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# USING ARTIFICIALLY INDUCED LOCOMOTION IN THE MEASUREMENT OF ACTIVE RESPIROMETRY RATES FROM SKIPJACK TUNA, KATSUWONUS PELAMIS

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## PREFACE

This report was prepared under contract (Purchase Order No. 81-ABA-2116, August 6, 1981) by Walter N. Ikehara, graduate student, University of Hawaii. The contract objective was to build a system for inducing locomotion in restrained tuna through brain stimulation and to test the feasibility of using this system for measuring metabolic rates at high sustained swimming speeds. The description of the methodologies employed provides a guide for future research activities in this area. Since the report has been prepared under contract, the statements, findings, conclusions, and recommendations herein are those of the contractor and do not necessarily reflect the view of the National Marine Fisheries Service.

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September 30, 1982

## INTRODUCTION

The purpose of this contract was to design and construct an apparatus for utilizing artificially induced locomotion (AIL) (Kashin et al. 1981) in the measurement of oxygen uptake at various activity levels from restrained, live skipjack tuna, Katsuwonus pelamis. The AIL technique involves electrically stimulating the midbrain of the skipjack tuna causing rhythmic, coordinated swimming motions to be generated. In other fish, this technique has been shown to produce coordinated swimming behavior in a free-swimming fish when stimulating electrodes are implanted (Kashin et al. 1974).

In the past, attempts have been made to measure oxygen uptake rates at high swim speeds from free-swimming skipjack tuna with little success. The methods included, chasing skipjack tuna in annular tanks at high swimming speeds, forcing skipjack tuna to swim in water tunnels while increasing the speed of water flow, and cannulating the gills of actively swimming tuna in small tanks. None of these methods were particularly successful, mainly because the skipjack tuna either failed to swim properly in a forced situation or the measurement of dissolved oxygen in rapidly flowing water proved to be infeasible.

Although some respirometry data have been obtained at near minimum swimming speeds (Gooding et al. 1981), no measurements have been made at higher swimming speeds. When Kashin et al. (1981) showed that simulated locomotory activity could be induced in restrained skipjack tuna, the possibility arose that this method could be used to obtain respirometry data from actively "swimming" skipjack tuna in a restraint system where oxygen uptake measurements and other physiological measurements are facilitated. This contract was funded to incorporate AIL into a restraint system with the capability of measuring oxygen uptake rates, tail-beat frequencies, and other physiological parameters.

## OBJECTIVES

1. The first task was to produce AIL in restrained skipjack tuna and to investigate the stimulus parameters needed to modulate the frequency of tail beats so that varying "swim speeds" could be produced.

2. A restraint system was also required to hold the anterior half of the fish stable and motionless so that the stimulating electrodes could be placed accurately in the midbrain of the fish and be free from motion artifacts. Simultaneously, the posterior half of the fish was to be unrestrained to permit locomotory activity to occur. Other apparatus would be constructed to record the frequency of tail beats for calculation of equivalent swim speeds (Yuen 1966).

3. Next, an apparatus had to be designed and built for the measurement of dissolved oxygen in water before and after passage over the gills. No cross-contamination of the pre-gill and post-gill water could be allowed. The rate of waterflow through the gills was to be monitored so that the oxygen uptake could be calculated.

## METHODS AND MATERIALS

### 1. Artificially Induced Locomotion

Several live skipjack tuna were anesthetized with sodium thiopentol injected intramuscularly at a dose of about 20 mg/kg wet weight. The anesthetized fish were transported onto a temporary restraint cradle and strapped securely. A stainless steel tube was inserted into the mouth of the fish and oxygenated seawater was supplied for respiration. After a short period for recovery, the surgery to expose the brain was begun. Small injections of Lidocaine, a local anesthetic, were made into the muscle overlying the skull of the skipjack tuna. The skin over the skull was incised and folded back and the underlying muscle bundles were retracted. Using a bone rongeurs, the skull bone directly over the cerebellum was cut away, and the fatty tissue and dural membranes were removed exposing the entire cerebellum to view.

Screw clamps were placed on the snout of the fish, clamping it tightly to the stainless steel perfusion tube. The micromanipulator was positioned over the skull opening, and the stimulating electrodes were attached to the manipulator. In bipolar stimulation, two sharpened tungsten electrodes with uninsulated tips were used as stimulating electrodes. In monopolar stimulation, only one tungsten stimulating electrode was used and a stainless steel syringe needle inserted into the anterior dorsal muscle was used as the indifferent electrode. The monopolar electrode seemed to be slightly more effective than the bipolar electrodes and caused less trauma. The electrodes were connected to the output of a Grass S9D Stimulator, and the entire unit was grounded by a stainless steel plate immersed in the seawater.

The most effective area of stimulation seemed to be centered on the midline of the brain, slightly less than half the length of the cerebellum posterior to the anterior edge. The monopolar electrode tip was placed between 6 and 7 mm deep. The lateral position of the stimulating electrode was rather critical, as deviations from the midline as small as 0.5 mm caused uneven tail flexures (Figure 1).

The effective stimulus parameters varied with individual fish but can be summarized as follows. The effective stimuli were repetitive, biphasic, of positive polarity, from 2 to 4.5 volts, of 2 to 5 ms duration, at 60 to 150 pulses per second. The frequency of tail beats could be modulated by increasing the frequency and slightly decreasing the duration. The threshold of stimulation varied with individual fish. The period of stimulation varied from several seconds to several minutes. The tail-beat responses at higher tail-beat rates generally began decreasing in amplitude after a few minutes. Long periods of stimulation are not recommended because lesions could be produced.

If the electrode is placed correctly, the fish will show no tail movement while not being stimulated, although opercular movements will occur. When the stimulator is turned on, the tail will begin moving in a coordinated fashion appearing like normal tail beats. When the stimulator is turned off, the tail beats will cease (Figure 2).

Figure 1.--The placement of the stimulating electrode. The photograph shows the stimulating electrode entering the cerebellum. A retractor is holding muscle bundles apart. The pineal window can be seen at the center right of the photograph. Anterior is to the right.

It is important that only fish in good condition be used for this experiment. The fish should be handled rapidly and with care, as the time that the fish is not respiring should be minimized. The gill perfusion water should be saturated with oxygen. Excess anesthetic should not be used, and the fish can be temporarily quieted by covering the eyes with an opaque material.

2. Live Restraint System

A restraint system was designed and built to hold the fish securely in position for the electrode placement yet permit movement of the tail for induced swimming motion. A rectangular plexiglass box formerly used for stasis respirometry measurements (Brill 1979) was modified by the addition of several new partitions (Figure 3). A plexiglass partition with sliding plexiglass clamps shaped to fit the lateral surface of the skipjack tuna's body was fitted into the box about one-third of the way back from the anterior wall of the box. The clamping surfaces were padded with foam rubber to protect the fish's body. The clamps held the fish's body just posterior to the pectoral fins and could be secured tightly enough to isolate the anterior half of the fish from the motion of the tail. Another partition

Figure 2.--A skipjack tuna being stimulated with the AIL technique. This photograph from the Kashin et al. (1981) experiments shows a skipjack tuna beating its tail in response to AIL stimulation. The electrode holder and the micromanipulator can be seen at left. Electromyographic recording electrodes can be seen at right.

placed just posterior to the anterior wall of the box had an opening shaped to fit around the snout of the fish, padded with firm foam rubber so that it clamped and sealed the snout. Additional screw clamps around the opening could be used to secure the fish's snout against the padded stainless steel perfusion tube protruding into the box from the anterior wall. The box and clamping partitions were designed for a skipjack tuna ranging in size from 1 to 2 kg in weight, the size of skipjack tuna usually maintained in captivity at the Kewalo Research Facility.

In this manner, the anterior portion of the fish could be held securely, sealed against contamination of exhaled water by inhaled water, yet permit collection of exhaled water with a cannulated manifold fitted into the opercular openings. The posterior portion of the box was left open to permit free tail movement and drainage of exhaled water. The box was bolted to a plexiglass water table on the electrophysiology table.

The tail-beat frequency can be monitored by the same method used in Kashin et al. (1981) utilizing a linkage to the caudal peduncle and a potentiometer used in a voltage divider circuit fed to a chart recorder.

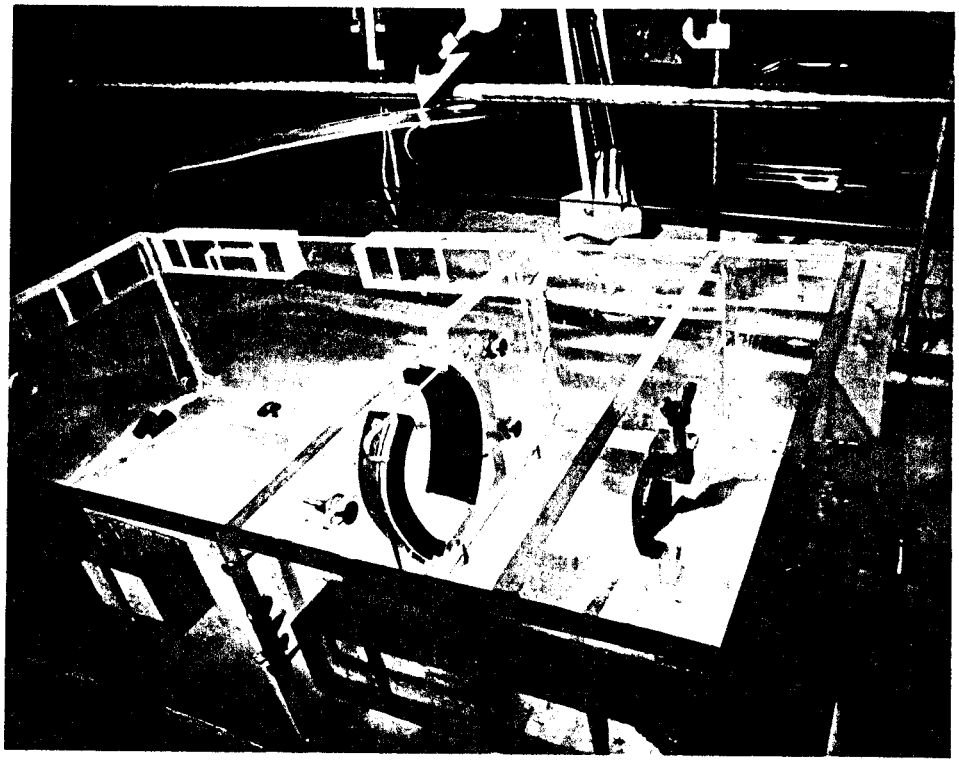

Figure 3.--The plexiglass box used in the restraint system. From right to left, the stainless steel perfusion tube with jaw seals, the first partition with the snout seal and snout clamps, and the second partition with the sliding body clamps can be seen. The compartments can be sealed at the snout and body partitions. Part of the electrophysiology table and support apparatus can also be seen.

3. Oxygen Measurement Apparatus

An apparatus was designed and constructed to permit the sampling of water before (inhalant) and after passage (exhalant over the fish's gills). A plexiglass chamber was constructed to hold a Yellow Springs Instruments (YSI) oxygen probe so that the inhalant flow passed over the membrane of the probe. An outlet was also provided for taking samples in a BOD bottle for Winkler titration (Figure 4).

The exhalant water, after passage over the gills, was sampled by a manifold with three cannulae in each opercular opening. Six cannulae, three from each side of the fish, led to a cylindrical plexiglass chamber designed so that the water forcefully passed over the oxygen probe membrane and also could be sampled into a BOD bottle for later Winkler titration. The exhalant probe chamber was placed lower than the opercular manifolds so that the water siphoned out of the opercular chambers. This caused a rapid flow through the cannulae, effectively collecting most of the exhalant flow (Figure 5).

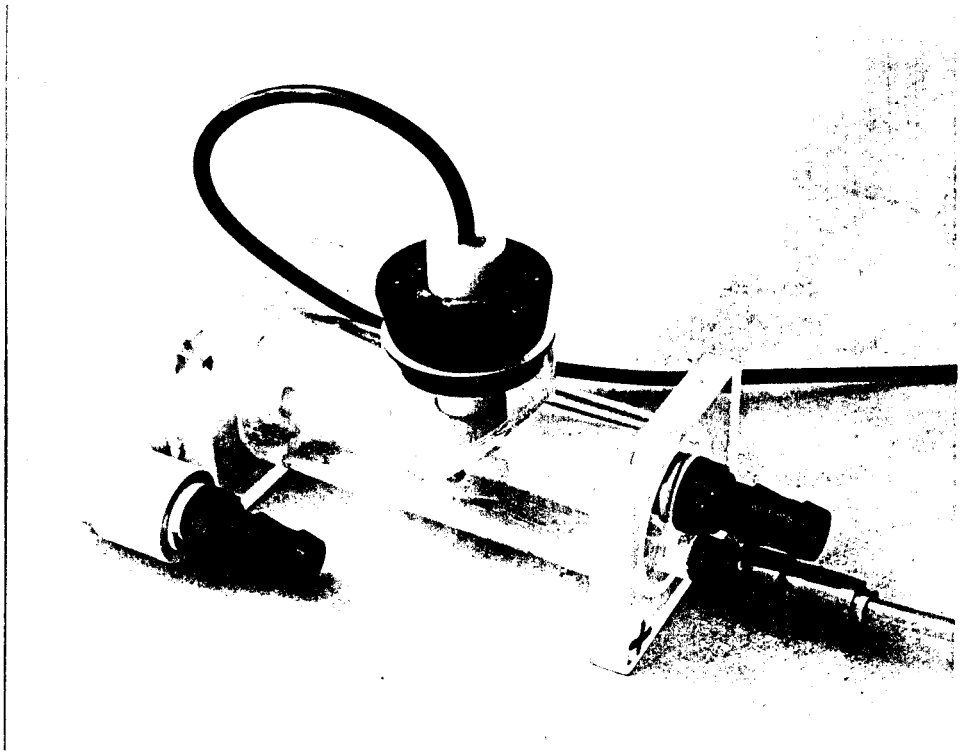

Figure 4.--The inhalant oxygen probe chamber. Oxygenated seawater enters the upper port on the right, passes through the oxygen probe, and exits from the left. The tube at the lower right is the Winkler sampling port. The stopper with the oxygen probe can be removed and replaced with a solid stopper.

The oxygen probe was connected to a YSI Model 57 Dissolved Oxygen Meter. This unit was calibrated with saturated air and was provided with salinity and temperature compensation. As a check on the meter, a Winkler titration apparatus was to be used to assay samples taken at the same time the oxygen meter was used.

The seawater for the perfusion system was oxygenated with an in-line airstone fed by a regulated oxygen tank. To prevent oxygen bubbles from contaminating the inhalant water, a large plastic barrel was used as an open reservoir. The water flowed by gravity from the reservoir through an in-line flowmeter to the inhalant probe chamber, then into the perfusion tube inserted into the fish's mouth. Leakage of inhalant water was prevented by a foam block shaped to seal the area between the fish's mouth and the perfusion tube. (See Figure 6 for a schematic diagram.)

Since all the inhalant water was monitored by the flowmeter and dissolved oxygen measurements could be made before and after passage over the gills, the total oxygen uptake of the fish could be calculated.

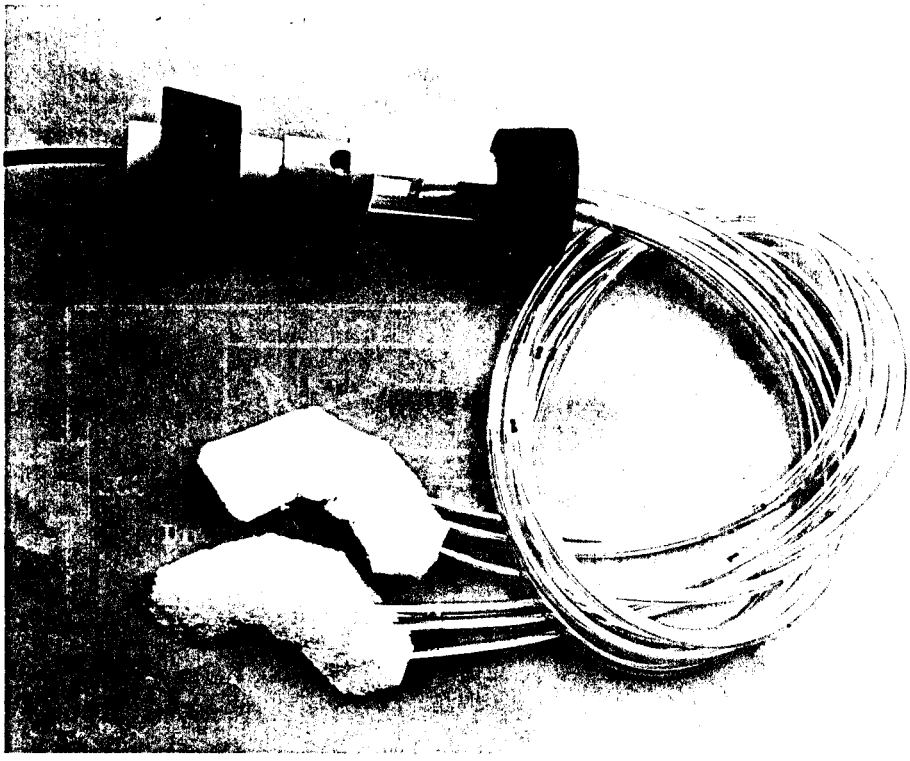

Figure 5.--The exhalant oxygen probe chamber with cannulae. Exhaled seawater is taken up by the cannulae held by the foam opercular manifolds at the lower left, passes over the oxygen probe, and is drained through an outer cylinder (not shown) equipped with a Winkler sampling port. The design of the probe chamber causes seawater to pass forcefully over the probe membrane, eliminating the need for stirring.

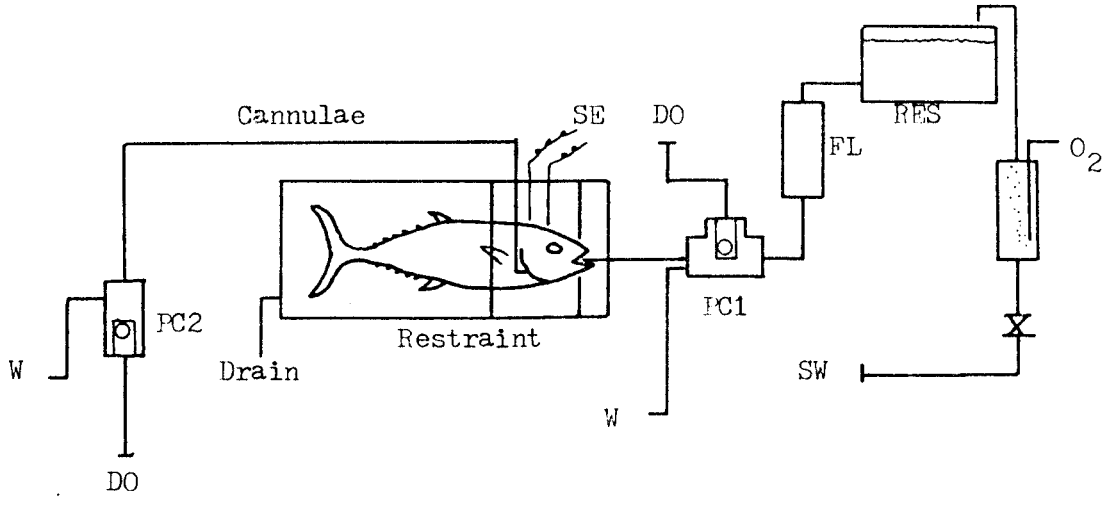


Figure 6.--The restraint system and the oxygen measurement system. In order of direction of flow: SW = seawater input, $O_2$ = oxygen, RES = open reservoir, FL = flowmeter, PC1 = inhalant oxygen probe chamber, DO = dissolved oxygen probe, W = Winkler port, SE = stimulating electrodes, PC2 = exhalant probe chamber.

## SUMMARY OF RESULTS

1. The experiment of Kashin et al. (1981) was successfully reproduced on three skipjack tuna. The parameters for electrical stimulation were studied and effective ranges were found. The frequency of tail beats could be varied by changes in stimulus parameters. The optimal location for stimulating electrodes was mapped in relation to the brain. A monopolar stimulating electrode was found to be slightly more effective than bipolar electrodes.

2. A restraint system was designed and constructed to hold the fish's head immobile and yet permit free tail movement. Access to the fish's head and brain was unimpaired.

3. An oxygen measurement system was designed and constructed to utilize polarographic oxygen electrodes for dissolved oxygen measurements of inhalant and exhalant seawater. Water samples for Winkler titration analyses could also be collected. A cannula system was designed and tested for collecting exhalant water from the opercular openings.

4. No measurements were obtained of oxygen uptake rates at various activity levels from skipjack tuna. Although experiments were planned with restrained fish, various factors such as the lack of suitably sized fish and lack of time prevented the accomplishment of these experiments.

## DISCUSSION

One obstacle to obtaining valid active respirometry measurements in the restrained AIL system is the time needed, after introduction of the fish to the box, for stress-related phenomena to subside. Barrett and Connor (1964) found that blood lactate levels in skipjack tuna were low immediately after capture, but increased to a high level in about 30 to 90 minutes after capture. Approximately 1 to 2 hours were required for lactate levels to fall to initial capture levels. It is possible that any active respirometry measurements made before such time would be biased by stress phenomena. Any respirometry rates obtained via the restrained AIL technique would have to be compared to resting respirometry rates measured after the fish was still and had stabilized, near the end of the experiment. If the fish survived for long periods in the restraint system, then resting respirometry rates could also be measured before active measurements were begun. However, the accumulation of lactate will inevitably occur as the fish is exercised in the restraint system.

The stamina of the fish under stimulation may also become a problem. The skipjack tuna I used were not able to maintain a high rate (i. e., higher than "cruise" rate) of tail beating for long periods of time. In most cases, they fatigued within a few minutes of the onset of stimulation. It is possible that the fish may not last long enough to obtain multiple measurements. This effect may also be due to the fact that weakened fish were used in the initial stimulation experiments, as healthy fish were reserved for behavior studies. If healthy fish are used for AIL experiments, then stamina may not become a problem.

It is important that the restraint system, and all support systems for the apparatus, be as rigid as possible. The skipjack tuna can generate considerable inertial forces when actively tail beating. If there is compliance in the system, the electrodes will move in relation to the brain, damaging the midbrain and brainstem. An implanted electrode system would be desirable, if the optimum location for the electrodes can be determined in advance, however, some adjustability in the location of electrodes would still be desirable.

The system developed for the AIL technique and oxygen measurement is also applicable for the measurement of other physiological parameters. For example, it should be possible to flood the posterior compartment of the restraint box and seal it so that power measurements can be made by measuring the rise in temperature of the water as the fish beats its tail. Heat flow sensors can be attached to the fish for heat flux measurements and electromyographic recordings can also be made from an active fish.

## ACKNOWLEDGMENTS

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## REFERENCES

Barrett, I., and A. R. Connor.
1964. Muscle glycogen and blood lactate in yellowfin tuna, Thunnus albacares, and skipjack tuna, Katsuwonus pelamis, following capture and tagging. [In Span. and Engl.] Bull. Inter-Am. Trop. Tuna Comm. 9:219-268.

Brill, R. W.
1979. The effect of body size on the standard metabolic rate of skipjack tuna, Katsuwonus pelamis. Fish. Bull., U.S. 77:494-498.

Gooding, R. M., W. H. Neill, and A. E. Dizon.
1981. Respiration rates and low-oxygen tolerance limits in skipjack tuna, Katsuwonus pelamis. Fish. Bull., U.S. 79:31-48.

Kashin, S. M., A. G. Feldman, and G. N. Orlovsky.
1974. Locomotion of fish evoked by electrical stimulation of the brain. Brain Res. 82:41-47.

Kashin, S. M., R. W. Brill, W. N. Ikehara, and A. E. Dizon.
1981. Induced locomotion by midbrain stimulation in restrained skipjack tuna, Katsuwonus pelamis, L. J. Exp. Zool. 216:327-329.

Yuen, H. S. H.
1966. Swimming speeds of yellowfin and skipjack tuna. Trans. Am. Fish. Soc. 95(2):203-209.