Epidermis structure in the brook trout *(Salvelinus fontinalis)* and its Arctic char (*Salvelinus alpinus*) hybrid

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Abstract

Brook trout (*Salvelinus fontinalis*) is a species of fish native to North-East America. Brook trout are also commercially raised in large numbers for food production. Skin infection and/or parasite outbreaks can have a serious economics effect on aquaculture businesses. For this reason, it has been hybridized with the more resistant Arctic char (*Salvelinus alpinus).* The aim of this study was an examination of the epidermal structure and dynamic in brook trout and its Arctic char hybrid which is less sensitive to skin infection. The samples of fish (72 brook trout, 72 brook trout × Arctic char hybrid) from fish farm in Pravíkov (49°19'10"N, 15°5'40"E) were collected five times during the year 2011. Absolute and relative epidermal thickness (in relation to body size) and relative proportion of secretory cells in a given volume of epidermis were measured. The epidermis structure of brook trout and brook trout × Arctic char hybrid both display similar seasonal dynamics, with a decrease in absolute and relative epidermal thickness and a reduction in the relative percentage of mucous secretory cells over the summer. On the other hand, the lower absolute (mean 103 μm (range 84–146 μm) in brook trout; 88 μm (range 68–115 μm) in hybrids) and relative epidermal thickness (mean 4.8 (range 3.6–6.8) in brook trout; 4.4 (range 2.9–6.4) in hybrids) and lower volume of secretory cells was observed to the hybrid (mean 28% (range 19–33%) in brook trout; 23% (range 10–30%) in hybrids). It can interrelate with their higher resistance to infection and/or parasite outbreaks.

Fish, goblet cell, sacciform cell

The only char species native to Europe is the Arctic char (*Salvelinus alpinus*), which is found across northern Europe and in the Alpine and Pyrenean mountain ranges (Kottelat and Freyhof 2007). The Arctic char is closely related to salmon and lake trout (*Salvelinus namaycush*), having many characteristics of both; and in North America, where the geographical distribution of Arctic char and the related brook trout (*Salvelinus fontinalis*) overlap, natural intergeneric hybrids (commonly known as 'Sparctic char'; Jannson 2013) have been recorded (Wilson and Bernatchez 1998). Most char species are presently endangered in their native habitats due to habitat loss (loss of spawning grounds and river connectivity), introduction of trout species (competition and cross-breeding) and pollution (especially acid rain). Numerous attempts have been made to farm char species, not only to supply individuals for restocking, but also as a possible food source. Early research on developing Arctic char as a farmed species took place in Canada in the 1970s and, since then, Arctic char aquacultural enterprises have been set up in many other countries. Three different char species are presently farmed in Europe: Arctic char, lake trout and, most important of all, the brook trout. Commercial char farms are intensively managed, as the species' have strict requirements, including very high water quality and low spawning temperatures. For example, arctic char range in the wild is limited by temperature as successful spawning can only take place at temperatures lower than 12–14 °C (Haffray et al. 2009).

Intensive management also encourages the keeping of fish at high densities in the tanks as a means of reducing costs. This, in turn, increases the likelihood of exposure to and

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spread of pathogens, including bacteria, parasites and viruses (Laidler et al. 1999). High fish densities also increase the risk of mechanical injury, meaning that char become highly susceptible to Monogenea infection and, especially, furunculosis *Aeromonas salmonicida* (Egidius 1987; Cipriano and Bullock 2001; Haffray et al. 2009). Such infection and/or parasite outbreaks can have a serious economic effect on aquacultural businesses. This has prompted fish farmers to research means of reducing the risk of infection, including selection and hybridisation. One such attempt has been the induced hybridisation of brook and Arctic char.

The skin structure of fish reflects its adaptation to the physical, chemical and biological properties of the aquatic environment and the natural history of the organism. The epidermis, therefore, can be highly variable and structural differences, especially in thickness and the occurrence of secretory cells, are found not only between species but also related to sex, sexual activity and ploidy (Knoz and Halačka 1991). Mucus produced by the different epidermal cells forms a natural semipermeable barrier that enables the exchange of nutrients, water, gases, odorants, hormones, and gametes. At the same time, mucus plays a critical role in the prevention of colonisation by parasites, bacteria and fungi by also acting as a biological barrier (Austin and McIntosh 1988; Mozumder 2005). Any change in the skin structure caused by hybridisation, therefore, has the potential to affect the ability of fish to fight infection and parasite infestation. In this study, we examined the skin structure of brook trout and brook trout × Arctic char hybrids, and discussed the findings in the light of char biology and aquacultural practice.

Materials and Methods

Sample fish were taken from the Pravíkov fish farm (BioFish Ltd., Czech Republic), which specialises in farming salmonid fish using a system of low pressure diffusers (Jokumsen and Svendsen 2010). The farm is located in the Bohemian-Moravian highlands, near the town of Kamenice nad Lipou (15.0946147° E, 49.3194111° N) at an altitude of almost 600 m. Water is sourced from either a borehole or the forested stream.

The brook trout and brook trout × Arctic char hybrids examined were reared under identical conditions (i.e. food, stocking density and hydro-chemical parameters) in two separate 34 m^3 trays connected to a recirculation system with a total volume of approximately 1 000 m^3. The fish were fed Biomar EFICO ENVIRO 920 extruded trout mixture. The breeding trays were initially stocked with 10 000 individuals, corresponding to a density of 295 ind/m^3. Water temperature fluctuated during monitoring between 4.4 (February) and 17 °C (August), with dissolved oxygen content ranging between 76.8 and 99.2%. Fish samples were collected × 5 during the year 2011 (Table 1). The experimental project was authorized under no. 13321/2009-30.

Prior to analysis, each fish was killed with an overdose of anaesthetic (2-phenoxy-ethanol), and its weight (g) and length (SL, mm) measured. All fish were juvenile (only 8 brook trout from November were subadult (5 males, 3 females), therefore the influence of sex was not evaluated). Skin samples of approximately 5 × 5 mm were taken from the dorsal part of the head and fixed in Bouin's solution for 2–3 days; whereupon the sample was transferred to 70% ethanol. The sample was subsequently embedded in paraffin and cut into serial slices 7 µm thick, perpendicular to the surface. These slices were then stained according to Mallory (Ross 2011) for a general view.

Histochemical staining with Alcian blue at pH 2.5 (Kiernan 1981) and periodic acid-Schiff stain (PAS; Horobin and Kevill-Davies 1971) was used to assess the glycoprotein content in secretory cells. Absolute epidermal thickness (A) and the relative proportion of secretory cells in a given volume of epidermis (B; calculated using a 10 µm morphometric web) were measured and used to compute the absolute values for secretory cells in the epidermis using the formula A × B/100 (Halačka et al. 1991, 2010). The relative thickness of the epidermis was calculated as the ratio of body size and absolute epidermal thickness (SL/A).

Statistical analyses were performed with Statistica for Windows® 9.0 (StatSoft, Tulsa, OK, USA). Results between two treatment groups were compared by Student's *t*-test. Results from different treatment groups were compared by one-way analysis of variance (ANOVA) and post-hoc analysis of means using Scheffe's test.

Results

The growth rate over the monitoring period was relatively balanced between the two groups, with brook trout showing a slightly greater weight gain over the year (Table 1).

After common epidermal cells, the secretory goblet cells represent the dominant

Table1. Temperature, number of individuals, the mean size (SL [mm]), weight (g), weight gain (g, in brackets) and total monitored fish (Σ) in each group of fish used in the experiment.

Date	Febr	April	May	Sept	Nov	Σ
Temperature	4.4°C	8.7°C	11.3°C	14.7°C	6.5°C	
S. fontinalis	25	10	10	12	15	72
	159 / 72	190 / 121	216 / 220	251 / 309	283 / 463	
		(49)	(99)	(89)	(154)	
S. fontinalis × *S. alpinus*	25	10	10	12	15	72
	148 / 55	185 / 109	199 / 170	235 / 221	273 / 316	
		(54)	(61)	(51)	(95)	

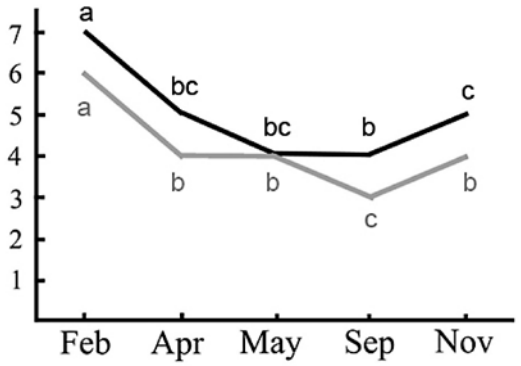

Fig. 3. Relative epidermal thickness in brook trout (black) and its hybrid brook trout × Arctic char (grey). Significant differences are marked by letters ($P < 0.05$).

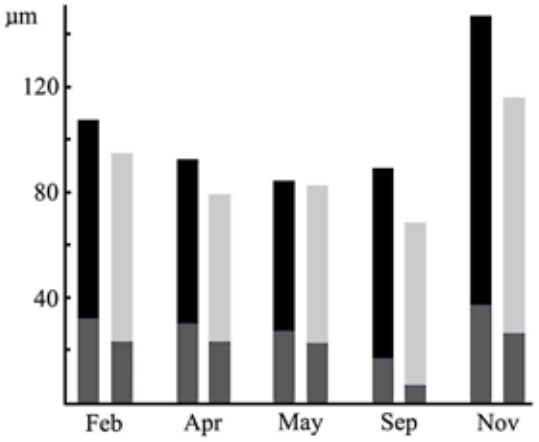

Fig. 4. Absolute epidermal thickness and volume of common epidermal cells and secretory cells in brook trout (left black/grey columns) and its hybrid brook trout × Arctic char (right light-grey/grey columns); black – common epidermal cells in brook trout, light-grey – common epidermal cells in hybrid, grey – secretory cells.

component of the epidermis; and particularly so in the upper half (Plate IV, Fig. 1), where they secrete a protective mucosal layer over the fish body (Plate IV, Fig. 2). The secretory content of these goblet cells showed a positive reaction to both Alcian blue and PAS, indicating a mixture of neutral and acidic glycoproteins. Pigment cells (melanophores) were not found in the epidermis, but were frequently present in the underlying dermis.

Skin thickness differed both between the groups and over the reporting period. Brook trout had thicker skin, both in absolute ($P < 0.001$) and relative ($P < 0.057$) terms, compared to hybrid individuals of the same age. In both groups, epidermal thickness gradually thinned between summer and autumn, and then gradually thickened again over winter (Fig. 3). The relative number of secretory cells showed similar dynamics, with the lowest values observed in September and an increase from November onward. Throughout the year, the secretory cell volume was higher in brook trout than in hybrids ($P < 0.001$), with the mean secretory cell volume in brook trout at 28% (range of 19–33%) and 23% (range of 10–30%) in hybrids (Fig. 4).

Discussion

Differences in epidermal thickness within the same fish species is normally described in relation to sex and, if information is available on changes over time, this usually focusses on differences related to the onset of spawning. Males frequently have a thicker epidermis than females (e.g. see brown trout *Salmo trutta* m. *fario*), and particularly so during the spawning season (e.g. brown trout) (Pickering 1977; Knoz et al. 1990) and Arctic char (Witkowski et al. 2004). On the other hand, Stoklosowa (1966, 1970) described a thin epidermis with just one layer of small epithelial cells in male sea-trout *S. trutta trutta* during the sexually active period. Witkowski et al (2004) described a similar situation for brook trout.

While the existence of interspecies differences in epidermal thickness (whether taxonomic or ethological) has long been suspected, actual comparisons have often been hampered by methodical variability or absence of supplementary information. Studies dealing with this phenomenon, particularly in Salmonidae, are still an exception rather than the rule. Fast et al. (2002), for example, compared mid-body epidermal thickness of rainbow trout (*Oncorhynchus mykiss*; SL - ?; 92 μm), coho salmon (*O. kisutch*; SL - ?; 39 μm) and Atlantic salmon (*S. salar*; SL - ?; 33 μm); Halačka et al. (2007) compared cranial skin from brown trout (SL 20 cm; 110 μm), rainbow trout (SL 27 cm, 170 μm) and brook trout (SL 25 cm, 130 μm). Based on these results, the epidermis of *Salmonidae* sp. appears to be relatively thin compared to representatives of other taxonomic groups. Further examples of Central European species with significantly thicker skin include crucian carp (*Carassius carassius*; SL - 23 cm, 240 μm), common carp (*Cyprinus carpio*; SL - 39 cm; 404 μm), and burbot (*Lota lota*; SL - 36 cm; 360 μm). Similar epidermal thicknesses, however, can be found in numerous smaller fish species, such as European bullhead (*Cottus gobio*; SL - 12 cm; 150 μm), stone loach (*Barbatula barbatula*; SL - 10 cm; 98 μm), and gudgeon (*Gobio gobio*; SL - 13 cm; 98 μm) (all cranial skin samples taken outside the spawning period [Knoz and Halačka 1991]).

The relatively thin salmonid epidermis may be associated with skin respiration, which contributes a large share of the total oxygen supply in fish (Jakubowski 1960). This is supported by the epidermal thinning in our observations during summer, when there is an increased need for skin respiration due to lower concentrations of dissolved oxygen and an increase in activity. In winter, the observed skin thickening is likely to be connected with increasing the skin's mechanical resistance and factors associated with entering the spawning period. This latter point may well explain the significant increase in epidermal thickness noted for brook trout which, unlike the hybrids, were already showing signs of sexual maturity.

The basic epidermal morphology of brook trout and its Arctic char hybrid is no different from other salmonids, with mucous goblet cells the dominant secretory cells present. In only a few species (e.g. brown trout or coho salmon), a secondary, morphologically similar cell type known as the sacciform cells has been observed. Unlike the goblet cells, the secretory content of these cells is usually eosinophilic, showing no reaction to PAS and Alcian blue. While these sacciform secretory cells were not visually observed in our brook trout and Arctic char hybrids; unambiguous confirmation will only be possible through observing differences in skin ultrastructure with an electron microscope (Harris and Hunt 1975; Knoz and Halačka 1991; Fast et al. 2002).

The presence of epidermal melanophores has yet to be observed in any salmonid species, even in those with expressive skin pigmentation, such as marble trout (*S. marmorata*; Sivka et al. 2012). In some fish species melanophores are present, however, the factors dictating the presence of epidermal melanophore cells are not yet fully understood. The epidermal melanophores are found e.g. in *Cottus gobio* and *C. poecilopus* inhabiting the same area with salmonids (Halačka et al. 2012).

The volume of secretory cells found in the epidermis varies by species, both overall and also within their different types (i.e. goblet cells [GC], club cells [CC], sacciform cells [SC]). Low values have been found in grayling (*Thymallus thymallus*; 9% GC), rainbow trout (11% GC) and gibel carp (*Carassius gibelio*; 7% GC, 14% CC), while higher values have been observed in brown trout (29% GC) and pike (*Esox lucius*; 30% GC), and the highest values in European and Siberian bullhead (40–50% GC, 1–10% sacciform cells) and spined loach (*Cobitis elongatoides*; 22% GC, 44% CC) (Knoz and Halačka 1991; Halačka et al. 2010). Our results of 28% GC for brook trout and 23% GC for the Arctic char hybrid, therefore, can be considered as relatively high.

Secretory cells differ not only in volume but also in the composition of their secretions. The goblet cells which are found in the epidermis (also gills and the gastrointestinal tract) of most fish species are responsible for the production of a mucosal film on the skin surface. This forms a very important part of the fish immune system, serving as a primary anatomical and physiological barrier against external hazards (Esteban 2012). In addition to its basic glycoprotein component, the mucus also contains a huge range of other important substances, including humoral non-specific inhibitors, lysins (protease and lysozyme) and specific antibodies (Ellis 1999; Ebran et al. 2000). Numerous authors have investigated the possible correlation between the amount or composition of goblet cell secretions and defence against infection and parasitism. Buchmann and Uldal (1997), for example, have demonstrated a connection between the density of superficial mucous cells with susceptibility to infection by *Gyrodactylus derjavini* in rainbow trout, brown trout, and in Baltic and Atlantic salmon. However, Sterud et al. (1998) found no such relationship for brook trout. In addition, Roberts and Powell (2005) noted a relationship between mucus acidity (higher sulphation) and infestation with the protozoan inflexibility parasite *Neoparamoeba pemaquidensis* in Atlantic salmon. Similarly, Olafsdottir and Buchmann (2004) have confirmed the protective role of mucus production by both an association between reduced mucous cell discharge and reduced host resistance to *Gyrodactylus* infection and migration of parasites to body parts with a lower density of mucous cells as the infection progresses. It would appear, therefore, that in at least some cases, higher goblet cell frequency equates with higher mucus production, which in turn means higher resistance against infection and parasitism. Whether this is due purely to mucus composition alone, or whether there is also a mechanical aspect (i.e. constant replacement of the mucous layer may also remove infectious agents from the body surface), is not yet clear. On the other hand, Dupont and Crivelli (1988) reported that mucus containing free proteins, glycoproteins and mucopolysaccharides can attract species-specific parasites. Similarly, Lindenstrøm et al. (2006) suggested that continual

mucous secretion facilitates gyrodactylid proliferation in East Atlantic salmon as it serves both as a chemo-attractant and as a food source; whereas Poulin et al. (1999) suggested that mucus acts as a chemo-attractant for diplostomus parasitism.

A wide variety of aquatic organisms, including numerous fish species, release chemical cues that serve as alarm signals (Mirza et al. 2001). This alarm cue, which is contained within the epidermal club cells characteristic for the Superorder Ostariophysi (Smith 1992), is released upon mechanical damage, such as would occur during a predation event. Recent findings suggest that such damage-related alarm cues are produced by a range of fish families, including gobiids, poeciliids, gasterosteids, percids, cottids, cichlids, and salmonids (Brown and Godin 1997, 1999; Chivers and Smith 1998; Nordell 1998; Berejikian et al. 1999; Chivers et al. 2000). These species do not have epidermal club cells and the alarm function is taken over by one of the other types of epidermal cell. In the case of brook trout, for whom the existence of alarm substances has been confirmed experimentally, the goblet cells appear to have taken this function. In laboratory experiments, brook trout exposed to char skin extract have been shown to exhibit a significant reduction in movement and/or altered their foraging behaviour (Mirza 2001, 2002). In situations of intensive artificial breeding, the probability of skin abrasion, and hence release of alarm substances, is relatively high. The reduced representation of secretory cells in the epidermis of hybrids found in this study may be advantageous, therefore, as it reduces the potential risk of a reduction in feeding activity of fish in the tank/group. On the other hand, the increased risk of mechanical injury means that brook trout hybrids may be more susceptible to Monogenea infection and, especially, furunculosis. To understand this complex issue more fully, further studies are needed on both the quantity and composition of the mucus produced, and on the influence of individual active ingredients on different types of infectious and parasitic illnesses.

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