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Marker technique for investigating gut throughput rates in coral reef fishes

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Abstract *Artemia* sp. shells were evaluated to determine their accuracy for tracing the passage of algal filaments through the gut of the damselfish *Pomacentrus amboinensis* Bleeker, 1868 (family Pomacentridae), an omnivorous coral reef fish. An automatic faeces-collection apparatus enabled the quantitative collection of markers and faeces in the laboratory. Defecation rates were similar for light and heavy doses of *Artemia* sp. shells and controls, indicating no detrimental effects of *Artemia* sp. shells on the gut throughput rate of *P. amboinensis*. In addition, similar rates and patterns of the passage of *Artemia* sp. shells and the algal markers *Enteromorpha* sp. and *Lynghya* sp. indicated that *Artemia* sp. shells provide a reliable representation of the throughput rate of algal filaments. The mean throughput time of *P. amboinensis* was 4.6 h \pm 0.3 SE, with a modal recovery time of 4 h. Laboratory throughput estimates were validated by comparing the distribution patterns of *Artemia* sp. shells in the dissected gut of specimens administered markers in the laboratory and field. In addition, the retention of markers in the stomach of *P. amboinensis* suggested a likely site of prolonged processing.

Introduction

Studies of gut throughput rates provide essential information on both ecological and physiological aspects of digestion and nutrition in fishes. Although throughput rates have been studied widely and successfully in terrestrial animals, throughput studies in aquatic systems have been restricted mainly to aquacultural and food fishes, with a major emphasis on gastric emptying rates

(Wetherbee et al. 1987). In studies of reef trophodynamics, throughput rates provide a basis for estimating daily rates of food intake and nutrient turnover by reef fish (Polunin 1988), and when combined with assimilation studies they also provide a key to examining energy budgets and nutrition (Targett and Targett 1990). More importantly, with respect to the focus of the present study, gut throughput rates can provide a basis for investigating complex digestive processes such as those found in herbivorous reef fishes (Horn and Messer 1992).

Several techniques have been developed to estimate gut throughput rates in terrestrial and aquatic animals. These methods include the use of markers added to the diet, visual observations of the time from the first bite until the first appearance of faeces, comparisons of cumulative faeces production over time with gut fullness, x-radiographic techniques, and mathematical modelling of gut emptying through serial slaughter methods and stomach pumping (Fänge and Grove 1979; Warner 1981). In terrestrial studies, the use of markers added to the diet has proven to be the most precise, practical and informative method for determining gut throughput rates (Uden et al. 1980; Martínez del Rio et al. 1994). Most importantly, markers can be used to estimate the mean gut throughput time (Warner 1981). This parameter provides the most valuable description of the passage of digesta through the gut (Penry and Jumars 1987; Martínez del Rio et al. 1994).

Although markers have been used widely and successfully in terrestrial studies, problems associated with the administration and collection of markers in the aquatic medium have restricted their application. In order to understand gut function in fishes, a reliable marker technique is required. This marker should be easy to use in the laboratory as well as in the field. It should be quantifiable in the faeces or dissected gut to provide full marker distributions, from which mean or modal throughput times may be calculated. The marker should also be indigestible, chemically stable and of small size to provide minimal disruption to digestive function. Most importantly, the marker should trace the path of natural

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dietary components through the gut to obtain accurate throughput estimates. The aim of this study therefore, was to evaluate the properties of *Artemia* sp. shells as a marker for tracing the passage of algal filaments through the gut of an omnivorous coral reef fish.

Materials and methods

Experimental apparatus

Each specimen of *Pomacentrus amboinensis* was maintained in an inverted 2-litre plastic softdrink container (Fig. 1). Six experimental containers were suspended in each of two adjacent 150-litre glass aquaria in a single, dedicated, closed seawater system. For the purpose of analyses, the system was a single unit. The inverted softdrink containers funnelled the faeces from each fish into a 25 mm clear plastic hose which led to a collection tray at the bottom of each tank (Fig. 1). Each container was covered in black plastic to reduce stress and to minimise interactions between experimental subjects. The plastic also reduced algal growth inside the containers which may trap faeces.

The collection trays consisted of a plastic grid composed of $20 \times 20 \times 30$ mm deep squares mounted on a glass base. Each cell on the grid collected the faeces from one individual for either 1 or 2 h (depending on the experiment). Collection trays were enclosed within clear Perspex boxes to prevent disturbance of the faeces when moving the tray. The tray was automated using a 240 V motor and electronic timer (Fig. 1) connected to the collection trays via a fine stainless steel cable and pulley system. This system allowed trays to be advanced by one cell every hour over 12 h or one cell every 2 h over 24 h.

Optimal water quality was maintained by incorporating a central biological and mechanical filtration unit with UV sterilisation, which provided a steady flow of water to each experimental container at 15 to 20 litres h^{-1} . Inflowing water entered the containers near the surface and was projected around the walls to create a circular current to keep faecal pellets off the sides of the containers, allowing them to fall directly into the collection trays. Water flowed out of the containers near the surface to ensure that faeces were not lost in the outflow. An aquarium heater maintained the experimental tanks at between 25.5 and 26.5 °C. Natural illumination provided daylight from $\approx 06:00$ to $18:30$ hrs.

Experimental subjects

Ambon damselfish, *Pomacentrus amboinensis* Bleeker, 1868 (family Pomacentridae) (45 to 60 mm standard length, SL), were collected from Orpheus Island, central Great Barrier Reef ($18^{\circ}37'S$;

$146^{\circ}30'E$). All fish were acclimatised for a minimum of 2 wk in the experimental setup before experiments began. Any fish which did not feed readily by the end of the acclimatisation period was not included in the experiment.

General experimental procedure

Artemia sp. eggs were hatched over 36 h in aerated seawater. Empty shells were carefully siphoned out to separate them from nauplii. The shells were then aerated in seawater for 2 h, followed by a repeat of the siphoning process to obtain a near pure sample of *Artemia* sp. shells. Shells were rinsed for 2 min in fresh water to prevent coagulation, and stored frozen until needed.

Fish were presented with a continuous supply of fresh algae (*Enteromorpha* sp.) in a plastic mesh cylinder suspended from the top of each container. Fish began feeding at approximately 06:00 hrs, which allowed a preliminary 2 to 3 h feeding period prior to the introduction of markers. At a set time, a measured dose of *Artemia* sp. shells mixed with seawater was added to each container. The mesh feeders were removed prior to administration of the marker to prevent *Artemia* sp. shells mixing with the algae and thus possibly being consumed later. Water flow to each container was also stopped for 5 min to allow fish time to consume the *Artemia* sp. shells before they were flushed out or fell through to the collection trays. Once the remaining marker had been flushed out, the mesh feeders were replaced and fish resumed feeding on algae ad libitum. Fish were then left undisturbed for the entire experimental period while markers and faeces were automatically collected. At the conclusion of the experiment, the faeces-collection trays were enclosed within the Perspex boxes and carefully removed from the tanks.

Faeces were removed sequentially from each cell of the collection tray and examined under a stereo dissecting microscope to record the number of *Artemia* sp. shells. This produced marker-recovery distributions from which mean throughput times could be calculated. Faecal samples from each cell were filtered through pre-dried and pre-weighed paper, then dried at 60 °C to constant weight.

Marker trials

Precision of the marker

An initial marker trial was conducted to determine the precision of the marker and to estimate the gut throughput rate of *Pomacentrus amboinensis*. Twelve *P. amboinensis*, (48 to 56 mm SL, 4.0 to 7.2 g), were used, with *Artemia* sp. shells introduced at 09:00 hrs and faeces and markers collected hourly for 12 h.

A second trial was conducted to determine if there was any significant retention of material in the gut of *Pomacentrus amboinensis* overnight. Six fish (46 to 55 mm SL) were administered markers at 15:00 hrs. Markers and faeces were collected hourly until 02:00 hrs. The last cell of the collection tray collected markers and faeces excreted between 02:00 and 09:00 hrs. Food was removed after dark so that none was present the following morning. Any faeces present in the last cell would therefore be a product of the previous day's feeding.

Effects of *Artemia* sp. shells on digestive function

The effects of *Artemia* sp. shells on digestive function were examined through the administration of two marker treatments, consisting of a light (25 to 100 *Artemia* sp. shells per fish) and a heavy (200 to 600 *Artemia* sp. shells per fish) marker dose. These were compared to a control treatment where no marker was fed. A randomised block design was employed to minimise variability between subjects. Twelve fish (47 to 53 mm SL, 3.4 to 6.4 g) were used, with four fish randomly allocated to each of the three treatments. Light and heavy marker doses were introduced to each bottle at 09:00 hrs following the general experimental procedure.

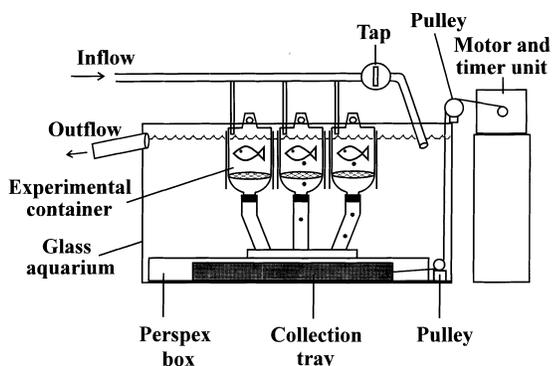


Fig. 1 Experimental apparatus, lateral view, showing 3 of the 12 experimental containers (Modified after Galetto and Bellwood 1994)

Seawater without *Artemia* sp. shells was introduced to the control subjects. Markers and faeces were then collected hourly for 12 h. Following a 2 d clearing period, treatments were reallocated for a further two trials (ensuring that no fish received the same treatment twice). All fish received an ad libitum diet of *Enteromorpha* sp. algae throughout the experiments.

The cumulative percent of faeces recovered over 12 h was calculated to examine any chronic effects of marker dosage on the rate of faeces production over time. However, any direct adverse effects of *Artemia* sp. shells on faeces production would most likely reach a maximum when the bulk of the marker was leaving the gut. Since the modal marker recovery time had been determined from initial marker trials to be 4 h, the mean weight of faeces accumulated after this time was calculated for each treatment and compared using a one-way analysis of variance. Marker recovery curves and mean throughput times for light versus heavy marker doses were also compared to examine the effects of the marker dosage on the marker-throughput pattern.

Marker properties of *Artemia* sp. shells for tracing algal filaments

The accuracy of *Artemia* shells for tracing the path of algal filaments through the gut of *Pomacentrus amboinensis* was examined by two marker-comparison experiments. *Artemia* sp. shells were compared against dietary markers consisting of filaments of the green algae *Enteromorpha* sp. and the cyanophyte *Lyngbya* sp.

In the first experiment, fish were fed ad libitum on a diet of the green algae *Cladophora* sp. Six fish were allocated to each of the treatments (*Artemia* sp. shells vs algal marker). Markers were introduced at 09:00 hrs following the general experimental procedure. Filaments of the algal marker *Enteromorpha* sp. were cut into 1 cm lengths to aid in quantification of the faeces, and were introduced using mesh feeders for a period of 15 min. Markers and faeces were then collected hourly for 12 h. Two fish which did not eat the algal marker were not included in the analyses. Quantification of the algal marker was achieved by counting the total number of filaments of *Enteromorpha* sp. present in each faecal sample. In faecal samples, *Enteromorpha* sp. could be identified as thick uniseriate filaments amongst the much thinner, multiseriate filaments of *Cladophora* sp.

Following a 10 d clearing period to allow fish to void any remaining marker and to recover from any possible marker effects, *Artemia* sp. shells were compared to the second algal marker, *Lyngbya* sp. Fish were fed ad libitum on a diet of *Enteromorpha* sp. throughout the experiment. The algal marker *Lyngbya* sp. was cut into 5 mm lengths to aid in identification and quantification of the faeces. In faecal samples, *Lyngbya* sp. could be identified as fine, black, hair-like filaments amongst the much thicker green filaments of *Enteromorpha* sp. Six fish were allocated to each treatment. One fish did not eat the *Artemia* sp. shells and was excluded from the analyses.

Field trials

Field marker trials were conducted at Orpheus Island (central Great Barrier Reef) to directly compare the results in the laboratory with the field as a test of the laboratory data, and to investigate the effects of time of day and fish size on gut throughput rates. The effects of time of day on throughput rates were examined by administering markers at a recorded time during two separate periods: 08:55 to 09:35 hrs and 14:45 to 15:40 hrs. Effects of fish size on throughput rates were investigated by examining three size classes of *Pomacentrus amboinensis* in field trials: <40, 40 to 50 and > 50 mm SL (ca. <2.6, 2.6 to 5.4 g and > 5.4 g). The numbers of specimens used for each time of day and each size class are shown in Table 1.

Groups of 4 to 5 fish were selected which were site-attached to small pieces of coral or rubble in open sandy expanses. Each site was marked with plastic tape and mapped for later reference. At a recorded time, *Artemia* sp. shells were introduced into the water

Table 1 *Pomacentrus amboinensis*. Number of specimens per time of day (morning 08:55 to 09:35 hrs; afternoon 14:45 to 15:40 hrs) and size class (SL standard length) used in field-marker trials

Size class	Morning	Afternoon	Total
Small (< 40 mm SL)	15	22	37
Medium (40 to 50 mm SL)	5	10	15
Large (> 50 mm SL)	5	12	17
Total no. specimens	25	44	69

column upcurrent of the sites. A limited quantity of *Artemia* sp. shells was used to ensure that the number of shells ingested was kept below 100 per fish. Once the markers had been consumed, fish were left undisturbed for an average of 1 h 30 min (± 8 min range). Fish were caught as quickly as possible using fence nets, killed immediately upon capture, and placed on ice within 40 min. Fish were then dissected, and the entire alimentary tract was removed and divided into the stomach and ten equal intestinal portions. The stomach and all ten intestinal segments were then opened under a stereo dissecting microscope and the number of *Artemia* sp. shells in each segment recorded.

A similar procedure was applied to 16 laboratory specimens (45 to 56 mm SL) to enable a direct comparison of gut marker distributions with 22 field specimens of similar size. Markers were introduced at 09:00 hrs, after which fish continued to feed ad libitum on *Enteromorpha* sp. for 1 h 30 min before being sacrificed and dissected.

Statistical procedures

Marker distributions were compared using repeated measures analyses of variance on the SASTM statistical package (Version 6.08 for Windows). All marker distributions violated sphericity tests due to the non-independence of samples through time. Therefore, Greenhouse-Geisser adjusted probabilities were used as replacements for normal *p*-values. Mean throughput times were compared using Student's two-sample *t*-tests and one-way analyses of variance on the STATGRAPHICSTM statistical package (Version 6.0). Data used in ANOVA's and Student's *t*-tests were not found to violate assumptions of normality and homoscedasticity.

Results

Precision of marker

The initial marker trial provided a full marker-recovery distribution over a 12 h period for *Pomacentrus amboinensis* (Fig. 2). The time of first appearance of *Artemia* shells was ≤ 1 h. This was followed by a sharp rise to a modal recovery after 4 h. The recovery distribution then followed a negative exponential decay until the time of last appearance at ≥ 11 h. The mean throughput time (\pm SE) for *P. amboinensis* (48 to 56 mm SL, 4.0 to 7.2 g) was estimated to be 4.6 ± 0.3 h.

The marker recovery distribution for the overnight trial (Fig. 3) was very similar to the daytime trial (Fig. 2). The mean throughput time (\pm SE) for the overnight trial was calculated to be 4.7 ± 0.6 h. The faeces output curve for the overnight trial (Fig. 4) showed a peak in faeces production between 16:00 and 17:00 hrs followed by a steady decrease. After feeding

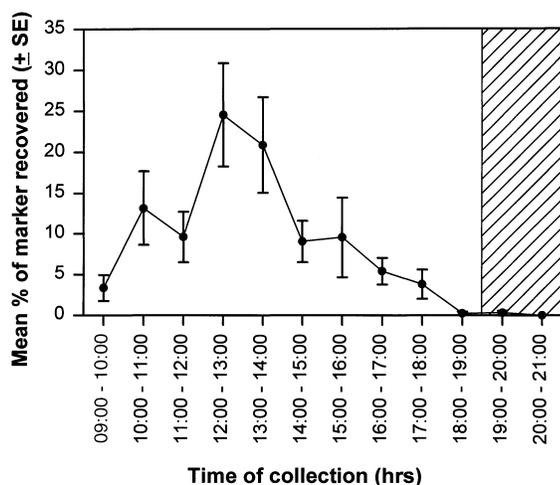


Fig. 2 *Pomacentrus amboinensis*. Hourly marker-recovery distribution over 12 h ($n = 12$; marker introduced at 09:00 hrs) (Hatched area period of darkness)

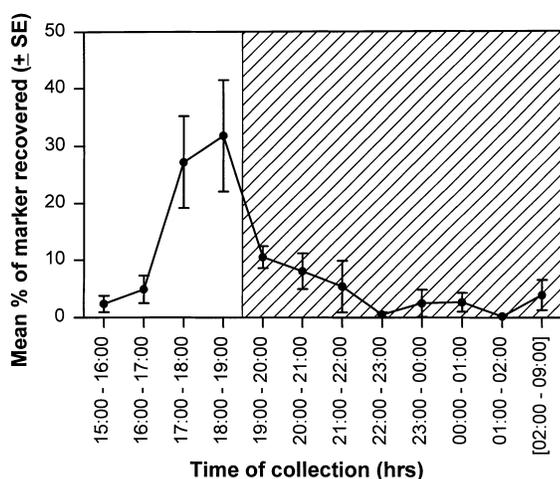


Fig. 3 *Pomacentrus amboinensis*. Overnight marker-recovery distribution ($n = 6$; marker introduced at 15:00 hrs). Samples collected hourly except from 02:00 to 09:00 hrs when samples were pooled over total time period (Hatched area period of darkness)

had ceased at $\approx 19:00$ hrs, faeces continued to be produced at decreasing rates until approximately 02:00 hrs, after which time virtually no faeces were collected.

Effects of *Artemia* sp. shells on digestive function

The cumulative production of faeces over 12 h was similar for each of the light and heavy marker doses as well as the control subjects (Fig. 5). In addition, the mean dry weight of faeces accumulated after 4 h (Table 2) showed no significant difference between treatments ($df = 4$, $F = 0.51$, $p = 0.73$). A repeated-measures analysis of variance showed that the differences observed in marker recovery distributions (Fig. 6)

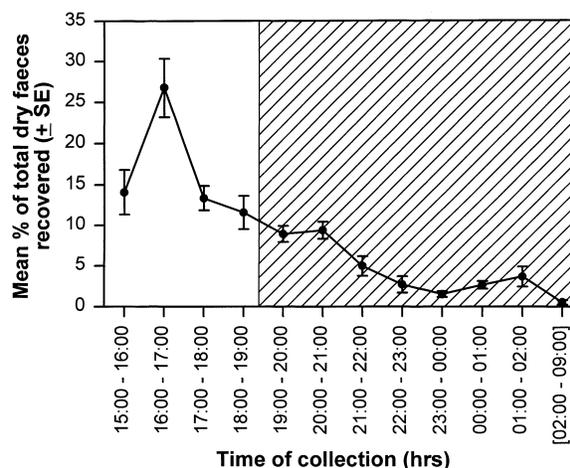


Fig. 4 *Pomacentrus amboinensis*. Overnight faeces production ($n = 6$; marker introduced at 15:00 hrs). Samples collected hourly, except from 02:00 to 09:00 h when samples were pooled over total time period (Hatched area period of darkness)

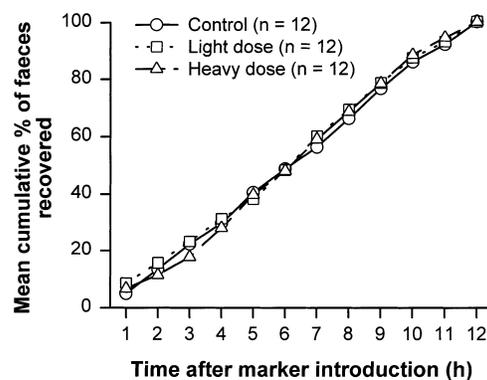


Fig. 5 *Pomacentrus amboinensis*. Cumulative faeces production over 12 h for light and heavy marker treatments vs controls (marker introduced at 09:00 hrs)

were not significant ($p = 0.09$; Table 3). In addition, the mean throughput time (\pm SE) for the light dose (4.8 ± 0.4 h) was not significantly different from that observed for the heavy dose (4.5 ± 0.2 h; $df = 2$, $F = 0.20$, $p = 0.82$). However, it appears that there may be a slight shift in the recovery distributions for heavy doses compared with light doses, indicated by a modal throughput time of 4 h for heavy marker doses compared with 3 h for light doses (Fig. 6).

Table 2 *Pomacentrus amboinensis*. Faeces produced after 4 h in light and heavy marker treatments vs controls ($n = 12$; marker introduced at 09:00 hrs; $p = 0.73$)

Treatment	Mean faeces dry wt (g, \pm SE)
Control	0.35 \pm 0.04
Light	0.32 \pm 0.05
Heavy	0.34 \pm 0.04

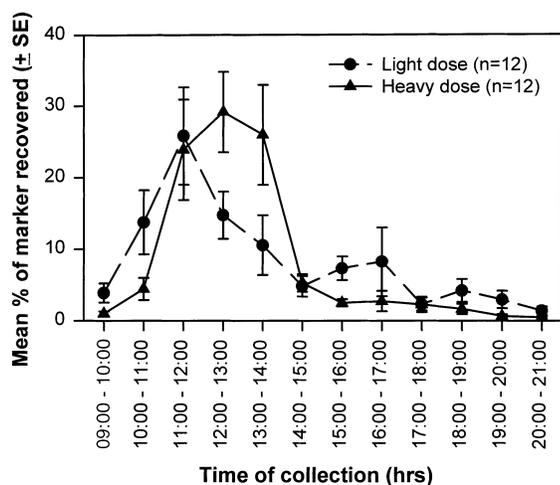


Fig. 6 *Pomacentrus amboinensis*. Hourly marker-recovery distribution over 12 h for light vs heavy marker treatments (marker introduced at 09:00 hrs)

Marker properties of *Artemia* sp. shells for tracing algal filaments

Marker-recovery distributions were similar for both *Artemia* sp. shells and *Enteromorpha* sp. (Fig. 7). A repeated-measures analysis of variance revealed no significant difference between the recovery distributions of the two markers ($p = 0.25$; Table 3). In addition, the mean throughput time (\pm SE) calculated for *Artemia* sp. shells (4.29 ± 0.20 h) was not significantly different from that obtained for *Enteromorpha* sp. (5.00 ± 0.51 h; $df = 8$, Student's $t = -1.337$, $p = 0.22$), although the modal recovery time was shorter for *Artemia* sp. shells (Fig. 7).

The marker-recovery distributions obtained for *Artemia* sp. shells and the second algal marker (*Lyngbya* sp.; Fig. 7) likewise showed no significant difference in marker-recovery distributions when examined using a repeated-measures analysis of variance ($p = 0.10$; Table 3). In addition, mean throughput estimates (\pm SE) for *Artemia* sp. shells (3.91 ± 0.13 h) were not significantly different from those obtained for *Lyngbya* sp. (3.97 ± 0.47 h; $df = 9$, Student's $t = -0.0989$, $p = 0.9244$). However, the modal recovery for *Lyngbya* sp. was earlier than for *Artemia* sp. shells (Fig. 7). Overall, even though there may be slight differences in the recovery distributions of *Artemia* sp.

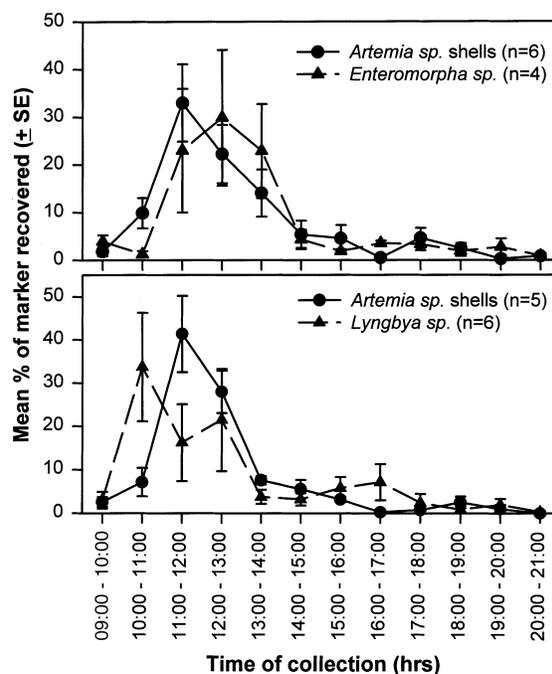


Fig. 7 *Pomacentrus amboinensis*. Hourly marker-recovery distributions for *Artemia* sp. shells vs *Enteromorpha* sp. and *Artemia* sp. shells vs *Lyngbya* sp. (markers introduced at 09:00 hrs)

shells compared with the two algal markers, the marker-recovery distributions of *Artemia* sp. shells lay between those of the two algal markers.

Field trials

Marker distributions in the gut of field specimens were very similar to those obtained for laboratory specimens using similar techniques (Fig. 8b). A repeated-measures analysis of variance revealed no significant difference in the gut marker distributions ($p = 0.30$; Table 3). In both cases, a significant proportion of marker was retained in the stomach, producing a bimodal distribution of the marker in the gut.

A two-way repeated-measures analysis of variance revealed no significant interaction between time-of-day and fish size effects on marker distributions ($p = 0.44$; Table 4) and no significant effects of time of day on marker distributions ($p = 0.49$; Table 4). The shape of

Table 3 *Pomacentrus amboinensis*. Results of repeated-measures analyses of marker-recovery distributions (*Experiment a* light vs heavy marker doses; *b* *Artemia* sp. shells vs algal marker *Enteromorpha* sp.; *c* *Artemia* sp. shells vs algal marker *Lyngbya* sp.;

d repeated-measures analysis results for gut-marker distributions in laboratory vs field trials; *Adjusted p* Greenhouse–Geisser-adjusted probabilities for non-independent repeated measures; *MS* mean square)

Experiment	Source of variance	(df)	MS	F	Adjusted p
a	Time \times dose	(11)	0.03	2.29	0.09
b	Time \times marker	(11)	0.02	1.47	0.25
c	Time \times marker	(11)	0.04	2.40	0.10
d	Gut segment \times trial	(10)	169	1.23	0.30

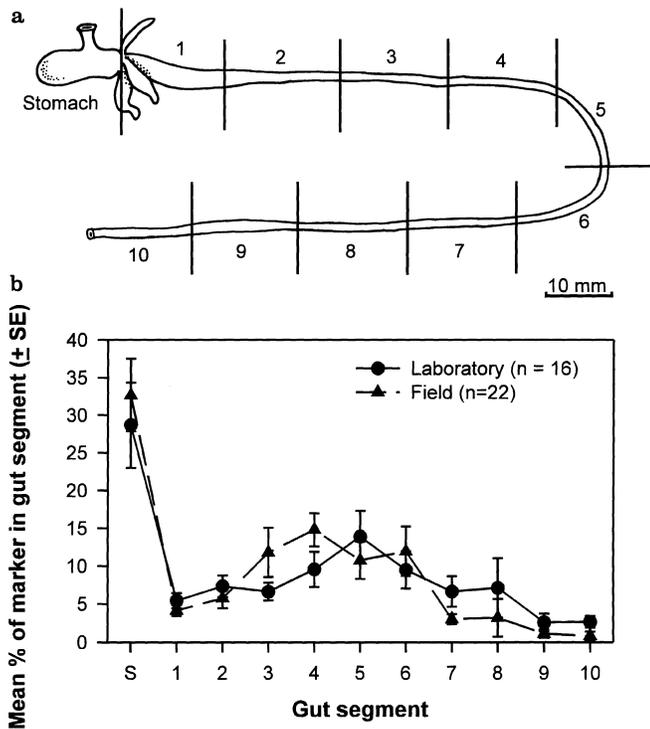


Fig. 8 *Pomacentrus amboinensis*. **a** Dissected gut of 66 mm standard-length fish showing size and location of stomach and 10 equal intestinal portions (segments) used in analyses (total gut length = 183 mm); **b** distribution of markers in guts of field vs laboratory specimens 1 h 30 min after marker introduction (S stomach)

Table 4 *Pomacentrus amboinensis*. Results of two-way repeated-measures analysis testing for differences between effects of time of day, fish size and interaction on marker distributions in guts of field specimens (Adjusted *p* Greenhouse–Geisser-adjusted probabilities for non-independent repeated measures)

Source of variance	(df)	MS	F	Adjusted <i>p</i>
Gut segment × time of day	(10)	182	0.85	0.49
Segment × size	(20)	348	1.62	0.12
Segment × time of day × size	(20)	214	1.00	0.44

the marker distributions were broadly similar for each size class (Fig. 9), although smaller fish showed a mode in marker distributions further down the gut than the two larger size classes, suggesting a slightly faster throughput time. However, this difference was not statistically significant ($p = 0.12$; Table 4). A macroscopic examination of the guts revealed no morphological differences between fish within the size ranges sampled.

Discussion

Artemia sp. shells provide a reliable marker of algal filaments in *Pomacentrus amboinensis* in both the laboratory and field. *Artemia* sp. shells provide precise estimates of mean and modal throughput times in the

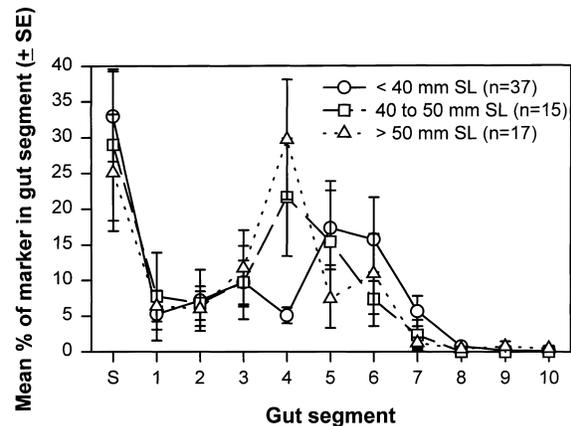


Fig. 9 *Pomacentrus amboinensis*. Distribution of markers in guts of field specimens from each of three size classes 1 h 30 min after marker introduction [morning and afternoon samples were not significantly different (Table 4) and were pooled] (S stomach; SL standard)

laboratory. Mean throughput times are considered to be the most accurate and useful description of the passage of the digesta through the gut (Warner 1981). Initial trials of the marker and faeces collection apparatus provided a mean (\pm SE) gut throughput estimate of 4.6 ± 0.3 h for *P. amboinensis* ranging in size between 4.0 and 7.2 g. These results are comparable to estimates for other genera of pomacentrids. Polunin and Koike (1987) estimated the throughput time for *Plectroglyphidodon lacrymatus* to be 5 to 6 h, while Lassuy (1984) gave an estimate of 4.5 h for a 1.5 g juvenile *Stegastes lividus*.

In addition to precise mean throughput estimates, the *Artemia* sp. shells marker-technique also provided a full marker-recovery distribution for *Pomacentrus amboinensis* over a 12 h period. This distribution provides precise, empirical data, ideal for the application to gut-modelling theories such as those of Penry and Jumars (1986), who investigated digestive systems using chemical reactor models. When markers are introduced to each type of reactor, distinctive marker-recovery curves are produced (Penry and Jumars 1986; Martínez del Río et al. 1994). The most striking feature of the marker-recovery distribution obtained for *P. amboinensis* is that it does not indicate a simple plug-flow of the marker through the alimentary tract (i.e. the marker does not pass through the gut in a single band). A plug-flow alimentary tract would be represented as a single narrow peak in the marker-recovery curve (Penry and Jumars 1986). This type of flow is assumed in all studies which use the time of first or last appearance of the marker as an estimate of throughput rate (e.g. Bardach 1961; Randall 1961; Fris and Horn 1993). In the present study, the first appearance of the marker was ≤ 1 h and the last of the marker was not recovered until at least 10 h after administration, while the bulk of digesta passed through between 3 and 5 h. If this marker output is indicative of other reef species, this raises serious doubts as to the accuracy of techniques which use the time of first or last appearance to estimate throughput rates. This empha-

sises the question of precision. Our technique provides precise estimates of mean and modal passage rates. However, the throughput time of an individual marker particle is inherently imprecise.

Overnight retention of digesta is thought to occur in some herbivorous species to aid in microbial fermentation (Fishelson et al. 1985). Since the overnight marker-recovery curve (Fig. 3) and mean throughput times were similar to those obtained in the daytime trial (Fig. 2), it appears that the gut of *Pomacentrus amboinensis* continues to empty at a steady rate once feeding has ceased, with no significant retention of digesta overnight. The lack of overnight retention is further supported by the overnight faeces production (Fig. 4). Virtually no faeces were produced between 02:00 and 09:00 hrs, indicating an empty gut after 02:00 hrs. These results are consistent with observations of the pomacentrid *Stegastes nigricans*, which has no particulate digesta in the gut prior to feeding in the morning (Bellwood unpublished data).

Light and heavy doses had negligible effects on mean throughput rates, whilst the shapes of marker-recovery curves were only slightly different (Fig. 6). The shapes of marker-recovery curves depend on the rate and pattern of mixing of markers with digesta in the gut (Penry and Jumars 1987). Therefore, slight differences in the position of modes and the shape of marker-recovery curves were most probably a result of the incomplete mixing of heavy marker doses with digesta. The comparison of *Artemia* sp. shells with the algal markers *Enteromorpha* sp. and *Lynghya* sp. also produced consistent results. The overall shape of marker distributions and mean throughput rates were not significantly different for each marker, with the marker recovery distributions for *Artemia* sp. shells, lying between those of the two algal markers. *Artemia* sp. shells, therefore, would appear to provide a reliable overall description of the passage of algal filaments through the gut of *Pomacentrus amboinensis*.

Gut-marker distributions obtained from laboratory and field trials were similar (Fig. 8b), suggesting that the conditions imposed by the experimental apparatus did not significantly alter throughput rates of *Pomacentrus amboinensis*. The laboratory data, therefore, may be taken as an accurate representation of the throughput rates of fish in the field.

The lack of any significant effect of fish size on throughput rates (Fig. 9) was unexpected. Lassuy (1984) showed a doubling of throughput time for the herbivorous damselfish *Stegastes lividus* with an increase in body size from 1.5 g to between 18 and 45 g. In contrast, in the present study, virtually no difference in throughput rates was observed for fish ranging between 0.9 and 13 g. Although this may have been due to interspecific differences or the smaller size range sampled in this study, it appears likely that fish size has a relatively small influence on throughput times in *Pomacentrus amboinensis*.

The application of the *Artemia* shells marker-technique to a wide range of studies appears promising. This

technique provides a valuable tool for the investigation of digestion in reef fishes. Gut-marker distributions obtained through this technique may be used to identify regions where digesta are retained. These regions may indicate likely sites of prolonged mechanical, digestive or fermentative action. Previously these areas have been identified based on morphological inference or speculation based on gut contents (e.g. Rimmer and Wiebe 1987). The present technique enables the realised function of the various regions of the gut to be examined. For example, the retention of markers was observed in the stomach of both laboratory and field specimens, indicating a likely site of storage and/or prolonged processing. In addition, *Artemia* sp. shells provide a precise estimate of the mean gut retention time of algal filaments, suggesting possible modes of digestion. The relatively fast mean passage rate (4.6 h) for *Pomacentrus amboinensis*, combined with no significant overnight retention, suggests that microbial fermentation which requires prolonged retention of digesta is unlikely to play a significant role in this species (cf. Horn 1989). The application of the technique to species in which microbial fermentation is likely to play a greater role (Clements and Choat 1995) would provide invaluable information on the nature of microbial fermentation in marine fishes.

In addition to studies of digestion, the technique also shows great promise in its application to studies of reef processes, particularly trophodynamics. In any study of reef system processes, from the production and transport of sediment (Bellwood 1995a, b) to nutrient flux (Hatcher 1981; Polunin et al. 1995), one of the key parameters is the passage rate of material by fish. Estimates of passage rates, if combined with assimilation studies, would also provide a more detailed appraisal of energy budgets and nutrition in coral reef fishes. A precise estimate of fish gut throughput represents a key step in our understanding of rate processes on coral reefs.

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