Helgoländer wiss. Meeresunters. 20, 620–628 (1970)

Remarks on the rearing of gobies *(Pomatoschistus minutus* and *P. lozanoi)* for experimental purposes

M. FONDS

Netherlands Institute for Sea Research; 't Horntje, Texel, The Netherlands

KURZFASSUNG: **Bemerkungen über die Aufzucht von Gobiiden *(Pomatoschistus minutus* und *P. lozanoi)* für experimentelle Zwecke.** Im Zusammenhang mit variationsstatistischen Untersuchungen über die Wirbelzahl von *Pomatoschistus (Gobius) minutus* PALLAS (33 vertebrae) und *P. lozanoi* DE BUEN (32 vertebrae) ergab sich die Notwendigkeit, diese Fische unter verschiedenen äußeren Bedingungen vom Ei bis zu einer Größe zu züchten, bei der die Wirbel einwandfrei zu erkennen sind. Erste diesbezügliche Versuche in den Jahren 1963–1965 waren wenig erfolgreich, denn von den geschlüpften Larven überlebten nur etwa 2–10 %; sie erreichten eine Maximallänge von 10 mm. Unter verbesserten Aufzuchtbedingungen gelang es im Jahre 1966, die Überlebensrate auf 66 %, bei einigen Versuchen sogar auf 90 % zu steigern. Die Hälterungsbedingungen werden geschildert und Angaben über die Fütterung der Larven bzw. Jungfische gemacht. Die Variabilität der Wirbelzahl wurde bei verschiedenen Inkubationstemperaturen (10°–20° C) und bei Kreuzungen zwischen Tieren mit 32 und 33 Wirbeln analysiert.

INTRODUCTION

Two closely related species of common gobies occur along the Dutch coast at 1 to 30 m water depths: *Pomatoschistus (Gobius) minutus* PALLAS (33 vertebrae, variation 32–34) and *P. lozanoi* DE BUEN 1923 (32 vertebrae, variation 31–33).

The rearing of gobies from eggs has been considered to be either very difficult or impossible (LEBOUR 1919, TAVOLGA 1950, KINZER 1960). We have developed rearing methods yielding 66–90 % survival. These methods will be described here and their usefulness documented on the basis of investigations concerned with numerical variation in vertebral counts.

In order to investigate the vertebral variation of *Pomatochistus minutus* and *P. lozanoi*, the larvae were raised from egg to young fish of about 10 mm total length.

REARING METHODS

Spawning

In order to obtain eggs for incubation, mature *Pomatoschistus minutus* and *P. lozanoi* were kept in aquaria of 40 × 25 × 25 cm with appropriate spawning con-

ditions. The gobies readily spawned from March to July when offered sand bottom and big mollusc shells of the genera *Ostrea, Mya* or *Cyprina* at water temperatures between 10° and 16° C.

The male goby digs a pit in the sand under a shell and cleans the inner shell surface which forms the ceiling of its nest. By means of a specific courtship behaviour a ripe female is guided into the nest and deposits her eggs on the cleaned inner surface of the mollusc shell. The male guards the eggs of one or more females until the larvae hatch (Fig. 1A). The pelagic larvae are 2.5 to 3 mm long.

The pear-shaped eggs, of approximately 1 × 0.5 mm, are attached to the inner shell surface by means of threads on the chorion at the animal pole. They are neatly and closely arranged in a rounded flat layer of 3 to 5 cm diameter, containing 2,000 to 4,000 eggs. One female can produce 3 to 4 batches of eggs in the course of 4 to 6 weeks. Very similar nesting, courting and spawning behaviour of different gobies species have been described by GUITEL (1892), NYMAN (1953), TAVOLGA (1954) and KINZER (1960).

In 1963, whole batches of eggs were used for incubation and rearing at different experimental conditions. In later years, the gobies were offered plastic discs on which they readily deposited their eggs. These discs were later cut into small pieces each containing about 100 to 200 eggs (Fig. 1B). In this way a single batch of eggs yielded the material necessary for a number of experiments.

Incubation

Within 4 h after spawning the eggs were brought to incubation in 2 l Erlenmeyer flasks (Fig. 1C) with weakly aerated seawater of different temperatures (10° to 22° C); salinities (8 ‰ to 17 ‰ Cl) and oxygen contents (air pressure from 0.5 to 2 atm). Antibiotics were added in concentrations of 50 I.U. penicillin G + 50 μg streptomycin sulph. per ml seawater.

After the embryos had reached the "black eye" stage (stage 20, TAVOLGA 1950) they were gradually transferred to one and the same temperature at which they were reared. They hatched after 14 to 4 days at water temperatures of 10° to 22° C, respectively. After hatching, the larvae were counted and transferred to the rearing jars.

Rearing

In 1963, rearing was attempted at 12 different temperature-salinity combinations in 12 asbestona basins (120 × 60 × 30 cm H) with closed water circuits.

In 1964 and 1965, high glass rearing jars of 20 × 20 × 40 cm H were used which were provided with a bottom filter of shellgrit and sand, operated by an airlift in the centre. A detailed description of bottom filters with airlifts has been published by FLÜCHTER (1964) and GREVE (1968).

In 1966, round, black polythene rearing jars of 35 cm diameter and 40 cm high,

were employed with the same seawater system as used in 1965. In this closed system of approximately 1 m³ volume, the circulating seawater was cooled, aerated with ozone-containing air and filtered through 50 cm charcoal and shell grit (Fig. 1D). The supply of seawater was directed upward into the upper water layer of the rearing jars, parallel to the wall so as to create a horizontal circular water current without too much vertical turbulence (Fig. 1E). The outflow of the seawater

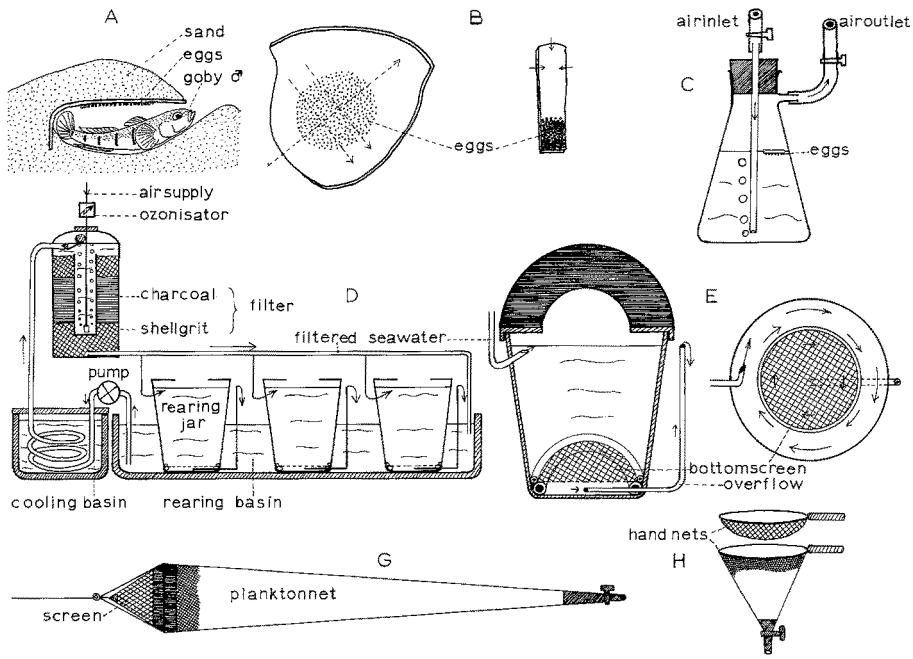

Fig. 1: *A* Male goby guarding eggs in its nest, made of a plastic disc. *B* Plastic disc with egg masses cut into pieces. *C* Incubation Erlenmeyer flask. *D* Rearing basin with rearing jars, cooling bath and ozone-charcoal filter from sideview. *E* Rearing jar from sideview and topview. Arrows indicate the water current. *G* Plankton net of 0.5 × 3 m with a screen at the opening. *H* Hand plankton nets, used for sieving and washing the zooplankton

from the rearing jars was drawn from the bottom as an overflow, protected by a plankton-gauze screen covering the bottom. This screen of 300 μ mesh-size was pressed onto the bottom of the jar by means of two tightly fitting polythene rings (Fig. 1E). The lid of the rearing jar had a circular opening of 30 cm diameter, covered by transparent plastic foil. The black covering rim of the lid caused a shadow on the wall of the jar, which prevented extensive filamentous algal growth and provided better dark-light contrast for the larvae to see their food.

Antibiotics were added to the seawater in concentrations of 20 I.U. penicillin G + 10 μg streptomycine sulph. per ml once in 14 days, together with water in order to compensate for evaporation and to keep the salinity at 16 ‰ Cl.

Food

The larvae were fed daily with North Sea zooplankton, caught at high tide off Den Helder harbour every 2 or 3 days. Several types of plankton nets were used; nets of 0.5 m opening diameter and 3 m long were the most practical for fishing from a small motor boat. The nets had mesh sizes of 56, 90 or 200 μ and the opening of the net was screened off with coarse plankton gauze of 200, 400 or 800 μ mesh size (Fig. 1G), to keep out unwanted plankton like *Noctiluca miliaris*, *Pleurobrachia pileus* and Medusae.

The plankton was stored in a pair of black polythene jars and left in the light for a few hours, so that the phytoplankton and detritus could settle. The phototactic zooplankton, swimming in the upper water layers, was then sieved through a coarse mesh net, collected in a fine mesh hand net (Fig. 1H) and washed with filtered seawater in order to remove fine detritus and phytoplankton. Antibiotics were added to the water containing the cleaned zooplankton; half of the plankton was fed to the *Gobius* larvae and the other half was kept in a black jar, with filtered seawater and weak aeration, to be used as food for the next day.

The cleaned zooplankton, collected with the 56 μ mesh size net and sieved through a 90 μ mesh size hand net, contained mainly small copepod nauplii with a body size of approximately 50–80 × 100–180 μ. With larger sizes of mesh the plankton contained more copepodits (120–200 × 240–600 μ), copepods (200–500 × 400–1,000 μ), nauplii and cyprids of balanoids (300–400 × 400–800 μ), polychaete larvae (*Polydora*, 120–200 × 700–1,000 μ) and small noctilucas (400–700 μ). If the plankton contained too many large polychaete larvae, these were washed out by sieving quickly several times through a 200 μ or a 400 μ mesh size hand net.

The larvae of the gobies were fed 56 μ mesh size net zooplankton during the first 2 weeks, sieved through 90 μ and 200 μ, respectively. The next two weeks, they were fed 90 μ mesh size net zooplankton, sieved through 200 μ and 400 μ mesh, together with *Artemia salina* nauplii (250 × 500 μ).

RESULTS

Survival rates and growth

Survival and growth rates of larvae, reared in 1963 to 1966, are shown in Table 1. In 1963 to 1965, rearing methods yielded an estimated survival of 2 to 10 % of the hatched larvae. In 1966, a batch of *Pomatoschistus lozanoi* eggs (divided into 12 experimental groups) yielded 1,585 young fishes from 2,400 hatched larvae with a survival rate of 66 %. From the original 2,800 eggs 2 % failed to develop, and approximately 12 % of the embryos died shortly before or after hatching. Some of the experimental groups yielded 150 to 200 young fish in a rearing jar and a survival rate of approximately 90 %.

In 1963, growth rate of the young fish (as given by increase in mean total length in mm per 100 days) showed a positive correlation with rearing temperatures

Table 1

Growth and survival of gobies larvae

Year	Number of young fish	Length variation (mm)	Mean length (mm)	Age (days)	Temperature (°C)	Salinity (‰)	Survival (%)	Increase in length (mm) in 100 days	Mean number of vertebrae (n)
1963	78	14–32	19.3	108	12	11	4	15.6	32.56 (78)
	96	16–40	24.5	108	15	17	10	20.3	32.12
	12	19–30	24.2	88	18	11	2	24.7	32.16
	6	23–29	26.0	94	18	14	1	25.0	32.0
	29	19–34	26.3	84	21	17	2	28.3	32.55
1964	34	20–26	23.0	60	16	16	7	34.1	32.44
1965	154	5–14	9.3	32	14.5	16	7	21.2	32.65 (118)
1966	1585	8–15	11	37	16	16	66	23	31.90

Table 2

Growth and development of *Pomatoschistus lozanoi* larvae

Length (mm)	Approximative age (days)	Vertical size open mouth (μ)	Vertical size full stomach (μ)	Development
2.5–3	1–2	160–200	80–120	Chorda, yolkrests; no finrays; dorsal larval pigment
4–5	9–14	± 240	100–160	Development of the tail skeleton; dorsal larval pigment disappears
6	16	± 320	160–200	Chorda rings, tail straight; development of D2, A, and C rays; development of tail pigment
7	18	300–370	160–250	Development of vertebrae, tail turned up; D2, A, and C rays
8	23–27	± 320	200–300	Vertebrae and urostyl formed; development of D1-, P-, and V-bases; developing pigment under vertebrae 1, 2, 3
9	28–32	300–400	250–300	Constriction of the vertebrae; development of D1 rays; development of dorsal vertebral pigment
10	27–35	± 480	300–400	Development of V rays
11	32–39	400–500	320–500	Development of P rays; development of dorsal yellow pigment
12	35–39	—	400	D1, V, and P rays; development of sensory papillae on the head
13–15			± 500	Development of neural and haemal arches; development of scales on the body

of 12° to 21° C, but no clear correlation with chlorinities of 11 ‰ to 17 ‰ Cl. Growth of the larvae in all rearing experiments was in the order of 20 to 30 mm per 100 days at 15° to 16° C, with a maximum of 34 to 39 mm per 100 days for the largest fishes.

In 1966, many larvae were sampled during rearing to examine microscopically their development, food intake and causes of mortality. The progress in development of *Pomatoschistus lozanoi* larvae is exemplified in Table 2; some larval stages are illustrated in Figure 2. At 16° C, the first young fish changed to bottom life at an age of 31 days, and a total length of approximately 12 mm.

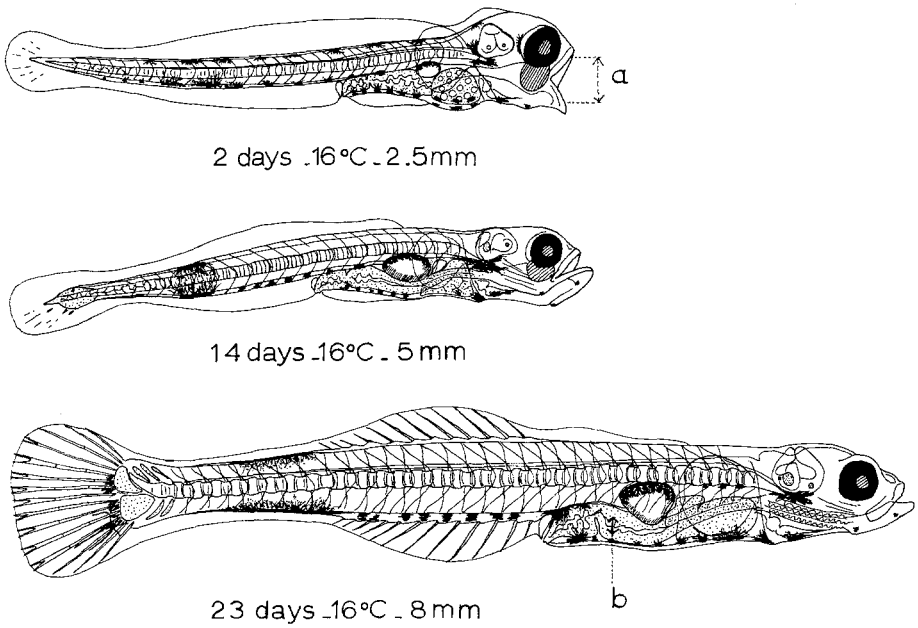

Fig. 2: Three stages in the development of *Pomatoschistus* larvae, reared in 1966. The arrows at *a* and *b* indicate the measured sizes of mouth and stomach, as given in Table 2

Young larvae had mostly copepod nauplii and copepodits (80–160 × 250–500 μ) in their stomachs. Larvae of 7 mm length or more also ate copepods, nauplii and cyprids of balanoids, polychaete larvae and larvae of *Artemia salina*. After formalin fixation of the young fishes, 93 individuals had 1 to 5 *Artemia salina* eggs in their stomachs.

Most of the young fish found dead or dying did not reveal clear causes of death. In 18 larvae a reduced liver or swim bladder was found or crystals in the urine bladder. Six dying larvae showed infection with protozoan parasites resembling *Oödinium* (NIGRELLI 1936). Four larvae were found with a nematod inside their body cavity, probably *Contracaecum aduncum* (MARKOWSKI 1937, PUNT 1941), and one of these gobies apparently died from a perforated stomach wall.

Numerical variation in vertebral counts

The vertebral count of offspring from *P. lozanoi* (32 vertebrae) found in 1966, was 31 to 33 vertebrae with a variation of the mean vertebrae number in the various experimental groups from 31.73 (n = 206) to 32.13 (n = 122) relative to the incubation temperatures of 10° to 20° C. Crossing of fishes with 32 vertebrae and 33 vertebrae yielded offspring with a mean vertebral number of 32.55 (1963; n = 107), 32.44 (1964; n = 34), and 32.65 (1965; n = 118).

DISCUSSION

The main bottleneck in rearing gobies seems to be the bacterial contamination of the seawater; the larvae are very susceptible to bacterial diseases. Adult gobies often die in the aquaria from fin rot or skin lesions and, judged from the symptoms, *Pseudomonas ichthyodermis* could be one of the main agents (SINDERMANN 1966).

For rearing gobies in the laboratory the same precautions should be taken as recommended for maintaining tropical sea fish in show aquaria (DE GRAAF 1969). To suppress the development of bacteria in the seawater application of ozone or ultraviolet light (HERALD et al. 1962) and antibiotics (OPPENHEIMER 1955, RUSTAD 1960, SHELBOURNE 1964) is necessary. A TUV lamp could be placed at the outflow of an ozone charcoal filter, to combine both methods.

Many antibiotics were tried out against bacteria in seawater by OPPENHEIMER (1955) and LAGARDE (1967), but only combinations of streptomycin and penicillin G or chloramphenicol, have been found useful for rearing purposes. According to LAGARDE 2.5 mg streptomycin + 2.5 mg chloramphenicol per litre of seawater will suppress bacterial growth for at least 20 h. OPPENHEIMER recommends 50 ppm streptomycin + 50 ppm penicillin for treatment of marine fish eggs. The 3 antibiotics mentioned here are stable in aqueous solution for at least 1 week and they exert suppressing effects on many pathogene bacteria in concentrations of 5 to 10 ppm (MANTEN 1963).

A second difficulty in rearing gobies is the provision of a proper food source, since the young larvae of 2.5 to 6 mm will not take *Artemia salina* nauplii during the first 2 weeks of their life. Although sieved natural zooplankton can be used as food, this may involve mortality caused by parasites (ROSENTHAL 1967).

To avoid laborious fishing for plankton and the introduction of parasites it would be much better to use cultivated food (ciliates, copepods) or artificial food. Since fish larvae very often eat *Artemia salina* eggs (ROSENTHAL 1969) it should be possible to rear them with artificial food pellets with a diameter of 100 to 300 μ.

It would be better to use rearing jars with plankton-gauze bottoms, suspended in a large basin. The water in this basin can then be treated rigorously (filtered, sterilized) without disturbing the fish larvae; water circulation in the rearing jars can simply be arranged by airlift from the basin. To give the fish larvae short prophylactic treatment against fungi (*Ichthyosporidium*, SPROSTON 1944) or protozoan parasites (*Oödinium* and *Cryptocaryon*, NIGRELLI 1936, 1966) the rearing jars with young fish

can be taken out of the basin and submerged in a treating bath for a short period of time. If the larvae cannot stand short air exposures, the jars can be lifted with a shallow water basin underneath.

Pomatoschistus species have a lifespan of approximately 1 year. They reach maturity within less than 1 year and readily spawn under laboratory conditions. Hence they provide excellent material for the study of marine fish eggs and larvae.

SUMMARY

1. During an investigation on the numerical variations in vertebral counts of *Pomatoschistus minutus* (33 vertebrae) und *P. lozanoi* (32 vertebrae), larvae of these closely related gobies were reared from eggs to young fish.
2. From 1963 to 1965, rearing was not very successful; it yielded 34 to 220 young fish with estimated survival rates of the larvae between 2 and 10 %. Rearing methods were improved and yielded 66 % survival in 1966; 1,585 young fish were reared from 2,400 larvae; in some experimental groups survival was approximately 90 %.
3. The seawater of the closed rearing system used in 1966 was treated with ozone; antibiotics were added in concentrations of 20 I.U. penicillin G + 10 μg streptomycin per ml seawater.
4. The larvae were fed natural zooplankton, washed and sieved through plankton gauze of different mesh sizes.
5. A description is given of the rearing method used in 1966 and the development of *Pomatoschistus lozanoi* larvae.
6. Growth rates of the larvae were in the order of 21 to 39 mm increase in length per 100 days at 14° to 16° C. The larvae changed to bottom life 31 days after hatching at a total length of approximately 12 mm.
7. Eggs of *Pomatoschistus lozanoi* (32 vertebrae), incubated at temperatures of 10° to 20° C until attainment of the "eyed egg" stage, yielded offspring (hatched and raised at 16° C) with a mean vertebral number of 31.90 (n = 1,585) and a variation in mean vertebral number for the various experimental groups from 31.73 (n = 206) to 32.13 (n = 122 vertebrae). Crossings of fishes with 32 vertebrae and 33 vertebrae yielded offspring with a mean vertebrae number of 32.44 (n = 34), 32.55 (n = 107) and 32.65 (n = 118), respectively.

LITERATURE CITED

ARNOLD, G. P., 1969. The orientation of plaice larvae (*Pleuronectes platessa* L.) in water currents. *J. exp. Biol.* **50**, 785–801.

BUEN, F. DE, 1923. *Gobius* de la Peninsula Ibérica y Baléares. *Mem. Inst. esp. Oceanogr.* **3**, 123–266.

FLÜCHTER, J., 1964. Eine besonders wirksame Aquarienfilterung und die Messung ihrer Leistung. *Helgoländer wiss. Meeresunters.* **11**, 168–170.

GRAAF, F. DE, 1969. Handboek voor het tropisch zeewater aquarium. Strengholt, Amsterdam, 336 pp.

GREVE, W., 1968. The "planktonkreisel", a new device for culturing zooplankton. *Mar. Biol.* **1**, 201–203.

GUITEL, F., 1892. Observations sur les moeurs de *Gobius minutus*. *Archs Zool. exp. gen.* **1892**, 499–550.

HERALD, E. S., DEMPSTER, R. P., WOLTERS, C. & HUNT, M. L., 1962. Filtration and ultraviolet sterilization of seawater in large closed, and semi-closed aquarium systems. *In:* Communications. 1er Congrès international d'Aquariologie, Monaco 1960. *Bull. Inst. océanogr. Monaco,* No spécial B, 49–60.

KINZER, J., 1960. Zur Ethologie und Biologie der Gobiidae unter besonderer Berücksichtigung der Schwarzgrundel *Gobius jozo* L. *Zool. Beitr.* **6**, 207–290.

LAGARDE, E., 1967. Utilisation des antibiotiques dans la réalisation des cultures de Foraminifères sous faible volume. I. Etude de l'action des antibiotiques sur les microflores hétérotrophes marines. *Vie Milieu* **18** A, 27–35.

LEBOUR, M. V., 1919. The young of the Gobiidae of the neighbourhood of Plymouth. *J. mar. biol. Ass. U.K.* **12**, 48–80.

MANTEN, A., 1963. Chemotherapeutica. Antibiotica. Antimycotica. *In:* Lammers, Nelemans & Siderius: Algemene Pharmacotherapie. L. Stafleu & Zoon, Leiden, 59 pp.

MARKOWSKI, ST., 1937. Über die Entwicklungsgeschichte und Biologie des Nematoden *Contracaecum aduncum* (RUDOLPHI 1802). *Bull. int. Acad. pol. Sci. Lett.* (Sér. B 2) **1937**, 227–247.

NIGRELLI, R. F., 1936. The morphology, cytology and life history of *Oödinium ocellatum* BROWN, a Dinoflagellate parasite on marine fish. *Zoologica, N.Y.* **21**, 129–164.

— & RUGGIERI, G. D., 1966. Enzoötics in the New York Aquarium caused by *Cryptocryon irritans* BROWN, 1951 (= *Ichthyophthirius marinus* SIKAMA, 1961), a histophagous ciliate in skin, eyes and gills of marine fishes. *Zoologica, N.Y.* **5**, 97–101.

NYMAN, K. J., 1953. Observations on the behaviour of *Gobius microps*. *Acta Soc. Fauna Flora fenn.* **69**, 1–11.

OPPENHEIMER, C. H., 1955. The effect of marine bacteria in the development and hatching of pelagic fish eggs, and the control of such bacteria by antibiotics. *Copeia* **1955** (1), 43–49.

OPPENHEIMER, J. M., 1954. Transplantation experiments on the eggs of *Gobius*. *Pubbl. Staz. zool. Napoli* **25**, 18–25.

PUNT, A., 1941. Recherches sur quelques Nematodes parasites de poissons de la mer du Nord. *Mem. Mus. r. Hist. nat. Belg.* **98**, 1–79.

ROSENTHAL, H., 1967. Parasites in larvae of the herring (*Clupea harengus* L.) fed with wild plankton. *Mar. Biol.* **1**, 10–15.

— 1969. Verdauungsgeschwindigkeit, Nahrungswahl und Nahrungsbedarf bei den Larven des Herings, *Clupea harengus* L. *Ber. dt. wiss. Kommn Meeresforsch.* **20**, 60–69.

RUSTAD, D., 1960. Improved rearing of young plaice by application of penicilline. *K. norske Vidensk. Selsk. Forh.* **33**, 1–4.

SHELBOURNE, J. E., 1964. The artificial propagation of marine fish. *Adv. mar. Biol.* **2**, 1–83.

SINDERMANN, C. J., 1966. Diseases of marine fishes. *Adv. Mar. Biol.* **4**, 1–89.

SPROSTON, N. G., 1944. *Ichthyosporidium hoferi* (PLEHN & MULSOW, 1911), an internal fungoid parasite of the mackerel. *J. mar. biol. Ass. U.K.* **26**, 72–98.

TAVOLGA, W. N., 1950. Development of the gobiid fish *Bathygobius soporator*. *J. Morph.* **87**, 467–492.

— 1954. Reproductive behavior in the gobiid fish *Bathygobius soporator*. *Bull. Am. Mus. nat. Hist.* **104**, 431–459.

Author's address: Dr. M. FONDS
Nederlands Instituut voor Onderzoek der Zee
P. B. 59
Texel, Netherlands