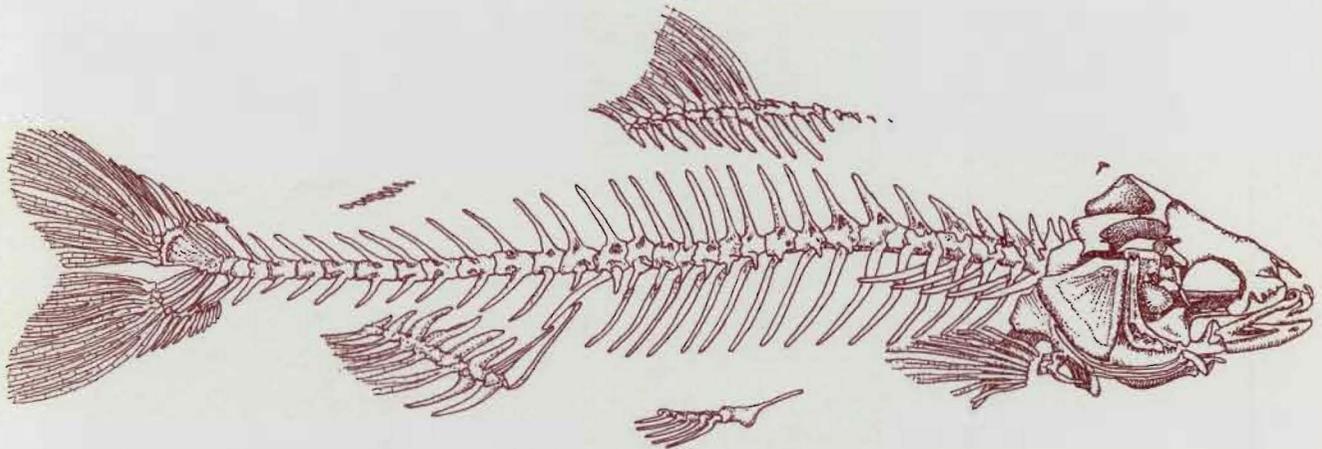
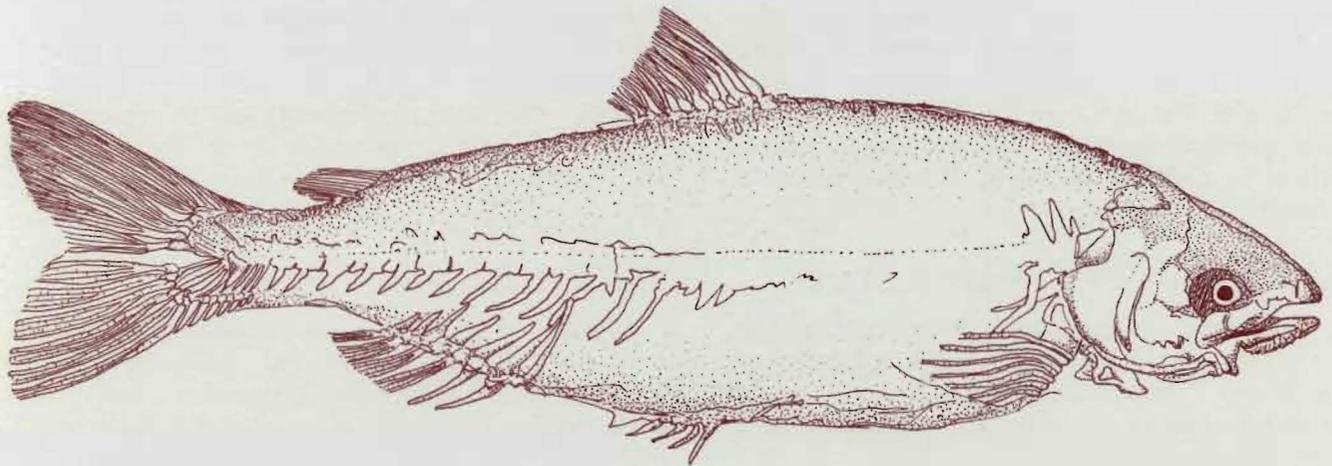
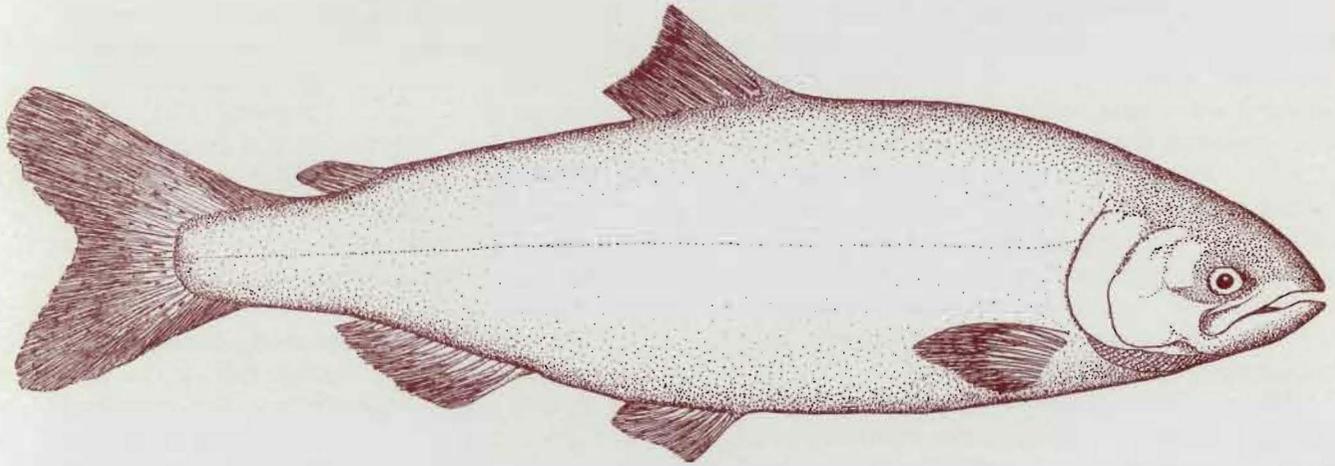


VIBRIOSIS

A COMMON DISEASE OF PACIFIC SALMON
CULTURED IN MARINE WATERS OF WASHINGTON
Extension Bulletin 663



VIBRIOSIS

A COMMON DISEASE OF PACIFIC SALMON CULTURED IN MARINE WATERS OF WASHINGTON

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The bulk of cultured fish production in the United States and other parts of the world is from fresh water. However, there is a growing emphasis on culture of marine fish in Japan and other countries that have sheltered coastal waters (1,2,3). On the west coast of North America, marine production of Pacific salmon, genus *Oncorhynchus*, is increasing (4). Growing salmon is particularly important in the state of Washington, where the commercial production of cultured pan-sized Pacific salmon was 350 metric tons in Puget Sound in 1974.

The majority of marine cultured salmon are grown in floating netpens or net enclosures, and the remainder are grown in diked tidal ponds or pumped seawater raceways. Any of these methods confine the fish in densities that can reach 40 kilograms per cubic meter (2.5 pounds per cubic foot). Close confinement of large numbers of livestock can cause the spread of infectious diseases, and this is true of marine fish culture also. The most common diseases of cultured marine fish are caused by bacterial infections of which the most prevalent is vibriosis.

Description of Causative Agent

The organism responsible for vibriosis in fish is the bacterium *Vibrio anguillarum*, a member of the family Vibrionaceae (5). It is related to the organisms that cause cholera and a shellfish food poisoning in humans.

V. anguillarum is a gram-negative, motile rod, usually slightly curved when observed from fresh isolates. The cells may range in size from 0.5 x 1.4 microns to 1.0 x 2.7 microns (6). The motile organisms (Figure 1) can frequently be seen in wet slide mounts of fresh spleen smears from infected fish (900X magnification). It is a salt-requiring organism but can be found in estuaries with water salinities as low as 5 parts per thousand.

Although vibriosis is generally considered to be a disease of cultured marine fish, it can also occur in wild populations. It has been identified as a disease in wild populations of herrings and, most recently, has caused extensive mortalities of wild fish off the coast of Norway (7).

Vibriosis can occur at any time of the year, reportedly in water temperatures as low as 4°C (39°F). However, it is most prevalent in the temperate zones during the warmer summer months. Outbreaks (epizootics) can be expected when the water temperatures reach 14°-20°C (57°-68°F).

Clinical History, Symptoms, and Presumptive Diagnosis

Symptoms of vibriosis are not usually evident until the fish have been in salt water for two weeks or more and are held in densities common to the net-pen culture of salmonids—8 to 16 kilograms per cubic meter (0.5 to 1 pound per cubic

Figure 1. Typical comma-shaped bacterial cells of *Vibrio anguillarum* and larger red blood cells. Specimen is from a spleen smear of an infected fish (900X magnification).

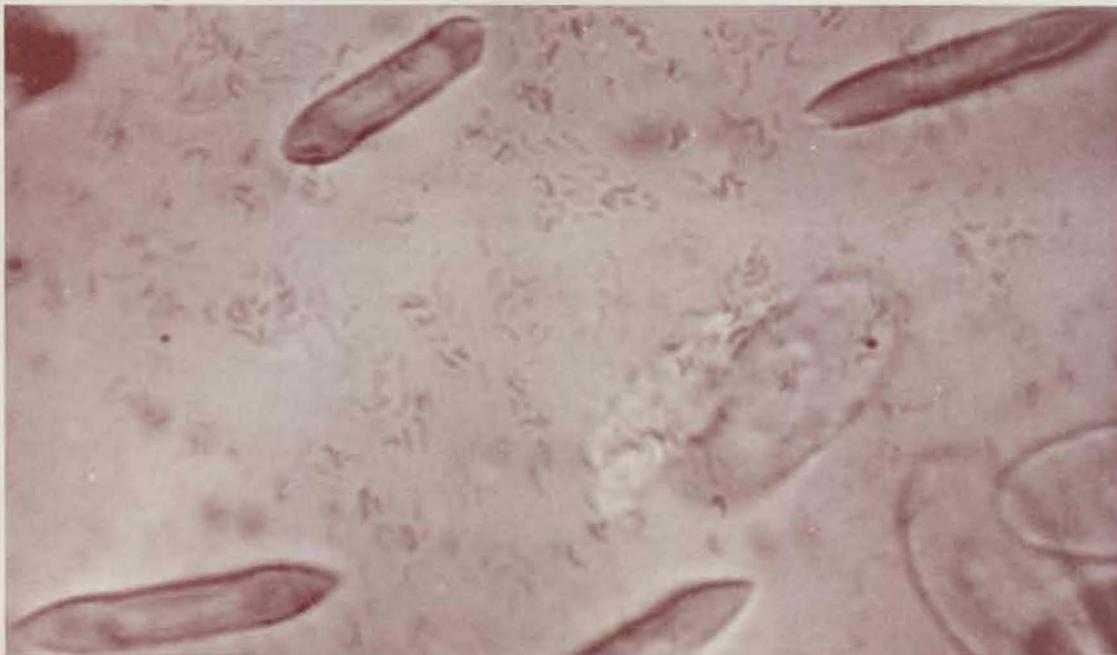




Figure 2. Characteristic lesions of vibriosis, below the lateral line—especially behind pectoral fin and above anal fin. Lesions are pink or red.

foot). Diminished feeding activity is one of the first noticeable symptoms. Lethargic fish gather around the edges of the pens, and others swim in erratic, spinning patterns. Diseased fish show hemorrhages around the base of the pectoral and anal fins or a bloody discharge from the vent (Figure 2).

When a fish is opened for necropsy, diffuse pin-point hemorrhages of the intestinal wall and liver may be evident. The spleen is frequently two to three times normal size.

A presumptive diagnosis of vibriosis can frequently be made when the history, symptoms, and external and internal lesions are considered. But vibriosis may be confused with furunculosis (*Aeromonas salmonicida*) on the basis of these criteria alone.

A positive diagnosis requires the isolation of the *Vibrio* organism from a smear taken from the kidney (Figure 3), spleen (Figure 4), liver (Figure 5), or external lesions. The kidney is the organ least likely to be contaminated when dissecting the fish and is the organ most frequently used to provide material for smears.

Methods Used in Necropsy

The object is to open the body cavity of the fish with a minimum of contamination from the gut. The basic equipment required is shown in Figure 6 and consists of sharp pointed scissors, forceps, paper tissues, a container of 70% alcohol,¹ and a V-notched styrofoam block to hold the fish in a suitable position for dissection.

Swab the fish with a paper tissue soaked in 70% alcohol. Insert the sharp point of the scissors into the body cavity at a point behind the gills (Figure 7). Maintain the point of the scissors just under the body wall and open the body cavity back to a point just in front of the vent. Repeat this process on the other side (Figure 8). Cut the skin and abdomen just behind the gills (Figure 9), then grasp the intestinal wall with the forceps just in front of the

liver and stomach, pulling it loose from the body (Figure 10). The entire intestinal tract can now be cut and pulled aside (Figure 11), exposing the air sac covering the kidneys. Carefully remove the air sac to expose the kidneys (Figure 12).

Isolation of Bacteria from Tissue

Diagnostic equipment is shown in Figure 6 (with the necropsy equipment) and consists of an alcohol lamp, a bacteriological loop (or sterile swabs), marking pen, a sterile petri dish of trypticase soy agar² and a supply of discs impregnated with a vibriostatic agent.³

"Flame" the loop and after allowing it to cool, aseptically transfer a small amount of kidney material or blood to the trypticase soy agar plate

1/ This equipment is available from biological supply houses.

2/ Prepared sterile media petri dishes can be ordered from bacteriological laboratory supply houses. Prepared media should be specified to contain 1.5% NaCl (the dehydrated media contains only 0.5% NaCl). Plates can be prepared by purchasing the trypticase soy agar in powder form, adding 1% NaCl, and dissolving in water as per the manufacturer's instructions. The dissolved media is poured into narrow-mouthed flasks, plugged with cotton, and sterilized in a pressure cooker at 10 pounds pressure for 20 minutes. Allow the flasks to cool to about 50°C (122°F), remove the plug, flame the flask mouth over the alcohol lamp, and pour into sterile, disposable plastic petri dishes. Cover each dish immediately after pouring. When the agar has set, stack the plates, wrap in foil, and store in a refrigerator for future use.

3/ 2,4-diamino-6,7-di-iso-propyl pteridine phosphate. This product is not presently available in Washington; it can be obtained from Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, Long Island, N.Y. It is often referred to in the literature as 0/129. The compound is a light powder. Our procedure for preparing the discs is as follows: half fill a sterile screw-cap vial or test tube with sterile tap or distilled water (boiling for 15 minutes is usually sufficient). Gradually add small quantities of the 0/129 powder until a precipitate forms. Shake vigorously to dissolve. Add more powder and repeat as long as the water will absorb the 0/129. Pour the saturated solution into a vial of one-quarter inch Bacto sterile blank concentration discs (available from biological supply houses). Plug the vial with a small amount of cotton and allow the water to evaporate by warming overnight in a 40°C (104°F) oven. Replace the screw cap and refrigerate until needed. Remove the discs with a forceps passed through the flame.

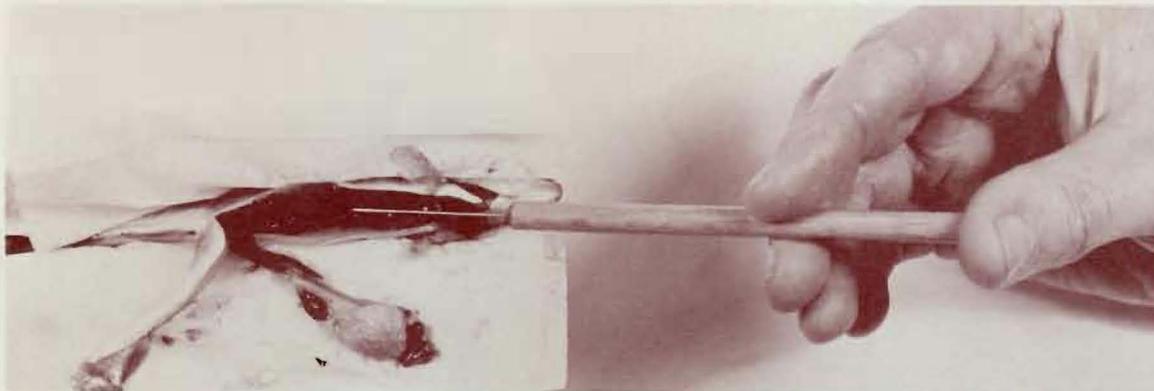


Figure 3. The exposed kidney lies along the backbone. It is protected by a thin membrane and the air bladder.



Figure 4. The spleen is located behind the stomach.

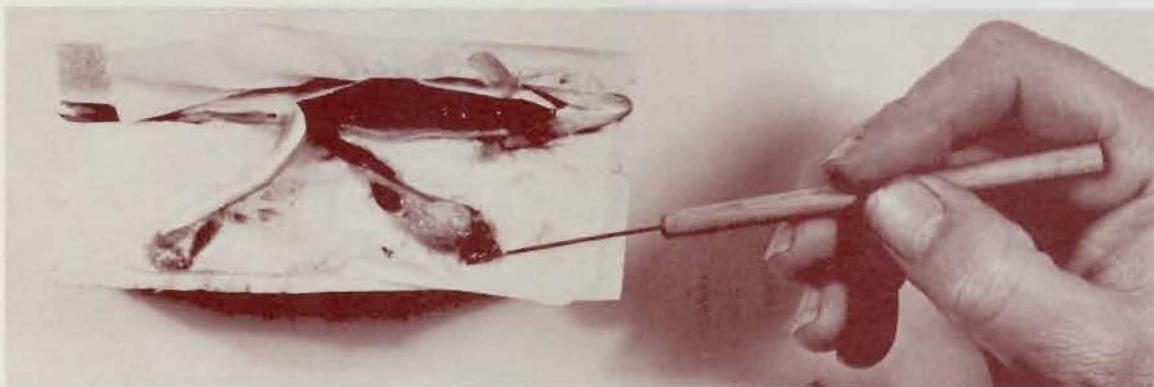


Figure 5. The liver is a large organ in front of the stomach and may range in color from light brown to dark red.

(Figure 13). Smear or "streak" the material back and forth across the entire plate with the loop or a sterile swab (Figure 14). Remove a 0/129 disc with the forceps and place it flat on top of the agar. Press the disc down lightly with the tip of the forceps. Replace the plate cover and incubate the prepared plate at room temperature, preferably at least 20°C (68°F), for two to four days to allow for growth of bacterial cultures.

If bacterial growth appears and there is no zone of inhibition around the 0/129 disc, the growth is not *Vibrio*. If a zone of inhibition appears around the disc (Figure 15), a rapid slide agglutination test should be performed. Place

one drop of specific *V. anguillarum* anti-serum⁴ on an ordinary microscope slide. Mix a partial loop of the unknown bacterial growth from the agar plate with the antiserum and observe for macroscopic agglutination (Figure 16). If no clumping occurs within several minutes, the organism is not a *V. anguillarum*. When the history and symptoms suggest the possibility of vibriosis and the cultured bacteria show a positive agglutination on the rapid slide agglutination test, *V. anguillarum* is the probable cause of disease.

4/ Specific antisera for several strains of *Vibrio anguillarum* are available from BioMed Research Laboratories, Seattle. There are two strains in the Puget Sound area that require separate antisera, as they do not cross-agglutinate in the rapid slide agglutination test.



Figure 6. Equipment used for necropsy and isolation of the *Vibrio* organism. Top left to right: tissue paper, alcohol lamp, styrofoam block with a V-cut to hold fish in position. Bottom: scalpel, scissors, forceps, inoculating loop (must be flamed and cooled before each use), vial of discs impregnated with vibriostatic compound (0/129), beaker with 70% alcohol, disposable petri dish containing trypticase soy agar with 1.5% sodium chloride, marking pen, and several types of disposable sterile cotton tipped applicators.



Figure 7. Opening body cavity with sharp pointed scissors.

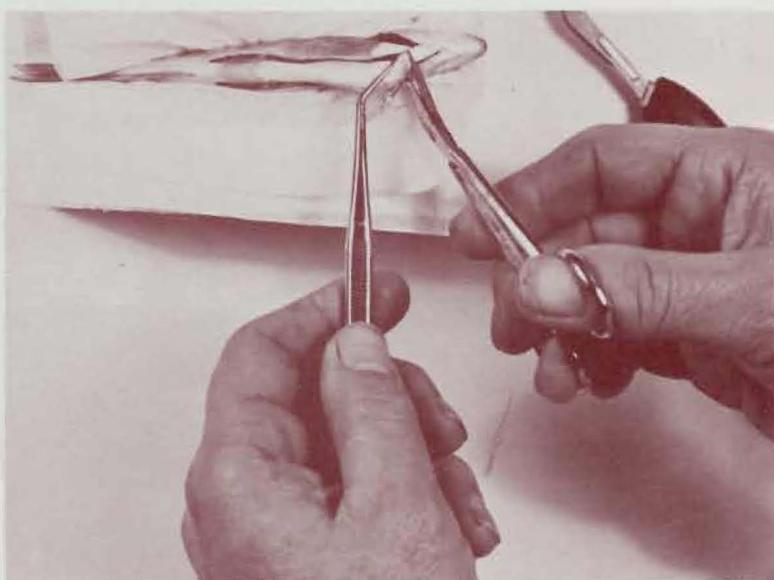


Figure 9. Cutting abdominal wall behind gills.



Figure 8. Opening body cavity on opposite side.

Treatment

The most effective treatment at this time is oral administration of oxytetracycline (Terramycin) in the feed at a rate of 4 grams per 45.4 kilograms (100 pounds) of fish per day for 10 days.

In the future, vaccines should be available. National Marine Fisheries Service and other agencies are developing vaccines to prevent vibriosis.

Large-scale tests show an injected heat-killed bacterin in marine cultured coho salmon has reduced the summer mortality due to vibriosis in one sector of Puget Sound to less than 10%. Tests of orally administered vaccine in Oregon indicate that they are effective in substantially reducing vibriosis in both coho and chinook salmon.

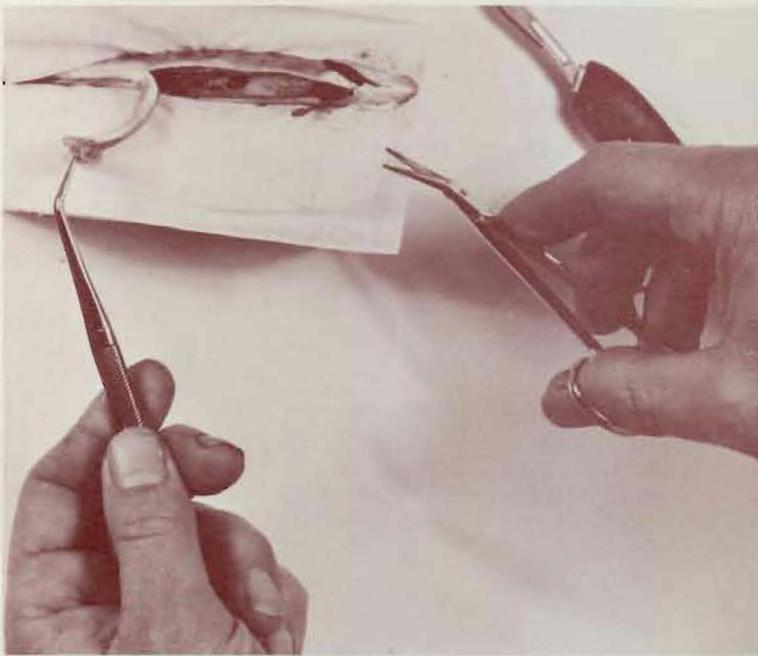


Figure 10. Pulling abdominal wall back to expose intestinal tract.

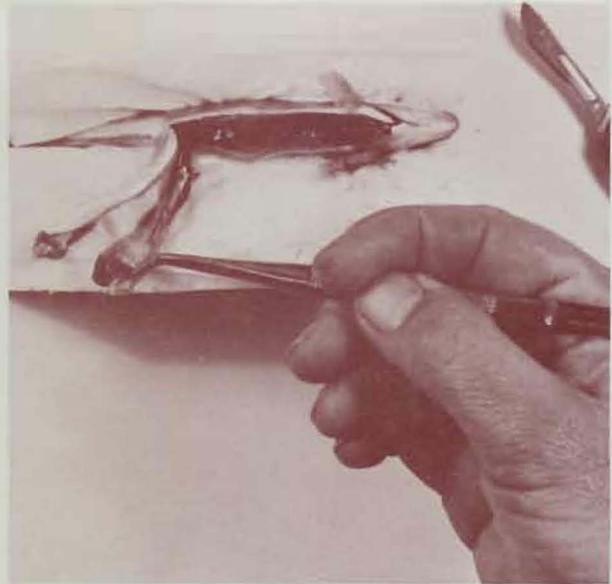


Figure 12. Pulling back intestinal tract and air sac to expose kidney.

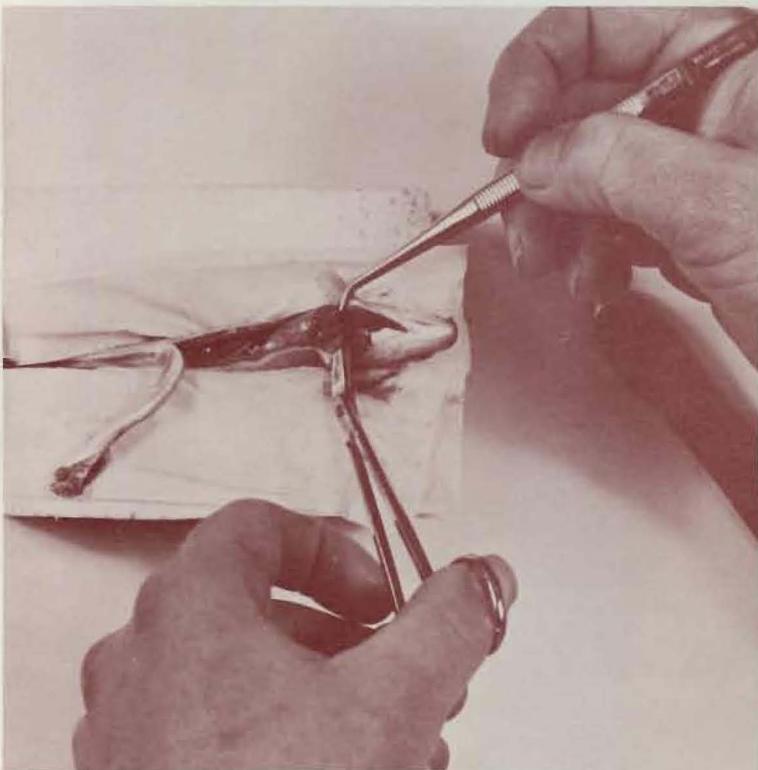


Figure 11. Cutting intestinal tract.

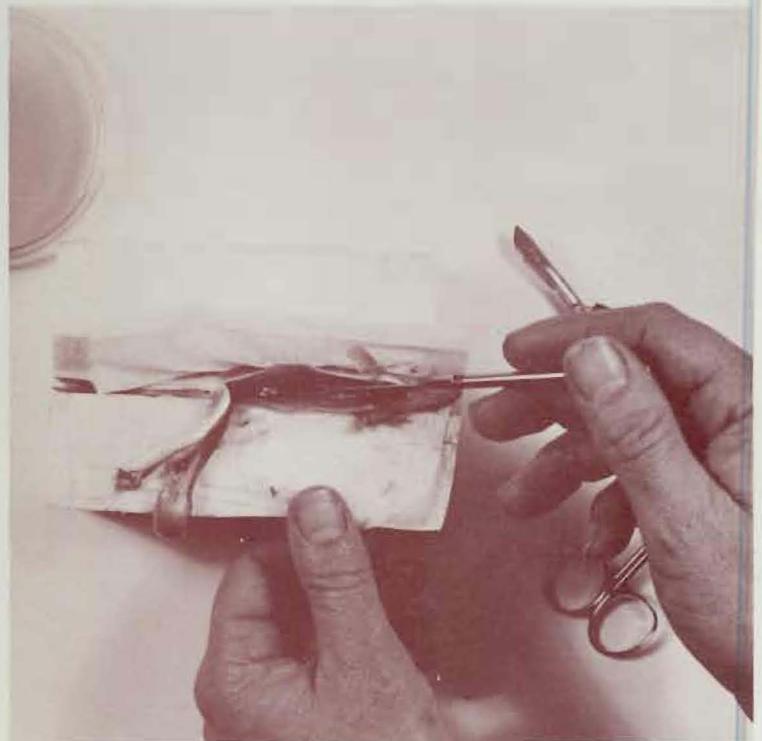


Figure 13. Transferring kidney material or blood to trypticase soy agar plate with a flamed (and cooled) inoculation loop.

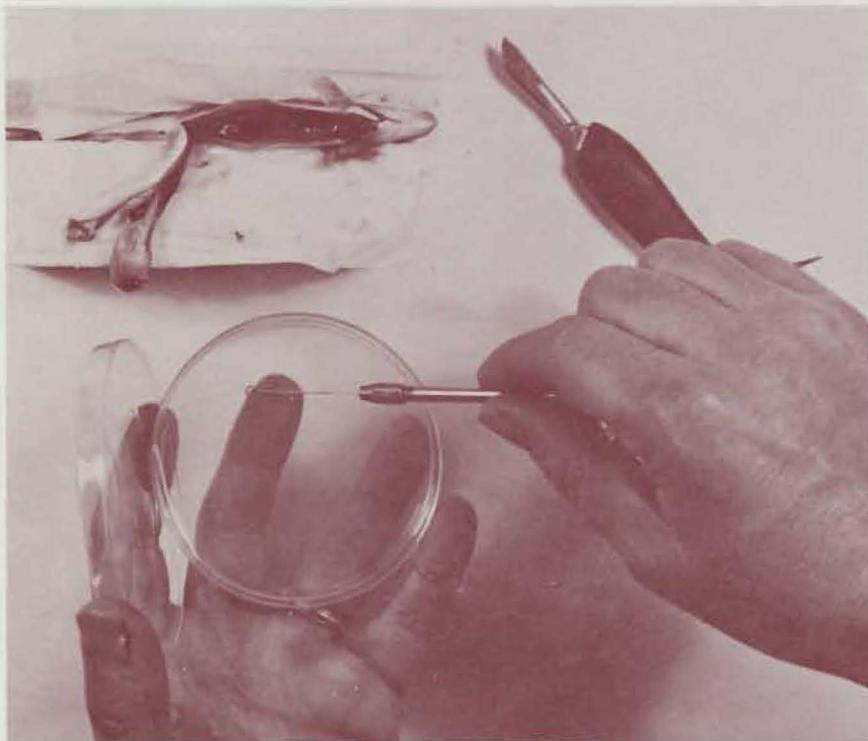


Figure 14. Streaking trypticase soy agar plate with kidney material.

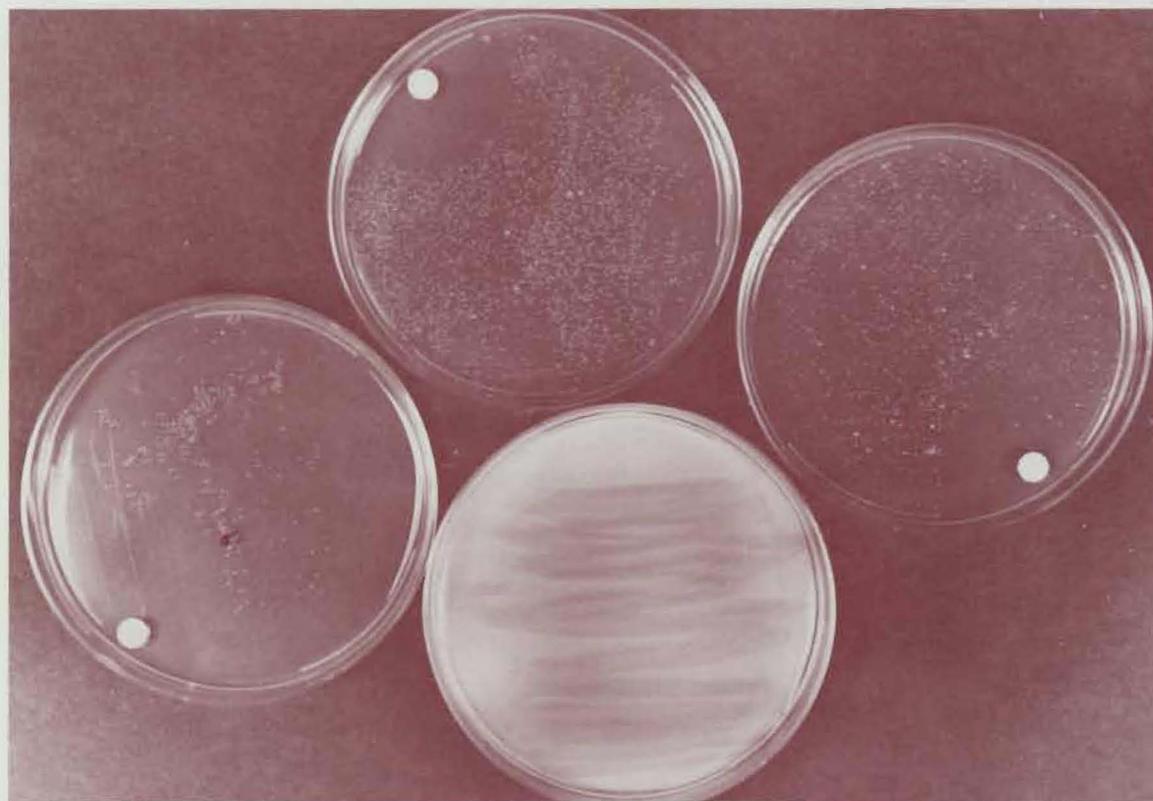


Figure 15. Three petri dishes with varying numbers of *Vibrio* colonies showing zones of inhibition around the 0/129 discs. Bottom plate is demonstration of freshly streaked plate.

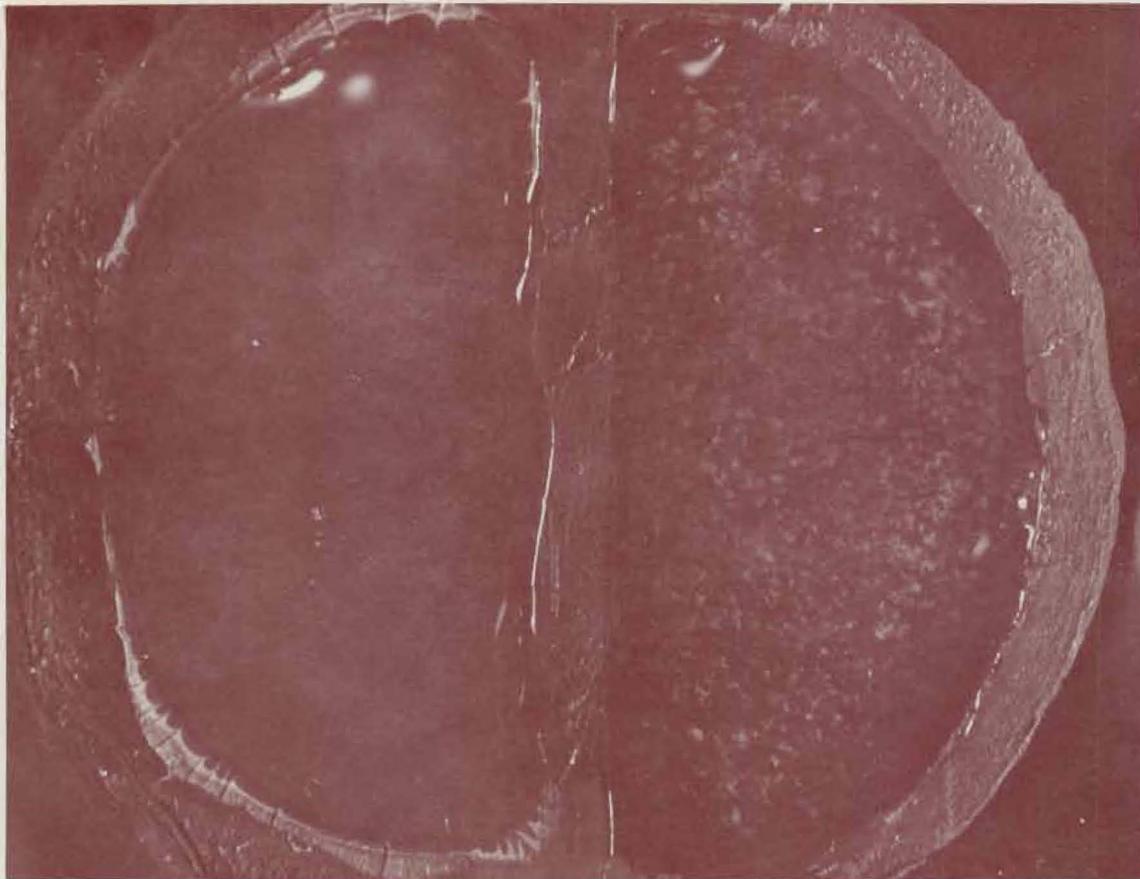


Figure 16. Positive agglutination test for vibriosis is shown on right, negative response on left.

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Photos are by Gunnar Safsten and Alan Ruger, National Marine Fisheries Service Technicians.

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