Completion report: Sea Grant project number R/FMD-170

“Larval diet, visual behavior, and juvenile pigmentation in hatchery-reared summer flounder.”  PI: J.A. Bolker

a. Accomplishments

The initial objective of this Sea Grant project was to measure possible effects of larval diet on both visual behavior and albinism in hatchery-reared summer flounder, a species being developed for aquaculture. We sought to determine whether measurements of visual acuity in larval and juvenile fish could serve as a behavioral assay to predict albinism. However, initial work by M.S. student Michael Baron revealed that testing visual performance at larval stages was not feasible due to the very small size of the larvae and the difficulty of eliciting normal behavior under appropriate experimental conditions. Moreover, by the time the project began, our summer flounder supplier (Great Bay Aquaculture of Newington, NH) had succeeded in eliminating albinism from their cultured fish by changing to a larval diet enriched in vitamin A. Although various forms of malpigmentation remain problematic in the industry, our particular source of fish no longer offered appropriate material (or would benefit from) studies relating to the incidence of albinism, as had been proposed in the original grant application.

Although it eliminated the problem of albinism, the change to a Vitamin A-enriched diet at GBA resulted in a dramatic increase in the frequency of abnormal blindside coloration (ambicoloration). Since the change in malpigmentation coincided with the change in diet, it was assumed that the enriched diet had a relatively direct effect on coloration. One strong candidate for the active factor was retinoic acid (RA), a highly biologically active derivative of Vitamin A. Prompted by the apparent effects on pigmentation of vitamin A enriched feed, Baron directly examined the effects of RA on summer flounder pigmentation; his results, further analyzed by Martinez and Bolker (see below and publications list) support the idea that RA stimulates ambicoloration.

b. Findings

We reared summer flounder larvae (Paralichthys dentatus) in water containing 0-20 nM RA in order to assess its effects on postmetamorphic pigmentation and on skeletal development. RA exposure disrupted pigmentation development: treated tanks had a smaller percentage of normally-pigmented fish than did controls, with increased numbers of both hypo- and hyperpigmented individuals. Exposure also affected the development of several skeletal features: RA treatment correlated with a significant increase in the severity of defects in jaws, fins, hypurals, and vertebrae compared to control groups.

Our results document pervasive and complex impacts of RA on pigmentation and skeletal development in larval summer flounder. We observed a strong size-dependence of pigmentation effects: a higher percentage of the smallest fish were pigment-deficient regardless of their metamorphic status, and RA exposure tended to enhance pigmentation in the largest cohort (exclusive of incompletely metamorphosed individuals).
This finding helps resolve an apparent conflict between earlier studies in Japanese flounder. In our study, which began with a heterogeneously-aged population (stages C-F), RA exposure was associated with both positive and negative disruptions of pigmentation; the prevalence of under-pigmentation in the smallest size class and excess pigmentation among the larger fish is consistent with the stage-dependence of RA effects implied by previous work.

We documented a high “background” frequency of developmental defects, especially hyperpigmentation and vertebral deformities, even among our control groups. Thus, within the hatchery population, even fish not deliberately exposed to RA exhibit some typical symptoms of RA exposure or VA overdose. This finding underscores the need for careful control of VA supplementation in larval diets, as well as the utility of identifying specific morphological hallmarks of RA toxicity. Finally, it points to the importance of better understanding of the etiology and functional significance of RA-induced developmental defects in cultured flatfishes.

2. Publications and manuscripts

_In review:_


[I’ve asked Mike Baron to email you directly his list of presentations based on his thesis work.]

3. Names and majors of students supported

Michael P. Baron. M.S. in Zoology, 2003 (full support)
Gabriela M. Martinez, Ph.D. in Zoology, 2004 (partial support, as research assistant in final stages of project)

4. Title of thesis

M.P. Baron: Factors affecting pigmentation development in summer flounder. (M.S., Department of Zoology, University of New Hampshire)
Sea Grant Extension Report (Project # R/FMD-170 to J. Bolker)

Prediction of adult pigmentation patterns from pre-metamorphic winter flounder larvae

Anecdotal reports from scientists culturing winter flounder at the UNH Coastal Marine Lab suggested that adult pigmentation patterns were recognizable in pre-metamorphic larvae. Specifically, pigmentation anomalies such as albinism or excess pigmentation were said to be readily distinguishable in larvae. These reports were intriguing since the current flatfish pigmentation literature suggests that adult pigmentation patterns are only definitively established during metamorphosis. We therefore proposed a preliminary study to explore, and if possible confirm, the reports of earlier predictability.

The original study design was conceptually simple. We proposed to select (1) individual larvae that were still in pre-metamorphic developmental stages and had normal larval pigmentation patterns (defined prior to the study), and (2) larvae that exhibited either a lack of pigment or excess pigment. We then planned to rear these populations in separate tanks but under the exact same light and diet regimes as other larvae at the CML. We also planned to document pigmentation patterns by photographing all of the selected larvae prior to the start of the investigation and again at the conclusion of the study. We selected approximately 150 larvae that were normal, albino or excessively pigmented and photographed them using a digital camera. We then placed all of the larvae in a circular rearing tank and reared them for six weeks.

Because the larval stages of almost all organisms are fragile and winter flounder larvae are particularly delicate, colleagues we consulted at the CML recommended that the above design because it would impose the least disturbance on the fish, and ensure high survival rates. Moreover, as the CML is used by many UNH faculty and students, space was limited. Therefore separate rearing and tracking of individual fish was not conducted. This proved to be a serious problem in the end because even though we had photographed the larvae prior to the start of the study it was impossible to determine which fish was which at the conclusion of the study; we were thus unable to draw conclusions regarding pigmentation establishment prior to metamorphosis. However, with regard to survival rates during the course of the study we only lost seven fish.

Since the first study was inconclusive, we redesigned the experiment and ran it again. The key to the study was having the ability to track and rear individual fish on a regular basis; a critical limitation was the lack of space in which to rear the fish at the CML. We were given the use of a running seawater table 345cm x 80cm x 18 cm in which to conduct the study. We built two rearing cages, each of which held 72 plastic tripour beakers with rectangular cutouts covered with nitex mesh to allow for gentle water circulation. Larvae exhibiting normal, deficient or excessive pigmentation were again selected manually from the main tanks and individual fish were photographed and then placed into each cup.

Each cage was fitted with tubing so that there was an even circulation of water in each cup. This proved to be more difficult than anticipated, and required constant monitoring. Cages were cleaned daily because the water used was piped directly from Portsmouth Harbor (unfiltered), and large amounts of sediment accumulated at the
bottom of the cups daily. Larvae in our study were reared on the same light and diet regimes as the rest of the stock. The experiment ran for a total of 6 weeks to ensure that all of the fish went through metamorphosis. However, despite our best efforts, only 5% of the larvae survived. Although it appeared that the original pigmentation patterns persisted—for example, larvae that were initially pigment-deficient remained so—the numbers were too small in all categories to draw statistically sound conclusions.

That adult pigmentation patterns may be distinguishable prior to metamorphosis in winter flounder remains an intriguing idea. It goes against what is commonly believed in the flatfish literature, and if proved to be correct could have significant implications for culturing of these fish as well as for our understanding of the mechanisms and processes that occur during metamorphosis and pigmentation development. However, we believe that in order to proper investigation of this question will require access to a facility allowing better control of factors such as water quality, circulation and aeration, and adequate space to rear large numbers of larvae in individual containers.