richards et al., 2003; McCormick et al., 2009b). Expression of these ATPase isoforms have been shown to change in a predictable manner throughout the smoltification process (McCormick et al., 2013). What is more, different osmoregulatory roles have been associated with these different isoforms. The α-1a NKA isoform (FW NKA) has been associated with expression in the freshwater environment and is associated with a solute uptake role. Conversely, the α-1b NKA isoform (SW NKA) expressed predominately in the saltwater environment has been associated with solute secretion across the gills (McCormick et al., 2009b). Increased SW NKA expression has been linked with increased saltwater tolerance in salmonids (Nilsen et al., 2008; Steffanson et al., 2009).

Conventional ATPase monitoring provides a total ATPase sum of all isoforms active in the gills at time of assessment. SmoltVision, a realtime RT PCR-based analysis offered by PHARMAQ Analytiq, is the only commercially available assay that can differentiate between isoform expressions. This assay was developed by Uni Research AS (Bergen, Norway) at the University of Bergen, and in 2015 PHARMAQ Analytiq acquired the rights to the assay and named it “SmoltVision”. Relative mRNA levels of three different smoltification markers are measured in SmoltVision; FW NKA, SW NKA, and a cofactor. Based on data gathered through 2015 and 2016 PHARMAQ Analytiq developed an algorithm that is used to compare and interpret data from new samplings. A combination of visual traits, behavior, temperature, light regime, and relative mRNA levels reveal what phase the smoltification is currently in. Commercial field trials have shown the novel ability of the SmoltVision assay to differentiate between saltwater and freshwater ATPase isoform expression to be a beneficial tool for understanding and predicting saltwater tolerance.

Methods

Two different field trials were conducted where SmoltVision was compared to other available smoltification assessment methodologies, one in Norway and the

other in Canada. The real-time RT-PCR for the SmoltVision assay was conducted according to Handeland et al. (2014); Na+, K+ ATPase (total NKA) activity was measured according to McCormick (1993), and blood chloride analysis performed via silver chloride coulometric titration (Cotlove et al., 1958) after the fish were subjected to a 24-hour, 34-ppt, saltwater challenge. Fish were euthanized prior to sampling by a sharp blow to the head. 5 mm x 5 mm portions of gill filament tissue for the ATPase and SmoltVision assays were sampled from the bend in the second gill arch.

Norwegian field trial

Eleven total NKA and 9 SmoltVision measurements were taken on a population of 119-gram S0 Atlantic salmon in a flow-through hatchery from July-September, 2016. Salinity was maintained at 0 ppt, while water temperature ranged between 12 and 16°C. A 5-week 12L:12D (winter) photoperiod was used just prior to the first sampling after which 24L:0D (summer) was maintained. The population was expected to be transferred in early August, but was held back due to suboptimal smoltification and a Costia infection. A formalin treatment was subsequently delivered to the population in early August after which the population was carefully monitored until seawater transfer at the end of September.

Canadian field trial

Three concurrent total NKA SmoltVision and blood chloride measurements were taken on two populations of 80-gram S1 Atlantic salmon in surface-water-fed flow-through hatcheries. Fish groups were cultured at geographically distinct hatcheries, but were of the same broodstock origin and had been subjected to similar environmental and production conditions up until the smoltification assessment. Two-week sampling intervals were maintained at both hatcheries over a 4-week period from December (’16) - January (’17). Water temperature over the sampling period at hatchery 1 ranged from 4.5 to 6°C, while at hatchery 2, temperature ranged from 1.5 to 3.0°C. A 6-week 12L:12D (winter) photoperiod was used at both hatcheries that ended in early December after which 24L:0D (summer) was maintained until transfer in mid-January. First smoltification assessments were taken approximately 2 weeks after the initiation of the summer photoperiod and the last assessments were taken 3 days prior to seawater transfer.

Results and discussion

The Norwegian S0 population was held in the hatchery for nearly 2 months longer than expected due to smoltification issues associated with Costia infections and formalin treatments. In late August through early September, an increasing smoltification trend was seen in the total NKA values (Figure 1a). If total NKA alone was used as the smoltification proxy, one would be inclined to think the fish were ready for seawater transfer in early September. However, when observing the SmoltVision results (Figure 1b) over this same time period, it is evident the increase in total NKA is related to an increase in the freshwater component (FW NKA). The Costia infection may have affected the ability of the fish to smoltify, thus producing this prolonged transitional period. This phenomenon has been observed with the gill-borne poxvirus and is thought to be possible with other diseases and parasites affecting the gills of salmonids (Gjessing et al., 2017). Although low-dose formalin treatments administered during smoltification are thought to be safe for normal smolt development (Powell et al., 1996), these same treatments have been associated with small changes to the gill structure of the salmonids (Speare et al., 1997). It could be surmised, then, that gill ionic function may be affected by formalin treatments administered at higher dosages or to fish with previously compromised gills. Either the Costia or the formalin treatment may have negatively affected smoltification within this population of fish.

From early September until transfer, the total NKA remained high, misrepresenting the smolt status of these fish. Through this same time period, however, changes are occurring within the mRNA expression of the fish. It was noted that the FW NKA had started to come down but that the SW NKA and cofactor both had not yet begun to increase. These results indicated that the fish were still transitioning and not ready for seawater transfer. The subsequent transfer of these fish to sea resulted in high mortality and non-performers. SmoltVision thus proved to be a more useful tool at predicting seawater tolerance within this group of fish.

In the Canadian case study, hatchery 1, having the warmer surface water relative to hatchery 2, progressed normally through smoltification. This was seen in both in the increasing total NKA and decreasing chloride values (Figure 2a). Threshold values were passed on both of these tests. The SmoltVision results for hatchery 1 (Figure 2b) showed a similar smoltification trend over the time series with a decreasing FW NKA and increasing SW NKA and cofactor. The last sampling point showed the SW NKA high relative to the FW NKA, indicating a seawater tolerant smolt. Where things progressed normally in hatchery 1 total

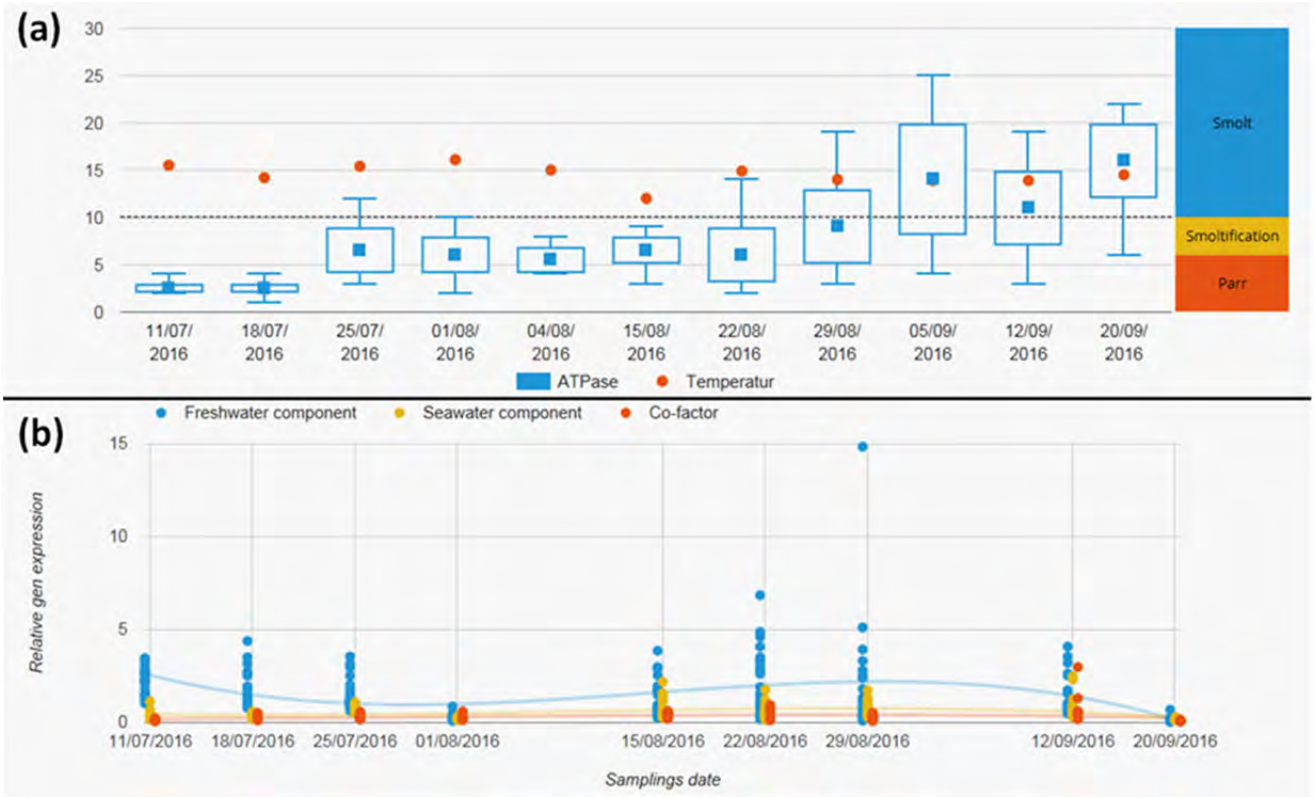

Figure 1
Norwegian field trial smoltification data from July - September, 2016. (a) Total NKA results. (b) SmoltVision results.

NKA and chloride values confirmed SmoltVision and were useful smoltification proxies unto themselves.

Throughout the sampling period, hatchery 2 (on average) experienced 2.5°C cooler water. Although total NKA and chlorides indicated average passing values by the last sampling point (Figure 2c), the trend over the time series was hard to decipher. Over the last two samplings chlorides were indicating a stalling or desmoltification trend, while total NKA remained unchanged. With chlorides and ATPase used alone here, one may be inclined to expedite seawater transfer of these fish given that the chlorides and total NKA were passing but indicating possible desmoltification. The SmoltVision results (Figure 2d) showed a much different picture. It is evident that the FW NKA was decreasing over the time series and that both the SW NKA and cofactor were increasing. SmoltVision thus provided assurance of smoltification progression. The last sampling indicated a FW NKA that was still high relative to the SW NKA, thus providing a clear signal to hold onto these fish and wait for further smoltification until seawater transfer. When comparing Figure 1b to Figure 1d, hatchery 2 was approximately two-weeks behind hatchery 1 in terms of smolt development. This was likely related to the cooler water and thus a reduced thermal sum over the trial

period. Cooler water has been shown to slow smoltification in other research (McCormick et al., 2002; Handeland et al., 2013).

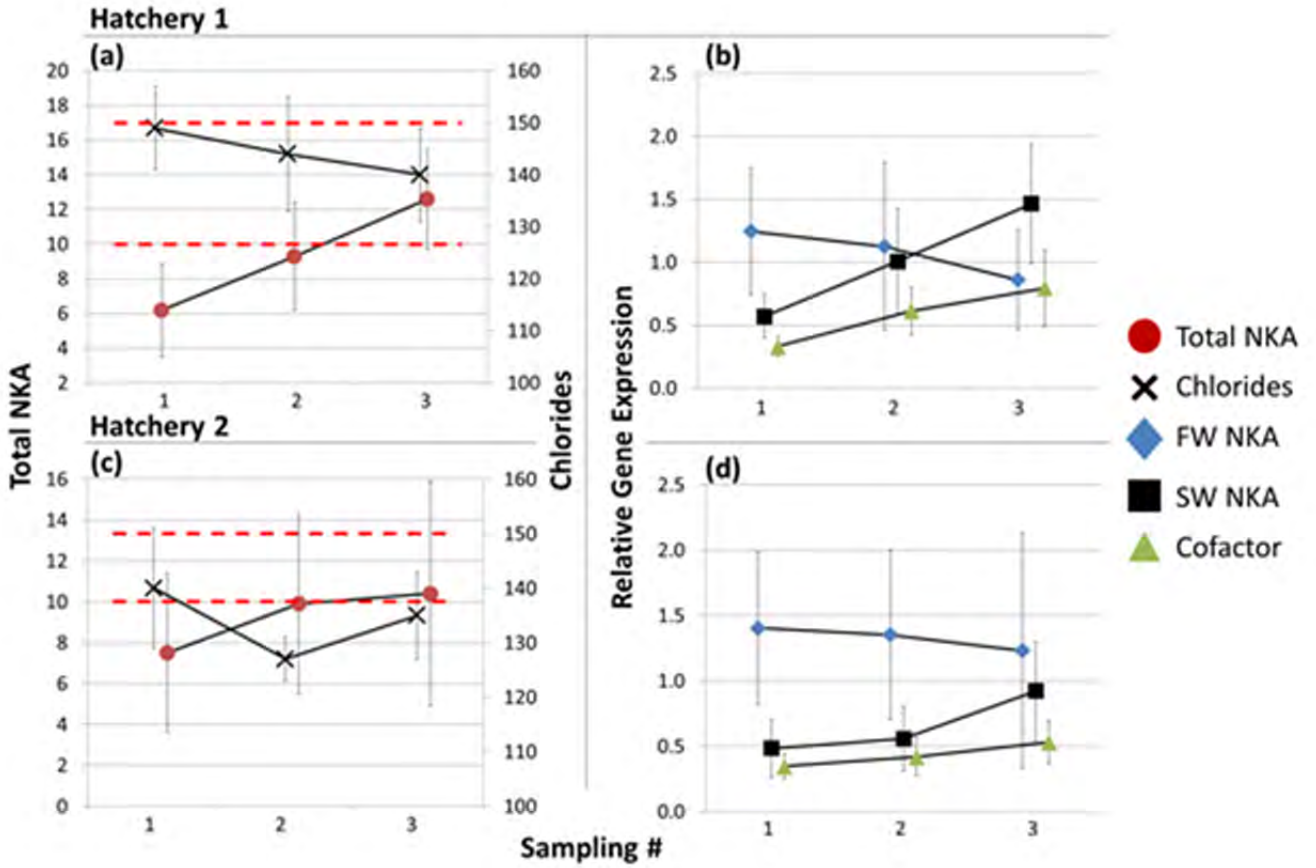

Figure 2
Canadian field trial smoltification results. All data are means ± S.D. (n = 15). (a-b) Hatchery 1 results; "warmer" water hatchery. (c-d) Hatchery 2 results; "cooler" water hatchery. (a) Hatchery 1 total NKA and chloride results. (b) Hatchery 1 SmoltVision results. (c) Hatchery 2 total NKA and chloride results. (d) Hatchery 2 SmoltVision results. (a and c) Total NKA threshold set at 10 and indicated by a red dotted line, chloride threshold set at 150 and indicated by a red dotted line.

Both groups of fish (hatchery 1 and hatchery 2) were transferred to sea 3 days after the final sampling to the same sea site. Post-transfer mortality was negligible (60-day, < 0.5%) for both groups. This was thought to be due to the brackish surface water (0 - 5 m, 25 ppt), which is normal for this particular site during the winter months. If salinity were high at time of entry, it is likely the fish from hatchery 2 would have experienced higher mortality and non-performers.

In sum, the novel ability of SmoltVision to differentiate between FW NKA and SW NKA isoforms provided the following advantages over traditional smoltification assessment methodologies within these field trials:

1) Correctly identified “false” smoltification.
2) Better predicted seawater transfer timing.
3) Provided assurance of progression through smoltification.
4) Served as a welfare indicator by helping to identify other factors present (formalin treatments, Costia infections and suboptimal water temperatures) affecting smoltification.

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GILL HEALTH IN SALMONID AQUACULTURE

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Abstract

Gill disease in marine and freshwater salmon aquaculture is a significant health challenge in all regions. The causes can be infectious, such as from parasites, bacteria or viruses, non-infectious, as from harmful algae and nematocysts in zooplankton, or a mixture or both infectious and non-infectious components. This article reviews recommendations for successful gill-health management and is based on practical farm and veterinary experience in salmon aquaculture.

Introduction

The global impact of marine gill disease is now equal to, or surpasses, in many geographic regions, that associated with sea lice in salmonid aquaculture; however, in many cases this remains un- or under-diagnosed. In freshwater salmonid aquaculture, gill disease is also a highly significant challenge in many regions, with surveys in Norway confirming that non-specific gill disease is either the first or second most-important disease in freshwater systems (Hjeltnes et al., 2016). The impacts from gill disease (both infectious and non-infectious) are multiple and, in addition to direct mortalities, there are indirect mortalities when bath treatments may be undertaken (for instance for sea lice). Also, there is poor growth, poor performance and increased food conversion ratios; increased susceptibility to other infectious diseases; and reduced capacity to tolerate rapid changes in the rearing environment or management and husbandry changes (Rodger, 2007; Mitchell and Rodger, 2011). This article summarises the presentations by the author at the CAHS Gill Health Workshop in Campbell River in February 2017.

Gill function and 10 key aspects for successful health management

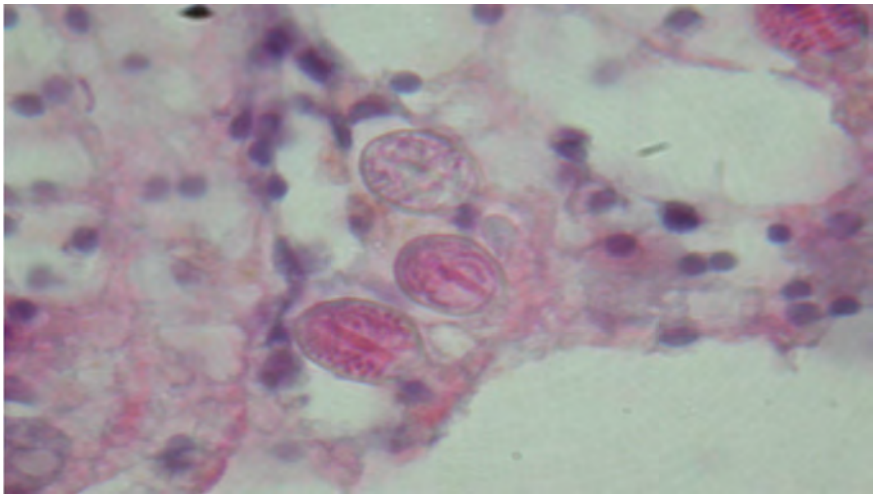

Figure 1
Histological section of nematocysts in the tentacle of the jellyfish *Pelagia noctiluca* (H & E x1000).

The gills have multiple essential functions for healthy living in fish and these include gas exchange (of both oxygen and carbon dioxide) for respiration, ion exchange for acid–base balance and osmoregulation in the body, excretion of nitrogenous waste products (mainly ammonia) and neurosensory capacity for oxygen and carbon dioxide levels. Damage to, or a disorder of, the tissues and functions of the gills will therefore impact on all these and lead to multiple effects for the fish. There are 10 keys aspects that can be applied to successful health management of gill disease and these can be outlined as follows:

Figure 2
Amoebic gill disease (AGD) gross pathology in Atlantic salmon gills showing typical areas of raised, mucoid, grey/white areas on filaments.

1. Know the causes of gill disease. Gill disease can have single or multiple aetiologies and these include harmful algal blooms (HABs) via physical, toxic, or deoxygenation effects; harmful zooplankton swarms due to the effects of nematocysts (Figure 1); parasites e.g. amoebic gill disease (AGD) (Figure 2); bacteria e.g. *Candidatus Branchiomonas cysticola* (Figure 3); viruses e.g. salmon gill pox virus (SGPV) (Figure 4); and chemical damage e.g. hydrogen peroxide (Rodger et al., 2011; Gjessing et al., 2017; Wiik-Nielsen et al., 2017). It is important to know what condition is affecting your stock to enable the most appropriate remedy or procedure to reduce the impact of this.

2. Know the status of the gill health of your livestock. This is especially important prior to any treatment, movement, grading, or other management procedure that could be stressful to the fish. The clinical situation with gill health can

change rapidly, especially during periods of high water temperature with clinical AGD developing within a week of the first signs of gross pathology. The presence of subclinical gill disease coupled with a sudden management procedure can induce significant stress and impact on livestock.

3. Monitor gill health regularly. This is the action that is undertaken by most marine farms now in Western and Southern Norway, Scotland, Ireland, and Tasmania through weekly clinical examination (gill scoring) under anaesthesia from every pen and routine checks via histology and PCR at least every month but more frequently during higher risk periods. In addition, environmental data [temperature, salinity, oxygen, carbon dioxide (in RAS units)], phytoplankton and harmful zooplankton, as well as levels of biofouling on pens should also be continuously monitored as these will all have a bearing on gill health and methods to control disease.

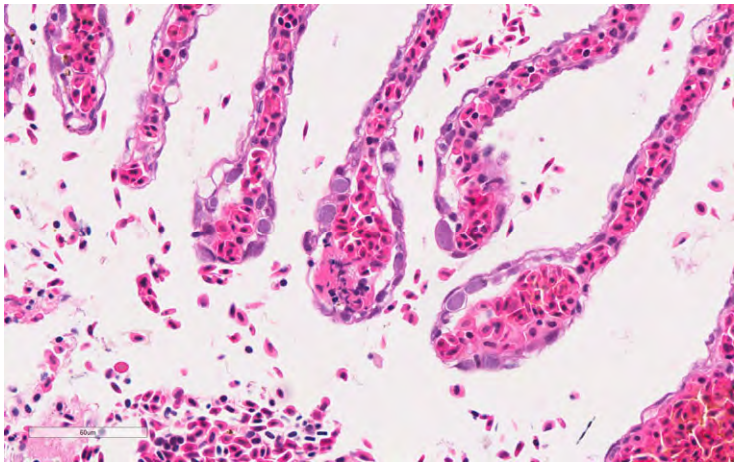

Figure 3
Cysts in the gills of Atlantic salmon caused by the bacteria *Candidatus Branchiomonas cysticola* (H & E).

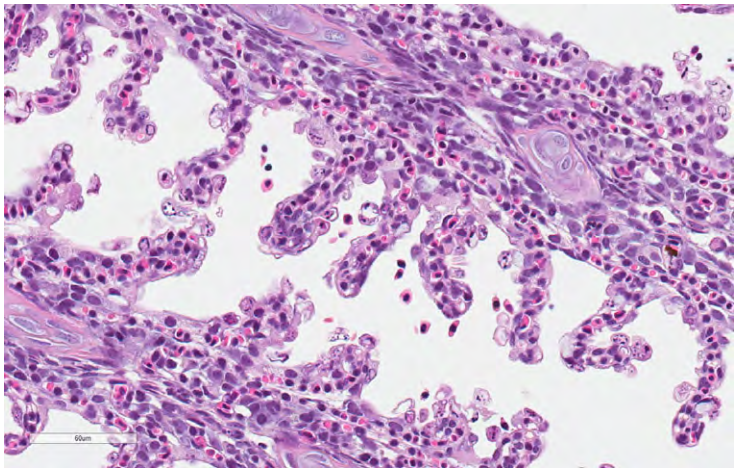

Figure 4
Histological section of Atlantic salmon gill affected by salmon gill pox virus (SGPV) exhibiting epithelial cell apoptosis and sloughing (H & E).

4. Undertake pre-bath gill (and health) assessments. Prior to any bath treatments for sea lice, amoebae, fungus, etc., examine the gills through clinical, histological, and PCR methods so that you are informed about gill condition and can adjust treatment dose and time accordingly. Further, fish in marine (and in some freshwater sites) should be screened for myopathy viruses and pathology (PCR and histology) to ensure there is no sub-clinical myopathy due to salmonid alphavirus (SAV), piscine reovirus (PRV), or piscine myocarditis virus (PMCV). If these viruses or pathology are present then treatment may require to be postponed, if practical, until the fish are through the viraemic phase of the disease, which is when they are most vulnerable to clinical impact.

5. Screen for other conditions which impact on gill health. Deformities and malformations such as mandibular osteopathy and opercular shortening reduce the ability of the buccal pump to operate normally in the fish and the efficacy of normal respiration is reduced.

6. Know when to treat. Treatment for AGD is more beneficial for the livestock when undertaken early in the disease progression for reasons such that the fish are better able to cope with the stress of treatment due to less gill pathology and a lower parasite burden (which reduces the reinfection risk). A longer time interval to retreatment, when required, is the usual result. However, it is important that an accurate diagnosis is obtained prior to any proposed treatment; if the fish do not have AGD but are rather affected by a proliferative gill disease, then bath treatments would do more harm than good. Is the gill score/condition changing rapidly, and how is the general health of the fish? What are the water quality parameters? It is important to have these established to adjust any treatment times and dosage.

7. Be prepared to treat. For AGD the most effective treatments remain freshwater baths (< 3 ppt for three hours) and hydrogen peroxide (1400 ppm for 20 to 30 minutes, but dosage depends on water temperature and fish condition), and both of these require considerable advance planning and preparation in terms of equipment, the bath itself, staff training and experience, and fish examinations. Both types of bath treatment can lead to mortalities as a result of low oxygen during or after treatment, physical damage at crowding or pumping, the presence of concomitant diseases such as systemic viral infections, and degree of gill damage present.

Figure 5
Freshwater bath treatment in Atlantic salmon farm in Tasmania.

8. Control oxygen and carbon dioxide. Prior to any bath treatment it is best to superoxygenate the bath water and maintain high levels throughout treatment time and post-treatment. Carbon dioxide levels will accumulate in the fish body, especially with larger fish and during long bath treatments; if gill pathology is significant, treatment times may have to be reduced. Carbon dioxide levels will also rise in the freshwater bath during longer treatments, and this can lead to a fall in water pH levels, especially if the water has naturally low levels of calcium carbonate. Additional buffering may be required (Figure 5).

9. Focus on genetic selection for gill health. It has been demonstrated that there is genetic heritability in the ability of salmon to tolerate challenge with gill amoebae (Taylor et al., 2009). This is reflected in farm experience where different stocks and individuals have very different reactions to AGD (Rodger, 2014). Broodstock companies have identified AGD as a disease that can be targeted via genomic selection. In regions where AGD is an increasing challenge, this should continue to be a focus, to ensure only tolerant stocks are grown in high-risk sites.

10. Accurate disease diagnosis is essential. The causes of gill disease can be single or multiple and the clinical situation is constantly evolving with opportunistic organisms and other agents becoming involved in gill disease secondary to primary insults. Therefore, it is important to monitor gill health on a continual basis and after primary disease diagnosis, because the situation is dynamic and liable to change. In addition to fish examinations and surveillance, the environmental parameters and observations are also vital in the decision-making process for whether and when to treat. On-site microscopy, point-of-care rapid tests (mobile PCR), plus histopathology and bacteriology are all important tools that should be utilized.

There are multiple challenges with gill disease such as: the problems becoming less seasonal and more of an all-year condition; restrictions on access to, and options for, treatments; and climate change and environmental pressures through algal and zooplankton blooms. All lead to the need for constant surveillance and increased monitoring of gills, fish health, and the rearing environment.

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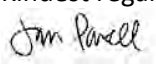
Corporate Description - BC Centre for Aquatic Health Sciences

The BC Centre for Aquatic Health Sciences (BC CAHS) exists to improve the health of aquatic ecosystems through applied scientific knowledge. We are a non-profit organization in Campbell River BC, Canada dedicated to improving the health of aquatic animals and ecosystems through applied scientific knowledge. We have a state-of-the-art laboratory in the heart of the wild and industrial aquaculture communities. We serve a variety of stakeholders in fisheries and aquaculture, First Nations and the enhancement communities. We investigate aquatic animal health issues that are important to the sustainability of fisheries and aquaculture production ranging from ecosystem, whole animal work to advanced molecular biology techniques. We have been in operation for over 12 years and are a stand-alone, fee-for-services operation that is independent of government, industry or other outside interests. We operate on a science-based model and adhere to a rigorous academic ethic.

As part of our ongoing commitment to stakeholders, our mandate and mission supports outreach in the areas of which we practice. Information not shared is of little use to others and causes progress to stagnate. To help disseminate important advances and identify future research directions, BC CAHS partners with the DFO ACRDP and a variety of sponsors to hold formal, education workshops on topics of aquaculture relevance concerning the health and welfare of aquatic ecosystems. The topics for the workshops are chosen by user groups and BC CAHS finds the experts, makes all the logistical arrangements and hosts the workshop in conjunction with the ACRDP, the BC Salmon Farmers Association and other industry stakeholders. These workshops are free for attendees and the proceedings open to the public. The results are an enlightened and engaged audience who help us deploy new information that is the state-of-the-art for the topic and identify the research gaps and priorities.

We are pleased to share the results of our resent Gill Health Workshop with you.

Kindest regards,



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