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The culture of benthic polychaetes and harpacticoid copepods on agar

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Abstract

A method is described whereby it has been possible to rear a capitellid polychaete, *Capitellides* (= *Capitella*) *giardi* Mesnil, and a new species of laophontid harpacticoid, *Microlaophonte sp.*, through many generations. Cultures were maintained on a layer of agar in clear polystyrene containers with the minimum attention. No decrease in viability of the populations was observed over a three year period. It has been possible to elucidate the life history of both species and to make many useful behavioural observations. The effects of temperature and salinity on the growth and mortality of the polychaete have been studied in detail.

Introduction

It was noted by Kinne (1970) in his opening address of the International Helgoland Symposium on the cultivation of marine organisms that less than 2 % of all known marine organisms could then be reared through their whole life cycle under controlled conditions. No dramatic improvement on this figure has occurred in the 6 years since that conference although many useful technical advances have been made, particularly in the cultivation of organisms used as a direct food source by man.

The utilization of benthic polychaetes and copepods as a primary food-source is unlikely ever to be of more than marginal importance (*e.g.* the consumption of the epitokes of the palolo worm by samoan natives). Rather their value lies in the fact that they occupy a vital position in the benthic marine food chains between the primary producers and the larger carnivores such as the demersal fish. Gut content analyses of many commercially exploited bottom feeding fish testify to the importance of these groups in the overall economy of the marine benthos (Wheeler, 1969 ; Odum and Heald, 1972). Therefore the culture of these organisms in the laboratory in order to elucidate their life cycles is a desirable objective, as is the development of a method by which the organisms can be reared in sufficient numbers to enable the effects of various environmental parameters on the reproduction, growth and mortality to be accurately assessed. Once the behaviour of such food organisms under controlled environmental

conditions has been ascertained a more meaningful forecast of the way in which they fit into the complex food webs of particular environments can be made.

If the animal cultures can be produced cheaply and maintained easily then their use as a food source for commercially desirable macrobenthic organisms is a distinct possibility. ''Standard'' laboratory populations that can be readily transferred to other institutions are of great value particularly for the short-term and long-term toxicity testing of pollutants. Both Akesson (1970) and Reish (1973) have stressed the value of such populations in marine pollution research.

In this paper a method is described whereby it has been possible to establish and maintain laboratory populations of the capitellid polychaete, *Capitellides* (=*Capitella*) *giardi* Mesnil and a new species of laophontid harpacticoid, *Microlaophonte sp.* with the minimum of attention.

Material

The few original specimens of *C. giardi* and *Microlaophonte sp.* were obtained in August, 1972 from Fell and Hendrix (Rosenstiel School of Marine and Atmospheric Science, University of Miami). The two species had appeared initially as contaminants in their agar plate cultures during investigations on the fungi and nematodes of the southern Florida red mangrove forest detrital system. Further specimens of *C. giardi* were collected by the author in August 1973 from intertidal habitats (including mangrove) containing quantities of organic detritus on the caribbean island of St. Lucia.

The small capitellid worm species (large adults 20 mm long) was originally described from northern european waters where it is to be found in detrital accumulations in such habitats as mussel byssus threads and *Laminaria sp.* holdfasts. The specimens obtained from the New World show some variation from their european conterparts in the number of capillary and hooked setae present along the body but these anomalies may be due to differences in prevailing seawater temperatures rather than to hereditary factors.

The species identity of the small laophontid harpacticoid (adult 0.5 mm long), is still uncertain but the taxonomic position is at present being clarified by a colleague at the British Museum (Natural History).

Culture method

Previous methods

A number of species of benthic polychaetes have been reared successfully in the laboratory by various workers for the purpose of elucidating their life histories. Little success has been achieved with errant carnivorous forms usually because of the difficulties of providing an adequate supply of live food, but many surface-dwelling omnivorous and herbivorous worms are far less exacting in their requirements. Powdered dry algae such as *Ulva sp.* and *Enteromorpha sp.* have been used frequently for food as have such plants as nettles, lettuce and spinach. Other worms have thrived on liver powder, dried mussel and commerically available aquarium fish-foods.

Throughout the world, several species of benthic harpacticoids have been cultured in laboratories using similar techniques to those for polychaetes. The animals were invariably maintained in seawater and supplied with a variety of foods including flagellates and diatoms, dehydrated and fresh algae, wood and dried mussel.

All the methods employed to date suffer to some extent from the fact that food doses must be carefully regulated to prevent the seawater becoming fouled with uneaten food. An excess of food often leads to a rapid deoxygenation and contamination with harmful bacteria and protozoa. To obviate this, frequent changes of seawater are required, or alternatively, a continuous-flow system needs to be adopted. Filtration of the water and/or aeration will sometimes alleviate the situation as will sterilisation with heat, ultraviolet light or antibiotics. More often than not, little attempt is made to duplicate the substratum conditions existing in the natural habitat when benthic species are laboratory reared. The artificiality of the culture systems, therefore, usually precludes the possibility of making significant behavioural observations.

Both *C. giardi* and *Microlaophonte sp.* can be reared in seawater using *Ulva sp.* and *Enteromorpha sp.* as a food source, with all the attendant problems outlined above. A less time-consuming method for the mass culture of non-selective bottom feeders has been sought by me for a number of years and the possibility that both a polychaete and a harpacticoid copepod could survive in or on a semi-solid organic medium such as agar (Fell and Hendrix, personal communication; Hopper *et al.*, 1973) was intriguing and warranted further investigation.

Choice of substratum

Examination of living animals that tunnel through opaque deposits has always presented a problem to marine biologists intent on investigating these organisms. Sophisticated techniques of X-ray radiography for examination of burrowing invertebrates have been employed by some workers (Howard, 1968; Howard and Elders, 1970), but the techniques suffer from being expensive. An alternative method using a naturally occurring mineral, cryolite (Na_3 AlF_6), as a medium in which the burrowing activities of aquatic organisms can be studied has been recently proposed by Josephson and Flessa (1972). The mineral has the advantage of having a refractive index close to that of seawater thus presenting a transparent medium through which the animals can be seen. The medium, however, has certain disadvantages, being somewhat toxic in the long term; also the problem of feeding the infauna still remains.

The presentation of a translucent, wholly organic, substratum in which the animals could forage would possibly solve the dual problem of providing them with a medium that would support their nutritional requirements as well as allowing examination of the animals *in situ*. Both *C. giardi* and *Microlaophonte sp.* normally crawl over and tunnel their way through the uncompacted organically rich surface layers of debris that have accumulated in sheltered tidal areas. They continue to feed throughout the tidal cycle, protected from desiccation by the semi-liquid nature of this type of sediment at low water.

Various organic solidifying agents have been employed in the past for rendering media sufficiently solid for culturing bacteria, fungi and nematodes. The two most com-

monly used are gelatine and agar. Gelatine, a proteinous material, suffers from lack of gel strength at temperatures above 25 °C and therefore is not suitable for rearing animals from tropical and subtropical areas. It suffers from the additional problem of being susceptible to liquefaction by proteolytic bacteria which constitute a large proportion of the marine bacterial flora (Zobell, 1941).

Agar (composed of polysaccharides) on the other hand does not liquefy at such low temperatures. Only about 3-4 % of marine bacteria digest agar (Zobell, 1941) and the period of time before liquefaction takes place is relatively greater than for gelatine. Agar would thus appear to be the most suitable organic solidifying agent on which to rear *C. giardi* and *Microlaophonte sp.*

Medium consistency

Investigations into the burrowing capabilities of *C. giardi* showed that when the gel was prepared to the manufacturer's specifications (1.2.-1.5 % solution of agar in water), it was helpful to furrow the surface to facilitate the worm's entry (Fig. 1A). *Microlaophonte sp.* found it more difficult to forage through normal strength gel even when it had been extensively scored; consequently gels were prepared at half the recommended strength (''sloppy'' agar) as a standard substratum for both species. Hopper *et al.* (1973) found this gel consistency most useful when culturing marine nematodes and other meiofaunal elements from red mangrove detritus.

Nutritional requirements

Initial experiments were carried out on plain agar using adult specimens of *C. giardi* and *Microlaophonte sp.* that had been retained in filtered sea-water sterilised with a combination of 200 µg/l streptomycin sulphate and 200 µg/l benzyl penicillin for 3 days prior to placing on the surface of a sterile agar plate. Both species made repeated excursions over the medium but on the whole showed little inclination to establish themselves and feed normally. The experiments were terminated after 3 weeks when mortality had reached a high level (50 %). These observations suggest that bacteria are important in the nutrition of both these benthic species (Fenchel, 1969). In one or two plates where a fungus was able to establish itself within the agar the polychaetes seemed able to derive enough nutriment from this source to sustain themselves but they rarely reached sexual maturity.

A second set of experiments showed that the two species could be reared succesfully if they were transferred to the agar without pretreatment with antibiotics, especially if the agar had been conditioned with natural seawater for a few days beforehand. Presumably preconditioning allowed time for microorganisms present in the seawater to establish themselves on the agar. The animals were seen to ingest large quantities of agar and pass it rapidly through their guts. Examination of agar and faeces using scanning electron microscopy techniques suggested that the agar changed little structurally on passage through the gut. It is likely, therefore, that the animals derive little nourishment directly from this source, but rely more upon the microorganisms (bacteria fungi, diatoms, and Protozoa) present in the agar to fulfil their nutritional re-

quirements. Newell (1965) demonstrated that a similar type of microbial feeding occurs in the small gastropod, *Hydrobia ulvae* (Pennant).

It is usual when culturing microorganisms and nematodes (Von Thun, 1966; Lee *et al.* 1970; Gerlach and Schrage, 1971) to provide them with organic nutrients to promote their growth. Peptone is one of the most commonly used protein sources and is often combined with a little ferric phosphate (Difco marine agar 2216) for the culture of heterotrophic marine bacteria, or mixed with beef extract (Oxoid blood agar base) for the growth of pathogenic and non-pathogenic bacteria. Both these media were tried in the hope that the additional nutrient would promote the growth of beneficial organisms, but unfortunately the cultures were rapidly overcome by deleterious bacteria. Further experiments with other media revealed that corn meal agar (Difco or Oxoid) was the most successful fortified agar for culturing both *C. giardi* and *Microlaophonte sp.* and confirmed the preliminary observation of Fell and Hendrix (personal communication). This medium is nutritionally impoverished compared with many others and is most usually employed for stock culture of fungi. The lack of nutrients is sufficient to suppress the prolific growth of harmful bacteria. The low pH (6.5-7.0 when prepared with seawater) is also useful in preventing excessive bacterial growth but does not have any deleterious effect on the development of either the polychaete or the harpacticoid. Since the growth rates achieved by both species were generally higher in corn meal agar than in plain agar, corn meal agar was used normally for the stock cultures.

Medium preparation

Agar gels were prepared by rehydrating with seawater. Commercially available agars vary somewhat in the ease with which they will dissolve in seawater and in the clarity of the resulting medium; however Difco and Oxoid products were found to be satisfactory. When possible filtered natural seawater from the English Channel off Plymouth was used to make up the media, as salt precipitation from artificial seawaters sometimes proved troublesome at normal salinities. Seawater was aged at room temperature for 2-3 weeks before use. Zobell (1941) found that more consistent results were obtained in culturing marine bacteria when seawater was treated in this manner.

Salinity tolerance experiments performed on both species showed that they survived well in seawater from 15-45 ‰ (Table I) over a 2 week period. It was decided therefore, to use media with a salinity of either 20 ‰ or 35 ‰ for normal mass culturing to ensure that salinity would have no effect on survival.

Rearing containers

Bernhard and Zattera (1970) drew attention to the toxicity of natural rubbers and certain plastics to marine organisms in experimental systems. Once it has been established that no chemicals toxic to either the worms or the copepods were present in the walls of containers manufactured from polystyrene, it was decided to adopt these clear lightweight vessels in preference to glass dishes for the rearing work. Compartmented plastic boxes have been used in several laboratories in recent years for rearing larval crusta-

TABLE I

Percentage survival of *C. giardi* and *Microlaophonte sp.* after 2 weeks at various salinities. Percentages are means from 10 experiments containing at the onset 20 adults and 20 larvae

Salinity (‰)	*C. giardi* % survival		*Microlaophonte sp.* % survival	
	Adults	Larvae/Juveniles	Adults	Nauplii/Copepodites
2.5	0	—	0	—
5	54	—	60	—
10	88	93	91	95
15	95	95	94	97
20	100	98	96	100
35	94	98	100	98
45	98	96	100	100
50	44	55	90	98
55	0	—	0	—

ceans (*e.g.* Rice and Williamson, 1970). These boxes, which have fitted lids, are available in a number of sizes. The smallest boxes, which fit conveniently on to the stage of a low power microscope, were used for experiments on *Microlaophonte sp.* (Table II). The larger containers, although convenient for holding stock populations of *C. giardi*, were not easy to handle under the microscope and thus disposable petri dishes (sometimes with three equal sized compartments) were used for culturing the worm when microscopic examination was required. Petri dishes proved most useful for rearing stocks of *Microlaophonte sp.*

Rearing techniques

Stock cultures of *C. giardi* were started by introducing one or two gravid adults or adults plus their recently laid egg batches on to the scored surface of the agar. No attempt was made to clean the old agar from around the worms before transfer, thus an inoculum of bacteria, fungi, diatoms, protozoa and nematodes was introduced to the new agar.

The adults started exploratory movements within a few minutes and after a few days had established themselves in mucus tubes lined with faecal packets within the agar (Fig. 1G). It was found that the presence of a layer of seawater over the agar was unneccessary and sometimes was detrimental to the well-being of the worms. This may have been due to the build up of anoxic conditions in the seawater as a result of respiration of the infauna, and to the accumulation of water soluble nitrogenous metabolites.

Cultures of *Microlaophonte sp.* were initiated by pipetting a few females carrying egg sacs on to the furrowed agar plates. In this case it was advantageous to introduce enough seawater along with the animals to form a thin water film over the surface of the agar. The presence of seawater over the agar was not absolutely necessary but conferred a greater degree of freedom of movement on the harpacticoids. These ani-

TABLE II

Approximate dimensions and capacity of containers used for rearing

	External dimensions (cm)	Number of compartments	Surface area of compartments (cm^2)	Depth of agar (cm)	Volume of agar (ml)	Function
Compartmented boxes	33.5 × 22.5 × 5.8	24	27	2.8	75	*C. giardi* stock
	28 × 17 × 4.5	18	22	2.0	44	*C. giardi* stock
	10.6 × 10.6 × 2	25	4	0.5	2	*Microlaophonte sp.* experiments
Petri dishes	9.3 diam. × 1.7	1	60	0.3	18	*C. giardi* stock and experiments
				0.5	30	*Microlaophonte sp.* stock
	9.3 diam. × 1.7	3	19	0.3	5.7	*C. giardi* experiments

mals are naturally more active than the tube-forming worms and preferred to crawl over the surface of the agar rather than burrow into it.

The cultures of both species were equally easy to maintain. Once the animals had been plated out, the containers were sealed with tape and stacked in glass-fronted cabinets at room temperature (20-25 °C). Illumination was by means of fluorescent lights programmed for a 12 hr day 12 hr night cycle. Once a culture had been started no further attention was required for periods of up to 3 months, by which time the supply of agar was becoming exhausted and a good deal of liquefaction had taken place. Liquefaction resulted from microbial action and possibly from digestion of the agar by gut enzymes of the worms and harpacticoids. It was beneficial to the survival of *C. giardi* if excess liquid was withdrawn from the cultures at 2-3 week intervals. Measurements on liquid from exhausted plates showed, rather surprisingly, that there had been a slight shift upwards in pH from an average of 6.5-7.0 to 7.0-7.5. The increase in pH probably resulted from the release of ammoniacal excretory products by the cultured organisms. The efficiency of autotrophic nitrifying bacteria, which normally break down these excretory products, was probably impaired by the low oxygen tensions existing in the containers.

The presence of colonies of protozoa [*Keronopsis rubra* (Ehrenberg) Kahl ; *Oxyrrhis marina* Dujardin] and nematodes [*Diplolaimella ocellata* (Butschli) Gerlach] had no apparent ill effects on the cultures. In fact these organisms may well have helped to promote the well-being of the cultures by feeding on faecal material and dead animals.

When the plates became overcrowded or the agar was exhausted the populations were sub-cultured. If sub-culturing was delayed the first noticeable feature was a drop in egg production followed by a cessation of growth. Continued neglect led after a few weeks to an increase in mortality ; *C. giardi* showing a tendency to crawl from the agar remnants and die on the walls and lids of the containers.

Viability of the cultures

In the 3 years since the original specimens were obtained, *C. giardi* has passed through approximately 18-20 generations in the stock cultures. There is as yet no evidence for any decrease in fertility of successive generations nor for the development of any abnormal physical characteristics in the adults. During this period only three physically deformed larvae were found. The abnormal larvae which had a single head and two bodies (Fig. 1E) grew normally until metamorphosis and then died. Similar abnormal larvae have been found in the closely related *Capitella capitata* (Fabricius) by Foret (1972) and Reish *et al.* (1974).

Microlaophonte sp., which has passed through approximately 50 generations in culture, has similarly not shown a decrease in viability in this time, nor a change in the ratio of males to females [*cf.* Battaglia (1970) for the harpacticoid family Tisbidae]. There have been no noticeable changes in external morphology through successive generations [*cf.* Barr (1969) for *Tisbe furcata* Baird]. One specimen (an adult male) has been found with a foreshortened seta on one of its caudal rami (Fig. 2E).

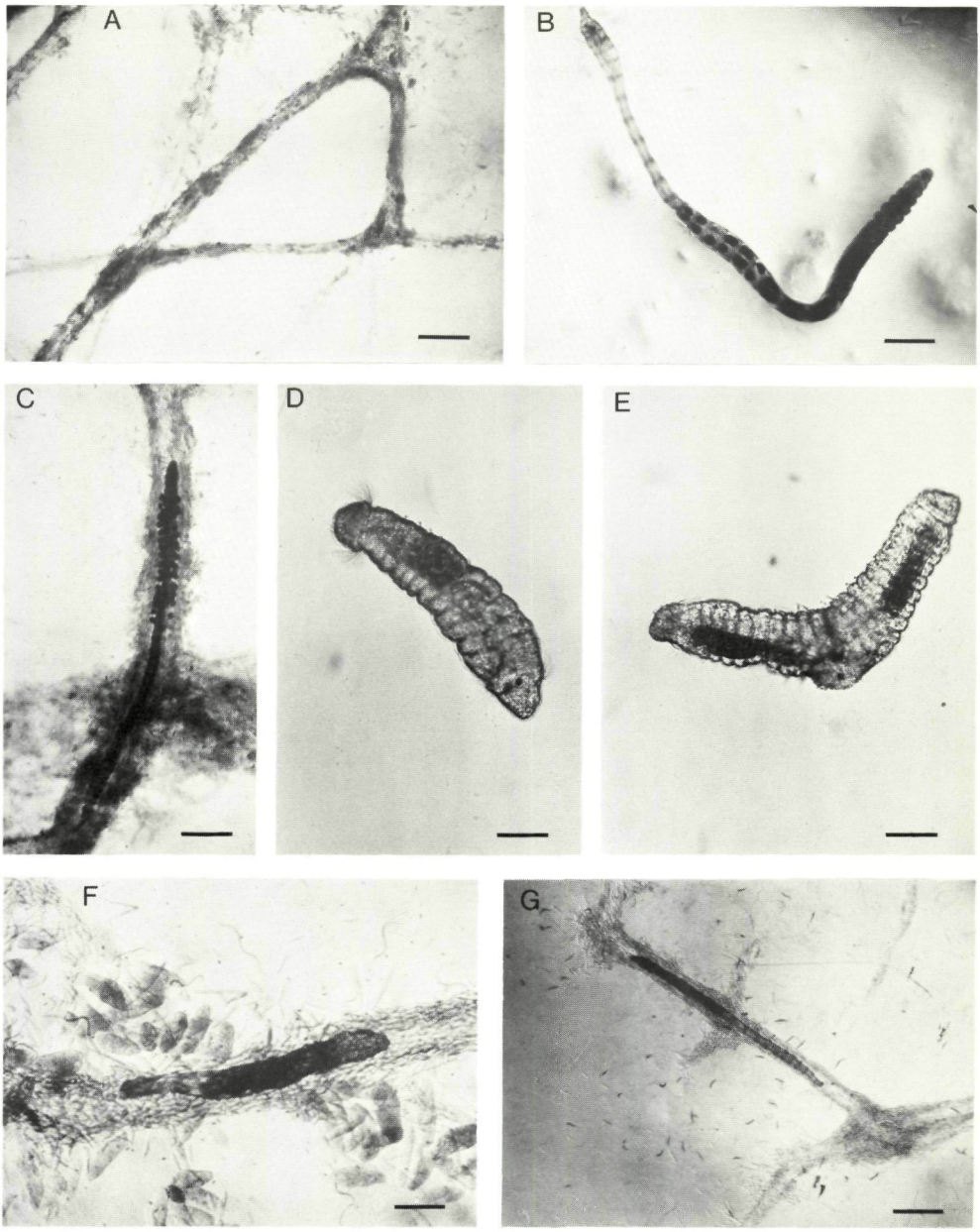

FIG. 1. *C. giardi*. A. Worm tubes following the score lines in normal strength agar (bar = 1 mm). B. Young adult at the late gravid stage for the first time (bar = 0.8 mm). C. Old adult brooding eggs in its tube (bar = 2 mm). D. Metatrochophore larva (bar = 85 μm). E. Abnormal metatrochophore larva with two bodies (bar = 85 μm). F. Early juvenile forming its faeces-lined tube amongst old faecal packets of an adult. Contaminating nematodes are clearly visible (bar = 180 μm). G. Immature adult in its burrow in "sloppy" agar. Contaminating ciliates are in evidence (bar = 1 mm).

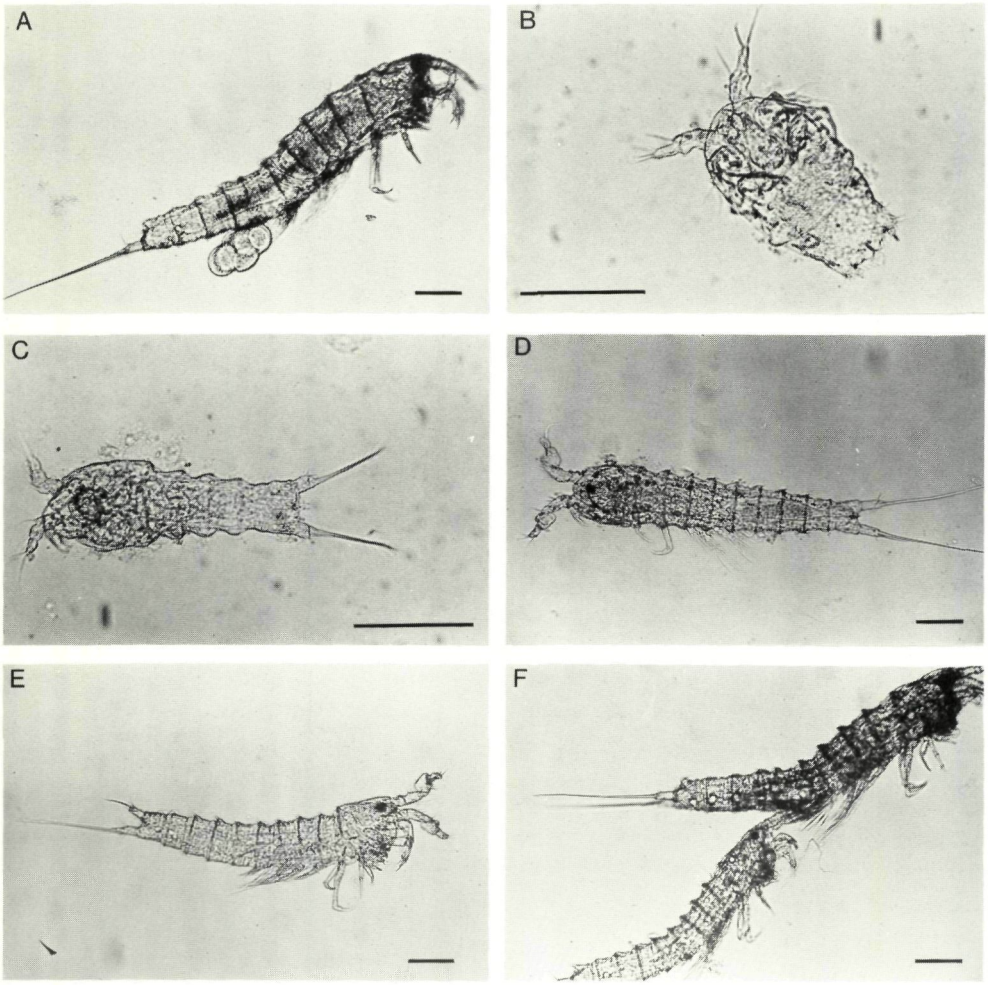

FIG. 2. *Microlaophonte sp.* (bar = 75 μm). A. Female carrying an egg sac. B. Late naupliar stage. C. Copepodite stage. D. Mature male. E. Mature male with an abnormally short seta on one of its caudal rami. F. Male clasping a female near her genital opening.

Life cycles

Culturing *C. giardi* on agar has allowed many observations to be made *in situ* which it is hoped can be directly related to its normal behaviour in the field. Its burrowing technique, for instance, has been observed through the translucent gel as have the ventilating movements of the adult whilst brooding its young in the tube beneath the surface. The fact that no seawater is needed above the medium enables the container to be inverted for closer observation of a specimen which has tunnelled deep within the agar.

The results of detailed investigations into the behaviour and life history of both *C. giardi* and *Microlaophonte sp.* will be published elsewhere and only a brief description of development in the agar cultures is given here.

C. giardi *life cycle at 25 °C*

Each worm produces an average of 24 large eggs at the first spawning (Fig. 1B). The large yolky eggs are laid within the tube of the adult in a ring around its body (Fig. 1C) and carefully brooded through cleavage and larval stages *in situ*. Whilst the eggs are developing the adult makes frequent body movements, possibly preventing the eggs from adhering to one another. A pear-shaped trochophore larva, typical of many polychaetes, is produced. It has well developed prototroch and telotroch ciliary bands which cause some movement which is discernible through the walls of the tube. The trochophore develops into a metatrochophore by the addition of segments between the prototroch and telotroch (Fig. 1D). The two eyes, which were first formed during development of the trochophore, become more obvious as they develop dark pigmentation. Under normal conditions the metatrochophore remains quiescent within the tube of the adult but if the tube is torn open in seawater the larvae will emerge and swim efficiently using their ciliary bands. It would seem therefore that if the adult chose to expel the metatrochophores from the tube a benthic free-swimming larval existence would be possible. However, even if a layer of seawater is retained above the agar the larvae do not normally leave the tube of the adult until after metamorphosis. Metamorphosis is marked by the loss of the ciliary bands and the eyes, the adoption of a worm-like movement, and the opening of the gut to the exterior. Until this stage the larvae have relied on their yolk reserves to sustain themselves. The average time taken for development from the early cleavage stages of the egg to metamorphosis of the larva is 2 weeks (Fig. 3).

The juvenile worms (Fig. 1F) burrow their way out of the adult tube and start feeding, producing small mucus tubes which they line with faecal packets (enclosed in peritrophic membranes) in the manner of the adult. The worms rapidly increase in size, the first individuals becoming gravid within 2-3 weeks of metamorphosis. On average the life cycle (egg batch to egg batch) is completed within 6.5 weeks at 25 °C (Fig. 3).

There is no sexual dimorphism between adults. It has been generally accepted that many capitellid species copulate to transfer gametes; however copulation was never observed in this species. In fact every individual from an egg batch eventually produced eggs, suggesting that the worms either undergo a sexual reversal at some stage in their

development or that they are functional hermaphrodites. The suggestion that they are hermaphroditic (or parthenogenetic) is supported by the fact that worms isolated just after metamorphosis all produce viable eggs.

Individuals are capable of laying more than one batch of eggs, producing on average five broods in their lifetime. Furthermore, an individual is able to achieve a gravid condition whilst brooding a previously spawned egg mass. However, it will not spawn until the earlier batch has metamorphosed (2 weeks at 25 °C).

The worms continue to grow after sexual maturity and the number of eggs produced increases. Some of the larger specimens produce between 50 and 100 eggs per spawning. An individual lays on average 300 eggs over an 8 week period.

Microlaophonte sp. *life cycle at 25 °C*

Adult females, readily distinguished from other stages by their red colouration, extrude 6-10 spherical eggs into an egg sac held medially on the ventral surface of the abdomen (Fig. 2A). The egg sac is retained for a short time (1-2 days) and then deposited on the agar. Usually an attempt is made by the female to position the sac in a crevice and not leave it exposed on the agar surface. Nauplii hatch from the eggs within a day or two and crawl over the surfaces of the substratum making no attempt to swim up into the overlying water. Development follows the pattern normally found in harpacticoids with 6 naupliar stages and 5 copepodite stages before the adult stage (Fig. 2B,C). Mature males are shorter and slimmer than the females and lack the red pigment (Fig. 2D). They are also distinguished from females by modified antennules, which are used by them to attach themselves ventrally near the female genital opening during copulation (Fig. 2F). Once mated the female produces a succession of egg batches at 1-2 day intervals. The average number of egg batches produced by a female during its lifetime has not yet been established but at least 10 egg sacs were deposited by an isolated female kept under continuous observation for a 3 week period.

The life cycle is completed in approximately 2 weeks at 25 °C. In well established cultures females outnumber males in an approximate ratio of 2 : 1.

Effect of temperature on development of *C. goardi*

Methods

Hopper *et al.* (1973) made some preliminary observations on the effect of temperature on the life cycle of *C. giardi* during their work on the nematodes of mangrove detrital systems. Their observations however were made at salinities optimal for the nematodes under investigation (15 ‰) and not for the polychaete. At 15 ‰ *C. giardi* is approaching its haline stress zone (Table I), and thus may not grow at its optimum rate even when temperatures are favourable. The temperature growth experiments were thus conducted at a higher salinity of 20 ‰ and the results crosschecked at 35 ‰.

Agar consistency is another factor which may influence growth rate. Experiments were usually conducted using an agar substratum of half the recommended strength (as

in the stock cultures) but results were checked against those with agar of normal gel strength.

Growth experiments were conducted at 5 °C intervals from 10-35 °C. A total of 20 petri dish cultures were started at each temperature using an adult with its recently laid egg mass. Once the adult's usefulness for brooding the developing egg mass had ended (at larval metamorphosis) it was removed to prevent confusion arising should it lay more egg batches. A further set of experiments were carried out at 20 °C by implanting one late juvenile per plate instead of an egg mass.

For convenience, development of the worm was divided into a number of easily recognisable stages : 1) early cleavage, 2) late cleavage, 3) trochophore, 4) metatrochophore, 5) early juvenile, 6) late juvenile, 7) immature adult, 8) early gravid, 9) late gravid. The experimental cultures were examined at weekly intervals and the number of specimens at each growth stage recorded. Experiments were continued until the majority of individuals had completed their life cycle or until mortality had reached a high level.

Results

At 10 °C and 35 °C no egg batches developed beyond cleavage stages and the adults accompanying them died. In a few isolated cases adults survived for more than 2 weeks suggesting that these temperatures are near the lower and upper lethal limits for the species.

At 15 °C some growth took place, several worms developing beyond metamorphosis to early juveniles before dying (Fig. 3). In all cultures, however, growth was slow and mortality high. Once the larvae had metamorphosed to the early juveniles stage and had exhausted their yolk reserves they seemed to be incapable of establishing themselves within the agar.

Worms were able to complete their life cycle in an average time of 11 weeks at 20 °C (Fig. 3). The majority of specimens reached adulthood within 5 weeks but no individuals became gravid until a further 2 weeks had elapsed. The time taken for the most rapidly developing animals to pass from the egg to sexual maturity is in the region of 7 weeks but the most tardy specimens took from 11-12 weeks.

Growth rates were not influenced by the development stage at which the experiments were started. For example, cultures at 20 °C started at the late juvenile stage rather than at egg cleavage stages took on average 11 weeks to pass through the life cycle.

Life cycles were completed in less time as the temperature increased, the mean generation time being 6.5 weeks at 25 °C and 5.3 weeks at 30 °C (Fig. 3).

The mean generation time of worms cultured at 25 °C in normal strength agar (6.3 weeks) was not significantly different from that in ''sloppy'' agar (Fig. 4). Similarly worms cultured at 25 °C in agar with a salinity of 35 ‰ completed their life cycle in 6.7 weeks which was not significantly different from the generation time in agar with a salinity of 20 ‰ (Fig. 4).

An interesting feature of all the temperature growth experiments carried out between 20 °C and 30 °C was the very low mortality rate in the cultures. The percentage mortality in any one culture rarely exceeded 5 %.

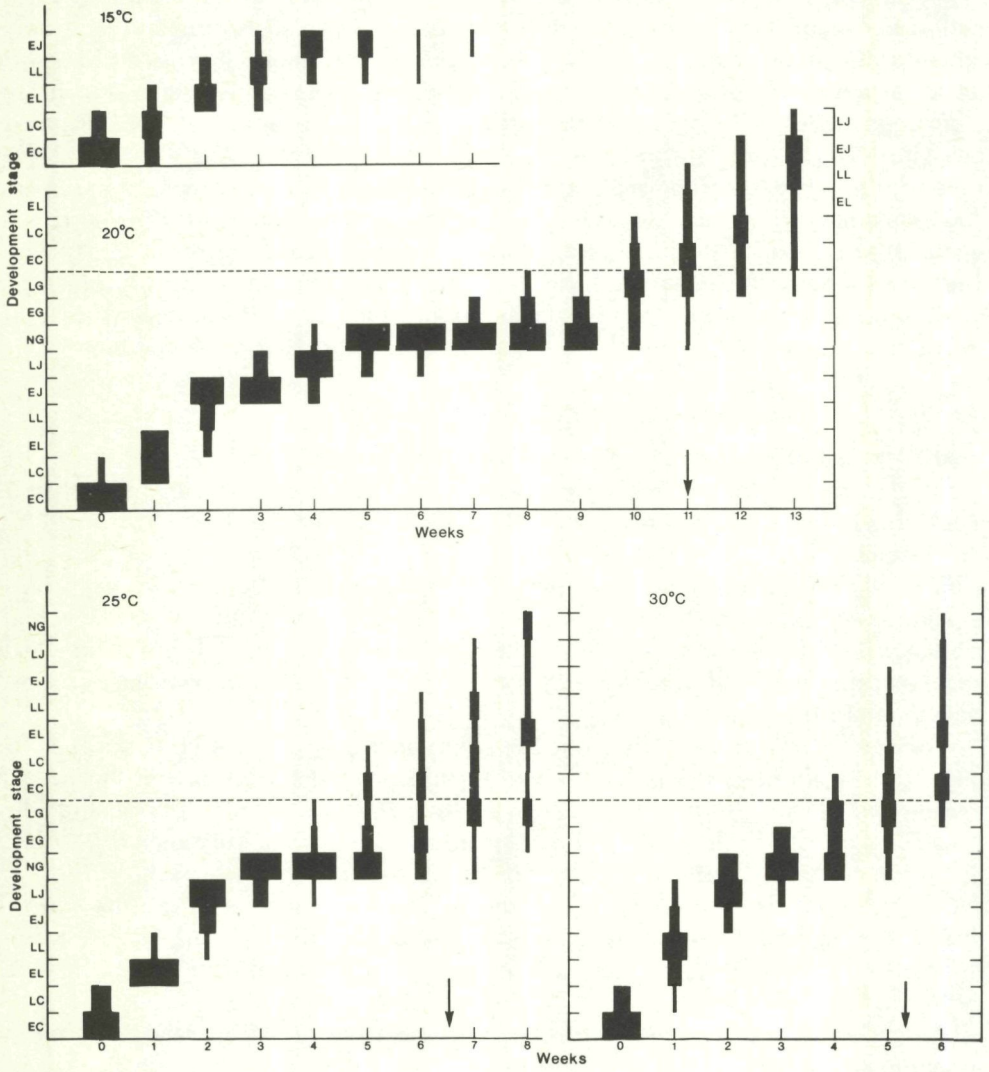

FIG. 3. Graphical representation of the effect of temperature on the development and mortality of *C. giardi* in agar culture at 20 ‰. Time is in weeks that have elapsed since the inception of the experiments. Area of the blocks represents the number of worms at each development stage. An arrow marks the time taken by 50 % of the worms to complete their life cycle. EC : early cleavage ; LC : late cleavage ; EL : early larvae ; LL : late larvae ; EJ : early juveniles ; LJ : late juveniles ; NG : non-gravid adults ; EG : early gravid ; LG : late gravid.

Effect of salinity on development of *C. giardi*

It has already been shown that at a temperature of 25 °C the cultures of *C. giardi* develop at approximately the same rate in agars with a salinity of 20 ‰ and 35 ‰ (Figs 3 and 4). It was not possible to prepare gels at higher salinities without considerable salt precipitation. This led to inconsistencies in the distribution of salts from one plate to the next and made it impossible to produce reliable information on the development rates as the gel salinities approached the animals' upper haline stress zone (Table I).

The life cycle times recorded for *C. giardi* by Hopper *et al.* (1973) although only of a preliminary nature were nonetheless consistently longer than those obtained in the present temperature growth experiments. Since the work by Hopper *et al.* (1973) took place at a salinity of 15 ‰ it is possible that the lower salinity was slowing the rate of development in their experiments even though the mortality was low at those salinities (Table I).

A total of 20 petri dish cultures were initiated at a lower salinity of 10 ‰ using early and late juveniles and several adults in a late gravid condition obtained from the stocks at 20 ‰ (Fig. 4). The worms established themselves in the agar with very little trouble and commenced feeding normally. The rate of progression from juvenile to adult was, however, very slow and worms introduced into the agar as juveniles did not to achieve sexual maturity before termination of the experiments after 6 weeks.

The fate of worms placed on the agar in a late gravid condition was most interesting. A few specimens remained in a gravid state for a number of weeks before eventually spawning but the majority reabsorbed their eggs and reverted to the non-gravid adult condition, presumably as a result of the salinity shock received on transfer to the lower salinity. These sexually mature worms eventually redeveloped eggs and spawned in the usual way.

Development of egg masses was not impaired by the reduced salinity, development to metamorphosis being as rapid as in cultures at higher salinities (Figs 3 and 4).

Mortality was greater amongst juveniles and adults at 10 ‰ than at salinities of 20 ‰ and 35 ‰ averaging 18 % in the experimental cultures. It was noticed that liquefaction was much less apparent in plates held at this salinity suggesting that the micro-organisms were less prolific. It is feasible, therefore, that death was caused by starvation rather than by the salinity *per se*, especially as the non-feeding larval stages developed at normal rates.

Conclusions

Akesson (1970) in his well reasoned case for the use of the polychaete, *Ophryotrocha labronica* La Greca *et* Bacci, as a laboratory test animal, established criteria by which such animals could be recognised. Both *C. giardi* and *Microlaophonte sp.* also display many characteristics which make them suitable for use as laboratory test animals: 1) they can be easily reared through many generations without loss of viability, 2) they

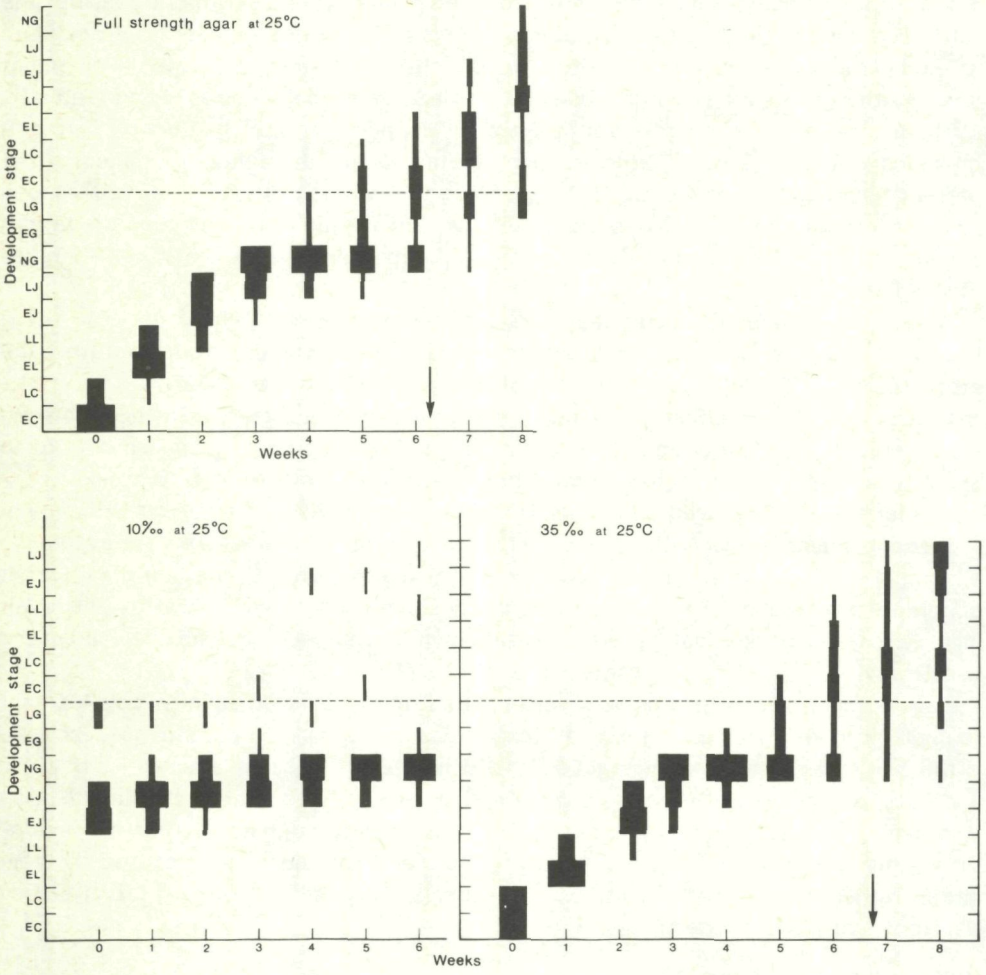

FIG. 4. Graphical representation of the effect of agar concentration and salinity on the development and mortality of *C. giardi* in agar culture at 25 °C. For explanatory key see Fig. 3.

have a short generation time and reproduce throughout the year providing various easily recognisable development stages whenever required for experiments, 3) they are small, allowing culture of large numbers in a small space, 4) being herbivores/microbial feeders they can be fed cheaply and easily on an agar substrate, and can be reared in high densities, 5) even at high population densities they have a low mortality rate and survive well at low oxygen tensions, 6) sexually mature animals produce small egg batches in rapid succession, 7) since they are eurythermal they can be transported without special precautions in agar-filled petri dishes to other laboratories.

The ease with which these standard populations can be transferred between laboratories makes them particularly suitable for experiments that require comparative results on a worldwide basis. It is hoped, for instance, that in the next few years international standardisation of toxicity tests will become a reality. Reish (1973) has argued that laboratory bred populations of test organisms, even though they may show some genetic drift from the wild stock, are more suitable for toxicity testing of pollutants than animals which have recently been transferred from their natural habitat to the laboratory. Indeed, Perkins (1972) has drawn attention to the pitfalls associated with using animals from the field for marine toxicity studies in the laboratory.

Long term sublethal effects of pollutants on marine organisms are often very difficult to detect (George, 1970 ; 1971) and it is an advantage to use well-established laboratory populations with a short life cycle for such work. Both Åkesson (1970) and Bellan *et al.* (1972), transusing laboratory reared polychaete populations, have detected subtle changes in growth rate and reproductive success caused by pollutants. Effects may not be noticeable until the second or third generation of the test animal, as discovered by Reish *et al.* (1974) when working on the effects of sublethal concentrations of zinc on laboratory populations of *Capitella capitata*.

It is hoped that the cultures of *C. giardi* and *Microlaophonte sp.* will prove equally useful in the future for pollution studies. Furthermore, there appears to be no reason why many more animals with similar nutritional requirements should not be brought successfully into culture using the techniques described in this paper.

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