

SENSITIVITY OF RESTRAINED SKIPJACK TUNA (*KATSUWONUS PELAMIS*) TO ABRUPT INCREASES IN TEMPERATURE*

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Abstract—1. Restrained skipjack tuna (*Katsuwonus pelamis*) signaled by deceleration of heart rate that they could perceive abrupt temperature increases (1°C/sec) as small as 1°C.

2. A thermal stimulus directed into the oral-branchial cavity was more effective in eliciting responses than stimuli delivered to the nasal cavity or the dorsal, anterior quadrant of the body surface. This finding is discussed in relation to the current belief that thermal sensors in fish are generously scattered over the skin surface.

INTRODUCTION

A TEMPERATURE-DETECTING mechanism that facilitates behavioral thermoregulation is adaptively important to fishes, because thermal heterogeneity is characteristic of the aquatic environment and temperature dramatically affects biological systems. The temperature sense of tunas must be especially acute if they are to perceive and make directed responses to the very gradual horizontal temperature gradients and fronts of the open ocean (0.001–0.1°C/100 m). That tunas do make directed responses to these gradients of temperature can be inferred from the close correspondence between certain sea-surface isotherms and the limits of tuna distribution (Blackburn, 1965).

Although the sensitivity of nonpelagic marine fish to environmental temperature change has been well documented (0.03–0.10°C, summarized by Murray, 1971), only casual and indirect observations have been made on temperature perception in tunas. Gooding & Higgins noted that skipjack tuna (*Katsuwonus pelamis*) stayed above a 15°C discontinuity layer in a vertical temperature gradient

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(R. M. Gooding & B. E. Higgins, Southwest Fisheries Center, National Marine Fisheries Service, NOAA, Honolulu, Hawaii 96812, personal communication). Laurs *et al.* (1970) reported that a sonic-tagged albacore (*Thunnus alalunga*) seemed to avoid a thermal front of $0.1^{\circ}\text{C}/100\text{ m}$. Green (1967) found that purse seining for tuna was most effective when a temperature discontinuity layer (thermocline) of at least $0.5^{\circ}\text{C}/\text{m}$ deterred escape through the bottom opening of the seine. In these cases temperature seems to be the main orienting cue, but no systematic attempts have been made to eliminate other concomitant cues such as pressure, light, turbidity, oxygen and olfactory quality of the water.

In this paper, we (1) test whether restrained skipjack tuna will respond to abrupt temperature changes, (2) compare sensitivity of the oral-branchial cavity, the olfactory cavity and the skin and (3) provide a first estimate of temperature threshold for a pelagic oceanic fish. Classical heart-rate conditioning was the method used. This technique has been successfully used on fish—most recently by Rommel & McCleave (1972) to determine perception of electric fields by eels. We are the first to use this technique to evaluate temperature perception. Application of the test for our purposes required demonstration that the observed responses (abrupt heart rate decelerations) were time-related to the temperature stimulus. The chief advantage of the method is speed of application—2 hr is sufficient to demonstrate temperature-related responses. The fragility of skipjack tuna in captivity obviates any long-term experimentation or methods that require extensive handling.

MATERIALS AND METHODS

Forty-one skipjack tuna (average weight 1.5 kg) were tested. Stocks were held at the Kewalo Basin Tuna Research Facility of the Southwest Fisheries Center, National Marine Fisheries Service, Honolulu, Hawaii. Techniques of maintaining tuna in captivity have been outlined by Nakamura (1972).

Experimental sessions were conducted at night, when the fish were easier to capture and tolerated restraint better. Two fish were tested each night in sessions that lasted approximately 2 hr per fish. For training, we secured the fish in a trough constructed from polyvinyl chloride pipe and padded with plastic foam. Velcro* strapping and an injection of a muscle relaxant (gallamine triethiodide, 1 mg/kg) were necessary to keep the fish sufficiently immobile so that muscle potentials did not interfere with the electrocardiogram (EKG) recording. Water for respiration was delivered at 8 l./min via a rubber tube secured in the fish's mouth. We kept a skipjack tuna alive for 8 hr under these conditions and had no mortalities during the usual 2-hr training sessions. During testing, the fish were isolated; all recording and stimulus preparation was done from an adjacent room to eliminate disturbances to the fish.

Temperature of the stimulus water and the heartbeat were continuously monitored.

We recorded temperature with a fast-responding copper-constantin thermocouple (Omega Engineering, Model SPSS-020U-6). The temperature signal was amplified in the upper channel vertical amplifier of a dual-channel oscilloscope (Textronix, Model 5030) and recorded (Brush, Model 220, two-channel paper recorder).

The EKG was recorded via fine wires inserted under the skin and adjacent to the heart. The signal was amplified and filtered (100–1000 Hz) to improve signal-to-noise characteristics (PAR Instruments, Model 113) and displayed visually on the lower channel

* Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

of the oscilloscope. The oscilloscope sweep was triggered by each heartbeat. The "gate output" of the oscilloscope was used to reset the analog integral of the output of a free-running multivibrator. These two circuits (multivibrator and integrator) were "patched" on an analog operational manifold (Teledyne Philbrick Instruments). The resultant sawtooth output, along with the stimulus temperature, was recorded with the paper recorder. Each sawtooth represented one heartbeat and the height of each "tooth" was directly proportional to the interbeat interval.

We used two different types of unconditioned stimuli (US): (1) water perfusing the gills momentarily shut off or (2) an electric shock. The unconditioned response (UR) in each case was an immediate cessation in the heartbeat followed by several irregularly spaced beats. For each fish one of three conditioned stimuli (CS) was applied: temperature stimuli directed to (1) the oral-branchial cavity (mouth), (2) the right nasal capsule (nose) or (3) the dorsal, anterior quadrant of the body (back). For the mouth stimulus, the temperature of the water flow through the oral-branchial cavity to the gills was altered. Water flows and maximum temperature rises under the three stimulus conditions were 100 ml/min up to $+10^{\circ}\text{C}$ for the nose and 8 l./min at $+6^{\circ}\text{C}$ for both mouth and back. Ambient temperature of the perfusion water was between 24 and 25°C . Stimulus flows were continuous; temperature was altered by heating a stainless steel segment inserted into the supply line. Application of heat ended approximately 30 sec before the temperature change reached the fish and thus reduced the possibility that the fish received other cues associated with changing the water temperature.

The US was applied when the temperature reached $+6^{\circ}\text{C}$, except for two mouth-stimulated fish (B and F) in which the US was delivered at $+5^{\circ}\text{C}$. This resulted in a 10-sec delay between the initial temperature rise and the US. Fish were trained for thirty trials; intertrial intervals were 2-4 min.

RESULTS

Of the forty-one fish examined, eight were tested with temperature stimuli delivered to the mouth; six of these were responsive. Ten fish were tested with stimuli delivered to the back; three were responsive. Twenty-three fish were tested with stimuli delivered to the nose; eight were responsive. Over 50 per cent of the fish tested were considered unresponsive. In most of these cases, no heartbeat decelerations were observed during temperature change; in others, the heart rate was so irregular it was impossible to discern responses.

Figure 1 illustrates typical heart-rate responses. In Fig. 1a the temperature stimulus was delivered to the nose and a response (decrease in heart rate) occurred at about 5°C . After the temperature reached 6°C , the US was applied and the UR (another decrease in heart rate) followed. In Fig. 1b the stimulus was delivered to the mouth and a decrease appeared almost simultaneously with the initial temperature rise. In this example spontaneous responses occurred before and after the trial. The question arises whether the simultaneous decrease was in response to the temperature stimulus or spontaneous. Because abrupt spontaneous decreases in heart rate were characteristic of most fish tested, a careful statistical evaluation of the data was necessitated to determine whether observed responses were in fact controlled by the thermal stimulus.

To distinguish spontaneous responses from responses to temperature, the frequencies of responses in the intertrial period were compared to the frequencies of responses during the period when the temperature was rising prior to the US

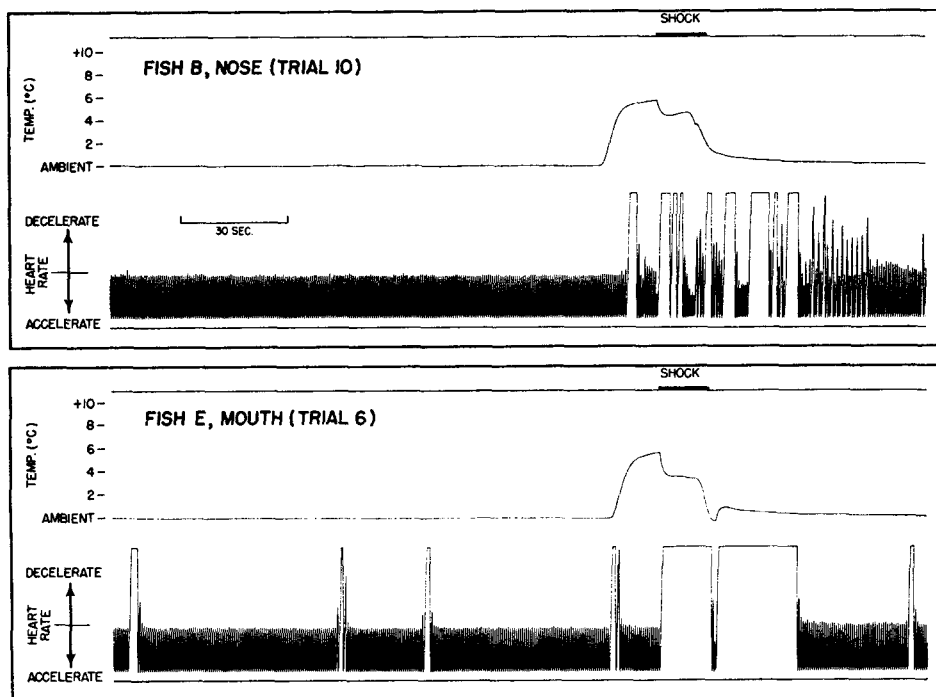


FIG. 1a. A recording from a skipjack tuna showing deceleration in heart rate, accompanying a temperature rise in the naris and then an electric shock (trial 10, fish B, nose). b. A similar record except that the temperature change occurred in the mouth and spontaneous decelerations occurred before and after the trial period (trial 6, fish E, mouth).

(Kolmogorov-Smirnov one-sample test; Siegel, 1956). Two of the six responsive fish stimulated in the mouth and one of the eight responsive fish stimulated in the nose had significantly greater frequencies of response during the temperature stimulus period than during the intertrial period ($P \leq 0.05$; Table 1). Combining the data for the six analyzed fish stimulated in the mouth indicated that the frequencies of response were significantly greater during the stimulus period ($P \leq 0.05$). The similarly combined data for the eight analyzed fish stimulated in the nose were also statistically significant ($P \leq 0.05$). Combined data for all seventeen analyzed fish (in Table 1) yielded a highly significant difference in frequencies of response between the intertrial period and the period during the temperature change ($P \leq 0.01$). We conclude that restrained skipjack tuna responded to water temperature change with a decreased heart rate.

To obtain an estimate of threshold of response, the temperature stimuli were grouped by 1°C intervals. Frequencies of response were then plotted for individual fish as a function of stimulus temperature (Fig. 2).

TABLE 1.—Time (sec), NUMBER OF RESPONSES AND RESPONSE FREQUENCIES OF SKIPJACK TUNA BEFORE TEMPERATURE STIMULATION (INTERTRIAL) AND DURING STIMULATION (TRIAL)

Stimulus position	Fish	Intertrial (No temperature change)			Trial (During temperature change)			Level of significance
		Time (sec)	No. of responses	Responses per sec	Time (sec)	No. of responses	Responses per sec	
Mouth	A	4476	86	0.019	717	42	0.059	Significant at 0.01
	B	4581	100	0.022	414	26	0.063	Significant at 0.05
	C	4803	65	0.014	295	4	0.014	—
	D	5040	134	0.027	380	26	0.068	—
	E	3052	121	0.040	237	19	0.080	—
	F	4258	81	0.019	510	19	0.037	—
Total		26,210	587	0.022	2553	136	0.053	Significant at 0.05
Nose	A	3074	109	0.035	589	42	0.071	Significant at 0.05
	B	6414	45	0.007	1508	19	0.013	—
	C	2081	58	0.028	594	15	0.025	—
	D	3055	42	0.014	446	11	0.025	—
	E	3942	100	0.025	732	19	0.026	—
	F	2919	35	0.012	1224	16	0.013	—
Total	G	4839	65	0.013	566	13	0.023	—
	M	3303	53	0.016	347	12	0.035	—
		29,627	507	0.017	6006	147	0.024	Significant at 0.05
Back	A	4866	138	0.028	361	9	0.025	—
	B	7137	172	0.024	517	21	0.041	—
	C	4545	88	0.019	347	18	0.052	—
Total		16,548	398	0.024	1225	48	0.039	—
Grand total		72,385	1492	0.021	9784	331	0.034	Significant at 0.01

Level of significance of differences in distribution of observed values (responses) and distribution of "effort" (time) was determined by the Kolmogorov-Smirnov one-sample test (Siegel, 1956).

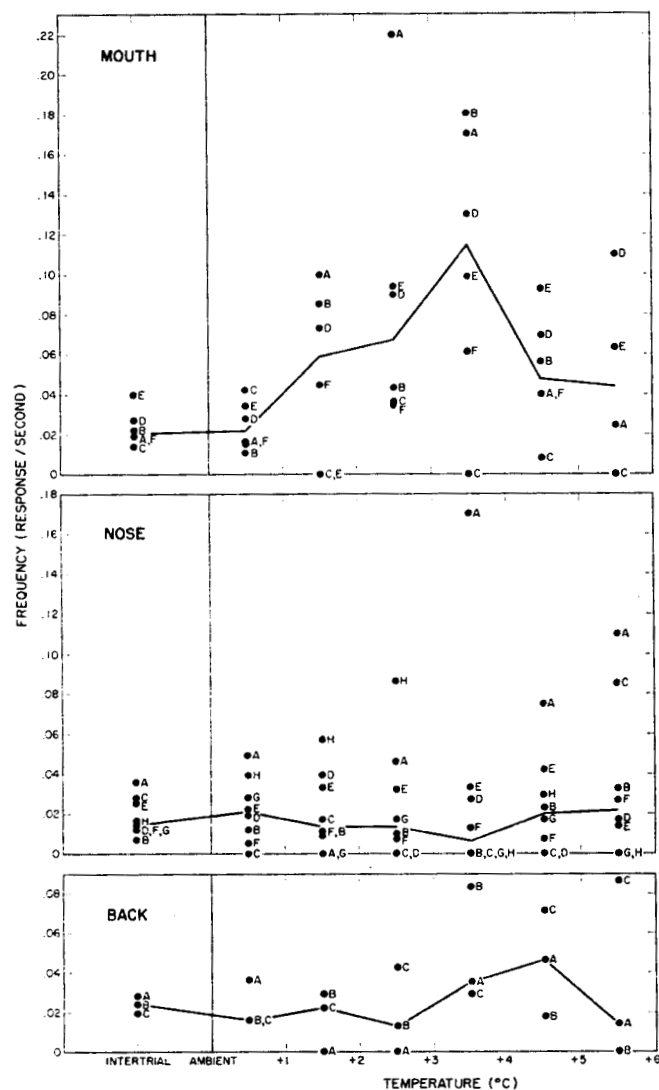


FIG. 2a. Response (heart beat rate decelerations) frequencies of six skipjack tuna before temperature stimulation (intertrial period) and during temperature stimulation by 1°C intervals. The temperature stimulus was applied to the mouth.
 b. Response frequencies of nine skipjack tuna to temperature stimuli in the nose.
 c. Response frequencies of three skipjack tuna to temperature stimuli on the back.

Temperature stimuli delivered to the mouth produced the most dramatic results (Fig. 2a). Frequencies of response varied significantly with temperature (Friedman two-way AOV by ranks, $0.01 < P < 0.02$; Siegel, 1956). Median

response frequency of the six fish was maximal in the +3 to +4°C interval, but an increase in the median was apparent in the +1 to +2°C interval.

The median frequency of response of the nose-stimulated fish did not vary significantly with temperature (Fig. 2b, Friedman test). Yet the one fish with a significant temperature effect (fish A) had the greatest frequency of response in the +3 to +4°C interval—similar to the mouth-stimulated fish.

Three responsive fish tested with stimuli directed to the back provided too little data to test statistically. However, there was a hint of increased responsiveness in the +3 to +5°C interval (Fig. 2c).

DISCUSSION

Temperature sensitivity

Work by Bull (1936), interpreted by Harden Jones (1968), demonstrated that a large variety of marine fishes in an unrestrained training situation and with prolonged conditioning can perceive temperature changes of about +0.03°C when the change occurs within 1–10 sec. In contrast, skipjack tuna stimulated in the mouth demonstrated perception in the +1 to +2°C temperature band (Fig. 2a). This change occurred within 1–2 sec. Under our experimental conditions skipjack tuna seemed to be less sensitive to rapid temperature changes than other fishes, by about two orders of magnitude. If this first estimate of temperature sensitivity is realistic, skipjack tuna would be able to perceive a relatively abrupt thermocline (0.5°C/m) but not the very gradual horizontal gradients (0.001–0.1°C/100 m) of the open sea unless they possess a mechanism for long-term thermal “memory”. Then, successive comparisons could be made that are sufficiently spaced in time in order for the necessary temperature difference of approximately 1°C to accrue. This would take about 20 min for a 0.50-m skipjack tuna swimming in a 0.1°C/100 m gradient at about two body lengths per sec.

By the methods employed skipjack tuna can perceive a temperature change of *at least* +1°C if the change occurs in 1 sec. Free-swimming unstressed fish can, no doubt, do better. Several factors contributed to reduce the performance of our fish:

- (1) No fish died during the 2-hr testing period and heart rates were (for the most part) constant; nevertheless, it was obvious that restraint is a highly artificial situation for tuna involving a barrage of unfamiliar, confusing and conflicting stimuli. These extraneous stimuli serve to mask and divert attention from the temperature change. Heart rates were generally higher (100–150 beats/min) than those observed in free-swimming tuna (75–125 beats/min), which suggests an excited state. Yet, previous experiments with hardier tilapia (*Tilapia mosambica*) using similar techniques yielded similar thresholds.

- (2) Because the temperature rise (CS) was gradual and not a step function, latency of response is confounded with sensory thresholds. It is conceivable that perception occurred at a smaller ΔT but the delay in organizing responses produced the apparent 1°C thresholds. However, the fish demonstrated almost no

delay in responding to the US. Furthermore, there was no consistency in latencies of response when different temperature slopes were employed. A slower rate of temperature change did not result in lower thresholds.

(3) Although actual conditioning of the fish to the temperature stimuli was desired, our results were not dependent upon it. It was sufficient to demonstrate that the observed responses were in some way controlled by the temperature stimulus. The tuna probably responded because the stimuli were of sufficiently high intensity. The fish did not have to learn an association between the noxious US and a weak CS. For this reason, responses were observed in many cases (especially with mouth stimuli) on the first trial. Perhaps repeated pairings of very weak temperature stimuli and the US would have eventually yielded lower thresholds. Bull (1936) had to train his free-swimming subjects for 2 months to obtain 0.03°C values. He found that as training progressed fish would respond with shorter latency after the onset of the temperature change. Rommel & McCleave (1972) required at least eighty trials to determine electric field thresholds. Yet, we observed no improvement in performance (response occurring at progressively lower temperature) when responding fish were trained for over thirty trials. Furthermore, if responses were not present after about fifteen trials, they did not appear after more trials. We strongly suspect that the declining condition of our restrained tuna made successful conditioning increasingly more difficult as the experimental session progressed.

Location of sensors

Thermal stimuli delivered to the oral-branchial cavity of skipjack tuna produced relatively the best responses. But, current belief holds that free-nerve endings in the skin over the entire body surface serve as the temperature sensors in fishes (Murray, 1971). This contention is based on little evidence. Response latencies from free-swimming fish preclude mediation by anything but surface receptors (Bull, 1936; Dijkgraaf, 1940). The unpublished cocaine treatment studies of Sullivan & Fisher (Sullivan, 1954) are most frequently cited as the definitive evidence of scattered, cutaneous thermal sensitivity; trout, whose body surfaces were desensitized by cocaine, failed to select temperature in a gradient.

Yet when temperature stimuli have been directed exclusively to the skin surface (summarized by Murray, 1971), response thresholds were higher by at least two orders of magnitude than those of free-swimming fish. Our results corroborate a lack of sensitivity in the skin surface. Free-swimming fishes have low temperature thresholds; our tuna seemed most sensitive to thermal stimuli directed into the mouth. In both situations, receptors in the oral-branchial cavity are exposed to temperature changes.

Alternatively, temperature perception (mouth vs. skin) in skipjack tuna may be different from that in other fishes. Free nerve endings in the skin of skipjack tuna are not found distal to a 150- μ deep layer of chromatophores. Other teleosts have endings virtually at the skin surface (J. I. Kendall, Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822, personal communication).

Perhaps denervation of the outermost skin surface has occurred concurrently with other adaptations of the skin of the tuna for continuous, fast swimming. Tactile sense may have been lost or downgraded in importance. Certainly the importance of a tactile sense should be less in a pelagic fish than in fish living near solid objects. In tuna, contact with any object except prey is very unusual; if it happens, damage to the tissues usually occurs. Another sensory restriction commensurate with a pelagic lifestyle does exist in tunas. In contrast to all other teleosts so far examined, skipjack tuna lack color vision (Tamura *et al.*, 1972). Skipjack tuna live in a virtually monochromatic environment.

From the data presented, we conclude that skipjack tuna do perceive abrupt temperature changes of at least $+1^{\circ}\text{C}$ with the greatest sensitivity within the oral-branchial cavity. This provides a first estimate of temperature threshold for a pelagic oceanic fish and is the first time cardiac conditioning has been used to evaluate thermal responses. Ongoing work is examining thresholds in free-swimming tuna and thresholds of temperature decreases of restrained tuna.

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Key Word Index—Temperature perception; tunas; thermoregulation; classical conditioning
