

Sequential Observations on the Digestive Process in the Squid, *Illex illecebrosus*

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Abstract

Color and pH of stomach and caecal contents and percent organic matter of stomachs of squid, sampled at 2-hour intervals for 16 hours after feeding on live fish, were observed and considered as possible indices of the "recency of feeding" for studies on feeding periods in nature. Caecal fluid color is the easiest index to record in routine sampling, but this may be subject to considerable variation. The pH of stomach contents is well correlated with time after feeding and is potentially applicable under field conditions.

Introduction

The squid is an actively swimming predator with a digestive system adapted to the intake and rapid processing of large meals. Storage and physical breakdown of food occurs in the stomach, with simultaneous enzymatic digestion and absorption occurring in the caecum and possibly the digestive gland. Undigested material normally passes directly from the stomach into the intestine when organic matter has been removed (Bidder, 1966). However, few details of the physical and chemical changes associated with the passage of food through the digestive system have been recorded, and even the reported rates of digestion vary widely (Bidder, 1950; Boucaud-Camou *et al.*, 1976; Karpov and Cailliet, 1978 and Boucher-Rodoni, 1975).

A need to develop criteria for establishing the progress of digestion in *Illex illecebrosus* in relation to chronological studies of feeding patterns has been noted (Amaratunga, MS 1980), and the present study was undertaken to evaluate possible indices. There is, as yet, no satisfactory way of estimating the time that a meal has been in the stomach of a squid. Studies on *I. illecebrosus* (Boucher-Rodoni, 1975) and several European squid (Bidder, 1950) indicate that characteristic changes in the colour of caecal fluid are associated with particular digestive phases. Changes in the appearance of gut contents have also been used to stage digestion (Bidder, 1950; Karpov and Cailliet, 1978). In the present experiments, these two indices are compared to pH changes and changes in the percentage of organic material in the stomach at intervals after a meal, as a first step toward the development of an index of the recency of feeding which can be applied in routine field surveys.

Materials and Methods

The squid used in this study were captured in a box trap at Herring Cove, Nova Scotia, on 8 and 15 August 1979. Approximately 50 squid were taken for each of two replicate experiments. The squid were transferred to a 45 m³ outdoor tank at the Aquatron Laboratory in Dalhousie University, using the techniques previously described (O'Dor *et al.*, 1977). A continuous flow of seawater through the tank maintained the temperature at $10 \pm 1^\circ \text{C}$. Live fish (*Fundulus* spp.) were introduced into the tank to familiarise the squid with this food source and the method of feeding. The squid were then left without food for 44 hours to allow complete clearance of gut contents. All squid showing external signs of damage were removed before the experiments were begun.

Each experiment commenced with the removal of two unfed squid. Analyses of these squid, as described below, were performed immediately. The remaining squid were then fed live *Fundulus*, introduced by hand, singly or a few at a time to ensure that all were eaten. Feeding behaviour was noted, but records of the number of fish taken by individual squid were not possible. Feeding was terminated after about 45 minutes when the attack rate slowed.

In each experiment two animals were removed for analysis as soon as the feeding operation was complete. Additional animals were taken at 2 hour intervals. Not all animals had fed, and in the first experiment squid were removed until two with material in their stomachs were obtained for each interval. Because the last squid taken at 14 hours still had material in their

stomachs, sampling during the early period was limited in the second experiment and additional late samples were taken. For analysis all data from the two experiments were pooled.

Animals were removed with a long-handled dip net, usually when they were near the surface to minimise disturbance to the animal and to the population. The animals were then transported in buckets to the laboratory, and analyses commenced within 5 minutes. Squid were immediately weighed (± 1 gm) and decapitated. After mantle lengths were taken, a ventral incision of the mantle exposed the internal organs, and the sex, maturity and stomach fullness were determined as described by Amaratunga and Durward (1979). The squid used had an average weight of 248 gm (174–388 gm) and average dorsal mantle length of 226 mm (185–250 mm), 20% were male and most were sexually immature.

Intact guts (stomach and caecum) were photographed for color and consistency. After ligation, the stomach was removed and weighed with its contents. Measurements of the pH of stomach and caecal contents were taken (pH meter 22, Radiometer, Copenhagen). Stomach contents were then diluted in seawater for examination under a binocular dissecting microscope for qualitative changes, and representative photographs taken. Because of the fragmented nature of gut contents, particular attention was paid to hard structures (scales, bones, etc.). Formalin was then added to the stomach contents which were stored for analysis of organic content.

The percentage of organic material in the preserved stomach contents was determined from weight loss during combustion. Stomach contents were placed in aluminium weighing pans and dried at 80°C until weights stabilized through loss of moisture. Whole *Fundulus* and collected faeces were similarly dried for comparison. The samples were weighed to nearest 0.01 mg, ashed at 520°C in a muffle furnace for at least 3 hours and reweighed immediately. The difference between ash and dry weights was taken as a measure of organic content. Empty weighing pans were assessed for weight loss by the same procedures.

Results

The behaviour of the feeding population of *I. illecebrosus* was similar to that described by O'Dor *et al.* (1980). Healthy, actively swimming squid remained in a tight school throughout captivity. School formation was only temporarily broken during feeding. Larger squid fed first, darting out to seize fish and returning quickly to the school. Some squid were observed to take more fish (4–6) than could easily be

held within the arms. Encounters with less-encumbered squid often resulted in the loss of one or two fish. When the larger squid had obtained enough food, they retired to the school, allowing the smaller squid to feed. The seized fish were typically held in the arms, the head was bitten off at first, and pieces of flesh were then bitten off and eaten. Ingestion of the meal required 5–15 minutes. Examination of waste material from the tank after similar feeding experiments indicated that vertebrae, tails, eyes and internal organs were mostly rejected. This selectivity was confirmed by the infrequent occurrence of these items in the gut contents.

Specimens sampled immediately after ingestion had stomach weights ranging from 3 to 11% (average 6.4%) of body weight, indicating that typical meals may be in this range. Stomach fullness was generally classed as 2 or 3 (Amaratunga and Durward, 1979) during most of the digestion period, although lower indices were observed in the final hours of the experiments. Stomach weights relative to body weights did not show a consistent decline because of the inclusion of fluid (Table 1). However, the pH of both the stomach and the caecum decreased gradually with time after ingestion, the latter being consistently slightly lower than the former (Fig. 1). In both cases, pH was well correlated with time after ingestion. The pH of fluid from the stomachs of four fasted squid was 5.50 ± 0.17 (mean \pm standard deviation).

The logarithm of % change in organic matter of the stomach contents with time after ingestion is shown in Fig. 2. The dashed line represents the logarithm of the average organic content (5.8%) of two samples of faecal material. If the rate of digestion decreases exponentially, as is commonly assumed for fish (Fänge and Grove, 1979), the intersection of the regression line and the dashed line indicates a likely time of about 28 hours for intestinal elimination. The rate of digestion is 0.032 log % organic matter per hour. The ratio of %

TABLE 1. Average changes in relative stomach weight, pH of stomach and caecum, and % organic matter of stomach with time after ingestion of a meal by *I. illecebrosus*. (Numbers of observations in Fig. 1 and 2.)

Time after ingestion (hours)	% stomach weight of body weight	Stomach pH	Caecum pH	% stomach organic matter
0	6.39	6.26	6.16	46.8
2	5.03	6.07	5.92	43.9
4	5.46	5.97	5.82	40.6
6	2.63	5.93	5.78	21.0
8	3.81	5.81	5.69	18.0
10	3.23	5.80	5.75	14.4
12	3.77	5.62	5.60	24.7
14	4.58	5.50	5.45	23.7
16	4.82	5.49	5.50	—

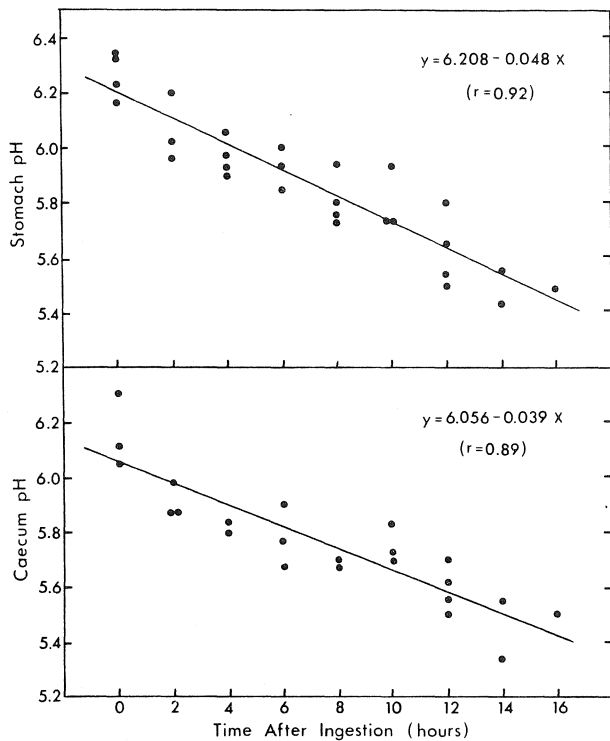


Fig. 1. The pH of stomach and caecum contents of *I. illecebrosus* sampled at 2-hour intervals after feeding.

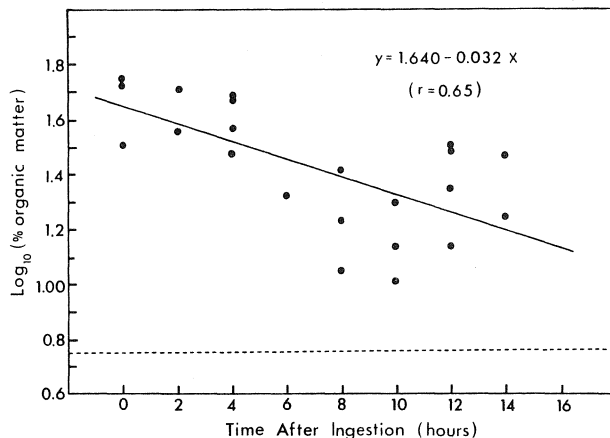


Fig. 2. Logarithm of percent organic matter of stomachs of *I. illecebrosus* sampled at 2-hour intervals after feeding. (The dashed line represents the average organic content of faecal material.)

organic matter in faeces to that at zero time from the regression (43%) gives a rough estimate of the assimilation coefficient for squid of 0.86.

External color changes in the stomach and caecum over the course of digestion are recorded in Table 2. The color of the stomachs was predominately that of the particulate contents and tended to vary very little

TABLE 2. Color changes of stomach and caecum contents of *I. illecebrosus* sampled at 2-hour intervals after feeding.

Hours after feeding	Stomach color	Caecum color
Pre-fed	clear	clear
0	grey-black	burgundy
0	grey-black	burgundy
2	grey-black	muddy brown
4	grey-black	red-brown
4	black	red-brown
6	grey	brown
6	blue-grey	yellow
8	grey	tan-brown
10	blue-grey	yellow
10	blue-grey	yellow-clear
12	silver-grey	brown-yellow
12	silver-grey	brown-yellow
14	silver-grey	brown-yellow
14	silver-grey	yellow-brown
14	yellow	yellow
16	yellow-clear	yellow-clear
16	clear	clear

until evacuation when a yellow or clear fluid remained, but the caecal fluid showed progressive changes. Caecal fluid from squid before feeding and at the end of digestion (16 hours) was clear. Intermediate color changes were distinct and consistent. A deep red or burgundy color appears immediately after ingestion, changing to varying shades of brown for most of the period of digestion and to light yellow in the final stages.

The stomach contents underwent qualitative changes indicative of the stage of digestion (Table 3). Fish flesh in recently fed squid were in 3–5 mm chunks due to the action of the beak, and bones and scales retained their cellular covering. As digestion progressed, the chunks became rounded and the soft tissue disappeared from the bones and scales. Connective tissue and hard structures became prominent in later stages of digestion. In the final stage, only connective tissue, bleached bones and scales remained. Particulate matter was absent in the stomachs of all animals taken at 16 hours.

TABLE 3. Changes in stomach contents of *I. illecebrosus* resolved into 4-hour intervals of digestion after feeding on fish.

Interval (hours)	Contents of stomach
0–4	Flesh in chunks; scales with chromatophores; bones bound in tissue.
4–8	Flesh rounded, connective tissue; scales with some chromatophores; bones separate from tissue.
8–12	Connective tissue; scales bleached; bones bleaching.
12–16	Little connective tissue; scales bleached; bones bleached.

Discussion

These experiments resemble those of Boucher-Rodoni (1975), who studied *I. illecebrosus* at similar sizes and temperatures and reported considerably more rapid digestion. The thawed fish fillets that she used as food may have been easier to digest because of cell disruption by ice crystals. Alternately, stresses caused by social interaction in the school or by the need to disrupt the school to capture individuals in the present study may have slowed digestion (Boucher-Rodoni studied isolated individuals). The slower rate of digestion is probably more realistic, because squid normally school, pursue live food and are themselves pursued by predators.

An additional factor related to the natural feeding situation is variable meal size. The meals in Boucher-Rodoni's (1975) experiments ranged only from 5 to 10% of body weight. In the present experiments, stomachs taken just after feeding ranged from 3 to 11% of body weight. Other studies have shown that meals as large as 15% of body weight are common (Hirtle *et al.*, in press), and meals up to 25% of body weight have been recorded for free-feeding fasted squid (O'Dor *et al.*, 1980). It is difficult to see how meals of 15–25% of body weight could be digested in the same time as meals of only 5% of body weight. Earlier observations (O'Dor *et al.*, 1980) support this view, because squid often skipped regular feedings after taking a large meal, and food in their digestive tracts was visible through the mantle up to 24 hours later.

Two possible biases in the present experiment relate to the variable meal size. Animals with no trace of food in their digestive tracts had to be rejected on the assumption that they had not fed. This was reasonable for the first 8–10 hours but may have biased the data at later times because all traces of small meals might already have been eliminated. The second bias relates to the greater difficulty in catching the larger, more active animals which tend to take the largest meals (O'Dor *et al.*, 1980). This could result in an underestimate of meal size and an over-representation in later data for animals that had taken larger meals. With stress and meal size as uncontrolled variables, the estimate of digestion time from the present experiment may be somewhat exaggerated.

Estimates of feeding time for squid under natural conditions will have to allow for not only the variables of stress and meal size but also those of temperature and body weight which affect digestion rate (Boucher-Rodoni, 1975; Boucher-Rodoni and Mangold, 1977), and such estimates are likely to err by several hours under all but the best of circumstances. Karpov and Cailliet (1978) developed an index for *Loligo opales-*

cens, involving a matrix of stomach weight data and stomach content condition to assign times since feeding. The assessment of the degree of digestion from the condition of particular food items in the stomach is feasible (Table 3) and is no doubt enhanced by taking account of meal size, but the procedure is very laborious and requires instruments and techniques not practical for routine sampling at sea by inexperienced observers.

The simplest index would appear to be the color of the caecal fluid (Table 2). The red to burgundy shade is characteristic of a recent meal (<4 hours), and the brown shades of the mid-digestive period prevail for 4–12 hours, or perhaps longer for very large meals. Yellow or clear fluid indicates that digestion is near completion and that the meal was probably taken 10 or more hours earlier. This index is easy to apply but is not particularly accurate, as some highly colored foods (e.g. euphausiid eye pigment) may interfere. General application of such an index would require careful experiments with realistic food items.

A second alternative, seemingly with some potential, is an index based on pH measurements. The decline in pH of stomach fluid with time after ingestion had the highest correlation ($r = 0.92$) of any variable measured. As stomach pH in fasted squid is around 5.5, it appears that a meal probably raised the pH to a value between this and the neutral pH expected in the tissues of the food. If continuous production of acid by digestive organs gradually overcomes the buffering capacity of the meal, minimal pH's would be likely when most of the meal has been digested. Low levels of acid production during fasting would then be expected to give intermediate pH values. This pattern is consistent with present observations and with the scanty information available for other cephalopods (Bidder, 1966). Such a numerical index, if shown to be reliable, would be of great value. A field trial, comparing the estimates of time since feeding predicted from the condition of stomach contents, the color of caecal fluid and the PH, would be a useful next step. If proven to be practical, portable PH meters that are robust and readily available could be used in field studies.

Because the concern of this study has been the estimation of how long a meal in the stomach has been there, emphasis has been placed on events prior to gastric evacuation. In fish, this phase represents only the beginning of a prolonged digestive process (Fänge and Grove, 1979). In cephalopods, the simultaneous digestion and absorption occurring in the stomach and caecum respectively mean that much of the digestion is over when gastric evacuation occurs (Boucher-Rodoni and Mangold, 1977), and the role of the intestine is apparently quite restricted (Boucaud-Camou *et*

al., 1976). Boucher-Rodoni's (1975) estimate of the total duration of digestion in *I. illecebrosus* (12–13 hours) was based on a projection from the percent of the meal remaining in the stomach at times less than 7 hours to the time when all of the meal would have been gone. Lack of information on meal size prevents a direct comparison of the results with the present study, but a similar projection based on the decrease in the organic content of the meal with time indicated that digestion was probably complete in about 28 hours. This may be an overestimate due to the late capture of some large animals with large meals. A digestion time of 12–13 hours may be a reasonable estimate for small meals, but larger meals may take as long as 36 hours to digest completely.

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