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Assessment, causes and consequences of short opercula in laboratory-reared Atlantic salmon (Salmo salar)

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Abstract

*Opercular deformity is a common morphological abnormality of laboratory and other cultured fishes, observed in a wide variety of species but with an unclear aetiology. Following observations of short opercula in stocks of Atlantic salmon (*Salmo salar*) reared in our laboratory, we developed a photographic key to score individual fish on a scale of 1 to 5. Inter-rater reliability was assessed as 'almost perfect'. This visual method is quick and simple to use, can be used to score live fish* in situ *in tanks as well as sampled fish, does not require sophisticated equipment and provides quantitative information to investigate the aetiology of short opercula. Opercular size was scored for a cohort of in-house reared Atlantic salmon, in a time series of random samples of ≥ 30 fish (mean weights ranging from 0.8 to 299 g) over 14 months. Short opercula were first recorded during the parr stage, prevalence and severity increased as the fish grew, and the deformity was asymmetrical, occurring predominantly on the left side. Therefore, among the many potential causal factors, nipping is suggested as the primary cause of short opercula within our culture system, with asymmetry due to the clockwise current. We also present evidence that short opercula are associated with gill damage which supports this deformity being a welfare issue that merits assessment.*

Keywords: *animal welfare, Atlantic salmon, fish welfare, opercular damage, welfare assessment, welfare indicator*

Introduction

Abnormalities of the operculum (gill cover) of cultured fish have been documented since the early 1900s (Osburn 1911). A diverse range of freshwater, marine and diadromous bony fishes, cultured in tropical and temperate waters, for food, laboratory, ornamental and conservation purposes are known to be affected (Table 1).

The operculum is composed of four distinct and articulated bony plates: the opercle, preopercle, interopercle and subopercle (Ortiz-Delgado *et al* 2014). Opercular abnormalities can be classed into three categories: opercular plate reduction (resulting in short opercula), opercular plate folding, and concave depression (Lindesjoo *et al* 1994; Koumoundouros *et al* 1997; Beraldo *et al* 2003; Boglione *et al* 2013; Ortiz-Delgado *et al* 2014; Conceicao & Tandler 2018). Deformities can occur unilaterally or bilaterally (Koumoundouros *et al* 1997; Kazlauskienė *et al* 2006; Ortiz-Delgado *et al* 2014; Pettersen *et al* 2014; Skipnes 2014; Larsen *et al* 2018; Noble *et al* 2018).

Skeletal deformities in fishes have been attributed to many causes (for reviews, see Boglione *et al* 2013; Berillis 2015). A definitive aetiology of opercular deformity has yet to be established, with a wide range of causal and risk factors being proposed for the different species and rearing systems. These include: nutritional deficiency (Al-Harbi 2001; Fraser & De Nys 2005; Darias *et al* 2011; Baeverfjord *et al* 2019); consumption of excessively large prey or hyperventilation (Beraldo *et al* 2003); feeding regime (Larsen *et al* 2018; Noble *et al* 2018); poor water quality (Lindesjoo *et al* 1994; Andrews 2011; Barkstedt *et al* 2018); inappropriate incubation temperatures (Georgakopoulou *et al* 2010; Fraser *et al* 2015); turbulence and water current velocity (Divanach *et al* 1996; Al-Harbi 2001; Beraldo *et al* 2003; Ortiz-Delgado *et al* 2014; Larsen *et al* 2018); gas supersaturation (Jensen 1988); genetic factors (Sadler *et al* 2001; Kazlauskienė *et al* 2006; Amoroso *et al* 2016; Peruzzi *et al* 2018); bacterial and parasite infections (Noble *et al* 2018); steroid exposure during early development (Lalone *et al* 2012); and aggression and physical injury (Noble *et al* 2012; Ortiz-Delgado *et al* 2014).

Opercular deformity has been identified as a potential fish welfare issue for laboratory fish (eg Beraldo *et al* 2003; Knight & Goodwin 2016) and farmed fish (eg Noble *et al* 2018; Royal Society for the Prevention of Cruelty to Animals [RSPCA] 2021). Within aquaculture, opercular abnormalities are further recognised as economically important due to potential impacts on market value (downgrading of aesthetic value of the product; Divanach *et al* 1996) and biological performance (and hence

Table 1 Examples of fish species reported with opercular abnormalities.

Order	Common name and species	References
Acipenseriformes	Beluga sturgeon (*Huso huso*)	Ruban *et al* (2006)
Salmoniformes	Atlantic salmon (*Salmo salar*)	Bruno (1990), Sadler *et al* (2001), Kazlauskienë *et al* (2006), Taylor *et al* (2012), Pettersen *et al* (2014), Skipnes (2014), Amoroso *et al* (2016), Larsen *et al* (2018), Peruzzi *et al* (2018)
	Coho salmon (*Oncorhynchus kisutch*)	Stevenson (2007)
	Rainbow trout (*Oncorhynchus mykiss*)	Boglione *et al* (2013)
Cypriniformes	Zebrafish (*Danio rerio*) Goldfish (*Carassius auratus*)	Andrews (2011)
	Common carp (*Cyprinus carpio*)	Al-Harbi (2001)
	Bluehead sucker (*Catostomus discobolus*) Flannelmouth sucker (*Catostomus latipinnis*) Razorback sucker (*Xyrauchen texanus*)	Barkstedt *et al* (2018)
	Shortnose sucker (*Chasmistes brevirostris*) Lost River sucker (*Deltistes luxatus*)	Plunkett & Snyder-Conn (2000)
Characiformes	Black widow tetra (*Gymnocorymbus ternetzi*) Penguin tetra (*Thayeria boehlkei*) Spotted headstander (*Chilodus punctatus*)	Andrews (2011)
Perciformes	Perch (*Perca fluviatilis*)	Lindesjoo *et al* (1994)
	Common seabream (*Pagrus pagrus*)	Boglione *et al* (2013)
	Gilthead seabream (*Sparus aurata*)	Koumoundouros *et al* (1997), Beraldo *et al* (2003), Georgakopoulou *et al* (2010), Beraldo & Canavese (2011), Ortiz-Delgado *et al* (2014)
	Barramundi (*Lates calcarifer*)	Fraser & De Nys (2005)
	Pacific threadfin (*Polydactylus sexfilis*)	Helsley *et al* (2001)
Cichliformes	Angelfish (*Pterophyllum scalare*)	Andrews (2011)
	Mozambique tilapia (*Tilapia mossambica*)	Handwerker & Tave (1994)

production cost). For laboratory fish, if opercular deformity affects biological performance, it may introduce variation into response variables, therefore countering the goal of standardising experimental animals (Vatsos 2017).

Short opercula appear to be the most common form of opercular abnormality and need to be quantified, for research into the issue, and as a fish welfare indicator in both the laboratory and field. However, much variation exists between published opercular scoring systems (Koumoundouros *et al* 1997; Kazlauskienė *et al* 2006; Ortiz-Delgado *et al* 2014; Pettersen *et al* 2014; Skipnes 2014; Larsen *et al* 2018; Noble *et al* 2018; RSPCA 2021). For example, some schemes record the severity of short opercula whereas others simply record unilateral or bilateral prevalence. Here, we present a scoring key to quantify short opercula which we have developed and used at the Cefas Weymouth Laboratory, UK with Atlantic salmon (*Salmo salar*). We also present additional observations relevant to its causes and consequences.

Materials and methods

Ethical statement

No ethical approval was required for this study. Salmon with short opercula would have undergone routine euthanasia in keeping with this institution's standard husbandry practice.

Study animals

Atlantic salmon are reared at the Cefas Weymouth aquarium facility for experimental use as alevin, parr, pre- and post-smolt. Eyed ova are purchased from commercial hatcheries and held in stainless steel mesh trays until hatching. All stages are reared in flow-through circular fibreglass tanks (nominal volumes 60, 300, 1,000 l), initially in dechlorinated mains freshwater (*circa* pH 7.6; hardness 257 mg l^{-1}) and after smoltification (> 60 g) in UV sterilised natural seawater (*circa* 35‰; pH 8.0). Commercial pelleted diets are provided at recommended

Table 2 Environmental conditions for rearing Atlantic salmon (*Salmo salar*) from eyed ova to post-smolt at Cefas Weymouth, UK.

Developmental stage		Ova	Yolk-sac alevin	First-feeding alevin	Parr ('summer conditions')	Parr ('winter conditions')	Pre-smolt ('summer conditions')	Smolt/post-smolt
Stage duration (DD; degree days)		400 post fertilisation	~ 300 post hatch - absorption of yolk sac assessed daily			350–800	350–450	
Approximate bodyweight (g)		< 0.2	< 0.2	< 1	1–8	8–18	18–60	> 60
Salinity (‰)		< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	35
Temperature (°C)		8	8–12	10–12	10–12	6–8	12–14	8–14
Photoperiod (h L:D)		0:24	0:24	24:0 (100–500 lux)	24:0 (100–500 lux)	12:12 (100–500 lux)	16:8 (100–500 lux)	16:8 (100–500 lux)
Feeding		n/a	n/a	20 h auto-feeder plus 4 manual feeds	20 h auto-feeder plus 3 manual feeds	3 manual feeds	3 manual feeds	3 manual feeds
Maximum stocking density (kg m^{-3})		n/a	10	10	20	20	25	30
Environmental enrichment	**Stippled matting**		✓					
	Black-out shading	✓	✓					
	Partial shading			✓				
	Clockwise current				✓	✓	✓	✓

rations, and environmental conditions during rearing of each developmental stage are summarised in Table 2.

The water inflow rate is varied in relation to biomass. During ova and alevin stages, the inflow is directed towards the side of the tank to reduce water disturbance. Once the fish are approximately 2–3 g the flow is introduced through a directional spray-bar to generate a clockwise current. Additional aeration is provided, introduced via a vertical pipe 'streamer' to maintain the clockwise current (Ellis *et al* 2019). The directional current aids tank self-cleaning and provides a cue for polarised school swimming (anti-clockwise against the current) (Timmons *et al* 1998) which is believed to reduce aggression in salmonids (Jobling & Wandsvik 1983; Jobling *et al* 1993; Murray *et al* 2016). The magnitude of the directional current is adjusted to promote polarised forward swimming.

Smoltification is assessed via visual smolt score (RSPCA 2021), condition factor and plasma osmolarity testing using a sample of fish (n = 10) held in 35‰ seawater for 24 h. When judged ready, the stock is transferred to seawater either by changing water supply or moving tank.

Scoring short opercula

After observations of short opercula in fish reared at Weymouth, images were collected from humanely killed (anaesthetic overdose followed by pithing) stock fish. The images were ordered and selected to produce a five-stage scoring key based upon the area of the entire operculum missing (Figure 1). Although the images selected may indicate that opercular reduction occurs uniformly from the distal edge, it should be noted that reduction can occur at just the bottom or top edge of the operculum; where this is observed, the fish are scored on the estimated area of operculum missing (Figure 2). Although the images are of the left opercula, they are equally applicable to the right.

Following development of the five-stage scoring key, operators were trained using two or three examples of each score. To assess inter-operator reliability of the scoring system, 90 reared Atlantic salmon (mean weight 20.3 g) that had been humanely killed and displaying a range of opercular sizes were independently scored (left [L] and right [R] opercula) by four different operators. The results were assessed using Fleiss' Kappa, a statistical index for assessing agreement across more than two scorers for categorical data (R Development Core Team 2013).

Figure 1

A five-stage key for scoring short opercula in Atlantic salmon (*Salmo salar*). The overlaid green lines in the lower images indicate the margin of an intact operculum.

Figure 2

A humanely killed Atlantic salmon (*Salmo salar*) showing opercular reduction at the dorsal edge. This operculum would be scored as 2, ie < 25% of the operculum is missing.

Table 3 Assessment of inter-operator reliability for the five-stage key for scoring short opercula in Atlantic salmon (*Salmo salar*). Summary data from four different operators independently scoring 90 fish.

Operculum score	Left operculum		Right operculum	
	Count	%	Count	%
1	276	77	360	100
2	43	12	0	0
3	12	3	0	0
4	16	4	0	0
5	13	4	0	0
Total	**360**	**100**	**360**	**100**

Application of the scoring system

The opercular scoring system has been applied to stock fish that have been euthanased due to deformity, stocking density management or size grading, at Cefas Weymouth, for five years. As a case study, we provide a time series of opercular scores for one cohort of stock Atlantic salmon. A series of 12 random samples (≥ 30 fish per sample) of fish at various ages (95–521 days post hatch [dph]); mean weights ranging from 0.8 to 299 g) were scored to assess changes in prevalence and severity. When this stock was held across several tanks (≤ three), equal numbers of fish were sampled from each tank. Although this sampling was random, it must be noted that the stock would have been subject to additional size grading and removal of small/deformed fish. The data were assessed to determine whether short opercula were predominantly on one side, using McNemar's test for paired nominal data (Petrie & Sabin 2000; R Development Core Team 2013). Further *ad hoc* observations of damage to the gills are also reported.

Results

Assessment of inter-operator reliability

To assess inter-operator reliability of the five-stage opercular scoring key, only the scores of the left opercula were assessed; all right opercula in the 90 fish sample were scored as 1, indicating perfect agreement in the absence of evident short opercula (Table 3). For the left opercula, although scores of 1 dominated, the full range of scores (1–5) were recorded. Disparity between operators occurred for three of the 90 fish, where scores were in adjacent categories (4,4,4,5; 2,3,2,2; 3,4,3,3). Fleiss' Kappa statistic ($K = 0.958$) for the left opercular scores confirmed 'almost perfect agreement' between operators.

Application of the scoring system

Once operators are familiar with the method, they can score dead fish quickly (< 10 s per fish for both opercula). One issue that has emerged is the scoring of small fish (< 5 g), where the distal part of the opercula is slightly translucent and may need manipulation to confirm presence (Figure 3).

Assessment of opercula has demonstrated that opercular plate reduction is the dominant type of deformity in our stocks. Opercular plate folding has also been observed, albeit rarely (Figure 4). We have not observed the third type of deformity, concave depression, in our Atlantic salmon stocks.

For the cohort of fish for which opercular size was tracked over time: no short opercula were noted in the first sample (95 dph; 0.8 g); short opercula (Score 2) were first recorded in the 2nd sample (157 dph; 6.4 g) and in all subsequent samples; opercula with more severe reduction (ie scores > 2) were recorded from the fourth sample (326 dph; 40 g) and in subsequent samples (Figure 5). Assessment of the pooled data from all the samples (426 individual fish; Table 4) confirmed that the prevalence of short opercula was higher for the left operculum than the right (McNemar's test; $\chi^2 = 41.397$; $P < 0.001$; Figure 5).

Observations of gill damage associated with reduced opercula

During sampling we have observed that fish with reduced opercula often show evidence of gill damage. Although we have not collected quantitative evidence (due to the lack of a method to score gross reduction in gill filament size), we provide photographic examples (Figures 6 and 7). These examples illustrate the intuitive notion that damage to the gill filaments is associated with the presence of a short operculum, and that the area/extent of such gill damage is related to the area of operculum missing.

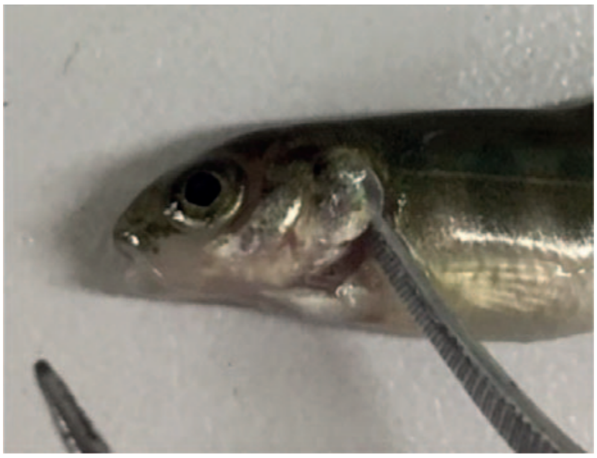
Discussion

Scoring system

The scoring system described here has been used within our aquarium facility to assess euthanased fish over the last five years and proved to be a quick and reliable method for quantifying short opercula. It is also used to standardise removal of affected individuals during manual grading/sorting of live fish and routine visual checks of stocks *in situ* within tanks. In our salmonid tank systems, viewing is restricted to above which limits lateral views, although opercula are readily visible via in-tank cameras (Ellis *et al* 2019). We consider the benefits of the method to include:

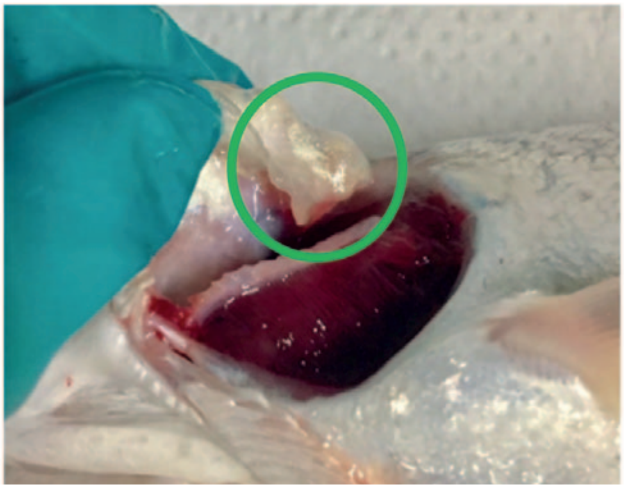
- Simplicity with a high level of consistency between operators;
- Low cost, not requiring sophisticated equipment and suitable for use in both laboratory and field environments;
- Recording of both the laterality and extent of the abnormality;
- Formalised quantitation of an abnormality that will enable standardised recording (Jirkof *et al* 2020);
- Suitable to use on live animals, both *in situ* (if visible) or out of water. (Use on live fish is limited by visibility and opacity of the distal edge of the operculum in small fish; Figure 3); and
- Suitable for other species; we have extended in-house use to stocks of rainbow trout (*Oncorhynchus mykiss*). Transfer to non-salmonid species is expected to be simple, where close lateral viewing within transparent-walled tanks would aid use with live fish.

Figure 3



Euthanased Atlantic salmon (*Salmo salar*) (3.6 g) showing translucent distal margin of the operculum. The forceps can be seen through the operculum, illustrating the need for careful visual assessment in small salmon.

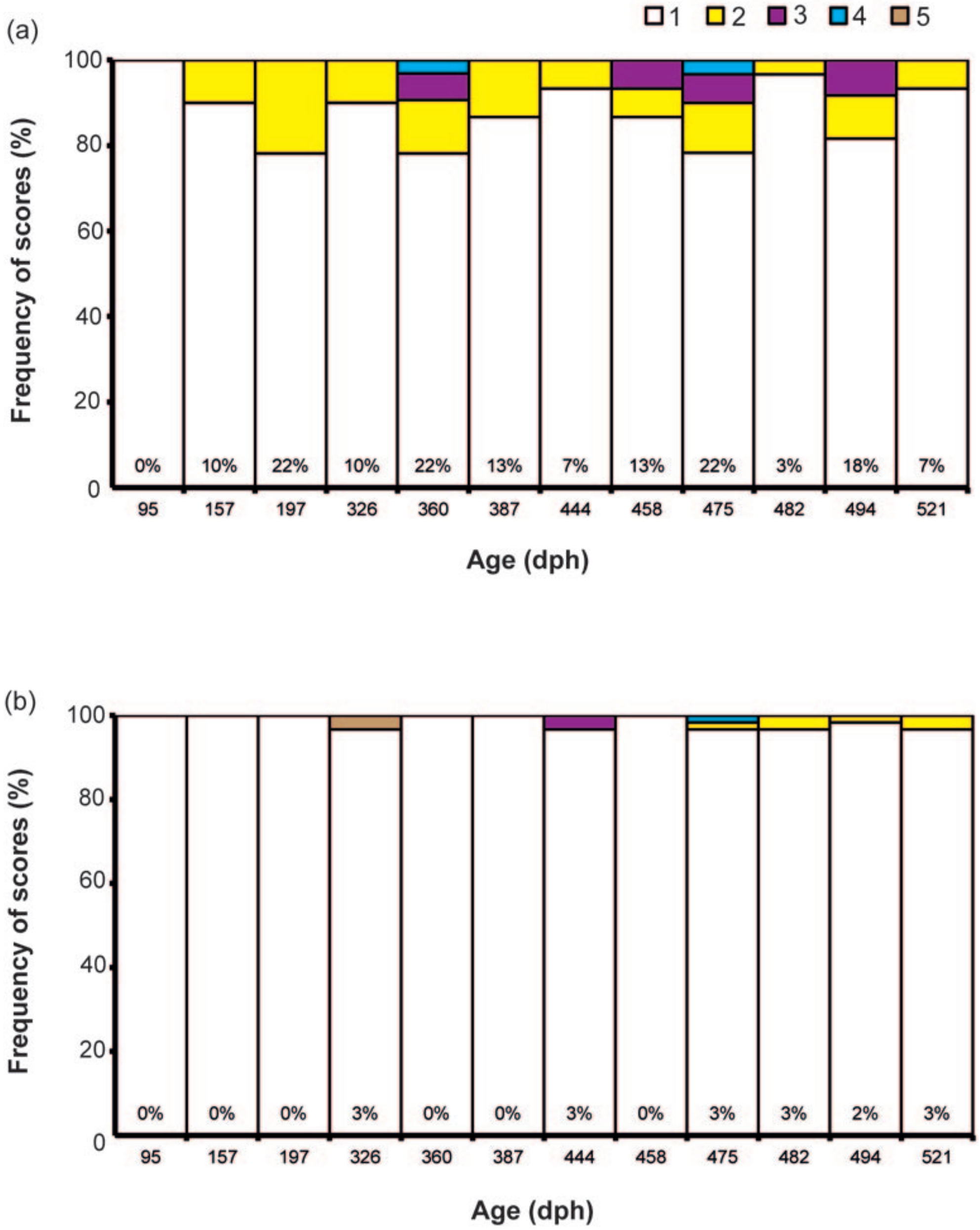
Figure 4



Euthanased Atlantic salmon (*Salmo salar*) showing opercular plate folding. The area of folding has been circled.

The methods proposed previously for scoring opercula vary in the information recorded. Kazlauskienė *et al* (2006) assessed laterality but not severity. Some methods (Pettersen *et al* 2014; Skipnes 2014; Noble *et al* 2018;

Figure 5

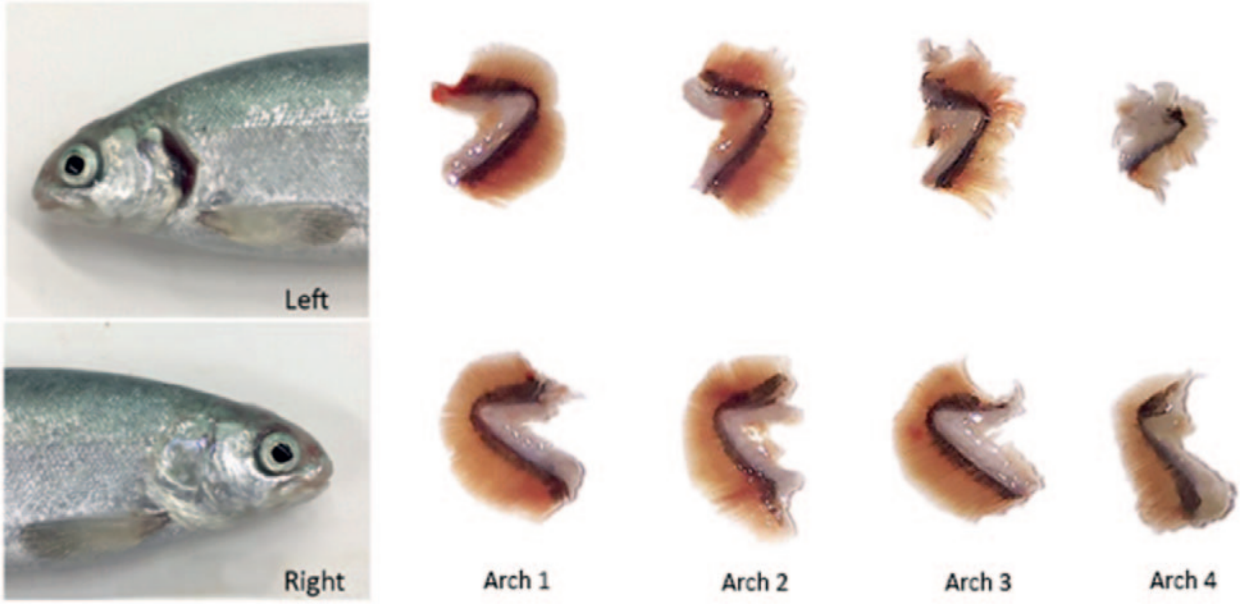


Relative frequencies of scores (1–5) of (a) left and (b) right opercula for a single stock of laboratory-reared Atlantic salmon (*Salmo salar*), sampled at different ages. Prevalence (%) of short opercula (Scores >1) indicated for each sample. Stock fish were not sampled between 197 and 326 days to avoid physical damage and stress to conspecifics during smoltification, a sensitive ontogenetic period.

Table 4 Summary of observations of short opercula in a cohort of laboratory-reared Atlantic salmon (*Salmo salar*), sampled 12 times over a 14-month period. Data used to assess symmetry of short opercula using McNemar's test.

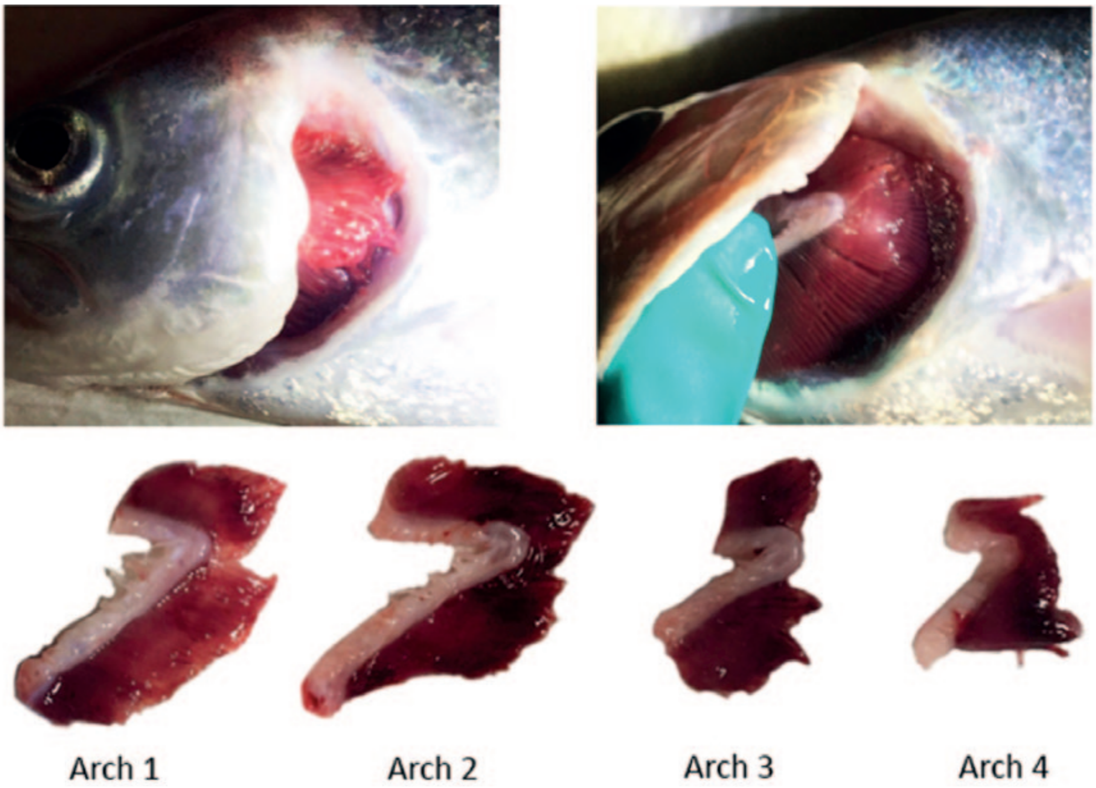
		Right operculum		**Total**
		Normal (score 1)	**Short (score ≥ 2)**	
Left operculum	**Normal (score 1)**	365	4	**369**
	Short (score ≥ 2)	54	3	**57**
	Total	**419**	**7**	**426**

Figure 6



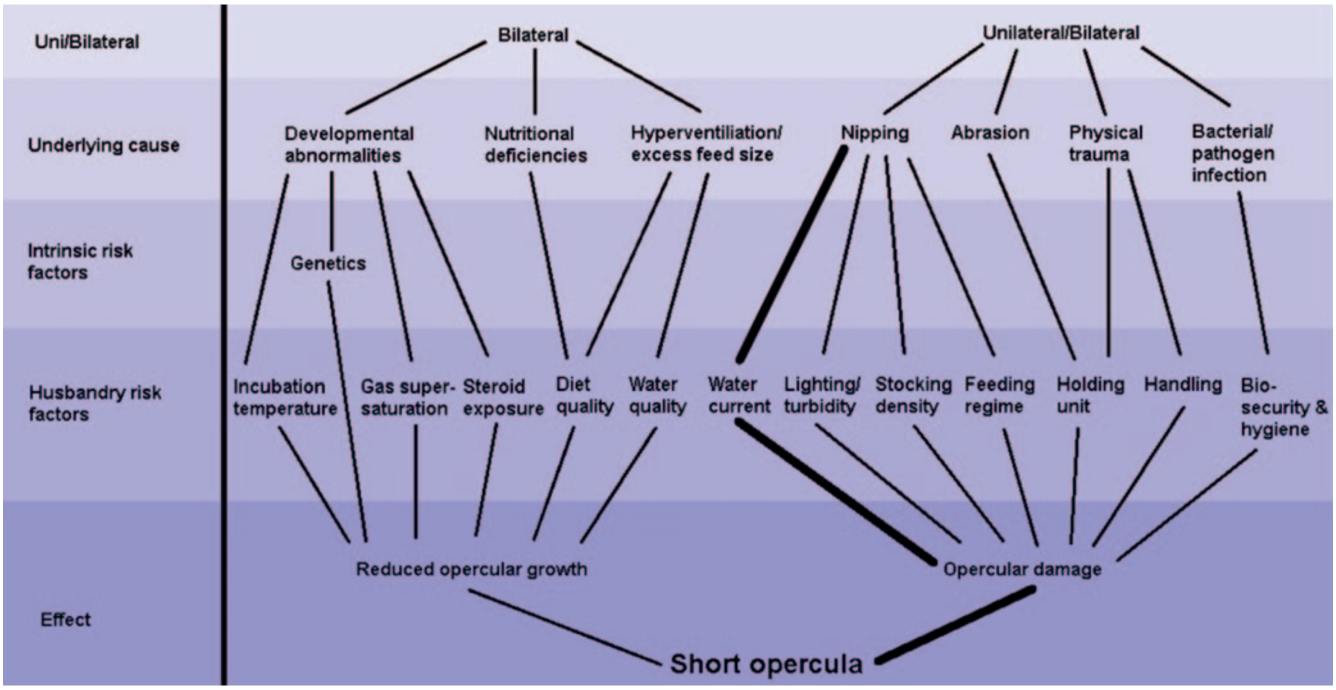
Euthanased Atlantic salmon (*Salmo salar*) with a left operculum score of 3 and right operculum score of 1. Gill filament damage is evident on arches 2–4 under the left operculum, while the gill arches under the right operculum were judged as normal. Gills photographed under water, causing a pale colouration.

Figure 7



Euthanased Atlantic salmon (*Salmo salar*) with a left operculum score of 5. Gill filament damage (reduction/clubbing) was evident to all left arches, including complete loss of filaments on arch 3. Photographs taken in air, so gill filaments clumped.

Figure 8



Initial web of causation drafted to illustrate multifactorial aetiology of short opercula in fish, after Smith (1997) suggested for furunculosis. Pathway thought to explain observations described in this paper highlighted in bold, although contribution from other husbandry risk factors cannot be deduced. Pathways for other forms of opercular deformity (plate folding and concave depression) excluded.

RSPCA 2021) assess severity, but only whether the operculum is intact, reduced or completely absent. Other methods (Koumoundouros *et al* 1997; Ortiz-Delgado *et al* 2014) require laboratory analysis and morphometric measurements which would be impractical to use in large-scale systems or the field. Here, we have demonstrated that a five-category scale for quantifying short opercula enables consistent scoring, tracking progression within laboratory stocks (Figure 5) and the implementation of interventions.

The number of categories chosen for any scoring system is a balance between the detail gathered and the time and resources used. A five-category scale is similar to that used for fin scoring (Hoyle *et al* 2007) and Larsen *et al* (2018) independently developed a similar scoring method for opercula. For routine, on-farm welfare assessment (ie non-research use), three-category scales may be preferred as they are considered easier and quicker to use (Noble *et al* 2018; RSPCA 2021). Nevertheless, a five-category scale may aid examination of aetiology on farms after problems are demonstrated via a three-category scale.

Aetiology of short opercula

As highlighted in the *Introduction*, a definitive aetiology of opercular deformity has yet to be established, with a wide range of causal and risk factors being proposed for different species and rearing systems. A further range of factors have been suggested in the grey literature. We have attempted to categorise this multifactorial aetiology and illustrate pathways within a draft 'web of causation' (Figure 8).

The data from our facility indicate that short opercula first appear during the parr stage and that the syndrome is asymmetric, with a greater prevalence on the left side. In our case study cohort (Figure 5), fish were not sampled between 0.8 (no opercula reduction evident) and 6.4 g, when the deformity was first observed. We have recorded reduced opercula in other stocks at 2.2 and 2.4 g. In our system, opercular reduction therefore starts around 2–3 g which is the similar size that directional water flow is introduced. We dismiss pathways associated with:

- Developmental abnormality, because short opercula are first observed in the parr stage and prevalence (and severity) increases over time; and
- Reduced opercular growth rate, due to the asymmetry, the absence of infection in our biosecure facility, close management of the water supply and quality, and provision of high quality formulated commercial diets.

Development in the parr stage (after the alevin stages) and asymmetry point towards opercular damage with nipping as the cause in our laboratory system. We observe an association between the provision of a clockwise current and the development of short opercula. Fish orientate into the current which promotes polarised anti-clockwise swimming behaviour. Consequently, the right operculum is orientated towards the tank wall and the left towards the centre of the tank. We therefore dismiss abrasion with the tank wall as the main cause. This leaves nipping as the probable cause. Turnbull *et al* (1998) observed aggressive nipping directed at the head in Atlantic salmon, and we have observed such behaviour in our stocks. Nipping may also be non-aggressive (Ellis *et al* 2008), either triggered by a feeding response to movement of the operculum, or occurring accidentally during feeding when the opercula flare during ingestion. All three potential types of nipping could be asymmetric due to fish orientation, with the right opercula receiving fewer nips due to proximity to the tank wall. Further nipping could also account for the damage to gill filaments; it may even be that the visible red gills promote additional nips at the area. Quantitative behavioural observations are needed to confirm nipping as the causal pathway but would be labour intensive and possibly require additional regulated experimentation.

Understanding the pathway of opercular reduction is necessary to implement mitigations. In our system, we conclude that nipping is the main cause, and the directional current is a linked risk factor. However, we cannot judge the relative importance of the various risk factors listed within the opercular damage pathway related to nipping (Figure 8). Any factor that affects fish behaviour (eg lighting/turbidity, stocking density and feeding regime; Ellis *et al* 2002) may affect nipping and hence opercular reduction. Local measures aimed at reducing nipping include:

- Ensuring full feed rations are provided spread through the light period;
- Minimum and maximum limits on stocking density;
- Tight size grades;
- Low light intensity; and
- Identification and removal of aggressive individuals where possible.

Animal welfare implications

The opercular plate forms an important part of the buccal pump system and provides physical protection to the gills (Beraldo *et al* 2003). It has been suggested that impairment of the buccal system may mean fish need to swim to pass water over the gills, resulting in increased metabolic costs and reduced growth while increasing susceptibility to low dissolved oxygen levels and handling procedures (Speare & Ferguson 2006; Branson & Turnbull 2008). The abilities to feed and 'cough' to clear the gills of debris may also be affected by an impaired buccal pump system (Drost *et al* 1988; Branson & Turnbull 2008). Reduction in opercular size has been suggested to expose the gill lamellae to physical injury (Pettersen *et al* 2014). We have provided evidence for this latter suggestion; that shortened opercula are associated with injury to, and loss of, gill filaments. Loss of gill tissue will impair gas exchange, as will lamellar clubbing which has been seen in fish with short opercula (Figure 7; Ribelin & Migaki 1975). It has also been suggested that short opercula leave the branchial arches more exposed to parasites (Boglione *et al* 2013; Conceicao & Tandler 2018). Short opercula are therefore a legitimate fish welfare issue, being associated with injury and various potential routes for reduced biological performance. Short opercula should therefore be considered when addressing the 3Rs of laboratory fish use (Russell & Burch 1959), as a potential source of variation in responses, and in assessment of welfare and the severity of experimental procedures (Hawkins *et al* 2011; Jirkof *et al* 2020). It would, however, be premature for us to link opercular scores to severity bands.

Conclusion

Scoring via a five-point scale is a simple, reliable and low-cost way to assess short opercula in fish. The method can contribute to severity assessment, standardisation of laboratory fish, and ultimately aid determination of aetiology and mitigations to improve welfare.

Declaration of interest

None.

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