

Green water: Optical rather than nutritional effect. pp. 266-269.

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INTRODUCTON

Many early publications on the rearing of marine fish larvae (May, 1971) indicated that phytoplankton cultures enhance survival rates. These green water trials usually involved an array of live planktonic food organisms, so that the actual dietary intake of the fish larvae could not be determined precisely, but the premise was usually accepted that the green algae provided a nutritional enhancement of other food organisms (zooplankton), whether the fish larvae directly consumed the algae or not. Recently, however, that premise of nutritional enhancement has come into question. Studies on first feeding of Atlantic halibut (*Hippoglossus hippoglossus*) larvae at the Austevoll Station in Norway (Naas, et al., 1992) demonstrated major improvements in survival when green water was employed rather than clear water, hut nutritional effects were considered minor in comparison to the effect on behavior. That is, the halibut larvae in clear water concentrated at the water surface and near the tank walls, whereas in green water the larvae spent most of the time in the water column, searching for prey.

The most likely alternative possibility to nutritional effects is that green water alters behavior through its optical qualities. Research at Western Kentucky University (Salgado, 1992) involved rearing fathead minnow (*Pimephales promelas*) larvae in constant darkness versus constant light (all trials in clear water). Replicate trials in both treatments were fed either live or ultrasonically killed brine shrimp nauplii. Dead nauplii provided only olfactory cues in darkness, whereas live larvae provided cues by their swimming movements. Visual cues were available in lighted conditions. Lighting and feeding regimes were, at a later stage in development, reversed in all treatment combinations and the relative food intake measured. The larval fathead minnows which had been reared in darkness displayed superior olfaction and acoustico-lateralis (distant touch) capability, compared to more visually oriented larvae which had been reared in constant light. Since day and night periods occur in nature and since feeding in nature tends to occur during daylight, the superior olfaction and distant touch capability of larvae from the dark treatment

may have involved some artifacts of experimental design, since larvae in nature would be expected to have well-balanced sensory capabilities.

Therefore, it was hypothesized that green water may obscure visibility to the extent that a better balance in the use of sensory modalities would be induced in fish larvae under those conditions, in comparison with clear water and unlimited visibility.

The family Hexagrammidae includes the greenlings and lingcod of the northeast Pacific Ocean. Larvae of this family have a tendency to sink to the bottom of a laboratory tank during periods of darkness, so that the practice was adopted at the Vancouver Aquarium of rearing them in constant light. Prior to the availability of fatty acid supplements for brine shrimp, efforts to rear greenling or lingcod larvae on brine shrimp nauplii alone met with complete mortality of larvae prior to completion of caudal fin formation. The increase in energy budget with formation of caudal fin rays, which enable more rapid swimming, frequently results in a stage of high mortality if dietary quality has been compromised. Greenlings also tend to show an extreme tendency to accumulate against tank walls, near the surface, where feeding behavior appears to be disrupted by "wall-nosing" behavior. With Selco enrichment of brine shrimp nauplii, lingcod (*Ophiodon elongatus*) and kelp greenling (*Hexagrammos decagrammus*) larvae were successfully reared to the juvenile stage, albeit with under 5% total survival from hatching to juvenile in clear water conditions. The very low survival to the settled juvenile could have been due to the persistent wall-nosing behavior and associated reduction of feeding in clear seawater.

METHODS

The whitespotted greenling (*Hexagrammos stelleri*), a fall-spawning species, was used in 1993/1994 in a rearing trial with a 2,000 liter tank, employing algae paste to create green-water conditions. In winter/spring of 1994, replicate 80 liter tanks were used for rearing kelp greenling (*Hexagrammos decagrammus*) and lingcod (*Ophiodon elongatus*) larvae in clear versus green water. Paired 2,000 liter tanks of clear versus green water were used to rear kelp greenling larvae in spring of 1984. All treatments were fed twice daily with excess numbers of Selco-enriched brine shrimp nauplii, floated at the surface in a layer of slightly warmed, diluted seawater. For green water treatments, green algae paste (*Nannochloropsis oculata*) was added with the brine shrimp, 8 ml per 2,000 liter tank and about 0.5 ml per 80 liter tank (algae paste, 9-10 billion cells/ml, from Innovative Aquaculture, fax (250) 755-9531). The 2,000 liter tanks received approximately two tank volumes per day through-flow of seawater, creating peripheral circular currents and causing algae and brine shrimp to be diluted (flow to waste) between feedings.

In all trials, tank bottoms were siphoned every 48 hours. All bottom-siphoned larvae were discarded and recorded as dead. Live larvae siphoned from the bottom were significantly smaller than larvae from surface water, and were presumed to be near death from poor nutritional condition. Preservations for assessing growth and development rate were not included in total numbers for determining survival rates.

Green water was attempted in an uncontrolled fashion with species for which previous observations had indicated problems with wall-nosing behavior. Tropical damselfishes (~*Amphiprion percula*, *Pomacentrus mollucensis*), painted greenling (*Oxylebius pictus*) and grunt sculpin (*Rhamphocottus richardsoni*) were reared with green water.

RESULTS AND DISCUSSION

In clear water, larval hexagrammids tended to swim cross-current into tank walls, and showed poor survival rates. The use of algae paste to reduce visibility resulted in slower swimming, active feeding and formation of schools, with higher survival rates. With the damselfish larvae, addition of algae paste caused immediate changes in larval distribution, bringing larvae away from glass walls of tanks and closer to the tank surface. Grunt sculpin and painted greenling larvae could not be reared in clear water in 80 liter trout tanks, evidently owing to wall-nosing by the painted greenling larvae and upward swimming at the surface tension film by grunt sculpin larvae; in both cases the larvae became distributed more in midwater, with apparent increases in frequency of food intake, and with survival to the juvenile.

With whitespotted greenling larvae in green water, 75% of hatched larvae survived to the pelagic juvenile stage at 65-days age (posthatch), and 36% of the hatch survived to the settled juvenile stage at 120-days age. Mortalities among individuals which persisted in wall-nosing were associated with lower growth rates and significantly lower contents of those fatty acids which can be oxidized to provide maintenance energy, whereas the more structural essential fatty acids remained at constant levels (J.N.C. Whyte, Pacific Biological Station, Canada Department of Fisheries and Oceans, unpublished data).

The green water was not, however, a panacea. On one date, 10% of total mortality of the whitespotted greenlings occurred as a result of reduced flows having increased algal density, apparently harming water quality. On an earlier occasion (8-days posthatch), increased mortality occurred at a lower rate and was apparently related to passive sinking behavior and loss of balance in swimming when reduced flows led to high overall algal density.

Increased tank flushing rapidly led to improved behavior at that time. Too high a flushing rate, however, kept water too clear to result in altered behavior. The occurrence of schooling behavior, itself associated with slower swimming and high rates of food ingestion, was exclusively associated with the green water conditions, and provided a criterion for adjusting flow rates (so that turbidity would remain high enough to induce schooling). It should be noted that on some mornings, which was when water clarity was greatest, the whitespotted greenling larvae would largely have resumed wall-nosing, so that extra algae paste would be required to adjust turbidity and induce schooling.

On a few occasions, green food coloring in skim milk (Vogel, 1981) was used rather than algae paste to increase turbidity. The effects of colored milk on behavior of whitespotted greenlings were comparable to those of algae, except that the milk settled out from the water relatively rapidly and the fish larvae tended to become stained green. It was evident, however, that the turbidity effect of the algae, and not some biochemical signal, was responsible for the alteration of behavior, presumably on a visually mediated basis.

The role of distant touch in schooling (Pitcher et al., 1976) is taken to indicate that the larvae tend to switch from more exclusively visual orientation to a balance between various sensory modalities when vision is occluded by turbidity. The immediate acceptance of frozen krill in green water, upon physical contact at the mouth, was presumably based on gustation rather than vision, whereas pelagic juveniles in clear water only gradually accepted frozen krill, striking from a distance by vision. The work by Salgado (1992) on larvae feeding in light versus darkness could be explained on the same basis, that in clear water in continuous light, the larvae would rely exclusively on vision, with the artifacts that they fail to undertake schooling and they tend not to encounter food items as frequently when they are nosing into walls. The wall-nosing may indicate an innate tendency of greenlings to cross current shears when in clear water, in order to increase chances of encountering water masses with phytoplankton blooms and the associated blooms of edible zooplankton.

In replicated trials with clear versus green water in small, 80 liter tanks, overall survival of kelp greenlings and lingcod was low, although consistently higher in green water than in clear water. None of the small tank replicates, however, yielded survival to the juvenile stage, probably because contacts with tank walls were much more frequent with the higher wall surface-to-tank volume ratio in small tanks, even with green water. Also, the water turnover rate was higher in small tanks, so that algae and food densities did not remain high for

as long in small tanks as in large tanks. Schooling never occurred in small tanks.

In the trial with kelp greenling larvae in two 2,000 liter tanks with clear versus green water, survival was not significantly greater in green water, perhaps because a relatively low-viability hatch was used which yielded about 50% mortality during the first three days posthatch. At 46-days age, the green water tank had a total of 6.2% surviving versus 4.39% in the clear water, whereas at 3-days the difference had been 54% (greenwater) versus 42% survival (clear). In that trial, the growth and development rates were significantly higher in the green water treatment than in the clear, along with marked differences in behavior, the larvae in clear water tending more towards wall-nosing and never exhibiting schooling.

The amounts of fatty acid which algae impart to nauplii cannot explain the large differences in fatty acid content between wall-nosing versus schooling greenling larvae. The differences are most likely attributable to differences in encounter and ingestion rates of prey. The obvious differences in behavior, with larvae in clear water swimming morerapidly and spending major proportions of time nosing into walls, suggest that reduced overall survival in clear water results from anomalies in behavior related to the combination of clear water and the effects of confinement in a tank. The smaller the tank the worse the effects of confinement, mainly involving contacts with tank walls which disrupt feeding. It must be noted, however, that whereas green water yielded successful production of juveniles of painted greenlings and grunt sculpins in small tanks, no juveniles were produced for kelp greenlings or lingcod in small tanks (in either green or clear water). Similarly, the altered behavior of damselfishes in green water was not sufficient to result in any significant growth and development. Low viability of hatch remains a source of chronic mortality problems in any one rearing trial, regardless of species or turbidity. Use of algae paste to create green water provides only one tool in a larval rearing strategy.

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