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Factors affecting growth of killifish, *Aphanius dispar*, a potential biological control of mosquitoes

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Abstract

Preliminary experiments were carried out on the growth of killifish, *Aphanius dispar*. The motivation of the study was to obtain information for growing the fish on a commercial scale for their use as biological control agents of mosquito larvae. Growth of fry in the laboratory was found to be exponential, where the degree of variance in size among the fry increased with age. Grading the fry was shown to be effective in reducing the significant differences in their growth rates observed prior to grading. The effects of temperature, salinity and feeding rate on the growth of wild adult fish were also investigated. There were significant increases in growth rates of adult fish as temperature was increased from 18°C to 23°C. Further increases in temperature, to 27°C, did not further affect growth. Significant differences were also found among growth rates of fish kept at salinities from 8 to 56 ppt. Growth was found to be lowest at 40 ppt, and increased steadily as salinities approached the two range limits. Growth rates were found to increase significantly as feeding rates increased from 0% to 4% body weight (BW)/day. Further increases up to 10% BW/day did not result in further increases in growth. Regressing mean growth rates on feeding rates showed 1.6% BW/day to be the maintenance feeding rate at which fish neither gained nor lost weight.

Keywords: Aphanius dispar; Temperature; Salinity; Feeding; Growth; Grading; Killifish

1. Introduction

Mosquitoes are known to transmit diseases such as encephalitis (Lichtenberg and Getz, 1985), yellow fever and filariasis (Jones, 1978), and malaria (Fletcher et al., 1992); the latter being responsible for more than 3 million deaths per year (Hildebrandt,

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1996). Using insecticides to control mosquitoes has a number of drawbacks including being costly, causing environmental pollution and resulting in mosquitoes (and other insects) eventually becoming resistant to them (Zaman, 1980). A known alternative to chemicals in the fight against mosquitoes is using fish, which not only prey on mosquito larvae, but have also been found to repel ovipositon (Ritchie and Laidlawbell, 1994).

Native fish should be used for biological control of mosquitoes as opposed to introducing exotic species (Rupp, 1996). The mosquito fish *Gambusia affinis* is a known piscivore (Walters and Lenger, 1979), but it has been introduced into many parts of the world for antimalarial control (Haas and Pal, 1984). The negative effects of introducing *Gambusia holbrooki*, for example, have been shown to cause a decrease in the abundance of frogs (Webb and Joss, 1997). The introduction of *G. affinis* was also found to markedly reduce population densities of invertebrate predators of mosquitoes (el Safi et al., 1985). Using native fish, however, necessitates the establishment of research programs on the local species for their mass production (Haas and Pal, 1984).

Most species of killifish are strong candidates as biological control agents, having an affinity for mosquito larvae as well as being very eurythermal and euryhaline (Whitehead et al., 1986). In comparison to commercially grown fish, however, little is known on the biology of cyprinodonts for efficient large-scale production. Literature is available for certain ornamental species (e.g., *Aphysemion* and *Notobranchius* spp.), the information here, however, being mainly at the hobbyist level and usually not relevant for large-scale culture programs.

The killifish, *Aphanius dispar* (Rüppell, 1828) is found in the waters surrounding the Arabian Peninsula, the Persian Gulf, the Red Sea and the eastern Mediterranean Sea (Goren, 1983). *A. dispar* is omnivorous and mosquito larvae are included in its diet (Goren, 1983; Haas and Pal, 1984). The fish are very eurythermal and euryhaline (Whitehead et al., 1986) and tolerate certain degrees of organic pollution (Homski et al., 1994), inorganic pollution (Haas, 1982) and relatively low levels of oxygen (Homski et al., 1994). Positive evaluations of the fish for mosquito control have been reported in Israel (Homski et al., 1994), Eritrea (Fletcher et al., 1992), Oman, (Haas, 1982), and Somalia (Nasir, 1979).

Essential for using fish to control mosquitoes on a large scale is the ability to breed the fish in captivity (Haas and Pal, 1984). Whereas information exists on the reproduction of *A. dispar* in its natural habitat (see Haas, 1982; Lotan, 1982; Goren, 1983), relatively little is known on the fish for efficient mass-breeding programs. One investigation showed how different environmental factors affect oocyte maturation in the ovaries of *A. dispar* (Frenkel and Goren, 1997). Another study provided further information on the reproduction of *A. dispar*, including preventing predation of eggs and newly hatched fry by adult fish (Frenkel and Goren, 1999).

Once the suitable candidates for biological control can be bred successfully under controlled conditions, further investigations are needed to provide information for maximizing growth rates of the fish. Efficient, low-cost fish-culture programs are essential, especially in developing countries where tropical diseases are one of the main obstacles for development (Guiguemde et al., 1994). Thus, the suitability of *A. dispar* as a biological control agent against mosquitoes was further investigated by determining the effects of a number of environmental factors on the growth of the fish.

2. Materials and methods

Fish were caught in their natural breeding grounds in the salt ponds at Atlit (ca. 10 km south of Haifa, Israel) in December 1992, using a small seine net. They were brought to the Zoological Garden of Tel Aviv University, where they were transferred to 200-l acclimation tanks, each equipped with a biological filter, aeration and water heater. The salinity of the water was matched to that of the salt ponds. Methylene blue was added to the water to prevent the onset of fungal infections, and tap water was dripped into the tanks, over a period of 3 days, gradually converting the tank water to 100% freshwater. This latter procedure was adopted in order to prevent infection by *Amyloodinium ocellatum*, a dinoflagellate whose normal reproduction and infection of fish is seriously inhibited at salinities below 1 ppt (Paperna, 1984). A minimum of 2 months was given for the fish to become acclimated before starting the experiments. For standard experimental conditions see Frenkel and Goren (1997).

Two groups of fry were grown in the laboratory. Four males and six females were placed in each of two 250-l aquaria ($100 \times 50 \times 50$ cm^3). For specific breeding tank conditions, see Frenkel and Goren (1999). In both groups, the adults were allowed to spawn for 2 weeks and then removed. In the first group of fry, 15 fry were netted weekly and weighed (and not returned) from week 2 after hatching until week 11. In the second group, the fry were left undisturbed for a total of 118 days, after which all were removed and weighed. Both groups received dry food (in excess) as well as *Artemia* nuapli hatched in the laboratory.

Two subgroups of 20 fish each were then taken from the second group of fry. The average initial weight of one subgroup (0.377 ± 0.011 g) ($\pm$S.E.) was close to that of the original group; the average initial weight of the other subgroup (0.219 ± 0.009 g) being approximately half of that. The two subgroups were then reared separately in two 125-l aquaria ($100 \times 50 \times 50$ cm^3) for a total of 42 days and then they were weighed. The initial growth rate of the fry (day 0–day 118) was calculated as their weight at the end of the growing period divided by the duration of the period (starting weights taken to be zero). Because initial weights of the two subgroups were different (day 118) ($=$ (ln final weight $-$ ln initial weight) $\times$ 100/time), growth rates of these fry were calculated as specific growth rates (SGR) (Schreck and Moyle, 1990). Mann–Whitney *U*-tests (Sokal and Rohlf, 1981) were used to compare growth rates for both periods (before and after separation).

In the experiments dealing with the effects of environmental factors, each factor was studied separately, while the remaining factors were maintained according to the predetermined standard conditions. Only females (initial weight: 0.4–0.8 g) were used, since the territorial behaviour of the males was considered to potentially affect the results. The experiments lasted for 30 days. Experimental treatments were: (a) temperature: 18, 20, 23, 25 and 27°C; (b) salinity: 8, 16, 24, 32, 40, 48 and 56 ppt; (c) feeding rate: 0%, 1%, 2%, 4%, 6%, 8% and 10% body weight (BW)/day. There were 15 females in each treatment. In the feeding-rate experiment, an algae controller ('Algen-Killer', 0.1 ml/l, HOBBY, Germany) was administered to each aquarium to insure that fish with lower feeding rates would not be able to supplement their lack of food with algae. Algae could normally be found growing on the bottom of the aquaria.

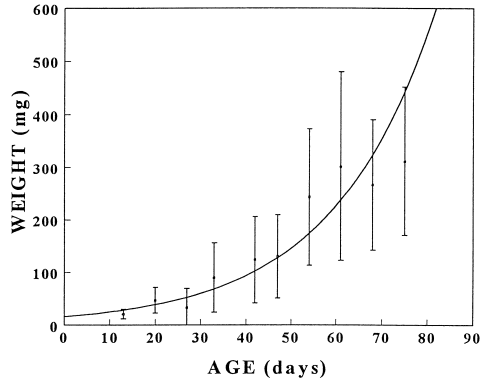

Fig. 1. Growth rate (mean ± S.D.) of *A. dispar* fry hatched in the laboratory at standard conditions (food: ad libitum) (for each point, $n = 15$).

At the end of each experiment all the fish were weighed. The SGR was calculated for each fish (see following) and a group average was calculated for each treatment. (The fish selected for each treatment group were of uniform size, with a low level of variation, and their initial weights were taken to be the group average at the beginning of the experiments.) A one-way ANOVA (SPSS 4.0, mainframe) was used for determining significant differences among SGRs. A pairwise *t*-test (SPSS 4.0) was used for determining significant differences between individual treatments.

3. Results

3.1. Growth rate of fry

The growth curve of the first group of fry, in which the fry were sampled weekly, appears in Fig. 1. The heterogeneity in size of the fry population was found to increase

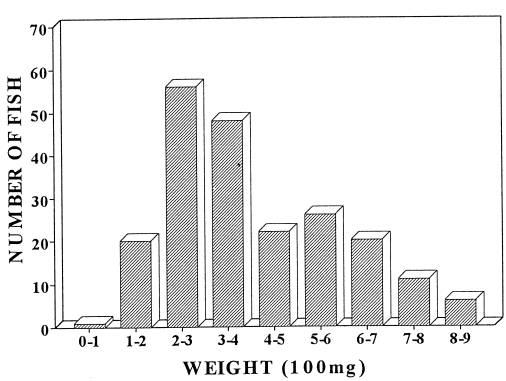

Fig. 2. Frequency distribution of *A. dispar* fry hatched in the laboratory at standard conditions (food: ad libitum) after a total of 118 days.

Table 1
The effects of grading on the growth rate of two groups of killifish, *A. dispar*: IGR, initial growth rate (before separation, days: 0–118), ($n = 20$); SGR, specific growth rate (after separation, days: 119–160), ($n = 20$); CV, coefficient of variation of the average weight of the groups after the growing period
Means in the same column with different letters are significantly different ($P < 0.05$).

	Days: 0–118		Days: 119–160	
	IGR (mg/day)	CV (S.D./mean)	SGR (mg/day)	CV (S.D./mean)
Group I	1.86 ± 0.0763a	0.041	0.676 ± 0.481a	0.205
Group II	3.19 ± 0.0932b	0.029	0.596 ± 0.480a	0.196

with their age. Age was regressed onto the average group weight indicating the growth of the fish over the examined time period to be exponential:

$$\text{weight(mg)} = 15.642\, e^{0.0446 \times \text{days}} \quad r = 0.953\ (P < 0.001)$$

3.2. Grading the fry

The weight distribution of the second group of fry at day 118 appears in Fig. 2. The distribution was skewed to the left and showed a high level of variation. The average growth rate was ca. 400 mg over 118 days. Of the two subgroups of fry isolated from the original group, growth rates of the smaller fish (I) and the large fish (II) were found to be significantly different ($P < 0.01$) for the period before separation. After separation, however, growth rates of the two subgroups were not significantly different. The results are presented in Table 1.

3.3. Effects of environment factors on the growth of adults

3.3.1. Temperature

The results of the experiment appears in Fig. 3. Significant differences ($P = 0.0002$) were found among SGR means. Fish at 18°C lost weight and the mean SGR at 20°C was

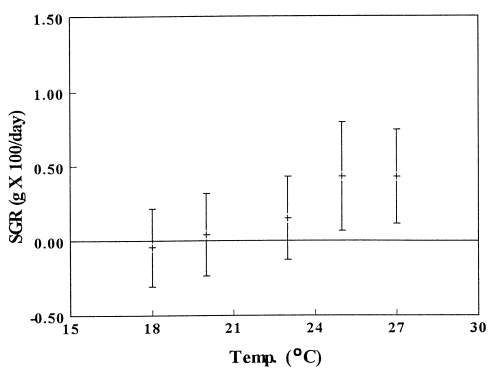

Fig. 3. Specific growth rates (mean ± S.D.) of mature female *A. dispar* held for 30 days at temperatures of 18°C to 27°C ($n = 15$ for each treatment).

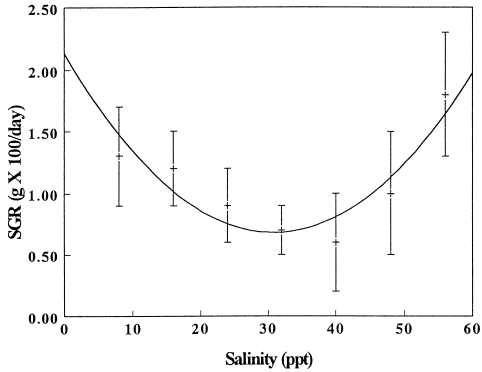

Fig. 4. Specific growth rates (mean ± S.D.) of mature female *A. dispar* held for 30 days at salinities of 8 to 56 ppt ($n = 15$ for each treatment).

just slightly above zero. SGR means were found to increase with temperature up until 23°C. Further increases in temperature were not found to cause further increases in growth.

3.3.2. Salinity

The results of the experiment appear in Fig. 4. There were significant differences ($P = 0.0001$) among SGR means. Growth rates were lowest at 40 ppt, and tended to increase towards the two outer ranges.

3.3.3. Feeding rate

The results of the experiment appear in Fig. 5. There were significant differences ($P = 0.04$) among SGR means. Growth rates increased with an increase in feeding rate up to 4% BW/day. Above that, there were no further increases in SGRs. Fish that were

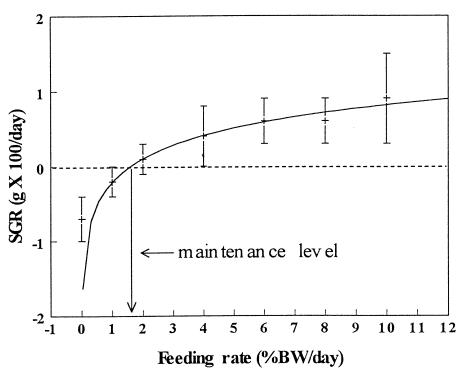

Fig. 5. Specific growth rates (mean ± S.D.) of mature female *A. dispar* held for 30 days at feeding rates of 0% to 10% BW/day ($n = 15$ for each treatment).

starved and those that received 1% BW/day lost weight. Feeding rate was regressed on to SGR means, yielding the following equation:

$$\mathrm{SGR}\,(\mathrm{g}\times 100/\mathrm{day}) = -0.208 + 0.442\ln(\text{feeding rate}) \quad r = 0.987\ (P = 0.001).$$

The maintenance level feeding rate (at which fish neither gained nor lost weight) was calculated from the regression equation to be 1.6% BW/day.

4. Discussion

4.1. Growth rate of fry

Growth of fish is normally found to be exponential over periods of a year or less (Schreck and Moyle, 1990), as was found for fry raised in this study. SGRs were calculated for growth experiments. SGRs were based on natural-log transformations and, hence, enable comparison of growth rates between groups of fish whose starting weights may not be the same (Weatherley, 1972). This is particularly advantageous when investigating fish caught in the wild, as in this study, which normally possess inherently greater variation in growth than domestic populations.

4.2. Grading the fry

Intraspecific variation in fish can be expressed in their morphological, physiological or biochemical characteristics (Kirpichnikov, 1981) as well as in the growth rate (Huet, 1986; Hepher, 1988). As the age of the killifish fry increased, so did the variation in size of the group. In addition, the size frequency distribution of the fry was found to be skewed to the left. Kirpichnikov (1981) noted that variations in body weight frequently deviate from the normal distribution of fish populations due to additional individuals which possess malformations, or poorer than normal growth rates. Larvae produced under artificial conditions are not exposed to the dangers of predation and unfavorable environmental variables that may significantly reduce survival rates of deficient, slow-growing and unstable fish.

When growing the fry, more than adequate amounts of food were given a number of times per day and, hence, lack of food in this study was not taken to be a factor affecting growth rates. Crowding effects were also ruled out since preliminary experiments had shown that stocking rates of up to seven times that of the standard set for the study did not effect growth rates of similar sized fish (Frenkel and Goren, unpublished data). Social interaction was taken to be a possibility, being known to potentially induce stress in the fish (Sumpter, 1992), and consequently suppress feeding activity and growth (Berne and Levy, 1993).

Two subgroups of fry were isolated from the overall population where one subgroup's mean weight was approximately twice that of the other. After a period of isolation (6 weeks) from each other, under identical conditions, the growth rates of both groups in

this study were not found to differ significantly. The technique of separating groups of fish according to size in fish culture is known as grading. Grading perch, *Perca fluviatilis*, grown in intensive rearing tanks, was shown to neutralize the high variability in their growth (Melard et al., 1995). In gilthead sea bream, *Sparus aurata*, frequent size grading of post larvae and fry not only reduced size differences but also substantially increased survival (Popper et al., 1992).

4.3. Effects of environmental factors on the growth of adults

A. dispar is known to tolerate large temperature ranges (Lotan, 1982; Goren, 1983; Haas and Pal, 1984; Whitehead et al., 1986); the fish have been observed in small shallow ponds withstanding daily temperature fluctuations of more than 20°C (Lotan, 1982). In this study, the SGRs of the fish were found to increase when incrementally increasing the temperature from 18°C to 23°C. Temperature is known to effect the growth of fish (Love, 1980), being manipulated in fish culture to improve both growth rates and food conversion ratios (Zanuy and Carrillo, 1985). The temperature for maximum growth exists at the balance point between efficient absorption of food and higher energy consumption at higher temperatures and reduced absorption efficiency and energy requirements at lower temperatures (Love, 1980). At increased temperatures, however, higher energy expenditures for catabolism may reduce the energy available for growth (Iwata et al., 1994). Although significant increases in growth rates in this study were observed as the temperature rose from 18°C to 23°C, further increases in growth were not reported with additional increases in temperature.

Like many other cyprinodonts, *A. dispar* is very euryhaline (Lotan, 1973; Goren, 1983; Haas and Pal, 1984), occurring naturally in a range of salinities from fresh water up to almost 400% seawater (SW) (Lotan, 1982). In laboratory experiments, fish were observed to survive direct transfer from 300% SW to freshwater, demonstrating electrolyte concentration and water content changes in plasma and muscles over a 96-h period (Lotan, 1973). The results of this study showed significant differences among SGRs of the fish held at different ambient salinities, where growth rates were lowest at 40 ppt while steadily increasing towards the two outer ranges.

Changes of salinity within the tolerance range of a particular species of fish may affect its routine metabolism (Nordlie et al., 1991), and food consumption and conversion rates (Marshall, 1970), as well as its growth (Hepher, 1988). Although patterns have been suggested for responses in metabolic rates to changes in ambient salinity, Nordlie et al. (1991) reported being not at all surprised that the extremely euryhaline cyprinodont *Cyprinodon variegatus* did not fit into any of them. *C. variegatus* was compared to *A. dispar* (which occupies habitats analogous to those of *C. variegatus*), where both similarities and differences in metabolic rate response to different salinities were found. Further examples of how individual species of killifish may vary in their responses to changing salinity include *Fundulus grandis*, which were found to have significantly higher growth rates (in their early stages) at 5, 20 and 35 ppt than at 0, 60 and 80 ppt (Perschbacher et al., 1990); and *C. mascularis*, which grew best at 35 ppt, with reduced growth observed below 15 ppt and above 55 ppt (Kinne, 1960).

A. dispar is omnivorous, feeding on crustaceans, mosquito larvae and filamentous algae (Goren, 1983). The fish were found to be suitable for controlling copepods in *Gracilaria* cultures (Friedlander et al., 1996), whereas gut contents of one population in the wild showed the fish to feed predominately (> 90%) on unicellular algae (Haas, 1982). The fish are evidently capable of withstanding long periods without eating. Those fish that were starved in this study all lost weight; however, no mortalities were reported over a period of 30 days.

Positive growth rates for *A. dispar* were noted at all feeding rates greater than 1.0% BW/day, although no significant increases in growth were observed beyond 4% BW/day. The pattern of increasing growth rate with feeding rate up until a threshold level is widely reported for commercially grown fish, where the high cost of feed motivates studies for determining the most cost-effective growth. In Atlantic salmon fry, *Salmo salar*, for example, raised for 4 weeks at 17–18°C, significant increases in mean body weight were found with each feeding rate increment from 2.5% to 6.5% BW/day, after which there were no further increases (Poston and Williams, 1991). The optimum feeding rate for growth is dependent, however, on the ambient temperature. For *Piaractus mesopotamicus* fingerlings reared at feeding rates of 1%, 3% and 5% BW/day, optimal growth was found at temperatures of 20, 24 and 27°C, respectively (Borghetti and Canzi, 1993).

The minimum feeding rate determined for maintaining the same body weight of *A. dispar* in this study, like that for the optimum growth rate, was also dependant on the temperature. Ambient temperature directly affects oxygen consumption and, therefore, the energy demands for maintenance (Hoar et al., 1983). The direct dependence of maintenance levels on temperature was demonstrated in a study on young inland silversides, *Menidia beryllina*, where maintenance requirements were found to be positively correlated with increases in the ambient temperature (Letcher and Bengtson, 1993).

5. Conclusions

A. dispar is indigenous in many countries where biological control may be implemented. Many of these countries, however, have limited resources making efficient production techniques of the fish critical. While the results presented here are seen to be an important contribution in the pursuit of implementing *A. dispar* for the biological control of mosquito larvae, further studies are needed to make their growth even more cost-effective, such as experiments on food-conversion ratios and how the interdependence of different factors affect their growth.

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