Diel variability of feeding activity in haddock (Melanogrammus aeglefinus) larvae in the East Shetland area, North Sea

Abstract Investigations of factors affecting feeding success in fish larvae require knowledge of the scales of variability of the feeding process itself and the indices used to assess this variability. In this study, we measured short-term (diel) variability in feeding rates of wild haddock (Melanogrammus aeglefinus) larvae four times per day during a 10-d cruise in the northern North Sea. Feeding activity was evaluated using indices of gut fullness, prey digestive state and biochemical measurements (trypsic enzyme activity). The gut fullness and the enzyme activity indices indicated moderate to high rates of food consumption throughout the cruise. Time series analysis of the three indices showed significant diel variability in all indices and enabled identification of significant lags between food uptake and peak digestive enzyme activity. The typical pattern of food consumption and digestion was characterized by maximal ingestion of prey early in the evening (19:00 hrs) and peak digestive enzyme activity at 01:00 hrs. The time scale over which enzyme activities reacted to prey ingestion was ca. 6 h, and is consistent with expectations from controlled laboratory experiments with other larval fish species. Significant diel variability in trypsic enzyme activity suggests that attempts to relate this measure of feeding success to other variables (e.g. food concentrations) should take care to accommodate natural cycles in feeding activity before making statistical comparisons.

Introduction

Variations in food supply are often considered to be a major factor regulating feeding, growth and survival rates in larval fishes (reviews by Heath 1992; Leggett and DeBlois 1994; Cushing 1995). High feeding and growth rates potentially increase larval survival by reducing the vulnerability of larvae to those predators which prey on small and weakly swimming prey (Bailey and Houde 1989; Leggett and DeBlois 1994). As a result a large research effort has been dedicated to establishing linkages between larval feeding, growth, or recruitment and food conditions during the larval stages (Heath 1992; Ferron and Leggett 1994). This effort has included analyses of larval gut contents (e.g. number of prey per gut, gut fullness, taxonomic composition of prey in the gut) to identify relationships with zooplankton concentrations and taxa observed at the time of capture (Sundby et al. 1994; McLaren and Avendano 1995; Theilacker et al. 1996; Lough and Mountain 1996).

A common result from these studies is that larval fishes generally ingest most of their prey when the water column is illuminated by solar radiation (Blaxter 1986). As a result, larval feeding indices based on analyses of gut contents usually show a significant diel variability in which feeding is lowest at night and highest during the day, with peaks occurring often at sunrise and sunset (Last 1978; Kane 1984; McLaren and Avendano 1995; McLaren et al. 1997). These patterns are so widespread that they are frequently used to support the suggestion that larvae are principally visual feeders.

Recently, biochemical methods to measure larval feeding activity have also become available (review by Ferron and Leggett 1994). These techniques measure the activity of a proteolytic enzyme (trypsin) whose
concentration in the gut and activity responds quickly (within a few hours) to changes in larval ingestion rate (Pedersen et al. 1987; Pedersen and Hjelmeland 1988). Because of its relatively short response time to variable food conditions, trypsin enzyme activity appears to be suitable for assessing short-term changes in feeding activity in wild populations of fish larvae (Ferron and Leggett 1994; Ueberschärf 1995).

However, the magnitude, timing and latency of variations in trypsin enzyme activity to changes in food ingestion rate are poorly known for natural larval fish populations. In the only diet field investigations of larval trypsin enzyme activities, Ueberschärf (1995 and unpublished data) has shown that highest enzyme activities in sardine and sprat larvae occurred from midnight to early morning. These findings indicate that peak enzyme activities occur at different times than peaks in gut fullness and are consistent with a latent period after the timing of peak food consumption rate. The results also suggest that, as with larval gut content analyses, a knowledge of the timing and dynamics of food uptake and digestion will be necessary if enzyme activities are to be interpreted in relation to external forcing variables such as photoperiod and prey concentration.

In this study we evaluate the short-term (sub-daily) variability in gut fullness, prey digestive state and digestive enzyme activity during a 10-d field study of larval haddock feeding and growth. Our objective is to identify the time lags and scales of variability in larval feeding and digestive processes. Understanding the timing and magnitude of these lags and scales can be useful when testing hypotheses involving processes governing short-term larval feeding and growth rates.

Materials and methods

The study was conducted as part of a larger Scottish–Danish field investigation of environmental and parental influences on gadoid recruitment in the northern North Sea. A full description of the larval and hydrographic sampling is presented by Gallego et al. (1999). Samples of Melanogrammus aeglefinus larvae were collected in time series fashion at 6-h intervals for a period of 10 d (8 to 19 May 1996); larvae were also collected 29 April to 6 May 1996 in the same area, and a small number of these larvae were included in size-based analyses of enzyme activity. All sampling during the time series study was conducted within 1 to 2 km of a drifting buoy. Within each 6-h time block, hydrographic (CTD, turbulent dissipation rate, light, nutrients) and biological (chlorophyll, zooplankton, particles, larval fish) variables in a drifting water mass were measured.

Haddock larvae were collected from depth-stratified tows; most larvae used in analyses were from the 25 to 50 m layer or the 50 m to bottom layer. Sample sizes were initially five larvae per haul but after 36 to 48 h, these were increased to a maximum of ten as onboard sampling and sorting procedures quickly became more efficient. Since biochemical changes occur in small fish larvae between capture and preservation (Lochmann et al. 1996), precautions were taken to minimize the probability that post-capture trauma during net handling and subsequent sample sorting would influence our results. All sample sorting glassware and hardware were kept at < 4 °C and all ichthyoplankton samples were sorted in ice baths to slow rates of physiological deterioration. The maximum time allowed to elapse for sample processing and larval sorting/identification was set to 15 min. Hence within 15 min of arrival of the sampler on the ship’s deck, all larvae were removed from the net codend, identified, sorted, photographed on a video camera system, placed in pre-labelled individual vials which were vented with nitrogen gas, and preserved in liquid nitrogen to immediately arrest physiological processes. This protocol placed a priority on obtaining a modest number of high quality larvae rather than a large number of possibly physiologically deteriorated larvae. As a result sample sizes were occasionally less than ten per haul when larvae were less abundant. Larvae were transferred from liquid nitrogen to a shipboard ~80 °C freezer after 48 h. When the ship returned to port, larvae were transported to the laboratory by airfreight in boxes containing dry ice. Larvae were stored at ~80 °C until analyses began.

Laboratory processing of haddock larvae

Larvae were first examined under a dissecting microscope to estimate gut fullness and the state of digestion of prey contained in the gut. To ensure unbiased evaluations, the investigator had no prior knowledge of the sampling history (e.g. sampling date, time within day) of the larvae. Gut fullness was evaluated qualitatively on a scale from 0 (empty) to 4 (full), and prey digestive state was evaluated qualitatively on a scale from 0 (prey freshly ingested and easily recognizable as discrete particles) to 3 (prey carcasses no longer distinguishable and resemble homogeneous slurry). Larval standard lengths were measured, and larvae were placed inside an Eppendorf vial (1.5 ml volume).

Each larva was then beheaded so as to preserve otoliths for future analysis. Larvae were beheaded inside the cap by positioning the larva near the mouth of the cap and cutting with a scalpel in a way which allowed otoliths to remain undisturbed in the dismembered head and gut contents to remain intact. Following beheading, the head and body were carefully rinsed down to the bottom of the cap using 500 μl Tris-HCl buffer (pH 8, 0.02 M CaCl2 × 2H2O; temperature 0 to 1 °C) and shaken for about 10 s using a vortex. The larval head was removed and placed back into the original labelled cap and refrozen at ~70 °C. Preliminary attempts to recover and extract otoliths from the dismembered heads and analyse otolith micro-structure have proven to be successful (personal observation). The body was homogenized using a special pestle which fits to the shape of the cap (Eppendorf). Finally the homogenate was centrifuged 60 min at 4110 × g (temperature 0 to 3 °C). The supernatant was used to assay the trypsin enzyme activity.

Tryptic enzyme assays were performed on an individual basis according to the highly sensitive fluorescence method as described in Ueberschärf (1988) with modifications as described in Ueberschärf and Ferron (1995). The assays were carried out in tempered microcuvettes (constant 30 °C) using a KONTRON spectral fluorimeter (Model SFM 25) with a computer driven cuvette holder with four places. Comparison of trypsin enzyme activity measurements as applied in this study and a radioimmunoassay technique which quantitatively measures both trypsinogen (the trypsin precursor produced in the larval pancreas) as well as the activated trypsin showed that the activity measurement is not significantly affected by the precursor of trypsin in larval homogenates under the described conditions (Ueberschärf et al. 1992). Moreover, activation of trypsinogen in rabbit pancreas juice requires 30 min at 30 °C (Glazer and Steer 1977). Since all fish larval homogenates in this study were held on ice prior to incubation and since all measurements were conducted within 15 min, activation of stored trypsinogen will contribute minimally to the activity measurements. These considerations indicate that the trypsin enzyme assay closely reflects activity in the gut and is not likely to be affected by either precursors or their conversion to the active form during tissue processing.

Data analyses

Scatterplots of time series were used to visually display the data for trends. Statistical analyses involved one-way analyses of variance (ANOVA) in which time of day was treated as a categorical