REARING OF PLAICE (*PLEURONECTES PLATESSA*) LARVAE TO METAMORPHOSIS USING AN ARTIFICIAL DIET

J. W. ADRON, A. BLAIR, AND C. B. COWEY[1]

ABSTRACT

Newly hatched larval plaice were grown to metamorphosis using an artificial diet. The overall survival rate to metamorphosis was of the order of 20%. This compares with a survival rate of 38% in control larvae fed *Artemia* in a similar tank system.

The preparation of the artificial diet is described. The main protein component was freeze-dried cod muscle and the diet contained 70.7% crude protein, 9.7% lipid, 7.9% ash, and 5% digestible carbohydrate.

This food in particle sizes ranging from 180 to 355 μ was introduced automatically into the inflowing water of a cylindrical tank containing 200 yolk sac larvae. Water temperature was 10±2°C. After about 13–14 days a relatively high larval mortality occurred, leaving approximately 70 established feeding larvae. Unfed larvae in an identical control tank did not survive beyond this time.

Once feeding had been established larval mortalities were occasional and sporadic. Thirty-five days after commencement of the experiment some larvae began to metamorphose, and 56 days from the start some 35 metamorphosed fish were transferred to a separate tank. These fish have since continued to feed and grow on the same diet.

The rearing of marine flatfish from egg to metamorphosis and subsequently to more adult stages was achieved under laboratory or hatchery conditions using live food (*Artemia salina*) about 10 yr ago ((Shelbourne, 1964). The experimental animal was plaice, *Pleuronectes platessa*. Since then other flatfish (lemon sole, *Microstomus kitt;* Dover sole, *Solea solea*; and turbot, *Scophthalmus maximus*) have been similarly reared to metamorphosis, using either the same food organism for sole or a combination of organisms such as rotifers followed by *Artemia* for turbot.

While such methods have been applied successfully on a pilot scale the ability to rear these fish on an artificial diet may confer certain advantages such as: (1) the ability to change the composition of the food and so ultimately arrive at a composite ration approaching the optimal requirement of the larva; (2) continuity of a food supply of standard quality (the large scale production of live food other than *Artemia* involves cultivation of several organisms, e.g. rotifers, and food for rotifers. Thus the whole cultivation program must be carefully synchronized and there must be certainty that production of food will keep pace with the increasing demands of the growing larval fish. Moreover, *Artemia* themselves may vary in nutritional quality and may contain variable amounts of pesticide residues (Bookhout and Costlow, 1970)); (3) elimination of the need to wean metamorphosed larvae from a natural to an artificial food. There can be little doubt that the availability of compounded foods has contributed greatly to the growth of fish farming procedures for freshwater fish such as trout, salmon, and channel catfish in several countries. All these species of fish have large eggs which give rise to large fry so that, compared with the early rearing of marine fish larvae, few technical problems arise.

The present paper describes a partially successful attempt to rear plaice from egg to metamorphosis using artificial food under small scale laboratory conditions.

EXPERIMENTAL

The apparatus used is shown in Figure 1 and Figure 2. The larval rearing tank was cylindrical and measured 26 cm in diameter with a depth of 23 cm. It was contained in an outer vessel which was normally full of seawater. The bottom of the tank was formed from a circular piece of rigid polyvinyl chloride pipe (Durapipe[2]) which fitted closely

[1]Institute of Marine Biochemistry, St. Fittick's Road, Aberdeen AB1 3RA Scotland.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Manuscript accepted September 1973.
FISHERY BULLETIN: VOL. 72, NO. 2, 1974.

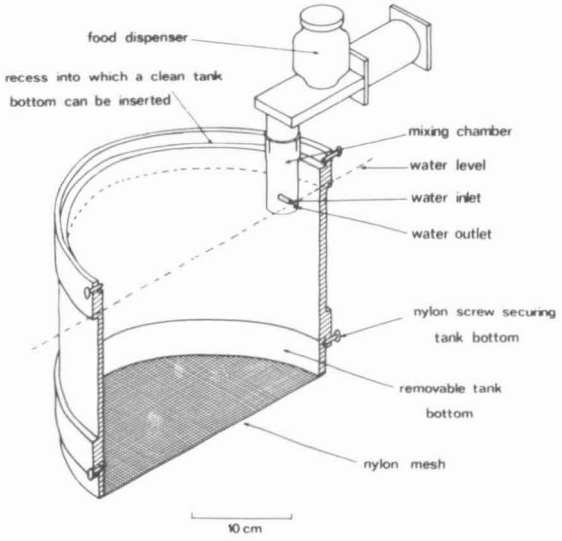

FIGURE 1.—Vertical section through the larvae rearing tank. In use the tank is suspended within a larger plastic vessel.

FIGURE 2.—Photograph of one of the larvae rearing units in use.

round the cylindrical tank and was firmly clipped onto it by a bayonet attachment. This circular piece of Durapipe was covered with 0.75 mm aperture nylon mesh to retain larvae and possibly permit uneaten food to escape. In the event the food tended to swell after being in the water for some time and only a portion of it passed through the screen at the bottom of the tank. To facilitate tank cleaning and hygiene, therefore, an exactly similar cover could be fitted over the top of the cylindrical tank and the tank slowly and carefully inverted in the outer vessel so that the bottom cover was now at the top and could readily be removed together with adhering uneaten food. This cleaning arrangement is not ideal, but by permitting the removal of much of the uneaten food from the bottom of the tank, it allowed positive control over tank hygiene and water quality. These factors have been a major obstacle to cultivation of larval fish on artificial diets in earlier experiments. As the larvae grew larger it became more and more possible to siphon uneaten food from the bottom of the tank without endangering or losing larvae.

Food was dispensed into the tank with the inflowing water by means of an automatic feeder of the type described by Adron (1972). This feeder was held on a clamp stand adjacent to the tank and could readily be removed when the tank was being cleaned by inversion. Approximately 15 mg dry food was introduced into the water via a mixing chamber at 10 min intervals, the flow rate of water being about 150 ml/min. The experiment was carried out at a temperature of $10° \pm 2°C$ with a light intensity at the water surface of 250 lux provided by fluorescent light.

The composition of the food used is shown in Table 1. Freeze-dried cod muscle and shrimp meal were prepared as in Cowey, Pope, Adron, and Blair (1972). Freeze–dried whole hen's egg was prepared by drying homogenized hen's egg in a bulk centrifugal freeze drier. The gelatin (pre-

TABLE 1.–Composition of diet used for rearing larval plaice.

Component	g/100 g dry diet
Freeze dried cod muscle	53.4
Freeze dried whole hen's egg	10.0
Shrimp meal	10.0
Cod liver oil	4.0
Encapsulated vitamin mixture	3.3
[1]Vitamin mixture	2.8
[1]Mineral mixture	1.0
Glucose	5.0
α-tocopherol	0.4
Sunset yellow F.C F.	0.1
Furanace	0.8 mg
Gelatin	10.0
[2]Water	150 ml

[1]Cowey et al. 1972
[2]Removed finally by freeze drying

pared from swine skin 300 bloom) and α-tocopherol (500 mg α-tocopherol per g) were obtained from the Sigma Chemical Co., Ltd. Sunset yellow was a gift from Imperial Chemical Industries; it was included in the diet to simulate the color of *Artemia* nauplii. Furanace, a broad spectrum antibiotic developed specifically for fish (Shimizu and Takase, 1967) was obtained from Dainippon Pharmaceutical Co., Ltd. Half of the vitamin supplement was encapsulated in hydrogenated coconut oil to prevent the leaching out of the vitamins into the seawater. Lest this procedure rendered vitamins unavailable to the larvae, the other half of the supplement was added without further treatment. To encapsulate the vitamin mixture 28 g were homogenized in 100 ml of di-ethyl ether in which 5 g of hydrogenated coconut oil MP 32-34°C (Loders and Nucoline Ltd., London) had been dissolved. The homogenate was dried in a Bucchi Rotary evaporator.

Freeze-dried cod muscle, freeze-dried whole egg, and shrimp meal were finely ground together in a hammer mill. To these ground components were added the vitamin mixture, mineral mixture, and glucose. The cod liver oil and α-tocopherol were mixed together and then thoroughly mixed with the dry components, mixing being carried out in a Hobart food mixer. The furanace and sunset yellow were dissolved in ⅔ of the allotted water and mixed with the dry components. The gelatin was dissolved in the remaining water at 50°C before being mixed with the other dietary components. While still warm the moist diet was pressed into slabs 5 mm thick and cooled to room temperature. The slabs of diet were then dried in a bulk freeze-drier, ground with a pestle and mortar and graded with sieves, to give sizes of 250 μ-355 μ and 180 μ-250 μ. For the first 2 days of feeding the larvae were given only diet of 180 μ-250 μ; for the next 8 days increasing quantities of the 250 μ-355 μ size were mixed with the 180 μ-250 μ size until only the 250 μ-355 μ size was offered. By analysis the diet contained protein (N × 6.25) 70.7%, lipid 9.7%, and ash 7.9%.

Two hundred newly hatched larvae were put into each of three tanks on 2 April 1973. These larvae were obtained from eggs kindly supplied by White Fish Authority, Hunterston; the eggs had been artificially fertilized on 12 March. Food was introduced into one of the tanks on 5 April; *Artemia* nauplii were added to the second tank, while the third tank was kept as an unfed control mainly because unfiltered seawater was being used, and it may have contained enough natural food to maintain a number of the larvae.

For the first few days of the experiment relatively few mortalities occurred in any of the tanks but then 10–12 days after hatching, a rapid mortality occurred in the unfed control, together with relatively high mortalities in the tanks fed artificial and natural (*Artemia* nauplii) diets. By 15 April no larvae remained alive in the unfed control tank, while the numbers surviving in the two tanks receiving food were about 70 in the tank receiving the compounded artificial diet and about 100 in the tank receiving *Artemia* nauplii. It seems possible that this high mortality corresponds with the complete utilization of the yolk and that the fish surviving in the tanks receiving food correspond to Shelbourne's "established feeders."

After about 15 April mortality rates fell to a low level in both the remaining tanks; some of the deaths in the tank receiving the artificial food were a direct consequence of tank-cleaning operations. Fish began to metamorphose in both these tanks as early as 6 May and by 28 May some 35 metamorphosed plaice from the tank receiving the artificial compounded diet were transferred to a conventional tank in our recirculated system where they continued to eat the same powdered diet. This represents a 17.5% survival of metamorphosed fish from hatched larvae. Of the larvae which were reared on *Artemia* some 76 survived to metamorphosis (last day of May), representing 38% of the original newly-hatched larvae. No abnormalities of pigmentation were discernible in the larvae, possibly because of the relatively uncrowded conditions in which they were reared.

The survival rates with the artificial food were much lower than with larvae fed *Artemia*; this may be due to the greater acceptability of the live moving diet as compared to the inert artificial food; the higher number of "established feeders" obtained when feeding *Artemia* perhaps supports this view. Both our survival rates are considerably lower than those achieved by Shelbourne (1963) using *Artemia*, his most successful regime giving about 67% survival to metamorphosis (including mortalities between fertilization and hatching). However, with a temperature regime somewhat similar to that used by us (his water bath 4) Shelbourne obtained survival rates not greatly different from our "*Artemia*" tank, i.e. 55% survival (when egg incubation was carried out in the pres-

ence of antibiotics) and 30% survival (eggs irrigated with seawater free of antibiotics during incubation).

Despite the lower larval survival rate when fed artificial food it is felt that this rate is sufficiently high to demonstrate the technical feasibility of using non-living food for rearing marine fish larvae. Moreover, eggs are produced in enormous numbers by marine fish and are extremely cheap so that a 20% survival rate to metamorphosis is an acceptable level. If such a survival rate can be achieved with the more exacting larvae of highly esteemed species (Dover sole, turbot) it would give a considerable impetus to the cultivation of these fish.

DISCUSSION

Reviewing marine fish larval rearing, May (1970) named the provision of a suitable food (i.e. "one which the larvae will consume and grow on and which can be supplied in sufficiently large quantities") as the prime obstacle. Several attempts have been made over the years to rear marine fish larvae on non-living, composite foods but none of these have yet been successful (Fishelson, 1963; Blaxter, 1962; Ivanchenko and Ivanchenko, 1969). In practice we have had no great difficulty in getting plaice larvae or at least a relatively high proportion of them to ingest the food and develop on it. The main obstacle has been one of tank hygiene and it remains the overriding problem. This too is recognized by May (1970) who comments on the use of non-living food: "uneaten food accumulates on the bottom of the rearing container and decays rapidly, fouling the water". Although the present set-up does permit control of the quantity of uneaten food in the water, any improvements in tank design which release uneaten food completely while retaining the larvae are desirable. Various modifications of tank design to this end are under consideration. The problem is particularly acute in the early stages as the food particles tend to swell in the water and fail to pass the screen at the bottom of tank. As larvae increase in size, and a screen of larger mesh size can be substituted at the bottom of the tank, the problem becomes less acute.

Some bacteriological control of water may be attained by sterilizing the incoming water by means of ultraviolet light and such a device should be incorporated into future experiments.

Microencapsulation of the food may offer a further means of improving tank hygiene. The microcapsules currently available seem to sink very rapidly through a water column. This militates against their chances of being consumed by larval fish in a rearing tank. The development of neutrally buoyant microcapsules, however, could lead to rapid strides in the controlled cultivation of larval marine fish.

The use of an antibiotic in the diet calls for some comment. The relatively free use in animal feeds of those antibiotics which are commonly employed in human medicine has obvious social dangers. Attention has yet again been focused on these dangers by Williams-Smith (1973). It must, therefore, be emphasized that furanace was used in very low concentrations and that it has been developed specifically for use in fish. Thus any resistant strains which could result from its use should still be sensitive to antibiotics currently in use in clinical medicine.

The diet used was designed empirically with the objects of providing a relatively large intake of high quality protein, marine oil, and a luxus of B vitamins all allied to reasonable water stability. The diet is by no means ideal and there is clearly scope for improvement in this ration in many ways. However, it does provide a basic experimental formula from which more nearly optimal diets may evolve.

LITERATURE CITED

ADRON, J. W.
1972. A design for automatic and demand feeders for experimental fish. J. Cons. 34:300-305.

BLAXTER, J. H. S.
1962. Herring rearing-IV. Rearing beyond the yolk-sac stage. Mar. Res. Dep. Agric. Fish. Scotl. 1, 18 p.

BOOKHOUT, C. G., AND J. D. COSTLOW, JR.
1970. Nutritional effects of *Artemia* from different locations on larval development of crabs. Helgoländer Wiss. Meeresunters. 20:435-442.

COWEY, C. B., J. A. POPE, J. W. ADRON, AND A. BLAIR.
1972. Studies on the nutrition of marine flatfish. The protein requirement of plaice (*Pleuronectes platessa*). Br. J. Nutr. 28:447-456.

FISHELSON, L.
1963. Observations on littoral fishes of Israel. II. Larval development and metamorphosis of *Blennius pavo* Risso (Teleostei, Blenniidae). Isr. J. Zool. 12:81-91.

IVANCHENKO, L. A., AND O. F. IVANCHENKO.
1969. Transition to active feeding by larval and juvenile white sea herring (Clupea harengus pallasi NATIO Maris-albi BERG) in artificial conditions. Dokl. Akad. Nauk SSSR 184:1444-1446.

MAY, R. C.
1970. Feeding larval marine fishes in the laboratory: a review. Calif. Mar. Res. Comm., Calif. Coop. Oceanic Fish. Invest. Rep. 14:76-83.

SHELBOURNE, J. E.
1963. A marine fish-rearing experiment using antibiotics. Nature (Lond.) 198:74-75.
1964. The artificial propagation of marine fish. *In* F. S. Russell (editor), Advances in Marine Biology, vol. 2, p. 1-83. Academic Press, N.Y.

SHIMIZU, M., AND Y. TAKASE.
1967. A potent chemotherapeutic agent against fish diseases: 6-hydroxymethyl-2-[2-(5-nitro-2-furyl) vinyl] pyridine (p-7138). Bull. Jap. Soc. Sci. Fish. 33:544-554.

WILLIAMS SMITH, H.
1973. Effect of prohibition of the use of tetracyclines in animal feeds on tetracycline resistance of faecal *E. coli* of pigs. Nature (Lond.) 243:237-238.