The estimation of melanophore responses in a pleuronectid fish

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Summary. A description is given of a simple method of estimating physiological responses of epidermal and dermal melanophores in a pleuronectid fish.

In teleosts, physiological and morphological responses of melanophores have been observed after background changes. Physiological responses involve movement of pigment-bearing melanosomes within integumentary melanophores; such activity usually having rapid (min), neurally controlled, and slower (h or days), hormonally controlled, phases in teleosts[1]. Morphological responses involve changes in melanophore density[2] after prolonged exposure to light or dark backgrounds.

During a study of cryptic patterns in winter flounder, *Pseudopleuronectes americanus*, it has proved necessary to use a simple method to estimate physiological responses of melanophores in different areas of skin. In teleosts, measurement of such responses has been based on cellular activity of individual melanophores or on reflectance changes from fish skin. The melanophore index[3] (MI) has proved a convenient arbitrary scale for teleost cellular responses; points 1 and 5 being assigned, respectively, to maximum melanosome aggregation and dispersion. MI methods have been criticized on the grounds that melanosome movement may be caused by disturbance associated with microscopic examination of live fish[1], or with handling fish before fixation[1], when histological preparations of skin are used. Such melanosome movement may continue for a short time after immersion in fixative[4]. To overcome this problem, whole fish have been plunged into liquid nitrogen[4,5], but this procedure is unsuitable for large fish[4]. Reflectance measurements use photoelectric methods[6] or the derived Ostwald index[7] in which the overall colour of the fish dorsal skin is matched, by naked eye, against points from the Ostwald gray series. Objections to using reflectance as a measure of melanophore activity include the possibility that xanthophore and iridophore activity may affect reflectance[8] and, also, such methods cannot be used to compare the activity of different layers of melanophores.

There are a number of macroscopic descriptions of chromatic activity in pleuronectid species[9–11]. These include an account[11] of responses of the cryptic pattern of *P. americanus*; although 'photographic enlargement' was used to study 'significant' responses, the melanophores were not described. Winter flounder upper skin is gray with white spots and dark areas[11], usually 2–4 dark bands in specimens caught off Newfoundland. The present study has established that *P. americanus* possesses epidermal and dermal layers of melanophores (figure 1). Mucous secretion sometimes partly obscures chromatophores on scale-slips mounted in sea water for microscopic examination. It is not known how variation in mucous cell activity may affect reflectance from the skin of this species.

In the present investigation, microscopic examination of scale-slips (figure 2) from fish adapting to different backgrounds, has proved convenient for studying different stages of physiological activity of melanophores. Scale-slips are frozen immediately after removal, using solid CO_2 (–78 °C), and placed in Bouin's fixative. They are then dehydrated, cleared and mounted, unstained. The epidermal and dermal melanophore index (EMI and DMI) are determined for a standard area of each skin sample. Reference examples of melanophores with complete melanosome aggregation and dispersion have been prepared by freezing localized areas of skin of white- and black-adapted fish in situ, using a thin rod of solid CO_2.

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Table 1. Comparison of EMI and DMI values, after different pre-fixation procedures, for scale-slip melanophores from winter flounders during adaptation to a constantly illuminated white background

Pre-fixation procedure	MI after different periods of adaptation to a white background: 0 min EMI	0 min DMI	10 min EMI	10 min DMI	5 days EMI	5 days DMI	10 days EMI	10 days DMI
Frozen at –78 °C immediately after removal from fish	4.8 (4.0–5.0)	4.8 (4.4–5.0)	2.8 (1.3–4.0)	3.2 (2.6–3.8)	1.0	1.4 (1.0–2.1)	1.0	1.3 (1.0–1.6)
None (fixed immediately)	4.7 (4.1–5.0)	4.7 (4.2–5.0)	3.0 (1.2–4.5)	3.2 (2.3–4.0)	1.1 (1.0–1.6)	1.6 (1.1–2.2)	1.0	1.6 (1.2–2.2)
Young's marine teleost Ringer (5 min) at sea water temperature (5–7 °C)	4.7 (4.0–5.0)	4.7 (4.0–5.0)	4.1 (3.2–5.0)	4.3 (3.6–5.0)	1.6 (1.0–2.9)	2.6 (1.8–3.8)	1.8 (1.0–2.5)	2.6 (1.7–3.7)

Each MI-value is the mean, followed by the range in parenthesis, for 10 scale-slips from the extensive gray areas of skin.

Scale-slips are easily removed, the skin separating readily at a fibrous dermal septum (figure 1), usually without observable injury to melanophores. Winter flounders are relatively quiescent in aquaria, and water levels can be lowered to facilitate sampling of scale-slips without handling the fish, usually, without evoking any 'startle' movement.

MI-values for white and black-adapted winter flounders (table 1) compare favourably with those for other teleosts (table 2) estimated by different methods. Using fixation, without pre-freezing, resulted in a slightly higher DMI for white-adapted fish (table 3), a larger proportion of dermal melanophores having slightly dispersed melanosomes (MI, 2.0). Except for black-adapted fish, the EMI and DMI were higher after immersion in a balanced salt solution (table 1), indicating that partial dispersion of melanosomes may normally follow scale-slip removal in this species.

Osborn[11] reported excitement pallor of dark areas, on otherwise white-adapted winter flounders (20 °C), when specimens were prodded. In the present experiments (5–10 °C), although similar responses have been observed, the bands usually pale to vestigial markings after 3 days white-adaptation and the experimental procedure sometimes evokes slight excitement darkening. In an experiment, designed to evaluate the effect of the experimental technique on the chromatic condition of fish, observer movement and removal of scale-slips evoked slight darkening of bands in 4 white-adapted fish (n = 10). Compared with most of the upper skin, MI-values of scale-slips from the bands of these 10 fish (table 3) showed more variation. This probably indicates the extent of excitement response to observer movements. Scale-slips removed from the bands 2 min later, when excitement response following the first sampling was maximum, had a higher MI and a wider range (table 3). 2 min after transfer of white-adapted flounders to a black background the dark-band EMI and DMI are usually 4.0–5.0 in contrast to values in table 3.

Table 2. Comparison of MI values of 4 teleost species after white and black background adaptation

Species	MI after white adaptation		MI after black adaptation	
	EMI	DMI	EMI	DMI
Ictalurus melas[5]	1.1	1.26	5.0	5.0
Anguilla anguilla[1]	1.0	1.3	4.6	4.9
Gasterosteus aculeatus[12]	–	1.3	–	4.7
Phoxinus phoxinus[13]	–	1.25	–	4.8

Table 3. Comparison of EMI and DMI values of scale-slip samples taken 2 min apart, without change of background colouration

Cryptic pattern component	Sample	MI after 72 h adaptation to a constantly illuminated background			
		White background		Black background	
		EMI	DMI	EMI	DMI
Extensive grey area	1st	1.4 (1.0–2.0)	1.6 (1.3–2.0)	4.8 (4.5–5.0)	4.7 (4.5–5.0)
	2nd (2 min after 1st)	1.3 (1.0–2.1)	1.6 (1.3–2.3)	4.7 (4.5–5.0)	4.7 (4.3–5.0)
Dark band area	1st	1.5 (1.0–2.3)	1.9 (1.3–3.0)	5.0	4.9 (4.4–5.0)
	2nd (2 min after 1st)	1.7 (1.0–3.0)	2.4 (1.4–3.5)	4.9 (4.6–5.0)	4.9 (4.4–5.0)

Each MI-value is the mean, followed by the range in parenthesis, for 10 scale-slips.

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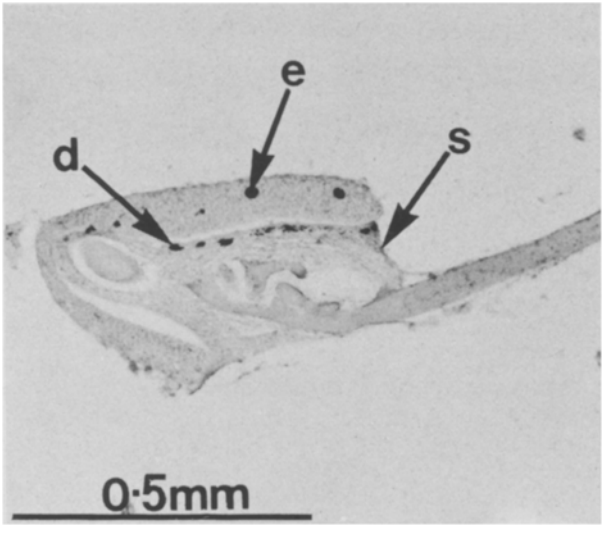

Fig. 1. Longitudinal section through a scale-slip. d, dermal melanophores; e, epidermal melanophores; s, fibrous septum in dermis.

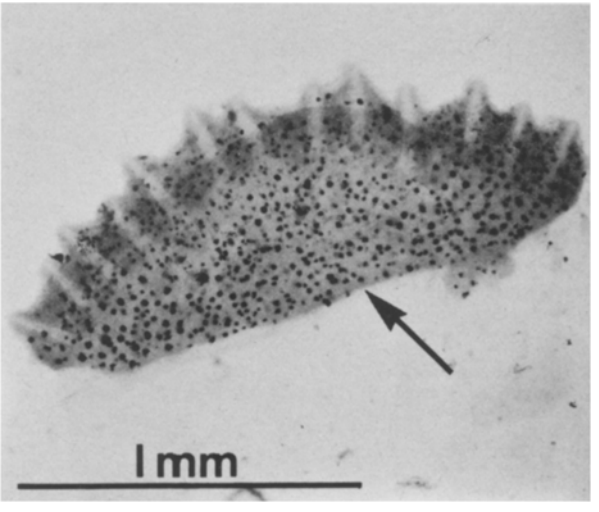

Fig. 2. Scale-slip preparation. Position of dermal septum is indicated.