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Growth and Behavior of *Trichoplax adhaerens*: First Record of the Phylum Placozoa in Hawaii[1]

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ABSTRACT: I report here the first record of *Trichoplax adhaerens* F. E. Schulze (phylum Placozoa) in Hawaii. Individuals were found on glass slides placed in the seawater system of the Pacific Biomedical Research Center on Kewalo Basin, Oahu, during November and December 1986. Records of growth rates of Hawaiian placozoans, in the laboratory at about 23°C, showed a doubling time ranging from 1 to 3 days, both in numbers (by fission) and in biomass (estimated by area). In a test for substrate preference, the animals favored conditioned glass slides (left in seawater for several weeks) over clean glass slides. A strong reaction to ultraviolet was observed.

PLACOZOANS, though discovered and described a century ago (Schulze 1883, 1891), were established as a phylum relatively recently (Grell 1971). Their recorded distribution is scant and patchy, probably because most biologists are not familiar with them, and it is entirely tropical or subtropical, with two exceptions. *Trichoplax adhaerens* was found in late summer at two temperate sites—the Marine Biological Laboratory, Woods Hole, Massachusetts, in 1973 and The Laboratory, Plymouth, England, in 1974—both during late summer (R. L. Miller, personal communication). Placozoans could possibly reach these sites in the Gulf Stream; however, nonlocal organisms, including many from subtropical habitats within the known range of placozoans, are regularly introduced into these laboratories. Other records include the Mediterranean (Adriatic), the Red Sea (Gulf of Eilat), the northwestern Atlantic (Bermuda and the southeastern coast of North America: Grell 1980), and the Pacific (Western Samoa: K. J. Marschall, personal communication, 1970; Izu Peninsula, Honshu Island, Japan: Sudzuki 1977). Several more reports can be added to this list, one for the Caribbean (Quintana Roo, Mexico: K. G. Grell, personal communication, July 1988) and several for the Pacific (Heron Island, Great Barrier Reef, Australia: A. T. Newberry, personal communication, July 1987; Townsville, Australia: K. G. Grell, personal communication, July 1988; Guam, Palau, and Madang, Papua New Guinea: V. B. Pearse, unpublished observations, July–August 1988). This scattering of sites suggests that placozoans probably occur in warm coastal waters around the world (see also Grell 1981).

I report here their presence in Hawaii and describe some observations on their behavior and growth rates in mixed laboratory culture.

MATERIALS AND METHODS

Collection and Maintenance

To collect placozoans, clean glass microscope slides were placed in shaded 150-liter tanks with running seawater (25°C) drawn from the ocean fronting the laboratory at Kewalo Basin, Honolulu, Oahu. At intervals of several days, slides were transferred into slide racks in 1-liter plastic boxes filled with unfiltered seawater and maintained in the laboratory at room temperature (23 ± 1°C) with aeration and added food [two drops of commercial liquid yeast suspension ("Marine Invertebrate Diet," Hawaiian Marine Imports, Inc.) per liter of seawater]. Every few days, the seawater was changed, food was

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added, and the slides were examined under a dissecting microscope. Slides were exposed during November and December 1986.

Growth Measurements

To measure growth, each slide on which I found a placozoan was isolated in a petri dish filled with seawater and kept at room temperature. The slide was supported clear of the bottom on bits of plastic clay, and the number of placozoans on both sides of the slide, as well as on the bottom of the dish, was recorded every few days. Tracings were made of all individuals in small cultures (< 50) by means of a camera lucida calibrated with a stage micrometer. The area of each tracing was then estimated using a digitizing pad (VIAS Image Analysis System, Ted Pella Inc., Redding, California, run on an IBM-PC).

RESULTS

Occurrence of Placozoans

Placozoans were collected from seawater on glass slides only after the slides had been in seawater for 2–3 weeks, long enough to acquire a light coating of bacterial and algal growth and scattered other small organisms of various kinds (e.g., foraminiferans, tintinnids, vorticellids, sponges, hydroids, turbellarians, rotifers, gastrotrichs, nematodes, archiannelids, harpacticoid copepods, kamptozoans, ascidians). More placozoans were found on slides that had been placed in close proximity to an assemblage of sessile animals (ascidians, sponges, etc.) and seaweeds than on slides that were in relatively clean tanks, but some placozoans were found in both kinds of situations.

When first seen, the placozoans were of sizes varying from about 200 μm to 1000 μm. I saw only crawling, dividing stages and never observed budding or sexual forms (Grell 1984).

Behavioral Observations

Two sets of six glass microscope slides—new, acid-cleaned slides and slides that been conditioned for several weeks in seawater—were placed in a large culture of placozoans and examined after about 24 hr. The number of placozoans on the two sets differed by an order of magnitude. Clean slides bore a mean of only 5 placozoans per slide (range, 2–10; $n = 31$ on all six slides), whereas conditioned slides bore a mean of 51 placozoans (range, 36–81; $n = 303$).

Encounters between placozoans and other small animals on the slides produced no dramatic interactions. Nematodes were often seen crawling freely around and under placozoans without any noticeable response by either animal. Small archiannelids (*Dinophilus* sp.), turbellarians, and harpacticoid copepods, on encountering a placozoan, displayed no strong reaction, but simply changed direction and crawled off. The only predation that I observed was by ciliates that gathered around wounded or degenerating placozoans. K. J. Marschall (personal communication, 1973) observed a small nemertean apparently preying on placozoans in an aquarium in Western Samoa, but I know of no other report of interspecific interactions involving placozoans.

Feeding by placozoans has been described by Grell (1981), and like Grell I noted cleared areas on the slides where a placozoan had been resting and moved away. Although Wenderoth (1986) reported phagocytosis of yeast cells by placozoans, the amount of yeast added to my cultures did not support growth in placozoans on clean slides and did not add measurably to growth by placozoans on "conditioned" slides. In cultures of both kinds, with and without added food, there appeared to be no difference in the numbers of placozoans recorded after 10 days. Wenderoth (1986) noted that yeast cells were relatively resistant to intracellular digestion in placozoans.

In individuals at rest, one edge of the body is often raised and folded, such that the apposed cells of the lower epithelium form a temporary flagellated groove. Because the beating of the flagella does not appear to be coordinated like that of the cilia in the food-gathering grooves of many larval types (see Strathmann 1987), it seems unlikely that the flagellated grooves of placozoans function in the same way. Possibly this behavior serves to circulate water beneath the placozoan.

I observed no responses to visible light or to gravity. The animals displayed no gradient with respect to distance from a light source. Equal numbers of placozoans were found on the top and bottom surfaces of horizontal slides, as well as on the upper and lower halves of vertical slides. However, I did not make observations around the clock; K. J. Marschall (personal communication, 1970) has cinemagraphic documentation of a daily vertical migration behavior by placozoans on the glass wall of an aquarium.

A strong reaction was noted to ultraviolet radiation. When an ultraviolet source (Olympus BH2-RFL) was switched on, within moments the placozoans detached from the substrate and rolled or twisted vigorously into contorted shapes. The response continued for up to 15–20 sec, until the ultraviolet source was switched off.

Growth Measurements

Data on increase in numbers (Figure 1) indicate that the interval between fissions was quite variable, ranging from 1 to 3 days, but did not change significantly over the time period followed. The most variable period was the first few days; this may be related to the different sizes or stages of the founders of each culture.

Because of the flattened form and relatively uniform thickness, the projected area of a placozoan provides an estimate of its volume or biomass. So determinations of area from tracings in addition to counts together express the two major components of growth. Data on increase in both numbers and area in the cultures suggest an initial phase of rapid growth in area, without fission, followed by a phase in which the rates of doubling in both numbers and area are steady and approximately equal (Figure 2). The mean size of individuals remained fairly constant over most of this latter phase at 0.14 mm^2 (SD = 0.08; $n = 216$), about half that seen during the first couple of fissions. The range of sizes (or variability around the mean) increased with time. Rounded or discoid individuals ranged from about 200 μm to 1000 μm across. In older

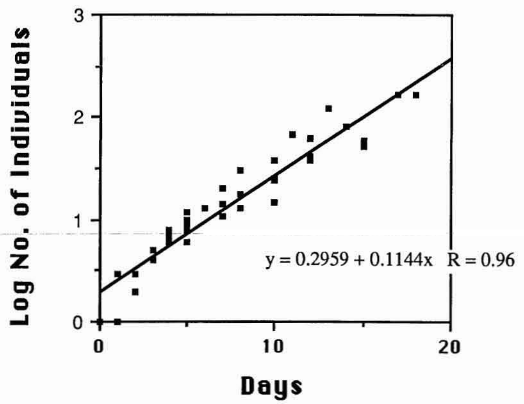

FIGURE 1. Growth in number of placozoans (log values) with time. The data represent 8 cultures, each grown from a distinct isolate with a final combined total of 505 individuals.

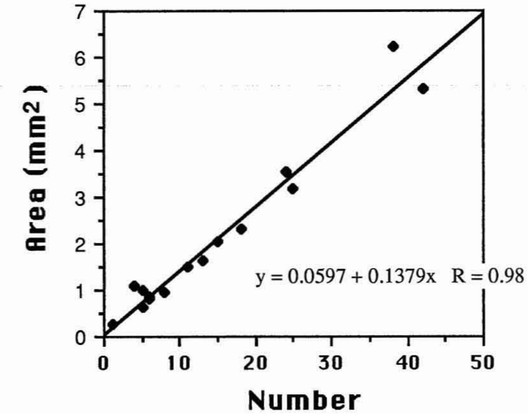

FIGURE 2. A regression of total area (determined from camera lucida tracings) against numbers of placozoans counted in 15 cultures, ranging in age from 1 to 15 days. The total number of separate individuals was 221.

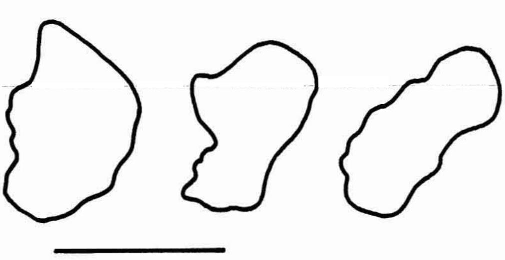

FIGURE 3. Successive tracings of a moving individual, 0.16 mm^2, 0.11 mm^2, and 0.13 mm^2 in measured area, respectively, showing as much as 32% difference. Scale bar = 500 μm.

cultures, elongate forms appeared, measuring up to 5000 μm in length.

The projected area of a single individual can change considerably as it moves along. For example, the area of successive tracings made of one individual (Figure 3) differed by as much as 32%. Another individual, traced while feeding and again when it was moving along (Figure 4), increased in projected area by 41%. However, such individual differences are presumably negligible when areas from a whole culture are being combined and compared with those from another, unless the two populations differ, for example, in number of individuals feeding.

DISCUSSION

As nothing is known about the natural habitat of placozoans beyond their general association with material collected from reef communities, observations of their behavior and development in the laboratory (though these must be interpreted with even more caution than in the case of better-known animals) may provide the beginnings of understanding of these simplest of animals.

The difference in the number of placozoans found on clean versus conditioned slides cannot be accounted for by differential growth rates of the placozoans, given the brief time period, but seems to represent a genuine substrate preference. However, I have no information about the stage that makes this choice. In this experiment, as with the original isolates, the placozoans may either have crawled onto the slides, or settled as budded swarmers (Grell 1984). The preference for conditioned versus clean glass substrate may explain in part why it always took a considerable time before any placozoans were found on slides set in seawater (and why they were found on clean slides only when these were placed in dense cultures).

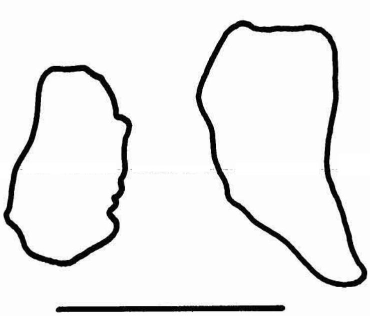

FIGURE 4. Successive tracings of an individual in transition from feeding to moving: 0.09 mm^2 and 0.14 mm^2 in measured area, respectively, a difference of 41%. Scale bar = 500 μm.

There are reports of their feeding on a variety of single food sources [living *Cryptomonas* (Grell 1981) and heat-killed yeast (Wenderoth 1986) and nauplii of *Artemia* (Grell 1983)], but the placozoans also appeared to grow well on the mixture of organisms that settled on my slides. In addition to the extracellular and intracellular digestion of particulate matter described by Grell and by Wenderoth, uptake of dissolved organic material may well play a major role in the nutrition of placozoans.

The strong response of placozoans to ultraviolet radiation suggests that their habitat may be cryptic, at least in shallow depths. Their lack of obvious mechanical protection and apparent immunity to a variety of potential predators suggest the possibility of chemical defenses.

Growth in placozoans comprises both increase in biomass and increase in number. Although the latter has been reported to be by fission and by budding (Grell 1984), I never observed budding. A rate of one fission every 27 to 30 hr, as reported by Ruthmann (1977) from placozoans cultured on flagellates (*Cryptomonas*), is within the range I measured; however, no data on the sizes or rates of biomass increase were given in Ruthmann's report. The mean size of the animals I observed (0.14 mm^2 or about 425 μm diameter) was much smaller than the average of 2 to 3 mm across mentioned by Grell (1984).

The increasing range and variability in size with time seems to be related to the tendency early in the history of a culture for individuals to have a rounded or discoid shape and to undergo binary fission into equal parts, whereas later on there occur many elongate individuals that fragment into multiple small parts. The production of elongate individuals has been observed by others and is discussed by Schwartz (1984).

Because the placozoans studied in Hawaii appeared similar in all respects to those in published descriptions and photomicrographs, I have assumed that they belong to *Trichoplax adhaerens*, the single well-established species in the phylum Placozoa. The delineation of species in this group will have to await more complete knowledge of the biology.

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