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Influence of larval feeding history on the body condition of *Amphiprion melanopus*

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The cellular condition of liver hepatocytes and the height of gut epithelium cells of larval *Amphiprion melanopus* were sensitive indicators of feeding condition. Muscle fibres of the trunk showed marked separation in fish fed every third day just prior to settlement. Low feeding regimes also caused reductions in growth, increases in larval duration and reductions in size at metamorphosis. Gut epithelium cell height was also influenced by fish standard length and age. This study suggests that gut epithelium cell height is a useful index for an examination of the importance of starvation of larvae in tropical waters; however, size and age standardization is required prior to comparisons of wild caught fish through time or with laboratory samples.

Key words: coral reef fish; body condition; larvae; histological indices.

INTRODUCTION

Fishes on coral reefs are characterized by large fluctuations in recruitment (Doherty, 1988; Sale *et al.*, 1994). These fluctuations in cohort strength may be governed in part by environmental conditions affecting growth and development of larvae (Meekan & Fortier, 1996; Theilacker *et al.*, 1996). Periods of low food availability may not only cause death through starvation (Blaxter & Ehrlich, 1974), but can lead to malnutrition (Ehrlich *et al.*, 1976), lower growth (Theilacker & Watanabe, 1989), reduced performance (Margulies, 1993) and ultimately lower probabilities of survival through increased predation (Neilson *et al.*, 1986; Rice *et al.*, 1987; Miller *et al.*, 1988). Recent studies have shown that the body condition of newly settled fish within and between recruitment episodes is highly variable (McCormick & Molony, 1993; Kerrigan, 1996), but at present the causes of this variability are unclear.

The body condition of newly settled fish may provide an important insight into the environmental conditions experienced by fish during their larval phase (McCormick, 1998). A variety of histological and morphological measures of body condition have been used successfully to discriminate between fish of different feeding histories (Theilacker & Watanabe, 1989; Margulies, 1993; Theilacker *et al.*, 1996). However, the temporal response of fish to food intake is likely to be species specific, and experimental studies are required to facilitate interpretation in relation to feeding history for species targeted for research.

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The objective of the present study was to examine the response of a common tropical reef fish to varying levels of food availability during the larval phase, and whether differences in feeding history could be quantified by currently recommended techniques (Ferron & Leggett, 1994; Theilacker *et al.*, 1996; Suthers, 1998). This was the first step toward determining the contribution of variable food availability to recruitment fluctuations. Specifically, our study examined experimentally the influence of larval feeding history on the body condition of larvae and post-settlement juveniles of the coral reef fish *Amphiprion melanopus* Bleeker (Pomacentridae). A comparison was made between the body condition of fish reared under conditions of constant food availability and two cyclical levels of food availability (fed every second day, and fed every third day). *A. melanopus* was chosen as a model species because of its typical bipartite life cycle, its relatively short larval phase and high growth and development rates, which facilitate successful experimental manipulation. This study represents one of the first to examine the importance of starvation in tropical waters for coral reef fish larvae through to metamorphosis and settlement.

MATERIALS AND METHODS

REARING

Amphiprion melanopus were hatched and reared at the James Cook University aquarium facility. Adult *A. melanopus* were provided with a terracotta pot as artificial substratum to lay their eggs on. Eggs were laid in clutches of 500–1000 and took *c.* 8–10 days to develop. A single clutch of full-sibling eggs was hatched into a 140-l glass tank that was coated in an external black gel-coating and temperature was maintained at $28 \pm 1^{\circ}$ C. Air passing through an airstone maintained water circulation. A culture of *Nannochloropsis* sp. algae was added to the tank each morning at a density such that the airstone on the bottom of the tank was not visible (emulating a secchi disc). The algae was required to diffuse light and reduce reflections off the aquarium that attract the highly phototactic fish as well as increase contrast for feeding and act as food for the zooplanktonic prey items (Naas *et al.*, 1992). Fluorescent lights were used to simulate a 14-h summer daylight cycle. The hatching tank was maintained as a semi-closed system that was flushed nightly when the lights were off. Salinity was maintained between 33 and 34·7 psu.

Upon hatching, *A. melanopus* can feed immediately, and so at daylight (controlled by lighting), larvae were fed rotifers (*Brachionus* sp.) at a density of $\sim 5\ \mathrm{ml}^{-1}$, boosted with yeast (Daintitch, 1993) and *Nannochloropsis* sp. algae. There were *c.* 350 larvae in the hatching tank. On the third day post-hatching larvae were transferred from the single hatching tank to the experimental set-up consisting of six individual tanks.

EXPERIMENTAL SET-UP

To determine the effect of periodic feeding on body condition of larval *Amphiprion melanopus*, fish were subjected to three different feeding regimes. The treatments consisted of: fed *ad lib* (fed every day); fed 1/2 (fed every second day); and fed 1/3 (fed every third day). Feeding treatments were allocated randomly to each of six tanks (two tanks per treatment). Individual larvae were distributed randomly among the experimental tanks (54 per tank). All fish were fed upon transfer to the experimental setup, to reduce any stress from food limitation combining with the disturbance of transfer. Feeding manipulations began the day after transfer.

The experimental set-up comprised six black aquaria ($61 \times 30 \times 38$ cm or 70 l), each equipped with a 300-W water heater, an airstone, a water inlet and outlet and fluorescent lighting. Fly-screen mesh covered the outlet pipes to retain larvae. Temperature was maintained at $28 \pm 1^{\circ}$ C. Every morning tanks were inspected by torchlight and dead fish

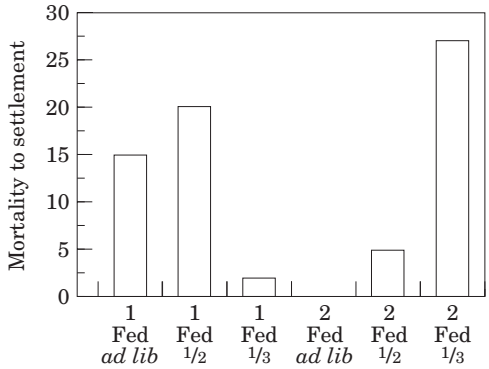

FIG. 1. Number of dead *Amphipion melanopus* among three feeding treatments, with two replicate tanks per treatment, from 3 dph until settlement. Fed *ad lib*, Fed every day; fed 1/2, fed every second day; fed 1/3, fed every third day.

and debris were siphoned off the bottom of the tanks. *Nannochloropsis* sp. algal culture was added prior to turning the lights on, again to a density such that the airstone at the bottom of each tank was no longer visible. *Artemia* nauplii were then added at a density of *c.* 2 ml^{-1} (Schoedinger & Epifanio, 1997) as per the requisite feeding schedule. *Artemia* densities were checked three times daily. In order to check prey densities, four 10-ml aliquots were taken from different areas and depths of the tanks with a pipette, and the number of *Artemia* nauplii were counted and the average per ml taken. More *Artemia* were added if necessary. Fish were fed 12–24-h-old *Artemia* from 1–4 days post-hatching (dph), and 12–48-h-old *Artemia* for fish >4 dph. *Artemia* nauplii were decapsulated following the methods of Daintitch (1993). Fish were reared until they had all settled.

SAMPLING

Prior to feeding, 10 fish were sampled from each tank at 5 and 8 days post-hatching. Eight days post-hatching coincided with settlement for the continuously fed treatment. Fish were caught randomly with a dip-net, put into vials of salt-water and then submerged in ice until dead. Fish were then fixed in marine Bouin's for 2–3 h, then flushed and stored in 70% alcohol.

Standard length was measured on all preserved specimens, using a stage graticule and an ocular micrometer (Wild) on a dissecting microscope. Measurements were recorded to the nearest 0·1 mm. Lengths were adjusted for shrinkage due to death (L_S) and preservative (L_{SP}) using the regression equation:

$$L_S = 0{\cdot}8611 + 0{\cdot}9506 L_{SP},\ r^2 = 0{\cdot}972,$$

calculated from measuring 40 live larvae and remeasuring them 120 days after preservation in marine Bouin's.

Mortalities were recorded throughout the experiment (Fig. 1), however patterns were not related to the feeding treatments and for this reason will not be examined further.

HISTOLOGY

Fish were processed for histology with standard histological techniques (Windsor, 1994). Whole fish were embedded in paraffin wax and sectioned in the sagittal plane at 5 μm, mounted and stained with Mayer's haematoxylin and eosin.

CONDITION MEASURES

Gut epithelium cell height

To investigate the response of the intestinal tract to feeding history, cell height of the epithelium lining the gut was measured. Randomly selected cells from the mid-gut were

TABLE I. Proportion of the variance explained by hierarchical levels of sampling to quantify epithelium cell height in 5 and 8 dph *Amphiprion melanopus*

Levels	Treatment (3)	Tank (2)	Fish (10)	Slides (4)	Sections (4)	Individual measures (10)
5 dph	50·5	1·8	3·1	4·1	3·2	37·2
8 dph	48·8	0	8·8	6·1	3·6	32·7

Number of levels measured for each factor are given in parentheses.

measured from the top of the basement membrane to the top of the microvilli forming the brush border using a high-power microscope at × 400 magnification, equipped with an ocular micrometer and calibrated with a stage graticule. The heights of 10 cells were measured from each section, four sections were sampled per slide and four slides were sampled per fish. This was not always possible due to damaged tissues (an artefact of sectioning); however, a range of 80–160 measurements was taken per fish to diminish variation from cutting any cells at an angle (Table I).

Hepatocyte vacuolation

Hepatocyte vacuole density was measured as an indicator of lipid and glycogen stores within the liver (Theilacker, 1978). These were measured using a Weibel eyepiece attached to a high-power microscope at × 400 magnification. Point count systems such as the Weibel 42-point system works on the basis of probability that each section is representative of the whole, and a reliable sample can be built up from point counts of tissue intersecting with marks on the graticule from a number of sections (Wild technical manual). The number of points corresponding to vacuoles as a percentage of the 42 points on the eyepiece, gave an estimate of hepatocyte vacuole density. Three density estimates were made per section with three slides sampled per fish, thereby giving a minimum of nine density estimates per fish.

Trunk muscle fibre density

The densities of the fibres in the main muscle blocks of the trunk (*Musculus carinatus dorsalis*, *M. carinatus ventralis*, *M. latero-dorsalis* and *M. latero-ventralis*; Takashima & Hibiya, 1995) were measured to gauge the degree of muscle fibre shrinkage and separation in response to periodic feeding. A Weibel grid was used to obtain a count of presence/absence of muscle fibres throughout the trunk at each point on the graticule, in the same way that liver vacuole density was measured, and expressed muscle fibre density as a percentage. Twenty measurements were taken for each fish.

VIDEO ANALYSIS OF STRIKE RATE, STRIKE SUCCESS AND STANDARD LENGTH

The effect of feeding history on the predatory ability of larval fish was examined by filming larvae within each experimental tank for 30 min using a VHS portable camcorder. Tanks were filmed on the day they were due to feed. A partition was placed inside the tanks, and moved gently towards the front of the tank (within *c.* 10 cm of the front glass), moving the larvae with it. The video camera was positioned directly in front of the experimental tank to record feeding behaviour in two dimensions.

Strike rate and strike success were measured on a 63-cm high-resolution colour monitor by following individual fish over a series of 1-min intervals and recording the number of strikes made and the number that were successful. A minimum of five 1-min intervals were examined per tank.

Video footage was used to measure standard length non-destructively at settlement in the fed 1/2 and fed 1/3 treatments. A scale-bar was recorded on the tanks and the

recorded image was calibrated to the nearest 0·1 cm. In order to prevent distortion caused by screen curvature, a 35 × 18-cm rectangle was marked in the middle of the television screen and only fish within this area and next to the front of the aquarium were measured. Further, only fish that were fully in focus were measured to prevent distortion by the depth of field. Ten fish were measured per tank, except from a tank in the fed 1/3 treatment where eight were measured.

DATA ANALYSIS

The hypothesis of no difference in indices of body condition among feeding treatments, and between treatments and tanks was analysed with a nested analysis of variance (ANOVA). Type III sums of squares were used because of the unequal number of fish within each treatment. Significant differences among treatments were then analysed with one-way ANOVA at the tank level followed by the Tukey's HSD multiple comparison procedure to identify which treatments differed. The assumptions of normality and homogeneity of residuals were examined using residual analysis. Liver vacuole density from 5 days post-hatching was skewed and so was transformed using log $(x+1)$ transformation. To remove the confounding effect of standard length on gut cell height in the test of cell height differences among treatments, residuals of a standard length by cell height regression were used in the ANOVA.

Multivariate analysis of variances (MANOVA) was used to test the hypothesis that there was no difference at the treatment by tank level in the four measures of body condition. Prior to running the MANOVA, data were checked for multivariate normality and correlations were performed on all the indices under test to check variables for co-linearity. Within the 8-day post-hatch sample standard length was correlated highly with both liver vacuoles (correlation coefficient 0·84) and gut epithelium cell height (correlation coefficient 0·75) and so was removed from further analyses. The nature of the significant differences found by MANOVA was examined using canonical discriminant analysis (CDA; Tabachnick & Fidell, 1996). This identifies the trends in the dataset (canonical variates) that discriminate maximally among the identified groups (feeding levels). Trends in the original variables (cell height, liver vacuole density, standard length and muscle density) are represented graphically as vectors, which are defined by the correlations of the original variable with the canonical variates. The strength of each of the variables in discriminating among groups is described by the length of these vectors. These vectors were plotted on the first two canonical axes together with treatment centroids and 95% confidence limits (Seber, 1984).

RESULTS

STANDARD LENGTH

Amphiprion melanopus hatched at a mean length of 4·75 mm with a range of 4·38–5·13 mm. At 5 dph the mean size of the fed *ad lib* treatment was 5·89 mm whilst size was significantly less in the fed 1/2 (mean L_S 5·13 mm) and fed 1/3 (mean L_S 5·24 mm) treatments [$F_{2,51}=39{\cdot}2062$, $P<0{\cdot}00001$, Fig. 2(a)]. At 8 dph, standard length of fish in the fed *ad lib* treatment (mean 7·54 mm) was significantly larger ($F_{2,50}=217{\cdot}62$, $P<0{\cdot}0001$) than that of fish from the fed 1/2 (mean 5·91 mm) and fed 1/3 (mean 5·85 mm) treatments [Fig. 2(b)].

GUT EPITHELIUM CELL HEIGHT

To determine the extent to which fish size was contributing to the patterns in the measured variables among treatments, the relationship between the variables and fish length was examined. The only variable correlating with fish length over the size range of fish included in this study was gut epithelium cell height. A regression of gut epithelium cell height on standard length showed that there was

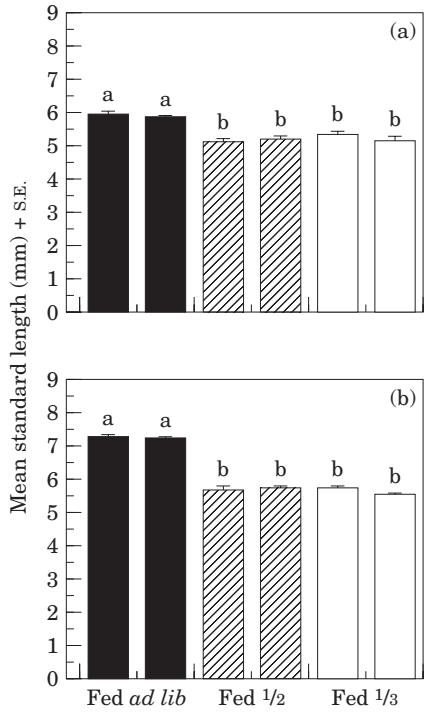

FIG. 2. Comparison of the mean standard length of *Amphiprion melanopus* among three feeding treatments, with two replicate tanks per treatment. Fish were sampled at 5 dph (a) and 8 dph (b). Tukey's (HSD) groups are given as superscripts.

a significant relationship between these indices of condition that is related to feeding, with 13% of the variation at 5 dph and 56% of the variation at 8 dph being explained by these linear relationships.

A breakdown of the variation in epithelium cell height explained by the various levels of the sampling programme found that most of the variation was at the levels of feeding treatments and differences in cell heights within histological sections from individual fish (Table I). This suggests that subsequent sampling programmes can be limited to measuring cell height from a single section of the mid-gut of *Amphiprion melanopus*.

The height and condition of the epithelium cell height was significantly different between feeding treatments (nested ANOVA 5 dph, $F_{2,50}=22{\cdot}24$, $P<0{\cdot}016$; 8 dph $F_{2,50}=63{\cdot}47$, $P<0{\cdot}004$) and decreased with reduced food [Fig. 3(a) and (b)]. At 5 dph there was a significant difference between the fed *ad lib* and the less fed treatments [$F_{2,51}=13{\cdot}3436$, $P<0{\cdot}00001$, Fig. 3(a)], and at 8 dph there was a difference among all of the treatments [$F_{2,50}=43{\cdot}199$, $P<0{\cdot}00001$, Fig. 3(b)]. Fed *ad lib* fish had epithelium cells with a high degree of supranuclear vacuolation [Fig. 4(a)], compared with the cells of those with periodic feeding, which had densely packed cells with little or no vacuolation and visible cellular atrophy [Fig. 4(b)]. The relationship between gut epithelium cell height and fish standard length was corrected for by using the residuals of this relationship in the analysis and compared with the uncorrected results. At 5 dph,

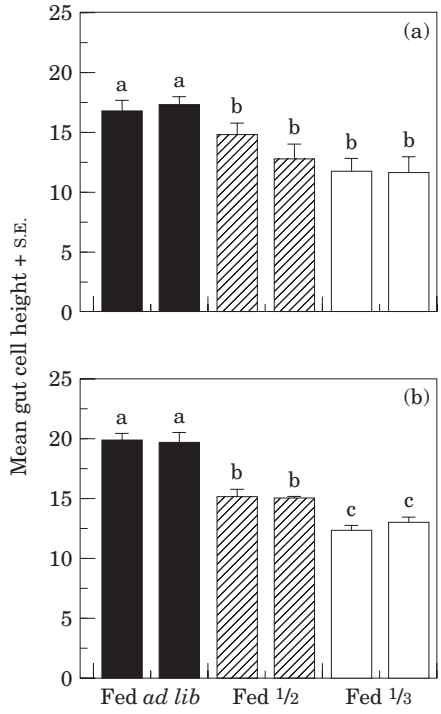

FIG. 3. Comparison of the mean gut epithelium cell height of *Amphiprion melanopus* among three feeding treatments, with two replicate tanks per treatment. Fish were sampled at 5 dph (a) and 8 dph (b). Tukey's (HSD) groups are given as superscripts.

the differences in corrected cell heights mirrored the raw cell height analysis (fed *ad lib*>fed 1/2>fed 1/3). However, at 8 dph, correction for the relationship between cell height and L_S changed the result substantially. There was a trend for fish from the fed 1/2 treatment to have larger cell heights for a given L_S than fish from the fed *ad lib* treatment, while fish from the fed 1/3 treatment had the smallest cell heights. There were no tank effects in either test (P>0·36).

HEPATOCYTE VACUOLE DENSITY

Liver vacuolation responded rapidly to the feeding levels [Fig. 4(c), *cf.* 4(d)]. Fed *ad lib* fish had livers with large clear intracellular spaces throughout the hepatocytes [Fig. 4(c)]. Livers from fish fed periodically had little vacuolation, and the cells were packed densely and stained very darkly [Fig. 4(d)]. At 5 dph, two days into the feeding experiments, a decrease in vacuole density was already apparent [Fig. 5(a)] and there was a significant difference among treatments (nested ANOVA, $F_{2,50}$=14·44, P<0·029). The fed *ad lib* treatment had a mean density of 31·5%, which was significantly higher than the two less fed treatments (fed 1/2 7·78% and fed 1/3 8·861%; $F_{2,51}$=86·8183, P<0·00001). At 8 dph the overall density of vacuoles had increased but the proportionate difference among treatments was similar [Fig. 5(a), *cf.* 5(b)]. The mean vacuole densities at this stage were 57·5, 28·9 and 11·27% for fed *ad lib*, fed 1/2 and fed 1/3 respectively

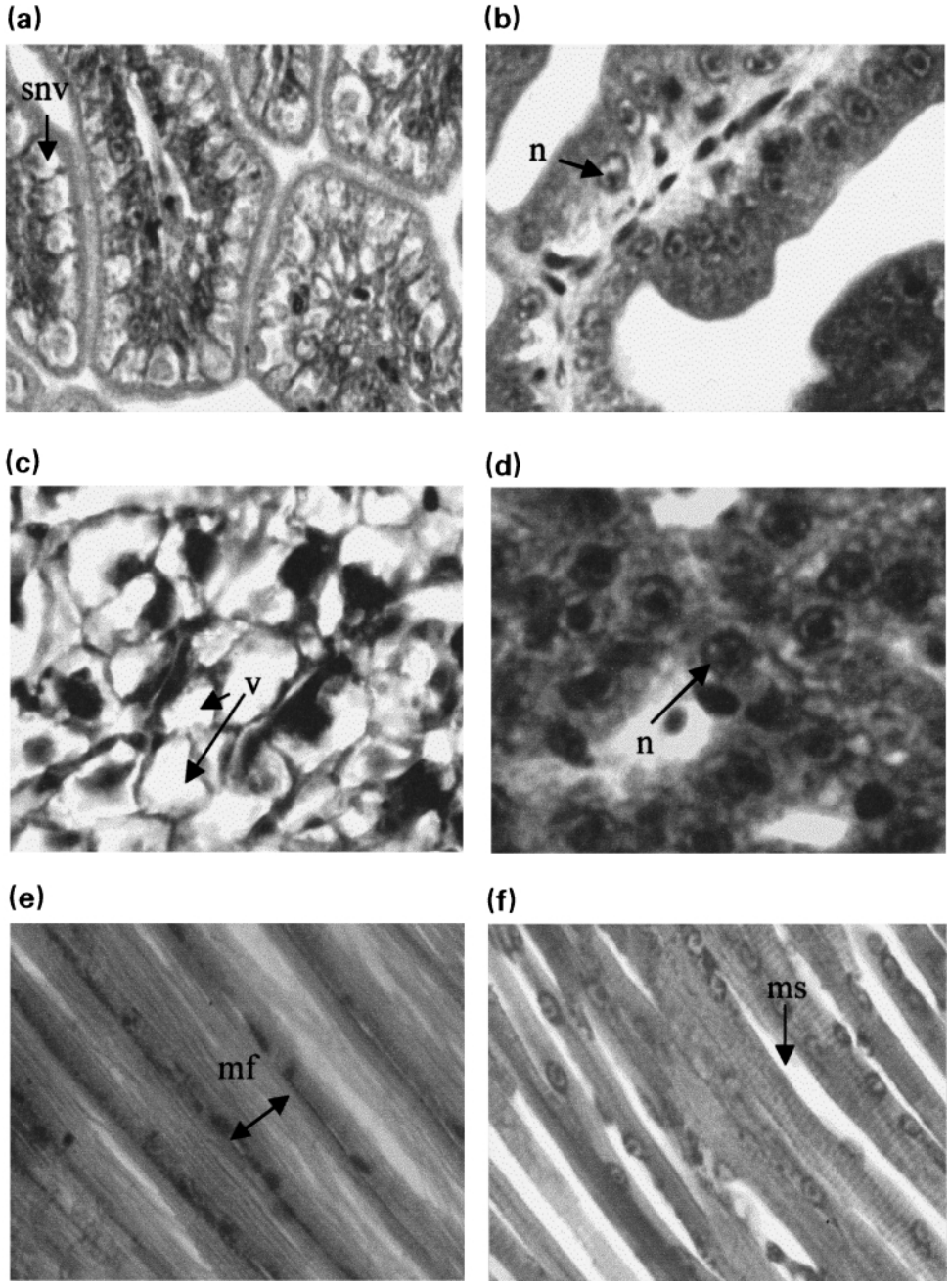

FIG. 4. Comparison of body tissues of *Amphiprion melanopus* among three feeding treatments. Fish were sampled at 8 dph. Tissues were sectioned at 5 μm, × 400 magnification, stained with haemotoxylin and eosin. (a) Gut epithelium cells, fed every day; (b) gut epithelium cells, fed every third day; (c) liver, fed every day; (d) liver, fed every third; (e) trunk muscles, fed every day; (f) trunk muscles, fed every third day. snv, Supra-nuclear vacuolation; n, nucleus; v, vacuoles; mf, muscle fibre; ms, muscle separation.

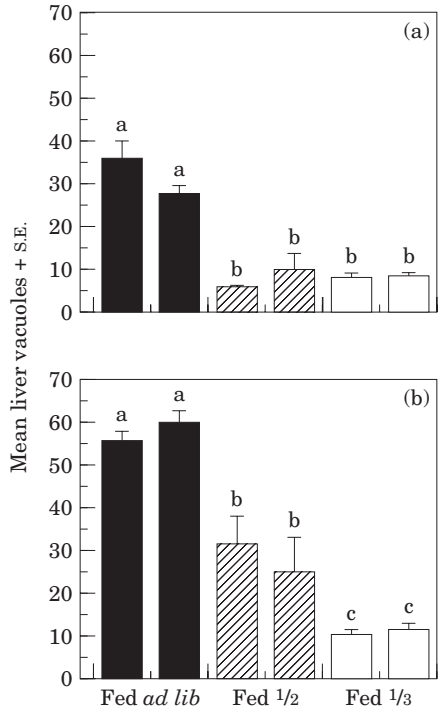

FIG. 5. Comparison of the mean liver vacuole density of *Amphiprion melanopus* among three feeding treatments, with two replicate tanks per treatment. Fish were sampled at 5 dph (a) and 8 dph (b). Tukey's (HSD) groups are given as superscripts.

[Fig. 5(b)] and all these feeding levels were significantly different ($F_{2,50}=70{\cdot}984$, $P<0{\cdot}0001$).

TRUNK MUSCLE DENSITY

The density of muscle fibres in the trunk of fish responded numerically in a similar way to gut cell height and liver vacuole density [Fig. 6(a) cf. 6(b)]. Muscle fibres within the myomeres became separated by wide spaces as a result of periodic feeding [Fig. 4(e), *cf.* 4(f)]. After 2 days under the experimental feeding regimes (5 dph), there was a significant difference caused by the feeding treatments (nested ANOVA, $F_{2,50}=14{\cdot}44$, $P<0{\cdot}029$). Muscle density was significantly higher in the fed *ad lib* than the less fed treatments ($F_{2,51}=26{\cdot}8188$, $P<0{\cdot}00001$); however, there was little difference between the two less fed treatments [Fig. 6(a)]. After 3 more days under these conditions (8 dph) there was a highly significant difference among all treatments ($F_{2,50}=17{\cdot}235$, $P<0{\cdot}00001$). The mean muscle fibre density at 8 dph for the fed *ad lib* treatment was 73·56%, and fed 1/3 treatment dropped to 63·04% [Fig. 6(b)]; however, the mean muscle fibre density of the fish in the two tanks within the fed *ad lib* treatment differed significantly—68·8% compared with 80·34%.

LARVAL DURATION AND SIZE AT SETTLEMENT

Metamorphosis was indicated by the appearance of adult pigmentation, which coincided with settlement as identified by a shift from the position of the fish high

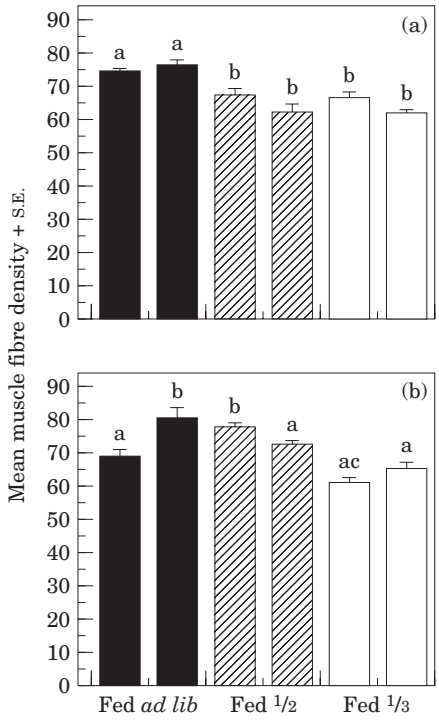

FIG. 6. Comparison of the mean trunk muscle fibre density of *Amphiprion melanopus* among three feeding treatments with two replicate tanks per treatment. Fish were sampled at 5 dph (a) and 8 dph (b). Tukey's (HSD) groups are given as superscripts.

in the water column to an association with the bottom of the tank. Larval duration was prolonged in fish that were fed less frequently [Fig. 7(a)]. Fish that were fed *ad lib* settled after 8 or 9 days, while fed 1/2 fish settle after 10 or 11 days. The fed 1/3 fish did not settle until 13 or 14 dph. The difference in larval duration, caused by the feeding levels was up to 6 days, or 75% of the fish's average larval life.

Fish from the fed *ad lib* treatment not only had a shorter larval duration but were larger at metamorphosis than those from the less fed treatments [Fig. 7(b)]. Fed *ad lib* fish had an average standard length of 7 mm at settlement, fed 1/2 fish were 6 mm, while fed 1/3 were 5·8 mm L_S [Fig. 7(b)].

STRIKE RATE AND STRIKE SUCCESS

Strike rate of *Amphiprion melanopus* was highly variable when compared among treatments, whereas strike success decreased dramatically with reduced feeding levels (Fig. 8). Fed *ad lib* fish had between 74% and 100% strike success, while the less fed treatments were successful in only 50% (fed 1/2) and 64% (fed 1/3) of their strike attempts.

OVERALL EFFECTS OF LARVAL FEEDING HISTORY

To summarize the effect of feeding regimes on the body condition of larval *Amphiprion melanopus*, MANOVA was employed. This analysis identified significant interactions at the tank × treatment level at 5 and 8 dph (for both

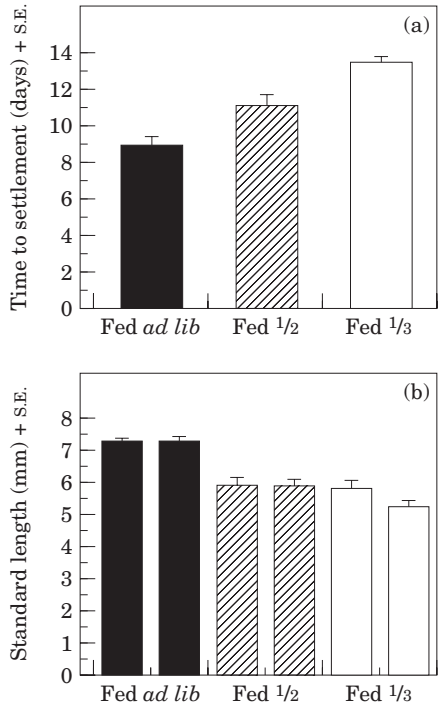

FIG. 7. Comparison of the effects of three feeding treatments on (a) mean time larval duration, and (b) standard length at settlement of *Amphiprion melanopus*.

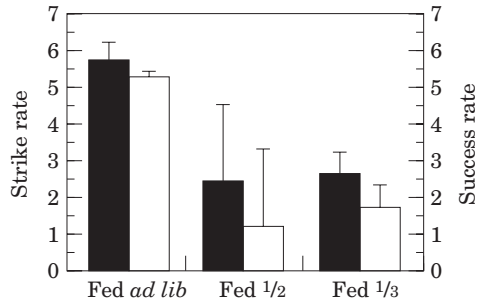

FIG. 8. Mean strike rate and strike success for *Amphiprion melanopus* under three feeding treatments, recorded at 11 dph. ■, Strike rate; □, strike success.

samples, Pillai's trace, $P<0{\cdot}0001$). Then canonical discriminant analysis was used to display the nature of these interactions [Fig. 9(a) and (b)]. At 5 dph, fish from both tanks in the fed *ad lib* treatment differed from fish in the other two treatments [Fig. 9(a)], while fish from fed 1/2 and fed 1/3 were not significantly different from each other. This difference between fish from high and low feeding regimes was largely due to differences in liver vacuole density, which appear to have the fastest response to low food availability [Fig. 9(a)]. Fish length also contributed to this difference among treatments. This pattern was more distinct three days later. The 8-dph samples showed further separation

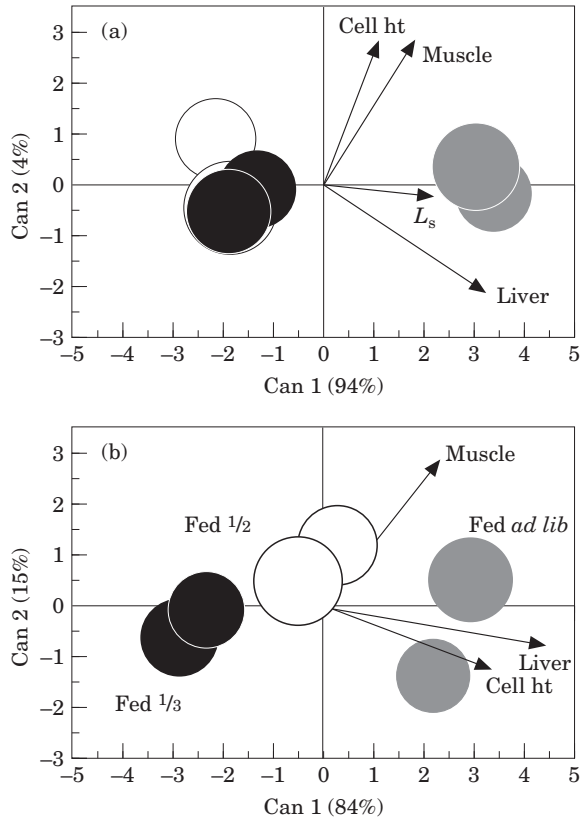

FIG. 9. CDA of mean measures of body condition of *Amphiprion melanopus* among three feeding treatments, with two replicate tanks per treatment. Each circle signifies one tank. Samples were taken at 5 dph (a) and 8 dph (b). Treatment by tank 95% confidence clouds are plotted together with the direction and importance (as indicated by vector length) of trends in condition indices. Cell ht, Epithelium cell height; Liver, liver vacuole density; Muscle, trunk muscle density; L_S, standard length.

among treatments, with all three treatments differing from one another in the measured attributes [Fig. 9(b)]. Liver vacuole density was still partly responsible for these differences among treatments, but epithelium cell height was now also important. Trunk muscle density was largely responsible for differences found among tanks within the treatments.

DISCUSSION

Larval *Amphiprion melanopus* had a rapid physiological response to limited food availability. All measures used in this study responded to the feeding levels, though on differing time scales. Many of the changes noted were similar to those found in other studies of malnourished fish larvae including: (a) changes in the structure of the liver hepatocytes, with reduced vacuolation (Ehrlich *et al.*, 1976; O'Connell, 1976; Theilacker, 1978; Margulies, 1993); (b) reductions in height of epithelial cells of the mid-gut (Ehrlich *et al.*, 1976; O'Connell, 1976; Theilacker, 1978, 1986; Margulies, 1993; Theilacker & Porter, 1995; Theilacker *et al.*, 1996);

(c) degeneration of trunk muscle tissue, with separation of the muscle fibres (O'Connell, 1976; Theilacker, 1978; Margulies, 1993); (d) reductions in growth (Rice *et al*, 1987); (e) increases in larval duration and reductions in size at metamorphosis under sub-lethal food limitations (McCormick & Molony, 1992). Subsequent comparison of these results with the condition of wild caught larvae may allow assessment of the recent feeding history of late stage larvae and the proportion exposed to poor feeding conditions.

Liver hepatocyte vacuoles are areas of short-term storage of glycogen and lipid (Wheater *et al.*, 1979). In this study, the hepatocyte vacuoles were particularly sensitive to low food levels, with a reduction in density with decreasing food availability. Glycogen from the hepatocyte vacuoles is the first energy component to be mobilized by fish when faced with starvation (O'Connell & Paloma, 1981; Black & Love, 1986; Margulies, 1993). The liver is also the main site of nutrient metabolism, including glycolysis, gluconeogenesis and lipid metabolism (Segner *et al.*, 1993). A decrease in vacuole density with reduced food suggests a reduction in the storage products, and therefore a reduction in the immediate energy available to the fish. Deterioration of the liver tissue in the fish from the lowest feeding treatment indicates that the fish's metabolism may be affected. There was an increase in vacuole density in all treatments between the samples at 5 and 8 dph. This suggests that while the experimental feeding regimes caused differences in lipid and glycogen storage among the different levels of feeding, the fish either increased assimilation efficiency with age, or developed compensatory mechanisms to offset the effects of low food availability (Love, 1980).

A decrease in gut epithelium cell height corresponded to reduced feeding levels in the larval phase. Part of this difference in cell height can be explained by differences in somatic growth among feeding treatments and the relationship between cell height and standard length. At both 5 and 8 dph, fish in the less fed treatments were smaller and consequently had smaller gut cell heights. However, once all treatments were corrected for standard length then the fed 1/2 treatment had larger cell heights than the fed *ad lib* treatment. This may represent compensatory mechanisms, in order to offset the effects of limited food (Love, 1980), augmenting efficiency through increasing the surface area available for absorption of nutrients. The much lower food supply in the fed 1/3 fishes may restrict the energy available for compensatory growth and therefore explain the consistently smaller cell height in these fishes. These differences show that it may be important to correct for standard length when comparing cell heights between field and laboratory samples so that any identified differences represent real differences and not simply size- or age-related trends. This has not been performed using residuals in any of the studies that have used histological indices to date, although corrections for the gross effects of standard length have been employed by dividing histological indices by standard length (Theilacker & Watanabe, 1989).

The visible degeneration in cells in fish from the lowest feeding level implies a reduced surface area for absorption of nutrients, and a reduction in the ability of the fish to assimilate food, as the epithelium cells are the site of endocrine and exocrine secretion into the intestinal lumen (Trier & Madara, 1981). The visible reduction in the vacuoles of the gut epithelium is generally one of the first responses to starvation, as these stores (presumably lipid deposits) have an

important role in energy provision when food is limited (Ince & Thorpe, 1976). Proteolysis of the intestine, as a response to short-term starvation, is generally one of the most immediate responses (Love, 1980), and for this reason cell height has been used as a reliable indicator of sub-optimal feeding levels (Theilacker, 1978; Theilacker & Watanabe 1989; Theilacker *et al.*, 1996). It has been employed as the single quantitative index to diagnose reliably nutritional status in *Theragra chalcogramma* (Pallas) (Theilacker & Porter, 1995), *Clarias gariepinus* (Burchell), *Coregonus lavaretus* (L.) and *Scophthalmus maximus* (L.) (Segner *et al.*, 1993). A qualitative decrease in the thickness of the intestine has also been used to gauge feeding condition (*Engraulis mordax* Girard, O'Connell, 1976; *Trachurus symmetricus* (Ayres), Theilacker, 1986). Based on the data from this study, gut epithelium cell height (corrected for standard length) also appears to be a reliable index of recent nutritional status for *Amphiprion melanopus*.

Generally, following rapid metabolism of liver lipid in a poorly fed fish, the protein of the muscle is mobilized (Love, 1980; Black & Love, 1986). In the present study, the visible degeneration and separation of the trunk muscle fibres in fish from the lowest feeding level suggests the occurrence of protein catabolism. Relative to other histological measures, trunk muscle fibres exhibited the slowest response to limited food. O'Connell (1976) and Margulies (1993) reported similar responses to food limitation for larval anchovy and scombrids, respectively. This may be because fish maintain muscle by diverting energy from being stored in the liver as glycogen, straight to the muscle in the form of blood glucose during periods of starvation (Love, 1980).

Physical and structural changes in the fish are not the only disadvantages caused by sub-optimal feeding history. Reduced feeding can also impair searching and feeding abilities (Richards & Lindeman, 1987), including feeding rate (Yin & Blaxter, 1987). *A. melanopus* larvae exposed to periodic feeding had less success in capturing prey than fish that were fed every day. This is perhaps an effect of the reduced muscle condition, affecting the fish's ability to swim (Margulies, 1993) and strike proficiently. Alternatively, many studies have shown that recent experience frequently plays a large role in capture success (Colgan *et al.*, 1986; Fuiman & Higgs, 1997), whereby fish that have not encountered prey recently do not strike as proficiently as fish with recent experience with that particular prey type. Fish from this study were filmed feeding after food had been withheld for 1 (fed 1/2) or 2 days (fed 1/3), so possibly lacked the recent experience to strike successfully.

Although low feeding levels did not directly increase the mortality rate of *A. melanopus* in this laboratory-based study, the scope of the response to different feeding levels suggests there is potential for larval feeding history to have a significant impact on the numbers recruiting to wild populations. The responses of *A. melanopus* to limited feeding found in this study will affect the fishes ability to attain food (Coates, 1980), avoid predators (Neilson *et al.*, 1986; Rice *et al.*, 1987; Miller *et al.*, 1988; Mesa *et al.*, 1994) and locate and defend territories in size-based hierarchies once settled (Jones, 1987).

This study forms the first step in an assessment of the importance of food availability to fluctuations in recruitment strength in this tropical fish species. Studies of temperate and tropical scombrids have shown that starvation induced mortality can account for most or all of the mortality that occurs during the

early larval stages (Hewitt *et al.*, 1985; Theilacker, 1986; Margulies, 1993). Like these studies, the degree of liver vacuolation and height of the cells of the gut epithelium proved to be sensitive means of quantifying body condition, and these provide a relatively simple way of quantifying the condition of wild caught larvae for future studies.

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