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

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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 aeglifinus*) 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 (tryptic 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 tryptic 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.

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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, tryptic enzyme activity appears to be suitable for assessing short-term changes in feeding activity in wild populations of fish larvae (Ferron and Leggett 1994; Ueberschär 1995).

However, the magnitude, timing and latency of variations in tryptic enzyme activity to changes in food ingestion rate are poorly known for natural larval fish populations. In the only diel field investigations of larval tryptic enzyme activities, Ueberschär (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 aeglifinus* 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* $CaCl_2 \times 2H_20$; 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 tryptic enzyme activity.

Tryptic enzyme assays were performed on an individual basis according to the highly sensitive fluorescence method as described in Ueberschär (1988) with modifications as described in Ueberschär et al. (1992). The assays were carried out in temperated microcuvettes (constant 30 °C) using a KONTRON spectral fluorometer (Model SFM 25) with a computer driven cuvette holder with four places. Comparison of tryptic 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 (Ueberschär 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 tryptic enzyme activity 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

variable; if differences between sampling times were significant ($P < 0.05$), a Tukey–Kramer multiple comparison test was used to identify group differences. Exploratory time series analyses involved both auto- and cross-correlations (Chatfield 1989) between feeding-related variables. These analyses identified correlations at different lags within and between variables.

Results

Hydrography

Descriptions of the hydrographic conditions during the cruise are presented in MacKenzie et al. (1998) and Gallego et al. (1999). The main feature of relevance for the present study was the weakly stratified and nearly isothermal water column present during the cruise. Temperatures were 6 to 8 °C throughout the water column with little variation during the 10-d period.

Variation in feeding activity (gut fullness, prey digestive state and tryptic enzyme activity)

Univariate analyses

Melanogrammus aeglifinus larvae used for evaluating short-term variability in feeding success varied in standard length between 6.9 and 14 mm. Lengths of these larvae varied widely both within and between hauls (Fig. 1).

The gut fullness indices showed that nearly all haddock larvae had prey in their guts during all hauls (Fig. 2A). Most larvae had guts approximately 50 to 75% full. Highest gut content indices occurred at the start and end of the cruise (8 and 9 May and 16 to 19 May), and lower indices occurred on 13 and 14 May. When the measures of gut fullness are grouped according to the time of day at which the larvae were captured, there was some evidence of a diel pattern; gut fullness was highest for larvae collected at 19:00 hrs (one-way ANOVA test: $P = 0.05$; Fig. 3), and larvae collected at other times of day had nearly equal levels of prey in the gut.

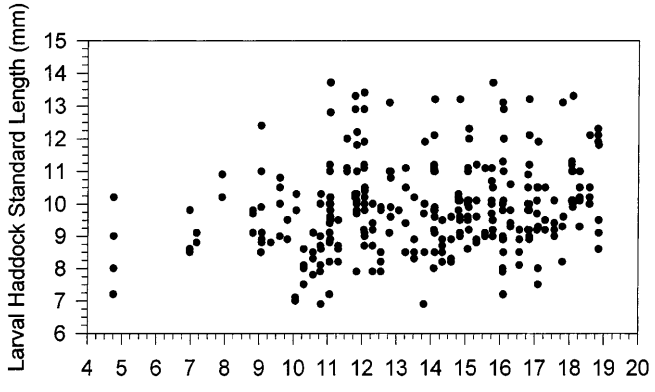

Fig. 1 *Melanogrammus aeglifinus*. Time series of standard length of haddock larvae used in tryptic enzyme activity analyses. Each dot represents one larva. All larvae were captured east of Shetland during 4 to 19 May 1996

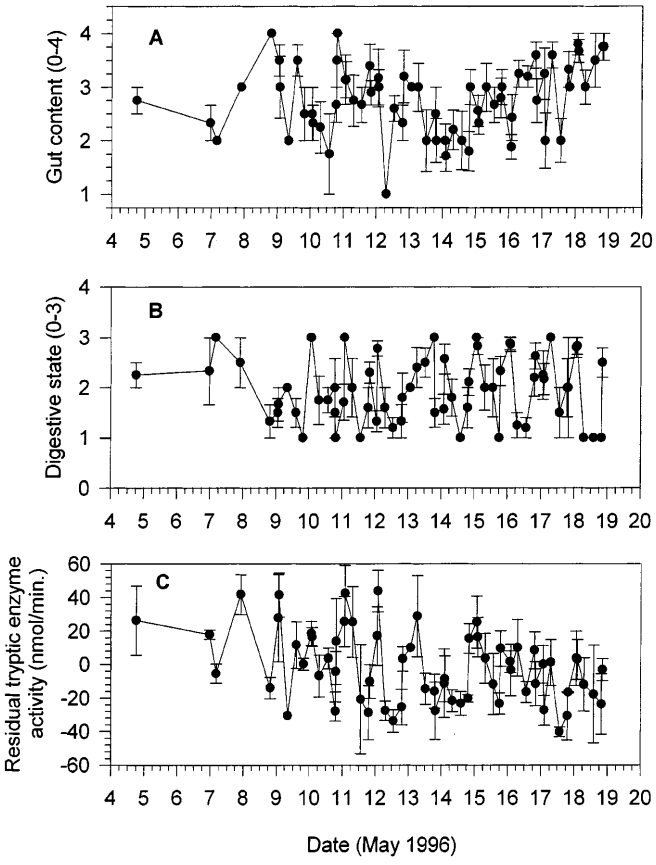

Fig. 2 *Melanogrammus aeglifinus*. Time series of gut fullness (**A** qualitative index increasing from 0 to 4 with increasing gut fullness), digestive state of prey in larval gut (**B** qualitative index increasing from 0 to 3 with increasing digestive state of prey), and length-corrected tryptic enzyme activity (**C** enzyme activities were standardized for differences in larval length using the regression relation in Fig. 5) for wild haddock larvae captured east of Shetland, 4 to 19 May 1996. Values are means and standard errors of a maximum of ten larvae per haul

The digestive state of prey within the gut showed stronger evidence of a diel pattern. Prey in the gut were in a relatively undigested state in larvae captured at 13:00 hrs (Fig. 4). The state of digestion increased over the next two time periods (19:00 hrs and 01:00 hrs) and reached its maximum during the haul at 01:00 hrs. Digestive state then decreased at 19:00 hrs and reached its minimum at 13:00 hrs.

A quantitative measure of feeding activity is represented by the activity of tryptic enzyme in the gut. Larger larvae had on average higher tryptic enzyme activities than smaller larvae (Fig. 5). The time pattern of residual variation from this relationship showed two scales of variation. First, larvae collected on some days during the cruise had higher enzyme activities than other days (Figs. 2C, 6); for example, during most of the period 12 to 15 May, activities were low, and activities were higher during the first part of the cruise. The sec-

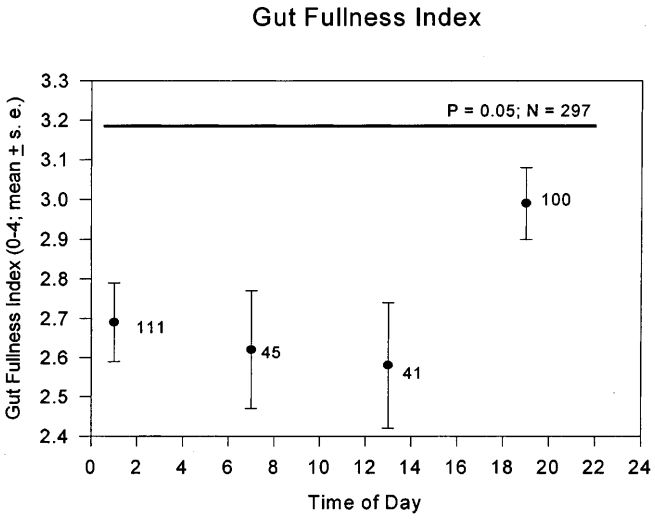

Fig. 3 *Melanogrammus aeglifinus*. Diel cycle of mean and standard error of gut fullness of wild haddock larvae. Gut fullness was assessed using a qualitative index from 0 (empty) to 4 (full). Means are based on observations regularly made four times per day during a time series study from 8 to 19 May 1996 east of Shetland. Numbers beside dots indicate sample sizes. Horizontal line joins groups which do not differ significantly ($P > 0.05$; Tukey–Kramer multiple comparison test)

ond scale of variation is the diel cycle (Figs. 6, 7). This pattern shows that larvae captured at 01:00 hrs had the highest activity levels, and that activity levels declined at 07:00 hrs and again at 13:00 hrs, when lowest activities were observed. Activities started to increase again at 19:00 hrs until reaching peak levels again at 01:00 hrs. The overall cycle shows that enzyme activity has a highly significant regular periodicity (one-way ANOVA test: $P < 0.0001$; Fig. 7). However, the time series of individual observations within each of the four time periods

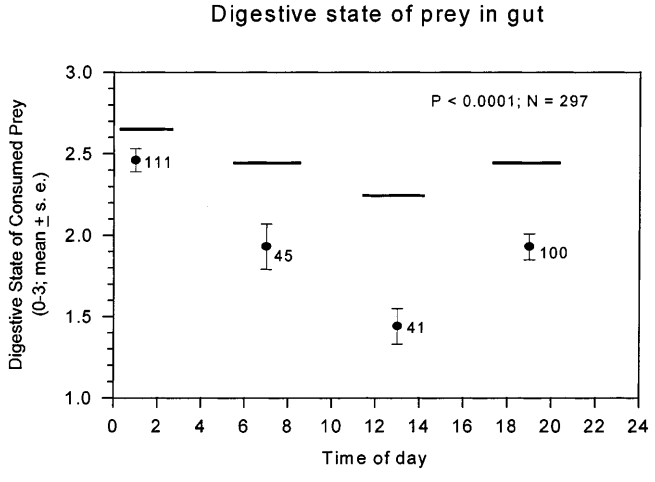

Fig. 4 *Melanogrammus aeglifinus*. Diel cycle of mean and standard error of digestive state of prey in the guts of wild haddock larvae. Digestive state of prey in larval guts was assessed with a qualitative index from 0 (freshly ingested, prey easily recognizable as discrete particles) to 3 (integrity of prey carcasses no longer distinguishable, prey appearance resembles homogenous slurry). Numbers beside dots indicate sample sizes. Horizontal lines join groups which do not differ significantly ($P > 0.05$; Tukey–Kramer multiple comparison test)

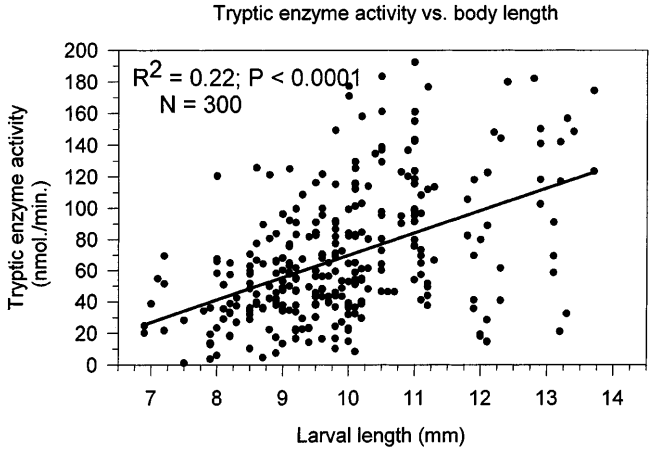

Fig. 5 *Melanogrammus aeglifinus*. Tryptic enzyme activity relative to body length for 300 haddock larvae captured in the northern North Sea, 4 to 19 May 1996. Each dot represents an individual larva. The equation for the regression line is $y = 14.3x - 73.6$

demonstrates that some larvae in the same haul had much higher enzyme activities than other larvae (Fig. 6).

Bivariate analyses

Auto- and cross-correlation analysis of the three feeding indices confirms their cyclic nature and inter-relations. Tryptic enzyme activities show statistically significant positive autocorrelations at 24-h intervals (Fig. 8A). Prey digestive state had a statistically significant negative lag at 12 h and at some other multiples of 12 h (Fig. 8B). Gut fullness shows only weak autocorrelation because this index had only marginally significant differences throughout the day (Fig. 8C). Cross-correlation of prey digestive state (dependent variable) with tryptic enzyme activity showed a positive correlation at lag 0, and a negative correlation at a negative lag of 12 h, i.e. variations in prey digestive state were inversely related to those in tryptic enzyme activities 12 h earlier (Fig. 9). Cross-correlation of tryptic enzyme activities (dependent variable) with gut fullness showed that variations in enzyme activities showed a weak positive correlation at lag 0, and significant negative correlation at a negative lag of 6 h, i.e. variations in enzyme activities were inversely related to those in gut fullness 6 h earlier (Fig. 10).

Discussion

Gut content and tryptic enzyme activity assays showed that haddock larvae were generally feeding at moderate to high rates during the cruise. Nearly all larvae had prey visible in their gut regardless of time of day, and had moderate to high levels of tryptic enzyme activity, particularly near midnight. However, fixed-interval time series sampling during the 10-d cruise showed that strong diel cycles appeared in the enzyme activity measurements,

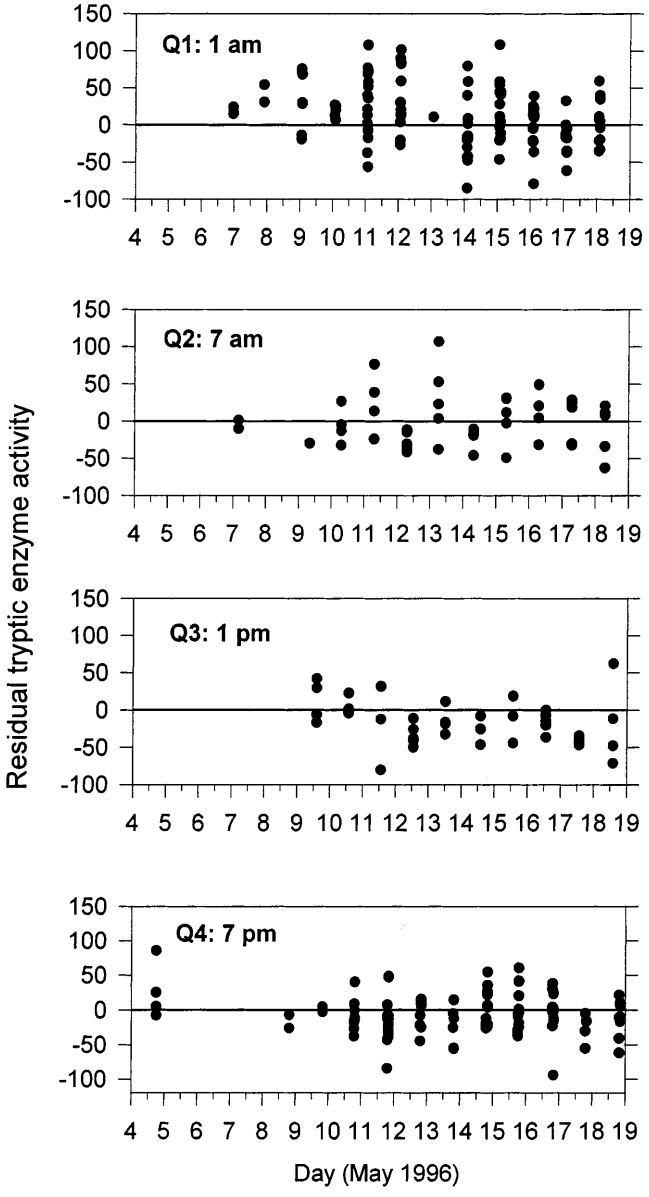

Fig. 6 *Melanogrammus aeglifinus*. Time series of size-corrected tryptic enzyme activity in individual wild haddock larvae captured east of Shetland, 4 to 19 May 1996. All measurements have been corrected for differences in body length using the relation shown in Fig. 5; each dot represents the enzyme activity for an individual larva. The four panels show time series of enzyme activities for each of the four sampling periods during the study

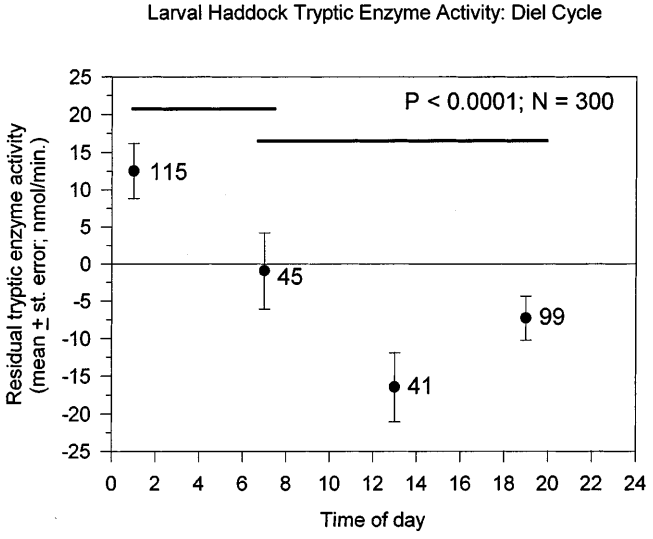

Fig. 7 *Melanogrammus aeglifinus*. Diel cycle of mean and standard error of tryptic enzyme activities of wild haddock larvae. All measurements have been corrected for differences in body length using the relation shown in Fig. 5. Means are based on observations regularly made four times per day during a time series study from 8 to 19 May 1996 east of Shetland. Numbers beside dots indicate sample sizes. Horizontal lines join groups which do not differ significantly ($P > 0.05$; Tukey–Kramer multiple comparison test)

the state of prey digestion, and to a lesser extent the gut fullness indices. The diel cycles in tryptic enzyme activity have been reported before, but for only sardine and sprat larvae (Ueberschär 1995 and unpublished observations) and only over shorter time periods (36 to 48 h); nevertheless in both of these previous cases, peak activity occurred between midnight and dawn which is consistent with the timing of peak activity observed in haddock larvae in the northern North Sea. The diel cycle observed for haddock larvae is the longest yet observed and highlights the rapidity and regularity of the response of larval haddock physiological processes to feeding activity.

One of the most important external factors responsible for diel variability in feeding activity is variation in light conditions (Blaxter 1986). Haddock larvae, like most fish larvae, are visual feeders, and their ability to find prey will be reduced at night; this is probably the major factor responsible for the diel variation in feeding indices seen in this study. Our observation of the timing of the main larval haddock feeding periods is generally consistent with those reported for other populations of haddock and cod larvae (southern North Sea: Last 1978; Georges Bank: Kane 1984; Scotian Shelf: McLaren and Avendano 1995; McLaren et al. 1997). All of these previous studies found highest incidences of feeding (gut fullness) at or shortly after sunset. Our study, conducted in spring at much more northerly latitudes and therefore having a longer daily photoperiod than those listed above, also found that haddock larvae had highest incidences of feeding at the sampling time (19:00 hrs) closest to sunset (21:00 to 21:30 hrs). The true peak in gut fullness may have been shortly after sunset (instead of that measured at 19:00 hrs) but the timing of our 6-h sampling resolution would not identify such a peak. Given the sampling resolution in our time series, the peak in tryptic enzyme activity at 01:00 hrs, after allowing for response time latency (see details below), probably indicates that food consumption rates peaked a few hours earlier and most likely sometime between 19:00 and 22:00 hrs.

The cross-correlation analyses of the available time series showed that both prey digestive state and gut fullness were positively correlated at lag 0 with tryptic enzyme activity. This result indicates that at any given time during the study higher levels of prey in the gut

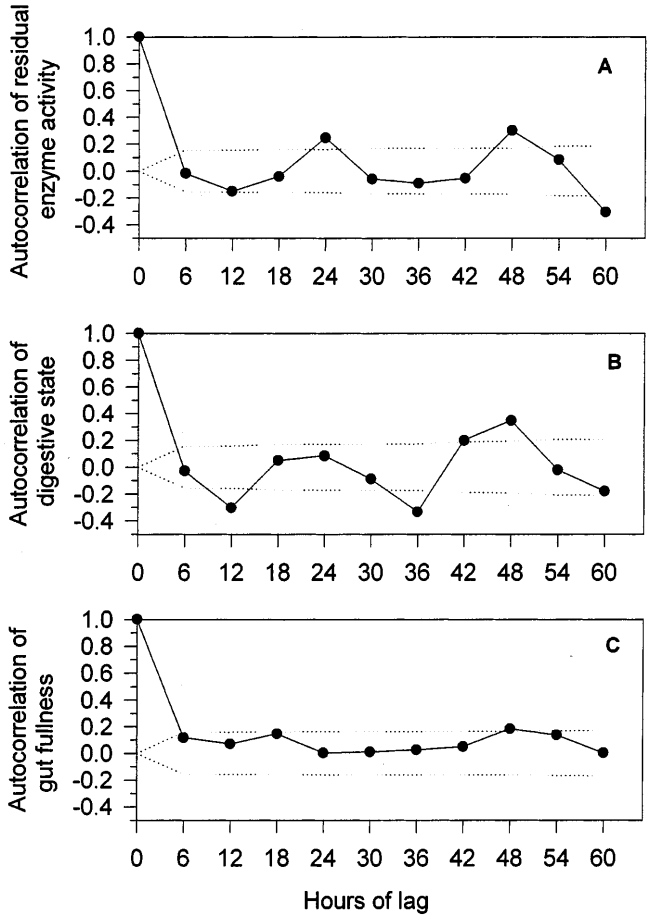

Fig. 8 *Melanogrammus aeglifinus*. Autocorrelation plots of length-corrected tryptic enzyme activity (**A**) digestive state of prey in larval guts (**B**) and gut fullness (**C**) for wild haddock larvae captured during a time series study east of Shetland, 8 to 19 May 1996 (*solid lines* autocorrelations; *dotted lines* minimum significant autocorrelations). Horizontal axis represents the number of hours by which the time series is shifted against itself to produce the observed autocorrelations

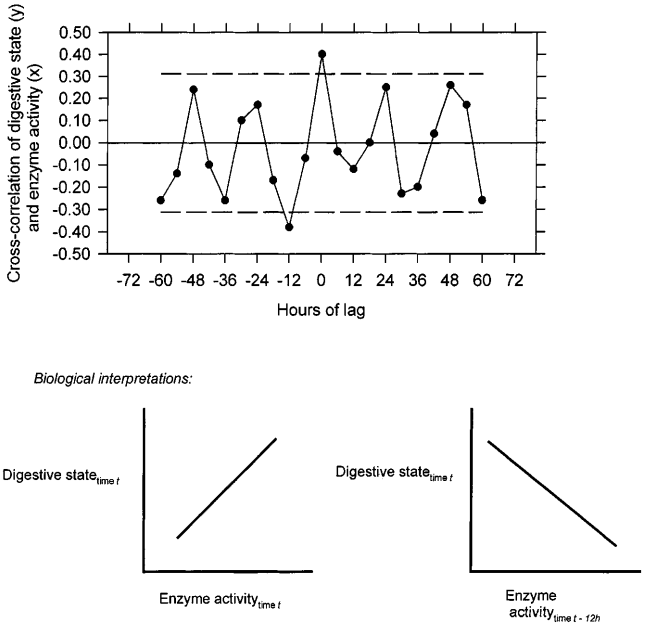

Fig. 9 *Melanogrammus aeglifinus*. Cross-correlation plot of prey digestive state (dependent variable) and length-corrected tryptic enzyme activity in guts of larval haddock captured east of Shetland during a field study 8 to 19 May 1996 (*solid line* cross-correlations; *dashed lines* minimum significant cross-correlations). Horizontal axis represents the number of hours by which the enzyme activity time series is shifted forward or backward relative to the digestive state time series to produce the observed cross-correlations. *Lower panels*: schematic representations of observed cross-correlation relationships

and the state of prey digestion were both associated with higher tryptic enzyme activity levels. Cross-correlation analyses also showed that variations in enzyme activities were inversely related to those in gut fullness 6 h earlier. For example, low gut fullness was followed by high enzyme activities within 6 h. This suggests that, on average, within the 6 h time lag between a minimum in gut fullness and a peak in tryptic enzyme activity, larvae ingested new prey, secreted trypsinogen and activated this enzyme to initiate digestion. Similarly, larvae with full guts reduced their enzyme activities to minimal levels within 6 h, suggesting that much of the prey had become digested. The expected gut clearance time for small fish larvae is variable (range 1 to 22 h; Govoni et al. 1986) and depends on many variables (e.g. larval size, meal size, continuous or ceased feeding; Govoni et al. 1986). In an extensive analysis of gut contents in wild haddock and cod larvae, Lough and Mountain (1996) assumed a gut clearance time of 4 h but recognized that it

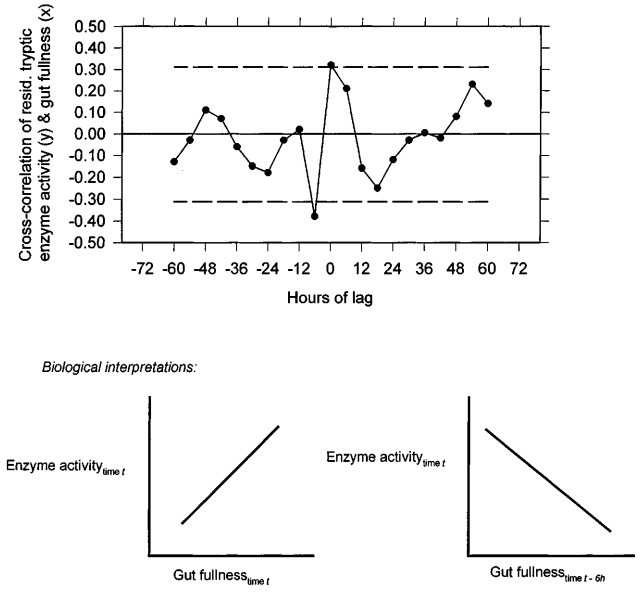

Fig. 10 *Melanogrammus aeglifinus*. Cross-correlation plot of length-corrected tryptic enzyme activity (dependent variable) and gut fullness of larval haddock captured east of Shetland during a field study 8 to 19 May 1996 (*solid line* cross-correlations; *dashed lines* minimum significant cross-correlations). Horizontal axis represents the number of hours by which the gut fullness time series is shifted forward or backward relative to the tryptic enzyme activity time series to produce the observed cross-correlations. *Lower panels*: schematic representations of observed cross-correlation relationships

could be 1 to 9 h. These two sets of estimates are therefore close to the shortest significant time lag (6 h) resolvable by our sampling schedule.

Another set of cross-correlation analyses showed that variations in prey digestive state were inversely related to tryptic enzyme activities 12 h previously. For example, highly digested prey in the gut were preceded 12 h previously by low tryptic enzyme activities. This time lag is longer than that involving gut fullness and enzyme activity, but is within the range observed in the laboratory (Govoni et al. 1986). The statistical correlations indicate that the relationship between prey digestive state and enzyme activity changes from being positive at lag 0 to negative after 12 h, indicating that the relationship between prey digestive state and enzyme activity is dynamic on time scales much less than 24 h. This time scale is consistent with general patterns of larval digestive physiology (Govoni et al. 1986; Pedersen et al. 1987; Ueberschär et al. 1992). The exact time lag is difficult to resolve given the 6-h sampling interval, natural variability in food consumption rates, gut clearance rates (Govoni et al. 1986; Lough and Mountain 1996) and physiological processes (Pedersen et al. 1987; Ueberschär 1995), and the simple qualitative index that we used to assess prey digestive state.

These statistical findings with wild haddock larvae indicate that larval haddock digestive processes in nature respond quickly to changes in food ingestion rate and reflect the sequence of physiological processes associated with larval feeding and digestion. Larval fish produce an inactive form of trypsin (i.e. trypsinogen) in the pancreas where it is stored until prey is consumed (Hjelmeland et al. 1984; Pedersen et al. 1987; Pedersen and Andersen 1992). After ingestion, trypsinogen is secreted into the larval intestine where after some time it is converted into its active form, trypsin. The time required for activation of trypsinogen in larval fish, adapted to temperatures as low as 6 to 8 °C is unknown, but probably much longer (perhaps 3 to 3.5 h, assuming a Q_{10} value of 2.3) than the 30 min required at 30 °C (Glazer and Steer 1977). The amount of trypsinogen molecules released depends on meal size (Pedersen et al. 1987; Pedersen and Andersen 1992; Ueberschär et al. 1992), larval size and other factors. Trypsin molecules then digest the prey which progressively loses its recognizable appearance and is absorbed across the intestinal wall. The time scale for secretion of trypsinogen after prey ingestion has been investigated in laboratory calibration experiments for several larval fish species and is approximately 0.5 to 12 h, depending on factors such as larval age (Pedersen et al. 1987; Pedersen and Hjelmeland 1988; Ueberschär 1995).

This time scale compares favorably with the tryptic enzyme activity response time and prey digestive state patterns seen in our field study of haddock larvae. We observed that tryptic enzyme activities on average decrease within 6 h (the shortest time scale resolvable by our sampling design) of peak gut fullness (Fig. 10), and that variations in prey digestive state corresponded inversely to those in tryptic enzyme activities 12 h previously (Fig. 9). In the absence of consumption of new prey, laboratory calibrations show that gut trypsin levels and tryptic enzyme activity in herring larvae fall substantially within 4 to 8 h (Pedersen and Hjelmeland 1988) or even faster. We note that a similar pattern, although with slightly longer latency, was seen in haddock larvae collected in the field.

The diel pattern of tryptic enzyme activity in the wild haddock larvae corresponds therefore to that which generally could be expected from the laboratory calibrations involving other species. However, the laboratory responses appear to be somewhat faster than the field responses. The apparently slower response time in the field could be partly due to the minimal sampling time interval of 6 h which is longer than some of the response times seen in the laboratory calibrations. Shorter sampling intervals might have revealed faster response times and peaks at slightly different times of day.

However, an additional factor responsible for the modest difference in response times between the laboratory calibrations and the field observations could be due to the experimental designs that have been employed. In many calibration experiments larvae are either offered an ad libitum meal after several hours or days of starvation to measure the rates of enzyme secretion, or food is completely withheld for several hours or days to measure the rates of enzyme disappearance from the gut. These results describe the physiological capabilities of the larvae to produce, secrete and remove enzyme from the gut. In such cases, it is to be expected that physiological response times would be fast and the cause–effect relations to be clearly resolvable.

However in nature, feeding events during a day's photoperiod are probably much more opportunistic and variable partly because of small-scale patahiness (Davis et al. 1992). In these situations, a larval gut may not have the opportunity to either fill completely because of low prey availability, or to empty completely because of high prey availability; moreover even if complete filling or emptying does occur it may not occur at the same time within the photoperiod every day. Hence the irregular sequences of feeding and refractory periods that likely occur in nature (Rothschild 1991) would prolong the period over which digestive enzymes might be found in the gut and thereby dampen the clear physiological responses seen in controlled laboratory situations.

Our biochemical diagnoses of larval feeding activity showed highly regular diel variations consistent with other estimates of larval feeding incidence made on the same larvae and enable additional insight into larval physiological processes related to feeding and ingestion. Processes preventing high production and secretion of trypsinogen will result in poor prey digestion and assimilation during daily feeding events. For example, Kjørsvik et al. (1991) and Hjelmeland et al. (1984) showed that prolonged starvation of cod larvae resulted in degeneration of both the pancreas and liver and that

levels of trypsin and trypsinogen remain low in food-deprived larvae. Variability in some of these processes between individual larvae may be responsible for some of the residual variation in our statistical relationships. In addition, the amplitude of the diel variations is probably related to the amount of food ingested by the larvae in the previous 6 to 12 h. This latter topic is presently under investigation.

In summary, tryptic enzyme activity in haddock larvae from the northern North Sea showed strong diel variations over a 10-d period. The clear diel patterns in tryptic enzyme activity, and to a lesser extent gut fullness, in wild haddock larvae indicate that it is important to know the timing of the peak feeding period if relations of feeding success to other variables (e.g. prey concentration) are to be investigated (Ferron and Leggett 1994; McLaren et al. 1997). Knowing that larvae have regular feeding and physiological cycles, and the time scales (e.g. diel, multi-day) of their response to various factors, allows the investigator to choose sampling times effectively (Taggart and Frank 1990; Ferron and Leggett 1994; Chícharo et al. 1998).

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