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Susceptible species

Hyperpigmentation primarily affects common dab (*Limanda limanda* L.) from the North Sea and, less frequently, from adjacent waters such as the English Channel, the Celtic Sea and the Irish Sea. Hyperpigmentation has been recorded in dab since the onset of systematic fish disease surveys in the North Sea in the 1980s and has also been observed occasionally in other flatfish species from the same habitat, such as long rough dab (*Hippoglossoides platessoides*), lemon sole (*Microstomus kitt*), solenette (*Buglossidium luteum*) and European flounder (*Platichthys flesus*), but at apparently lower prevalence (Grütjen *et al.*, 2013).

Disease name

The term "hyperpigmentation" reflects the major macroscopic signs of the condition which consist of an increase in green to black or pearly-white pigmentation that can either be patchy or can affect large parts of the body surface.

Aetiological agent

Although virological, bacteriological, parasitological, histopathological and ultrastructural studies have been carried out (Noguera *et al.*, 2013), no aetiological agents were identified and reasons for the condition and its characteristic spatial patterns and temporal trends in the North Sea have not been resolved. It is believed that environmental factors are the causes of hyperpigmentation and several hypotheses as to the main aetiological factors have been discussed (Grütjen *et al.*, 2013; Noguera *et al.*, 2013).

Geographical distribution

Hyperpigmentation is especially common in the North Sea dab stock, with highest maximum prevalences (approximately 50%) recorded in the German Bight, at the Dogger Bank and off the Scottish coast in the period 2005–2009. The prevalence in northern regions of the central North Sea has always been lower and generally does not exceed 10%. A conspicuous and statistically significant, often almost linear, increase in prevalence was recorded in many North Sea regions since the 1980s. The condition has occurred less frequently in dab from the English Channel, The Celtic Sea and Irish Sea. Interestingly, hyperpigmentation is virtually absent in dab stocks from the Baltic Sea and Icelandic waters (Grütjen *et al.*, 2013).

Associated environmental conditions

From the long-term prevalence data available over a period of 30 years and from the lack of findings of any pathogen involvement, there is indication that the increase in prevalence of hyperpigmentation has a strong environmental link. Causes discussed include, e.g. an increased UV-B radiation affecting pelagic early life stages, changes in food composition associated with climate change and effects of contaminants

(Grütjen *et al.*, 2013; *Noguera et al.*, 2013). However, no conclusive evidence on the role of these factors exists to date.

Significance

Hyperpigmentation is considered as a disease because it could be demonstrated that it has an impact on the fitness of dab. Particularly specimens with a severe colour change have significantly lower condition factors and show a greater mortality if kept in captivity than their non-affected conspecifics (Grütjen *et al.*, 2013). Therefore, it cannot be excluded that hyperpigmentation exert population-level effects, particularly if it occurs at high prevalence.

Gross clinical signs

Hyperpigmentation is characterized by the occurrence of spots or diffuse patches of green to black pigment on the ocular side and, occasionally, pearly-white spots on the blind ventral side (Figure 1). Rarely, the ventral side is also affected by green to black spots (Figure 1). Histopathological findings in affected fish include hyperplasia of chromatophores (melanophores and iridophores) in the dermis (Figure 2). In highly pigmented dab, dermal lymphocytic infiltration, occasionally expanding into the epidermis and sub-dermal layer (Figure 2), was more frequent than in normal fish, suggesting an active immune response. Ultrastructure studies showed additional disruption of the epithelial layer, with loose melanin granules between cells and a number of single or aggregated melanocytes.

Control measures and legislation

Marketability of hyperpigmented specimens may be affected by EU Regulations No. 178/2002 (Article 14) on procedures in matters of food safety (EU, 2002), as hyperpigmented specimens may be regarded as "unfit for human consumption" based on sensory (visual) inspection.

Diagnostic methods

Diagnosis of hyperpigmentation can be done by macroscopic examination. It is recommended to use a grading system with three severity stages, based on the body area covered by the pigment anomaly, as defined by the Biological Effects Quality Assurance in Monitoring (BEQUALM programme (<u>www.bequalm.org</u>). Macroscopic examination can be supported by histopathology (H&E stain of the epidermis, dermis and underlying musculature) and ultrastructural studies as described by Noguera *et al.* (2013).

Key References

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- Noguera, P. A., Feist, S. W., Bateman, K. S., Lang, T., Grütjen, F., Bruno, D. W. 2013. Hyperpigmentation in North Sea dab (*Limanda limanda*): macroscopic and



Figure 1. *Limanda limanda*. Severe hyperpigmentation (grade 3) on the upper (A) and lower (B) body.



Figure 2. *Limanda limanda*. Histological appearance of hyperpigmentation. A: skin of normal pigmented dab. B and C: skin of hyperpigmented fish showing characteristic dermal hyperplasia of chromatophores (arrows). Note also mild epidermal disruption (B and C; asterisks) and subdermal infiltration (C; arrowheads).

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