Ventilation of Spiny Dogfish (*Squalus suckleyi*): Are These Five Gill Slits Working Together?

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Ventilation of Spiny Dogfish (*Squalus suckleyi*): Are These Five Gill Slits Working Together?

Shark's ventilation has long been characterised by a two-pump model, where the water coming from the spiracle and the mouth is released by the gill slits after the breathing process. This movement is created by the difference of pressure between the oro-branchial cavity and the parabranchial cavity, consisting of five gill pouches. In an attempt to compare the actual flow velocity coming from each of these five gill openings, experiments have been made on seven individuals of spiny dogfish, *Squalus suckleyi*. External measurements have been taken, revealing important differences between the fifth gill slit and the four others, in term of vertical length and width. Also, the introduction of dyed water just above the spiracle has revealed the utilisation of the four first slits. A particle image velocimetry analysis have confirmed the weak flow coming from this last slit. It has also exposed the possibility of the fourth slit's flow being less than the flow coming from the three first gill openings. This could be in relation with their feeding behavior, which mostly consist in suction action. The fifth slit could then be used to discharge water while the shark is feeding on preys.

Introduction

The ventilation cycle of sharks is characterized by a two-pump model where the water first enters the mouth and the spiracle and is then expelled from the parabranchial cavity through the gill slits. The gills of the elasmobranchs consist of five gill pouches, the barabranchial cavities (Goto *et al.*, 2013). The water's movement from the oro-branchial cavity to the gill openings is created by a differential pressure, allowing the flow to pass from the mouth to the actual gills where the breathing occurs (Ferry-Graham, 1999). It is then interesting to point out the fact that sharks are usually characterised by a series of at least five gill slits that allow the water to be expelled after the oxygen had been taken up by these gills. To contrast the majority of fishes that possesses only one gill opening, the operculum, it would be of great interest to understand the actual way of working of these five gill slits.

It has been pointed out by Hughes (1959) that adduction and abduction of the branchial region spreads serially from the first to last gill slit in the dogfish. It is also interesting to mention that water entering from one side of the mouth leaves by the three posterior gill slits of the same side. The water entering from the spiracle leaves through the anterior slits of the same side (Hughues, 1959). But there is actually no studies that have been made concerning the actual flow coming from each of these gills, from a quantitative point of view.

An interesting technic that have been used for the past few years, especially associated with locomotion in fish, is the particle image velocimetry (PIV). This technic allows to quantify the actual flow by analysing the movement of particle in a fluid, using a light-sheet created by a laser. That kind of method has already been used to describe the burrow ventilation in the tube-dwelling shrimp *Callianassa subterranean* by Stamhius and Videler (1998). This technic could then be useful in describing the flow coming from each of the five gill slits in the spiny dogfish, *Squalus suckleyi*, in an attempt to compare the water velocity. That would allow to better understand the actual way of working of these five pouches during the ventilation cycle of sharks.

Materials and Methods

External measurements

The dogfish were captured with a trawl on the 20th of June 2014 in the San Juan Channel (48°35'10.9''N 123°02'18.7''W) and the Lopez Upright Head (48°34'45.4''N 122°53'03.2''W), near the San Juan Island, Washington state. They were then held in two circular tanks with a constant flow of sea water, taken directly from the Pacific Northwest Ocean. A total of seven spiny dogfish were used for these experiments, one female and six males. For each sharks, the total length was measured by taking a picture of their dorsal view associated with a scale and by using ImageJ software (version 1.48). The gill slits horizontal widths were also obtained using the same technic, when the slits were opened at their maximum. The vertical heights of the five gill slits were obtained with pictures of the lateral view of the sharks and shown as percent of total length. Cluster analysis were used to regroup the different measurements taken on the gill slits using R Studio (version 3.1.0) and the package vegan (Oksanen *et al*, version 2.10).

Dye experiments

Dye experiments were made to visualize the flow coming from each gill slits. The sharks were placed in a glass tank (121 cm x 60 cm x 45 cm). The water was changed for every sharks, and they were not immobilized in any way. They had to lay in the bottom and stop moving prior to these experiments to be able to record videos using a Casio Exilim EX-FH20 high speed camera (30 fps) from dorsal and lateral views of the gill slits. The fact that the sharks have to stop moving by themselves instead of immobilizing them tend to mimic a behavior more natural. That allows to give a better idea of the real flow coming from each slits.

Once the shark had calm down in the glass tank, an appreciable quantity of dye (McCormick food dye) mixed with sea water was introduced by a pipette positioned just above the spiracle. The subsequent colored flow was then assessed by recording high speed video for at least five cycles of ventilation to allow a good amount of dye passing through the spiracle and flowing out the slits.

Particle Image Velocimetry

The quantitative flows from the five gill openings were assessed using particle image velocimetry (PIV). The sharks were again placed in the same glass tank filled with sea water. The particles used to visualize the flow were artemis cysts eggs (Great Salt Lake, Sanders Brine Shrimp Company, Ogden, Utah). An important amount of these eggs was added in the water, enough to be able to see them moving while shark's ventilation. Once the shark was laying in the bottom of the tank, a light sheet was created using a red laser (Black&Decker Crosshair Auto Laser Level, 9.0 V) positioned right in front of the five gill slits. A video of the dorsal view of the fish was recorded with the high speed camera for the next twenty seconds. These videos were then analysed using the software Matlab (version 7.10) with the PIVlab package (Thielicke and Stamhuis, 2010). Once the videos have been analysed, the flow coming from each gill were compared for all five sharks using a boxplot made on R with the package sfsmisc (Maechler *et al*, version 1.0-26).

Results

External morphology

The external morphology of the gill slits of the spiny dogfish tends to show that there is variation between the height of these openings (Table 1). The fifth one tends to be bigger than the others four. Also, the distance between the fourth and the fifth gill slits is reduce compare to the distance between the others adjacent slits for first to fourth. It is also interesting to mention that the fifth gill slits is much arched than the others and surrounded the pectoral fin.

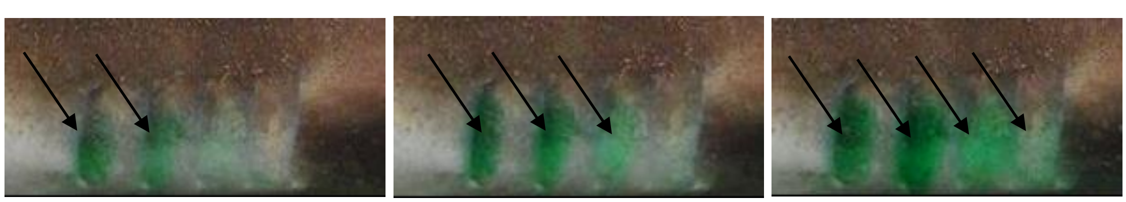
Table 1. External gill measurements for seven individuals of *Squalus suckleyi*

Shark	Sex	Total lenght (TL mm)	Vertical height of gill slit (% TL)				
			1st	2nd	3rd	4th	5th
1	male	739.4	1.4	1.5	1.6	1.6	2.1
2	female	723.0	1.8	1.9	1.8	2.0	2.2
3	male	600.3	1.3	1.3	1.4	1.6	2.1
4	male	596.0	1.6	1.6	1.8	1.7	1.9
5	male	613.3	1.9	1.9	1.9	1.8	2.1
6	male	691.7	1.7	1.7	1.7	1.8	2.1
7	male	666.4	1.4	1.5	1.6	1.8	2.4

Ventilation cycle of the five gill slits

The dye experiments were useful to demonstrate the ventilation cycle of the five gill slits. As the shark begins to assimilate the food dye by its spiracle, the four first gill slits tend to open simultaneously. The first one tends to expelled dye rapidly (Fig1A) after what the second, the third and the fourth slits begin to work each turn (Fig1B-1C). The fifth gill slit tend to never reject any dye (Fig1D) and is also not opening very much (Table 3).

A **B** **C**



D

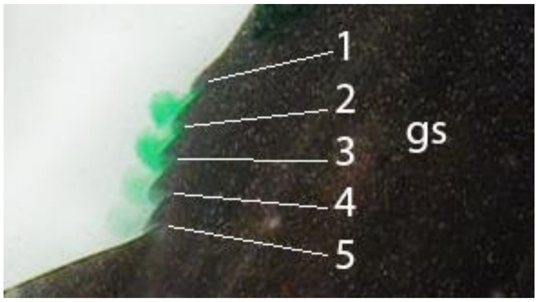


Fig. 1. Gill ventilation for respiration of *Squalus suckleyi* **(A-C)** In lateral view for shark one. Dye introduced by the spiracle and slowly expelled from first to fourth gill slits. **(D)** In dorsal view for shark five. The first four gills are used to expel the dye coming from the spiracle, but the fifth one remain unused. Abbreviation: gs, gill slit.

Table 2. Horizontal width for seven individuals of *Squalus suckleyi*

Shark	Horizontal width (mm)				
	1st	2nd	3rd	4th	5th
1	3.68	3.82	4.16	3.58	0.81
2	3.87	3.80	3.89	2.93	1.20
3	3.32	3.50	3.19	2.89	1.33
4	3.15	3.82	3.52	3.55	0.99
5	3.16	3.69	3.91	3.44	0.89
6	4.04	4.00	3.70	3.69	0.72
7	2.70	2.98	2.73	2.33	0.61

Flow from the gill slits

The particle image velocimetry analysis show that the flow coming from each gill slits tends to be the same, except for the fifth opening. The last gill slit expel water with a weaker flow than the others four, and that for the entire cycle of ventilation. The flow around gill one to four tend to be the same during the entire cycle of ventilation, from the beginning (Fig.2A) until the slits are opened at their maximum (Fig.2B).

A **B**

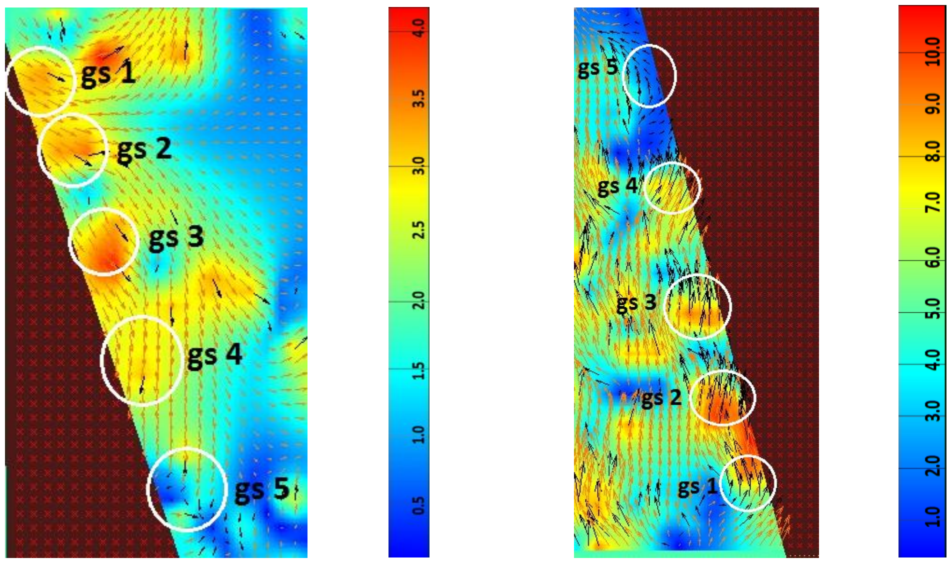


Fig. 2. Result of the particle image velocimetry (PIV) showing the flow coming from each five gill slits. **(A)** PIV of shark seven at the beginning of the cycle of ventilation. **(B)** PIV of shark six when its gill slits are open at their maximum. Color bars represent the velocity magnitude ($*10^{-3}$ mm/sec). Abbreviation: gs, gill slit.

Over all, the fifth slit seems to remain poorly used, with a flow coming from it much lower than the others (Fig.3). For the five sharks that have been used for the particle image velocimetry, the analysis reveal a drop in the water flow for this fifth gill. What is also interesting is to point out the fact that the flow from the fourth gill slit tend to be less important compare to the others.

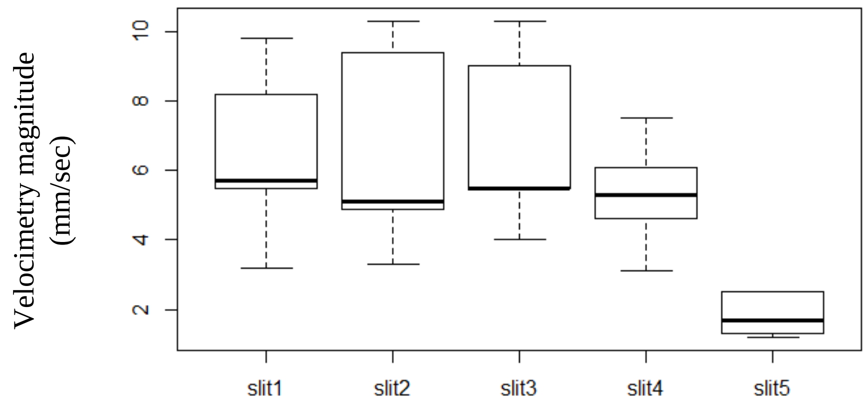


Fig.3. Boxplot showing the amplitude of velocity magnitude of the water coming from each gill slits of five *Squalus suckleyi*.

Discussion

Two cluster analysis have been made to compare the external morphology of gill slits of the seven spiny dogfish. The results tend to demonstrate that for the vertical length and the width of the gill openings, the fifth slit is slightly different from the others. In fact, its height is much more important than for the others, but its opening tends to be smaller. It means that this last slit is apart from the four others slits, which look more alike each from another in terms of external morphology.

These differences in term of morphology could in part explain the fact that, during dye experiments, only the four first slits were expelling colored water. It is not sure if the water was flowing out of the gills in a perfect synchronicity. The problem with this technic might come from the fact that the dye could have been dissolved with more water when passing through the fourth slit. That could be explained by the fact that the distance between the last slits and the spiracle is bigger compare to the distance between it and the first gill openings. I have to mention that at any time, even if I was dropping an important amount of dye, I have never seen colored water coming from the fifth gill slit.

The fact that the fifth gill slit doesn't seem to be working a lot has also been demonstrated by the PIV analysis. The flow coming from this opening is slightly less than for the others. Goto *et al.* (2013) had also reported the difference in term of utilisation and morphology of the fifth gill slit in the carpet shark family (Parascylliidae). They related it to the feeding behavior of these sharks, which are suction feeders. They concluded that this uniquely large fifth gill is functional for water discharge during suction feeding actions. It could then be interesting to also relate the unusual fifth gill of the spiny dogfish to its feeding behavior. In fact, *Squalus suckleyi* use both suction and ram behaviors to capture and manipulate prey, while only suction is used to transport it (Wilga and Motta, 1998).

It would be ideal to conduct further research on the actual feeding ventilation during the manipulation of prey by the spiny dogfish. More PIV should be done to confirm the hypothesis of the utilisation of the fifth gill slit during feeding. The fourth gill slit should also be investigated more, to try to understand if the shark is actually able to regulate and control its ventilation through the different gill openings.

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