Reference: *Biol. Bull.*, 139: 203–221. (August, 1970)

LIFE CYCLE OF THE HYDROMEDUSA *PHIALIDIUM GREGARIUM* (A. AGASSIZ, 1862) IN THE LABORATORY

EDWARD C. ROOSEN-RUNGE

Department of Biological Structure, School of Medicine, and University of Washington Friday Harbor Laboratories, Seattle, Washington 98105

It has been known for more than a hundred years that it is not difficult to raise hydroids from the eggs of *Phialidium hemisphericum* (Wright, 1858). There is no report of the raising of hydroids of the medusa known as *Phialidium gregarium* (A. Agassiz, in L. Agassiz, 1862) and its hydroid has not been identified until now. This would seem to be an excellent reason to undertake the observation of the whole life cycle of *P. gregarium* in the laboratory. There are, however, several equally cogent reasons. In the first place, the elaboration of a culture method which permits reliably raising hydroid colonies from single planulae, and medusae from single hydroid colonies, also enables us to study *individual* life cycles. Colonies found in nature and even colonies raised from batches of eggs are composites of many individual beings and the dynamics of their behavior are difficult to explain because the extent, the developmental stage and other specific conditions of the individual components are usually unknown. On the other hand, a hydroid "colony" derived from a single planula may be considered a single animal. By observing and comparing many of these animals, conclusions can be reached about age changes, responses to environmental conditions and about differences between animals under identical conditions which must be ascribed to genetic variability. Hydroids have been notorious for the taxonomic difficulties which they represent, and many descriptions of what are assumed to be single species show a wide and poorly delineated range of anatomical features. Detailed comparison of colonies arising from single planulae under controlled conditions can be expected to clarify some of the taxonomic issues. Finally, the breeding of medusae in clones derived from single eggs will permit more precise experimentation, for instance, on sex determination and on physiological parameters such as light sensitivity (Roosen-Runge, 1962) where results have been ambiguous until now because of genetic heterogeneity.

Materials and Methods

All observations and experiments on living animals were made between the end of July and the end of November. Medusae were caught from the dock of the Friday Harbor Laboratories. They were brought up with a small, white enamelled saucepan on a 3½ foot handle from a float approximately 2 feet above the water. Therefore, the animals came from the surface layer down to little more than 3 feet. Freshly caught medusae, 30–40, with a preponderance of males, were left in large bowls (1000 cc) overnight and the eggs collected around 10 A.M. Special care was taken to make sure that every parent animal belonged to the species *P. gregarium* as defined by Kramp (1962).

Several settling experiments were undertaken (see Results). The simplest method proved to be the most efficient. A dozen 3×1 inch microscopic slides were arranged on the bottom of a large bowl containing many hundreds of planulae. After 6–12 days, 50–66% of the slides were found to have at least one primary hydroid growing on them. Such slides were hung in plastic frames, each holding 5 slides into the tanks in which most of the hydroid colonies and many medusae were raised. More than 30 cultures were observed in detail for nearly 2 months and many more were used occasionally for short-term observations.

The tanks were modified after the "Plankton-Kreisel" described by Greve (1968). The principle of the "Kreisel" is a rotary circulation in which inflow into an outer compartment and outflow from an inner compartment are separated from each other by the sand on the bottom. The "airlift" of the inner compartment was omitted as unnecessary in an open seawater system. The Kreisels (Fig. 1) were made out of carboys by removing the tops. They were 31–32 cm

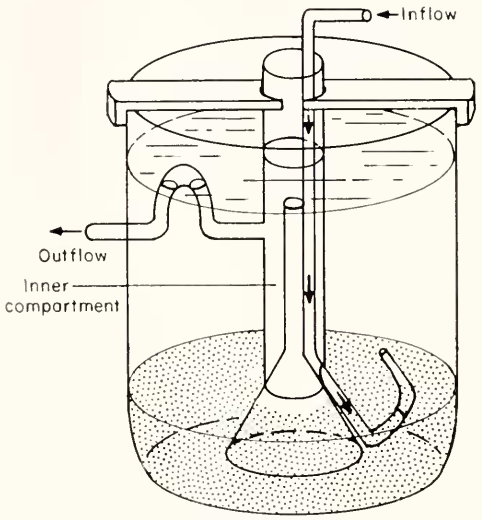


FIGURE 1. Sketch of the modified "Kreisel" used in raising hydroid colonies and medusae.

high and 26½ cm in diameter. A self-syphoning outflow was arranged so that the volume of water in the tank oscillated between approximately 15 and 12½ liters. Flow was kept between 5 and 20 liters per hour. Occasional plugging of the water lines caused irregularities and standstills which did not noticeably influence the experiments. The tanks were standing in 5–8 cm of seawater with continuous flow which assisted in maintaining a low temperature even when the circulation through the Kreisels stopped. At the inflow into the water tables, the temperature of the seawater varied from 10–13.5° C depending on the outside temperature. At an average temperature of the inflow of approximately 12.5° C, the water in the tanks had a temperature of 14° C at a flow of 14 liters per hour, 15° C at a flow of 9 liters per hour. In general, temperatures declined toward the fall.

For examination and counts, the individual cultures on their slides were re-

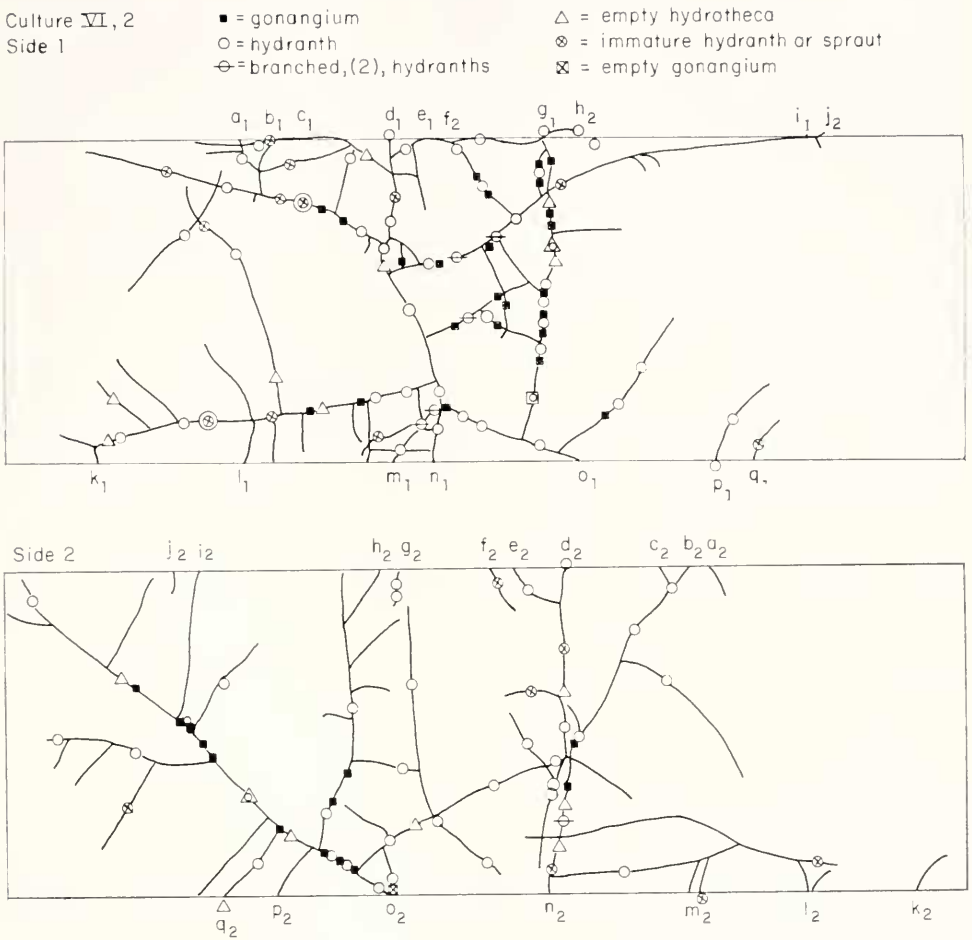


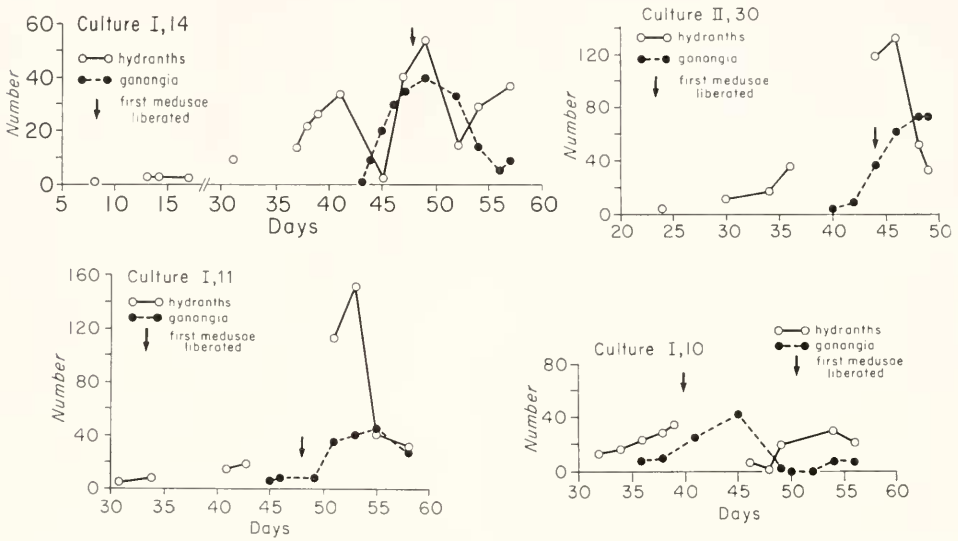
FIGURE 2a and b. Semidiagrammatic representation of a clonal hydroid culture of *Phialidium gregarium* on a 3×1 inch slide, after a direct tracing. The culture was drawn 34 days after the planula settled and 21 days after the first side branches appeared on the stolon. Figure 2a shows the side of the primary hydroid.

moved from the tank and inspected submersed in a Petri dish. Cultures which grew on both sides of the slides were supported by a plastic ring (diameter $\frac{3}{4}$ inch). Counts of hydroids and gonangia were made with the help of a 1×3 inch glass slide on which 14 areas 1.5×1.1 cm were drawn. This was placed directly under the culture slide and under the plastic ring.

Microscopic observations, photographs and measurements were made of living hydroids, gonangia and small medusae on a cold stage using an electric module (Cloney and Schaadt, 1970). On this stage the animals and their parts were kept very conveniently in depression slides at 10–13° C.

Results

Results will be presented by first recounting observations on phases of the life cycle: settling, primary hydroids, growth of a stolon and trophosome, formation of gonosome, and growth of planktonic medusae. Secondly, the morphological features of the hydroid will be reported in detail, and the hydroid be defined summarily. This definition will then be compared with descriptions of related species in the literature, and an attempt will be made to identify the whole animal, inclusive of both its hydroid and medusal phases, by suggesting a single proper name.



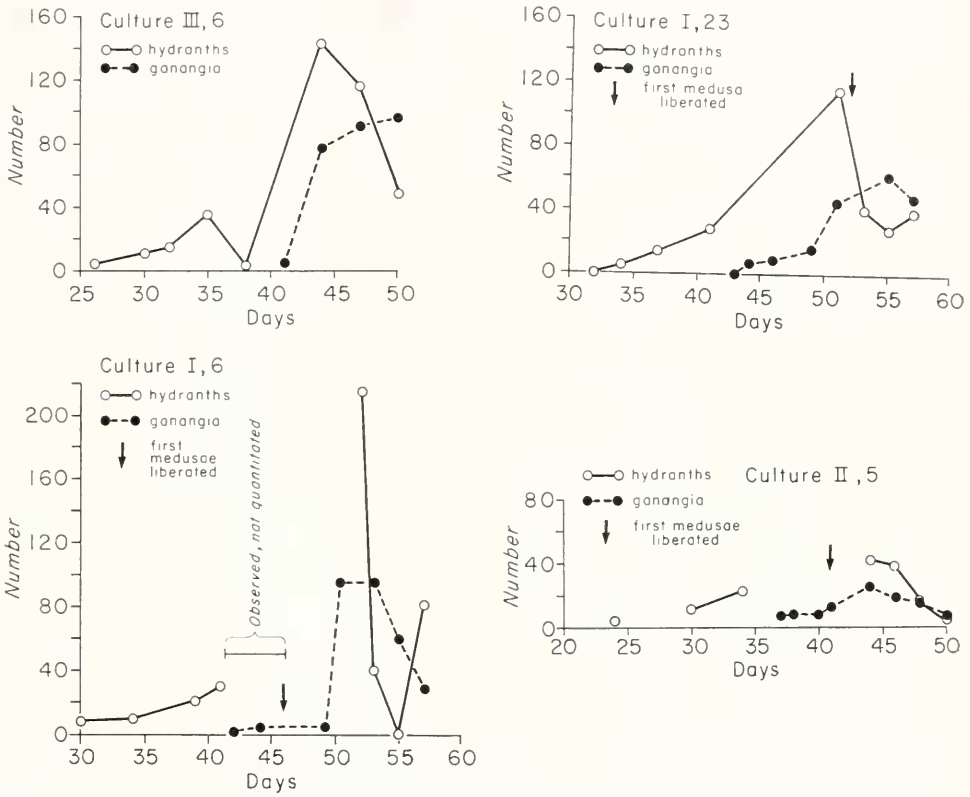
Figures 3–6. Graph of numbers of hydroids and gonangia during development of clonal hydroid cultures. The lines between points were drawn only when frequent observations justified the assumption of continuity of slope, even if no counts were made in the interval.

The hydroid culture—settling

The planulae of *P. gregarium* settle on many substrates with great ease; for instance, on glass, plastic, wood and algae. (The non-glycogen polysaccharide glue and the glandular cells which produce it have been discussed by Bonner, 1955.) They will settle on vertical as well as on horizontal surfaces. In one of the Kreisels in which large numbers of medusae were kept, several blank microscopic slides hanging vertically in the current began to show growth of hydroid colonies after a few weeks. In other settling experiments it was found that planulae often accumulated at the walls of the vessels near the waterline, and attempts were made to utilize this tendency by putting many slides in "staining jars" with the waterline running lengthwise along the middle of the slide. These experiments failed, perhaps, because the amount of water in these jars was very small.

In repeated experiments with large numbers of eggs the first planulae to become sluggish and settle were observed in the second half of the third day after

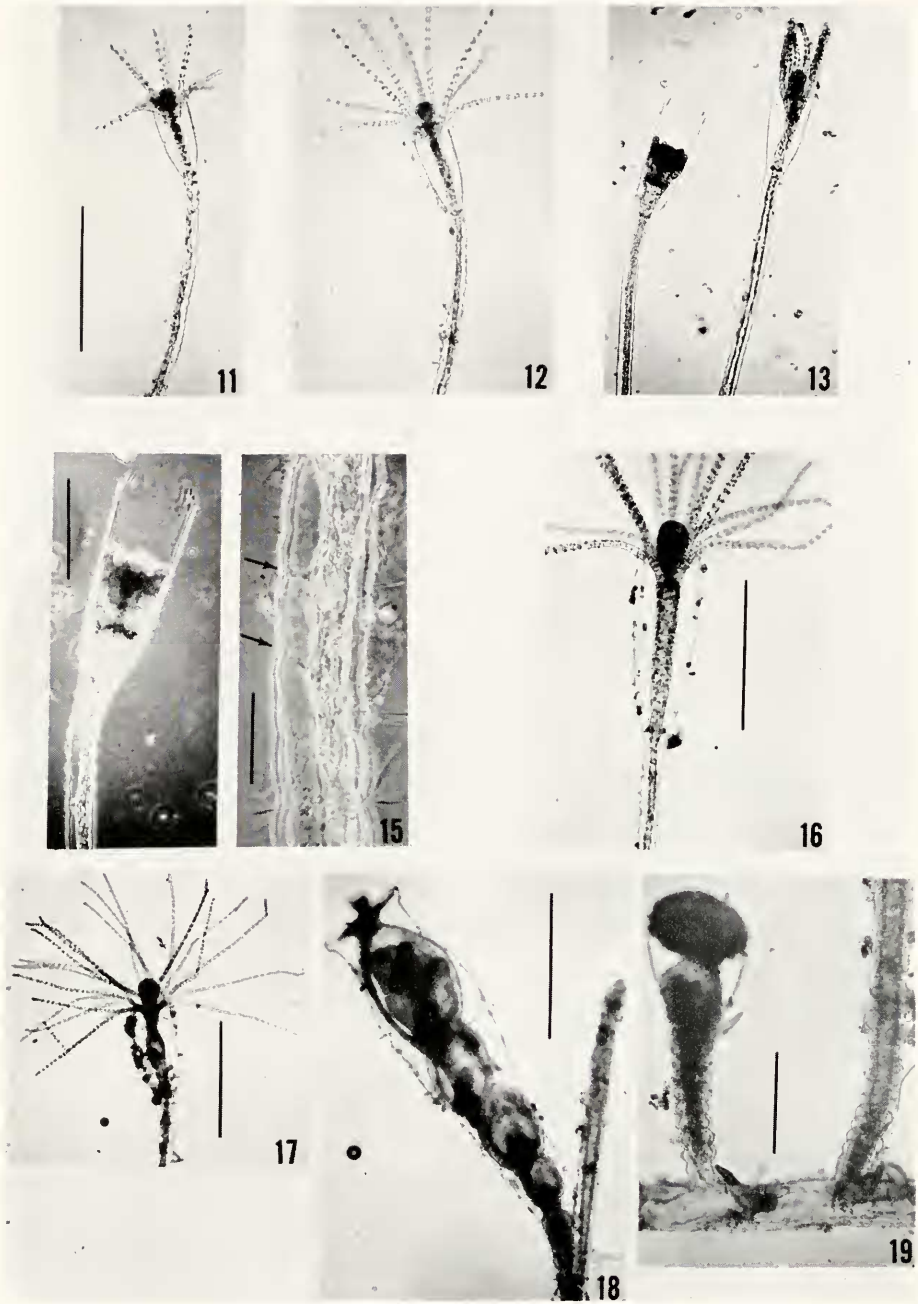
fertilization. Approximately half of the planulae settled on the fourth and fifth days, but even on the twelfth day a few planulae were still swimming. In this species which shows little selectivity in the substrate for its hydroid phase, the time of settlement appears to be in large part determined by an endogenous readiness of the planula.



FIGURES 7–10. Graph of numbers of hydroids and gonangia during development of clonal hydroid cultures. The lines between points were drawn only when frequent observations justified the assumption of continuity of slope, even if no counts were made in the interval.

Primary hydroids; first growth of stolon

Once a planula has settled it flattens, rounds off and spreads into a pedal disc with 4 to 6 lobes within 2 days. It then forms a stalk on which a hydranth develops. Hydranths capable of feeding usually are observed on the third or fourth day after settlement. The primary hydroid will be described in some detail below (Figs. 11–15). There appears to be a period of several days during which there is little visible change in the pedal disc. If a hydroid is fed abundantly during this period it may regress and the disc develop one or more new hydroids which are usually somewhat larger than the first.



FIGURES 11–13. Photomicrographs from living clonal cultures of *Phialidium gregarium*. (Figures 11–15 show primary hydroids.) The bar represents 500 μ (also for Figures 12 and 13.) Compare hydroids in Figures 11–13 with respect to number of tentacles, size, proportions of hydrotheca and features of annulations.

A stolon is visible usually a week after settlement. It grows frequently from one pole of the pedal disc, but sometimes from two opposite poles. Initially, the stolon advances in an almost straight line, even when it progresses in two opposite directions.

Secondary hydroids; branching of stolon

As the stolon grows, secondary hydroids are sprouting from it at rather regular intervals (1–3 mm in young cultures) which tend to become larger as the colony expands. Hydroids tend to become taller, develop more tentacles and branch more frequently (details are described below) but the degree to which this happens depends largely on the amount of food available. As long as colonies received only occasional feedings by pipette in addition to a small amount of planktonic organisms from the water supply, growth was slow and the hydroids which developed were only slightly more differentiated than primary ones. The first month of culture I, 14 (Fig. 3) serves an example. A primary hydroid was present on day 7 after fertilization. Three hydroids were counted on day 13, 14 and 17. When the culture was next examined in detail, on day 31, it had 10 hydroids. The culture had only subsisted and had, in fact, been somewhat damaged, so that the stolon was interrupted in several places. From that day on it was fed with very large numbers of brine shrimp several times daily and after 10 days there were 34 feeding hydranths, many of which were large and had 20 tentacles or more. Despite continued abundant supply of food (on day 41 the culture was completely saturated with brine shrimp under the microscope, each hydranth receiving many shrimp), within the next 4 days the number of feeding hydranths fell to 3, but in the following 4 days the number of feeding hydranths went up to 54. The eight examples in Figures 3–10 and all other cultures observed showed the same phenomenon. Boosting the food supply did always initially stimulate the growth of hydranths and, to a lesser extent, of stolon and hydroids. On the other hand, the hydranths were short-lived, if they fed heavily. A single hydranth which had ingested 6, 8 or even 10 freshly hatched brine shrimp usually regressed within 24–48 hours and only the empty hydrotheca was found in its place. Hydranths which were fed poorly often lived for many days. A lifespan of a week was observed several times. The stolon did not appear to regress after any amount of feeding. Neither did it easily respond to starvation. In cultures neglected for several weeks the stolon appeared to contain a living cenosarc

---

FIGURE 14. The bar represents 200 μ. Phase optics permit identification of teeth at rim of hydrotheca. At left a "rib" may be discerned running downwards from one of the teeth.

FIGURE 15. The bar represents 50 μ. Phase picture of the distal annulations on the stem of a hydroid. Cytoplasmic processes are shown extending from cenosarc to hydrotheca in the region of a constriction.

FIGURES 16 and 17. Secondary hydroids. Bar represents 500 μ in Figure 16, 1 mm in Figure 17. Compare the hydranths in Figure 11 and 12 with that in Figure 16 (same magnification) and 17 (less than half the magnification). The hydroid in Figure 17 was 7 mm tall in life.

FIGURE 18. The bar represents 500 μ. Gonangium with 4 gonophores, arising from a stem.

FIGURE 19. The bar represents 200 μ. An immature gonangium (left), approximately 1 day old, and the base of a hydrocaulus (right) arising from a stolon. Note annulations and the difference in thickness of hydrotheca and gonotheca.

throughout, even when only a few small hydroids were present. These results are, in general, similar to those of Crowell (1953) who studied growth and regression in hydroids of *Campanularia* kept at various levels of nutrition (brine shrimp).

During the initial slow growth of the colony the stolon did not branch. The earliest branching was seen 2 weeks after fertilization in a culture which had settled 8 days previously. At first branching there were as few as 3 hydroids and as many as 10 or more. Branches tended to occur at right angles (Figs. 22–25). When branching began the culture expanded rapidly. It reached the edge of the slide somewhere before another week had passed. The edge presented no obstacle (Fig. 23). Figure 2, for example, demonstrates that 3 weeks after the first branching of the stolon a colony may have grown around the edge of the slide at 17 different points. A culture of approximately the same age not confined to a slide, but growing on the wall of the tank, extended over a circular area with a diameter of approximately 7½ cm. It appears that for the first 2 months of a colony's life the area presented by the two sides of a 3 × 1 inch slide does not limit expansion to any appreciable degree.

Growth of gonosome

The earliest formation of gonangia occurred 19 days after fertilization. This was observed several times in mass cultures from many eggs of a single spawning. In the cultures raised from one egg the earliest gonangia were seen after 21 days. In the 8 cultures represented in Figures 3–10 the first gonangia were observed on days 36–45. When a gonangium was first unmistakably recognized, it was a small, dense, elevated structure of mushroom shape (Figs. 19, 22, at arrows) very different from the thin stalks of budding hydroids. Such early stages of gonangia appeared in locations where 12 hours before there had been no indications.

Figures 3–10 demonstrate that the first appearance of gonangia is regularly preceded by an increase in the number of hydroids which begins 7–4 days earlier. For instance, culture II, 30 (Fig. 4) showed an appreciable increase in the number of hydroids on day 34, culture I, 11 (Fig. 5) on day 41 or earlier; the first gonangia were observed on day 40 and 45, respectively. Culture I, 10 (Fig. 6) demonstrates two periods of gonangial growth, the first beginning on day 36, the second on day 52. The first was preceded by a burst of hydroid formation beginning on day 32, the second by one beginning on day 48. There was no clear correlation between the peaks of hydroid development and the beginning of gonangial growth. (Compare, for instance, Fig. 7 where a peak almost coincides with the appearance of gonangia with Fig. 8 where it comes much later, or Fig. 3 where it precedes.) Nor is every rise in the number of hydroids followed by the appearance of gonangia (Figs. 3, 7).

Gonangia develop either from the stolon directly (Fig. 22) or, more rarely, from the stems of hydroids (Fig. 18). When they spring from the stolon they do so almost invariably close to a hydroid stem and characteristically equidistant on both sides of it (Fig. 22). Their location within the culture is predictable in certain respects. They occur on parts of the stolon which have reached a certain minimal age (1–2 weeks?). They never are formed by new side branches of the trophosome but are most frequently found on the middle portions of long stretches of stolon which are by their very positions identified as relatively

mature. This is shown, for example, in Figure 2 which also demonstrates that the side of the slide on which the primary hydroid grew (Fig. 2a) has far more gonangia than the other side which is on the average younger. Because of the relatively short duration of the observations it cannot be stated whether there is also an upper age limit beyond which any part of the stolon becomes incapable of producing gonangia. In any case, this hydroid presents a pattern very different from one like *Podocoryne* (Braverman and Schrandt, 1969) in which a dense center is seen populated by sexual and nutritive polyps.

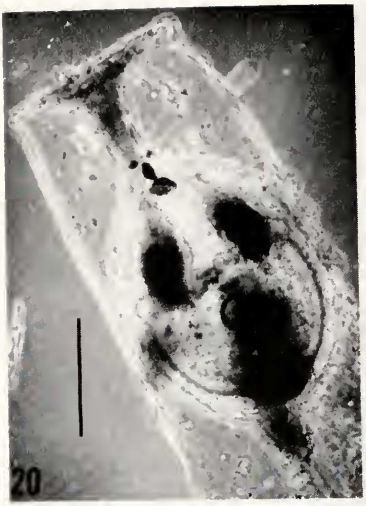
No hydroid culture was observed continuously for longer than 7 weeks. A few cultures were seen to have two phases of formation on gonangia during this period (Figs. 3, 6). The amounts of proliferation of stolon, hydroids and gonangia varied greatly from culture to culture, but appeared to be largely dependent on food supply in every case. There was no evidence that fluctuations in temperature or the lessening of daylight with approaching fall had any influence. At the end of September, 15 cultures, 2–2½ months old, were suspended from the dock at 15 foot depth for a wintering experiment. They were contained in a plastic cage which permitted open circulation. At the end of November, 6 of these cultures had been destroyed by some predator (nudibranch?). At this time, the cage was wrapped tightly in nylon screening to prevent further predation. Nine cultures were alive but much reduced from their state in September and showed only 1–6 hydranths of primary type, *i.e.*, very small with relatively few tentacles. At the end of March the cultures were once again examined. Six of them were alive and showed vigorous growth which apparently was of recent occurrence because detritus on the hydranths was minimal and empty hydrothecae were rare. The largest cultures had more than 30 hydranths with up to 20 tentacles. Three cultures showed gonangia in small numbers, in stages of development which indicated that their development had begun from 2–7 days previously.

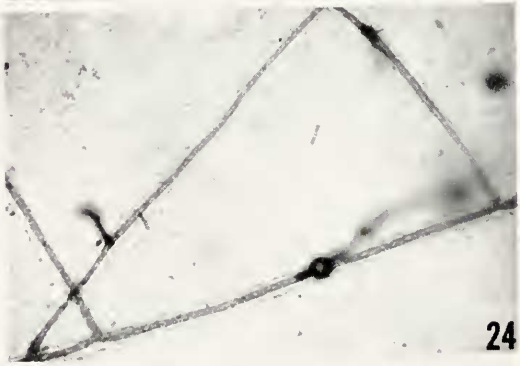
Liberation, growth and maturation of medusae

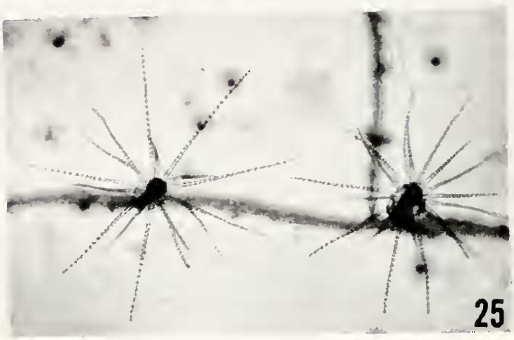
The first medusae were liberated from hydroid cultures 26 days after fertilization. This occurred usually after 35–45 days and once after 52 days. Many cultures had not begun the formation of gonangia when observation ceased in the fall, but none of these were more than 6 weeks old, and there is no reason to assume that they would not have produced medusae eventually. All cultures observed for 52 days finally did liberate medusae. There is no indication that productivity became less with the approach of fall.

The interval between the first appearance of gonangia and the first free medusae was always between 4 and 5 days (for instance, Figs. 3–10). After medusae first appeared the cultures continued to produce for 6–10 days, which reflects the duration of a single period of formation of gonangia. During such a period 70 to more than 250 medusae were produced.

Newly hatched medusae (Fig. 21) measured 1.2–1.4 mm in largest diameter. They had 4 tentacles and 8 lithocysts, conforming in this to the defining characteristics of the genus *Clytia* (Hincks, 1868). In addition, they displayed 4 immature tentacular buds. The gonadal Anlagen were very inconspicuous but were clearly defined under higher magnifications and phase optics. The tiny medusae were able to feed on single brine shrimp while they were still connected to the blastostyle,







FIGURES 20–25.

but even when unfed they expanded rapidly to a diameter of 2.5–3 mm during the first 2 days and after that time they had 8 tentacles. Freshly hatched medusae tended to remain "hung up" within the hydroid culture, even when it was vertically suspended, and were often seen somewhat entangled in the tentacles of hydroids. There was no evidence that they were ever ingested or harmed.

A few medusae were raised to near sexual maturity in 4 weeks. These were kept in fingerbowls, were handfed and transferred into fresh seawater daily. After 3 weeks they had 23 tentacles in the average with 8 tentacular buds, and their diameter was approximately 6 mm. After 4 weeks they measured approximately 1 cm and immature oocytes were discernible in the female gonads under the microscope. It was evident that the rate of their development depended greatly on feeding, water circulation and temperature. Certainly, the conditions for their development were not optimal in the present investigation. As long as extensive attempts have not been made to establish a better method of raising medusae, possibly in the Kreisel, the fastest time of their development to sexual maturity remains unknown, but one can predict with confidence that it will be less than 4 weeks.

It is of some interest that small medusae with tentacle numbers and diameters very similar to those raised in 3–4 weeks in the laboratory were frequently found in the Friday Harbor Bay in the first 10 days of September.

Morphology of hydroids

In the following paragraphs the range of variability of features conventionally used in taxonomic identification is presented point by point for the hydroid of *P. gregarium.*

Trophosome—size and branching

A great number of primary hydroids were observed and measured. They branched only very rarely. Variations in size were relatively small. The distance from pedal disc to rim of hydrotheca was in the average 1.4 mm, the range 1.25–

FIGURE 20. Photomicrographs from living clonal cultures of *Phialidium gregarium.* The bar represents 200 μ. Phase picture of the terminal part of a 3–4 day old gonangium (same magnification as in Figure 19) showing the lid of the blastostyle and one gonophore. The upper two oval black spots are tentacular bulbs, the larger irregular black spot below is the manubrium.

FIGURE 21. The bar represents 500 μ. Medusa of *Phialidium gregarium* just after hatching. Four tentacular bulbs (2 out of focus), 4 buds of tentacles (2 out of focus) and 4 of 8 lithocysts are clearly seen. The medusa is slightly flattened in a depression slide and consequently appears wider and lower than normal.

FIGURE 22. The bar represents 1 mm in Figures 22–25. Gonangia are shown (arrows), the lower two originating on both sides of a hydroid stem departing from the stolon to the left; the upper gonangium originates near another hydroid stem seen as a hook off the stolon slightly to the left below.

FIGURE 23. A damaged stolon (arrow) winding around the edge of its slide. The cenosarc is discontinuous; the theca extends intact around the edge and the stolon is continued out of focus, above and parallel with the one on the upper side of the slide.

FIGURE 24. A crossing of one part of the stolon over another one.

FIGURE 25. The focus is on two hydranths. A characteristic pattern of hydroids and stolon branching is shown.

1.55 mm. Subsequent hydroids in an adequately fed colony are gradually increasing in size. In the beginning of colony formation, each new hydroid formed along a stolon may be larger than the last, but with the beginning of branching of the stolon new hydroids are not always bigger, but often much smaller than the last ones formed, particularly on new branches of the stolon. Branching becomes more frequent with age in most colonies, but not in all. After a few weeks some cultures show as many hydroids branching as non-branching; others may have hardly any branches but only relatively tall single hydroids. The largest single hydroid measured was 7.52 mm tall from stolon to rim of hydrotheca with tentacles 1.6–1.8 mm long. Branched hydroids occasionally exceeded a centimeter in height. As no experiments were made with a continuous maximal food supply or with different types of food, it can only be surmised that under some conditions the size of the colonies may be greater than the largest observed in the present investigation.

The variability of the hydroids of *P. gregarium* with regard to size and branching, but also with regard to number of tentacles and annulations (see below) is, perhaps, characteristic for the genus *Clytia*. Berrill (1950) has pointed out that hydroids of *johnstoni* are similarly variable in contrast to hydroids of, for instance, *Campanularia* or *Obelia*.

Tentacles. Primary hydroids (Figs. 11–14) were observed to have 8–12 tentacles, 11 in the average. The tentacles were 400 μ or less in length. Large secondary hydranths (Figs. 16, 17) had up to 25 tentacles, frequently 20–22, and the tentacles were 1.6–1.8 mm long. It should be emphasized, however, that the same colonies which possessed many large hydroids usually had some small ones with low numbers of relatively short tentacles.

Hydrotheca. The size of the hydrotheca varied greatly. The length from diaphragm to rim measured in 10 primary hydroids was in the average 440 μ, the range 385–470 μ. The diameter at the rim, usually the largest diameter, was in the average 150 μ, range 110–185 μ. In 10 large hydroids from mature colonies the average of the same measurements was 1100 μ (range 1000–1185 μ) and 410 μ (range 390–430 μ). In general, secondary hydrothecae tended to be slightly less elongate in shape than the primary ones. Hydrothecae of primary hydroids had 8–11 teeth (Fig. 14) which were sometimes pointed, but often blunt or partially broken. Empty hydrothecae often showed no teeth at all. This variability was attributed to the fragility of the thin teeth. The tentacles usually are draped over the edge of the hydrotheca within the cusps between teeth. Their movements appear to wear the rim easily, particularly when brine shrimp are fed which are large in relation to the hydranth and cause great wear and tear of the tenuous perisarc. It was difficult to find intact hydrothecae on large secondary hydroids. Usually 10 or 11 teeth were counted, but one hydrotheca possessed 13 teeth. With phase optics 4 and occasionally 8 ridges could be seen to extend downward about $\frac{1}{3}$ of the length of the hydrotheca (one is visible in Fig. 14).

Annulations. As a rule annulations were found distally, on the stem immediately under the hydrotheca (Figs. 11–14, 16) and basally where the stem sprang from the stolon (Figs. 11, 12). In primary hydroids the distal annuli varied from 3–7 (rarely more) and were in the average 33 μ high (range 28–41 μ); basal annuli varied from 12–16 and were 31 μ high (range 27–43 μ). The zones of annulation often ended sharply and the part of the stem between them was

smooth (Fig. 13), but not infrequently the annuli were seen to become shallower and higher and give way to undulations throughout the center part of the stem (Fig. 11). Large secondary hydroids had a different pattern. Distal annuli were greater in number, 6–10, and much higher, 66 μ in the average (range 48–80 μ) (Fig. 16); basal annuli (Fig. 19) were less numerous, 9–14, and in the average, 48 μ high (range 35–53 μ). Cytoplasmic processes were seen which ran from cenosarc to perisarc, usually at the constrictions (Fig. 15, arrows). It was also observed that contractions of the cenosarc increased the curvature of the annuli. A thorough study of the way in which the hydrotheca is laid down and maintained may in the future bring a better understanding of the nature of the annuli, and clarify their value as a taxonomic feature. This topic has been discussed pertinently by Berrill (1950, 1961).

Gonosome

Origin, size and shape. The size and shape of a gonangium depends to a large extent on the state of its development. Small gonangia are funnel-shaped and the gonotheca is closely applied to the early gonophores. At this stage, it may appear that the pedicel is quite long and has from 5–12 annuli (Fig. 19). The pedicel is relatively shorter and has less annuli in more mature gonangia and in gonangia originating from a stem (Fig. 18). The envelope of the gonangium about the pedicel is smooth, an important distinction in comparison with the corrugated gonangium of *C. johnstoni*. A gonangium containing 5 medusae may grow taller than 3 mm and exceed $\frac{1}{2}$ mm in largest diameter which is at the equator of the largest gonophore and not at the upper rim (Fig. 20). The number of gonophores varies, however, from 2–7 and gonangia are consequently smaller or larger. Usually the majority arises directly from the stolon in the vicinity of a hydroid stem. Others arise on stems which often have 2 hydroids and may finally have also 2 gonangia. The frequency of these sites appears to vary distinctly between different colonies.

Summary definition of the hydroid of P. gregarium

Trophosome. Colonies of hydroids predominantly single but branched increasingly as a function of age and food supply. Height of primary hydroid less than 1.6 mm. Second hydroids up to 10 mm or more, depending on conditions. Hydrocaulus long, annulated at base and at top; intermediate portion generally smooth or undulating. Hydrotheca deeply campanulate, length to width 2.5–3.0:1 expanding very slightly above, with 8–13 teeth, 4 or rarely 8 fine ridges from rim downwards through upper $\frac{1}{3}$ of hydrotheca. Stolon branching predominantly at right angles.

Gonosome. Gonothecae ovate, outline smooth or somewhat undulating with a distinct collar at the opening, 3 mm or more in height when mature, arising from stolon with relatively long pedicel (up to 14 annuli) or from stem with shorter pedicel (4–8 annuli); 2–7 gonophores in each gonangium.

Taxonomic consideration

Now that the life cycle of the leptomedusa authoritatively identified (Kramp, 1962) as *Phialidium gregarium* (A. Agassiz) in L. Agassiz, 1862, page 353,

Oceania gregaria, has been observed in the laboratory, the question of the proper scientific name of the animal must be briefly considered. This question contains two problems: (1) the identity of the hydroid which has been shown to develop from the eggs of *P. gregarium*; and (2) the selection of one name for both the planktonic and the sessile phase of the species.

Identification of hydroid. Certainly the hydroid belongs properly to the genus *Clytia* established by Lamouroux (1816) as used by Nutting (1915), Fraser (1937) and others. For convenience sake the hydroid is called *Clytia X* in the following paragraphs. Hincks (1868) in defining the genus stated as its main characteristic the production of medusae with 4 radial canals, 4 marginal tentacles and 8 lithocysts. Nutting (1915, page 53) expanded the definition by describing the trophosome: "Colony often simple but always consisting of a creeping rootstock from which spring pedicels which are not regularly branched as a rule. Hydrothecae companulate, hydranths with trumpet-shaped proboscis." All these characteristics are observed in *Clytia X*.

It is to be expected that the species of *Clytia* which liberates medusae conforming as adults to the description of *P. gregarium*, will be found among the hydroid species described for the Pacific Coast and preferably for the Northwest Pacific Coast of North America. No more than two species of *Phialidium* are ordinarily found in Puget Sound and around the San Juan Archipelago, *P. gregarium* and *P. hemisphericum*. The last is not discussed here, but I can state on good, if not conclusive, evidence, that it occurs. Fraser (1937) listed not 2 but 12 species of *Clytia* from the Pacific Coast. Five of these, namely *C. attenuata* (Calkins, 1899), *C. bakeri* (Torrey, 1904), *C. hendersoni* (Torrey, 1904), *C. minuta* (Nutting, 1901) and *C. universitatis* (Torrey, 1904), may be excluded because of their large size and completely different growth habits. *C. inconspicua* (Forbes, 1848) may be eliminated from consideration because it is in all probability synonymous with *C. johnstoni* (Alder, 1856). *C. johnstoni* has distinctive corrugations on the gonotheca. It is a well described species (Hincks, 1868; Russell, 1953) and has been clearly established as the hydroid of *Phialidium hemisphericum* (Wright, 1858). Calkins (1899) found *C. inconspicua* near Port Townsend in Puget Sound, but stated that Alder (1856) and Hincks (1868) had found it in England. Hincks reported that Wright (1858) had raised this hydroid from jellyfish, which appears to establish the fact that it is the sessile phase of *P. hemisphericum* (Wright, 1858) and not of *P. gregarium* (A. Agassiz, 1862) which has not been found in Atlantic waters.

The question remains whether any one of the three species, *C. cylindrica* (Agassiz, 1862), *C. kinkaidi* (Nutting, 1915) and *C. longitheca* (Fraser, 1914), can be singled out as uniquely fitting the description of *Clytia X*. The answer is somewhat ambiguous. *C. cylindrica* was well described by L. Agassiz (1862), page 306. In his illustration the gonangium appears very similar to that of *Clytia X*, but is described as distinctly flattened while that of *Clytia X* is round. Agassiz found this hydroid on the New England Coast where *P. gregarium* has never been observed. Fraser (1937) stated that it was found at Friday Harbor, but his description while less clear than that of Agassiz adds another discrepancy, a short pedicel with only one or two annulations. *C. kinkaidi* is found in Puget Sound (Nutting, 1915) and is similar to colonies of *Clytia X* in its early phases;

it is described as not branching and as depicted by Fraser appears to be of much smaller size than *Clytia X*. It is especially similar in the features of ribs on the hydrotheca and of long gonangial pedicels. Finally, *C. longitheca*, reported for California and Vancouver Island, is also generally similar to *Clytia X* and uniquely so with regards to its long hydrotheca. Branching is not one of its features, however, and the gonangia apparently were never observed to spring from the stems but only from the stolon.

In summary, although the above three species show many general similarities, none can be unequivocally identified with *Clytia X*. It is probable that the descriptions in the literature partly pertain to *Clytia X* in at least one of its growth phases but they remain ambiguous and confusing. It is, therefore, proposed that the hydroid of *P. gregarium* be named *Clytia gregaria*. The specific *gregarium* or *-ia*, is, indeed, the senior name of the species, if it is granted that "Clytia X" is excluded from any species previously recognized. This designation has the inherent advantage of being immediately associated with the planktonic phase of the hydroid. *C. cylindrica, kinkaidi* and *longitheca* may come to be considered synonyms of this species once the full range of variability is demonstrated.

Identification of the animal. Once an animal has been observed continuously throughout its life cycle, there is every reason to bestow a single scientific name on it. Yet, in the case of hydrozoa with two life forms, this has become a difficult matter because the traditional pattern of the nomenclature appears to present almost insuperable obstacles which can be resolved only through an inordinate amount of scholarly work. For instance, *Clytia johnstoni* and *Phialidium hemisphericum* unquestionably refer to the two phases of the same animal, but authorities, such as Russell (1953), have not proposed one proper name for the animal, and the case of *C. gregaria* and *P. gregarium* may be very similar. On first sight, the genus *Clytia* appears appropriate for reasons of priority, but there are probably difficulties in establishing the genus *Clytia* (and *Campanularia* for that matter) beyond all doubt, a task which the present author considers beyond his competence. On the other hand, one might suggest that the species discussed in the present paper be named in both its forms *Phialidium gregarium* (A. Agassiz) in L. Agassiz, 1862, p. 353. This circumvents the possible difficulty of having to revise the nomenclature with respect to the genera *Clytia* and *Campanularia* which have been thoroughly entrenched for more than a century.

Discussion

It was demonstrated in the first part of this paper that the "Plankton-Kreisel" (Greve, 1968) facilitates the raising of a great number of hydroid cultures from single eggs. There is no claim that the Kreisel is the only or even the best method to do this. Rees and Russell (1937), for instance, have raised hydroids of *Amphinema, Rathkea* and *Mitocomella* in plunger jars and did not even find the renewal of water very necessary, although it proved useful for reviving unhealthy colonies. The hydroids of *Phialidium* grow quite easily in small vessels, particularly in mass cultures, if there is a continuous flow of water. Clonal cultures, however, appeared to be more visible, accessible and experimentally controllable in the Kreisels. For optimal culture conditions of small

medusae the Kreisel may well be uniquely suited, but the recent investigation offered only very limited experience for medusal culture.

The availability of many individual hydroid cultures created opportunities for observation and experiment which have only been explored tentatively. Only those of most immediate interest in connection with problems of the general biology of *Phialidium* will be discussed here. There has been much speculation as to what causes the waxing and waning of swarms of jellyfish. The medusae of *P. gregarium* appear each year around the middle of April at Friday Harbor and disappear almost completely by the middle of September. Medusae of the species tentatively identified as *Phialidium hemisphericum* (Wright, 1858) behave in approximately the same way, which is of interest because this species in contrast to *P. gregarium* is circumpolar and its behavior in different localities has been well described. At Plymouth, England, large medusae are found in spring and summer (Russell, 1938, 1953), in Danish waters in winter and in spring (Kramp, 1929). On the other hand, small medusae are seen at Plymouth and in the Atlantic in midwinter and, indeed, throughout the year (Lebour, 1922). The behavior of hydroid cultures in the laboratory suggests a reasonable hypothesis on the cause of the appearance and disappearance of medusal swarms, which can be tested in the future. The hypothesis states that hydroid colonies of the genus *Phialidium* grow and produce medusae in direct response to the food supply. They react with increased formation of hydroids to major increases in the supply of brine shrimp, and a major increase in hydroid formation is followed by liberation of medusae within 2 weeks. It is evident in the laboratory that small amounts of food are not effective as stimulus; only a sustained, heavy supply of suitable food did induce medusae in the numbers necessary for the remarkably dense swarms often seen in the summer. According to the hypothesis, there should be a considerable increase in the density of planktonic organisms on which the hydroids feed 5 to 6 weeks before swarms of mature medusae are found. At Friday Harbor this appears to be the case. Johnson (1932) showed that swarms of copepods, which presumably constitute the bulk of hydroid prey showed a first noticeable rise in March and continued to increase in bursts through May. March and April also saw a great increase in barnacle nauplii. Particularly relevant may be the behavior of the copepod *Calanus finmarchicus* which appeared on limited occasions in March, April and May, very suddenly and for only a few days in tremendous numbers in the 5 years investigated. The average plankton density in surface waters usually rose in March, reached its peak in June or early July and fell precipitously in August. It is obvious that these data which take no cognizance of vertical migrations or of the preference of the hydroids for certain food organisms are not satisfactory for detailed support of the hypothesis, but they fit in a general way. A rise in plankton density in March should bring a great increase in mature medusae by the second half of April. The increase should continue until August, partly because further waves of food supply may repeatedly stimulate old hydroid colonies, and partly because the eggs of medusae spawning from April on must give rise to new hydroids and these to new medusae which will mature first in July (6–8 weeks after fertilization). It appears that the life span of individual medusae does not exceed 3 months (all findings on medusae raised in the laboratory either from hydroids or from young marine specimens support this view) so that medusae

liberated at the probable peak of gonangial production in June, should disappear in September. Small growing medusae with 16–32 tentacles are, however, found until early September, but they occur infrequently and at best in small swarms of low density which must have a very low fertility.

It is obviously not a new and original finding that hydroid colonies are dependent on food supply for their growth. In fact, the results in the present paper appear to confirm, in general and in many details, the more extensive investigations of Crowell (1957) who explored the responses of growth zones in the hydroids of *Campanularia* to various nutritional levels, and who also used brine shrimp for food. He found that of all growth zones the tip of the main stolon is least affected by nutrition. Hydranth growth reacted strongly and within a very few days to restriction or increase in food. Gonangia, however, were produced only by the two best fed of 8 graded experimental groups.

From laboratory findings one would expect small numbers of medusae to be liberated even in times of relative scarcity of food because of short-term local abundance of one or the other food organism. There has been no thorough search around Friday Harbor for single medusae during the months from September to March, but occasional single specimens have indeed been found. It is important, however, to realize that in order to produce swarms of fertile density the rate of hydroid and medusal production must be extremely high, and that there is probably a critical limit below which such accumulations cannot occur. Swarms must originate in localities where production occurs with great temporal and spatial density. In laboratory cultures a few of the factors which further this can be observed. (1) The stimulus of food, provided it is of sufficient magnitude, appears to act on gonangial formation rather precisely and cause a great number of gonangia to arise within a relatively short time. (2) The liberation of medusae occurs most frequently between the hours of dusk and dawn, probably most usually in the early morning, which would tend to concentrate the release of medusae. (3) Small medusae tend to stay on the bottom and to be kept there in between the hydroids and gonangia, sharing the food supply with the hydranths. Water movement will flush large numbers of medusae out of the colonies within very short intervals of time. In addition, the medusae show a tendency to swim away at the end of the first week.

Finally, it is now possible to make an estimate within an order or two of magnitude of the productivity of a small hydromedusa such as *P. gregarium*. A female may produce an average of 50 eggs per day for 60 days (Roosen-Runge, 1962) or 3000 eggs in its lifetime. Each egg may produce minimally 300 medusae in a season. The maximal number is as yet unknown and may be many times as large. Potential productivity of each fertile couple is, therefore, at least 1 million medusae or more, which are, in the average, reduced by predators, tides and winds to another couple upholding the race.

This research was supported by Grant GB 8094 from the National Science Foundation. I wish to thank Dr. Paul L. Illg for his invaluable, critical assistance with the taxonomic part of this paper. Thanks are also due to Mr. J. Rankin for his skillful construction of the Kreisels and much other equipment, to Miss U. L.

Roosen-Runge for technical assistance and drawings, and to Mrs. D. Ringer and Mrs. J. Tada for secretarial help.

Summary

1. A method was described by which hydroid colonies, each on a microscopic slide, were raised from individual planulae of the leptomedusa known as *Phialidium gregarium*.

2. Growth and behavior of approximately 30 cultures were observed and quantitated through nearly 2 months. Cultures produced gonangia and medusae 3–7 weeks after fertilization of the egg.

3. Gonosome development was always preceded by a burst of hydroid development 5–9 days previously. This burst appeared to be initiated entirely by an abundant food supply (brine shrimp).

4. Medusae were liberated 4–5 days after gonangia first appeared. From the growth rate of medusae under the less than optimal laboratory conditions, it was estimated that sexual maturity may be reached in approximately 3 weeks. The lifespan of the medusae probably does not exceed 3 months.

5. A small number of cultures wintered in an open cage in the sea. At the end of November they were found in a greatly reduced state and possessed only a very few, very small hydranths. At the end of March they were in the early phases of vigorous trophosomal growth and the gonosome had just begun to flourish.

6. From the behavior of clonal colonies in the laboratory, the hypothesis was derived that the swarms of mature medusae in nature are the direct result of a "bloom" in zooplankton which occurred 5–7 weeks previously and stimulated first hydroid development and in consequence the formation of gonangia.

7. Morphological characteristics of the hydroid colonies were described and their variability under laboratory conditions recorded and discussed. It was shown that many taxonomic features such as size, branching, number of tentacles and annulations depend quantitatively on the age of the individual colony, its state of nutrition and on genetic factors.

8. The hydroid belongs to the genus *Clytia* (Lamouroux, 1816). A careful comparison was made with species of the genus as described for the Puget Sound region and California. It was concluded that none of these delineates unambiguously the morphological features of this hydroid. A tentative name for the hydroid was assigned according to priority principles as *Clytia gregaria*. It was suggested that *Phialidium gregarium* may be the most appropriate name for the species in both its phases.

LITERATURE CITED

Agassiz, L., 1862. The genera of the Campanularians. *Contrib. Nat. Hist. U. S.*, **4** (Chapter VII) : 353.

Alder, J., 1856. A notice of some new genera and species of British hydroid zoophytes. *Ann. Magaz. Natur. Hist., Series 2*, **18**: 353.

Berrill, N. J., 1950. Growth and form in calyptoblastic hydroids. II. Polymorphism within the Campanulariidae. *J. Morphol.*, **87** : 1–26.

Berrill, N. J., 1961. *Growth, Development and Pattern*. Freeman and Company, San Francisco and London, 555 pp.

Bonner, J. T., 1955. A note concerning the distribution of polysaccharides in the early development of Phialidium gregarium. *Biol. Bull.*, **108**: 18–20.

Calkins, G. N., 1899. Some hydroids from Puget Sound. *Proc. Boston Soc. Natur. Hist.*, **28**: 333–367.

Cloney, R., and J. Schaadt, 1970. Thermoelectric cooling stage for the compound microscope. *Acta Zool.*, in press.

Crowell, S., 1953. The regression-replacement cycle of hydranths of *Obelia* and *Campanularia*. *Physiol. Zool.*, **26**: 319–327.

Crowell, S., 1957. Differential responses of growth zones to nutritive level, age and temperature in the colonial hydroid *Campanularia*. *J. Exp. Zool.*, **134**: 63–90.

Forbes, E., 1848. A monograph of the British naked-eye medusae. Ray Society, London, 104 pp.

Fraser, C. M., 1937. *Hydroids of the Pacific Coast of Canada and the United States.* University of Toronto Press, Toronto, Canada, 207 pp.

Fraser, C. M., 1914. Some hydroids from the Vancouver Island region. *Trans. Roy. Soc., Can.*, **8**: 99–216.

Greve, W., 1968. The "Plankton-Kreisel," a device for culturing zooplankton. *Marine Biology*, **1**: 201–203.

Hincks, T., 1868. *A History of the British Hydroid Zoophytes, Volume 1.* John van Voorst, London.

Johnson, M. W., 1932. Seasonal distribution of plankton at Friday Harbor, Washington. *Univ. of Wash. Publ. Oceanography*, **1**: 1–38.

Kramp, P. L., 1929. The hydromedusae of the Danish waters. *Mem. Acad. Sci. Lettr. Denmark, Series 8*, **12**: 1–291.

Kramp, P. L., 1962. Notes on some Eastern Pacific species of *Phialidium* (Leptomedusae). *Pacif. Sci.*, **16**: 25–29.

Lamouroux, J. V. F., 1816. *Histoire des Polypiers Coralligénes Flexibles, vulgairement nommés zoophytes.* F. Poisson, Caen, France, 599 pp.

Lebour, M. V., 1922. The food of plankton organisms. *J. Mar. Biol. Assoc.*, **12**: 644–677.

Nutting, C. C., 1901. The hydroids of the Woods Hole region. *U. S. Commission Fish and Fisheries Doc.*, **455**: 325–386. [Washington, Government Printing Office]

Nutting, C. C., 1915. American hydroids, Part III. The Campanularidae and the Bonneviellidae. *Spec. Bull. 4, Smithsonian Institution, Washington.*

Rees, W. J., and F. S. Russell, 1937. On rearing the hydroids of certain medusae, with an account of the methods used. *J. Mar. Assoc., U. K.*, **22**: 61–82.

Roosen-Runge, E. C., 1962. On the biology of sexual reproduction of Hydromedusae, genus *Phialidium* Leuckhart. *Pacif. Sci.*, **16**: 15–24.

Russell, F. S., 1938. The Plymouth off-shore fauna. *J. Mar. Biol. Assoc. U. K.*, **22**: 411–439.

Russell, F. S., 1953. *The Medusae of the British Isles.* Cambridge Univ. Press, 529 pp.

Torrey, H. B., 1904. The hydroids of the San Diego region. *Univ. Calif. Publ. Zool.*, **2**: 1–43.

Wright, T. S., 1858. Description of new protozoa and observations on British zoophytes: 1. Coryne gravata, 2. Stauridia producta, 3. Campanularia johnstoni. *Edinburgh. New Philosophical Journal (New Series)*, **7**: 276–287.