This paper not to be cited without prior reference to the author

International Council for
the Exploration of the Sea

C.M. 1971/E:26
Fisheries Improvement Committee
Ref.: F (Dem. Fish. (N) Cttee)

Rearing the larvae of the lemon sole, Microstomus kitt Walbaum, on cultured foods

by
B. R. Howell
Marine Hatchery, Port Erin, Isle of Man

THÜNEN
Digitalization sponsored by Thünen-Institut

INTRODUCTION

The laboratory rearing of the lemon sole has been hindered by the lack of a suitable food for its early larval stages which are too small to ingest Artemia nauplii. Although Blaxter and Staines (1970) report rearing lemon sole larvae on natural plankton, a mass production programme would require a food readily available in large quantities.

The aim of this work, therefore, was to identify easily cultured organisms which would support the growth of early larvae to a size at which they could ingest Artemia nauplii. Five organisms were tested: the marine flagellate Dunaliella tertiolecta Butcher; an unidentified hypotrichid ciliate, the 'vinegar eelworm' Turbatrix sp.; the trochophore larvae of the mussel, Mytilus edulis L.; and the marine rotifer Brachionus plicatilis Muller. The latter organism is widely used in Japanese fish culture (Harada 1970) and allows growth of plaice and sole larvae to metamorphosis (Howell, in prep.).

CULTURE OF THE FOOD ORGANISMS

(a) Dunaliella tertiolecta was cultured in sterile sea water contained in 20 litre spherical glass flasks and enriched according to the formula of Provasoli et al. (1957). A pH of 7.5 to 8.0 was maintained by aerating with a mixture of air and carbon dioxide in appropriate proportions. Temperature was controlled at 21.0 ± 1.0°C and illumination provided by groups of 30W 'daylight' fluorescent tubes.

(b) Brachionus plicatilis was cultured in full salinity sea water in 60 x 30 x 30 cm open perspex tanks using D. tertiolecta as food, at a temperature of 18 ± 1.0°C. Cultures were gently aerated and continuously illuminated by light from the flagellate culture system. Each day 30 per cent of every culture was replaced with a fresh flagellate suspension and the rotifers concentrated using a 60 µm mesh nylon net.

(c) A hypotrichid ciliate of approximate dimensions 90 x 60 µm was a common contaminant of some of the rotifer cultures. A concentrated suspension of the organism was obtained by further passing the rotifer washings through a 25 µm mesh nylon net.

(d) Mussel trochophores Unripe adult Mytilus edulis were brought into the laboratory during late February and conditioned in tanks of well aerated, running sea water at a temperature of 10.0-12.0°C for a minimum period of two weeks. Conditioned mussels, when stimulated by violent shaking in a plastic bucket for 2-3 minutes, spawned within 12 hours on being replaced in lightly aerated static sea water at 15°C. Within 24-48 hours, trochophore larvae developed which could be concentrated using a 25 µm mesh nylon net.

(e) Turbatrix sp. Small quantities of this nematode were cultured in a vinegar solution.

(f) Artemia nauplii were hatched from eggs of San Francisco origin by a technique similar to that described by Riley (1966). Batches were hatched daily so that nauplii were always less than 1 day old when fed to the larvae.

LARVAL FEEDING EXPERIMENTS

Lemon sole larvae were obtained from pond-spawned eggs incubated under standard conditions in 60 x 30 x 30 cm black polythene tanks. Eggs incubated at 7.0 ± 1.0°C and 10.0 ± 1.0°C hatched after approximately 12 and 7 days respectively.

An experiment was carried out in 1 litre beakers to determine whether any of the available organisms had any value as a food to first-feeding larvae. Twelve 1 litre beakers were each stocked with 25 late yolk-sac larvae and each of the five foods offered ad lib. in duplicate beakers, with the remaining two beakers serving as unfed controls. Dead larvae were counted and removed daily. Only mussel trochophores and rotifers supported survival:

Food offered	Mean survival after 7 days (%)
B. plicatilis	88
Mussel trochophores	87
D. tertiolecta	6
Ciliates	10
Turbatrix sp.	10
No food	10

In a larger scale experiment, early larvae were offered a mixture of rotifers and trochophores. Two standard 60 x 30 x 30 cm rearing tanks were each stocked with 500 late yolk-sac larvae, gently aerated and irrigated with a flow of fresh sea water at a rate of 2-3 litres per hour. Illumination was provided for 12 hours per day by 40W 'daylight' fluorescent tubes giving a light intensity of 400 mc at the water surface. Temperatures varied between 9.5 and 13.5°C (Fig. 1). Dead larvae were counted and removed every 2 days. Periodically, representative samples of starved larvae were preserved in 5 per cent formaldehyde in a standard physiological saline, after narcotization in MS222, and later weighed and measured.

Food, initially a mixture of trochophores and rotifers, was first offered 8 days after hatching. An examination of the early samples, prior to preservation, indicated that the larvae were exercising a high degree of selection in their feeding behaviour. On days 12 and 20 all feeding larvae contained only trochophores in their guts, although both foods had been shown to be acceptable. By day 29 all larvae were feeding exclusively on rotifers so trochophores were no longer offered. Surviving larvae were successfully transferred to a diet of Artemia nauplii between days 40 and 52 (Fig. 1).

Cumulative survival values have been calculated from the mortality data, a correction having been made for those larvae removed in sampling. The pattern of mortality (Fig. 1) was similar to those obtained with captive populations of other flatfish larvae, a 'critical period' of comparatively high mortality being followed by a period of low, gradual mortality (Shelbourne 1970). However, the early mortalities of lemon sole larvae were severe compared with those of larval plaice and sole reared under similar conditions, only 32 per cent surviving to day 30. Thereafter, mortalities were low with the further loss of less than 10 per cent over the remaining 65 days of the experiment.

The lemon sole larvae grew from a mean length of 5.8 mm (0.23 mg) at the end of the yolk sac stage to a mean length of 19.5 mm (83.5 mg) (Fig. 2) when more than 70 per cent had reached stage 5, as described by Ryland (1966) for plaice larvae. Larval development showed important differences from that of the plaice. Whereas the larvae metamorphosed at a larger size, the migration of the left eye commenced earlier relative to the development of the heterocercal condition of the caudal fin. Asymmetry occurred before the upturning of the notochord with the left eye visible from the right side before the angle of notochord had reached 45°.

From these data the relationship between logarithmic transformations of larval length (L mm) and weight (W mg) is described by the equations:

$$\log_e W = 4.9031 \log_e L - 10.2377 \quad (S.E. = 0.1963) \quad (1)$$

$$\log_e L = 0.2019 \log_e W + 2.0904 \quad (S.E. = 0.0398) \quad (2)$$

(correlation coefficient = 0.995; n = 317).

Equation 1 is of the general form $W = a + kL^n$ where k is the ponderal index and the 'y-intersect', a, is assumed to be of negligible value. The value of the exponent, n, differs appreciably from the value of 3 applicable to three-dimensional growth situations where the material being laid down is of constant density. Although the data have not yet been analysed fully, it seems probable that this anomaly is the result of a continuous change in the value of k produced by changes in the body proportions of the developing larvae.

CONCLUSIONS

A technique for rearing lemon sole larvae to metamorphosis on cultured foods is described. Future studies will be aimed at reducing the heavy losses during the early stages, possibly by the provision of more suitable foods. It may also prove beneficial to increase the size of the food offered to the later stages although the harpacticoid copepod Tigriopus sp. and the oligochaete worm Lumbricillus sp. were unacceptable to larvae of 18.0 mm length.

REFERENCES

BLAXTER, J. H. S. and STAINES, Mary, 1970. Pure-cone retinae and retino-motor responses in larval teleosts. J. mar. biol. Ass. U.K., 50, 449-460.

HARADA, T., 1970. The present status of marine fish cultivation research in Japan. Helgoländer Wiss. Meeresunters., 20, 594-601.

PROVASOLI, L., McLAUGHLIN, J. J. A. and DROOP, M. R., 1957. The development of artificial media for marine algae. Arch. Mikrobiol., 25 392-428.

RILEY, J. D., 1966. Marine fish culture in Britain. VII. Plaice (Pleuronectes platessa L.) post-larval feeding on Artemia salina L. nauplii and the affects of varying feeding levels. J. Cons. perm. int. Explor. Mer, 30 (2), 204-221.

RYLAND, J. S., 1966. Observations of the development of larvae of the plaice, Pleuronectes platessa L., in aquaria. J. Cons. perm. int. Explor. Mer, 30 (2), 177-195.

SHELBOURNE, J. E., 1970. Marine fish cultivation: priorities and progress in Britain. In: Marine Aquiculture, Ed. William J. McNeil, Oregon State University Press, Corvallis, Oregon.

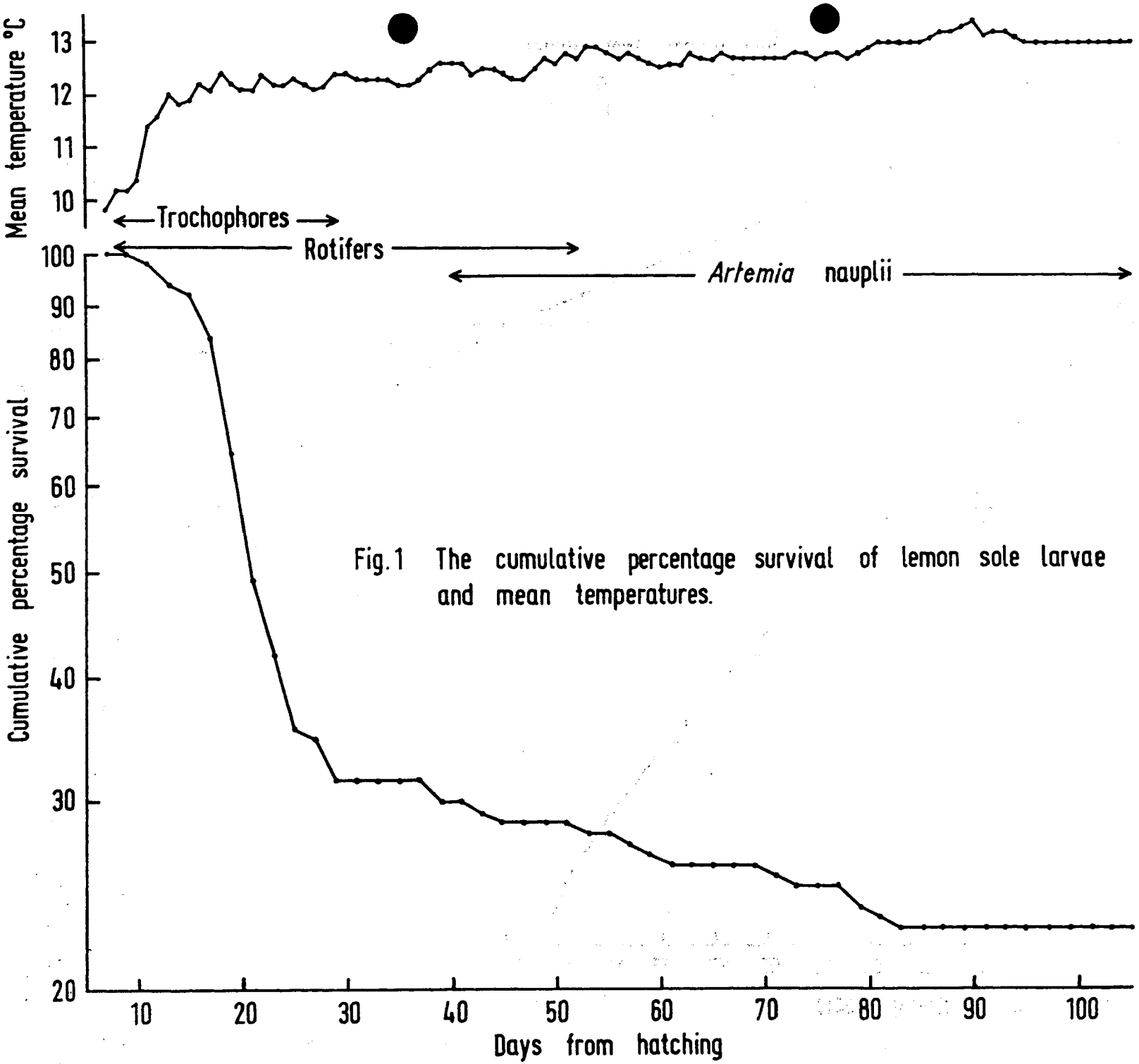

Fig. 1 The cumulative percentage survival of lemon sole larvae and mean temperatures.

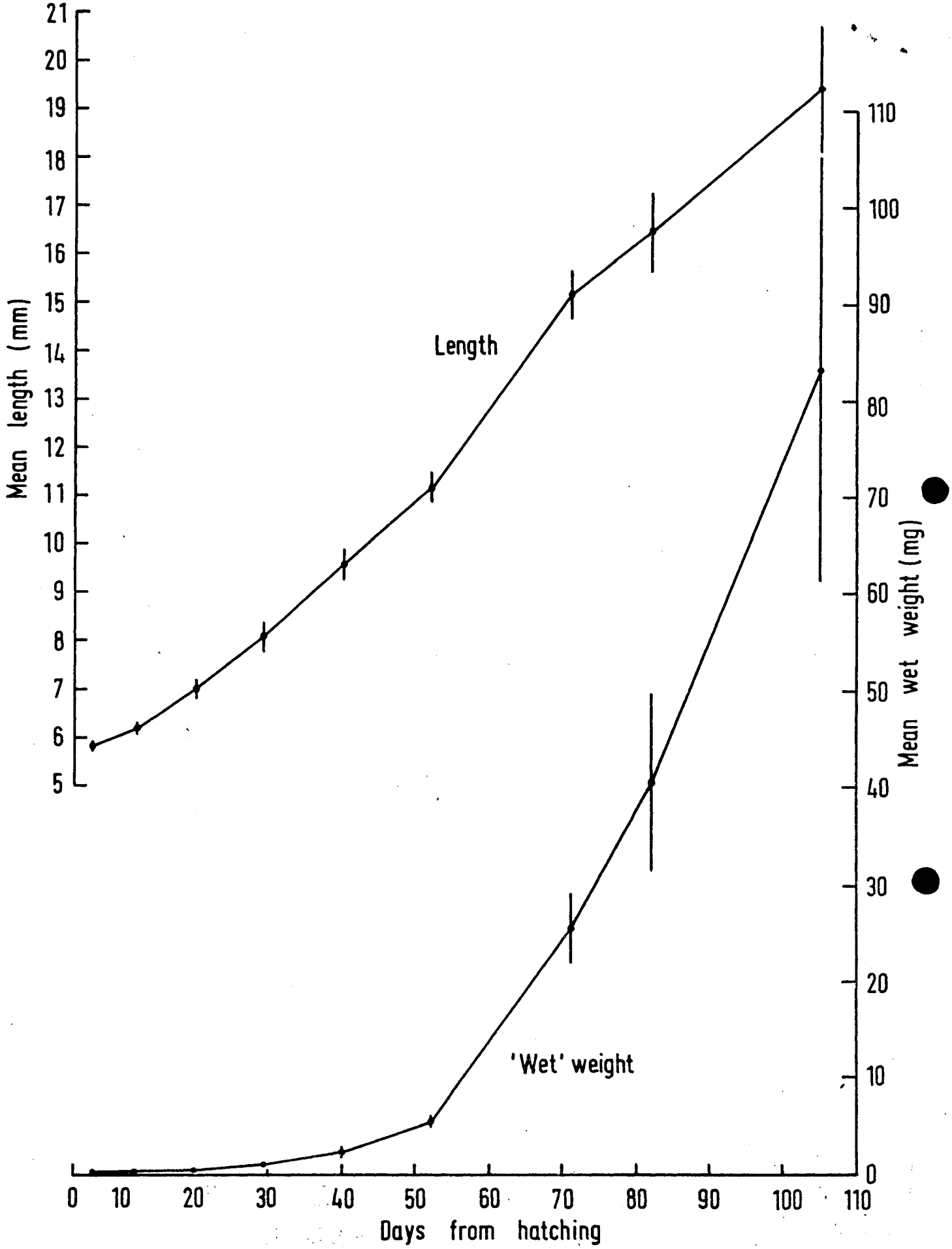

Fig. 2 Growth in length and weight of lemon sole larvae. (95% confidence limits shown by vertical lines.)