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Effects of feeding on the sustained swimming abilities of late-stage larval *Amphiprion melanopus*

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Abstract To date, all sustained swimming experiments on tropical reef fish larvae have been conducted using unfed larvae. Such studies may produce unrealistic estimates of sustained swimming abilities. We examined the effect of food on the sustained swimming ability of late-stage *Amphiprion melanopus*. Larvae were swum in a six-channel swimming flume at 7 cm s^{-1}, with "unfed" and "fed" channels. Fed channels had *Artemia* nauplii added four times per day for 10 min. Feeding larvae during swimming experiments significantly increased their average swimming distance from around 6.9 to 12.2 km, and the maximum swimming distance from around 11.8 to 28.7 km. Existing flume-based estimates of sustained swimming may be underestimating field abilities. With access to food, many larvae may have the potential to swim considerably greater distances than previously suggested.

Keywords Dispersal · Coral reef fish · Swimming · Larvae · Energetics · Feeding

Introduction

There is increasing evidence that some late-stage larval fishes have the ability to actively locate and settle on reefs (Swearer et al. 1999; Armsworth 2000). Recent studies have demonstrated that these late-stage reef fish larvae are good swimmers (Stobutzki and Bellwood 1994, 1997; Leis et al. 1996; Leis and Carson-Ewart 1997, 1998), at least in the last few days before settlement (Fisher et al. 2000). These studies indicate that prior to settlement, many species of larvae are capable of swimming against currents (at least for short periods) (Leis and Carson-Ewart 1997) and may potentially cover considerable distances (Stobutzki and Bellwood 1997).

Information on the swimming abilities of reef fishes comes from two different methodologies. One has been to follow late-stage larvae directly in the field (Leis and Carson-Ewart 1997, 1998). The other approach has concentrated on obtaining estimates of maximal performance measures of swimming ability measured using current flumes (Stobutzki and Bellwood 1994, 1997; Fisher et al. 2000). Flume-based sustained swimming experiments provide a measure of the maximum long-term swimming abilities of larvae and have provided empirical data on the ability of larvae to influence their dispersal over extended periods of time. Data obtained from sustained swimming studies have been recently incorporated into various models examining the effect of behavior on larval dispersal (e.g. Wolanski et al. 1997; Armsworth 2000). It is becoming increasingly important to obtain reliable estimates of the swimming abilities of larvae. However, existing flume-based estimates of sustained swimming may misrepresent the swimming abilities of larvae in the field, as all experiments on tropical reef fish larvae have been conducted using unfed larvae (Stobutzki and Bellwood 1997; Fisher et al. 2000). It is unrealistic to expect larval fish to undergo swimming migrations without feeding. Indeed, larvae have been observed to feed while swimming in situ (Leis and Carson-Ewart 1998). This study therefore aims to assess the effects of food on the sustained swimming ability of reef-fish larvae.

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Materials and methods

Laboratory-reared larvae of *Amphiprion melanopus* were used for this experiment to ensure that all larvae within a batch were in a relatively similar physical condition at the start of the experiment. Rearing procedures were modified from Job and Bellwood (2000). Larvae were reared in 200-L glass aquaria using cultured rotifers

and *Artemia* nauplii. Wild-caught plankton was not used for these experiments to ensure that all larvae within and among batches received similar quality food throughout development.

At 7 days post hatch, larvae were transferred to a six-channel experimental swimming flume (Stobutzki and Bellwood 1997). Two fish were placed in each channel and all fish were allowed to acclimatize for several minutes before the start of the experiment. Larvae were swum at a speed of 7 cm s^{-1}, a speed known to be sustainable for late-stage *Amphiprion melanopus* (Fisher et al. 2000). At the start of the experiment, all fish were swum for approximately 2 h without feeding. Stressed fish, not exhibiting sustained swimming behavior, were removed from the experiment to avoid confounding the results. After 2 h, swimming channels with fish still remaining were randomly allocated to a "fed" or "unfed" treatment. During feeding, the current flume in all channels was stopped for 10 min and newly hatched *Artemia* nauplii (Prime Artemia Incorporated Great Salt Lake brine shrimp eggs) were added to the "fed" channels. Remaining *Artemia* were removed from the water exiting the swimming channel using a 62-μm sieve. Feeding stops were performed four times per day, at 4-h intervals between 8 a.m. and 8 p.m. throughout the experiment. The experiment was then repeated for a further four replicate clutches of larvae. Experiments were terminated after a maximum of 6 days of continuous swimming, at which point any remaining larvae were sampled. The data were analyzed by calculating the mean and maximum time swum for fed and unfed fish for each clutch and compared using a paired t-test (Zar 1999). This test was selected because it permits a direct comparison of the two feeding treatments within each batch, thus avoiding confounding effects due to differences in quality or size between batches (cf. Bellwood and Fisher 2001). For graphical simplicity, however, the mean of the five clutches was plotted for both the maximum and mean swimming duration for each treatment. At the start of each experiment, the maximum sustainable speed (U-crit, see Brett 1964) was determined for each clutch. These were compared with the experimental speed to indicate the relative level of activity during sustained swimming. A sample of larvae was also obtained and fixed in Marine Bouin's, then stored in 70% ethanol. Using these samples, measurements were made of wet and dry weight, standard length, and body depth. A mean was obtained for each clutch and used as the value for that clutch. Means presented in the results represent the mean and standard error between the five clutches.

Estimates of the number of *Artemia* nauplii ingested by larvae during the 10-min feeding break were obtained from videos of larvae in the swimming channels. Of the films taken, only four individuals were clearly visible throughout the feeding stop and were therefore used for analysis. Ingestion was signified by a feeding strike. For late-stage larval anemone fishes, feeding success is close to 100% (Job and Bellwood 1996); therefore counts of feeding strikes provide a good estimate of individual consumption. Counts were converted to equivalent dry weights using published data for newly hatched Great Salt Lake *Artemia* nauplii (Garcia-Ortega et al. 1998). These were expressed as a percentage of mean dry body weight of the fish swum. An estimate of the energy obtained by larvae during feeding breaks was calculated by converting the known biochemical composition of newly hatched *Artemia* nauplii (Garcia-Ortega et al. 1998) to energy values following Henken et al. (1986). These were expressed as joules per gram of fish wet weight per hour to facilitate comparison with literature values. Energy values were adjusted for assimilation (Brett and Groves 1979) as well as metabolic and respiratory costs or "apparent heat increment" (Beamish and Tripple 1990).

Results

A total of 87 fish from 5 clutches were swum: 44 "fed" and 43 "unfed." The mean length of fish in each clutch at the beginning of the experiments ranged from 5.5 to 7.6 mm standard length (Table 1). The experimental speed (7 cm s^{-1}) as expressed in body lengths (bl) per second ranged from 9.2 to 12.8 bl s^{-1}, which is equivalent to approximately half (35 to 85%) of the maximum speed (U-crit) of each clutch (Table 1).

Feeding larvae during swimming experiments were found to significantly increase their average swimming duration ($t = 4.9$, $df = 4$, $p < 0.005$), with fed fish being able to swim about twice as long as unfed fish (Fig. 1A). Feeding increased the equivalent distances covered by larvae from around 6.9 to 12.2 km. The effect of food on the sustained swimming ability of larvae was even greater when only the longest swimming individual from each clutch for each treatment was considered. Feeding larvae significantly increased the maximum swimming duration of fed larvae to more than double that of unfed larvae ($t = 5.3$, $df = 4$, $p < 0.005$; Fig. 1B). This increased

Table 1 Swimming by *Amphiprion melanopus* larvae used in experiments. Standard errors refer to variation between clutches ($n = 5$). Maximum and minimum values are based on batch means

	Maximum	Minimum	Mean
Standard length (mm)	7.6	5.5	6.6 ± 0.5
Relative speed (body lengths s^{-1})	12.7	9.2	10.6 ± 0.8
U-crit (cm s^{-1})	19.9	8.2	16.3 ± 2.3
Experimental speed (% U-crit)	85	35	48 ± 10

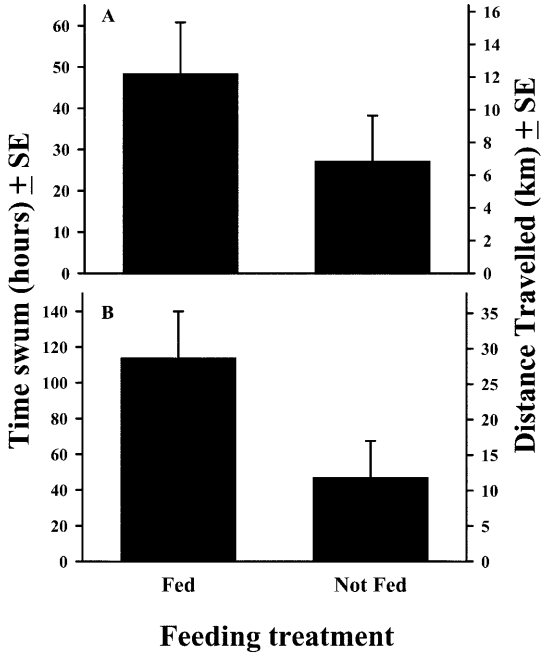

Fig. 1 Comparison of mean sustained swimming time and equivalent distance traveled between fed and unfed larvae ($n = 5$): **A** Mean time swum for each treatment for each clutch. **B** Maximum time swum for each treatment for each clutch. Standard errors are calculated from the overall mean of each treatment across all clutches. The two treatments were found to be statistically different using a paired t-test based on means from each treatment for each clutch ($t = 4.9$, $df = 4$, $p < 0.005$) as well as maximum time swum from each treatment for each clutch ($t = 5.3$, $df = 4$, $p < 0.005$)

the equivalent distance covered before exhaustion from 11.8 to 28.7 km.

Of the fed larvae swum, five were preserved and used for measurements. After 6 days of swimming these larvae increased their total standard length by 1.5 mm, which is equivalent to 3.0% per day. They also increased in wet weight by 10.4 mg, an increase of 10.4% per day from their original wet weight. During the 6 days of swimming, the fed larvae had also undergone metamorphosis, developing all the color patterns of a settled juvenile. Measurements were also made for two batches of larvae left undisturbed in rearing tanks. These larvae were found to grow by $4.0 \pm 0.2\%$ in length and $16.9 \pm 2.0\%$ in wet weight per day.

The number of *Artemia* nauplii ingested in a feeding stop and therefore the amount of energy obtained per day for fed larvae varied considerably. Larvae consumed a minimum of 692 and a maximum of 1,344 individual *Artemia* nauplii each day over the four feeding stops (Table 2). This is equivalent to between 86 and 167% of the mean dry weight of the larvae, and between approximately 2,252 and 4,374 J g^{-1} wet weight of larvae day^{-1} (Table 2).

Discussion

Fed larvae remaining at the end of the experiments showed no evidence of malnutrition, had undergone metamorphosis, and even after several days of swimming were not exhausted. This is in stark contrast to unfed larvae which were unable to maintain their position at the end of the experiment. The apparently healthy nature of fed larvae after swimming for several days is supported by the fact that these larvae grew by about 62% in body weight and 18% in standard length during the swimming bout (corresponding to 10.5 and 3.0% growth per day, respectively). These growth rates are comparable to those observed for larvae remaining in rearing tanks (16.9% in body weight and 4.0% in standard length per day). The values are also comparable with other values reported in the literature for larvae and juveniles of various taxa (cf. Kiorboe et al. 1987; Naas et al. 1992; Watanabe and Saito 1998). During swimming trials on unfed larvae, lipids, carbohydrates and proteins were all used extensively during swimming bouts (Stobutzki 1997). The magnitude of the increase in swimming performance due to feeding shown in our study, as well as the healthy condition of some larvae even after swimming for several days indicate that previous sustained swimming trials without feeding may simply be measuring the energy reserves of larvae. Stobutzki (1997) found a good correlation between swimming duration and initial energy reserves of larvae. It seems that other than food, there may be little restriction on swimming endurance of at least some species of reef fishes. This finding is particularly striking considering that in the present study larvae were swum at around half of their maximum speed.

By-products of swimming exercise in fishes include creatine and lactate (Franklin et al. 1996). Fish must deal with these effectively in order to sustain swimming for long durations. Franklin et al. (1996) found that larval fish appear to be able to clear lactate an order of magnitude faster than rates reported for adult fish, which may explain the apparently indefinite swimming capabilities of some larvae. Furthermore, the fact that larval fishes may have a greater ability to remove the by-products of sustained swimming than adults suggests that the remarkable endurance swimming observed in larval reef fishes may be an adaptive response to the unique demands of the pelagic environment.

The results of Stobutzki and Bellwood (1997) show that some late-stage larval fishes are able to swim considerable distances even without food. The greatest decrease in energy stores was seen in the heaviest species, which also swam for the longest duration (Stobutzki 1997). For these species, feeding during swimming events may not be as important, and these species may be able to find and settle on reefs without needing to feed during the settlement phase. For the smaller-bodied species, as in the *Amphiprion melanopus* larvae used in our study, energy reserves are less, and food is more likely to be a limiting factor for swimming endurance. It seems, therefore, that current estimates of swimming capabilities are likely to be most inaccurate for the smaller-bodied species. Given food, the swimming performance of smaller-bodied larvae may be considerably greater than is currently recognized. However, even for the larger-bodied species, feeding during swimming migrations may significantly increase the condition of larvae at settlement, which is likely to considerably improve chances of survival once they reach a reef and settle (Kerrigan 1996; McCormick 1998). Laboratory studies have indicated that feeding history can have marked effects on the condition of the liver, gut epithelium, muscle fibers, larval duration, and size at metamorphosis (Green and McCormick 1999). Therefore, even for larger-bodied species, feeding during swimming is likely to be important.

Table 2 Quantities and energetic value of food fed to *Amphiprion melanopus* larvae used in experiments. Standard error based on four individuals

	Number of *Artemia* ingested (number day^{-1})	Weight of *Artemia* ingested[a] (% larval body weight)	Equivalent energy[b] (J g^{-1} day^{-1})
Maximum	1,344	166.9	4,374
Minimum	692	85.9	2,252
Mean	977 ± 151	121.3	3,179

[a]Calculated from *Artemia* counts using values presented in Garcia-Ortega et al. (1998)

[b]Approximated from *Artemia* counts using values presented in Brett and Groves (1979), Henken et al. (1986), Beamish and Tripple (1990), and Garcia-Ortega et al. (1998)

The amount of food fed to larvae during the experiment was between 86 and 167% of the fish dry body weight each day. Larvae were able to obtain this food during a total of only 40 min of feeding time throughout the day, with larvae ingesting between 20.5 and 41.7% of their body weight in a single feeding stop (based on dry-weight estimates). These values are comparable with values obtained for other larval fishes (cf. Watanabe and Saito 1998). The amount of *Artemia* ingested provides the larvae with approximately 2,252–4,374 J g^{-1} fish wet weight day^{-1}. We compared this estimate with the estimated energetic cost of swimming from Stobutzki (1997). She found that settlement-stage reef fishes used between 154 (for a lethrinid swimming at 7.1 bl s^{-1}) and 1,090 J g^{-1} day^{-1} (for *Pomacentrus amboinensis* swimming at 9.9 bl s^{-1}), which is considerably lower than the amount supplied to the larvae during our experiment. It seems, therefore, that larvae were supplied with more than enough energy to maintain swimming, at least at the experimental speed larvae were swum.

Existing estimates of sustained swimming, based on larvae that were not fed during swimming experiments, may be unrealistic. Larvae are probably able to swim distances considerably greater than currently suggested. Models that examine the efficiency of different behavioral strategies on larval dispersal need to consider the effect that feeding may have on the conclusions drawn from such models. Even at high relative swimming speeds, only limited exposure to food appears to provide larvae with more than sufficient energy for swimming migrations and still allow larvae enough resources for growth. Sustained swimming may not be particularly detrimental to larvae and long-term maintenance of position seems possible, especially if larvae utilize boundary layers.

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