Life in green water: the effect of microalgae on the behaviour of Atlantic cod (*Gadus morhua*) larvae

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Abstract

In the intensive culture of marine fish larvae, microalgae is often added to the water along with microzooplankton or microparticulate food (the "green water" technique). Although using green water generally improves the survival and growth rate of larvae, the mechanism(s) through which the microalgae act to generate this effect remains unclear. We tested the hypothesis that the presence of microalgae in the culture environment affects the behaviour of 5 day post hatch Atlantic cod (*Gadus morhua*) larvae. Using silhouette video photography, the behaviour of cod larvae was observed (i) with rotifers (*Brachionus sp.* at 7 ml^{-1}) but no algae, and (ii) with rotifers (7 ml^{-1}) and 100,000 *Nannochloropsis sp.* cells ml^{-1}. Light intensities (and spectra) in the experiments were similar to those used in culture systems and were not measurably different amongst the treatments. The following variables were extracted from the observations of cod swim paths: durations of pauses (non-active displacement), lengths and durations of moves, and turn angles (decomposed into vertical and horizontal components). These components of the swim pattern were compared to determine if the presence of algae affected the behaviour of cod larvae, and in what manner. There were no significant/consistent differences amongst treatments. The results indicate that the effect of green water on the behaviour of cod larvae is subtle and that any improvement in growth and/or survivorship associated with green water is most likely a result of the indirect effects that microalgae might have on the nutritional quality of their food, and/or on larval physiology.

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Introduction

In the intensive culture of marine fish larvae, microalgae is often added to the water along with microzooplankton or microparticulate food (Reitan et al. 1997; Muller-Feuga 2000; Lee and Ostrowski 2001; Shields 2001). This is commonly referred to as the "green water" technique. Although using green water generally improves the survival and growth rate of larvae (e.g. Naas et al. 1992; Bengston et al. 1999), the mechanism(s) through which the microalgae act to generate this effect remains unclear. Evidence in support of several possibilities has been reported, including improvement of digestive functions (e.g. Cahu et al. 1998; Lazo et al. 2000), enhanced nutritional value of prey (because they feed on the microalgae before being eaten themselves)(e.g. Silva 1999), increased assimilation efficiency (e.g. Papandroulakis et al. 2002), and higher prey contrast (through the effect of the algae on the underwater light field) (e.g. Cobcroft et al. 2001).

Although the effect of microalgae on feeding incidence and intensity has been studied, the manner in which algal cells might alter the behaviour of marine fish larvae has not been thoroughly characterized. Thus, we tested the hypothesis that the presence of microalgae in the culture environment affects the behaviour of Atlantic cod (*Gadus morhua*) larvae.

Materials and Methods

Source of cod larvae. Larvae used in these experiments were obtained from a single spawning event between one male and one female cod (B08-03 in Table 1 of Browman et al. 2003). Females were paired with males in 1.2 m^3 circular tanks, allowing for every batch of eggs released by females to be collected individually. Complete descriptions of the brood stock, holding conditions, and methods of egg collection and incubation, are presented in Lambert and Dutil (2000) and in Ouellet et al. (2001). Eggs were incubated in black 60 l round-bottom tanks (at 6 ± 1.0 °C). Larvae were transferred to fresh 60 l black tanks at 3 days post hatch (DPH) (just prior to first exogenous feeding). The rearing basins were stocked with algae (*Nannochloropsis sp.*) at 100 000 cells l^{-1} (green water technique), and larvae were fed nutritionally enriched (INVE Aquaculture Super Selco®) rotifers (*Brachionus sp.*) at 7 ml^{-1}. Larvae were cultured at 6 °C on a 14 h L:10 h D photoperiod at a crepuscular-level light intensity of 1.20 μE sec^{-1} m^{-2} (diffuse light from overhead fluorescent lamps). The same light intensity (and light source) was used in the behavioural experiments.

Experiments on larval behaviour. Silhouette (shadow) video photography (SVP) was used to observe the behaviour of cod larvae. The physical characteristics of the system, its advantages, and details of the software used to extract the data, are presented elsewhere in this book (Browman et al. 2003). Larvae were tested at 5 DPH, two days after the initiation of exogenous feeding. Fourty larvae were placed into a 20 x 20 x 20 cm test aquarium and video taped for 30 min. in water (taken from the culture tank from which they were sampled) which had been vacuum-filtered through a 5 μm mesh sieve and to which rotifers (at 7 ml^{-1}) had been added. Next, algae were added to the aquarium (at 100,000 cells ml^{-1}) and the larvae were observed for another 30 min. Upon completion of this

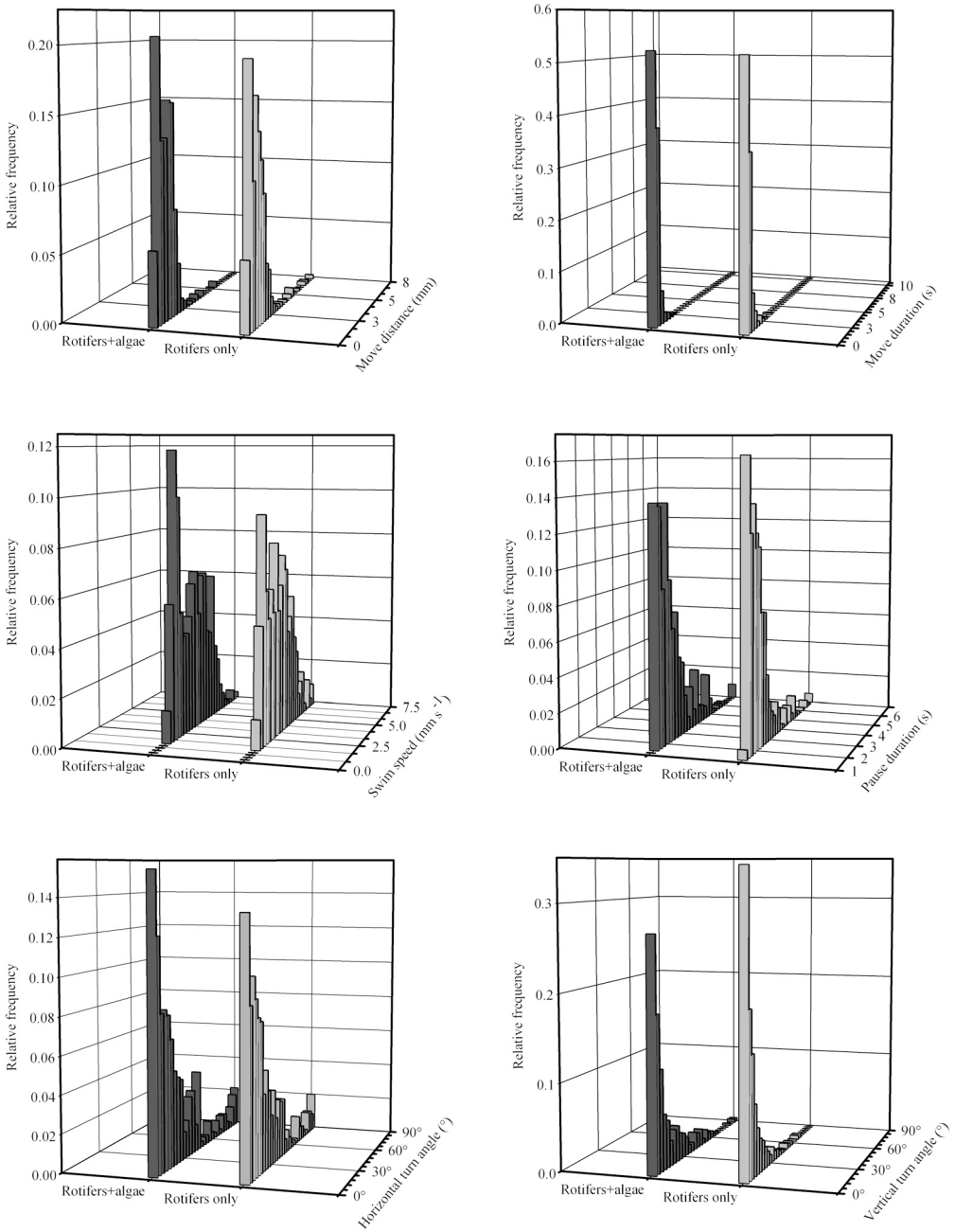

Figure 1. *Frequency distributions of behavioural variables (both replicates combined) measured on 5 day post hatch Atlantic cod (*Gadus morhua*) larvae feeding in the presence of rotifers only (*Brachionus sp.* at 7 ml^{-1}), and with rotifers and algae (*Nannochloropsis sp.* at 100 000 cells ml^{-1}).*

Table 1a. *Mean (±SE) values for each of the behavioural variables measured on 5 day post hatch Atlantic cod (*Gadus morhua*) larvae feeding in the presence of rotifers (*Brachionus sp. *at 7 ml^{-1}) only, and rotifers with algae (*Nannochloropsis sp. *at 100 000 cells ml^{-1}). Values for the two replicates (Rep) are presented seperately.*

Behavioural variable	Rep	N	Rotifers only	N	Rotifers+algae
Move distance (mm)	1	98	4.9 ± 0.2	298	3.8 ± 0.1
	2	122	3.7 ± 0.2	86	4.4 ± 0.3
Move duration (s)	1	98	0.6 ± 0.04	298	0.5 ± 0.02
	2	122	0.5 ± 0.02	86	0.5 ± 0.02
Swim speed (mm s^{-1})	1	98	8.3 ± 0.3	298	7.3 ± 0.2
	2	122	7.3 ± 0.2	86	8.0 ± 0.3
Horizontal turn angle (°)	1	87	41.1 ± 3.9	274	48.1 ±2.6
	2	110	40.1 ± 3.6	76	38.3 ± 5.6
Vertical turn angle (°)	1	87	14.4 ± 1.9	274	26.5 ± 1.8
	2	110	18.9 ± 2.1	76	19.2 ± 2.7
Pause duration (s)	1	91	1.2 ± 0.1	286	1.8 ± 0.1
	2	118	2.1 ± 0.2	79	1.8 ± 0.2

Table 1b. *Results of Kolmogorov-Smirnov two-sample tests evaluating for between-treatment differences in the behavioural variables measured on 5 day post hatch Atlantic cod (*Gadus morhua*) larvae feeding in the presence of rotifers (*Brachionus sp. *at 7 ml^{-1}) only, and rotifers with algae (*Nannochloropsis sp. *At 100 000 cells ml^{-1}).*

Behavioural variable tested	Kolmogorov-Smirnov statistical parameters	Rotifers only vs. rotifers and algae
Move distance	D	0.07
	$K_{0.05}$	1.36
	$D_{0.05}$	0.09
	Decision	Same
Move duration	D	0.05
	$K_{0.05}$	1.36
	$D_{0.05}$	0.09
	Decision	Same
Swim speed	D	0.07
	$K_{0.05}$	1.36
	$D_{0.05}$	0.09
	Decision	Same
Horizontal turn angle	D	0.05
	$K_{0.05}$	1.36
	$D_{0.05}$	0.11
	Decision	Same
Vertical turn angle	D	0.13
	$K_{0.05}$	1.36
	$D_{0.05}$	0.11
	Decision	Different
Pause duration	D	0.0848
	$K_{0.05}$	1.3581
	$D_{0.05}$	0.0937
	Decision	Same

sequence, a new group of 40 larvae were sampled (from the same culture tank), placed into a second test aquarium, and the same sequence was repeated. Thus, there were two replicates for each treatment.

The behaviour of cod larvae was evaluated by analysing a 5 min. segment of video images drawn from the middle of each treatment's 30 min. observation period (but never near the transition from one treatment to the next). Due to the nature of the imaging system, the behavioural observations cannot be attributed to any single larva - they swim in and out of the field of view and, therefore, it is impossible to tell one larva from another. Further, observations on any one larva in a tank containing 40 are not statistically independent and, therefore, all observations made on the 40 larvae in any given tank collapse down to a sample size of one. Thus, there were two replicate 30 min. observation periods for each treatment, each of which was subsampled for 5 min. The swim paths of cod larvae were extracted from the video taped images and analysed using custom software (see Browman et al. 2003). For each of the 5 min. subsamples, the longest 20 paths were identified and combined (recall that, for statistical analysis, all of these paths must be collapsed into one since they are not independent). Paths totalling approximately 2.6 m in length were used as the basis for the analysis. The following variables were extracted from the swim paths: durations of pauses (non-active displacement, as defined in Browman et al. 2003), lengths and durations of moves, and turn angles (decomposed into vertical and horizontal components). For each of these variables, the software produced a mean (± standard error, SE) for the various behavioural variables associated with the 20 longest paths from each of the two replicates. The frequency distributions of these variables (Fig. 1) were compared using Kolmogorov-Smirnov two-tailed tests to determine if the presence of microalgae affected larval behaviour, and in what manner.

Spectral irradiance ($W\ m^{-2}\ nm^{-1}$) was measured at 1 nm intervals (280 – 800 nm) inside the test aquarium - in filtered water and in water containing the same numbers of rotifers and/or microalgae as used in the experimental treatments – using a high-sensitivity/high resolution scanning spectroradiometer (OL-754, Optronic Laboratories Inc., Orlando, Florida, USA). Before measurements, the instrument was calibrated against a NIST-traceable 200 W tungsten-halogen standard lamp (OL 752-10) and its wavelength and gain accuracy were verified using a dual source calibration module (OL-752-150).

Results and Discussion

Earlier work demonstrated quantifiable changes in behaviour associated with the transition from endogenous to exogenous feeding (Skifetsvik 1992), that the behaviour of sick larvae was different from those that were healthy (Skiftesvik and Bergh 1993), and that larval growth rate is reflected in their behaviour (Browman et al. 2003). Thus, the variables that we measured should be sensitive enough to detect any change in behaviour associated with the presence of microalgae. Nonetheless, with the exception of vertical turn angle, there were no statistically discernible (Kolmogorov-Smirnov two-tailed comparisons, $P > 0.05$) between-treatment differences in the behavioural variables (Table 1, Fig. 1). This is possibly due to the fact that there were no measurable (that is, $> 10^{-6}\ W\ cm^{-2}$, the absolute detection limit of the OL754) differences in spectral irradiance amongst these treat-

ments indicating that, at least at the microalgal and rotifer particle counts used, there was no major effect on the ambient light quality or intensity. It is also possible that the high level of variability exhibited within one group of larvae, and/or the small number of replicates in this experiment, reduced our ability to discern any differences in behaviour. Alternately (or in addition), the water in which the larvae were placed in the rotifers-only treatment had the algae vacuum-filtered out of it: this filtrate water may have contained substances (e.g. microalgae-specific amino acids) that alter the behaviour of cod larvae. Finally, the result-conclusion may be different for other microalgal species. It would be useful to test all of these possibilities.

The results of this experiment (albeit as qualified above) illustrate that the effect of green water on the behaviour of cod larvae is subtle. Thus, any improvement in the growth and/or survivorship of cod larvae associated with green water is most likely a result of the indirect effects that the microalgae might have on the nutritional quality of their food, and/or on their physiology (reviewed by Reitan et al. 1997).

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