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Influence of dietary arachidonic acid combined with light intensity and tank background colour on pigmentation of common sole (*Solea solea* L.) larvae

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Abstract

The presence of abnormally pigmented fishes in flatfish culture is an unsolved problem. Both nutritional as well as environmental factors may cause pigment defects in flatfish larvae and genetics may determine the larval sensitivity. The essential polyunsaturated acid, arachidonic acid (20:4n-6, ARA) has been found to induce malpigmentation (hypopigmentation) in flatfish larvae. The aim of the present study was to reveal or unreveal possible physical and environmental pathways interactions on larval pigmentation investigating the combined effect of dietary arachidonic acid, light intensity as well as tank background colour. ARA had a highly significant influence on hypopigmentation (i.e >90 % of larvae hypopigmented) masking eventual effect of light and background . The combination of a high light intensity and transparent tanks increased hypopigmentation for larval groups not treated with ARA.

Experimental Setup

Triplicate tanks of larvae were fed *Artemia* enriched for 24 h by one of 2 experimental emulsions at 3-11 days post hatch (dph). One emulsion contained fish oil, while fish oil in two was partly substituted with arachidonic acid oil (Vevodar oil) according to table 1.Each triplicate tank fed one of the two diets were exposed to either 4000 lux or 100 lux surface light and a black foil (bl.) or transparent (wh) (acrylic glass) tank background giving a matrix of 2 x 2 x 2 treatments (24 tanks).

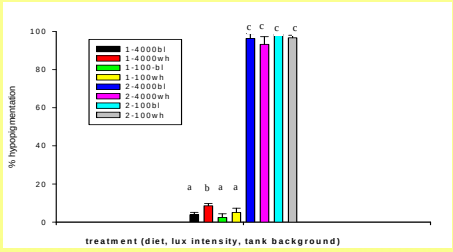
Emulsion: composition % inclusion	1	2	Light intensity (lux) and tank colour
Fish oil	89.0	65.0	
Arachidonic acid	0	24.0	4000 or 100 Lux
Soy lechithin	7.0	7.0	Black or transparent
E vitamin	4.0	4.0	

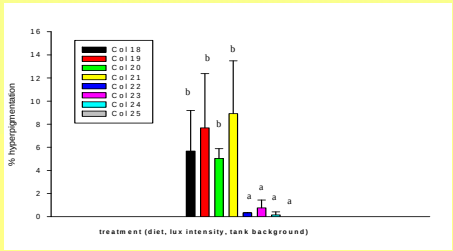
Larval Growth & Mortality

Illumination influenced significantly on the size (d.w) of the larvae (see table). Larvae exposed to 4000 lux surface light was largest (P<0.001) with no significant effect of diet and tank colour at 11 days post hatch.The calculated daily growth rate, SGR (Ln Wf-Ln Wi x100)/t was not significantly different between treatments (P= 0.055). Survival was not significantly related to treatment, but highest in black larval tanks at 21 days post hatch. The combination of a high light intensity and black coloured tanks may have influenced positively on survival and growth, probably as due to better prey visualization and an improved feed intake.

	1[1]-4000[2]-bl	1-4000-wh	2-4000-bl	2-4000-wh	1-100-bl	1-100-wh	2-100-bl	2-100-wh
d.w. (dph 11)	0.31 ± 0.08^{b}	0.32 ± 0.03^{b}	0.28 ± 0.02^{ab}	0.27 ± 0.05^{ab}	0.23 ± 0.07^{ab}	0.19 ± 0.02^{a}	0.24 ± 0.01^{ab}	0.19 ± 0.07^{a}
SGR (dph 11)	17.7±3.2	18.3±1.0	17.0±0.7	16.6±2.1	14.5±3.4	13.1±0.9	15.5±0.5	12.5±3.7
d.w. (dph 21)	2.02 ± 0.33^{bc}	2.28 ± 0.46^{c}	1.61 ± 0.15^{ab}	1.89 ± 0.24^{b}	1.31 ± 0.20^{a}	1.83 ± 0.31^{b}	1.32 ± 0.19^{a}	1.75 ± 0.20^{ab}
survival (3-21 dph)	43.4 ± 7.4^{abc}	30.6 ± 10.6^{a}	42.9 ± 3.7^{abc}	37.3 ± 7.7^{abc}	52.8 ± 4.1^{b}	28.6 ± 1.4^{a}	54.5 ± 5.7^{bc}	34.2 ± 6.8^{ab}

A different superscript in a horizontal row denotes a significant difference P < 0.05.
[1]) 1, 2 (i.e. diet 1 or 2
[2]) 100, 4000 (i.e. surface light intensity, lux)
[3]) bl., wh. (i.e. tank background, black or transparent/white





Larval pigmentation

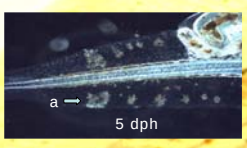
A strong linear correlation was recorded between tissue and dietary ARA. The degree of hypopigmentation at 35 days post hatch was highly significantly (P<0.001) related to tissue ARA level. Larvae fed *Artemia* enriched with ARA oil (emulsion 2) during premetamorphosis showed a highly significant higher proportion (P<0.001) of later hypopigmentation than larvae fed *Artemia* enriched by a fish oil based emulsion without ARA supplementation (emulsion 1) (Figure 1). 4000 lux light intensity combined with a transparent tank background increased hypopigmentation among the groups of larvae not supplemented with ARA (P< 0.04). Hyperpigmentation was significantly (P<0.001) more prevalent in the groups of larvae not treated with ARA. Hyperpigmentation was most likely related to other factors than light or background on a scale of each individual tank as indicated by a large replicate variation.

Pigment cell development

Pigment cell development (chromatophores, i.e melanophores (ml.); xanthophores (xt.)) were compared for two groups of larvae treated with ARA oil (i.e. 98 % hypopigmented) or fish oil (i.e. 93 % normal pigmented), but with similar light intensity (100 lux) and tank colour (black). The development of larval pigment cells during pre metamorphosis seemed similar for the two groups. Single larva melanophores were spread mainly on the fin area in these early larva stages. Xanthophores were dominant along chorda. The chromatophore density increased during early metamorphosis (especially xanthophores) but was lower for larvae treated with dietary ARA. At end of metamorphosis pigment cells underwent cytolysis - markedly for larva treated with ARA.

Number of chromatofores ($mm^{2\,-1}$)	Dph 5	Dph 9	Dph 14	Dph 16	Dph 18	Dph 23	Dph 25
1-100-bl	28±14	44±26	52±30	54±24	30±10	12±3	13±5
2-100-bl	32±16	51±24	40±16	18±12	24±6	2.7±0.7	4±2

Dph: Days post hatch



a) larval ml.
b) adult ml.
c) xt

Conclusion

The present study confirmed previous studies, that dietary ARA induces hypopigmentation when fed to sole larvae during premetamorphosis (i.e 7 days only). Consequently more than 90 % of larvae in these groups had this kind of malpigmentation. No combined effect of ARA and light intensity / tank colour was observed rejecting the hypothesis of an involvement of light in ARA induced malpigmentation. In groups of larvae not treated with ARA, visual light at 4000 lux surface intensity in combination with transparent tank colour increased hypopigmentation slightly, but significantly. Early chromatophor development was not related to dietary treatment, but during metamorphosis chromatophore concentration was lower for larvae treated with ARA and degeneration / cytolysis was higher for this group during post metamorphosis.

References

Denson, M.R.; Smith, T.I.J. (1997). Diet and light intensity effects on survival, growth and pigmentation of southern flounder *Paralichthys lethostigma*. J. World Aquacult. Soc. 28, 366-373.

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