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BREEDING OF MARINE TIMBER-BORER, *TEREDO NAVALIS* L., IN TANKS AND ITS USE FOR ANTI-BORING TEST. *

By

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Introduction

Because of the seriousness of destructive damages often caused by timber-borers on marine wooden structures as well as on wooden boats, a vast amount of informations has been accumulated, during a period of more than a century, on the biology of boring organisms and the possible methods to prevent the damage. Among the borers, Lamellibranch Teredinidae do most harm. Most of the biological observations and experiments on ship-worms, however, have been focussed on the anatomy, physiology and ecology of the worms burrowed in timber. As is well known, the shipworms have a free-swimming larval life, the duration of which depends on the stage of larval life when the broods are released from the mother shell. In certain groups, the eggs are spawned and fertilized in sea water, while in others the eggs are retained and fertilized in the gill filament of the parent and the larvae are released either in the early veliger stage or in the late veliger stage, in which case they metamorphose before long and find a suitable site for burrowing.

So far as we are aware, very little research has been reported concerning the larval life of the ship-worms, though the knowledge of life history, physiology and ecology of their larvae seems to be of fundamental importance in the search for the means to prevent the attack. Sigerfoos, in his classical reports (1886 and 1908), described observations on the early development of metamorphosing larvae, mostly in *Xylotrya gouldi.* Later Miyazaki (1935—1936) reported the development of *Teredo japonica* Clessin. Lebour (1938) also gave a short description of the development in three species of Teredinidae found near Plymouth coast. Recently Sullivan (1948) described the larvel shell forms of *T. navalis* collected in Malpeque Bay, Prince Edward Island, Canada. These reports are either on the larval life

* Contribution from Onagawa Fisheries Laboratory

within mother shell or on material collected from the sea. None of them gave a full and detailed description of the swimming larval life, based on the continuous observation of growing larvae. Under such circumstances, it is naturally desired to find the means for culturing the larvae under laboratory conditions so as to obtain knowledge of the life history and the behavior of the larval forms. Such knowledge will be of service to the development of anti-boring methods.

We succeeded in rearing the oyster larvae on non-colored naked flagellate in small vessels as well as in large outdoor tanks (Imai et al, 1949 and 1950). Wide applicability of the flagellate as food of larvae of Lamellibranchs suggested the use of the same culture method for the ship-worms. Having brought up a dense culture of their larvae in tanks, we could follow the larval development up to the metamorphosis. Furthermore, with a dense population of full grown larvae, it was possible to carry on an anti-boring test right in the tank. Previously this had been possible only in the sea. The results of breeding experiments and a method for testing in tanks are presented herewith.

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Artificial Breeding of *Teredo navalis* in Tank

The material used for experiment was *Teredo navalis* L., the commonest and most destructive teredinid in the subtropical region. This species and *T. yatsui* Moll are abundant in Onagawa Bay and its vicinities. *T. navalis* begins spawning in early summer when the temperature of the sea water reaches 18°C, and the spawning lasts until late fall. The eggs are retained and fertilized in gill chamber of the mother. Early veligers, approximately $85 \times 75\,\mu$, are released from the mother shell and enter a free swimming life. Artificial culture was started with such young veligers collected from the gill chamber by dissection. It was not difficult to get 20 to 50 thousand veliger larvae from an adult specimen with a length of 3~5 cm.

The principle of the rearing method was the same as that already described in the breeding experiment of oyster larvae (Imai et al. 1950). Outdoor tanks in Onagawa Fisheries Laboratory were used for that purpose. The tops of the tanks were covered with two layers of reed screen so that the intensity of light might be reduced to inhibit the excessive growth of diatoms and other colored algae. Sea water was pumped from the beach

and filtered through layers of gravel, sand and charcoal. No dilution with fresh water was necessary. Chlorinity was around 18‰ and pH around 8.2.

As a food organism, culture No. 34 of *Monas* sp., a non-colored naked flagellate, was used. Tank water was enriched with a small amount of soluble starch and then a few litres of dense *Monas* culture were added. A continuous growth of *Monas* resulted from the organic decomposition and the increase of bacterial population. One milligram of starch for each litre of water was usually sufficient to keep the *Monas* population at a density of a few thousands per cubic centimeter, which was considered the optimum density for larval growth. Additional enrichment was given whenever the population density dropped below about 500 per cc.

The density of larvae in the tanks was less than 100 per one litre of sea water. Gentle stirring was given twice a day. Culture conditions such as water temperature, pH, oxygen content and chlorinity were recorded together with *Monas* population and larval growth during the course of culture.

An example will illustrate the results of the cultures. On 26th of June, 1945, six hundred thousands straight-hinge larvae were collected and set free in Tank A with a capacity of 34,000 litres. After 30 days of culture, over four hundred thousands full grown larvae were counted in the tank. Shell growth in this culture experiment is shown in Table 1. The record showed that the water temperature had varied between 18.5 and 23.8°C, oxygen content between 4.2 and 4.8 cc. per litre and pH between 8.20 and 8.35 during the course of culture. 70 grams of starch were used for enrichment during the period. Occasionally population of *Monas* dropped as low as 350 per cc. but it was kept at a level between 1,000 and 3,000 per cc. during most of the period. A growth of a few zooplankton organisms was noticed but the growth of diatoms and other colored algae remained at a low level.

In this experiment, it had taken almost a month before the larvae reached full grown size, that is, over 200 μ in shell length, and began metamorphosis and boring. But it was proven afterwards that we could shorten the duration of larval life to less than 22 days if the temperature of tank water was kept at 23°C or higher.

In tank culture, *Teredo* larvae showed high viability as compared to oyster larvae. The mortality was fairly low even in conceivably unfavorable conditions. In one case of oyster breeding, the *Teredo* larvae were mixed in the tank by accident. In this mixed culture, *Teredo* larvae overcame the oyster, which were utterly eliminated in the tank.

Addition of new straight-hinge larvae at intervals to the tank made it possible to obtain a dense population of metamorphosing larvae continuously

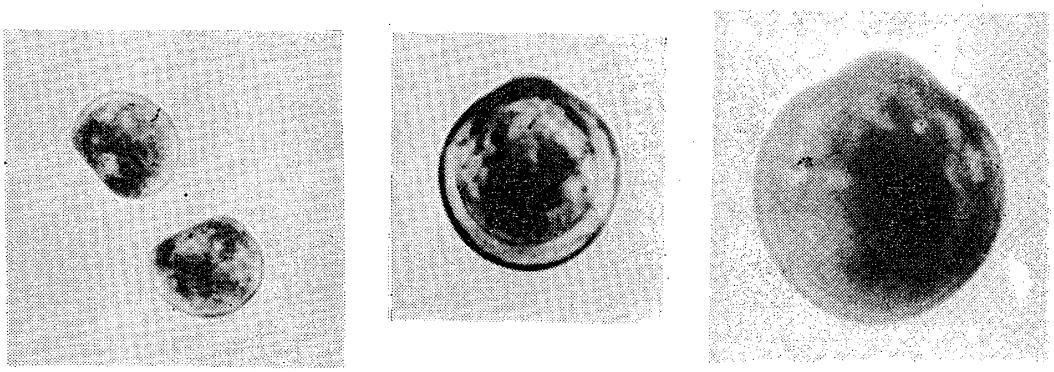
for nearly a month. Such advantage will favor the use of tank culture method in anti-boring test, as will be discussed later.

Development and Growth of *Teredo navalis.*

In *Teredo navalis,* fertilization occurs in the gill chamber of the mother shell as already stated. The resulting larvae are bred there until the straight-hinge stage is reached and then they are released into sea water and begin planktonic life.

Both male and female gonads are milky white in appearance. Straight-hinge veligers found in gill chamber already had some colors; the shell top is purple and the hinge line yellowish brown. The rest of shell is yellow. No color had developed in the liver at this stage. The gill chamber filled with veliger larvae is purple in general appearance. Larvae were endowed with swimming ability at bastula stage. Therefore the veligers, when discharged from gill chamber, can swim readily.

Veliger larvae, when released naturally, had a mean size of $85 \times 72\,\mu$. They were not exactly D-shaped, but somewhat circular in general appearance (Fig. 1, a.). Therefore they can be distinguished easily from other



a b c

Fig. 1. Development of larval shell.
a. $85 \times 72\,\mu$
b. $162 \times 169\,\mu$
c. $200 \times 215\,\mu$

straight-hinge larvae such as those of oysters and clams. When they reached a size of $100 \times 90\,\mu$ faint yellow color appeared in the liver. Intestine was still short and unlooped. The growth in height gradually exceeded the length, and at around $150\,\mu$ the shell was nearly spherical (Fig. 1, b). The shell at this stage showed deep purple color at the top, brown on hinge line and yellow along periphery. The liver was bright yellow. The intestine was elongated and looped. Neither foot nor gill filament was

developed. These organs, as well as otocyst, began to appear at around the 200×215 μ stage (Fig. 1, c). Finally, the larvae began metamorphosis at around 215×235 μ and their shells were modified to get ready to burrow. The foot became prominent and bulky and they crawled on the surface of timber substratum. Before long they started the boring action.

An example of larval shell growth in tank culture is shown in Table 1. Culture conditions have been referred to elsewhere.

Table 1. Growth of veliger larvae of *Teredo navalis* in tank.

Days of Culture	Average Shell Length in μ	Average Shell Height in μ
0	82	71
3	105	95
6	128	121
10	153	156
14	162	169
16	179	189
21	189	205
24	191	213
28	208	231
31	207	245
34	205	244

Morphological change during metamorphosis and action of boring could be followed easily under the microscope. It showed results similar to those of *T. yatsui* which was described in detail (Imai et al, 1944).

Since it was possible to determine the exact date of burrowing in tank culture, we could measure the rate of growth on an exact time basis.

Table 2. Growth and development of *Teredo navalis* after burrowed.

Days after Burrowing	Average Body-length in mm.	Average Shell-length in mm.	Average Number of Ridge in Ant. Lobe.	Development
5	—	—	4	
6	—	0.25	5	Pallet appeared.
10	0.45	—	5	
15	0.67	0.47	—	
20	2.0	0.9	10	
30	7.0	1.4	16.2	
40	10.4	1.76	19.3	Gonad developed.
45	15.6	—	21.8	Reached Maturity.
65	50.5	3.3	25.8	Spawning.

Infected timber was transferred to another tank and was brought under observation at definite intervals. Table 2 shows the result of observation which covered 60 days from August 1st. During this period the water temperature varied between 19.0 and 26.5°C. As is seen in the table, *Teredo navalis* reaches sexual maturity at about the 45th day after beginning to burrow, when the body length reached an average of 15 mm. or over.

Boring Activity of Metamorphosing Larvae under Various Environmental Conditions

With ample metamorphosing larvae at hand, it was possible to carry on experimental observations on the burrowing activity of larvae under different conditions of temperature and salinity. The experiments were carried in Petri-dishes of 500 cc. of capacity in which 10 larvae and one small test piece of Cryptomeria wood was introduced.

As a result of experiments it was shown that no burrowing occurred at temperatures either above 26°C or below 14°C. At temperatures above 30°C or below 8°C, high mortality was observed. Burrowing was most active at the temperature between 17° and 22°C.

As to salinity, it was shown that the larvae burrowed successfully at over 14‰ in chlorine. At 10‰ their burrowing ability was doubtful, though they showed metamorphosis. The larvae lived few days at 7‰ and few hours at 4‰, but eventually they all died.

Method of Boring Test in Tank

Protection of timber structure in the sea has been a great concern of ocean builders and wooden-ship builders since olden time. Several means of protection, chemical or mechanical, have been tried and a few of them have been recommended as effective and used for practical purposes. But their effectiveness seem to be short lived or of spotty success.

The difficulty in the development of anti-boring method may be due to several causes. Among them we can count the inconveniency of the method of anti-boring test. The ordinary test universally used is to place the test panel in the sea and to examine how long the timber remains free from the attack by borers. Such tests imply many difficulties and uncertainty. The kinds of borers, their density and activity, or to put it other way, the degree of exposure of a timber to the risk of infection, vary by time and place. As these panels are usually kept under a long period of observ tion, the influence of fouling organisms should also be taken into consideration. Certain fouling organisms often prevent infection. There are also economic

problems because the facilities for the test usually require much expense. When we count the risk of loosing the test panel in the sea, as frequently happens, it is certainly a great drawback.

Under such conditions it would be desirable if we could carry on the test under laboratory conditions. Our culture method in tanks provide for this type of test. Thus we could use tanks in which a dense population of full grown larvae for the purpose were reared. Small test pieces with a size of $3\times1.5\times0.5$ cm. were used. They were so small that we could set a hundred or more of them for varicus treatments at one time (Fig. 2).

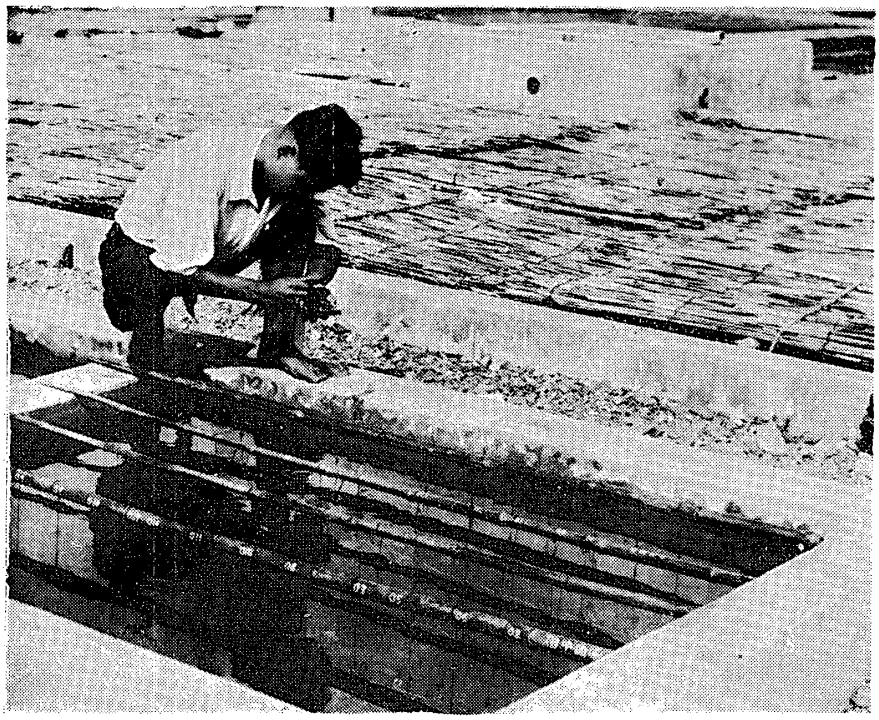


Fig. 2. Anti-boring test in tank.

Those pieces which had no anti-boring evidence at the start showed a distinct infection in a few days.

At present, because of the temperature condition, the test in tank can be carried only during the breeding season of *Teredo*, which extends from July to November in Onagawa Bay. However it seems possible to run the test through the whole year if facilities are provided to raise the temperature condition in tank water during cold weather.

We prepared the test pieces at various seasons of the year and kept them in running sea water so that we could finish up the test experiment in warm season. We also used supplementarily a crustacean borer, *Limnolia lignorum* (Rathke) whenever the test was needed during winter season.

Result of Experimental Test in Tanks

1. *Resistance of woods against infection.*

While it is believed that there is no wood immune to marine borer, it is well known that some woods are more resistant than the other. Hardness is one of the qualities related to the degree of resistance. In order to select a suitable kind of wood for test in tanks we made the resistance test with several woods readily available in our district, including Akamatsu (*Pinus densiflora* Sieb. et Zucc.), Momi (*Abies firma* Sieb. et Zucc.), Sugi (*Cryptomeria japonica* D. Don.), Hinoki (*Chamaecyparis obtusa* Endl), Nara (*Quercus serrata* Thunb.), Kiri (*Paulownia tomentosa* Kanitz), Buna (*Fagus crenata* Blume), Katsura (*Cercidiphyllum japonicum* Sieb. et Zucc.) and Mizuki (*Cornus controversa* Hemsl). They were all infected in a few days after immersion in the tanks. None of them escaped attack for more than 10 days. But certain differences in resistance were observed, and Sugi was the most readily attacked: it was infected heavily in four days. In the Sugi pieces the white part of wood was found more susceptible than the red part, and the cross-cut surface was attacked heavily than the transverse surface as is indicated by average number of burrows per sq cm. (Table 3).

Table 3. Burrows per Square Centimeter Surface.

	White Part	Red Part
Transverse Section	7.0	1.6
Cross Section	20.0	3.0

Fig. 3 illustrates how heavily the infection occurred in the tank. This is the piece of untreated Sugi log used for stoppage of the drain in the tank.

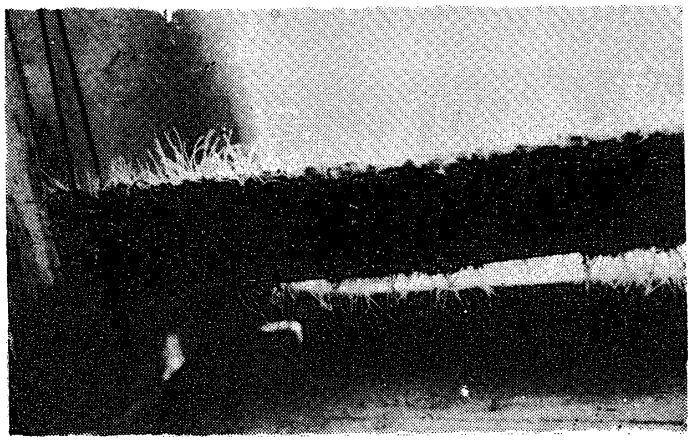


Fig. 3. Infected log of Sugi wood.

The picture was taken on the 50th day after infection. Pairs of siphons indicate the number of burrowed *Teredo*. When it was taken out of the water, the wood surface showed no sign of infection unless carefully examined under a magnifier. Since Sugi was found to be the most readily infested by *Teredo*, it was used for further experimental purposes.

2. *Test of anti-boring effect of various chemicals.*

Test pieces of Sugi $3\times1.5\times0.5$ cm. in size were dried by heat under reduced pressure, where they were treated with various chemical solutions. Then, dried in the air, they were used for the tests. So far the results of tests are by no means conclusive, and therefore only a brief summary will be given as to the anti-boring effect of various chemicals tested.

Among inorganic substances tested, copper compounds showed the highest resistance. Almost all pieces treated with 1 N solution of copper sulphate, chloride, nitrate or acetate were resistant to infection particularly when they were additionally treated with 1 N sodium hydroxide. Most of them were immune for more than 815 days, the whole period of test. Bismuth salts such as chloride and nitrate showed some resistance but their practical use is doubtful. Effectiveness of arsenous acid and oxide lasts only for nearly 50 days.

Among organic compounds tested, anilin showed good resistance especially when the pieces were further treated with potassium bichromate. It was effective over the 751 days of the test. Dinitrochlorobenzene was also effective over 700 days. Basic components of shale oil were found effective over 650 days. DDT also showed high resistance for over 400 days.

Following chemicals were found not resistant to marine borers. They are: among inorganic substances: magnesium nitrate, alminium fluoride, sodium fluoride, ferric chloride, ferric nitrate, ferric acetate, ferrous sulphate and zinc chloride; among organic compounds: monochlorobenzene, *o*-dichlorobenzene, *p*-dichlorobenzene, nitrobenzene, pyridine, benzene sulphonic acid, oil of Eucalyptus, terpentine oil, acidic components of shale oil, chlorinated rubber, cyclorubber, bakelite and waste liquor of sulphite pulp.

Summary

1. Veliger larvae of the timber borer, *Terede navalis* L. were reared successfully in tanks with non-colored naked flagellates as food. As a result, it was possible to follow the life history of larvae up to the stage of metamorphosis and burrowing into wood.

2. Early veligers which were released from the mother shell reached the full grown stage in 3 or 4 weeks depending on the water temperature.

Two months after the burrowing began, the animal reached sexual maturity.

3. The method of tank culture enabled us to perform anti-boring tests under experimental condition. The new method of test in tank will provide an excellent opportunity for the study of anti-boring methods.

4. In preliminary test performed, it was found that copper compounds, anilin-chromic acid, basic components of shale oil and DDT were effective in preventing the infection.

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