Melanin concentrations in vendace (*Coregonus albula*) and whitefish (*Coregonus lavaretus*) larvae from five boreal lakes with different optical properties

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The objective was to investigate correlation between melanin concentration in fish larvae and the optical properties of water in five study lakes. Melanin concentration was analyzed from vendace and whitefish larvae; sampled in May from lakes with different UV attenuation depth, water colour and transparency. Overall, vendace exhibited more pigmentation than whitefish. In both species larval pigmentation correlated positively with water colour, but the correlation was negative with UV-B attenuation depth. Based on our results, it is evident that ambient UV-B was insufficient to modify the degree of melanin pigmentation of coregonid fish larvae in Finnish lakes. We suggest that the main reason for the observed difference in larval melanin concentrations between lakes is that larvae adapt to the background colour, possibly to reduce visual predation.

**Introduction**

Flexible colouration of fish is an important element in avoiding predation and can be expressed as adaptation to background or to social communication and as mimicry (Bond 1979, Stepien 1987). Colour changes can be fast physiological changes (concentration and dispersion of pigments), or slow morphological changes involving actual change in quantity of pigment within the animal or its integument. Normally, both physiological and morphological changes proceed simultaneously (Brown 1973). The chromatophores responsible for the body colour of the fish are melanophores, erythrophores, xanthophores, leucophores, cyanophores and iridophores (Brown 1973, Hawkes 1974, Kelsh *et al.* 2000). Melanophores are specific effector cells
in the dermis of many poikilothermic vertebrates and play an important role in colour changes. In teleost fish, melanophores are under the control of sympathetic nervous and endocrine systems (Latey and Rangneker 1982). Morphological changes are regulated by melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH) (Baker et al. 1986, Eberle 1988).

Several environmental factors, such as light, background colour, pollution and nutrition may contribute to pigmentation of freshwater fish (Brown 1973, Latey and Rangneker 1982, Bolker and Hill 2000, Payne et al. 2001, Nguyen and Janssen 2002). Further, in aquatic organisms, melanin pigmentation seems to provide an efficient photoprotection mechanism against ultraviolet radiation (Hobaek and Wolf 1991, Hessen 1996, Cummins et al. 1999, Häkkinen et al. 2002). Ultraviolet radiation (UV), particularly UV-B (280–315 nm), is harmful to many organisms, and the ongoing thinning of the ozone layer (Taalas et al. 2000) may affect aquatic ecosystems and fish larvae (Hunter et al. 1979, Browman et al. 2000). In clear ocean waters, UV-B radiation penetrates down to 20 meters, whereas even in the clearest freshwater lakes the penetration is a few meters at most. However, in humic lakes UV-B penetration can be only a few centimeters (Smith and Baker 1979, Smith et al. 1992, Kirk 1994, Bukaveckas and Robbins-Forbes 2000, Huovinen et al. 2000). Studying several Daphnia species, Rhode et al. (2001) showed that the extent to which the daphnids tried to avoid ultraviolet radiation was inversely associated with their pigmentation. Similarly, translucent 1-day-old larvae of Clupea pallasi are more sensitive to UV-B than pigmented 7- and 14-day-old larvae (Speekmann et al. 2001). Our recent study reveals that boreal species of coregonids are UV-B tolerant and can cope with high UV-B doses, probably through morphological colour change by producing melanin for protection (Häkkinen et al. 2002).

The objective of this study was to examine correlation between the optical properties (UV penetration, water colour, and transparency) of lake water and larval melanin concentration. Two hypotheses were tested: (1) if fish pigmentation is associated with the depth of UV-B penetration in lakes and (2) if melanin pigmentation correlates with the colour properties of water, representing possible adaptation against visual predation. As far as we know, this is the first attempt to analyse melanin concentration from freshwater fish sampled from the field. Hence the study also provides basic information about the melanin pigmentation of freshwater fish larvae in different environments.

Materials and methods

Study lakes

During the springs of 2001 (Konnevesi, Paasivesi, Puruvesi) and 2002 (SW Pyhäjärvi and Pyhäselkä), larval vendace (Coregonus albula) and whitefish (Coregonus lavaretus) were sampled from five different lakes in southern and central Finland (Table 1). The lakes represent typical coregonid lakes in Finland. The lakes Paasivesi, Pyhäselkä and Puruvesi are part of the

<table>
<thead>
<tr>
<th>Lake</th>
<th>Location</th>
<th>Colour (mg Pt l⁻¹)</th>
<th>Transparency (m)</th>
<th>Trophic status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puruvesi</td>
<td>61°47'–62°03' N, 29°17'–29°48' E</td>
<td>5</td>
<td>7.5</td>
<td>oligo</td>
</tr>
<tr>
<td>Konnevesi</td>
<td>62°38' N, 26°21' E</td>
<td>20</td>
<td>3.5</td>
<td>oligo</td>
</tr>
<tr>
<td>SW Pyhäjärvi</td>
<td>60°54'–61°06' N, 22°09'–22°22' E</td>
<td>25</td>
<td>1.8</td>
<td>meso</td>
</tr>
<tr>
<td>Paasivesi</td>
<td>62°06'–62°13' N, 29°17'–29°31' E</td>
<td>40</td>
<td>3.9</td>
<td>meso</td>
</tr>
<tr>
<td>Pyhäselkä</td>
<td>62°22'–62°38' N, 29°32'–29°55' E</td>
<td>68</td>
<td>2.1</td>
<td>meso</td>
</tr>
</tbody>
</table>
Saimaa lake system, but are separate lake basins. The lakes included a range from oligotrophic to mesotrophic.

**Sampling**

Sampling was conducted immediately after ice-off in May, because in Finnish lakes the abundance of yolk-sac larvae of vendace and whitefish is highest at that time (Viljanen 1988, Viljanen and Karjalainen 1992). All larvae were either in developmental stage LDS 0 or LDS 1 (Luczynski et al. 1988). In every lake, 10 sampling points were randomly chosen and sampling was conducted in the same way in each lake. Bongo nets (width 60 cm, height 30 cm, mesh size 0.5 mm) were pushed in front (on both sides) of a vessel at a speed of 4–6 km h⁻¹. This yielded samples from near the surface (0–1 m) from both pelagic (bottom depth > 2 m) and littoral zones (bottom depth < 2 m), in the lakes Paasivesi, Puruvesi and Konnevesi. In the lakes Pyhäjärvi and Pyhäselkä fish were collected only from the pelagic zone. In addition, in the lakes Paasivesi and Puruvesi, sampling was made from different vertical depth layers 0–1 m, 1–2 m, 2–3 m and 3–4 m. Larvae were preserved in 10% neutral buffered formalin and frozen. Because the number of whitefish larvae was small, littoral and pelagic (0–1 m) samples were pooled within a lake in order to compare different lakes, without comparing vertical zones. In addition, in order to examine how environment affects pigmentation, we sampled newly hatched larvae (eggs fertilized in autumn 2001) originating from humic lake Pyhäselkä stock and incubated them in clear groundwater under laboratory conditions (Häkkinen et al. 2002). Newly hatched larvae were reared for two days in flow-through aquaria (40 × 20 × 25 cm; length, height and width respectively) and the photoperiod was set at 18L: 6D (only visible light, ca. 1500 lux).

**UV penetration and other optical measurements**

The spectral irradiance measurements of air and underwater UV-B and UV-A irradiance were conducted between late July and August in 2001 or 2002, depending on the sampling year of the study lakes. The field measurements were carried out around solar noon under clear sky (no cloud cover). Irradiance spectra were measured with a Macam spectroradiometer (SR 9910, double monochromator), equipped with long light guide and a planar cosine collector 30 mm in diameter. Irradiance from 290 to 800 nm was measured in steps of 1 nm at several water depths. The attenuation depth, i.e. the depth where the UV irradiance (ranging from 310 to 390 nm, at 10 nm steps) was reduced to 1% of the irradiance just beneath the surface was calculated with the equation of Kirk (1994: p. 7). The data for optical quality of the lakes (water colour and transparency) were gathered from the databases of the Finnish Regional Environmental Centres, except the data of lake Pyhäselkä, which were based on the analyses of Karelian Institute, University of Joensuu (A.-L. Holopainen unpubl. data).

**Determination of melanin concentration**

Pigment analysis took place two months after sampling. In order to determine the total body melanin concentrations of whitefish and vendace, spectrophotometric determination of total melanin (eu- and pheomelanin) in fish larvae was made, with slight modifications, by the method developed to assay melanin concentrations in mammalian hair (Ozeki et al. 1995, 1996, Häkkinen et al. 2002). Sepia melanin (Sigma) was used as the standard for total melanin. Soluene-350 was a product of Packard. Larvae were kept frozen at −20 °C until they were thawed for extraction. The formalin was washed off with distilled water. Heads were removed before homogenisation, so the melanin content of the eyes would not bias whole body data. For one sample, 3–4 larvae were pooled and homogenized in distilled water with an Ultra-Turrax device for three minutes (10–20 mg wet tissue/ml). As a standard, 200 µl of Sepia melanin was used. Samples (200 µl) were placed in capped test tubes (glass), to which Soluene-350 was added (1.8 ml). A blank tube contained 200 µl distilled water and 1.8 ml Soluene-350. After ultrasonication for 5 minutes (Branson
tubes were vortexed and placed in a boiling water bath for 30 minutes. The cooled tubes were re-vortexed and heated for an additional 15 minutes in boiling water. No centrifugation was made as no visible deposit appeared; however, we checked that centrifugation had no influence. Samples were analyzed for absorbances at 500 nm ($A_{500}$). The $A_{500}$ values are referred to as total melanin. Although melanin does not have a distinct absorption maximum at 500 nm, shorter wavelengths revealed much larger background noise due to proteins (Ozeki et al. 1995).

### Statistical analysis

The differences in larval melanin concentration between study lakes were analysed by one-way ANOVA and lakes were compared to each other with Tukey’s test. Further $t$-tests were used when comparing differences in melanin concentration between species within lakes. The effects of sampling depth on the melanin pigmentation of the larvae, and the correlations of melanin concentration with the water colour, transparency and UV-B attenuation depth in lakes were analysed by Spearman’s coefficient correlation.

### Results

#### Optical properties

The UV-B attenuation depth, i.e. the depth where the UV-B irradiance (310 nm) was reduced to 1% of the irradiance just beneath the surface, varied from 0.11 to 0.65 m in the five study lakes (Table 2). Lake Puruvesi was the clearest and lake Pyhäsälä the brownest lake. UV attenuation depth correlated negatively with the colour of water (Spearman’s correlation coefficient, $r = -0.9, p < 0.05$), but transparency did not correlate either with UV attenuation depth or the colour of water ($p > 0.05$). SW Pyhäjärvi had very low transparency, although in this lake UV penetration was second highest and the water was clear. One possible reason for the low transparency in SW Pyhäjärvi was the high algae population. The 1% penetration depth of the UV-A (wavelength 390 nm) ranged from 0.32 to 2.62 m (Table 2).

### Melanin concentration

Vendace larvae had more melanin than whitefish in every lake, although a statistically significant difference was observed between species only in lakes Konnevesi and Pyhäsälä ($t$-test, $p < 0.05$). Total melanin concentration of vendace larvae differed significantly between study lakes in both littoral (ANOVA, $F = 30.24, p < 0.001$) and pelagic zones (ANOVA, $F = 5.884, p < 0.001$). Larvae sampled from the littoral zone of lake Konnevesi had significantly more melanin than larvae in two other study lakes; the mean concentration was 122% and 151% higher than in the lakes Paasivesi (Tukey’s test, $p < 0.001$) and Puruvesi ($p < 0.001$), respectively (Fig. 1). Melanin concentrations in larvae sampled from pelagic zones in the lakes Pyhäsälä, Konnevesi and Paasivesi were high, whereas larvae from

### Table 2. UV attenuation depths (m) for different wavelengths where the UV irradiance is 1% of the value measured just beneath the surface.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Puruvesi</th>
<th>SW Pyhäjärvi</th>
<th>Konnevesi</th>
<th>Paasivesi</th>
<th>Pyhäsälä</th>
</tr>
</thead>
<tbody>
<tr>
<td>310</td>
<td>0.65</td>
<td>0.28</td>
<td>0.22</td>
<td>0.18</td>
<td>0.11</td>
</tr>
<tr>
<td>320</td>
<td>0.98</td>
<td>0.34</td>
<td>0.23</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>330</td>
<td>1.13</td>
<td>0.42</td>
<td>0.31</td>
<td>0.23</td>
<td>0.13</td>
</tr>
<tr>
<td>340</td>
<td>1.19</td>
<td>0.49</td>
<td>0.40</td>
<td>0.25</td>
<td>0.15</td>
</tr>
<tr>
<td>350</td>
<td>1.43</td>
<td>0.57</td>
<td>0.45</td>
<td>0.30</td>
<td>0.19</td>
</tr>
<tr>
<td>360</td>
<td>1.70</td>
<td>0.69</td>
<td>0.55</td>
<td>0.37</td>
<td>0.20</td>
</tr>
<tr>
<td>370</td>
<td>1.99</td>
<td>0.80</td>
<td>0.66</td>
<td>0.46</td>
<td>0.24</td>
</tr>
<tr>
<td>380</td>
<td>2.29</td>
<td>0.92</td>
<td>0.75</td>
<td>0.54</td>
<td>0.28</td>
</tr>
<tr>
<td>390</td>
<td>2.62</td>
<td>1.04</td>
<td>0.84</td>
<td>0.66</td>
<td>0.32</td>
</tr>
</tbody>
</table>
lakes Puruvesi and Pyhäjärvi resembled each other in having lower melanin (Fig. 1). Larvae from SW Pyhäjärvi were highly transparent and had significantly (p < 0.05) less melanin than individuals from lakes Pyhäselkä, Konnevesi and Paasivesi.

There were also significant differences between lakes in the melanin concentrations of whitefish larvae (ANOVA, F = 4.165, p < 0.01). The most pigmented larvae were from lake Pyhäselkä, with significantly higher melanin concentration than fish in lakes Puruvesi (Tukey’s test, p < 0.01) and SW Pyhäjärvi (p < 0.05). However, differences between the lakes Pyhäselkä and Konnevesi or Paasivesi were not statistically significant (p > 0.05). The least pigmented larvae, from lake Puruvesi, also differed from larvae sampled from lake Paasivesi (p < 0.05).

The data allow comparison between the littoral and pelagic zones in three lakes. In Paasivesi, the vendace larvae from the pelagic zone had significantly more melanin than those from the littoral zone (t-test, p < 0.05). In the other lakes, Puruvesi and Konnevesi, there was no difference in larval melanin concentration between horizontal zones (t-test, p > 0.05). Thus no consistent difference between horizontal zones was observed.

Larval melanin concentration correlated negatively with increasing UV-B (310 nm) attenuation depth in both vendace and whitefish (Fig. 2A) (Spearman’s correlation coefficient, r = –1.0, p < 0.001 and r = –0.9, p < 0.05, respectively). For vendace there was a positive correlation between melanin concentration and water colour (r = 0.9, p < 0.05), but not for whitefish (Fig. 2B). No correlation between larval melanin content and the transparency of the lake water was observed (Fig. 2C). On the other hand, in Paasivesi negative correlation was observed between the sampling depth and the melanin concentration of the vendace larvae (Fig. 3).
(Spearman’s correlation coefficient, $r = -0.413$, $p < 0.01$). However, no generalization is possible, as in Puruvesi ($p > 0.05$) there was no such correlation. On the other hand, the most heavily pigmented individuals were swimming near the surface both in Paasivesi and in Puruvesi (Fig. 3), implying colour adaptation.

There was a clear difference between vendace and whitefish larvae reared under laboratory conditions and larvae sampled from the field, even if both were from the same spawning stock (Fig. 4). Vendace larvae sampled from lake Pyhäselkä had significantly more pigmentation than larvae reared in clear groundwater ($t$-test, $p < 0.001$) and a similar trend was observed with whitefish ($t$-test, $p < 0.05$). Although the light regimes or other conditions are not comparable, this shows the influence that environment can have on fish colouration.

**Discussion**

Melanin protects against harmful effects of UV in two ways. Not only does it absorb and scatter light, it also effectively scavenges reactive oxygen species (ROS) (e.g. Sarna et al. 1984, Bustamante et al. 1993). Furthermore, the increased melanin content may be connected with decreased numbers of dermal DNA-dimers, caused by UV radiation in *Xiphophorus* spp. (Ahmed and Setlow 1993). In *Daphnia*, melanin pigmentation provides efficient protection against UV radiation (Zellmer 1995, Hessen 1996). Rautio and Korhola (2002) reported that in *Daphnia umbra* the highest pigment concentrations were found in populations in lakes with low concentrations of dissolved organic carbon ($< 2$ mg l$^{-1}$), while very shallow clear ponds (DOC $< 5$ mg l$^{-1}$) lacked *Daphnia*.

Our results show that UV-B radiation at the ambient level appears to be insufficient to determine the degree of melanin pigmentation of coregonid larvae in Finnish lakes. The melanin content in larvae correlated negatively with UV-B penetration, although it has been shown that UV-B can induce melanin production in fish (Lowe and Goodman-Lowe 1996, Häkkinen et al. 2002). For instance, in our previous study, UV radiation induced production of melanin in larval whitefish and vendace by 30% in 14 days. These morphological colour changes regulated by melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH) involve real changes in the quantity of pigment within the animal or its integument (Baker et al. 1986, Eberle 1988).

There was a significant positive correlation between water colour and larval pigmentation in vendace and whitefish. This might be due to adaptation to the colour of the background, i.e. to the water colour. The number of melanophores may increase or decrease, if fish stay long enough in the same environment with a certain background, depending on the colour of that substrate (Latey and Rangneker 1982, Stepie 1987). The actual concentration of melanin
may also change, and our results with pelagic vendace — and also with whitefish — support this hypothesis. However, the most heavily pigmented individuals came from the littoral zone of Konnevesi, not from lake Pyhäselkä where the water was the darkest. The comparison between laboratory-acclimated larvae and larvae sampled from nature (both originating from one lake) provides strong evidence for the influence of environment on pigmentation. One possible explanation for the correlation between pigmentation and the water colour can be that dark colouration in dark water and a transparent body in clear water protect against visually hunting predators (e.g. Hairston 1979). Predation can be an even more powerful selective factor than UV radiation (Hansson 2000). Further, there can be a direct correlation between the value of the ratio between incident light and reflected light, the albedo, and the degree of melanin formed (Sumner 1940, Brown 1973). On an illuminated black background, where the ratio is large, the animal becomes dark and on an illuminated white background, where the ratio is small, the animal becomes pale, irrespective of the total illumination. On the other hand, predators have contrast-increasing mechanisms such as polarization vision, coloured ocular filters, offset visual pigments and UV vision (Lythgoe 1984, Bowmaker and Kunz 1987, Loew et al. 1993, Browman et al. 1994, Shashar et al. 1998, Losey et al. 1999).

However, vendace larvae swimming near the surface had more melanin than larvae from deeper, which indicates that UV does contribute to pigmentation. Possibly, in lakes Paasivesi and Puruvesi there is a trade-off between pressures due to predation and UV radiation. This suggestion agrees with the observation of Hansson (2000) that pigmentation of copepods was higher in clear waters than in humic ones; however, in the presence of fish predator pigmentation decreased. Furthermore, melanin synthesis is costly (Hessen 1996) and for larvae that have no exposure to UV-B it is not worthwhile to produce melanin.

An alternative explanation can be that translucent larvae in lake Puruvesi have other protective mechanisms, such as avoidance behaviour (Speekman et al. 2000), other UV-B absorbing compounds, or effective DNA-repair mechanisms (Ahmed and Setlow 1993). After hatching, larval vendace and whitefish are positively phototactic and swim near the surface for 1–2 months (Shkorbatov 1966, Viljanen et al. 1995). However, there are indications that vendace larvae are able to avoid UV-B by changing behaviour (Ylönen et al. 2003). A UV-B absorbing compound was recently found in skin extractions of some freshwater fishes and concentration of the substance correlated with increasing UV resistance (Kaweewat and Hofer 1997). Furthermore, Zamzow and Losey (2002) showed widespread distribution of both UVA- and UV-B-absorbing compounds in the epithelial mucus of tropical reef fishes. Vendace also have a strong absorbance peak at 280 nm when extracted with methanol, while in whitefish larvae no absorbance peak occurred at UV-B range (Häkkinen unpubl. data). Thus, besides melanin other protective compounds may be important for high UV tolerance of these species.

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