The effect of green water and light intensity on survival, growth and lipid composition in Atlantic cod (Gadus morhua) during intensive larval rearing.

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Abstract

The effects of green water and light intensity on establishment of exogenous feeding, survival, growth, and lipid and fatty acid composition were investigated for larvae of Atlantic cod in two replicate experiments. In each experiment, six tanks received "low" irradiance (12-20 μWcm^{-2}) and another six tanks "high" irradiance (240-283 μWcm^{-2}). In three of the tanks within each light treatment, the alga Isochrysis galbana was added to make green water, and the other three tanks had clear water. Cod larvae were stocked into the tanks at day 3 post-hatch, and fed the rotifer Brachionus plicatilis. Larval survival and gut filling at initiation of exogenous feeding were significantly improved by use of green water, Feeding incidence was not significantly affected by any of the treatments,

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although low light conditions gave a marginal increase in feeding incidence. Growth was not affected by any of the treatments, but a tendency to density-dependent growth was observed within green vs. clear water, yielding some improvement in growth with use of algae. However, a significant signal from the algal treatment was detected in the fatty acid composition of the phospholipids and in the relative amount of triacylglycerols in the larval lipids of cod. Thus, the data demonstrate a nutritional vector of algae in larval cod rearing, and the possibly benefit of this on survival. Lack of effects of light intensity contradicts previous studies on larval cod, but point out possible genetic adaptations in this species to variable environmental conditions throughout its distribution area.

Keywords: Atlantic cod; Algae; Light intensity; Feeding incidence; Lipid classes; Fatty acid composition; Nutrition

1. Introduction

Identification of environmental key parameters for larval rearing is important for optimising juvenile fry production in marine fish culture. In particular, before new species are propagated in commercial hatcheries, protocols for the larval rearing environment should be developed from controlled experiments. Two important environmental parameters, light intensity and use of green water vs. clear water, have gained attention in the recent interest of bringing Atlantic cod (Gadus morhua) into intensive culture systems.

Different kinds of green water techniques are extensively used in culture of many other marine fish species (Fushimi, 2001; Lee and Ostrowski, 2001; Liao et al., 2001; Shields, 2001). One of the earliest reports on use of unicellular microalgae in marine fish cultivation concluded that the algae Chlorella sp. improved growth and survival in over 40 of different fish species studied (Jones, 1970). Different algal species used in green water rearing has later proved to be beneficial for larval performance among fish species like turbot Psetta maxima, Atlantic halibut Hippoglossus hippoglossus, sole Solea solea, sea bream Sparus aurata, sea bass Dicentrarcus labrax, striped mullet Mugil cephalus, grunion Leuresthes tenuis, and summer flounder Paralichthys dentatus (Alderson and Howell, 1973; Scott and Baynes, 1978; Scott and Middleton, 1979; Vásques-Yeomans et al., 1990; Naas et al., 1992; Reitan et al., 1993; Tamaru et al., 1994; Cahu et al., 1998; Bengtson et al., 1999; Papandroulakis et al., 2002).

The effect of microalgae in larval fish culture is not completely understood. The hypotheses listed in a recent review by Muller-Feuga et al. (2003b) include improvement of water quality and light contrasts, direct nutrition through active ingestion, indirect nutrition by enriching the live prey in the rearing tank, micronutrient stimulus for feeding behaviour or physiological processes, and regulation of opportunistic bacteria by antibacterial or probiotic action (Naviner et al., 1999). Rearing of cod juveniles in extensive (lagoon) systems has shown that cod larvae at initiation of exogenous feeding obtain "green guts" containing a diverse composition of algal material, and that cod larvae are capable of actively ingesting algal cells of a certain size by filter feeding (van der Meeren, 1991). Thus, the questions are if cod larvae will benefit from access to algae, or if clear water is adequate for intensive rearing under controlled environmental conditions?

Another parameter that easily can be controlled in intensive rearing systems is light. Light intensity has been reported to have profound influence on larval growth and survival in cod and haddock (Melanogrammus aeglefinus) (Puvanendran and Brown, 2002; Downing and Litvak, 1999b; van der Meeren and Jørstad, 2001). Light intensity may affect swim bladder inflation in fish larvae (Trotter et al., 2003), and optimal light intensity for larval feeding and growth varies among fish species (Boeuf and Le Bail, 1999). Also stock-dependent differences in optimal light conditions have been suggested for cod (Puvanendran and Brown, 1998). Light intensity may modulate larval food-searching activity (Batty, 1987), and thereby influence larval foraging success. Most fish larvae are visual feeders, and foraging is characterised by a series of

sequential events involving search, encounter, attack and capture (O'Brian, 1979). Detection of prey may be influenced by conspicuousness of the prey item (Wanzenböck and Schiemer, 1989), which in turn is dependent on contrast. In intensive rearing light intensity and tank colour may affect prey contrast (Naas et al., 1996; Downing and Litvak, 1999a), and use of algae may add visual contrast by changing turbidity (Boehlert and Morgan, 1985; Naas et al., 1992). In the sea, fish larvae can probably optimise light conditions and prey contrast by diurnal vertical migration. Larval fish in a "shallow" intensive rearing tank do not have this option, and optimal larval feeding environment has to be determined for the different larval stages and species.

The objectives of the present study were to determine if use of microalgae (green water) and light intensity, within ranges commonly used by commercial producers of cod juveniles, had any effect on initiation of exogenous feeding, survival, growth, and nutritional status in cod larvae.

2. Material and methods

2.1. Larval material

An experiment investigating two variables (green water or clear water, at two different light intensities) was carried out twice (Exp-1 and Exp-2) with Atlantic cod larvae

(Gadus morhua) obtained from off-season-spawned eggs collected in late autumn 2001 at Institute of Marine Research, Austevoll Research Station (IMR-Austevoll), Norway. The experimental design is described in Table 1. The parental fish were three and four-year-old reared cod from IMR-Parisvannet (Blom et al., 1991), and spawning season was shifted as described by van der Meeren and Ivannikow (2006). Eggs were incubated in 70 l black polyethylene incubators at ambient temperature (7-8°C) and salinity (35 ‰) of the water intake at 160 m depth. The incubators were modified from van der Meeren and Lønøy (1998), with submerged inlet of 25 mm PVC tube with six 10 mm holes to ensure gentle water movement, and airflow directed towards the surface to prevent aggregation of eggs. Dead eggs were removed every day, and the amount of larvae was estimated by tube sampling in the incubators at transfer to the experimental tanks on day 3 after 100% hatching (dph). In Exp-1 and Exp-2, 17 800 and 17 500 cod larvae were transferred to each tank, respectively.

2.2. Rearing system

Twelve black 500 l polyethylene tanks, 1 m in diameter, were used for larval rearing. The tanks were equipped with a 250 µm outlet sieve placed in the centre of the tank. Gentle aeration was applied around the sieves from a plastic tube to prevent cod larvae and live feed to be collected on the plankton net. Each tank was equipped with a peripheral cover to shadow the tank wall (Harboe et al., 1998). An air skimmer was used to clean the water surface from any lipid film entering along with the live feed.

Light was applied 24 h a day by a luminaire placed 70 cm above each tank, with two 18W Osram Biolux 72 fluorescent light tubes which have a broad spectrum between 450 and 670 nm. In the reduced light intensity treatment, one of the light tubes was disconnected, and three layers of 50% neutral grey plastic film were placed inside the protective transparent plastic cover of the luminaire. To shadow the tanks from additional roof lights, sheets of 3-layer woven black PE-plastic impenetrable to light were surrounding triplicate tanks with similar treatment (Table 1). Light intensities were measured with a model IL 1400A light meter (International Light Inc., Peabody, MA, USA) equipped with a SUL033 silicon photodiode broadband underwater detector (400-1000 nm). Light intensities were calculated from integrated readings over 30 sec. Since the detector was calibrated for measurements in air, underwater readings at the tank bottom was calculated as a fraction of air readings at surface, corresponding to percent light attenuation from readings just below surface and at tank bottom.

Live algae (Isochrysis galbana, Tahitian strain) were used to provide green water. A 250 l tank with conical bottom and air bubbling was filled every day with algal solution produced by standard protocols at IMR-Austevoll. The algae were continuously pumped from this tank to the respective larval green water tanks by using Iwaki EH/S membrane pumps (Iwaki Co. Ltd., Tokyo, Japan). In Exp-1, turbidity was used as a proxy for algal density (Table 1). Turbidity, given as NTU (Nephelometric Turbidity Unit), was measured by a Hach 2100P turbidimeter (Hach Company, Loveland, CO, USA). Due to breakdown of the turbidimeter, algal density in Exp-2

was determined by a Coulter Counter Multisizer particle analyser (Beckman Coulter Inc, Fullerton, CA, USA) (Table 1). However, algal densities in the rearing tanks were considered to be within the same range in the two experiments, as the same algal culture dilution procedures were used, and no modifications were made either to the algal production and supply systems.

Water was pumped from 160 m depth in the fjord outside IMR-Austevoll, and then sand-filtered and aerated. Temperature in the tanks was kept between 11.0 and 11.7 °C by a heat pump system, after a gradual increase from egg incubation temperature to rearing temperature the first 4 days after larval transfer. To prevent gas supersaturation in the water, a separate column degasser was used for each larval tank. Flow was initially set to 0.5 l min^{-1}, and was increased to 1.0 l min^{-1} at 14 dph, with another increase to 1.5 l min^{-1} in Exp-2 at 32 dph.

2.3. Larval feed

The larvae were fed rotifers (Brachionus plicatilis) grown on Rotimac (BioMarine Aquafauna Inc., California, USA). Only rotifers were used as feed during the experimental period, and the prey were given as meals (batch feeding). Table 2 gives details of the feeding schedule, which was based on the experience gained from previous start-feeding trials with cod (van der Meeren and Ivannikov, 2006). Rotifers were added to attain a certain prey density at each feed batch, resembling 8250 prey l^{-1} at transfer of larvae to the tanks, at least 4000 prey l^{-1} during early larval stages, and more than 6000 prey l^{-1} in older larvae. However, due to shortage in production of

rotifers, this feeding schedule was not possible to keep in Exp-1. Compared to the original plan, both number of feed batches and amount of rotifers at each batch had to be reduced (Table 2), and Exp-1 had to be terminated on 22 dph. Exp-2 had fewer problems and was extended to 36 dph.

2.4. Sampling and measurements

2.4.1. Feeding incidence and gut fullness

To ensure enough time to allow feed intake in all larvae capable of initiation of exogenous feeding, cod larvae were sampled to investigate feeding incidence (FI) and gut fullness at 8 h after transfer to the rearing tanks. Between 22 and 44 larvae were sampled with a 100 ml beaker at various places of the tank, including both larvae at surface and larvae drifting upward in the currents created from the aeration along the sieve. The larvae were anesthetised with a 10 mg metomidate l^{-1} solution (Mattson and Riple, 1989), and the gut content was inspected in the relatively transparent larvae with a Leica MS5 stereo Microscope with both light and dark field options (Leica Microsystems GmbH, Wetzlar, Germany). FI was determined as the fraction of larvae that had rotifers in their gut. Intestinal filling was assessed among the larvae that had been eating by a simple gut filling index (GFI) modified from Huusko and Sutela, (1997) that gives score 1 for a few but easily countable number of rotifers (< 10), score 2 for a medium filled gut (more rotifers than could easily be counted but not extending the gut wall), and score 3 for a completely filled gut (packed with rotifers and

extending the walls of the digestive tract). The following formula was used to assign average values of GFI in a rearing tank:

$$GFI = (N_1+2N_2+3N_3)/(N_1+N_2+N_3)$$

where N_1 to N_3 is the number of larvae classified with gut fullness scores of 1 to 3, respectively.

2.4.2. Growth and survival

Dry weight (DW) of 30 larvae was determined at transfer to the rearing tanks, and for each tank also at experiment termination. The larvae were anesthetised with metomidate, rinsed in distilled water, and dried for 72 h in a Heto FD8 freeze drier (Heto-Holten AS, Allerød, Denmark). Weight was determined by a Mettler Toledo UMX2 (Mettler-Toledo Inc., Columbus, OH, USA). Specific growth rate (SGR) was calculated according to the exponential model given by Ricker (1958):

$$SGR = (e^g - 1) * 100\ \% \qquad \text{where} \qquad g = (\ln DW_t - \ln DW_0)/(t-t_0)$$

From each tank, the number of surviving larvae was counted at end of the experiment.

2.5. Lipid analyses

To assess potential effects of algae on biochemical composition of the larvae, 20 larvae were collected for lipid analysis from each tank at the end of the experiment. Dry weight of the samples was estimated from wet weights and a factor of 15% dry matter according to Finn et al. (2002) for cod larvae.

2.5.1. Extraction and fatty acid analyses

The larval samples were homogenized (3 x 30 s) and extracted according to Hara and Radin (1978) in 75 ml HIP (hexane - isopropanol; 3:2, v/v) by using an ultra-turrax (Janke & Kunkel, IKA-Werke GmbH, Staufen, Germany). Details are presented in Pickova et al. (1997). To remove non-lipids, 32.5 ml 6.67% Na_2SO_4 was added to the homogenate. The samples were shaken, centrifuged and evaporated. The dried samples were dissolved in chloroform and stored in –80 ˚C. Before fatty acid analyses the total lipids were separated into triacylglycerols (TAG) and phospholipids (PL) on thin-layer chromatography (TLC) silica coated plates 20x20 cm 60 F 254 (Merck KGaA, Darmstadt, Germany). An amount of 2 mg lipids in hexane were applied on the TLC plate. Each sample was placed on a 2 cm band. The lipid classes were separated by placing the plates in hexane - diethyl ether - acetic acid (85:15:1, v/v/v) solvent for one hour. TAG and PL were then scraped off for further analyses. PL were extracted sequentially in 3 ml chloroform - methanol (1:1, v/v), 2 ml chloroform - methanol (2:1, v/v) and 2 ml chloroform. TAG fraction was extracted three times in chloroform (3 ml, 2 ml and 2 ml). The lipid extracts were transferred to new tubes and the solvents were evaporated under nitrogen gas until dry. The dried PL and TAG samples were

disolved in 0.5 ml hexane and stored at –80 ˚C until methylation (Dutta and Appelqvist, 1989).

2.5.2. Preparation of fatty acids methyl esters (FAME)

Larval PL and TAG were converted to methyl esters according to Appelqvist (1968). To the total lipids 60 µg internal standard was added for quantification of fatty acids. First 2 ml 0.01 M NaOH in dry methanol was added to PL, TAG. The samples were shaken and heated for 10 min on a heating block at 60^{o}C. Next, 3 ml BF_3 reagent (boron trifluoride-methanol complex) was added and the samples were reheated for 10 minutes. Thereafter, the samples were cooled under running water and 2 ml 20% NaCI before 2 ml hexane was added. The test tubes were shaken vigorously and centrifuged for 5 min at 2500 rpm (440 rcf) and 18 ^{o}C. The FAME were transferred to small test tubes and evaporated under nitrogen gas. The dried samples were disolved in hexane and stored at –80^{o}C until GC-analyses.

2.5.3. Gas chromatography

FAME were analysed with a gas chromatograph CP3800 (Varian AB, Stockholm, Sweden) equipped with flame ionisation detector (FID) and split injector and fitted with a fused silica capillary column BPX 70 (SGE, Austin, TX, USA), length 50 m. id. 0.22 mm, 0.25 µm film thickness. The samples were injected by a CP8400 auto sampler (Varian AB), split mode. The split ratio 1:10 was used. Column temperature was programmed to start at 158°C hold 5 min and then increase 2°C min^{-1} from 158°C to 220°C and remain at 220°C for 8 min.. Injector and detector temperature was 230°C

and 250°C respectively. Identification of the fatty acids was done by comparing retention times of the chromatograms of the samples with chromatograms of the standard sample GLC-68 A (Nu-Check Prep Inc, Elysian, MN, USA). Peak areas were integrated using a Star chromatography workstation software version 5.5 (Varian AB). The carrier gas was helium (22 cm sec^{-1}, flow rate 0.8 ml min^{-1}). Make-up-gas was nitrogen.

2.5.4. Lipid class analyses

The composition of lipid classes of the total lipids was analysed with TLC. As a stationary phase, glass plates pre-coated with silica gel TLC plates (20 x 10 cm; Silica gel 60; 0.20 mm layer, Merck KGaA) were used. The analysis was performed according to Olsen and Hendersen (1989) with slight modifications. Prior to use, the plates were pre-developed to full length with hexane - diethyl ether - acetic acid (85:15:1, v/v/v) as mobile phase and dried for 5 min in 110°C. The upper 1 cm of the silica gel was removed and the plates were activated in 110°C for 1 h and stored in a vacuum exicator until further use. The lipid samples were diluted in hexane to a concentration of 1 μg μl^{-1}. Samples were applied on a TLC plate with a Camag TLC sampler 4 (Camag, Muttenz, Switzerland) in 2-mm bonds with an application speed of 250 nl sec^{-1}. Nitrogen was used as a spray gas. Each sample was applied 4 times on the plates. The distance between the bands was 10 mm. The lipids were then separated in a Twin Through Chamber 20 x 20 (Camag) using 25 ml hexane - diethyl ether - acetic acid (85:15:1, v/v/v) as mobile phase; the chamber saturation was increased by placing a piece of filter paper in the chamber. The plates were removed from the chamber

when they had been developed 6.9 cm from the base line thereafter they were air dried at room temperature and sprayed with a solution of 3% cupric acetate in 8% phosphoric acid and then charred for 20 min at 160°C. Plates were scanned with Camag TLC scanner 3 (Camag) to identify lipid classes. The scanning was performed at a speed of 20 mm sec^{-1} and a data resolution of 100 μm $step^{-1}$ with a slit dimension of 6 x 45 mm at a wavelength of 350 nm.The lipid classes were identified by comparison with an external standard (TLC 18-4A, Nu-Chek Prep Inc). For data filtering, the mode Savatisky-Golay 7 and manual baseline correlation were used.

2.6. Data analysis

Differences among treatments and experiments were analysed with factorial ANOVA (Statistica 6.1, Statsoft Inc, Tulsa, OK, USA), followed by Tukey HSD homogeneity post-hoc test for identification of groups among the treatments. The unintentional differences in food availability between the experiments (see section 2.3.) clearly indicated that experiment number was as an important source to variation. Experiment number was therefore included as the third factor of the ANOVA in addition to the treatments (light intensity and use of algae). Data within treatments and experiments were checked for normal distribution and homogeneity of variances before ANOVA analysis. Whenever data in the form of percentages were tested (with the exception of SGR that is not limited mathematically to between 0 and 100%), data were transformed with the arcsine transformation as suggested by Sokal and Rohlf (1995). Differences among means were considered statistically significant at $P < 0.05$.

3. Results

3.1 Initiation of exogenous feeding

Independent of experiment or treatment, the larval feeding incidence was high and ranged from 72.7 to 100% between individual rearing tanks (Fig. 1). In both experiments, low light intensity together with algae yielded the highest feeding incidences with least variation. Exp-2 seemed to have better feeding incidence in all treatments than Exp-1, and within each experiment, low light intensity appeared to elevate feeding incidence compared to high light intensity. However, there were no significant effects in either of clear vs. algal water, light intensity, or experiment on larval feeding incidence (lowest P-value was found for the light intensity treatment: $F_{[1,16]} = 4.224$, $P = 0.056$, indicating a tendency to higher feeding incidence at low light intensity).

The cod larvae in all groups exhibited high gut filling at 8 h after the first introduction of rotifer prey. The index of gut fullness (GFI) ranged from 1.72 to 2.47 among individual tanks, with mean values for the treatments between 1.88 and 2.36 (Fig. 1). Although differences in GFI between treatments and experiments were small, an overall significant improvement in gut filling was found by adding algae to the rearing water ($F_{[1,16]} = 5.667$, $P = 0.030$).

3.2 Survival and growth

Larval survivals at termination of the experiments were between 16.5 and 62.5% in individual tanks, with averages within treatments and experiments ranging between 17.5 and 56.3% (Fig. 2). Adding algae to the rearing water significantly improved survival ($F_{[1,16]} = 26.695$, $P = 0.00012$), particularly in Exp-2. However, although low light intensity had a tendency to better survival in both clear and algal water within each of the experiments, no significant effect was attributed to either light intensity or experiment.

Growth rate is given in Fig. 2, and SGR was observed to vary between 4.8 and 9.3% increase in weight per day of individual tanks, with averages of treatments between 6.8 and 8.7% per day. No significant effects of either algal vs. clear water or light intensity were found, but SGR was significantly higher in Exp-2 than in Exp-1 ($F_{[1,16]} = 36.428$, $P = 0.00003$). Since SGR was not affected by the experimental treatments, but survival was affected by addition of algae in the water, Fig. 3 shows correlations between SGR and survival, grouped into tanks with either clear or green water. Within this grouping, there was a consistent tendency to reduction of SGR at increasing survival, but only green water in Exp-2 showed a significant negative correlation between SGR and survival (one-tailed t-test, $P = 0.029$).

3.3 Lipid composition

Average total lipid (TL) content of the cod larvae among the treatments varied between 19.5 and 27.4% of larval dry weight (Table 3). Similarly, the fraction of PL was between 12.6 and 14.8%, while TAG constituted 1.4 to 7.0% of larval dry weight. Fractions of PL and TAG relative to TL were in the ranges of 48.9 to 64.6% and 7.1 to 27.2%, respectively (Table 3). Between the treatments, the TAG/PL ratio was between 10.9 and 18.4% in Exp-1, and considerably lower than the 27.8 to 54.3% found in Exp-2. Except for weight-specific TL content, all lipid indices above showed significant differences between the two experiments. No significant effects of light intensity could be detected on lipid composition, but addition of algae in the water diverged for several of the lipid composition indices, specifically PL relative to TL and those indices containing TAG (see Table 3).

In Table 4 and 5, the larval composition of selected fatty acids is presented for PL and TAG, respectively. The displayed fatty acids were selected because they are the most abundant fatty acids in Isochrysis sp. (Reitan et al., 1993; Muller-Feuga et al., 2003a; Pernet et al., 2003). Also the most important essential PUFAs in fish larvae were included in Table 4 and 5, along with their ratios. In general, variation in fatty acid composition was less in PL compared to TAG. PUFAs showed highest levels in PL (56.1-55.8%) with DHA being most the abundant fatty acid (32.2-37.1%). In TAG, the DHA levels varied between 8.7 and 20.3%, and PUFAs between 32.5 and 56.7%, with the highest levels observed in Exp-2. The DHA/EPA ratio was lowest in TAG (1.8-3.0) compared to PL (6.8-9.5). In contrast, the EPA/ARA ratio peaked in the TAG

(2.0-5.8), while this ratio was between 0.7 and 0.8 in PL. The TAG fraction of the lipids contained more EPA than the PL fraction.

Whenever significant differences in the fatty acids occurred relative to treatments, they were always associated with algae vs. clear water, and only found in PL (Table 4, 5). Light intensity had no effect on fatty acid composition, except for DHA in the TAG fraction. Significant differences in the fatty acid profiles were also observed between the two experiments, in particular for TAG, but also for PL. Further, in TAG the variation among replicates was in general lowest in Exp-2.

4. Discussion

The most notable effect of adding algae to the rearing water of cod larvae was increased survival. Although not as apparent as improved survival, increased prey ingestion at initiation of exogenous feeding was also documented with use of algae. These effects were consistent in both experiments, but most prominent in Exp-2, which had best feeding conditions. The results are in concordance with a number of studies on different larval fish species reared in green vs. clear water (Jones, 1970; Naas et al. 1992; Reitan et al. 1993; Tamaru et al., 1994; Cahu et al. 1998; Bengtson et al., 1999; Papandroulakis et al., 2002; Baskerville-Bridges et al., 2004; Shaw et al., 2006). However, the positive effects of algae on larval feeding may be species specific and depend on physical factors like algal concentration and light intensity

(Baskerville-Bridges et al., 2004; Carton, 2005). Within the light intensities and algal concentrations used in the present study, addition of algae to the rearing water represented a clear benefit for the cod larvae. In Exp-1, the restricted feeding (once a day last 5 days before cessation of the experiment) seems to have exhausted the storage lipids, causing low condition in terms of reduced fractions of TAG relative to dry weight and total lipids, lower TAG/PL ratio, as well as the changes in proportions of various fatty acids. Regarding the latter, the changes in fatty acid fractions between the two experiments seem to be more prominent for TAG than for PL. Since TAG is a storage lipid, such variability in condition is not unexpected and have been observed in various species (Frazer et al., 1988; Håkanson,, 1989; Mourente and Vazquez, 1996). This suggests that the membrane-bound PL composition is predetermined more accurately, and being of large importance in many biological processes (Bell et al., 2003).

The use of microalgae in rearing of marine fish larvae has extensively been reviewed by Muller-Feuga et al. (2003b). Algae can have effects on the visual feeding environment of fish larvae and change turbidity and light conditions in a way that improves larval prey ingestion (Naas et al., 1992; 1996). Such modulation of larval feeding incidence and intensity has even been shown with the use of inert particles resembling the size of microalgae (Boelert and Morgan, 1985). Although Skiftesvik et al. (2003) failed to demonstrate any effects of green water on behaviour in cod larvae at 5-days post-hatch, the present results indicate that food ingestion increases in presence of algae. Cod larvae have previously been found to change swimming

activity in presence of algae, and selectively concentrate and ingest algae directly based on size (van der Meeren, 1991). However, Isochrysis is too small for the latter to occur, and probably did no have any significance on larval nutrition in the present study. The higher gut filling when algae were present at initiation of exogenous feeding therefore suggests some effects of algae on feeding intensity in young cod larvae. Feeding intensity was not recorded at older larval stages, and possible changes in larval prey ingestion rate along the course of ontogenesis could therefore not be evaluated for algal vs. clear water. Nor was any overall effect of potentially elevated feeding levels detected in larval growth rates. But when considering the possible effect of survival inducing density-dependent growth within the algae vs. clear water treatment, a slightly improved SGR appeared when algae were present in each of the experiments (Fig. 3). The relative moderate SGR levels compared to growth rates of cod larvae reared in nature-like systems (Blom et al., 1991; van der Meeren and Jørstad, 2001; Finn et al., 2002), along with possible density-dependent growth suggests that feeding protocols, including rotifer quality and quantity, can still be improved.

A significant nutritional signal of algal addition was found both in the lipid class distribution and fatty acid composition. The contribution of TAG to both larval dry weight and total lipid content was less in cod larvae reared in presence of algae. Both percentage of DHA and the DHA/EPA ratio of phospholipids were higher when algae were added, and Isochysis is known to be rich in DHA (Reitan et al., 1993; Muller-Feuga et al., 2003a; Pernet et al., 2003). Phospholipids are structural lipids that are

important for cell membrane functions. Fish larvae may have a certain need or an absolute requirement for dietary phospholipids during early development, due to lack of a fully functional digestive tract and their inability to synthesise phospholipids de novo (reviewed by Bell et al., 2003). Further, in contrast to Artemia sp., phospholipids from rotifers or copepods contain high levels of strongly bioactive polyunsaturated fatty acids (in particular the essential fatty acid DHA), and these may be incorporated directly into the larval cell membranes (Bell et al., 2003). Presence of algae facilitates the onset of hydrolytic functions of brush border cell membranes in European sea bass larvae, and triggers digestive enzyme production (Cahu et al., 1998). The indirect enhancement of larval nutrition by algal modulation of rotifer biochemical composition should therefore not be overlooked.

It was observed that the rotifers used in the present study turned green inside their gut when algae were added to the water, resulting in green material in the gut of the cod larvae. This was not found in the clear water groups. Since the lipids of Isochrysis are relatively rich in phospholipids and other polar lipids (Pernet et al., 2003), use of this alga for green water may therefore have been beneficial to the nutrition of the cod larvae, and thereby contributing to the enhanced survival. Enrichment of live prey usually implies use of neutral lipids like TAG, and microalgae may substitute the rotifer enrichment with more phospholipids, which in turn may reduce TAG in the fish larvae as observed in the present experiments.

The importance of dietary essential fatty acids provided by certain algae added to the rearing water, and further transfer to fish larvae through their prey, has previously been confirmed for larval survival and growth in several studies (Howell, 1979; Scott and Middleton, 1979; Reitan et al., 1993; 1997). Fish larvae may also achieve other essential nutrients this way because algae contain a vast number of highly bioactive compounds like amino acids, vitamins, pigments (including antioxidants), and possibly also minerals. Specific uptake of auto-fluorescent molecules in the larval hindgut, resembling algal pigments, supports this (Kjørsvik et al., 1991; Tytler et al., 1997). Use of algae in the rearing water may be considered as short-term enrichment of the live prey, and can alter both protein and lipid content of the larval food (reviewed by Reitan et al., 1997). Algae have also been considered as a stabilising factor on water quality, particularly with respect to bacterial composition and levels (reviewed by Muller-Feuga et al., 2003b).

Light intensity, within the ranges used in the present study, did not seem to affect growth or survival, although there was a tendency to better survival at the lowest light intensity. This contrasts the improved survival and growth found for cod larvae by Puvanendran and Brown (2002), although they extended the light intensity up to 2400 lux where the cod larvae performed the best. However, Puvanendran and Brown (1998) also showed that inter-population differences in optimal light intensity might occur in Atlantic cod. This calls for more investigations on genetic adaptations to environmental conditions (e.g. light optimum for larval feeding) throughout the distribution area of Atlantic cod. Further, ontogenetic changes in optimal light

intensity preferences may be possible (Monk et al., 2006). In comparisons of optimal light intensity between other larval fish species, the results are diverging (Barahona-Fernandes, 1979; Tandler and Mason, 1983; Batty, 1987; Daniels et al., 1996; Denson and Smith, 1997; Downing and Litvak, 1999a; Peña et al., 2004; Carton, 2005; Copeland and Watanabe, 2006), reflecting inter-specific differences in larval ecology, or variations in the success of matching rearing protocols with optimal feeding environments required by larval feeding ecology and development.

In intensive larval rearing, prey distribution and availability are much less forced by light intensity than in the natural environment of cod larvae where the larvae may find optimal feeding conditions in relation to diurnal vertical migrations of prey. Thus, larval feeding during intensive rearing, with subsequent growth and survival, may only to a small extent be dependent on what the larva perceives as optimal light intensity. Other factors of importance may be tank size, tank colour, turbidity, food quality, and prey densities. The influence of prey availability is very well illustrated by the differences in growth and survival observed between Exp-1 and Exp-2 of the present study. Similarly, differences in rearing system or protocols may mask the effect of light intensity when compared across different experiments, or between different species. Cod larvae can feed in light intensities from several thousands lux found at surface water on a clear sunny day, and down to 0.1 lux (Ellertsen et al., 1980). The present study does not indicate that intermediate light intensities in the range of 20-280 $\mu W\ cm^{-2}$ (roughly corresponding to 50-690 lux) do affect the cod larvae in a negative way. This suggests that cod larvae can manage well a broad range of light intensities,

commonly used by producers of cod juveniles today. However, it may be possible that suboptimal light conditions can add to chronic stress induced by other suboptimal factors, and further research should be carried out to clarify this. Considering the differences observed in light intensity preference between different cod populations (Puvanendran and Brown, 1998), similar experiments should be carried out to determine dissimilarities in preferences of light intensity among genetically distinct populations used in aquaculture.

5. Conclusion

Use of algae enhanced larval survival and supported initial larval feeding in Atlantic cod by increasing prey ingestion. However, it cannot be inferred that improved ingestion persisted and enhanced growth throughout the larval stages, although algae had a tendency to elevate growth rates slightly when the density-dependent effect of larval survival was considered. Another issue for larval rearing success is the importance of sufficient food supply, which has previously been reported for this species (van der Meeren and Ivannikov, 2006). But more essential, an algal effect on larval lipid composition was demonstrated, indicating the importance of the indirect nutritional benefits algae may have through the larval prey. The findings suggest to use green water in larval rearing of cod, and to carefully select algal species that would enhance lipid composition of the larvae. Effects of algal density were not addressed, and should acquire attention in future studies. Recently, requirement for labour-saving

procedures have made it more common to use algal paste instead of live algae in larval rearing. Thus, studies on paste vs. live algae should be initiated to clarify if paste exhibit similar benefits as live algae. Regarding light, the results suggest that larval survival and growth are sustained over a wide range of light intensities below what was previously believed to be optimal for cod (Puvanendran and Brown, 2002). In further optimising larval rearing protocols, this calls for more studies on effects of light, including combined effects of light and algal densities.

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Table 1.
Details of the experimental treatments and layout.

		Turbidity		Light intensity (µW cm^{-2})	
Tank no.	Water	Exp-1 (NTU)	Exp-2 (particles[d] μl^{-1})	Surface[a]	Bottom[b]
1 to 3	green[c]	1.31 – 2.07	66 - 184	259.3 – 283.0	55.6 – 61.1
4 to 6	clear	0.32 – 0.80	3 - 6	240.3 – 254.0	78.2 – 88.2
7 to 9	green[c]	1.26 – 2.30	81 - 199	11.7 – 20.2	2.0 – 3.9
10 to 12	clear	0.31 – 0.89	3 - 6	18.0 – 20.1	5.6 – 5.2

[a] Measured midway between the peripheral cover and the outlet sieve, perpendicular to the length axis of the light armature.

[b] Measured on the bottom vertical to the surface measurement.

[c] Isochrysis galbana (Tahitian).

[d] Particles > 3 µm diameter (average size among counts: 3.86 – 4.13 µm in clear water and 4.28 – 4.69 µm in green water).

Table 2.
Details of the daily feeding schedule for each rearing tank for Exp-1and Exp-2. Added food density represents the increase in prey density in a tank when a feed batch is added.

Larval age (Days post-hatch)	Rotifers (mill day^{-1})	No. of feed batches (N day^{-1})	Added food density (prey l^{-1} $batch^{-1}$)
Exp-1			
3	3.3	1	8250
4	1.7	1	4125
5-8	3.3	2	4125
9-13	5.0	3	4125
14-16	6.6	2	5500
17-21	2.6	2	3610
Exp-2			
3	3.3	1	8250
4-6	3.3	3	2750
7-11	4.1	3	3450
12-14	3.5	3	2900
15	5.6	3	4658
16-35	6.8	3	6225

Table 3.
Total lipid content (TL) and lipid classes (PL: phospholipids, TAG: triacylglycerols) in relation to larval dry weight (DW) in Atlantic cod at the experiment terminations (average of three replicate tanks ± SD). Data are shown for the different treatments in the two experiments, along with significant effects calculated from ANOVA analysis.

	Experiment 1				Experiment 2						
Water:	Algae		Clear		Algae		Clear		P values:		
Light:	High	Low	High	Low	High	Low	High	Low	Water	Light	Experiment
DW (mg)	0.26 ±0.02	0.28 ±0.01	0.28 ±0.03	0.22 ±0.04	0.67 ±0.19	0.53 ±0.02	0.76 ±0.01	0.70 ±0.03	n.s.	n.s.	< 0.001
TL/DW (%)	20.6 ±4.0	19.5 ±3.2	23.2 ±5.8	21.2 ±1.5	24.3 ±3.1	24.1 ±1.2	27.4 ±3.7	25.7 ±1.1	n.s.	n.s.	< 0.05
PL/DW (%)	13.0 ±2.3	12.6 ±2.0	14.8 ±4.7	13.1 ±0.2	14.6 ±2.1	12.9 ±0.1	13.4 ±2.0	12.8 ±0.4	n.s.	n.s.	n.s.
TAG/DW (%)	1.6 ±0.2	1.4 ±0.2	2.5 ±0.2	1.9 ±0.4	4.0 ±0.5	5.3 ±0.9	7.0 ±0.1	7.0 ±0.6	< 0.001	n.s.	< 0.001
PL/TL (%)	63.3 ±1.7	64.6 ±1.8	63.0 ±5.2	62.3 ±4.0	59.8 ±1.9	53.8 ±3.3	48.9 ±0.8	50.0 ±1.1	< 0.001	n.s.	< 0.001
TAG/TL (%)	8.1 ±1.6	7.1 ±0.2	11.4 ±3.0	9.0 ±1.7	16.6 ±2.7	22.0 ±2.6	25.9 ±3.0	27.2 ±1.3	< 0.001	n.s.	< 0.001
TAG/PL (%)	12.8 ±2.5	10.9 ±0.1	18.4 ±6.6	14.6 ±3.1	27.8 ±5.4	41.0 ±7.3	53.1 ±6.9	54.3 ±3.3	< 0.001	n.s.	< 0.001

n.s.: not significant

Table 4.
Selected fatty acids (%) of total phospholipids (PL) in Atlantic cod larvae at the experiment terminations (average of three replicate tanks ± SD). Data are shown for the different treatments in the two experiments, along with significant effects calculated from ANOVA analysis.

	Experiment 1				Experiment 2						
Water:	Algae		Clear		Algae		Clear		P values:		
Light:	High	Low	High	Low	High	Low	High	Low	Water	Light	Experiment
14:0	0.5 ±0.1	0.7 ±0.2	0.8 ±0.02	0.9 ±0.01	0.8 ±0.03	0.8 ±0.05	1.1 ±0.4	0.8 ±0.01	< 0.01	n.s.	< 0.01
16:0	13.8 ±0.6	15.6 ±1.0	16.5 ±0.8	17.2 ±1.0	15.5 ±0.7	15.2 ±0.2	16.1 ±2.0	15.2 ±0.5	< 0.05	n.s.	n.s.
16:1 n-7	0.3 ±0.02	0.2 ±0.2	0.1 ±0.01	0.3 ±0.3	1.4 ±0.1	1.3 ±0.01	1.3 ±0.1	1.4 ±0.04	n.s.	n.s.	< 0.001
18:4 n-3	0.2 ±0.05	0.1 ±0.1	0.1 ±0.1	0.7 ±0.7	0.3 ±0.1	0.3 ±0.05	1.4 ±2.0	0.2 ±0.01	n.s.	n.s.	n.s.
EPA	3.9 ±0.1	4.1 ±0.2	4.3 ±0.1	4.0 ±0.2	3.9 ±0.2	3.9 ±0.1	5.0 ±1.6	4.2 ±0.1	< 0.05	n.s.	n.s.
DHA	36.8 ±0.5	37.1 ±1.7	34.1 ±2.1	34.2 ±2.1	34.3 ±0.9	33.9 ±0.3	32.2 ±0.3	33.6 ±0.6	< 0.01	n.s.	< 0.01
DHA/EPA	9.5 ±0.2	9.0 ±0.3	7.9 ±0.6	8.5 ±1.0	8.7 ±0.6	8.7 ±0.3	6.8 ±2.3	8.0 ±0.3	< 0.01	n.s.	n.s.
EPA/ARA	0.7 ±0.02	0.7 ±0.04	0.8 ±0.04	0.8 ±0.1	0.7 ±0.1	0.7 ±0.03	0.9 ±0.3	0.7 ±0.01	< 0.05	n.s.	n.s.
n-3/n-6	3.1 ±0.1	3.8 ±1.3	2.9 ±0.2	3.0 ±0.2	2.7 ±0.04	2.6 ±0.02	2.9 ±0.1	2.6 ±0.03	n.s.	n.s.	< 0.05
SAFA	25.6 ±0.5	27.4 ±1.4	27.9 ±1.2	28.2 ±0.1	25.6 ±0.3	25.5 ±0.2	27.7 ±4.1	25.5 ±0.2	< 0.05	n.s.	n.s.
MUFA	15.7 ±1.2	15.3 ±0.3	15.5 ±0.4	15.7 ±2.4	16.9 ±0.3	17.3 ±0.3	15.8 ±0.4	16.8 ±0.2	n.s.	n.s.	< 0.05
n-3 PUFA	44.4 ±0.6	44.8 ±1.9	42.2 ±1.8	42.2 ±2.6	41.8 ±0.6	41.3 ±0.01	42.1 ±3.0	41.8 ±0.3	n.s.	n.s.	< 0.05
n-6 PUFA	14.4 ±0.3	12.6 ±3.1	14.4 ±0.3	13.9 ±0.04	15.7 ±0.1	15.9 ±0.1	14.4 ±0.7	15.9 ±0.1	n.s.	n.s.	< 0.05
PUFA	58.8 ±0.9	57.3 ±1.5	56.6 ±1.6	56.1 ±2.6	57.5 ±0.5	57.2 ±0.1	56.5 ±3.7	57.7 ±0.3	n.s.	n.s.	n.s.

Abbreviations: EPA: eicosapentaenoic acid (20:5 n-3), DHA: docosahexaenoic acid (22:6 n-3), ARA: arachidonic acid (20:4 n-6), SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n.s.: not significant.

Table 5.
Selected fatty acids (%) of total triacylglycerols (TAG) in Atlantic cod larvae at the experiment terminations (average of three replicate tanks ± SD). Data are shown for the different treatments, along with significant effects calculated from ANOVA analysis.

	Experiment 1				Experiment 2						
Water:	Algae		Clear		Algae		Clear		P values:		
Light:	High	Low	High	Low	High	Low	High	Low	Water	Light	Experiment
14:0	1.8 ±0.5	3.4 ±1.6	2.8 ±1.1	3.1 ±1.3	2.7 ±0.3	2.8 ±0.02	2.7 ±0.4	2.7 ±0.3	n.s.	n.s.	n.s.
16:0	17.8 ±4.6	21.2 ±7.5	20.1 ±11.1	13.3 ±0.3	11.0 ±2.8	9.9 ±0.9	11.6 ±0.7	9.9 ±0.6	n.s.	n.s.	< 0.01
16:1 n-7	4.6 ±4.0	2.3 ±0.9	2.6 ±1.1	2.4 ±0.5	3.5 ±0.8	3.7 ±0.1	3.7 ±0.1	4.2 ±0.1	n.s.	n.s.	n.s.
18:4 n-3	1.4 ±0.6	0.3 ±0.5	0.6 ±0.01	0.5 ±0.6	1.0 ±1.1	1.6 ±0.1	0.5 ±0.8	1.0 ±0.3	n.s.	n.s.	n.s.
EPA	7.0 ±5.4	6.5 ±3.2	5.4 ±2.8	13.2 ±11.0	8.1 ±1.3	8.5 ±0.2	7.7 ±0.2	9.2 ±0.2	n.s.	n.s.	n.s.
DHA	8.7 ±3.2	13.4 ±2.3	15.3 ±5.3	15.1 ±2.1	17.9 ±1.7	20.3 ±0.7	15.7 ±0.4	18.7 ±0.7	n.s.	< 0.05	< 0.001
DHA/EPA	1.8 ±1.2	2.5 ±1.2	3.0 ±0.6	1.8 ±1.7	2.2 ±0.2	2.4 ±0.1	2.0 ±0.01	2.0 ±0.1	n.s.	n.s.	n.s.
EPA/ARA	2.2 ±1.1	2.9 ±1.7	2.0 ±0.1	5.8 ±5.3	2.0 ±0.1	2.1 ±0.1	2.1 ±0.02	2.1 ±0.1	n.s.	n.s.	n.s.
n-3/n-6	1.6 ±0.9	1.6 ±0.1	1.8 ±0.1	2.3 ±1.3	1.5 ±0.2	1.8 ±0.04	1.5 ±0.2	1.7 ±0.1	n.s.	n.s.	n.s.
SAFA	37.7 ±12.9	38.4 ±10.3	37.8 ±18.1	30.2 ±1.7	20.8 ±4.7	19.1 ±0.3	21.3 ±1.3	17.4 ±1.9	n.s.	n.s.	< 0.001
MUFA	29.9 ±6.9	23.1 ±4.4	24.5 ±1.7	21.1 ±7.1	26.1 ±0.3	24.2 ±1.8	27.5 ±0.9	27.9 ±1.2	n.s.	n.s.	n.s.
n-3 PUFA	19.5 ±10.0	23.7 ±3.4	24.1 ±10.1	33.3 ±9.6	31.9 ±4.3	36.3 ±1.1	30.4 ±0.3	34.8 ±0.9	n.s.	n.s.	< 0.01
n-6 PUFA	12.9 ±1.6	14.8 ±2.6	13.6 ±6.3	15.4 ±4.2	21.2 ±1.1	20.4 ±1.0	20.8 ±2.4	19.9 ±0.8	n.s.	n.s.	< 0.001
PUFA	32.5 ±9.6	38.5 ±5.9	37.7 ±16.4	48.7 ±5.4	53.0 ±4.7	56.7 ±2.1	51.2 ±2.1	54.7 ±1.3	n.s.	n.s.	< 0.001

Abbreviations: see Table 4.

Figure legends:

Figure 1. Larval prey ingestion at initiation of exogenous feeding in Atlantic cod. Feeding incidence (left panel) and gut fullness index (right panel) are given for each experiment as average of replicate tanks within each treatment (algae vs. clear water and light intensity). Error bars are standard deviation. Bars with same letter are not statistically significantly different (Tukey post-hoc test). Note the discontinuous ordinate for feeding incidence.

Figure 2. Larval survival (left panel) and growth in terms of SGR (right panel) in Atlantic cod, given for each experiment as average of replicate tanks within each treatment (algae vs. clear water and light intensity). Error bars are standard deviation. Bars with same letter are not statistically significantly different (Tukey post-hoc test). Note the discontinuous ordinate for SGR.

Figure 3. Correlations between larval SGR and survival in Atlantic cod, grouped by presence of algae or clear water in the two experiments. Note the discontinuous ordinate.

Figure 1.

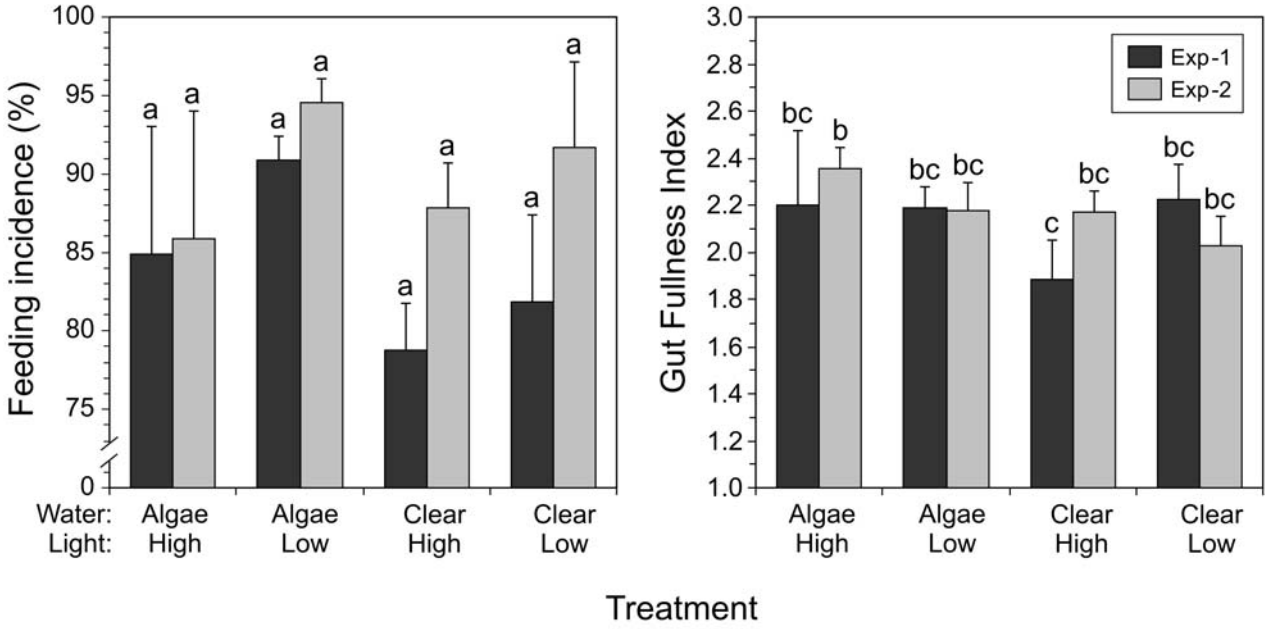


Figure 2.

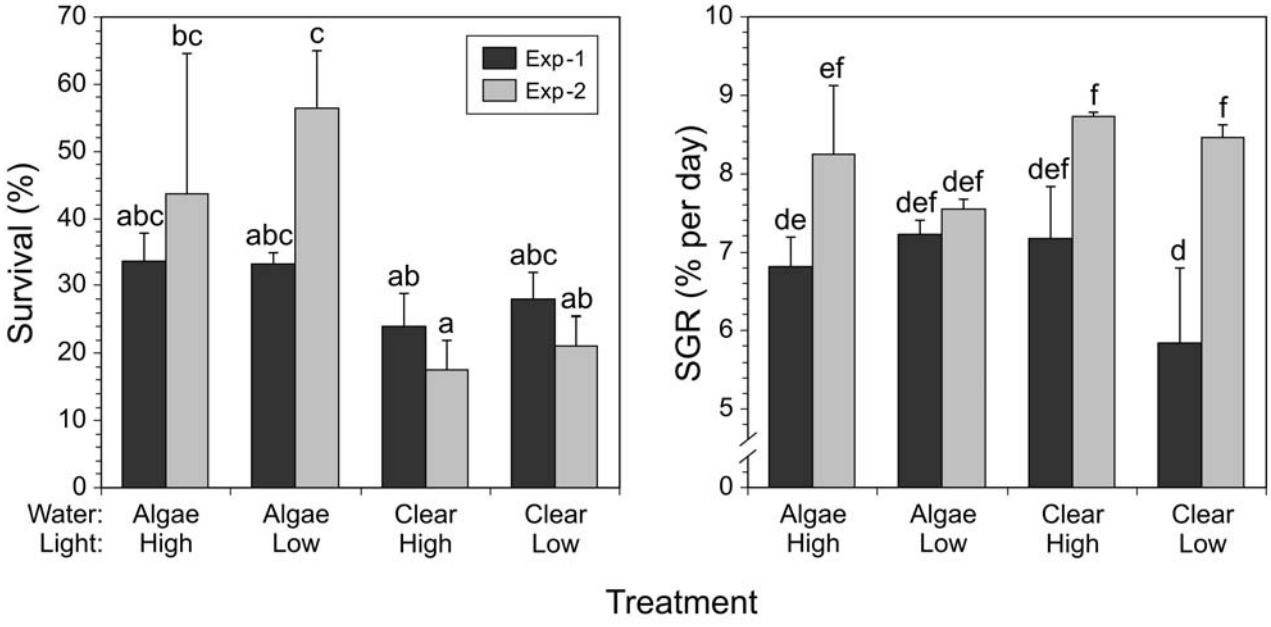
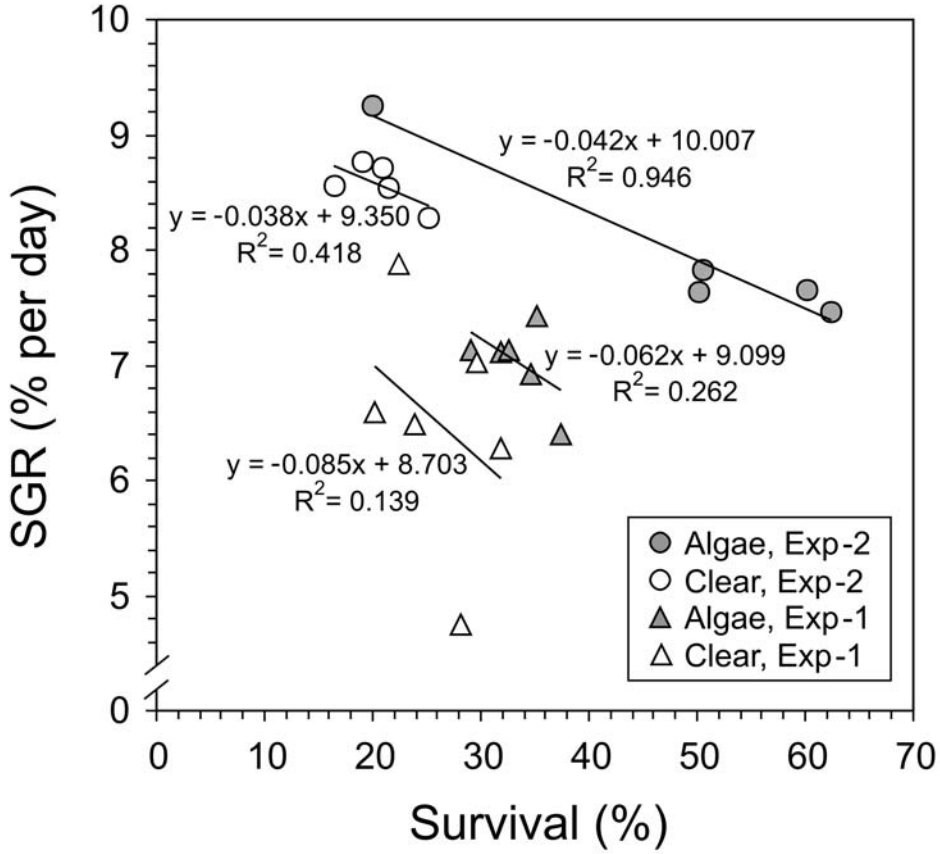


Figure 3.


y = -0.042x + 10.007
R2= 0.946
y = -0.038x + 9.350
R2= 0.418
y = -0.062x + 9.099
R2= 0.262
y = -0.085x + 8.703
R2= 0.139
Algae, Exp-2
Clear, Exp-2
Algae, Exp-1
Clear, Exp-1
SGR (% per day)
Survival (%)
0
5
6
7
8
9
10
0
10
20
30
40
50
60
70