WIADOMOŚCI PARAZYTOLOGICZNE
T. XXIII, NR 1-2-3: 1977

LABORATORY MAITENANCE OF A MARINE PARASITIC COPEPOD

ROBERT SHIELDS

Department of Biology, The City College, New York, U.S.A

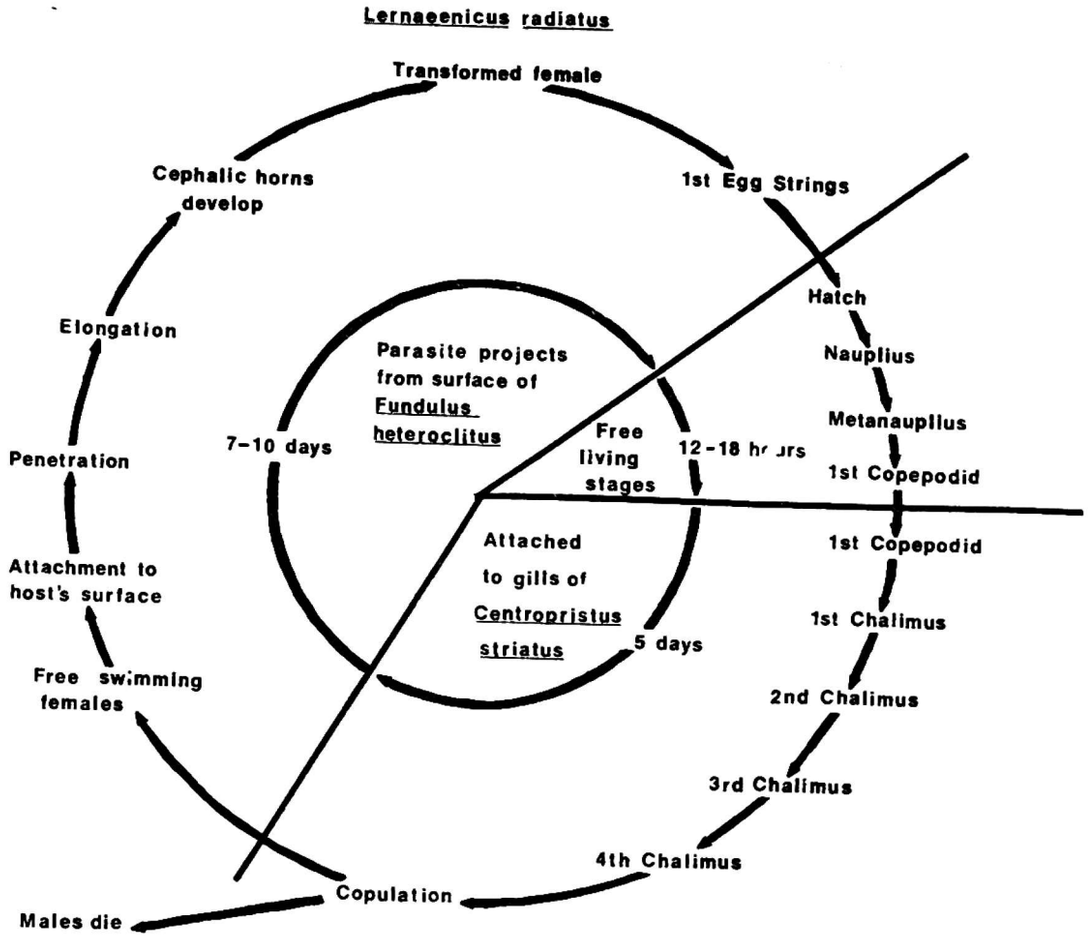
Introduction

The number of experimental studies utilizing any parasite seems to correlate closely to its ease of maintenance in the laboratory. Thus with parasitic copepods, success in rearing the freshwater species *Lernaea cyprinacea* has allowed a series of experimental studies dealing with all aspects of its biology (Tidd and Shields, 1963; Shields and Tidd, 1968, Shields and Sperber, 1974; Shields and Tidd, 1974). These results stimulated the search for a marine species which would lend itself to comparable laboratory manipulation. *Lernaeenicus radiatus* originally obtained from parasitized fishes in the Beaufort, North Carolina area (Pearse, 1947; Kabata, 1965) has proven such an organism (Shields and Stein, 1965; Shields, 1970).

Since the Genus *Lernaeenicus* has a world-wide distribution, it is hoped that publication of the following procedures will aid and encourage other investigators to establish laboratory populations. Not only are parasitic copepods excellent experimental organisms for the study of a diversity of basic parasitological phenomena, but such studies should allow us to more adequately assess the impact of this group on the world's fisheries.

Life history

Summarized in the following diagram (Figure) is the life history of *L. radiatus*. In the laboratory, we are only able to infest a single host species, the sea bass, *Centropristus striatus* with chalimus stages. However, we can experimentally infest *C. striatus, Fundulus heteroclitus* and *F. majalis* with adults. Field observations indicate an even greater number of species potentially may serve as host for adults of this species. Probably



the most critical factor in laboratory maintenance of parasitic copepods is host availability. A greater variety of hosts tends to enhance successful long term maintenance.

For convenience, procedures will be discussed in relationship to four phases of the cycle: egg hatching-lst copepodit; infestation by lst copepodits; chalimus development; penetration-egg formation.

Infestation by lst copepodits

It is rarely feasible to obtain a stock of laboratory reared fish, which would insure no prior exposure to the parasitic species to be maintained. Therefore, the best procedure is to isolate potential hosts and observe for a sufficient period to determine them to be free of the copepods. Since hosts previously infested with adults may have developed immunity, such hosts are never reinfested. Fortunately, since this is a point of rigid specificity, it is possible to constantly reinfest the sea bass with lst copepodits. Isolated *S. striatus,* 20-25 cm in length are infested by exposing a host to all of the lst copepodits (12-14 hrs post hatch) resulting from a single hatch. Since lst copepodits attach to the gills and subsequent chalimus development occurs on this tissue site the host is allowed to swim freely during the exposure period.

Chalimus development: isolation of hosts, temperature and water quality control

Sea bass following exposure to lst copepodits are isolated in aerated 8 liter rectangular tanks. Temperature control now becomes the most critical factor. Temperature must be maintained in the parasite's upper range. At 27-30°C chalimus development is completed and free swimming females appear in the water 4-5 days post infestation.

Since aeration and evaporation may lead to salinity changes, daily hydrometer readings should be taken and necessary adjustments made. Feeding should be done a few hours prior to these operations and unconsumed bits of food, which might serve as sites of contamination, should be siphoned from the bottom of the tank before readings are made. On alternate days half the water in isolation tanks should be replaced.

Egg hatching-lst copepodit: time, temperature and water quality

Obviously successful laboratory maintenance of a parasitic species requires that reasonable large populations of the organism be available at all times. Fortunately, within hours of egg string removal new eggs appear on *L. radiatus*. It is therefore possible to remove eggs simultaneously from all parasites on hosts and essentially synchronize development of new eggs. The initial set of egg strings is small and best allowed to hatch in situ. Time of removal of the second set then establishes subsequent collection times. The longer eggs are left to mature on the parasite the higher the percentage of successful hatch. Therefore, eggs are removed every 52 hrs. Since temperature affects egg maturation, visual cues prove a convenient means of confirming development. In the final hours prior to hatching eggs become transparent and pigments of the naupliar chromatophores become distinct.

Gently bubbling air through sea water containing the eggs increases hatchability and produces a tighter synchrony of hatch. When aerated large segments of the string hatch almost simultaneously and the entire string hatches in 2-4 hrs. Without aeration the strings tend to hatch sequentially. This release then may extend over an entire day with progressively fewer individuals successfully emerging.

Culture dishes (9 cm in dia.) maintained in a constant temperature cabinet (29°C) are used for hatching eggs and holding larvae during development to the infestive lst copepodit stage. Dishes are covered to prevent evaporation and Millipore filtered sea water used to reduce fungal contamination. Due to the short duration of the development (12 hrs) to the infestive lst copepodit stage, salinity does not pose a problem during this phase.

Penetration through egg formation

The killifish, *F. heteroclitus* is an excellent host for the completion of the life history of *L. radiatus*. A sea bass, approximately 80 hrs post infestation by lst copepodits, is placed in a tank (at least 25 gal) containing 25 killifish. It is best to screen partition the tank to prevent contact between *C. striatus* and *F. heteroclitus*. However the screen should have a sufficiently large mesh to allow free exchange of water and female copepods as they detach from *C. striatus*. Beginning with the 96th hr post infestation of *C. striatus*, exposed *F. heteroclitus* are examined for the presence of penetrating females. When penetration is observed the host should be isolated in a well aerated 2-4 liter aquarium. Killifish 10 cm or larger with less than 5 parasites subsequently tend to have lower host mortality rates. Should a host have a heavier burden parasites may be eliminated by cutting the protruding abdomen. Additional killifish should be added to the community tank, until new infestations cease. Development of adult parasites on sea bass is rare, if sufficient specimens of killifish are available as potential hosts during this period.

Under the previously stated temperature regime transformed females begin producing eggs 7-10 days post penetration. Laboratory specimens of *L. radiatus* have produced four successive sets of eggs. During this period hosts should always remain isolated. Cleaning activity has been observed in community tanks. Again water quality controls and temperature regulation are critical. In particular temperature must be maintained in the upper portion of the range. If the mean temperature is too low, egg production ceases or eggs produced fail to mature and degenerate, without hatching.

Although these procedures specifically apply to *L. radiatus* past experience with other parasitic copepods suggests they are basic guidelines applicable to many marine species.

Author's address:
139th Street at Convent Avenue, New York, N. Y. 10031
U.S.A.

LITERATURE

1. Kabata, Z.: *Proc. Zool. Soc. London*, 144, 351-360, 1965.
2. Pearse, A. S.: Parasitic copepods from Beaufort, North Carolina. — J. Elisha Mitchell sci. Soc., 63, 1-16, 1947.
3. Shields, R. J.: Rate of development of the marine parasitic copepod, *Lernaeenicus radiatus*. — Proc. Second Internat. Cong. Parasit., 56 (Sec. II). 583, 1970.

4. Shields, R. J., Stein, P. C.: *J. Parasit.*, 51, 51, 1965.
5. Shields, R. J., Tidd, W. M.: *Crustaceana*, (Suppl. 1) Studies on copepoda: 87-95, 1968.
6. Shields, R. J., Tidd, W. M.: *Crustaceana*, 27: 225-230, 1974.
7. Shields, R. J., Sperber, R. G.: *Crustaceana*, 26: 157-171, 1974.
8. Tidd, W. M., Shields, R. J.: *J. Parasit.*, 49, 693-696, 1963.

HODOWLA LABORATORYJNA MORSKICH WIDŁONOGÓW PASOŻYTNICZYCH

R. SHIELDS

Badania doświadczalne nad pasożytniczymi widłonogami ryb należą do niezwykle rzadkich, a to z braku populacji hodowlanych laboratoryjnie. Stosując podstawowe zasady hodowli słodkowodnego gatunku *Lernaea cyprinacea*, zdołano wyhodować także gatunek morski — *Lernaeenicus radiatus*. W warunkach laboratoryjnych używa się rutynowo 2 gatunków żywicieli: *Centropristus striatus* dla stadiow kopepodit i chalimus, a *Fundulus heteroclitus* dla dorosłych samic. Zagadnienia związane z metodami hodowli laboratoryjnej najwygodniej rozpatrywać w odniesieniu do następujących 4 faz rozwojowych: kopepodit I wykluwający się z jaja, infestacja przez kopepodit I, rozwój chalimus i produkcja jaj.

Ważne dla prowadzenia hodowli widłonogów pasożytujących u ryb są czynniki fizyczne i biologiczne. Cały ciąg form rozwojowych powinno się hodować w temperaturze bliskiej maksymalnej dla danego pasożyta. Jest to szczególnie doniosłe w przypadku samic, gdyż nieco niższe temperatury mogą spowodować zaprzestanie produkcji jaj lub nieprawidłowy rozwój i brak wylęgu larw. Zważywszy, że czas życia stadium wolnożyjącego jest krótki, dbanie o jakość wody w okresie życia pasożytniczego jest ogromnie ważne. Należy codziennie sprawdzać zasolenie i usuwać zanieczyszczenia. Równie ważne jest, aby zapewnić rybie maksimum ruchu, zwłaszcza gdy pasożyty noszą worki z jajami. Wreszcie, aby zredukować śmiertelność żywicieli, należy utrzymywać infestację przez dorosłe samice na możliwie niskim poziomie, a zarażone żywiciele codziennie karmić. Ponieważ rodzaj *Lernaeenicus* jest szeroko rozprzestrzeniony i przystosowany do różnych warunków lokalnych, przedstawiona tu metoda hodowli *L. radiatus* nadaje się prawdopodobnie i do innych gatunków.