CULTIVATION, LIFE HISTORY AND SALINITY TOLERANCE OF THE TIDEPOOL COPEPOD, *TIGRIOPUS CALIFORNICUS* BAKER 1912, IN ARTIFICIAL SEA WATER

HARRY W. HUIZINGA
Department of Biological Sciences, Illinois State University, Normal, Illinois 61761

ABSTRACT.—A simple method of cultivating the marine harpacticoid copepod, *Tigriopus Californicus* Baker 1912, using artifically prepared sea water enriched with Purine laboratory rat chow is described. The copepod feeds upon a mixed diet of unicellular algae, protozoa, bacteria and organic matter. The life history from egg through nauplius, copepodite and reproductive adult takes 18 to 21 days at 23 C. Life history stages are illustrated. The copepod is tolerant to a wide range of salinity (21.2 to 75.3 o/oo) and shows an optimum growth and reproduction in a salinity of 42.3 to 47.00 o/oo. Potential uses of this hardy and easily cultured organism in research and teaching are discussed.

The harpacticoid copepod, *Tigriopus californicus* Baker 1912, may be of interest to the invertebrate zoologist who is looking for a versatile marine organism to use in research and teaching. *T. californicus* lives in splash pools above the high tide zone along the rocky California coastline. It is a hardy organism that is able to withstand the wide fluctuations of temperature and salinity that are found in the unstable tidepool habitat (Monk, 1941).

The copepod is little-known, but potentially a valuable animal for use in various kinds of research. *Tigriopus* spp. have been used in ecological studies (Fraser, 1936), nutritional study (Provasoli, et al., 1959; Gilat, 1967), genetic study (Vacquier, 1962), radioactive tracer uptake (Chipman, 1958; Lear and Oppenheimer, 1962), as a food for marine killifish (Faye, personal communication) and as an experimental intermediate host for nematode parasites (Huizinga, 1966).

Most marine copepods are difficult to cultivate since they have exacting physiological requirements and must be fed a variety of specially cultured algae. However, *T. californicus* is a benthic filter-feeder and its nutritional needs are easily satisfied in laboratory culture with a diet of mixed unicellular organisms.

This paper describes a simple method for raising *T. californicus* in artificially prepared sea water culture. Observations are given on the life history, reproduction and salinity tolerance.

MATERIALS AND METHODS

Cultivation techniques. T. californicus can be obtained from Dr. Rimon Fay, Pacific Bio-Marine Supply Company, Box 536, Venice, California 90291. The animal is easily shipped via air mail in sealed polyethylene bags containing about one liter of sea water with an overlaying air space. Field collected copepods are placed into a standard 8 inch diameter finger bowl and left in the natural sea water for about one week to allow for equilibration to the laboratory conditions. They are fed Purina laboratory rat chow which is finely ground with a blender. A small amount of food (150 mg) is added at 4 to 7 day intervals to a bowl containing 1300 ml of sea water and approximately 500 adult copepods. The culture is covered and maintained at room temperature (20-25 C), without aeration. The initial water level is marked on the side of the bowl with a wax

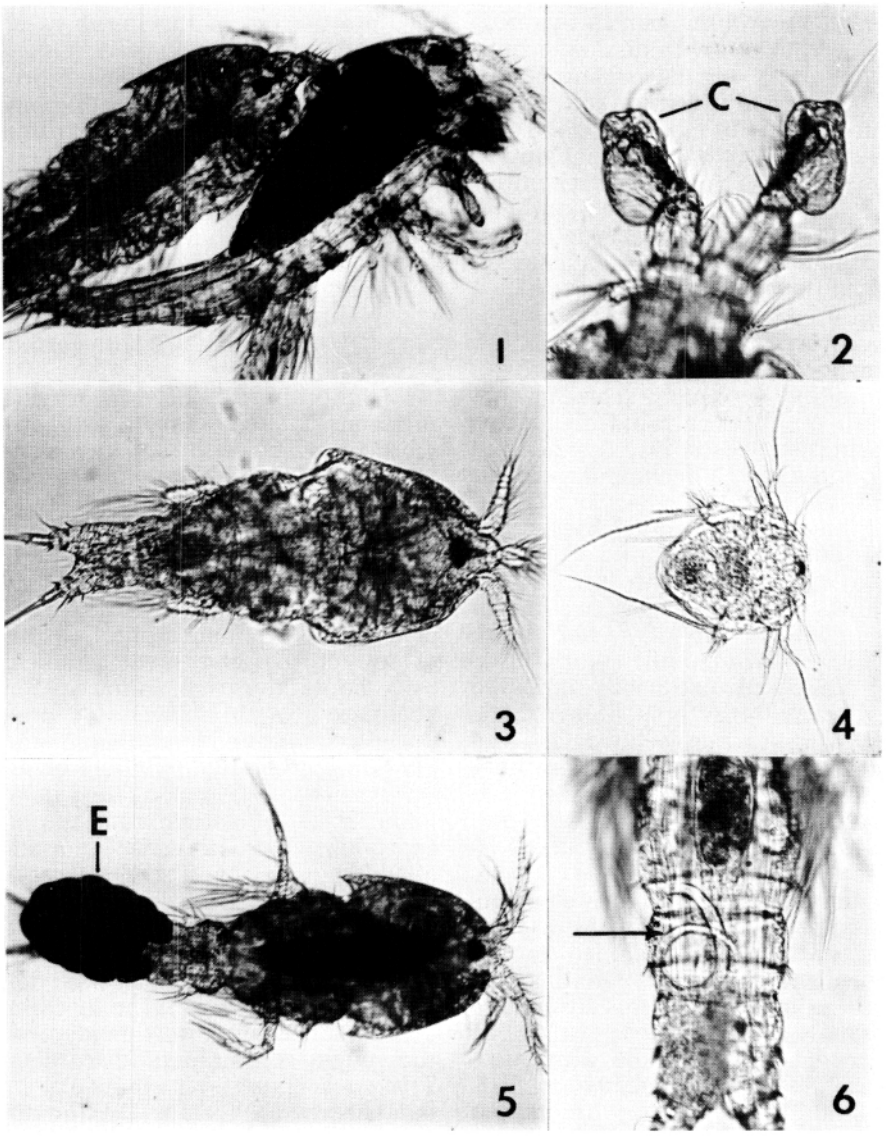

PLATE 1. Life history stages of the harpacticoid copepod, *Tigriopus californicus*.

FIGURE 1. Male (left) and female in copulation, magnification 58 X original.

FIGURE 2. Antennae of male, modified as clasping organs (c), 174 X.

FIGURE 3. Copepodite, 112 X.

FIGURE 4. Nauplius, 194 X.

FIGURE 5. Female with attached egg sac (E), 57 X.

FIGURE 6. Larval stage of the nematode *Contracaecum spiculigerum* (arrow) within the hemocoel of T. *californicus*, 220 X.

pencil, and water lost to evaporation is replaced with distilled water.

After the original culture begins to show multiplication of nauplii and copepodite stages (Figs. 3 and 4), subcultures are made at approximately two to three week intervals, depending upon the room temperature and growth characteristics of the culture. The frequency of subculturing is not critical, since a neglected culture will remain viable for several months with only a few copepods present. The animal can withstand extremely crowded conditions, but will attain a maximum population density in about 24 days, beyond which a decline of numbers will occur.

Subculturing is done by stirring the bottom debris and copepods into suspension with an artist's paintbrush and pouring one-half of the contents into a second bowl. Each bowl is then filled to the 1300 ml mark with artificially prepared sea water (42.6 gms of synthetic sea salts per liter of deionized tap water, salinity 35 parts per thousand, specific gravity 1.025, Dayno Sea Salts, Supreme Products, Lynn, Mass.). The resulting mixture is 50 parts original sea water and 50 parts artificial sea water. Trace elements were not added. Part of the original bottom debris is included in the subculture, since it contains the needed food organisms from the original field-collected sea water shipment (e.g. unicellular algae, bacteria and protozoa). The copepods will not thrive if they are placed into unconditioned artificially prepared sea water. A critical factor in the growth of *Tigriopus* is the quantity of microorganisms present. In my experience, a slightly cloudy medium indicates an optimum growth of microorganisms which are feeding upon the powdered food. If the culture becomes transparent and low in microorganisms, an immediate decline in the numbers of copepods will be observed. Overfeeding is not critical, since *Tigriopus* is tolerant of considerable putrifaction. The organism does equally well with or without aeration.

Adult copepods are harvested from cultures with a sieve that has a mesh of 500 microns. This allows the passage of smaller nauplii and copepodite stages and retains the larger adults which are transferred to other containers.

Attempts to raise *T. californicus* in a constant temperature plant incubator (25 C) with high intensity lighting (100-200 foot candles, 13 hours duration) failed. This light intensity stimulated excessive growth of undesirable red filamentous algae which caused an imbalance of the system and death of copepods. The fluorescent lighting of my office (20-40 foot candles, 8-10 hours duration) allowed for adequate growth of algae and produced good copepod cultures. A single-celled green alga (*Chlorococcum* sp.) was the predominant food item found in the intestinal contents of copepods along with lesser amounts of a blue-green alga (*Oscillatoria* sp.) bacteria, protozoa (*Oxyrrhis marina*, *Euplotes* spp.) and non-living particulate organic matter.

When a white fungus was observed to grow upon the bottom debris, subcultures were made and the fungal contaminant discarded. *Tigriopus* was resistant to infection, but the toxic effect of the fungus appeared to limit the growth of organisms serving as food for the copepod. Fungal contamination was minimized by autoclaving the powdered food and storing in a dry container.

The following method was used to study the life history of *T. californicus*. A gravid female (Fig. 5) was transferred from the stock cul-

ture with a capillary pipette into a 4 inch diameter petri dish containing 40 ml of filtered and conditioned sea water. Ten milligrams of powdered food (see above) was added. The dish was placed in a lighted room at a temperature of 23 C. Daily observations were made using a dissection microscope with bright illumination and a black background. A few drops of stock Harris hematoxylin stain, added to the dish at the termination of an experiment, caused copepods to adhere to organic matter on the bottom of the dish. The stained cope-

TABLE I.—Progeny from single egg sacs of isolated female *Tigriopus californicus*.

Isolated Female*	Day Killed	Numbers of Progeny		Adults	
		Nauplii	Copepodites	Male	Female
1	2	9	0	0	0
2	2	13	0	0	0
3	2	15	0	0	0
4	5	2	13	0	0
5	5	5	10	0	0
6	6	0	12	0	0
7	8	0	13	0	0
8	12	0	19	0	0
9	17	0	0	9	3
10	17	0	0	13	8

*Females were removed after one egg sac was laid.

TABLE II.—Salinity tolerance of *Tigriopus californicus* in artificial sea water.

Concentration Sea Salt, Gms per liter	Salinity o/oo	Maximum Survival in Days	Reproduction of Nauplii & Copepodites	Behavior	Overall Success of Culture
126.0	103.5	1	–	A,E**	4***
114.6	94.1	7	–	A,E	4
103.1	84.7	10	–	A,E,M	4
91.6	75.3	40*	+	A,E,M	3
80.9	65.9	40*	++	M,N	3
68.7	56.4	40*	+++	M,N	2
57.3	47.0	40*	+++++	M,N	1
51.6	42.3	40*	+++++	M,N	1
45.8	37.6	40*	++++	M,N	1
42.6	35.0	40*	++++	M,N	1
40.1	32.9	40*	+++	M,N	1
34.4	28.2	40*	+++	M,N	2
28.6	23.5	40*	+++	M,N	2
25.8	21.2	40*	+++	M,N	2
22.9	18.8	16	++	E,M,N	3
20.0	16.5	13	+	A,E,M	4
17.2	14.1	11	+	A,E,M	4
14.3	11.7	11	+	E,M	4
11.4	9.4	5	–	A,E	4
8.8	7.0	4	–	A,E,M	4
5.7	4.7	4	–	A,E	4
2.9	2.3	3	–	A,E	4
0.0	0.0	2	–	A,E	4

*terminated

**A = abnormal movement and feeding, E = epiphytes, M = mating, N = normal movement and feeding

***1. optimum, maximum numbers and survival, 2. average, moderate numbers and survival, 3. below average, limited reproduction and survival, 4. unsuccessful, poor to no reproduction, premature death.

pods continued to move in place for several minutes before death and were easily counted using a dissecting microscope and ruled petri dish.

Copepods were placed into various concentrations of artificial sea water to test their salinity tolerance (Table II). A stock concentration of sea water was prepared by adding 126 gms of synthetic sea salt per liter of deionized tap water and heating to boiling. Successive 10 per cent dilutions of the stock concentration were made with deionized water. Since the calcium sulfate, calcium manganate and calcium carbonate salts tended to precipitate from the supersaturated solution, they were stirred into uniform suspension before dilutions were made. The salinity and number of grams of sea salt per liter for each dilution were calculated based upon the manufacturer's specifications for oceanic sea water (Table II, Dayno Sea Salt, 42.6 gms per liter equals 35 o/oo).

Results

Life History. After the female copepod drops an egg sac, nauplii begin to hatch from the eggs within 24 hours (Fig. 4). These move along the bottom of the dish as they feed upon microorganisms. The first nauplius molts three times giving rise to three additional nauplii stages. The animal increases slightly in size after each molt. After 5 or 6 days, the final nauplius stage develops into the copepodite which resembles the miniature adult (Fig. 3). There is also a sequence of four copepodite stages over a period of 8 to 9 days. The final copepodite then develops into the adult copepod (Fig. 1). Development from the egg to adult takes about 15 to 18 days at 23 C.

Young male and female copepods begin to mate immediately. There is a striking sexual dimorphism and the male has specially modified antennae which are used to clasp the female during copulation (Figs. 1 and 2). The male pursues the female vigorously from behind and grasps her cephalothorax with a quick motion. He then rides the female for several minutes to hours during which the sperm are deposited in the seminal receptacle. The female goes about her usual feeding behavior, apparently unaffected by the male in tow. An inseminated female will begin to produce an egg sac in about 3 to 4 days. She will continue to produce egg sacs, in isolation from the male, by fertilizing the eggs with stored sperm from a single insemination. New egg sacs appear every 2 to 3 days. Isolated females were observed to lay three consecutive egg sacs. The average number of eggs per sac is 18. At room temperature, the entire life cycle from egg to egg takes 18 to 21 days. The numbers of progeny produced by isolated gravid female copepods (Fig. 5) at various time intervals are given in Table I.

Salinity Tolerance. T. californicus survived and reproduced for over 40 days in a wide range of salinity (21.2 to 75.3 o/oo, Table II). Above and below this range, the feeding, reproductive behavior and survival were abnormal. Epiphytic fungi grew upon the exoskeleton of abnormally sluggish copepods.

Copepods remained alive for one day in 84.7 o/oo which was nearly three times the concentration of normal sea water (35 o/oo), but they were sluggish and twitched convulsively. The copepod survived for two days in deionized water.

Moderate growth and reproduction of copepods was observed in the concentrations of 21.2 and 56.4 o/oo. Abundant cultures developed in the range of 32.9 to 47.0 o/oo. Based upon maximum reproduction

of nauplii and copepodites plus vigorous feeding and mating behavior, the optimum sea salt concentration was 42.3 to 47.0 o/oo.

Discussion

The simple method described here has been used in my laboratory for the continuous cultivation of *T. californicus* for over five years. The copepod is hardy and offers considerable potential for use in research and teaching, such as: an assay organism for environmental pollutants (Mileikovsky, 1970), model of population dynamics, study of nutritional requirements, and copepod reproductive behavior.

T. californicus is susceptible to experimental infection with *Contracaecum*, a larval nematode parasite (Huizinga, 1966, and Fig. 6 of this paper). The copepod is also capable of withstanding considerable salinity variation. It may therefore serve as an experimental intermediate host for various larval helminth parasites found in marine or estuarine environments.

The tolerance of the copepod to a wide range of salinity is not surprising in view of its natural habitat which is subject to evaporation and periodic dilution by rainwater. *Tigriopus* is also tolerant to considerable putrifaction and this is probably an adaptation to the tidepool which is normally exposed to fecal enrichment from marine birds.

T. californicus was observed to feed upon a mixed diet of unicellular algae, protozoa, bacteria and organic matter. Provasoli et al. (1959) and Gilat (1967) showed similarly that *T. brevicornis* and *T. californicus* require a diet of mixed algae and bacteria for successful reproduction in laboratory culture.

Since *T. californicus* is easily cultivated, it can be used conveniently in the classroom to demonstrate the life history and reproduction of a marine copepod. The following student exercise is suggested to compare the theoretical and observed reproductive potential. Since a female copepod produces on average of 18 eggs per sac, and assuming a 50-50 sex ratio, the F1 generation will contain about nine females. Upon maturity in 21 days, each F1 female will then produce at least one egg sac giving rise to a total of about 162 F2 progeny. Continuing with a similar theoretical line of mathematics and optimum growth conditions, the F3 generation will contain about 1458 progeny in about 60 days. The actual number of offspring will be several times the above calculated number, since a single female copepod usually lays several consecutive egg sacs. Mortality must also be taken into account. The student can perform total or sample counts of the copepod stages present in the culture over a several week period (as Table I, Results). This study can be used to illustrate the high rate of biological productivity shown by the primary consumer copepod which serves as an important food organism for marine fish.

Summary

1. *Tigriopous californicus* was cultured in a simple system using artificially prepared sea water enriched with powdered Purine laboratory rat chow. The copepod fed upon a mixed diet of unicellular algae, protozoa, bacteria and organic matter.
2. The life history of *T. californicus* from egg through four nauplii, four copepodites and the reproductive adult took 18 to 21 days at 23 C.
3. The copepod survived and reproduced in a wide range of salinities (21.2 to 75.3 o/oo). A salinity range of 42.3 to 47.0 o/oo

was optimum for growth and reproduction.

4. Potential uses of this hardy and adaptable organism in research and teaching are discussed.

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