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# Laboratory studies on larval growth of *Polydora ligni*, *Polydora ciliata*, and *Pygospio elegans* (Polychaeta, Spionidae)

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ABSTRACT: The spionid polychaete species *Polydora ligni*, *P. ciliata*, and *Pygospio elegans* were cultivated in the laboratory over several successive generations. A flow-through cultivation system for *Polydora* spp. is described. Duration of life cycles (time from hatching of the larva to first reproduction) and life spans (hatching to death) of these species were not significantly influenced by the degree of inbreeding nor by individual age of the parents. Minimum time from metamorphosis (15-setiger stage) to first hatching of offspring larvae (in the 3-setiger stage) at 18 °C was 33 days in *Polydora* spp. and 81 days in *Pygospio elegans*. Larval growth patterns are described in terms of number of setigers, body length, and biomass (dry weight, carbon, nitrogen, hydrogen), in relation to time after hatching. Regression models are proposed which link these measures of larval growth and, thus, may be used for conversions. Rates of development and growth show a high degree of variability in all three species, not only caused by variation in environmental factors such as temperature or food, but also among and within single hatches of larvae reared under identical conditions. Larvae were reared at constant temperatures (6 °, 12 °, and 18 °C). Temperature affected larval growth in *Polydora ligni* more than in *P. ciliata*, and least of all in *Pygospio elegans*. Only the latter species was able to develop at 6 °C from hatching to metamorphosis. This differential response may be explained by differences in the natural spawning season of these species. Eleven phytoplankton species were tested as to their food values. A "relative index of growth" is proposed which compares the slopes of two growth curves (one standard and one test condition). The flagellate *Dunaliella tertiolecta* was used as a standard food in these experiments. Most algal species were less suitable, and only the diatom *Thalassiosira rotula* was consistently better food for spionid larvae than *D. tertiolecta*.

## INTRODUCTION

Spionid polychaetes belong to the most common invertebrates of coastal, estuarine, and marine benthic environments. Three species, *Polydora ligni*, *P. ciliata* and *Pygospio elegans*, have a particularly wide geographical distribution with dense local populations, often indicating organic pollution (e.g. Hempel, 1957a; Muus, 1967; Blake, 1969; Hartmann-Schröder, 1971; Anger, 1977; Rice & Simon, 1980). During the reproductive season, their pelagic larvae may become very frequent, sometimes dominant members of coastal zooplankton communities (e.g. Smidt, 1944a, 1951; Thorson, 1946; Banse, 1955; Schram, 1968, 1970; Orth, 1971; Daro & Bofill, 1972; Daro & Polk, 1973; Rasmussen, 1973; Grassle & Grassle, 1974).

In the present study, these three spionid species were cultivated in the laboratory in order to obtain information on their larval development and growth, maturation, reproduction, and other aspects of their life cycles under controlled conditions. Particular attention was paid to larval growth in relation to the type of phytoplankton offered as food.

## MATERIAL AND METHODS

### Obtaining and handling of laboratory populations

*Polydora ciliata* and *P. ligni* larvae were isolated from plankton near the island of Helgoland (North Sea) in May and September 1977, respectively. They were reared at 18 °C in beakers (1 $dm^3$) with slightly aerated seawater, and the flagellate *Dunaliella tertiolecta* was given as food. Water and food were changed three times a week, collecting the larvae on a fine gauze screen (50 µm mesh size). When metamorphosis was imminent (at ca 15 setigers), the larvae were transferred to aerated aquaria (2 $dm^3$), again with *D. tertiolecta* as food, and with fine sand as a substrate for settlement. Both species produced larvae in the laboratory ($F_1$ generation) in the same year. Cultivation of these original populations and of their later offspring was continued for several years.

Juvenile and adult *Pygospio elegans* were collected in summer 1977 in a tidal mud flat (Königshafen) near List (Island of Sylt, North Sea) and transported to the laboratory at Helgoland.

### Cultivation techniques

After some preliminary methodical tests, the following standard cultivation techniques were employed. Freshly hatched, free-swimming larvae (3-setiger stage) were separated from the adults and transferred to beakers with 800 $cm^3$ filtered (1 µm) seawater and slight aeration (12 °C). *Dunaliella tertiolecta* was added to an initial concentration of ca 300–500 cells · $mm^{-3}$, larval density ranged from ca 100 to 1000 individuals · $dm^{-3}$. Water and food were renewed three times a week, and the larvae were checked under a stereo microscope for growth and possible contamination with fungus, protozoans or other organisms. Standard water temperature for adults was kept constant at 18 °C for *Polydora spp.* and at 12 °C for *Pygospio elegans*. Salinity varied seasonally between 29 and 33 ‰, and was mostly around 32 ‰. Light regimes simulated late spring (12 °C) and summer (18 °C) conditions, with day lengths of 17 and 15 h, respectively. For adult polychaetes, two different techniques of maintenance were used. Both yielded larval material for continued cultivation and experiments throughout the year.

(1) Small groups of ca 10 to 30 individuals were kept in glass bowls (50 to 100 $cm^3$) with filtered seawater and fine sand. A mixture of flagellates (*Dunaliella tertiolecta)* and diatoms *(Thalassiosira rotula)* were given as a standard food (10:1 by cell numbers, ca 500 cells·$mm^{-3}$). Water and food were changed three times per week, and the cultures were checked for presence of larvae. In this way, numerous inbreeding populations were cultivated over several generations (see below), providing data on maximum life span and life cycle duration, in relation to the degree of inbreeding. This method was applied in all three species.

(2) A flow-through system was developed which sustained mass-cultures of *Polydora* spp., but not those of *Pygospio elegans*. The latter species survived and grew better in standing (closed) cultures (see above). Figure 1 shows the design of this cultivation system which works as follows: unfiltered or pre-filtered seawater with ambient temperature flows from the main tap (1) into an aquarium containing a stratified sand-gravel filter (2). Part of the water is discarded immediately through an overflow (4) keeping the water level constant, while another portion flows through the filter into a second aquarium with 10 $dm^3$ capacity (6). There, a constant temperature of 18 °C is maintained by an aquarium heater (7) connected with a control unit (8) (Biotherm 80; K. Dietsche, Waldshut, W. Germany). The seawater is then pumped (9) through a rubber foam (10) serving as an additional filter (Eheim type 1018 rotary pump, W. Germany) into a PVC tube (11) with outlets and small taps (14). Simultaneously, algal culture is added by a peristaltic pump (12; Mini-S, Ismatec S.A., Zürich, Switzerland) at a rate of ca 3 $cm^3 \cdot min^{-1}$ from a 10 or 20 $dm^3$ supply tank (not shown in Fig. 1). The seawater which is filtered, temperature-controlled, and enriched with phytoplankton (final concentration: ca 10–20 cells · $mm^3$), then constantly flows into the culture aquaria (13; 5 $dm^3$ capacity; usually 5 per system). The final food concentration may be adjusted by varying the algal density in the stock culture (supply tank), the flow rate of algal culture (12), that of the diluted medium (14), or that of filtered seawater (compressing the elastic tube coming from the rotary pump, 9). Supplement feeds of diatom *(Thalassiosira rotula)* culture are given directly to the aquaria.

At the bottom of the culture aquaria (13) open petri dishes are placed (15) containing *Polydora* spp. in fine sand (only siblings from the same generation, but not necessarily from identical parents). Larvae produced by these polychaetes are transported by the overflowing water (4) into sieves (17; mesh size: 50 µm) standing in a large tray (18) with

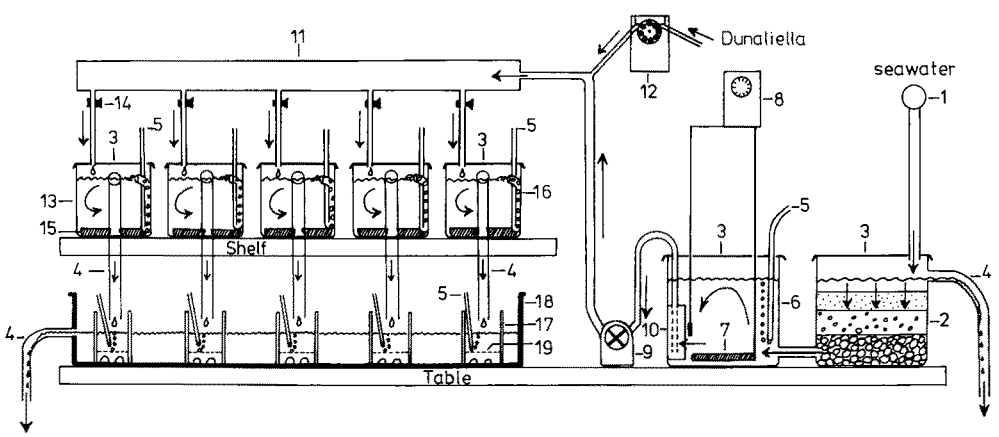


Fig. 1. Flow-through cultivation system for *Polydora* spp. 1 main seawater tap. 2 sand-gravel filter. 3 PVC cover. 4 overflow. 5 aeration tube. 6 heated aquarium. 7 aquarium heater. 8 temperature control unit. 9 rotary pump. 10 rubber foam filter. 11 PVC tube. 12 peristaltic pump. 13 culture aquarium. 14 outlet tap. 15 culture dish. 16 air lift. 17 sieve. 18 polyethylene tray. 19 nylon gauze (50 µm mesh size); arrows: direction of water flow; *Dunaliella:* food suspension coming from a supply tank (not shown)

constant water level. Larvae may be collected daily from these sieves for further cultivation or other experiments.

The system can also be operated discontinually: preliminary tests suggest that flow-through periods of 12 h per day suffice to successfully maintain *Polydora* spp. cultures and produce larvae throughout the year. An electric timer is necessary for this mode of operation, in order to switch the two pumps on and off. The algal supply is then sufficient for up to a whole week.

Cleaning and other necessary maintenance work is very limited in this system, and the frequent production of larvae through all seasons shows that it provides favourable culture conditions for *Polydora* spp. and, after appropriate modifications, possibly also for other polychaete species.

## Production and treatment of food

Algae needed as standard food or in comparative feeding experiments (see below) were cultivated in autoclaved seawater with F/2 medium (Guillard & Ryther, 1962). An addition of silicate (1.47 µg at·dm$^{-3}$) was given to diatom cultures. Before feeding phytoplankton to adults in closed cultures (not in the flow-through system) or to larvae, the algal cells were separated from their culture medium. Small species (*Dunaliella tertiolecta, Rhodomonas* sp., *Protococcus* sp., *Isochrysis galbana, Pavlova lutheri, Platymonas suecica, Amphidinium carterae*) were centrifuged for 5 min at 5000 rpm in a Labofuge 15000 (Heraeus-Christ, W. Germany). Larger species *(Thalassiosira rotula, Scrippsiella faeroënse, Prorocentrum micans, Gymnodinium splendens)* were screened on a nylon gauze (mesh size: 20 µm), before resuspension in filtered seawater. Cell concentrations were checked with a TA II Coulter Counter, or (in chain-forming species) with the Utermöhl method. In feeding experiments, algal cell concentrations were adjusted in such a way that approximately equal concentrations of carbon (ca 6 mg·dm$^{-3}$) were offered in tests of different phytoplankton species. The carbon content of algae was measured in a CHN analyzer (Model 1106, Carlo Erba Science) or, in some species, estimated from cell volumes (Strathmann, 1967).

## Measurement of larval growth

Larval growth was measured every two to three days in samples of at least 10 larvae taken at random from experiments. The larvae were pipetted on to a microscopic slide with small amounts of seawater in order to hamper movements of the larvae without deforming, squeezing or injuring them under the cover glass. Larval body length was measured under a microscope with an eye-piece micrometer, and the number of setigers was counted under appropriate magnification.

Larval growth data were used to evaluate relative differences between rearing conditions or food values of different algal species. Since each growth curve comprised ca 60 to >100 measurements of both body length and number of setigers, and several hundred experiments were carried out during this study, a "Relative Index of Growth" (RIG) is introduced to express results of such comparisons in a condensed way. It is based upon the following observations: larval growth under constant conditions of temperature and feeding, when expressed as number of setigers in relation to time after hatching, is

non-linear. Hatching takes place at the 3-setiger stage, metamorphosis and settling at ca 15 setigers. The number of setigers (S) increases as a power function of time ($t$, in days) after hatching. This function is computed as a linear least-square regression equation:

$$\ln (S - 2) = b + m \cdot \ln (t + 1), \quad (1)$$

where $b$ is the intercept with the ln $y$ axis, and $m$ is the regression coefficient (slope) of this linear equation. It may be re-transformed to the power function:

$$S = e^b \cdot (t + 1)^m + 2 \quad (2)$$

The time (in days) to reach a given number of setigers is then:

$$t = \sqrt[m]{\frac{S - 2}{e^b}} - 1 \quad (3)$$

Since all larvae hatch on day 0 with 3 setigers, $b$ must be close to 0, and its antilogarithm, $e^b$, close to 1. These terms may therefore be omitted in the above equations. The approximate time from hatching to metamorphosis, $t_M$, can thus be estimated by:

$$t_M = \sqrt[m]{13} - 1 \quad (4)$$

In all comparative experiments, e.g. on food value of several algal species, a control experiment under standard conditions (see above) was always included: 12 °C, *Dunaliella tertiolecta* (ca 150 cells · mm$^{-3}$ ≙ ca 6 mg C · dm$^{-3}$), aerated beaker (1 dm$^3$). Three times per week, when water and food were changed, larval growth was measured in the control and in other experiments. The slope ($m$) of the fitted growth equations was then used to calculate the RIG:

$$RIG = \left(\frac{m_{test}}{m_{control}} - 1\right) \cdot 100 \quad (5)$$

It represents the percent difference between growth rates, and thus may be used to characterize the quality of growth conditions in an experiment as compared to standard conditions (the latter are defined arbitrarily but they are well reproducible). The RIG will be −100 when there is no growth at all under test conditions, zero when it is equal to that under standard conditions, and positive when it is better.

Figure 2 shows, as an example, a test of the flagellate *Scrippsiella faeroënse* offered as a food organism to larvae of *Polydora ligni*. Larval growth is significantly reduced as compared to the control *(Dunaliella tertiolecta)*. From the $m$ values given in Figure 2 an RIG = −35·8 is obtained. The theoretical time of development from hatching to metamorphosis may be estimated from Eq. (4): 20 days in the control, and 113 days with *S. faeroënse* as food. Mortality under such unfavourable conditions, however, usually inhibits reaching of metamorphosis and so causes premature termination of the experiment.

In the following, growth curves such as those in Figure 2 are no longer given. All information contained in such comparisons will be summarized by only one value: the RIG. This index compensates variation in larval viability and growth rates occurring from hatch to hatch (reflected in varying $m$ values in repeated standard experiments), as it compares the slopes of two growth curves observed in larvae of identical origin, but reared in parallel experiments under different conditions. The index is based on growth

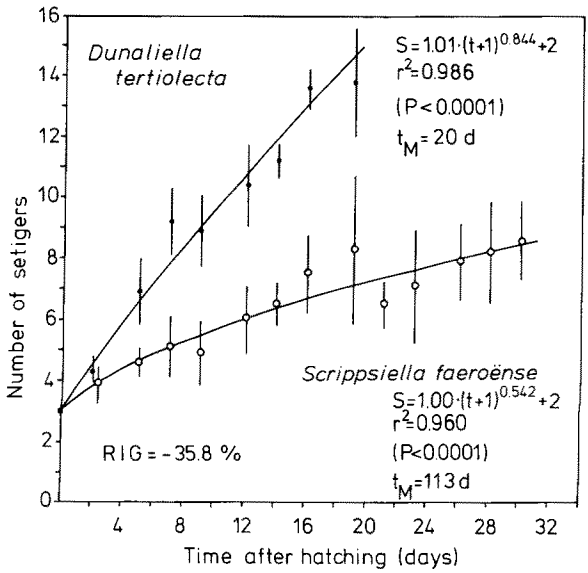


Fig. 2. *Polydora ligni*. Relationship between number of setigers (S) and time after hatching ($t$, in days) in larvae fed two different types of algal food. RIG: relative index of growth (see text); $t_M$: theoretical time from hatching to metamorphosis (in days; for calculation see text); $r^2$: coefficient of determination; P: level of significance for $r \neq$ «

in terms of increment in the number of setigers. Conversions to body length and biomass will be given below. Since, likewise, the quality of algal cultures, including that of the standard *(D. tertiolecta)*, may vary from experiment to experiment, most food organisms were tested at least five times in each polychaete species, and average RIGs will be given. These mean values can be statistically tested for differences from the theoretical standard (RIG = 0) (Sachs, 1984; p. 201). All experiments on larval growth in relation to food quality were carried out at a constant temperature of 12 °C.

## Measurement of biomass

Larval biomass in relation to body size (total length, measured microscopically) was measured in a total of 44 samples in *Polydora ligni* and *Pygospio elegans*: dry weight (W), carbon (C), nitrogen (N), and hydrogen (H). For each sample, 50 to 1500 larvae, depending on their body size, were counted under a dissecting microscope, pipetted into filtered (0.2 µm Millipore) seawater, and then transferred to a pre-weighed glass fiber filter (Whatman GF/C). After sucking through the seawater by means of a vacuum pump and briefly rinsing the sample with distilled water, the filters with the polychaete larvae were transferred to pre-weighed silver cartridges and then freeze-dried over night at ca $10^{-2}$ mbar in a GT 2 (Leybold-Heraeus) apparatus. W was measured on an Autobalance AD-2 (Perkin-Elmer) to the nearest 0.1 µg. C, N, and H contents were determined in an Elemental Analyzer model 1106 (Carlo Erba Science) using Cyclohexane-2,4-dinitrophenylehydrazone as a standard. Care was taken that total animal weight in the

samples was between 0.2 and 0.6 mg, which is the range of maximum accuracy in the CHN analyzer. Blank filters (without larvae) were treated identically to check for organic particulate substances in the seawater.

### Statistical procedures

Least-square regressions were calculated, with or without logarithmic transformation of data, and their regression and correlation coefficients were tested for significant differences from zero with the statistics $t$ and $F$, respectively (Sachs, 1984; pp. 339 and 329). Differences between two slopes of intercepts were tested with another $t$ statistic (Sachs, 1984; pp. 340–341). In non-linear regression, the coefficient of determination, $r^2$, is given.

Arithmetic mean values (of a "Relative Index of Growth"; see below) were tested against a theoretical mean value of zero, applying again a $t$ statistic (Sachs, 1984; p. 201).

## RESULTS

### Benthic life span and reproduction

Maximum benthic life span (exclusive embryonic and larval development) and reproduction were monitored in numerous cultures with groups of 10–30 polychaetes kept in glass bowls. If not stated otherwise, the following life cycle data refer to constant 18 °C conditions in *Polydora* spp. and to 12 °C in *Pygospio elegans.*

16 successive generations of *P. ligni,* 11 of *P. ciliata,* and 9 of *P. elegans* were cultivated in the laboratory. Most polychaetes had a life span of up to two years, with averages of 13 months in *P. ligni,* 11 in *P. ciliata,* and 9 in *P. elegans.* Maximum survival times in these species were 31, 24, and 45 months, respectively. At 6 °C, the oldest *P. elegans* survived for 52 months.

First reproduction (hatching of larvae) occurred in *Polydora* spp. ca one month (33 days) after metamorphosis and settlement, and after 3 months (minimum: 87 days) in *P. elegans.* This great difference is species-specific. It was not caused by differential temperature (18 ° vs 12 °C), as the minimum time observed in *P. elegans* at 18 °C was only one week shorter (81 days). Total minimum generation times are obtained when the duration of pelagic larval development (ca 2–3 weeks in *Polydora* spp., 4–5 weeks in *P. elegans*) are added to these time spans of minimum benthic existence: ca 12–14 weeks in the former and ca 15–17 weeks in the latter taxon.

When duration of the benthic life cycle (i.e. the time from settlement to first reproduction) was plotted against the generation number ($F_s$) produced, in order to detect possible effects of an increasing degree of inbreeding, no significant correlation was found in either species ($r = -0.05$ to $+0.16$). Maximum life span was not influenced by inbreeding either. Tests were also carried out on whether the individual age of parents might affect the viability or life cycle duration of the offspring. The occurrence of unviable broods (larvae growing extremely slowly under otherwise favourable conditions) and delayed reproduction, however, did not show any statistically significant correlation with parental age nor with the number of inbreeding generations (all $r$ values close to zero).

In general, the production of larvae ceased, independent of later survival time, after ca one year. The maximum age in which ultimate reproduction occurred, was 26 months in *P. ligni,* 17 in *P. ciliata,* and 19 in *P. elegans.* In one culture of the latter species maintained at a constant temperature of 6°C, however, larvae hatched in frequent intervals over a 24-month period, before reproduction finally ceased.

## Patterns of larval growth

The general relationship between larval growth (expressed as number of setigers) and age (in days after hatching in the 3-setiger stage) has been shown in the Material and Methods section (Fig. 2). The fit of the power function model [Eqs. (1) and (2)] was in general very good, with correlation coefficients *(r)* usually higher than 0.95 and only exceptionally below 0.90 (in all cases significantly different from zero; $P$ normally $<0.01$). This indicates that our model describes fairly accurately larval growth under constant conditions, and the comparison of slope coefficients *(m)* calculated from data in different experiments is a useful tool for the evaluation of experimental conditions (RIG; see above). The $m$ values in *Polydora* spp. larvae reared under standard conditions (12°C; *Dunaliella tertiolecta* given as food) were usually ca 0.80 to 1.0, and duration of larval development from hatching to metamorphosis ca 12 to 24 days [Eq. (4)]. In *Pygospio elegans,* development under the same conditions took ca 3 to 4 weeks, with $m$ values varying mostly between 0.75 and 0.85.

In the initial phase of this study, growth of larvae was measured regularly considering both the number of setigers and total body length (in identical individuals). 250 pairs of data obtained in each species were used to calculate the relationships between these two measurements of body size. Each pair represents two mean values from 10 measurements in different individuals. Thus, each regression (number of setigers, S, plotted against body length, L, in mm) is actually based on 2500 individual measurements of each criterion.

Best fit of observed and predicted data was obtained with an exponential model (Fig. 3). Since no statistically significant differences in either slope or intercept were found between the regressions for the two *Polydora* spp., their data were pooled. The curve for *Pygospio elegans* was almost parallel to that of *Polydora* spp., with consistently larger body size in individuals with an equal number of setigers (Fig. 3). This difference in intercepts was statistically highly significant ($P<0.0001$), whereas the slopes were similar, with a weakly significant difference ($P<0.05$). This indicates that the two *Polydora* spp. resemble one another with regard to body shape, whereas *P. elegans* differs from these two, in that its larvae have somewhat longer segments.

On hatching, the larvae of all three species have 3 segments and are ca 0.2 mm in length. During the following day, the size of the larvae increases, but not the number of segments. This initial stretching phase is indicated in Figure 3 by arrows. The larvae do not grow a fourth setiger until they have attained a length of ca 0.3 mm. The initial body size values predicted by the regression curves (Fig. 3) thus represent an average size during the first day of pelagic larval life.

Larval biomass was measured in only two species, *Polydora ligni* and *Pygospio elegans,* in relation to body length and number of setigers. When dry weight (W), carbon (C), nitrogen (N), or hydrogen (H) content was plotted against total body length, similar

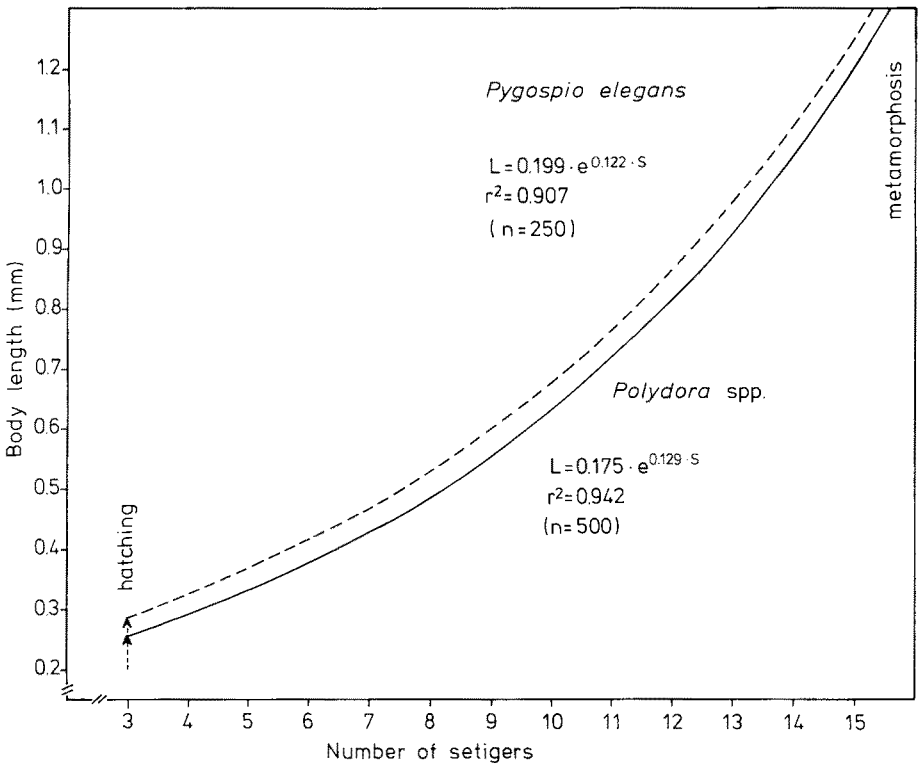


Fig. 3. *Pygospio elegans* (dotted line), *Polydora* spp. (pooled data of *P. ligni* and *P. ciliata*, solid line). Relationship between larval body length (L, in mm) and the number of setigers (S). $r^2$: see Figure 2; n: number of observations

(statistically not significantly different) relationships were found in these two spionids, so that their data could be pooled. Figure 4 shows the regression curves (power functions) and, as examples of single measurements, the C values. The regression equation and curves given in this graph should be approximately the same in all three species under consideration. Due to the difference in larval body shape, however, the relationship between the number of setigers and biomass is slightly different in *P. elegans*, which has higher biomass than *Polydora* spp. in larvae with an equal number of setigers. This can be seen in Figure 4, when the setiger scale is used instead of the body length scale.

Direct conversion of setiger numbers (S) to biomass values (in µg) is possible by substituting L in the equations given in Figure 4 for the appropriate equations in Figure 3, and re-calculating the constants:

*Polydora* spp.:

$W = 0.080 \cdot e^{0.291 \cdot S}$
$C = 0.038 \cdot e^{0.297 \cdot S}$
$N = 0.0093 \cdot e^{0.287 \cdot S}$
$H = 0.0060 \cdot e^{0.296 \cdot S}$

*Pygospio elegans:*

$W = 0.107 \cdot e^{0.275 \cdot S}$
$C = 0.051 \cdot e^{0.281 \cdot S}$
$N = 0.012 \cdot e^{0.272 \cdot S}$
$H = 0.0081 \cdot e^{0.280 \cdot S}$

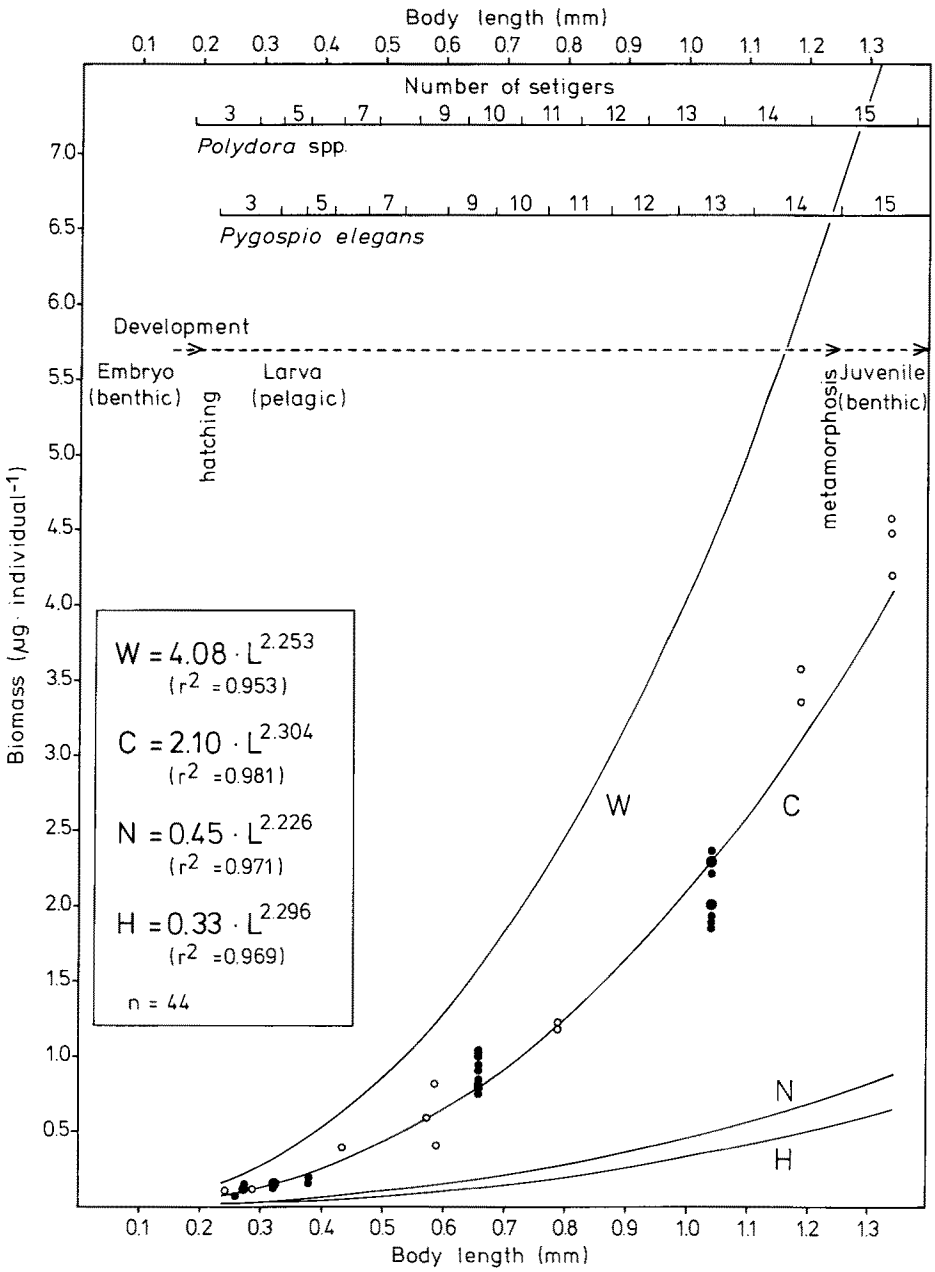


Fig. 4. *Polydora ligni, Pygospio elegans* (pooled data). Relationship between larval body length (L, in mm) and dry weight (W), carbon (C), nitrogen (N), and hydrogen (H) (all in µg). Single results shown, as an example, only in C values (solid circles: *P. elegans;* open circles: *P. ligni*). Number of setigers calculated from Figure 3. $r^2$: see Figure 2; n: number of analyses

Larval dry weight increases during development from ca 0.15 µg at hatching to ca 7 µg at metamorphosis, i.e. it multiplies by a factor of almost 50. Carbon, the main component of organic body substance, increases from ca 0.08 to 3.5 µg per individual. The C/N ratio shows an increasing trend during larval development, with average values of 4.2 at hatching and 4.8 at metamorphosis, respectively, indicating accumulation of more lipid and/or carbohydrates than protein.

Larval growth patterns at constant conditions (12 °C) are summarized in Figure 5. In the upper graph, increase in the number of setigers in relation to the time of pelagic development is shown, assuming average growth rates, with $m = 0.95$ in *Polydora* spp. and $m = 0.80$ in *Pygospio elegans*, and time to metamorphosis, $t_M$, of 14 and 24 days, respectively. This pattern of growth is an almost linear function of time, with a weak

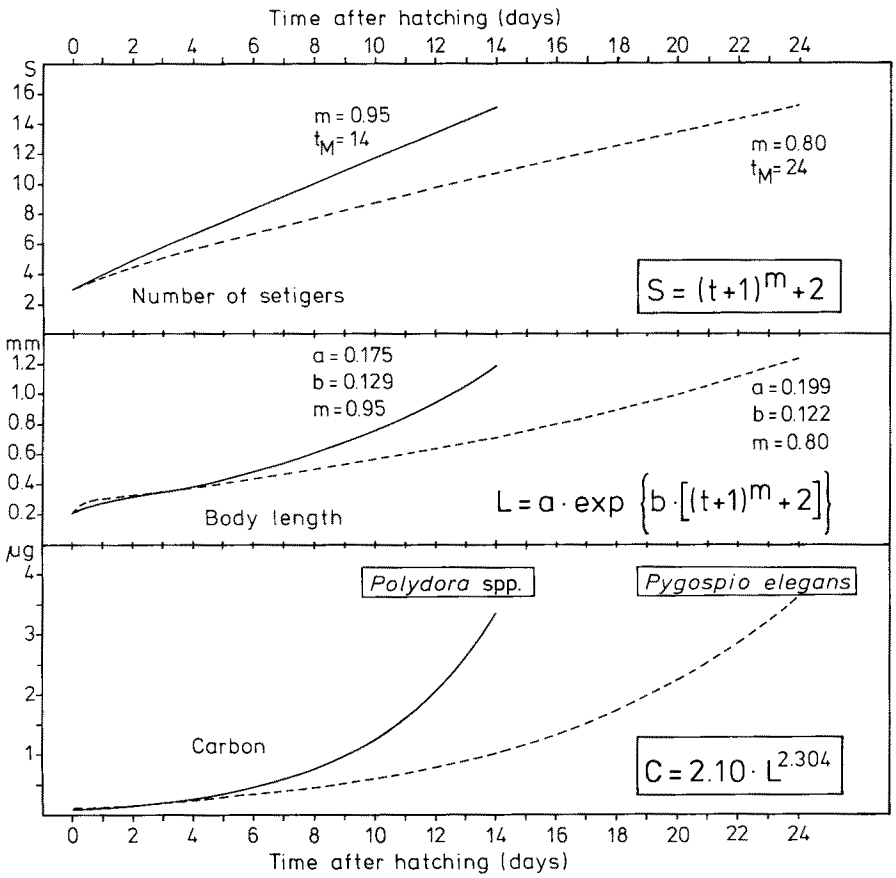


Fig. 5. *Polydora* spp. (solid lines), *Pygospio elegans* (dotted lines). Summary of larval growth patterns at constant 12 °C (average growth rates assumed, with slope, m = 0.80 and 0.95, respectively). Relationship between time after hatching (*t*, in days) and the number of setigers (S, upper graph), body length (L, in mm, middle graph), and carbon content (C, in µg per individual, lower graph; L in the conversion equation may be substituted for the equation given in the middle graph, in order to describe C as a function of *t*)

curvature during the first few days after hatching (cf. Fig. 2). Body length, in contrast, increases in an exponential way, after passing a brief initial "stretching phase" (Fig. 5, middle graph). *P. elegans* reveals a significantly slower larval growth than *Polydora* spp. but, due to the slightly larger average length of body segments ,has in the beginning of larval life a similar size. A general equation is given in this graph, describing body length as a function of time (constants from Fig. 3; *m* values as assumed above). This equation can be inserted in those linking biomass with body length (Fig. 4; Fig. 5, lower graph). Exponential growth of biomass is shown, taking carbon as an example.

## Influence of temperature

Temperature dependence of larval growth was evaluated in a total of 10 experiments with *Polydora ligni,* 8 with *P. ciliata,* and 15 with *Pygospio elegans,* at 6°, 12°, and 18°C. At the lowest temperature (3 experiments carried out with each species), metamorphosis was achieved only by *P. elegans* larvae, 50–60 days after hatching. *Polydora ciliata* reached maximally 8–11 setigers during the same time span, and *Polydora ligni* only 7–8. Experiments were usually terminated after ca two months since, due to mortality, not enough larvae were left for further regular measurements of growth.

At 12°C, *Polydora* spp. larvae reached the 15-setiger stage (here considered metamorphosis) 16–28 days after hatching (3 experiments with *Polydora ciliata,* 4 with *P. ligni*), whereas *Pygospio elegans* grew more slowly, with development times varying between 26 and 43 days (6 experiments).

Development from hatching to metamorphosis was in all species fastest at 18°C, but with different degrees of acceleration: *Polydora* spp. (4 experiments) needed only 9–11 days, *Pygospio elegans* (6 experiments) 20–40 days.

From the growth curves obtained at different temperatures, the theoretical time of development ($t_M$) may be calculated [Eq (4)]. When these values are plotted against temperature (T; °C), significant regression curves are obtained for all three species (Fig. 6).

These results indicate highly differing temperature adaptation in the species investigated, *Polydora* spp. appearing to be warmth-adapted and *Pygospio elegans* cold-adapted. No tests were carried out, however, to discover whether this was a true difference between species (or populations) or an artifact caused by different maintenance temperatures of the adult worms in the laboratory. In spite of high variability among larvae from different hatches, one safe conclusion may be made: the degree of temperature dependence in larval development duration is far higher in *Polydora* spp. than in *Pygospio elegans.*

## Food value of some phytoplankton algae

Eleven species of phytoplankton algae were tested as to their potential food value (Table 1). With few exceptions *(Amphidinium carterae, Scrippsiella faeroënse),* all algal species were used in at least five experiments per spionid species, each with *Dunaliella tertiolecta* as standard food. The RIG values are compiled in Figure 7. The algal species are ranked in order of their relative food value for *Polydora ligni* larvae (upper graph). There was a high variation in growth rates of polychaete larvae in the different

experiments in spite of identical type of food, reflecting variability in quality of both algal cultures and larvae. Despite this variability, a rather consistent tendency was found in most cases, in particular in the experiments with *P. ligni* larvae (Fig. 7). The flagellates *A. carterae, Protococcus* sp., *S. faeroënse, Isochrysis galbana,* and *Pavlova lutheri*

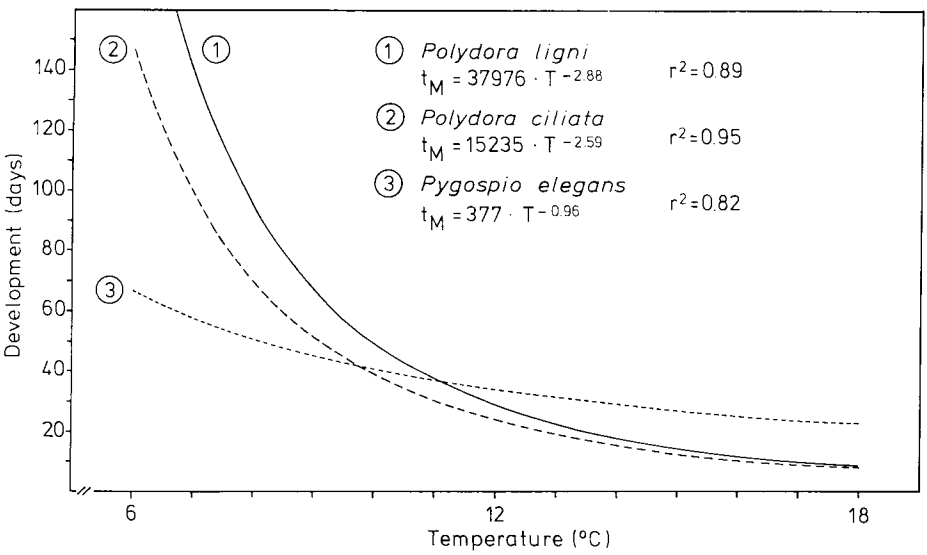


Fig. 6. *Polydora ligni* (1), *P. ciliata* (2), *Pygospio elegans* (3). Relationship between theoretical time of larval development from hatching to metamorphosis ($t_M$, in days, calculated from regression equations for larval growth obtained in single experiments, see text) and temperature (T, in °C). $r^2$: see Figure 2

Table 1. List of phytoplankton algae tested as food organisms for spionid larvae. Nomenclature and taxonomic position according to Parke & Dixon (1976). Carbon values from CHN analyses (present study, marked "a") or estimated from cell volume (Strathmann, 1967; "b")

| Species | Taxonomic position (phylum/family) | Size (µm) | Carbon (pg · cell$^{-1}$) |
|---|---|---|---|
| *Amphidinium carterae* | Dinophyta, Gymnodiniaceae | 10 × 15 | 73[a] |
| *Scrippsiella faeroënse* | Dinophyta, Calciodinellidaceae | 25 × 30 | 800[a] |
| *Gymnodinium splendens* | Dinophyta, Gymnodiniaceae | 40 – 80 | 5000[a] |
| *Prorocentrum micans* | Dinophyta, Prorocentraceae | 30 × 50 | 2400[a] |
| *Protococcus* sp. | unknown | 5 | 7[a] |
| *Isochrysis galbana* | Haptophyta, Isochrysidaceae | 6 | 11[a] |
| *Pavlova lutheri* | Haptophyta, Pavlovaceae | 5 × 8 | 15[b] |
| *Dunaliella tertiolecta* | Chlorophyta, Dunaliellaceae | 11 × 6 | 25[a] |
| *Platymonas suecica* | Chlorophyta, Prasinocladaceae | 5 × 6 | 30[b] |
| *Rhodomonas* sp. | Cryptophyta, Cryptomonadaceae | 12 × 8 | 45[a] |
| *Thalassiosira rotula* | Bacillariophyta, Thalassiosiraceae | 42 × 20 | 700[a] |

did not, in general, support larval growth as well as the standard food species, *D. tertiolecta*. *A. carterae* can apparently become toxic to spionid larvae, as in most experiments with *A. carterae* there was extremely high mortality and almost no growth at all. *Polydora ciliata* larvae, however, reached in some cases metamorphosis, though

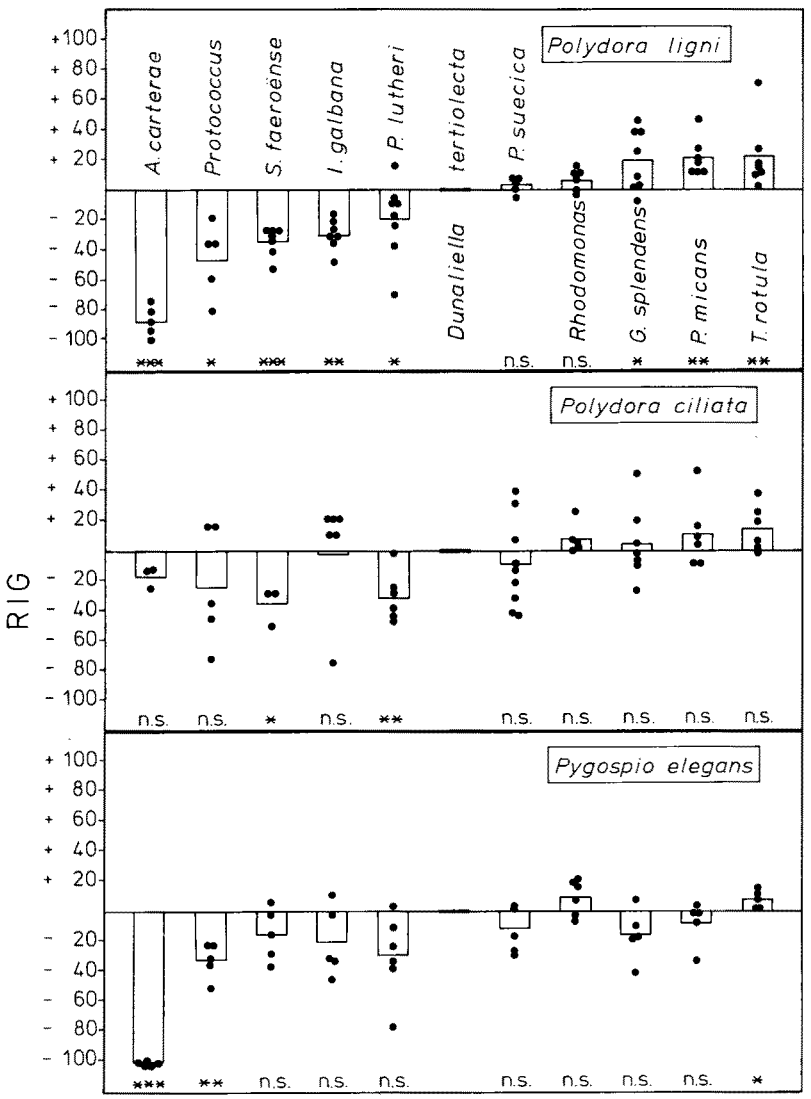


Fig. 7. *Polydora ligni* (upper graph), *P. ciliata* (middle graph), *Pygospio elegans* (lower graph). Relative index of growth (RIG, see text) in larvae offered different phytoplankton species as food. Bars: mean RIG; circles: single values. Level of significance (P) given for comparison of mean RIG with the standard (*Dunaliella tertiolecta*, RIG = 0): $\leq 0.001$ (***), $\leq 0.01$ (**), $\leq 0.05$ (*), or $> 0.05$ (n.s. not significant)

after some delay. With two other flagellates, *Platymonas suecica* and *Rhodomonas* sp., similar results to those with *D. tertiolecta* were attained. However, somewhat faster growth was observed in the *Rhodomonas*-fed larvae (in 12 out of 17 experiments, but mean RIG values statistically not significantly different from zero). The flagellates *Gymnodinium splendens* and *Prorocentrum micans,* and the diatom *Thalassiosira rotula* were the best food algae for *Polydora* spp. In *Pygospio elegans,* only *T. rotula* was significantly better food than *D. tertiolecta.*

The RIG gives the percentage by which the growth coefficient of Eq. (2), the constant $m$, is reduced or increased (see definition above). If average growth at 12 °C is assumed ($m$ ca 0.85–1.0 in *Polydora* spp., 0.75–0.85 in *Pygospio elegans*), effects of single phytoplankton species on larval growth curves and development duration to metamorphosis ($t_M$) may be estimated from the results in Figure 7. One example has been shown in Figure 2.

## DISCUSSION

The present paper represents a summary of a long-term laboratory study on the life cycles of three spionid polychaetes. Our observations on the benthic life-spans suggest that all three species may live for up to several years. In the field, where average temperatures are lower than in our culture conditions, this also should theoretically be possible, as long as there is no predation. The "normal" life-span, however, is probably between one and two years. In the western Baltic Sea, an average life-span of ca 10 months was estimated by Schütz (1966) for *Polydora ciliata.* Daro & Polk (1973) found in a Belgish population of the same species that most individuals died in their first year, and only a few survived during the winter.

The period from settlement and metamorphosis to first hatching of larvae of the following generation lasted at the minimum ca 1 month in *Polydora* spp. and almost 3 months in *Pygospio elegans.* This is in the range of observations already made in these and other spionids (e.g. Daro & Polk, 1973; Blake & Woodwick, 1975; Rice, 1975; Day & Blake, 1979). Thus, in the natural environment three generations per year may be produced by *Polydora* spp., and two by *Pygospio elegans.* This conclusion is supported by field observations (Hannerz, 1956; Daro, 1970; Daro & Polk, 1973; Rasmussen, 1973; Dean & Mazurkiewicz, 1975).

*P. elegans* developed exclusively through pelagic larvae hatching in the 3-setiger stage. This is typical of the Sylt population from where the present material originated (Anger, 1984), but the reproductive mode of this species can vary considerably (see recent reviews: Dean & Mazurkiewicz, 1975; Blake & Kudenov, 1981). It is likely that *P. elegans* actually represents a complex of sibling species (Anger, 1984). The same is probably true for *Polydora ligni* (Rice & Simon, 1980).

Larval growth patterns (number of setigers in relation to body length and biomass) as described in the present paper may be compared with the relatively sparse literature data on these species (Wilson, 1928; Hannerz, 1956; Blake, 1969; Orth, 1971; Podamo, 1974). There is good agreement between most of these data, so that our growth models may be considered realistic descriptions. According to our observations, the number of setigers is a better criterion for growth studies than body length. It may now be easily converted to estimates of size or biomass, applying the appropriate regression equation.

The size at hatching is apparently somewhat different in *Polydora* spp. and *Pygospio*

*elegans*, the latter being larger. There is considerable growth in body length during the 3-setiger stage: ca 170–280 µm in *Polydora ligni* (Blake, 1969), 200–260 in *P. ciliata* (Wilson, 1928), 250–330 µm in *P. elegans* (Smidt, 1951; Hannerz, 1956; Muus, 1967). It appears from these literature data as well as from the present investigation that when the larvae hatch and leave the maternal tube they may be any size in this range. Similarly, in all later stages there is length growth before a new segment is added. Thus, our regression curves (Fig. 3) show the approximate size range for any number of setigers during larval development.

Duration of larval development and, hence, also growth rates are strongly influenced by genetic and historical factors (origin, conditions during oogenesis and embryogenesis) as well as environmental factors such as temperature and food. Great variability of growth rates, even within a single hatch reared communally under constant conditions, hampers prediction of development and metamorphosis in spionid larvae. Numerous parallel experiments are therefore necessary to quantify effects of single factors. Our results on temperature effects, for example, should thus be considered preliminary, since there was high variability of data which was not caused by the factor examined. Our results suggest, however, that there are differences in the degree of temperature dependence, in particular at low temperature, in the three species compared.

*Pygospio elegans* showed the weakest response to changes of temperature in its development duration, *Polydora ligni* the strongest. *P. elegans* was the only species which was able to develop successfully to metamorphosis at 6 °C, with a relatively slight delay as compared to 12 °C. This is consistent with the ability of this species to reproduce during practically the whole year, even in winter (Leschke, 1903; Linke, 1939; Smidt, 1944a, b, 1951; Thorson, 1946; Hannerz, 1956; Giere, 1968; Gillandt, 1979; Hernroth & Ackefors, 1979).

*Polydora ciliata* reproduces in spring and summer, with maximum occurrence of larvae often found in spring, when water temperature is still low (e.g. Thorson, 1946; Hempel, 1957b, 1961; Dorsett, 1961; Schram, 1968; Meadows, 1969; Daro & Polk, 1973; Kühl & Mann, 1976; Kölmel, 1979; Harms & Anger, 1983). At constant 6 °C the larvae of this species exhibit significant growth, and they may be able to metamorphose within approx. 3 months. In nature, however, temperature increases during spring and early summer, and settlement should occur after a shorter time of pelagic development.

*P. ligni*, in contrast to the other species, showed very little larval growth at 6 °C. This is consistent with the season of reproduction which can be, in the field, in spring, but is mainly in summer when temperature is at its maximum (e.g. Smidt, 1951; Hannerz, 1956; Giere, 1968; Blake, 1969; Harms & Anger, 1983).

Metamorphosis is not clearly defined in spionid polychaetes; however, most authors consider stages with ca 14–17 setigers to be the transition from larval to juvenile life (e.g. Hannerz, 1956; Hempel, 1957b; Blake, 1969; Orth, 1971; Rice, 1975; Day & Blake, 1979). In the present study, the attainment of 15 setigers was considered metamorphosis. The process of settlement, i.e. examination of the substrate and first tube building, begins approximately at the 14-setiger stage, but swimming often continues or is resumed until ca 18 setigers are attained.

The food value of various phytoplankton algae to, in the main, molluscan larvae has been compared (e.g. Davis & Guillard, 1958; Walne, 1963, 1970; see also reviews by Epifanio, 1975; Ukeles, 1975; Kinne, 1977). Among the species tested in the present

study, *Pavlova (= Monochrysis) lutheri, Platymonas (= Tetraselmis) suecica,* and *Isochrysis galbana* supported the growth of bivalve and gastropod larvae in most cases better than our standard food, *Dunaliella tertiolecta. Polydora* spp. and *Pygospio elegans* larvae, however, usually thrived more successfully when *D. tertiolecta* was given as food. Only a few phytoplankton species yielded better results (Fig. 7), and only the diatom *Thalassiosira rotula* was consistently better food than *D. tertiolecta.* Since this was the only diatom tested in the present study, future investigations should include tests of further diatom species, in order to optimize culture conditions. Such comparisons should allow, in addition, conclusions on nutrition and development of the larvae in the field, provided their seasonal occurrence and concurrent composition of phytoplankton communities are known. The present results, for example, suggest that some dinoflagellates *(Amphidinium carterae, Scrippsiella faeroënse)* are poor food and may even be toxic to the larvae, whereas others *(Gymnodinium splendens, Prorocentrum micans)* may range among the best food items. In diatoms, cell or chain size might be a crucial factor, as ingestion can be inhibited by too large a particle size. Preliminary observations within the present investigation have shown, however, that even small larvae (5–10 setigers) may swallow large diatom cells such as *Coscinodiscus* spp. but these are not digested and eventually are excreted alive.

The present study has improved the technical basis for future experimental life cycle studies on spionid polychaetes, in particular those on larval development. In contrast to some other marine invertebrate groups, particularly molluscs and crustaceans, ecological, physiological, and biochemical aspects of larval life are in general poorly understood, and further laboratory studies conducted under controlled environmental conditions are necessary to improve our knowledge of this important polychaete family.

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