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National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
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OPERATIONS AND PROCEDURES MANUAL FOR VISITING SCIENTISTS AT THE KEWALO RESEARCH FACILITY

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INTRODUCTION

The Kewalo Research Facility (KRF) is part of the Southwest Fisheries Center's Honolulu Laboratory. The KRF is the only research facility in the world that maintains stocks of live tuna specifically for behavioral and physiological research, and it has been the site of active tuna research for 25 years. The KRF has hosted visiting scientists since its inception. This booklet is designed to orient prospective and visiting researchers to various aspects of working with tunas and to list certain obligations that must be fulfilled by visiting scientists.

Before work can begin a proposal must be submitted for review and approval to Mr. Richard S. Shomura, Laboratory Director, Dr. Richard W. Brill, Leader, Experimental Ecology of Tunas Program; and Mr. Randolph K.C. Chang, KRF Manager. The proposal should include a background discussion of the intended work, tentative procedures, fish and laboratory requirements (including space, equipment, and personnel), and the expected duration of the visit. The desired starting dates should also be stated, keeping in mind that the availability of tunas at the KRF is not the same year-round.

Also, there are forms that must be completed before work at KRF can begin. These will be sent upon approval of the initial proposal.

FACILITIES

The following accommodations (Figure 1) are available to visiting scientists at the KRF: 1) Office space (usually limited to a small work area with a desk), 2) the RV Kaahele'ale (a 33-ft research vessel outfitted for inshore ocean work, available only on a short-term basis), 3) holding and experimental tanks (tank space is limited but some flexibility is possible; however, Honolulu Laboratory research projects must have first priority).

The KRF is an excellent place for marine research. The seawater supplied to the holding tanks is filtered through crushed coral. It is therefore free of most fouling organisms and the water quality is well within levels set by the Environmental Protection Agency (Table 1). Holding tanks (Figure 1) are 8 and 6 m in diameter above ground pools and a large concrete oceanarium (20 × 15 × 3.3 m deep). Seawater is supplied to the pools at a flow rate sufficient to turn over the volume in the tanks in approximately 24 h. There are also three observation towers situated near the holding tanks.

AVAILABILITY OF LIVE TUNAS

The KRF routinely maintains three species of live tunas: skipjack tuna, *Katsuwonus pelamis*; yellowfin tuna (ahi), *Thunnus albacares*; kawakawa, *Euthynnus affinis*; and occasionally bigeye tuna, *T. obesus*. In addition, dolphinfish (mahimahi), *Coryphaena hippurus*, are being cultured at the KRF and at several other institutions in Hawaii. These fish are therefore available as research subjects as eggs, larvae, juveniles, or adults.

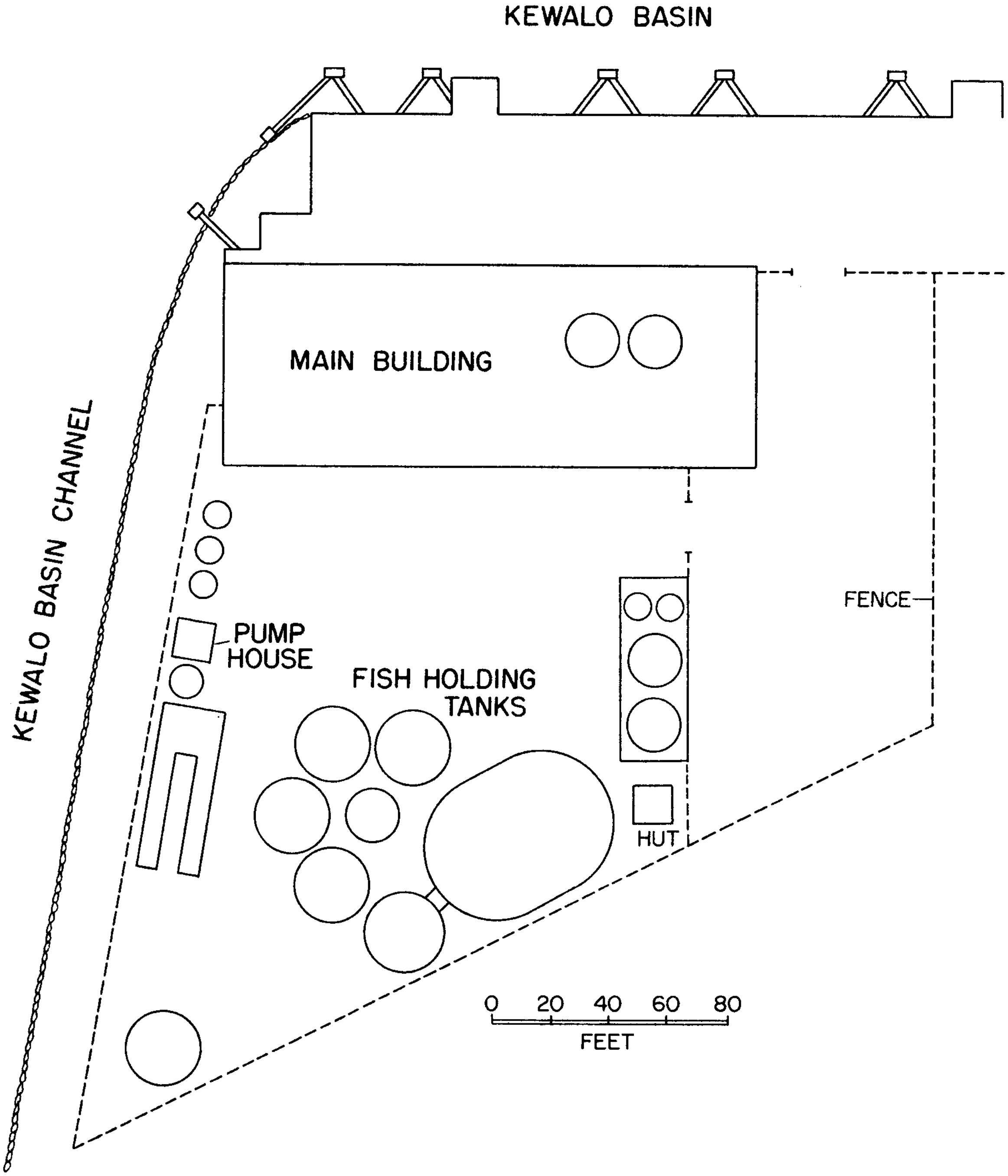


Figure 1.--Schematic map of the Kewalo Research Facility. Offices and laboratories are in the main building.

Table 1.--Analysis results of seawater from well at Kewalo Research Facility.

Contents	KRF well	Standard seawater	Units
Salinity S°/oo	34.566	35.0	°/oo
Cl	20,050	19,353	mg/ℓ
Ca	392	411	mg/ℓ
Mg	1,300	1,293	mg/ℓ
Na	11,300	10,762	mg/ℓ
K	400	399	mg/ℓ
Si	9	--	mg/ℓ
		Safe levels*	
Pb	<0.6	50	μg/ℓ
Cu	0.46	50	μg/ℓ
Zn**	135	4	μg/ℓ
Mn	18	100	μg/ℓ
Fe	0.61	1	mg/ℓ
Cr	1.6	100	μg/ℓ
Cd	<0.01	1.2	μg/ℓ

*As established by the Environmental Protection Agency.

**Zn values may be due to laboratory error.

The availability of live tunas at KRF is not the same year-round and this must be considered when scheduling a research project. There are a number of reasons why the fish may be unavailable. However, the seasonal commercial value of the tunas is the overriding factor. The commercial tuna fishing boats, locally referred to as aku boats, are the only suppliers of live tuna and it is only through their voluntary cooperation that we receive animals. The fishermen are paid for the fish that are delivered to the KRF, but they receive only a fraction of the amount that they can usually get in the market. This is especially true around the holidays (December-January).

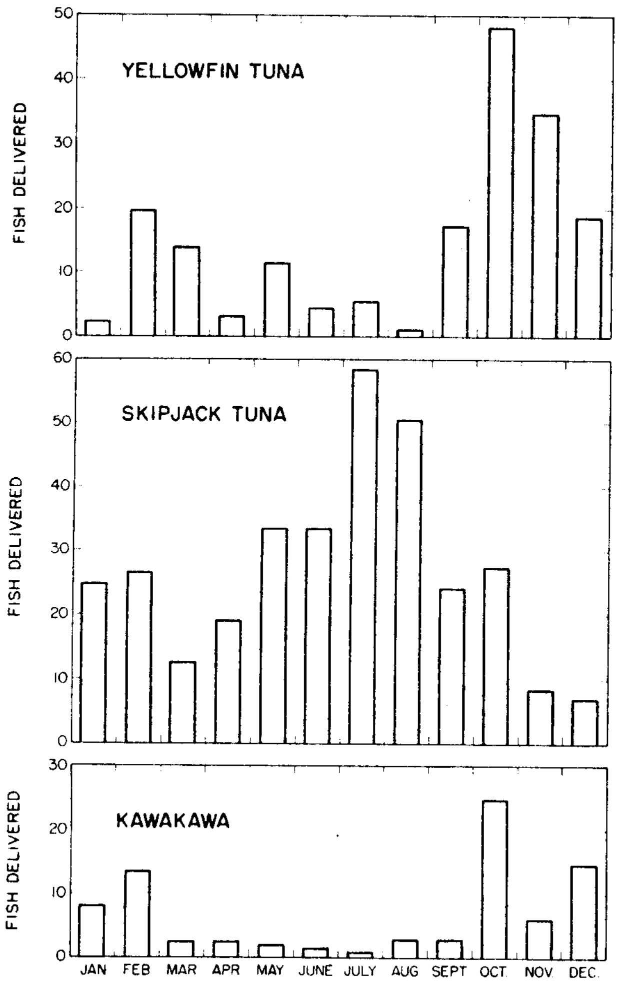
Figure 2 shows the monthly average number of skipjack tuna, yellowfin tuna, and kawakawa delivered to the KRF over a 5-year period (1977-81). These graphs do not take into account whether fish were on order, nor are they an indication of availability of fish to the commercial fishery. (Note: Kawakawa have little market value and they are not generally sought by the aku boats.) These graphs are intended to show when research time might be optimally scheduled. For example, a researcher intending to use skipjack tuna in late winter may spend long periods of time waiting for fish.

Once the KRF Manager is aware of a researcher's need for fish he informs the tuna boat liaison, Mr. Shoji Teramoto, who in turn contacts the aku boat captains. If fishing for market-sized fish is slow, captains will look for schools of small tuna for the KRF. Although tuna of a specific size can be requested, delivery cannot be guaranteed. The normal price for a load of live tuna (12-25 fish) is $300. The price for dead specimens is usually the market price ($50 for 10, for example). These prices are subject to change.

Figure 3 gives the fork-length frequency distribution for the tunas available at the KRF during 1981 and 1982. The relatively narrow size range available is due to the fact that tuna with fork lengths of 29 to 49 cm (approximately 0.5 to 2 kg body weight) have the best survival rates during capture and delivery operations.

Capture and delivery procedures are as follows. A feeding school of tuna is attracted to the boat by chumming with live bait and the tuna are caught on feather jigs with barbless hooks. The fish are then literally tossed into baitwells that have been flooded with seawater. When a sufficient number have been caught the boat returns to the KRF. News of their catch is received well in advance via radio and Mr. Teramoto is there to meet the boat with a transfer tank.

When the fishermen arrive at a dock near the KRF they offload the fish into a transfer tank. Chamois nets are used to get the fish out of the baitwells. The soft nets help to prevent eye and skin damage. The researcher may be present at the transfer, especially if there is some procedure that needs to be done shortly after the fish arrive. The transfer tank is then moved to the appropriate holding tank. At this point the fish are released by lowering the entire transfer tank into the holding tank. One edge of the transfer tank is lowered allowing the fish to swim out. Handling, while unavoidable, is kept to a minimum to avoid unnecessary injury to the fish.



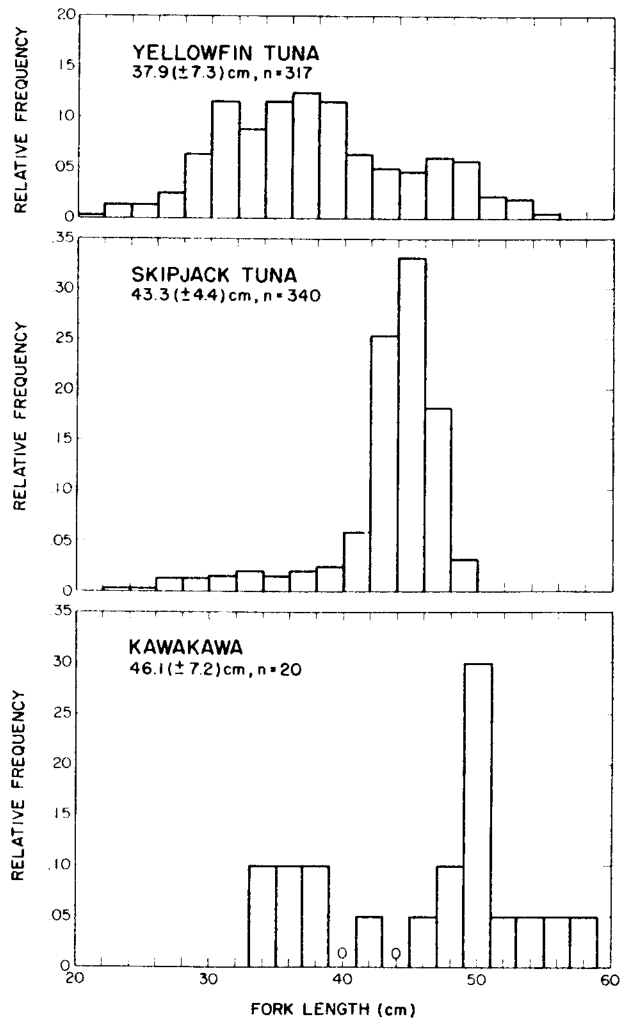


Figure 2.--Average number of tunas (by species) delivered to the Kewalo Research Facility over a 5-year period (1977-81). Skipjack tuna are the most readily available species. Kawakawa are not routinely sought by the commercial fishing boats that supply tuna to the Kewalo Research Facility and a special order must be placed.

Figure 3.--Size-frequency distribution for the species of tunas routinely available at the Kewalo Research Facility (1981-82). Tunas greater than 50-60 cm fork length rarely survive capture and delivery operations, although larger tunas are commercially caught around Oahu, Hawaii.

It is the responsibility of each researcher to see that tank space has been reserved for incoming fish. This is done at the weekly staff meeting.

CAPTIVITY PROBLEMS

The condition of the tuna at the time of arrival at the KRF holding tanks depends on a number of uncontrollable factors. Severe hook wounds, skin abrasions, and frayed fins are common and unavoidable during capture. The only surface a tuna "knows" is the interface between the sea and air. The walls of the tuna boat's baitwells are new and stressful, and if rough seas are added to crowded baitwell conditions, tunas with normally excellent avoidance responses may literally bounce off the walls.

Mortality statistics collected at KRF reveal the following:

1) Mortality in a delivery of fish may be from 0 to 100%.

2) Most mortalities occur on the second or third day after capture (Figure 4).

3) Many mortalities can be attributed to severe hook wounds and/or skin abrasions which appear to cause death either through osmotic imbalance or infection.

Each species of tuna has its own problems in captivity. Skipjack tuna are the most fragile and can be kept healthy for only a relatively short time. They are easily acclimated to captivity and usually feed readily but are subject to a condition known as puffy snout. All tunas are susceptible to puffy snout, which is probably stress related, but skipjack tuna are the most severely affected in the shortest period of time. Puffy snout is a deformation of the skull into pillowy looking blisters (Figure 5). Fish with severe puffy snout die from starvation because the blisters eventually cover the teeth and occlude the eyes and prevent the fish from feeding. A number of studies have been conducted to determine the cause of puffy snout, but no conclusive factor has been found. Puffy snout usually occurs within the first few weeks of captivity in most skipjack tuna.

It is often difficult to get yellowfin tuna to start feeding. Once they begin to feed, however, they do well. As an example, yellowfin tuna were maintained at KRF over 3 years and grew to weigh over 45 kg (100 lb). Yellowfin tuna may jump out of the holding tanks and are especially prone to do this during feeding. Puffy snout is only a slight problem. Mortalities of yellowfin tuna kept for extended periods of time are probably due to dietary deficiencies.

Kawakawa do very well in captivity and are prone to only minor puffy snout problems. They have also been kept at the KRF for as long as 3 years but their growth in captivity is not as dramatic as that of yellowfin tuna.

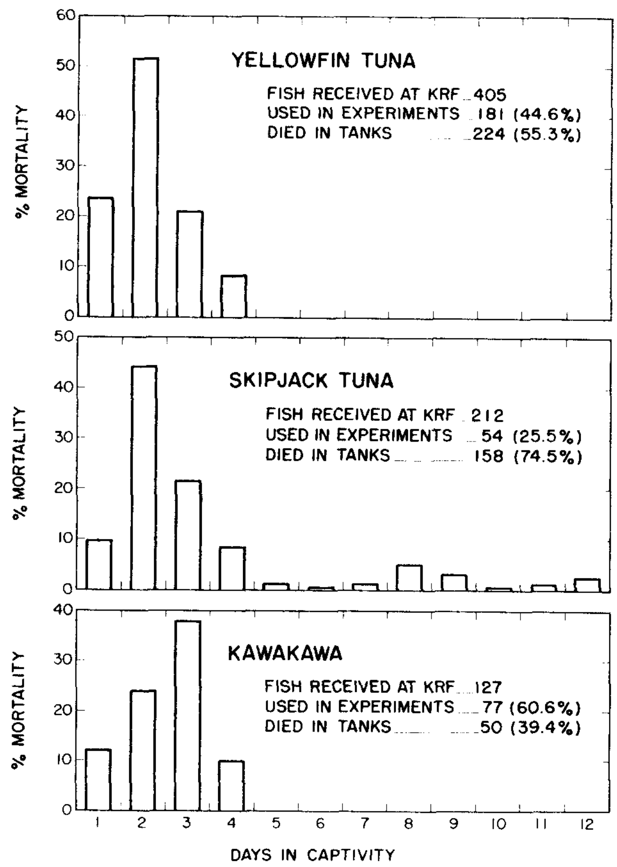


Figure 4.--Daily mortalities expressed as the fraction of fish dying before they can be used as experimental subjects (1980-81). After heavy mortalities on days 2 and 3, usually due to capture trauma, survival rates increase. Note: Data past day 4 for kawakawa and yellowfin tuna are available but now shown.

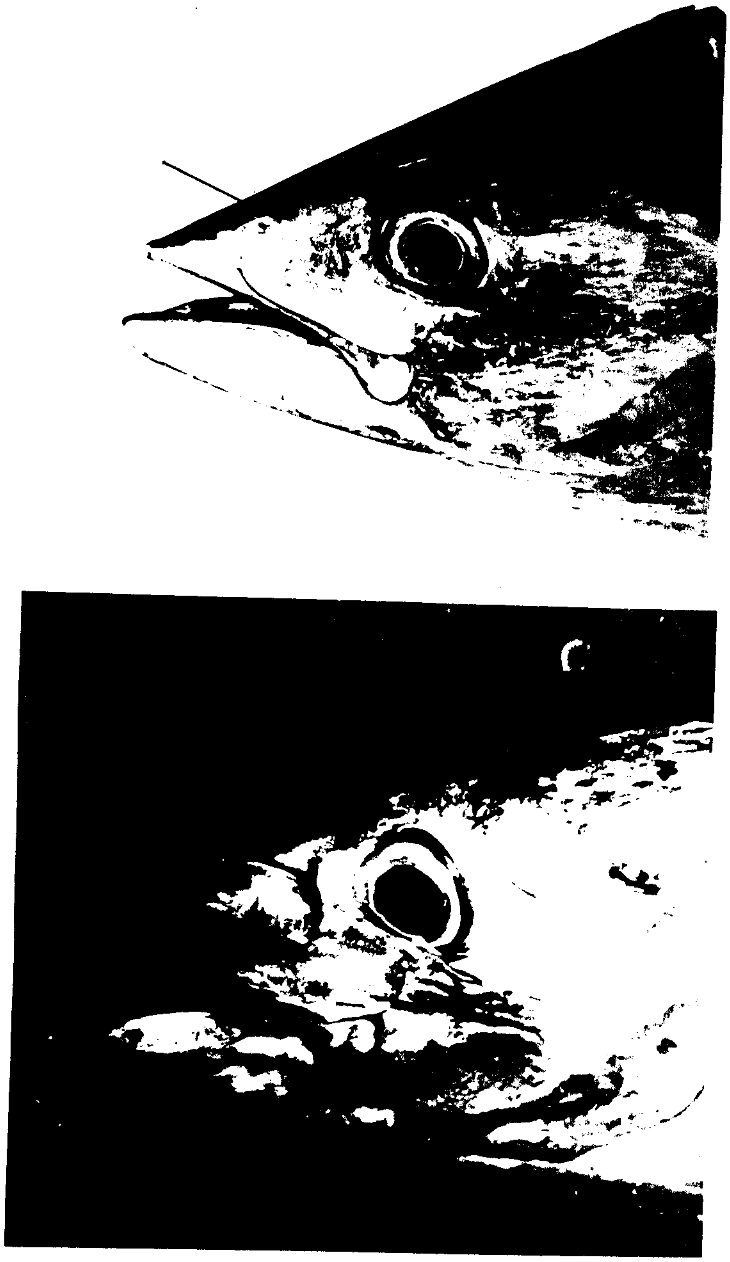


Figure 5.--Normal skipjack tuna (upper) and a skipjack tuna with advanced puffy snout. This condition can occur within weeks in captivity. The teeth and eyes are affected by puffy snout. Fish with severe puffy snout cannot feed and eventually die of starvation.

Fish will usually begin to feed by the second week of their captivity, but there is no set pattern. If fish do not feed by this time, starvation becomes a primary cause of mortality. The KRF keeps a supply of krill and nehu (Hawaiian anchovy, Stolephorus purpureus) to entice newly captured fish to feed. Yellowfin tuna often respond to krill first, whereas skipjack tuna will often begin feeding on nehu. Initially, feeding attempts must be made at least three times a day by throwing a few pieces of food in front of the school while splashing the water. (We humans suppose that this simulates a bait school.) Once one fish in a tank shows interest in feeding, the rest usually follow. It is also easier to get new fish feeding if there is an acclimated fish already in the tank. After fish get accustomed to food that no longer swims, daily feedings of thawed frozen foods begin.

Established fish are fed once a day until satiated. Tuna will often jump during feeding as the food hits the surface and will also smash into the sides of a tank in an effort to get food. For these reasons food must be thrown towards the center of the tank and away from the walls. An interesting aspect of tuna's feeding behavior is the appearance of vertical bars on the sides of the fish. These are quite striking in skipjack tuna and show up to a lesser degree in the other species.

Diets can be varied according to the needs of the researcher and his funding. Providing a diet that is closest to that of a wild fish is possible but expensive, because the food must be purchased at retail market prices. The KRF keeps a supply of frozen smelt, krill, and squid for daily feedings.

Once in the tanks the fish show behavioral characteristics that are similar to those of fish in the wild. Healthy, unstressed fish will begin schooling soon after being put into the holding tanks. One sign of fish in distress is a break in the schooling pattern. An unhealthy individual will swim independently of the school.

HANDLING PROCEDURES FOR BEHAVIORAL EXPERIMENTS

Tuna, as experimental animals, can be both incredibly fragile and unbelievably tough. They may survive a drop on the floor only to die due to a seemingly minor bump on the snout. They must always be handled as carefully and as gently as possible.

In transferring fish to be used in behavioral experiments the equipment developed by Dr. Calvin M. Kaya for spawning studies is used (Figure 6). The water level in the tank is lowered and an individual fish is separated from the school. Using a small seine, the fish is channeled into a large funnel that forces it to swim into a plastic sac (Figure 7). The sac containing the fish is handed out of the tank and literally run to its new destination. When working with fish in the holding tanks it is important to keep the uninvolved fish moving and away from nets and transfer equipment. Tunas must continuously swim to pass water over their gills and time spent immobile can cause severe hypoxia. Healthy tuna have a good avoidance response but in a crowded situation panic may override this.

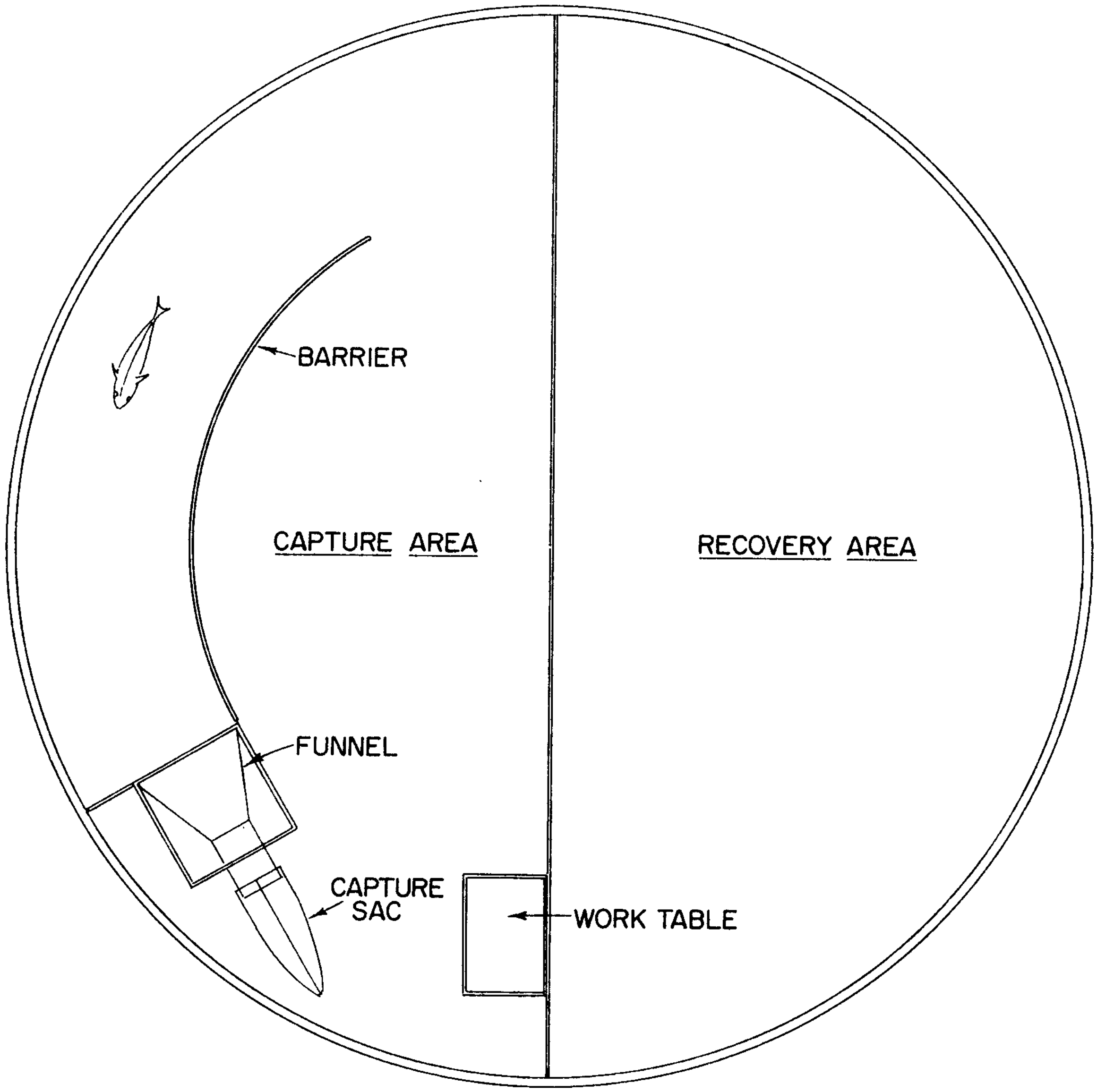


Figure 6.--A fish holding tank with capture system installed. Fish are channeled in the capture sac. At this point they can be transferred to the desired work site or put on the work table (see Figure 8) for taking biopsies or injecting drugs. Figure is from C. M. Kaya, M. K. K. Queenth, and A. E. Dizon, "A capturing and restraining technique for the gonadal biopsy, injection, and marking of small tuna in laboratory holding tanks." (Manuscript in preparation.)

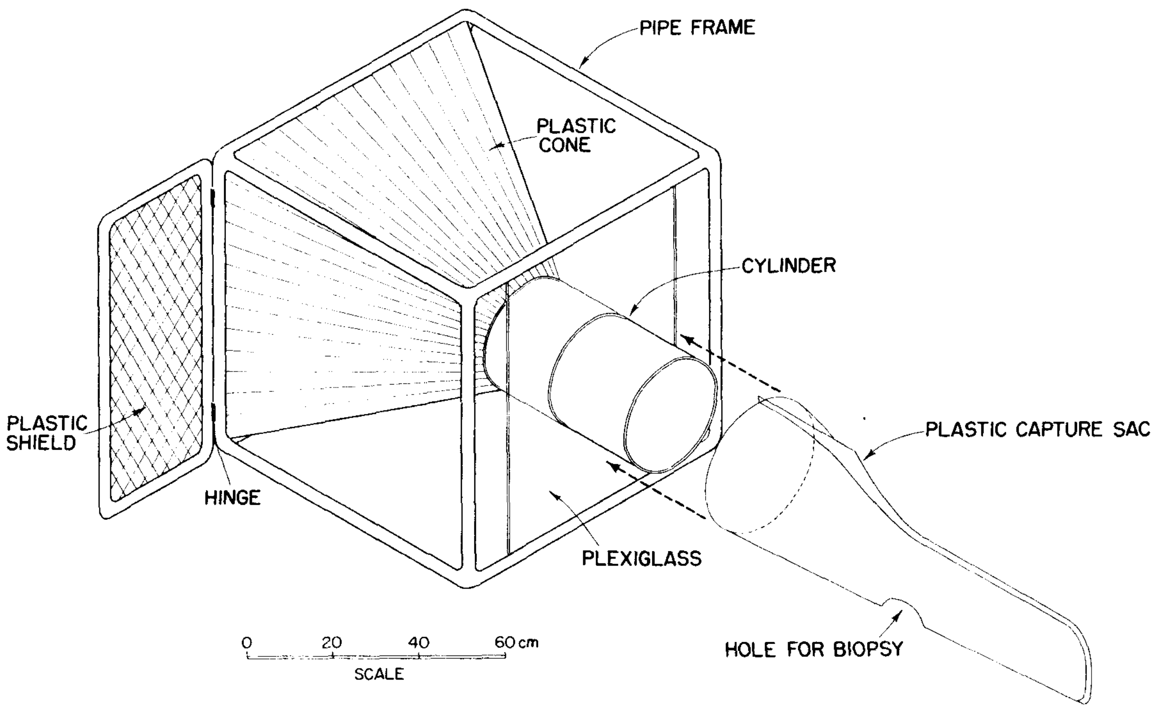


Figure 7.--Plastic funnel and capture sac. Figure is from C. M. Kaya, M. K. K. Queenth, and A. E. Dizon, "A capturing and restraining technique for the gonadal biopsy, injection, and marking of small tuna in laboratory holding tanks." (Manuscript in preparation.)

Behavioral work done at the KRF often requires a tuna to perform a task in response to a stimulus. Tunas learn fairly rapidly although their repertoire of responses is limited to activities that can be performed while moving. As an example, tunas can be trained to swim through a hoop or tunnel initially using bait to induce the fish to respond in the desired fashion. After a number of trials the fish will usually begin to respond freely.

Skipjack tuna are the most difficult to train primarily because their activity level is high and the period of time that they stay healthy is short. Yellowfin tuna are the easiest to train and they become almost petlike in their ability to perform. Kawakawa are also easily trained although they are so motivated by the food reward that they must often be fed first to slow down their activity.

HANDLING PROCEDURES FOR PHYSIOLOGICAL EXPERIMENTS

To obtain fish from the holding tanks that are to be used in acute physiological experiments, a large crowder net and a scoop net will suffice. These fish are not usually expected to perform or recover. Again, the fish that will be used in the experiment must be carefully isolated from the rest of the school. After a fish has been netted it may be injected with an anesthetic or paralytic agent and returned to a holding tank until the drug takes effect.

Both bath and injectable anesthetics have been tried but there are a number of disadvantages to the former. Large amounts of bath type drugs must be used in the volume of water that is needed for a swimming tuna. This makes such drugs as MS 222 and quinaldine impractical. These anesthetics also tend to irritate the gills which cause the fish to become overly excited and the tunas tend to smash into the tank walls before the drug can take effect. Drugs given in the food may or may not work as the fish will often reject the drugged bait. The most often used procedure for administering drugs is injection, but it also has some drawbacks. With intramuscular injections the drug may be pushed back out the injection site by muscular contractions, resulting in a reduced dosage. Intravenous injections are not practical because neither the ventral aorta nor caudal vein can be easily entered in a struggling tuna. Intraperitoneal injection is also difficult in a struggling fish.

Once on the operating table, the fish's gills are irrigated with oxygenated seawater and the fish is confined within a padded plexiglass trough to minimize struggling. Fish have survived up to 8 h in this situation and can be used in neurophysiological, cardiovascular, or other types of physiological experiments.

If biopsies or carefully placed injections are necessary there is a restraining table (Figure 8) that can be used within the holding tanks. The fish is captured using the previously described funnel device and plastic sac and then placed on the foam padded table while it is still in the plastic carrying sac. The fish can then be tightly restrained while biopsies are taken or injections given.

GENERAL PROCEDURES

There are some general procedures that should be followed by visiting researchers at KRF. The staff will be able to provide some assistance, but this is limited and visiting researchers are expected to do the following:

1) Take care of research animals if they require any specialized maintenance other than routine daily feeding and tank cleaning. Tank cleaning must be done every 2 or 3 days. The primary fouling organism is a brown diatom that proliferates on the walls and bottoms of the tanks. Time permitting, the KRF staff will assist with tank cleaning.

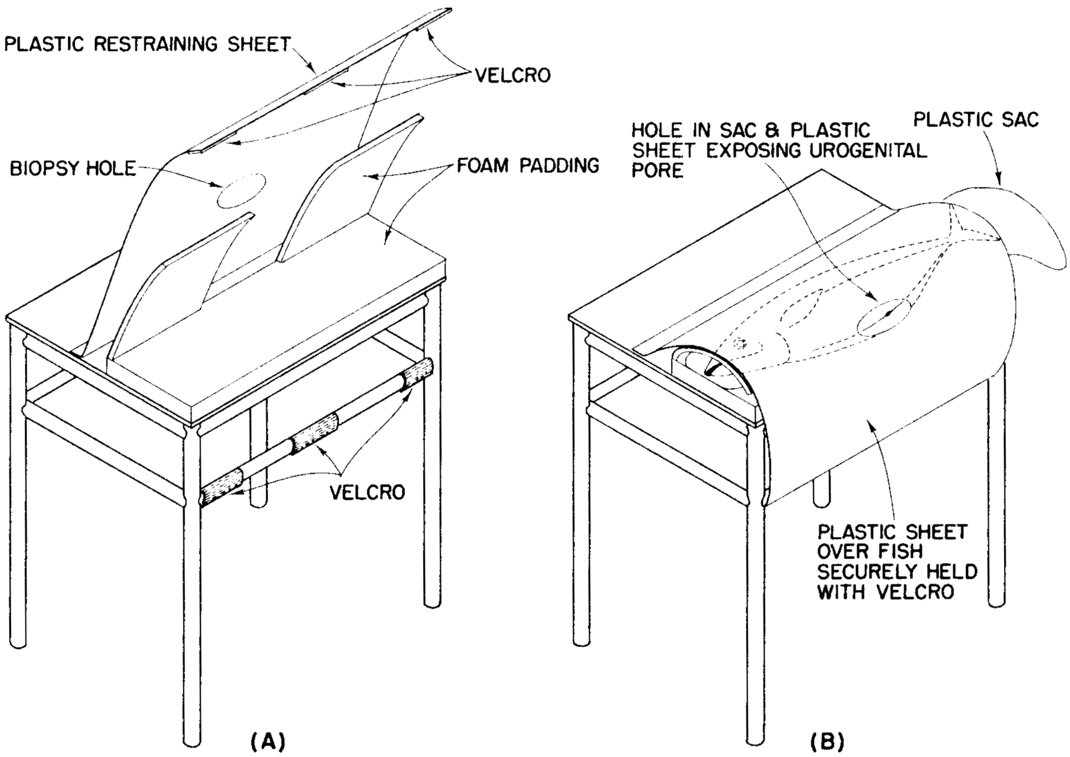


Figure 8.--Restraining table used for taking biopsies or injecting drugs. When this table is used in conjunction with the capture system shown in Figure 6, injection or biopsy procedures can often be done repeatedly. Figure is from C. M. Kaya, M. K. K. Queenth, and A. E. Dizon, "A capturing and restraining technique for the gonadal biopsy, injection, and marking of small tuna in laboratory holding tanks." (Manuscript in preparation.)

2) Maintain a clean and orderly work area.

3) Use appropriate safety equipment when using any power tools.

4) Label (Figure 9) and store all specimens in the appropriate storage areas.

5) Keep track of all specifically assigned animals by filling out a mortality log (Figure 10).

6) Attend KRF staff meetings each Monday at noon. At this time all researchers must be prepared to discuss requirements for the upcoming week such as fish, tank space, etc., and to give a brief summary of the past week's progress. The meetings are informal, but follow a set agenda. The minutes are posted after each meeting.

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FISH MORTALITY LOG

Tank GIVE DESIGNATED TANK NUMBER

Species SJ/ YF/ KK ____________ **Date of death** ____________

Date of capture IF KNOWN

Sex ___ **Male** ________ g **Fork length** ________ cm -TIP OF NOSE TO FORK OF TAIL

USUALLY DETERMINED FOR SJ,KK **Female** ________ g **Weight** ________ g

Not determined ________

Cause of death ____ Handling

____ **Used in experiment** LIST RESEARCHER AND EXPERIMENT TITLE

____ Starved

____ Unknown OR OTHER ____________

Puffy snout ____ Yes STATE DEGREE (MODERATE, BAD, EYES OCCLUDED)

____ No

Autopsy:

(1) Hook wounds ___ Upper ______ STATE DEGREE

___ Lower ______

___ Not applicable

(2) Frayed fins ___ Yes DETAILS ____________

___ No

(3) Bruises ___ Yes DETAILS ____________

___ No

(4) Eye damage ___ Yes DETAILS ____________

___ No

(5) Gross anatomy LIST ANY DETAILS THAT ARE OF INTEREST OR THAT MAY HAVE CONTRIBUTED TO THE ANIMAL'S DEATH.

____

Figure 10.--Sample mortality log. One of these logs must be filled out for every fish removed from a holding tank.

OBLIGATIONS OF VISITING SCIENTISTS

Visiting researchers must be prepared to finance their own work. Researchers in the past have often been funded through their home institutions or through grants. The KRF/Experimental Ecology of Tunas Program is willing to be part of joint proposals (National Science Foundation, Sea Grant, etc.) and will assist when needed to arrange funding. Budgets should include money to cover travel, per diem, fish, fish food, and any large purchases.

When work has been completed a report should be sent to the Program Leader that includes results, conclusions, and plans for publication. This should be submitted 2 weeks after the researcher has completed the work. Researchers should also submit a copy of any publications based on work at the KRF. It is required that the KRF be specifically acknowledged in publications that use data based on work done here.

The KRF, because of its unique location and its ability to get live tuna for study, has been and will continue to be the site of important marine research. Visiting scientists are always welcome.